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ORAL PRESENTATIONS

O1

Loss of function mutation in a mitochondrial chaperone protein leading to dyregulated ROS production, and autoinflammatory disease in a single kindred

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Introduction: Reactive oxygen species (ROS) are known to have many roles in the propagation of the inflammatory process. Indeed increased leucocyte ROS have been implicated in a number of autoinflammatory diseases (TRAPS, FMF, CAPS and FCAS2).
Objective: To use genetic mapping, next generation sequencing and functional studies to identify the genetic cause for a severe unclassified autoinflammatory disease mimicking Wegener's granulomatosis in a consanguineous family.
Patients and methods: Three affected children in a Pakistani family suffered from a severe and unusual autoinflammatory syndrome, presenting in the first year of life with recurrent fevers, erythema nodosum-like rash, severe oromucocutaneous ulceration, systemic inflammation, and massively elevated serum IgD, without mutation in MVK. One of the affected children also suffered from multifocal sterile osteomyelitis with bony lytic lesions and died at age 12 months from bronchopneumonia, and acute cervical myelopathy from cervical vertebral collapse. The two older children were resistant to treatment with corticosteroids, colchicine, several different DMARDs, anakinra and infliximab. Both were cured by allogeneic haematopoietic stem cell transplantation (HSCT) in their teenage years and remain well and off all treatment approximately 6 years later. We performed homozygosity mapping in the three affected siblings, two unaffected siblings and their unaffected parents; followed by targeted capture and re-sequencing of an identified region of homozygosity. To study protein function, we used small interfering and short hairpin RNA knockdowns in THP1 cells. THP1 cell lines were also generated over-expressing wild-type and mutant protein harbouring the same missense variation identified in the patients. ROS levels were measured by flow cytometry and production by electron spin resonance (ESR). Co-localisation studies were conducted in HEK-293T cells and peripheral blood mononuclear cells using confocal microscopy.
Results: Within the 5Mb region identified from the homozygosity mapping; a missense variant of interest in a mitochondrial chaperone-like protein was discovered. This segregated with disease in the family. This variant was rare or absent in ethnically matched and other healthy controls. Knockdown of this gene in macrophage-like THP1 cells led to increased mitochondrial ROS production; whilst wild-type protein overexpression led to reduced levels of mitochondrial ROS, which was not observed with mutant protein overexpression.
Conclusion: We describe a novel monogenic autoinflammatory disease caused by a loss-of-function mutation in a mitochondrial chaperone protein, leading to dysregulated mitochondrial ROS production and severe autoinflammatory phenotype, and cured with allogeneic HSCT.

O2

Whole exome sequencing in systemic juvenile idiopathic arthritis

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Pediatric Rheumatology 2015, **13(Suppl 1):**O2

Introduction: Systemic juvenile idiopathic arthritis (sJIA) shares clinical features with classic monogenic autoinflammatory diseases, characterised by fevers, arthritis and evanescent rashes. Disease exacerbations are associated with elevated serum cytokine levels including 1L-1β, IL-6, and IL-18; and clinical response to anakinra, canakinumab and tocilizumab suggests that cytokine dysregulation is a key pathophysiological mechanism. Macrophage activation syndrome (MAS) may complicate sJIA, rendering individuals clinically indistinguishable from their familial haemophagocytic lymphohistiocytosis (fHLH) counterparts; with NK and CD8+ cell dysfunction leading to sustained immune cell activation and cytokine storm. Whilst there have been HLA associations and polymorphisms noted on sJIA genome-wide association studies, in rare cases mutations have been found in genes encoding key components of the inflammatory response, which may contribute to disease pathogenesis.
Objectives: We aim to identify rare variants in innate immune genes that contribute to cytokine imbalance and lead to the inflammatory phenotype seen in sJIA patients.
Methods: We performed whole exome sequencing on probands with sJIA and their parents (trios). Recessive, compound heterozygous and de novo inheritance models were tested and variants evaluated for potential functional effect using a combination of SIFT, PolyPhen and MutationTaster. We confirmed candidate pathogenic variants by Sanger sequencing and cross-checked these variants with the Infevers database, known fHLH causing mutations and inflammatory pathways. We harnessed CRISPR-Cas9

gene editing technology to model mutations in appropriate cell lines, and investigate the pathogenic nature of identified novel variants.
Results: Exome sequencing was performed on a total of 16 trios and unaffected siblings, with data from 13 trios suitable for analysis. There were no rare variants or indels inherited in a dominant, recessive or compound heterozygous manner, and predicted to affect function in a known inflammatory pathway. Furthermore, rare variants with predicted functional effects in Infever genes or fHLH genes were not identified. In total, there were 26 de novo variants (median = 2, range 0 to 6 per trio), with 17 of these predicted to affect protein function (median = 1, range 0 to 2 per trio). At least one de novo variant affects a known innate immune gene, and the pathogenic nature of this variant is being confirmed in vitro.
Conclusion: Disease in a small subset of patients with sJIA may be accounted for by rare de novo variants, however this requires further in vitro confirmation. Alternatively, it is possible that some of these individuals may in fact represent a separate disease entity.

O3

Role of RNH1 in the regulation of RNase H2 function

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Ribonuclease H2 plays an essential role for genome stability as it removes ribonucleotides misincorporated into genomic DNA by replicative polymerases and resolves RNA/DNA hybrids. Hypomorphic loss-of-function mutations in the genes encoding the three RNase H2 subunits cause the type I interferonopathies Aicardi-Goutières syndrome (AGS) and systemic lupus erythemotosus (SLE). We showed that in patients with AGS and SLE mutations cause enhanced levels of ribonucleotides in genomic DNA. We analyzed the proteomic environment of the RNase H2 complex and identified RNase Inhibitor 1 (RNH1) as an interactor. We validated the interaction of RNH1 with RNase H2 on an endogenous level using co-immunoprecipitation. Furthermore, we demonstrated that a siRNA-induced knockdown of RNH1 in HeLa cells causes low level DNA damage, activation of p53 and up-regulation of type I interferon-stimulated genes. These findings suggest a role of RNH1 in the regulation of RNase H2 function and implicate RNH1 in AGS pathogenesis.

O4

Generation of inducible immortalized bone marrow derived cell lines expressing mutant procaspase-1 C284A on a caspase-1 knock-out background

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Pediatric Rheumatology 2015, **13(Suppl 1):**O4

Introduction: Caspase-1, belonging to the family of cystein proteases, is well-known for its involvement in IL-1β and IL-18 maturation. Although, caspase-1 is one of the best described caspase-family-members, open questions concerning autoinflammatory diseases, caused by procaspase-1 variants (e.g. ICE-Fever), remain. ICE-Fever patients suffer from chronic febrile episodes, although procaspase-1 variants show reduced enzymatic activity leading to limited IL-1β secretion. The paradox of reduced IL-1β secretion but increased inflammation led to the hypothesis, that *CASP1*-variants enhance alternative signaling pathways. However, the role of procaspase-1 variants and their interaction partners during IL-1β maturation or NF-κB activation is still poorly understood.
Objectives: Studies with macrophages and dendritic cells are mainly limited by their low numbers *in vivo* and their difficult maintenance *in vitro*. In order to unravel the pathophysiological mechanisms of caspase-1 related autoinflammation, we aimed to generate inducible immortalized cell lines from transgenic mice expressing an inducible SV40 large T-antigen and mutant procaspase-1 C284A on a caspase-1 and/or receptor-interacting-protein 2 (RIP2) knock-out background.
Materials and methods: We crossed transgenic mice expressing an inducible SV40 large T-antigen with caspase-1 and RIP2 knock-out mice and generated a murine cell system which is in parallel immortalizable and contains the procaspase-1 C284A variant. After isolation of heterogeneous bone marrow cells, we differentiated cells into macrophages or dendritic cells by the supplementation of different colony stimulation factors and immortalized them by the addition of doxycyclin. Immortalized cells as well as de-induced cells (withdraw of doxycycline) were characterized and used for further stimulation experiments, western blot analysis, microscopy and Co-IPs.
Results: Inducible immortalized murine caspase-1 and RIP2 knock-out cell lines were generated and stable in long-term culture. Subsequent analysis of corresponding surface markers verified the differentiation status of the generated cell lines. Immortalized cells and de-induced cells were characterized and used for further stimulation experiments, investigating the consequences of enzymatically impaired procaspase-1 variant C284A and their interaction partners. We further established cell lines usable for live cell imaging of procaspase-1 wildtype or variant C284A and its interaction partners RIP2 and ASC.
Conclusion: In summary, we successfully expanded bone marrow derived macrophages and dendritic cells containing caspase-1 and RIP2 knock-out using conditional immortalization. The generated de-induced cells demonstrate the characteristic immunophenotype of primary cells. They thus represent a physiological model for further studies on the effects of the procaspase-1 variant C284A on inflammatory signaling pathways.

O5

Colchicine trial in PFAPA Syndrome and MEFV-negative patients

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Pediatric Rheumatology 2015, **13(Suppl 1):**O5

Introduction: PFAPA Syndrome (Periodic Fever, Aphthous stomatitis, Pharingitis, and cervical Adenitis) is the most common periodic fever in childhood; the diagnosis is based on clinical criteria. Familiar Mediterranean Fever (FMF) is a monogenic autosomal recessive autoinflammatory disease, whose diagnosis is based on clinical elements, supported by MEFV genetic mutations. When there is only a mutation or no one, the patient undergoes a trial with colchicine for 4-6 months, and diagnosis is confirmed in case of clinical response and fever early recurrence after suspension. Current treatment of PFAPA is symptomatic. Febrile episodes show a rapid response to the administration of one or two doses of prednisone (1-2 mg/kg) or betamethasone (0.1-0.2 mg/kg). Total requirement of steroid increases over time, and the frequency of attacks worsens the quality of life of patients. In literature, the prophylaxis of PFAPA febrile attacks with colchicine (0.5-1 mg/day) has been tested only on a few patients, with controversial results.
Objectives: Considering the similarities between FMF MEFV-negative patients (MEFVneg) and PFAPA patients, we aimed to demonstrate that colchicine is effective in PFAPA too: positive response was evaluated in terms of reduction in frequency >50% and severity of attacks >50%.
Materials and methods: We conducted a prospective cohort study (from September 2012, still ongoing), comparing two groups: 67 MEFVneg and 51 PFAPA patients. 36 of the latter group underwent colchicine trial, after obtaining informed consent.
Results: We assessed the response of PFAPA patients to colchicine preventive treatment: good response was observed in 75% (27 patients of 36), and a non-response in 25% (9 pts). The effective treatment rate of MEFVneg is 100%, by definition. The average dose of colchicine administered in PFAPA was 1.14 mg/day, compared to MEFVneg (1.34 mg/day). The dose per kilogram of body weight is 0.020 mg/kg/day in both groups. We can state that the colchicine dose requirement in PFAPA coincides to the one of FMF patients.
Conclusion: Our study showed that colchicine regimen is effective in 75% of cases. Prophylaxis with colchicine should be offered to all PFAPA patients, instead of steroids or other symptomatic therapy (as paracetamol

or ibuprofen), before the treatment with anti-IL1β biologic drugs, with considerable savings in pharmacoeconomics.

O6

Functional analysis of macrophages in Behçet's disease

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Pediatric Rheumatology 2015, **13(Suppl 1):**O6

Introduction: Behçet's disease (BD) is an inflammatory disorder of unknown cause. The previous genome-wide association studies identified the associations between BD and several loci. Among them, *CCR1*, *MEFV*, and *IL10* encode genes highly expressed in macrophages, suggesting roles of macrophages in BD.
Objectives: To evaluate functional differences of macrophages between BD and healthy controls (HC).
Methods: We have differentiated peripheral monocytes into M1 or M2 macrophages under presence of either M-CSF or GM-CSF, cytokines involved in M2 or M1 macrophage polarizations, respectively. Real-time PCR, western blotting, ELISA, and flow cytometric analyses were performed to evaluate CD68, CD163, and heme oxygenase (HO)-1 expressions.
Results: Expression of CD163, and numbers of M1 and M2 macrophages from BD are found to be similar compared with HC. HO-1 expression in sera and macrophages tend to be lower in BD.
Conclusion: Lower HO-1 expression in BD suggests functional alteration of M2 macrophages in BD. Further experiments are required to elucidate mechanisms how M1 or M2 macrophages are involved in pathogenesis of BD.

O7

Phenotypic variability in patients with ADA2 deficiency due to identical homozygous R169Q mutations

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Introduction: Deficiency of adenosine deaminase-2 (ADA2) is a recently described autoinflammatory disorder with cutaneous inflammatory disease, febrile episodes, cytopenias, splenomegaly and early-onset stroke. Several homozygous and compound heterozygous mutations in *CECR1* have been reported in these patients; however, pathogenesis is still poorly understood.
Objective: To determine the genotype - phenotype association in patients with ADA2 deficiency due to identical homozygous R169Q mutations in CECR1.
Methods: We performed a cohort study in nine patients diagnosed with ADA2 deficiency due to a homozygous R169Q mutation in the Netherlands and Belgium. Clinical and diagnostic data were collected from clinical files. We performed genealogy and haplotype analyses and measured serum ADA2 activity. ADA2 activity values were correlated to clinical symptoms.
Results: Age of presentation differed widely between patients (range: 0 mths to 8 yrs). The main clinical manifestations were (hepato)splenomegaly (9/9); skin involvement (8/9) and neurological involvement (8/9, of whom 6 encountered stroke). Considerable variation was seen in type, frequency and intensity of other symptoms, which included aplastic anemia, acute myeloid leukemia and cutaneous ulcers. Common laboratory abnormalities included cytopenias and hypogammaglobulinemia. ADA2 enzyme activity in patients was significantly decreased compared to healthy controls (0.78 vs 5.41 IU/L, $p < 0.0001$). Within the patient cohort, ADA2 activity levels tended to be lower in patients with stroke compared to patients without stroke (0.30 vs 1.57 IU/L, p= 0.064). No common ancestor for all families could be detected by genealogy, however, based on allele frequency, a Dutch founder effect can be noted. Three patients underwent hematopoietic cell transplantation, after which ADA2 activity was restored and clinical symptoms resolved.
Conclusions: This study revealed large phenotypic variability in patients with ADA2 deficiency though they carried the same homozygous R169Q mutation in CECR1. Epigenetic and environmental factors thus seem important in the phenotype. A trend towards a relation between stroke risk and low ADA2 residual activity was seen. Furthermore, hematopoietic stem cell transplantation appears promising for those patients with a severe clinical phenotype.

O8

The phenotypic variability of PAPA syndrome: evidence from the Eurofever Registry

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Introduction: PAPA syndrome is a very rare autoinflammatory condition. Few data are nowadays available about the clinical characteristic, the response to treatment and the outcome of this disease.
Objective: To analyse the data of the PAPA patients enrolled to the Eurofever registry.
Methods: The data analysed in the study were extracted from the Eurofever registry, which is hosted in the PRINTO website (http://www.printo.it). The patients were included in the study in the presence of mutations in the PSTPIP1 gene or, in genetically negative patients, in the presence of at least two of the following clinical manifestation: recurrent pyogenic arthritis, pyoderma gangrenousm or skin abscess with negative cultural tests. Demographic data, clinical manifestations and response to treatment were analysed.
Results: In March 2015 baseline and clinical information were available of 3200 patients from 88 centers in the Eurofever registry. Of the 27 patients classified as PAPA syndrome, 4 were excluded from the study. 23 PAPA patients (M: F =10:13), from 6 different centers, fulfilled the inclusion criteria and were therefore analysed: 10 were of the same family, in 4 cases one parent was affected (2 included in the registry), while in other 6 patients the family history was negative(of these 3 patients were genetically negative, while the other 3 had de novo mutations). The mean age at enrolment was 26,22 years (8 paediatric and 15 adult patients). The mean age at disease onset was 5,7 years (range birth - 18 years). The mean age at diagnosis was 24,5 years (range 1,8 - 57,5), with a mean delay of 18,8 years (range 2 months - 50 years). The mutations found in the PSTPIP1 gene were V344I (1pt), E250K (1 pt), E257G (1 pt), E277D (1 pts), A230T (2 pts), and E250Q (11 pts).
The disease course was recurrent in 13 patients, while the other 10 presented a chronic disease course with periodic recrudesces. 20 patients presented an articular involvement during their disease course, while 15 patients presented clinical manifestations affecting the skin (folliculitis in 11, pyoderma gangrenosum in 4, skin abscess in 9 patients);7 and 2 patients presented only the articular and skin involvement respectively. 2 patients complained with suppurative hidradenitis while 10 out of the 23 patients presented clinical manifestations not typical of PAPA syndrome (psoriasis, osteolytic bone lesions, chronic renal failure, muscular abscesses, anaemia

and hepatosplenomegaly). 12 patients were treated with NSAID with poor response while steroids caused a complete or partial control of disease manifestations in 5 and 8 patients respectively. Four patients were treated with methotrexate with partial response. Etanercept was used in one patient with complete response, adalimumab in 4 patients (3 partial and 1 complete responders) and anakinra in 5 patients (2 partial and 3 complete responders).

Conclusions: The study analyses the largest series of PAPA syndrome patients described so far. The wide clinical heterogeneity and the usual presentation with a single manifestation might be responsible for under-recognition of the syndrome.

O10

Differential expression of miR-4520a is associated with gain of function mutations in Familial Mediterranean Fever (FMF)

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Pediatric Rheumatology 2015, **13(Suppl 1):**O10

Introduction: MicroRNA signature of THP1 cells revealed a 5.9-fold decreased expression of miR-4520a following siRNA-mediated knockdown of *MEFV* gene that encodes pyrin [1].

Objectives: We herein sought to validate the expression levels of miR-4520a in monocytes isolated from peripheral blood mononuclear cells (PBMCs) of FMF patients.

Methods: Dual luciferase assay was used to validate a predicted miR-4520a recognition element in the 3'UTR region of the Rheb gene. The expression levels of pyrin, miR-4520a and its putative target Rheb were validated in monocytes from FMF patients (n=9) and compared with healthy controls (n=8). Patients were off colchicine for two days (attack-free period) and monocytes were isolated from PBMCs. Total RNA together with the respective miRNA-enriched fractions were isolated from monocytes and used for mRNA and miR-4520a quantitation by real-time PCR using the 2-ΔΔCt method after normalizing to 18S RNA and RNU6B genes, respectively. Protein levels of pyrin and Rheb were detected by western blotting.

Results: The relative expression levels of miR-4520a were variable among FMF patients and not significantly different between patients and controls. However, when patients that did not harbor any mutations in *MEFV* were excluded from the analyses, the expression of miR-4520a was statistically different between FMF patients and controls ($p<0.05$), indicating an association between miR-4520a expression and mutations in the *MEFV* gene. Moreover, stratification of patients group by genotype revealed an intriguing difference in miR-4520a relative expression, with carriers of M694V variant (combined group of homozygotes, heterozygotes and compound heterozygotes) showing the highest increase ($p<0.05$). Subsequent comparison between the M694V group and healthy controls showed a significant increase in miR-4520a expression levels that remained significant even after bonferroni correction ($p<0.01$). Interestingly, one of the homozygote M694V patients with the highest fold change in miR-4520a expression (FC=7.8) experienced an FMF-attack while on study, with a concomitant decrease in miR-4520a relative expression (FC=0.45). Bio-informatic analyses showed that miR-4520a is predicted to target genes implicated in autophagy through regulation of Rheb/mTOR signaling. Expression levels of Rheb were confirmed by luciferase reporter gene assays providing further evidence that Rheb is a direct target of miR-4520a ($p<0.01$). Validation of pyrin and Rheb protein expression levels in monocytes from FMF patients is in progress.

Conclusion: Our findings provide initial evidence that Rheb is a valid target of miR-4520a and suggest that a dysfunctional pyrin due to gain of function mutations with a dosage effect [2], especially of M694V variant, may be associated with an increase in miR-4520a expression levels, thus contributing to deregulated mTOR signaling and subsequently IL-1β release [3].

References

1. Mashreghi M F, Latsoudis H, Gruen J, *et al*: *Ann Rheum Dis* 2014, **73**:344-345.
2. Chae J J, Cho Y-H, Lee G-S, *et al*: *Immunity* 2011, **34**:755-768.
3. Schmitz F, Heit A, Dreher S, *et al*: *Eur J Immun* 2008, **38**:2981-2992.

O11

Activation of the pyrin inflammasome through the RhoA signaling pathway in FMF and HIDS

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Pediatric Rheumatology 2015, **13(Suppl 1):**O11

Introduction: Mutations in the genes encoding pyrin and mevalonate kinase (MVK) cause the autoinflammatory diseases familial Mediterranean fever (FMF) and hyperimmunoglobulinemia D syndrome (HIDS), respectively. The inflammation of both diseases is mediated by interleukin-1β (IL-1β). Recently it has been reported that pyrin forms an inflammasome, a multiprotein complex that mediates the maturation of IL-1β by activating caspase-1, in response to bacterial modifications of RhoA. However, the precise molecular mechanism by which the pyrin inflammasome is activated is unknown.

Objectives: To investigate the molecular mechanism of pyrin inflammasome activation and the molecular pathogenesis of FMF and HIDS.

Methods: We studied IL-1β production in immune cells from wild type mice and from several knockout and knockin mouse strains, as well as from FMF and HIDS patients and healthy controls, in response to LPS and/or various other bacterial toxins, and in the presence of pharmacologic agents targeting the Rho GTPase or adenylate cyclase pathways. Protein interactions were studied by immunoprecipitation.

Results: The Clostridial TcdB and C3 toxins, which inactivate RhoA, activate IL-1β maturation by a pathway that is *Mefv-*, *Asc-*, and *Caspase-1*-dependent, but *Nlrp3-*, *Nlrc4-*, and *Aim2*-independent. Leukocytes from FMF patients or FMF knockin mice produce IL-1β in response to LPS without a second signal; this is inhibited both by the bacterial CNF toxin, which activates RhoA, and by colchicine. In addition, the constitutive IL-1β secretion from FMF patients' peripheral blood mononuclear cells (PBMCs) or macrophages of FMF-KI mice is potentiated by cAMP, which has a role in suppressing RhoA through PKA-mediated direct phosphorylation. RhoA inhibition-induced inflammasome activation is mediated by reduced activities of downstream RhoA-effector kinases, Rho-associated coiled-coil-containing protein kinase (ROCK) and protein kinase N1 (PKN1). Indeed, the kinase domain of the RhoA-effector kinases binds to pyrin directly and phosphorylates two serine residues (S208 and S242 of human pyrin). The phosphorylated pyrin is recognized by 14-3-3 proteins, which negatively regulate the pyrin inflammasome. The binding affinity of 14-3-3 proteins for the pyrin of FMF-KI mice is substantially lower than for wild-type mouse pyrin, which lacks a B30.2 orthologous domain. The constitutive IL-1β secretion from macrophages of FMF-KI mice as well as FMF or HIDS patients' PBMCs is attenuated by activating RhoA-effector kinases. Defects in prenylation, seen in HIDS, lead to RhoA inactivation and consequent pyrin inflammasome activation.

Conclusion: These data directly implicate Rho GTPase in the regulation of the pyrin inflammasome, and suggest that this pathway is also important in HIDS.

O12

Familial Mediterranean Fever in childhood: a single center experience

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Introduction: Familial Mediterranean fever (FMF) is an autosomal recessively inherited autoinflammatory disease which is clinically manifested with periodic episodes of fever, polyserositis and arthritis. Among the Turkish, Arabic, Armenian and Jewish population the incidence

rate ranges between 1 in 500 to 1000. The severity of the disease depends mostly on the MEFV gene mutation variations.

Objective: The objective of this study is to reveal a single center follow-up experience of a wide amount of childhood FMF patients from different regions around Turkey in terms of the demographic and clinical features, the genetic diversity and treatment response.

Material-methods: 708 children diagnosed with FMF and under treatment of colchicine for at least 6 months that are seen out patiently in our Pediatric Rheumatology Clinic between November 2014 and March 2015 were reviewed retrospectively with the data based on the patient records and also taken from the parents.

Results: 708 patients consisting 362 males and 246 females that are diagnosed with FMF were included in the study. The mean age of the patients by the time of the study was found to be 12,3 ± 4,4 years, while the mean age at the onset of the disease was 4,8 ± 3,4 years and at diagnosis was 7,3 ± 3,8 years. The consanguinity rate was resulted to be %29,2 and positive family history was detected in 370 (52,3%) patients.

In 634 (89,5%) of patients, episodes of abdominal pain for at least 6 hours due to peritonitis; in 629, (88.8%) periods of fever for at least 12 hours and in 122 (17,2%), chest pain probably due to pleuritis was found. 213 (30,1%) patients experienced erysipelas like erythema and 288 (40,7%) were diagnosed with findings of arthritis that last for at least a day. In 467 (66%) cases exertional leg pain and in 29 (4,1%) myalgia are among the complaints and enthesitis was reported in 26 (3,7%) patients. Pericarditis was developed only in 2 (0,3%) patients. The mean duration of the attack was found to be 64,8 ± 38,5 hours. In 38 (53,4%) patients with appendectomy was performed due to unresolved episodes of abdominal pain.

The patients were investigated about the MEFV gene mutations and M694V homozygote mutation was found in 154 (21,8%) and M694V heterozygote in 141 (19,8%) children. All the other mutations in exon 10 region (M680I, V726A, M694I) in a compound heterozygous manner with M694V mutation were detected to be in 90 (12,7%) cases and the rate of patients that is a carrier of only one copy of exon 10 region mutations other than M694V was %13. The rest of the mutations (in exon 2,3,5 regions) were revealed in 45 (6,4%) children. In 45 (6,4%) of patients none of the so far known main mutations were shown.

Amyloidosis had been developed only in two cases both of whom were suffering from the disease process for at least 10 years and showing defective compliance to the colchicine treatment. One of them was a 20 years old M694V homozygote mutation carrier and the other was happened to be compound heterozygous with M694V/M680I mutations.

All of the patients were under colchicine treatment, of which 57 (8,1%) were uncompliant. As the side effects of the treatment were evaluated, diarrhea was found out to be in the first rank with the rate of 47(6.6%), then comes the elevation in serum transaminase levels by 10 (1.4%). Only in one case leukopenia and also in another case alopecia developed.

The response to colchicine treatment was investigated and in 559 (79%) patients no episode occurred in the last year. In 149 (21%) cases periodic episodes were reported by the mean rate of 4,9 ± 4,4 per year (median 3/year, ranging between 1-24/year). After the follow up under treatment for at least 6 months the mean episode duration was decreased to 39,7 ± 29,4 hours (median 24 hours ranging between 6-168 hours).

The patients who are taking their colchicine treatment properly but still complaining about at least 6 episodes of any possible manifestations per year were considered resistant and were found to be in the rate of 47 (6,6%) in our cohort. In resistant cases, the most detected mutations were homozygous M694V (71.4%) in the first range and then compound heterozygosity with M694V/M680I.

Conclusion: The diagnosis of childhood FMF is frequently encountered in our country. The most severe clinical presentation occurs as a result of M694V and other exon 10 region mutations. With the absolute compliance to treatment; the episodes disappear and amyloidosis, the most dreadful complication of the disease, can be prevented.

O13

Investigation of the inflammatory cell migration process in familial Mediterranean fever

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Pediatric Rheumatology 2015, **13(Suppl 1):**O13

Introduction: Familial Mediterranean fever (FMF) is one of the most common autoinflammatory disorders and is characterized by episodic attacks of fever, along with inflammation. FMF pathogenesis is associated with various mutations in the MEFV gene, which encodes pyrin. Pyrin is expressed predominantly in neutrophils that have an important role in the innate immune response. Several proteins related with actin machinery have been identified as pyrin-interacting proteins in our *in vitro* cell migration models. Thus, in this study, we hypothesized that pyrin may have a key role in neutrophil migration during inflammation and decided to do functional analysis on pyrin silenced cell lines and primary neutrophils.

Objectives: In this study, we aimed to analyze the possible role of Pyrin in cell migration process in both neutrophil cell line and primary neutrophil cells isolated from FMF patients.

Patients and methods: HL-60 cells, a neutrophil-like cell line, were cultured and differentiated. MEFV gene was silenced with MEFV siRNA. The expression level of pyrin was assessed by using western blot. Cells were stimulated for migration using fMLP (N-formyl-Met-Leu-Phe) after that cells were co-stained with pyrin and actin to see polarization. Colocalizations were analyzed by drawing profile and correlation curves with the help of confocal microscopy. Blood samples were collected from 2 controls and 3 M694V/M694V patients. Neutrophil cells were isolated with Lympholyte-poly solution. A modified Boyden Chamber assay was used to detect chemotaxis range of the neutrophils. Equal numbers of cells were migrated towards the gradient of fMLP for 24 hours. Then the migrated cells were stained by 4 uM calcein-AM and visualized under fluorescence microscopy.

Results: According to the MEFV siRNA experiments, pyrin was silenced in HL-60 cells with %80 efficiency. In this high efficiency rate, when cells stimulated for migration, they showed less actin polymerization compared to control cells and appeared as round shape instead of having polarized shape. Chemotaxis experiments using primary neutrophils showed that the migration rates of patients' neutrophil cells were 6 times higher than the controls ($p<0.05$). The average of the migrated cell numbers in patient and control group was 38×10^3 and 5×10^3, respectively.

Conclusion: We have demonstrated that mutant pyrin causes an increase in the neutrophil migration rate. Besides, when pyrin is silenced, the ability of the cell migration is decreased and the cells get less polarized shape. Thus these results suggest a pro-inflammatory role of pyrin in the regulation of inflammation by influencing the cell migration process possibly at the early phase of the migration by interaction with actin. In conclusion, the studies described here provide a new insight to the potential role of pyrin protein in the process of neutrophil migration during inflammation.

O14

Molecular modeling of complete tertiary structure of pyrin and influence of mutations on it

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Introduction: Pyrin protein is the product of the MEFV gene, mutations in which cause the manifestation of Familial Mediterranean Fever (FMF). Complete tertiary structure of pyrin and the effects of mutations on it are still experimentally not studied. Mutations - E148Q, M680I, M694V, M694I, V726A, A744S and R761H of pyrin induce manifestation of the most widespread and severe forms of FMF. One striking feature of FMF is the phenomenon of complex allele mutations. In case of complex allele mutations, the pyrin protein will contain more than one mutated amino acids. From currently known complex allele mutations there are those that combine the most widespread and severe single mutations - M680I-M694I, E148Q-M694V, E148Q-M694I, E148Q-V726A, E148Q-A744S, E148Q-R761H, E148Q-V726A-R761H. In complex alleles, one mutation may have a modifying effect on the other one. The other striking feature of FMF is the fact that despite the fact that the FMF is considered as an autosomal recessive autoinflammatory syndrome, were detected mutations - T577N и

T577S for which was proved autosomal dominant inheritance. Understanding the correlation between the FMF phenotype and genotype is further obscured by the existence of complex allele and dominant mutations. Therefore, we suggest that computational modeling of native and mutated pyrin complete tertiary structure and their comparative investigation will help to understand the effects of abovementioned mutations on pyrin and on FMF manifestation in general.

Objectives: From abovementioned the goal of current study was to detect the effect of mutations on the complete tertiary structure of the pyrin protein for clarifying its functions in the autoinflammatory processes and in the pathogenesis of FMF.

To achieve our objectives the following tasks were set:

1) Develop a computer model of the complete tertiary structure of pyrn and validate the obtained model.

2) Develop a computer model of the tertiary structure of pyrin with single, dominant and complex allele mutations.

3) Analyze the impact of mutations on the tertiary structure of pyrin.

4) Compare the effects of dominant and complex allele mutations influence with the single recessive mutations effects in order to clarify the effect of dominant and complex allele mutations to the FMF.

Methods: Molecular modeling of pyrin native tertiary structure and its mutant variations was carried out using the software package ROSETTA 3.5 using de novo and threading modeling methods. For validation and to determine the correctness of the obtained model VADAR and RESPROX programs was used. Models visualization and analysis were performed using VMD program. These software packages have been used in the operating system Linux, by 24-nood computer cluster and HPC of M.V. Lomonosov Moscow State University.

Results: Using de novo and threading modeling methods was obtained 1000000 models of the pyrin protein tertiary structure. Then was carried out validation of the pyrin structure best model and assessment of its stereochemical correctness. As a result, it was found that obtained model have a resolution of 1,6 Å, which is a high resolution of stereochemistry correctness for a such large protein. In order to study the effect of mutations on the tertiary structure of pyrin were modeled tertiary structures for the abovementioned single, dominant and complex allele mutations, using homology modeling method. For each mutation 10000 models have been obtained. After which the analysis and comparison of the native and mutated tertiary structures of pyrin were carried out, which showed that mutations lead to structural rearrangements such as: transition loop - β-sheet, loop - α-helix, β-sheet - loop, β-sheeted - α-helix, α-helix - loop and α-helix - β-sheet. Also were observed rearrangements leading to elongation of β-sheets and α-helices, to a shortening of β-sheets and α-helices and to partition of one large β-sheet into two smaller ones.

Conclusion: From obtained results the following conclusions can be made:

1) Have been obtained the model of pyrin complete tertiary structure with a resolution of 1.6Å.

2) It was found that all the studied mutations lead to structural rearrangements affecting on the tertiary structure of pyrin.

3) in spite of the domain affiliation of studied mutations, they lead to structural rearrangements in other parts of the pyrin also.

O15

First report of *MEFV* duplication in FMF patient

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Introduction: Familial mediterranean fever (FMF) is a rare monogenic disease and the prototype of autoinflammatory disorders. It is caused by mutations in the *MEFV* gene and is autosomal recessively inherited. Most mutations are missense substitutions, small deletions are quite rare, and only three nonsense mutation has been described (http://fmf.igh.cnrs.fr/ISSAID/infevers/). Large rearrangements have been searched for in the frame of a collaborative project including 216 patients but were not identified.

Objectives: We report here the first case of *MEFV* duplication in a FMF patient.

Patients and methods: The proband is a 21 years-old woman who presented with classical FMF phenotype: recurrent fever, arthralgia, and abdominal pain with vomiting. Attacks lasted three days and biological inflammation was documented with elevated C-reactive protein. Her father is Armenian and her mother Malagasy, and both are asymptomatic. We performed Sanger analysis (ABI3130x, Life Technologies) of the *MEFV* gene, quantitative polymerase chain reaction (qPCR) (LighCycler, Roche) and deep-sequencing (Nextera Rapid Capture, Illumina) (MiSeq, Illumina). Microsatellite analysis (ABI3130x, Life Technologies) was also performed.

Results: We identified a well-known severe mutation: p.Met694Val, and a controversial variant: p.Glu148Gln. Parental testing confirmed that the variants were non-allelic. Sanger sequencing displayed unbalanced ratio of the mutated and wild type alleles. Mosaicism was excluded because all polymorphisms were found at the same 1:2 ratio. DNA contamination was ruled out through microsatellite analysis. We thus suspected a gene micro-rearrangement. qPCR and deep-sequencing revealed a heterozygous duplication of the entire wild *MEFV* gene. The two surrounding genes (*NAA60* and *OR1F1*) were not duplicated demonstrating that this rearrangement was confined to the *MEFV* region. qPCR analysis showed that the duplication was inherited from the mother.

Conclusion: We report here the first FMF patient with 1/3 dose of p.Met694Val. Interestingly, the patient's phenotype seemed not to be impacted by the "dilution" of the pathological variants.

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

O16

Endothelial biomarkers in patients with familial Mediterranean fever associated vascular disease and vasculopathy

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Objectives: FMF is an autosomal recessive disease, caused by mutations in the MEFV gene, encoding the pyrin protein associated with the interleukin-1β related inflammation cascade. In FMF overproduction of IL-1β and uncontrolled TNF-α release has been documented. These cytokines mediate activation of endothelial cells causing sustained inflammatory response and endothelial cell dysfunction[1]. Vascular injuries are frequently seen in FMF patients although the exact mechanisms are not well understood. We design this study to determine the role of endothelial biomarkers in pathogenesis of vasculopathy in FMF.

Methods: The study cohort included 50 FMF patients with vascular involvement (VI, mean age 39.7±12, male/female 23/27) - group 1, and 52 FMF patients without VI (31.6±11, 32/20) - group 2. A total of 50 healthy subjects between 16-67 years of age were enrolled as controls. All patients diagnosed in accordance with Tel-Hashomer criteria. VI was determined by physical examination, lab tests and instrumental modalities (ECG, Doppler echocardiography, CT-scan and MRI). The patients group with VI included 30 FMF patients with coronary heart disease (2 of them with myocardial infarction), 14 with pulmonary hypertension, 2 with stroke, 1 with poliarteritis nodosa, 1 with Henoch-Schonlein purpura, 1 with livedoid vasculopathy and 1 with Raynaud's phenomenon. Laboratory tests, including leucocyte count, erythrocyte sedimentation rate, fibrinogen, C-reactive protein, serum amyloid-A, nitric oxide and endothelin-1 were carried out on all patients in attack free period. CRP was determined by immunoturbidimetric method and SAA by ELISA. Serum NO and plasma ET-1 levels were measured by Griess reaction and enzyme immunoassay, respectively. Normal values of NO and ET-1 in our laboratory were <20μmol/L and 7.2±4pg/ml, respectively.

Results: Fibrinogen and leucocyte count were normal in patient groups (mean±SD, group 1 vs group 2) 4.46±1.1g/L vs 3.57±1 and 8.5±2.4 ($x10^9$)g/L vs 7.4±1.6, whereas ESR was increased 30.3±18.7 mm/h vs 19±12.6. Mean CRP and SAA were significantly higher in group 1 than in group 2 (21.06 ±16.8 mg/L vs 12,5±12.1, t=2.94, p<0.01; 30.56±63.3 mg/L vs 10.47±32,79, t=2,0013, P<0,05, respectively). The mean level of NO was lower in group 2 than in controls (3.12±0.75μmol/l vs 8.79±2.72 μmol/l, t=14.2, p<0.0001) and in group 1 than in group 2 (2.79±0.7 μmol/l vs 3.12±0.75 μmol/l, t=2.3, p<0.05), though it was within normal ranges. Although there were no correlations between NO, fibrinogen, leucocytes, ESR and SAA in both patients group, there were negative correlations between NO and CRP (r=-0.338, p=0,016, group 1; r=-0,398, p=0,003, group 2). There was a

significant difference in ET-1 levels between the controls and the patient group 2 (7.8.±1.99 pg/ml vs 16.2±7.32 pg/ml, t=7.97, p<0.0001) and between the patient group 2 and 1 (16.2±7.32 pg/ml vs 23.13±14.8 pg/ml, t=2.98, p<0.05). Also ET-1 levels showed positive correlations with CRP in patient groups (r=0,585, p=0,000, group 1 and r=0,285, p=0,041, group 2), and with SAA in group 1 (r=0,459, p=0,001). No correlations were found between ET-1, fibrinogen, leucocytes and ESR.

Conclusions: Endothelial biomarkers were found abnormal in patients with FMF. We have shown that levels of NO in FMF patients were normal, but lower compared with healthy controls. Expression of NO is induced drastically by multiple stimuli- interferon-α, tumor necrosis factor-α and IL-1β that induce iNOS (inducible nitric oxide synthase) expression in macrophages, leucocytes and vascular endothelial cells [1]. NO inhibits the biochemical effect of ET-1, however, ET-1 induces iNOS expression in endothelial cells. Impaired NO production may contribute to the pathogenesis of vascular injury in FMF.

We have revealed an increased ET-1 level in the patients with FMF associated vascular disease. We have also demonstrated that there were significant correlations between ET-1 levels and inflammatory markers, such as CRP and SAA, in the patients with FMF associated vascular disease. We conclude that continuous low-grade inflammation in FMF may cause vascular injury and endothelial dysfunction that may lead to vasoconstriction and ischemic complications in FMF patients with the development of cardiovascular, pulmonary, neurological and other vascular events. Last but not least, endothelin-1 may have a role in the pathogenesis of FMF associated vascular disease.

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Reference

1. Sprague AH, Khalil RA: Inflammatory Cytokines in Vascular Dysfunction and Vascular Disease. *Biochem Pharmacol* 2009, **78(6)**:539-552.

O17

NGS for the diagnosis of autoinflammatory diseases: the experience of Montpellier

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Introduction: Monogenic autoinflammatory diseases present with overlapping clinical features such as recurrent fever, biological inflammation, abdominal pain, arthritis, and sometimes disabling complications. Over 30 genes have been confirmed or hypothesized as causing diseases. Sanger sequencing is the gold-standard approach for genetic diagnosis, but cannot be exhaustive.

Objectives: We aimed at offering a quick and efficient service for these genetically heterogeneous disorders through NGS sequencing of all published and candidate (N=32) autoinflammatory genes.

Patients and methods: 34 patients were selected on clinical grounds by members of the French reference center for autoinflammatory diseases (CeReMAI). 9 of them were considered positive control samples as they had previously known variants (by Sanger sequencing) and were run in parallel for methodological validation.

A custom panel of 108kb was designed to capture the genomic regions of interest using the Illumina Nextera Rapid Capture enrichment DNA preparation kit. MicroV2 chip were then loaded with 12 samples on a MiSeq equipment for multiparallel sequencing. Annotation and filtering were performed with Seqnext software (JSI), and annotation was performed with Alamut Visual (Interactive Biosoftware).

Results: We obtained a 545X average coverage and only 2 exons were not captured. Minimal coverage was 40X. Results were concordant in 100% of the cases with Sanger analysis. We uncovered pathological mutations or variants of unknown significance in 17 patients. Of note recurrent genes were *CARD14, NOD2, PSTPIP1* and *SCL29A3*. About 20 new potentially pathogenic sequence variants were found in published genes.

Conclusion: This innovative sequencing approach demonstrated high performance (accuracy, the precision, analytical sensitivity and specificity) in evaluating mutations in known autoinflammatory genes. The current positive rate using sequential Sanger sequencing of 1-4 genes in the French network is quite stable around 10-15%. The NGS approach allowed diagnosis of approximately another 20% of patients.

O19

Severe autoinflammatory disease caused by mutation in a gene controlling actin cytoskeletal dynamics and cure with allogeneic haematopoetic stem cell transplantation

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Introduction: The actin cytoskeleton is crucial at many junctures of normal immune function, and consequently there are many immune specific regulators of actin dynamics. A growing number of primary immunodeficiencies are being defined as caused by mutations in the genes encoding these regulators. In addition to immunodeficiency, immune dysregulation and autoinflammation are increasingly recognised to arise from defects within this pathway.

Objectives: To use next generation sequencing, and functional studies to identify the genetic cause for a severe unclassified autoinflammatory disease mimicking Behcet's disease in a consanguineous family.

Patients and methods: Two affected children in a consanguineous Pakistani kindred suffered from an unclassified autoinflammatory syndrome presenting in the first year of life with: severe oral ulceration resulting in scarring; perianal cutaneous ulceration; severe sterile recurrent fevers; and intermittent episodes of thrombocytopenia associated with intercurrent (presumed) viral infections. Both children were partially responsive to corticosteroids; DMARDS were largely ineffective. TNF-alpha blockade was ineffective; IL-1-beta blockade with Anakinra was partially effective. Despite that the older sibling died at the age of 14.5 years from severe sterile systemic inflammation, thrombocytopaenia, and multi-organ failure. In view of this, the younger (index) sibling underwent allogeneic haematopoietic stem cell transplantation (HSCT) at the age of seven years, and is 100% engrafted 23 months later with complete cure of her illness.

DNA from the two patients, two unaffected siblings and their parents were genotyped for homozygosity mapping. One of the affected patients was exome sequenced, and the homozygous regions scrutinised. Variants of interest segregating with disease were confirmed with Sanger sequencing. Patient cells were analysed by flow cytometry and confocal microscopy to visualise polymerised actin levels and phagocytic activity of dendritic cells (DCs). Cellular motility was assessed using a Dunn chamber. These assays were also used to assess THP1 monocytic cell lines modified with shRNA and derived to macrophage-like and DC-like phenotypes. Wild-type and mutant sequence were tagged with mCherry fluorescent protein and overexpressed in HEK393T cells.

Results: The index case demonstrated normal T-cell activation to PHA but absent T-cell response to anti-CD3, highly suggestive of a defect in actin-regulated organisation of the immune synapse. A homozygous missense mutation was identified in a gene encoding a regulator of actin stability. CD20+ and CD56+ (but not CD3+) cells showed increased levels of polymerised actin. Dendritic cell and neutrophil motility was disrupted. The mutation identified resulted caused abnormal cellular localisation of the encoded actin-regulating protein.

Conclusion: We describe a novel monogenic autoinflammatory disease with sterile inflammation and intermittent thrombocytopenia in association with periodic fever. This resulted from a mutation in a regulator of the actin cytoskeleton, and was resistant to conventional immunosuppression, though ultimately cured by HSCT.

O20

Update on *CECR1* molecular diagnostics: new mutations in the deficiency of ADA2 (DADA2) and the North American polyarteritis nodosa (PAN) cohort

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Background: Loss-of-function mutations in *CECR1*, encoding adenosine deaminase-2 (ADA2), have been associated with a spectrum of vascular and inflammatory phenotypes, ranging from early-onset vasculopathy manifesting as recurrent stroke, to systemic vasculitis manifesting as polyarteritis nodosa (PAN) and Sneddon's Syndrome.
Objectives: Knowledge about the ADA2 genotype and phenotype has only recently started to emerge. We aimed to further characterize the genotype-phenotype spectrum associated with *CECR1* mutations.
Patients and methods: 19 newly diagnosed DADA2 patients and 92 DNA samples of the North American cohort with PAN were screened for mutations in *CECR1* by Sanger sequencing of all coding exons.
Results: 14/19 patients were homozygous or compound heterozygous for rare or novel missense mutations in the coding region of *CECR1*. In 5 patients, we have detected only one likely disease-associated mutation; further analysis is necessary to identify a possible genomic deletion or a second mutation in the non-coding region of *CECR1*. We have found three rare missense mutations that were not previously associated with DADA2: A357T, G358R, and L249P. More importantly, we have identified four novel mutations that cause DADA2: T129P, K55del, N370K and N423K. The R169Q mutation is a founder mutation in the Dutch population, while the G47R mutation is a founder mutation in the Middle Eastern and Pakistani populations.
In the PAN cohort, we identified 6/92 patients with mutations in *CECR1*. Three patients carry biallelic homozygous or compound heterozygous mutations, and three patients are carriers for a single mutation in *CECR1*. Four rare variants are reported in Ensemble or ExAC, but they have not been previously associated with PAN: P106S, F355L, V349I, and T65M. We have identified one novel mutation in the cohort with PAN: E328K.
Conclusion: The *CECR1* gene is highly polymorphic, and interpreting identified gene variants should be done cautiously. When possible, parental samples should be used to demonstrate proper inheritance of biallelic variants. Biochemical assays may help to complement molecular diagnostics. We have identified a significant number of patients who carry only a single novel or rare mutation in *CECR1*.These patients should be analyzed for the presence of structural or non-coding variants in *CECR1*. Alternatively, we will consider the possibility that single mutations may act as susceptibility alleles for complex forms of vasculitis. Our study expands on the role of ADA2 in the pathogenesis of PAN in non-founder populations.

O21

Clinical follow-up on a cohort of patients with deficiency of adenosine deaminase 2 (DADA2)

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Pediatric Rheumatology 2015, **13(Suppl 1):**O21

Introduction: We previously reported a syndrome of intermittent fevers, early-onset lacunar strokes, livedoid rash, hepatosplenomegaly, immune deficiency, and systemic vasculopathy, associated with loss-of-function mutations in *CECR1*. An additional report by others expanded the clinical spectrum to include patients with cutaneous polyarteritis nodosa.
Objectives: We now present clinical follow-up on our reported 5 patients and an additional 9 patients.
Patients and methods: We evaluated the 14 patients at the National Institutes of Health. All patients were enrolled in an IRB approved study. We performed whole-exome sequencing in the initial 3 patients and their unaffected parents and candidate-gene sequencing in the other 11 patients. Clinical information and radiographic and laboratory testing were obtained at each visit.
Results: All patients had 2 mutations in *CECR1*. 11/14 patients reported recurrent fevers.
10/14 patients had at least one stroke, with 8/10 before the age of 5 years. Magnetic resonance imaging showed evidence of acute or chronic small subcortical infarcts involving the deep-brain nuclei and the brain stem, consistent with small-vessel occlusions (lacunar strokes). Three patients had additional hemorrhagic strokes. In 10/10 patients, magnetic angiography showed no evidence of cerebral vasculitis.
All 14 patients demonstrated livedo racemosa. Erythematous papules or nodules were seen in 11 of these patients.
Hepato- and/or splenomegaly was observed in 10/14 with 3 patients demonstrating portal hypertension. One patient developed a perforated small bowel requiring resection.
Hypertension was noted in 2 patients. Prolonged QT was reported in 3 patients.
12/14 demonstrated hematologic abnormalities including anemia, leukopenia, and/or thrombocytopenia. Elevation of acute phase reactants was reported in 13/14 patients.
Low serum iron was noted in 8/10 patients tested.
10/13 presented with hypogammaglobulinemia, however, this may reflect prior treatment with cyclophosphamide in 3 patients.
Most patients had received a number of medications over the course of their disease. It was our practice to discontinue aspirin and/or anticoagulation in all of our DADA2 patients. We observed striking improvement in CRP, ESR, CBC, and serum iron in 10/12 patients receiving anti-TNF agents.
Conclusion: We have expanded the clinical picture of our cohort of patients with DADA2 to include multiple strokes, livedo racemosa, cutaneous PAN, portal hypertension, hematologic abnormalities, vascular pathology and mild immunodeficiency. In addition, we have demonstrated both clinical and laboratory improvement following treatment with anti-TNF agents.

O22

NLRP1 mutations cause autoinflammatory diseases in human

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Introduction: Inflammation is a vital and complex process in response to diverse tissue damaging stimuli such as trauma, injury and pathogen. NLRP1, NLRP3 and NLRC4 belonging to the intracellular proteins Nod like receptor family, are capable of sensing the inflammatory inducers and trigger the assembly of a large complex called the inflammasome. By inducing the caspase-1 activation, inflammasome plays a crucial role in the release of IL-1β and IL-18, two critical cytokines of the initial steps of inflammatory responsesand, in some cases, the induction of a pro-inflammatory cell death called pyroptosis.
Whereas mutations in *NLRP3* and *NLRC4* have been linked to two rare monogenic systemic autoinflammatory diseases (SAIDs), several polymorphisms in the *NLRP1* gene have been associated extensively to an increased risk of autoimmune disorders (e.g. vitiligo, psoriasis, type 1 diabetes, and rheumatoid arthritis). We identified for the first time two distinct *NLRP1* mutations in patients displaying a novel systemic autoinflammatory disease (SAID) and a novel syndrome combining autoinflammation and autoimmunity.
Objectives: The aim of our study was to unravel how mutation in NLRP1 impaired its function and triggered autoinflammation.
Materials and methods: Peripheral blood mononuclear cells from patients and healthy donors were analyzed to identify the immunologic components involved in these novel diseases, using flow cytometry and *ex vivo* NLRP1 inflammasome stimulation. The pathogenic effect of the *NLRP1* mutations in inflammation was investigated using *in vitro* functional assays in transfected HEK293T.
Results: An immunophenotyping in one patient revealed high numbers of granulocytes, CD64+ neutrophils, NK cells and immature blood B cells (CD20+CD27-CD38highCD24high). The level of caspase-1 in serum samples from patients was increased as compared to controls and unaffected parents. Moreover, patient's cells displayed constitutive production of IL-1β. Functional studies in HEK293T are ongoing in an attempt to confirm constitutive activation of the NLRP1 inflammasome suggested by our results on patient's samples.
Conclusion: We demonstrated for the first time that two mutations in the *NLRP1* gene are involved in autoinflammation in human. Our data,

combined to the literature, highlight the pleomorphic roles of NLRP1 in inflammation and immunity.

O23

Inflammatory Cytokine response in a cohort of patients carrying novel NLRP12 variants

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Introduction: The NLRP12 related autoinflammatory disorder (NLRP12-RD) is a rare autosomal dominant disease, caused by mutations in the NLRP12 gene, a member of NLRs family involved in negative regulation of NF-kB pro-inflammatory pathways. At present, few cases have been reported, although novel NLRP12 variants have recently been described.
Objective: To assess *ex vivo* the production of inflammatory cytokines, in patients carrying different NLRP12 variants not yet demonstrated as being associated to NLRP12-RD, in order to demonstrate the potential functional and pathogenic role of these variants.
Materials and methods: 30 children, carrying NLRP12 variants in heterozygosis, and 1 patient, carrying the F402L variant in homozygosis, were identified using Next Generation Sequencing [G39V (n=16), F402L (n=8), H304Y (n=3), T260M (n=2) and G448A (n=1)]; clinical information was collected for all patients. Patients with G39V were excluded from subsequent analysis, due to its high frequency both in our cohort (17.4%) as in the general population. Whole blood cultures from NLRP12 patients and Juvenile Idiopathic Arthritis (JIA) patients (n=10, pathological control) were stimulated *ex vivo* with TLRs ligands (LPS 1-10-100 ng/mL, Zymosan 10 μg/mL), with or without the further addition of ATP, for 5 or 22 hours. TNF-α, IL-1β and IL-6 were measured in supernatants by ELISA.
Results: Among all stimuli used, we observed a clear inflammatory response in cells stimulated with 10mg/ml of Zymosan. Indeed, we found that the TNF-α release, after stimulation with Zymosan, was higherboth at 5 and 22 hours of incubationin all NLRP12 patients, although carrying different variants, compared to JIA patients. Moreover, we observed a trend for higher IL-6 levels released from a significant number of NLRP12 patients both at 5 and 22 hours of incubation. Interestingly, we did not find differences in the IL-1β levels measured in media from NLRP12 patients, compared with JIA patients. When we investigated parents, carrying the same NLRP12 variant, we did not find any differences in cytokines released in response to stimulation with TLRs ligands, compared with those obtained from age-matched healthy donors. Moreover, no association of a specific clinical phenotype with different NLRP12 variants, and a particularcytokine release signature was observed.
Conclusion: We were not able to demonstrate a clear association of the investigated NLRP12 variants with the specific clinical phenotype or with the profile of pro-inflammatory cytokine released *ex vivo*. Other studies are needed before final conclusions on the pathogenic role of these NLRP12 variants can be drawn.

O24

A Next Generation Sequencing approach to the mutational screening of patients affected with systemic autoinflammatory disorders: diagnosis improvement and interpretation of complex clinical phenotypes

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Introduction: Systemic autoinflammatory diseases (SAIDs) are a group of monogenic disorders characterized by inflammation which occurs in the absence of pathogenic auto-antibodies, auto-reactive T lymphocytes or other infective causes. More than 50% of SAID patients recruited to our Unit does not show any mutation at gene(s) tested by direct Sanger sequencing in the routine diagnosis. Clinical misdiagnosis, mutations in untested gene regions and genetic heterogeneity are possible explanations.
Objectives: To improve both the molecular diagnosis and genotype interpretation of SAIDs, we aimed at the development of a Next Generation Sequencing based protocol, designed for the simultaneous screening of ten genes known to be involved in a remarkable proportion of SAIDs. Different bio-informatics approaches were taken into consideration in order to define the best pipeline for variants detection.
Patients and methods: A panel of gene amplicons specific for the diseases under study was designed through the Ion AmpliSeq™ designer software. Fifty SAID patients, already genotyped for the respective causative gene(s), were massively sequenced for the coding portions of MEFV, MVK, TNFRSF1A, NLRP3, NLRP12, NOD2, PSTPIP1, IL1RN, LPIN2 and PSMB8. Three different bio-informatics pipelines, Ion Reporter™, CLC Bio Genomics Workbench, and GATK-based in-house workflow, were compared.
Results: The approach we propose here for NGS-based diagnosis of SAIDs, has resulted technically suitable, with a very high mean coverage (336X) and nearly full detection of variants. Besides expected mutation, we could also identify many unexpected variants that were all validated by Sanger sequencing and compared to assess true and false positive detection rates of the three workflows. Finally, the overall clinical picture of 34 patients were re-evaluated in the light of the new mutations found.
Conclusions: The present gene panel has resulted suitable for molecular diagnosis of SAIDs. Besides causative mutations, most patients have turned out to carry variants of unclear significance that will need further investigation. Moreover, genotype-phenotype correlation drawn in 34 patients has confirmed a remarkably difficult interpretation of NGS data in patients with an undefined or complex inflammatory phenotype. This supports the need of evidence-based and validated clinical criteria as crucial tools to be used concurrently with the genetic analysis for the final diagnosis and classification of SAIDs patients. In addition to setting a first approach to mutational detection in SAIDs, patients left without a clear diagnosis will be identified as candidate for the successive NGS analysis of the whole exome.

O25

Pathological and immunological features of autoinflammatory syndrome associated with lymphedema (AISLE)

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Background: We recently described a new autoinflammatory syndrome associated with lymphedema (AISLE), and a frameshift mutation in the MDFIC gene was identified by homozygosity mapping and targeted sequencing using DNA samples of a consanguineous Turkish family as the cause of this syndrome. The same mutation in the MDFIC gene was identified in an Italian patient with similar clinical findings. Clinical findings of these patients are characterized by recurrent attacks of fever, erythematous/urticarial rash with hyperesthesia, myalgia, serositis, chylous serosal effusions, edema/lymphedema on the face and extremities. These patients gradually developed a permanent lymphedema in the same

areas affected during attacks. On the other hand, there is limited information about the functions of MDFIC gene, which include transcriptional regulator interacting with cellular transcription factors, including such as axin and wnt/b-catenin signaling pathway. Although, its expression has been documented in immune cells, no clear function has been described in immune mediated or inflammatory conditions.
Methods: We evaluated the clinical course and laboratory investigations of the patients. We investigated the gene expression patterns in peripheral blood mononuclear cells in one of the patients and his parents following LPS stimulation to understand involved pathways in MDFIC-related inflammatory changes using an Illumina gene expression kit. We also evaluated pathological specimens obtained from the same patient during surgical resection of the giant scrotal tissue affected from lymphedema. Detailed immunohistochemical analyses were done to define the characteristics of lymphatics in involved tissues.
Results: LPS stimulation resulted in a significant deviation in gene expression pattern in the AISLE patient compared to the expression patterns of one of his parents and un-related healthy controls. Expression of CCL8, IFITM3, IFIT1, IFIT3, IFIT2, ISG15, OAS3 genes showed more than two times decrease following LPS stimulation, and these gene expression changes were interpreted as a negative interferon signature. Pathological examinations revealed lower than expected number of lymphatic vessels as well as rare enlarged lymhatics in scrotal tissues with lymphedema. Regarding two of the Turkish patients' treatments, no clear response was observed to monoclonal anti-IL-1 beta canakinumab antibody. However, both patients are doing well with daily anakinra injections. The first patient experienced no adverse event during and following two operations for giant scrotal lymphedema, and a normal recovery period was observed.
Conclusions: Gene expression findings indicating a negative interferon response following LPS stimulation need to be confirmed in other two patients and cell line models. Current immunological and pathological findings suggest an interferon-signature mediated inflammatory response defect, which may affect lymphatic endothelial cells and their survival, leading to gradual decrease in the number of lymphatic vessels and development of lymphedema. Also differential response to anakinra but not canakinumab may suggest possible contribution of IL-1 alpha to the pathogenesis of AISLE.

O26

Severe peripheral blood lymphopenia without NK cell cytotoxicty deficiency is the rule in adult acquired HLH

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Introduction: Hemophagocytic lymphohistiocytosis (HLH) is characterized by hypercytokinemia and hemophagocytosis due to abnormal activation and proliferation of T lymphocytes and macrophages. Inherited forms are due to gene defects affecting CD8 and NK lymphocyte cytotoxicity, whereas in acquired forms complicating rheumatic, infectious or neoplastic diseases, lymphocyte subpopulation and function profiles remain poorly studied.
Methods: Between 2000 and 2014, we prospectively studied 71 adult patients (from 20 to 87 years) with acquired HLH defined by the International HLH Society criteria (group 1), compared with 87 patients suffering of rheumatic, infectious or tumoral diseases without HLH (group 2) and 66 healthy volunteers (group 3). Peripheral blood lymphocyte phenotype was studied by FACS analysis with special focus on NK cell activation markers and functions.
Results: Acquired HLH was mainly associated with infections (n = 33), lymphomas (n = 14), inflammatory pathologies (4 lupus, 2 others connective tissue diseases, 3 adult onset Still's diseases) or undetermined causes (n = 15). HLH patients had a global lymphopenia compared to group 2 and 3 affecting both T, B and NK cells ($p < 0.0001$) which was transient and corrected in case of HLH recovery. Among CD3 cells, both CD4 (mean: 333 vs 638/mm^3) and CD8 (272 vs 638/mm^3) were lower in HLH compared to group 2 ($p < 0.0001$). NK cell phenotype showed membrane CD69 and CCR5 up-regulation reflecting NK cell activation, but no differences for NKp30, p46, CD16, CXCRC1, CXCR3 and CCR7 membrane expression. NK cytotoxic functions consisting in perforin expression, LAMP degranulation ability, NK natural cytotoxicity or ADCC against K562 or P815 targets respectively, appeared not to be affected in acquired HLH and completely different from the severe functional defects despite normal cell counts, observed in patients with inherited HLH.
Conclusion: Compared to inherited forms of HLH and matched patients with rheumatic, infectious or neoplastic diseases, patients with acquired HLH present with a severe but transient global lymphopenia. Despite severe decreased NK cell counts, these cells appeared to express several activation markers and overall a normal phenotype. More importantly, NK cells demonstrated normal cytotoxic functions. Despite some reports showing mutations affecting T cell cytotoxicity genes in acquired HLH patients, our study shows that this disease is in most of the cases, not due to a defect of lymphocyte cytotoxicity.

O27

A significant role for tumor necrosis factor in *Nlrp3* inflammasomeopathies

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Introduction: The NLRP3 inflammasome is a protein complex responsible for caspase-1 dependent maturation of the pro-inflammatory cytokines IL-1β and IL-18. Gain of function missense mutations in NLRP3 cause the disease spectrum known as cryopyrin-associated periodic syndromes (CAPS).
Objective: To elucidate systemic autoinflammatory disease mechanisms involved NLRP3 inflammasomeopathies other than IL-1β and IL-18.
Materials and methods: Knock-in *Nlrp3*$^{L351P/+}$*/CreL/Il1b-/-/Il18-/-*mice (*FCAS Il1b*$^{-/-}$*Il18-/-*), *Nlrp3*$^{L351P/+}$*/CreL/* Casp1$^{-/-}$, (*FCAS Casp1*$^{-/-}$),*Nlrp3*$^{A350V/+}$*/CreL (MWS)* and *Nlrp3*$^{A350V/+}$*/CreL/Tnf*$^{-/-}$ *(MWS Tnf*$^{-/-}$*)* were generated in which Cre-mediated expression is limited to the myeloid cell lineage.
Results: Nearly all *FCAS Il1b*$^{-/-}$*Il18-/-*mice survived and grew normally until adulthood however, investigation of mice at > 6 months of age showed marked splenomegaly and elevated numbers of white blood cells as compared to *FCAS Casp1*$^{-/-}$mice and non-mutant *Il1b*$^{-/-}$*Il18-/-*littermates, suggesting a caspase-1 dependent phenotype independent of IL-1β and IL-18. To examine other potential inflammatory mediators, non-lethal *in vivo* LPS (5 ug/g) stimulation of FCAS Il1b$^{-/-}$Il18$^{-/-}$mice revealed significantly elevated levels of serum TNF at both 2 and 6 hours post induction when compared to *FCAS Casp1*$^{-/-}$mice and non-mutant *Il1b*$^{-/-}$ *Il18-/-*controls. To further investigate the role of TNF in *Nlrp3* inflammasomeopathies, *MWS* mice (which die within two weeks of birth) were bred on a TNF knockout background. MWS Tnf$^{-/-}$ pups were indistinguishable from non-mutant controls with all animals surviving to adulthood with normalization of both body and spleen/body weight comparisons. Serum analysis of *MWS Tnf*$^{-/-}$ pups showed attenuation of NLRP3 inflammsome related cytokines when compared to intact *MWS* pups. The skin of intact *MWS* pups exhibited strong neutrophilic and inflammatory macrophage infiltrations, which were normalized in *MWS Tnf*$^{-/-}$ animals. Interestingly, *MWS Tnf* $^{+/-}$ pups also showed an intermediate protective effect on all of the aforementioned comparisons. To determine if TNF ablation could be recapitulated with therapeutic intervention, *MWS* pups were treated with recombinant soluble TNF receptor (Etanercept 400ug/g sc EOD). Treatment provided similar phenotypic rescue and extended survival for an average of 22 days after cessation of treatment. Adult *MWS Tnf* $^{-/-}$mice at > 6 months of age were found to have splenomegaly and elevated numbers of white blood cells when compared to non-mutant *Tnf* $^{-/-}$littermates, implicating a role for other inflammatory mechanisms as mice age.
Conclusion: TNF plays an unexpected and significant role in murine inflammasomeopathies, which may have therapeutic implications for CAPS patients with incomplete responses to IL-1 targeted therapies.

O28

Altered expression of IL-10 family cytokines in CRMO result in enhanced inflammasome activation

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Introduction: Chronic recurrent multifocal osteomyelitis (CRMO) is the most severe presentation of the autoinflammatory bone disorder chronic nonbacterial osteomyelitis (CNO). The pathophysiology of CNO remains to be determined. We recently demonstrated reduced activation of mitogen-activated protein kinases ERK1 and 2 in monocytes from CRMO patients responsible for impaired activation of the transcription factor signaling protein (Sp-)1. This resulted in failure to express the immuno-modulatory cytokine IL-10. The *IL10* gene, together with its homologues *IL19* and *IL20*, is organized in the 145 kb spanning *IL10* cytokine cluster on chromosome 1q32. In most cells, including monocytes, IL-10 cytokine family members are co-regulated in response to certain stimuli. IL-10 and IL-19 mainly have immune-modulating functions, while IL-20 acts as a pro-inflammatory cytokine contributing to inflammatory bone-loss. The NLRP3 inflammasome is a multi-protein complex forming in response to innate stimuli, subsequently mediating the cleavage and release of IL-1β. Enhanced inflammasome activation in IL-10 deficient mice was linked with bone-loss. Convincing evidence of this mechanism playing a role in CNO, however, is lacking.
Objectives: The aim of our study was to determine i) IL-10 cytokine family expression patterns in CRMO monocytes, ii) molecular mechanisms underlying impaired cytokine expression, and iii) potential effects on inflammasome-dependent cytokine secretion.
Methods*Ex vivo* isolated monocytes from CRMO patients were cultured in the absence or presence of LPS. Expression patterns of cytokines were monitored on the transcriptional (mRNA) and protein level. Effects of impaired Sp-1 activation on cytokine expression were investigated through forced expression, chemical inhibition, or knock-down of Sp-1.
ResultsWe saw reduced expression of anti-inflammatory cytokines IL-10 and IL-19 and unaffected expression of IL-20 in CRMO monocytes when compared to controls. We for the first time demonstrate Sp-1 recruitment to the *IL19* promoter, governing IL-19 expression in monocytes. Impaired expression of IL-10 and IL-19 in CRMO monocytes was caused by reduced binding of Sp-1 to regulatory regions. Expression of IL-20 was independent of Sp-1. Reduced IL-10 and IL-19 secretion from CRMO monocytes mediated increased activity of the NLRP3 inflammasome, as assessed by IL-1β secretion. Addition of recombinant IL-10 or IL-19 reversed these findings.
ConclusionImpaired activation of Sp-1 in monocytes from CRMO patients contributes to reduced expression of IL-10 and IL-19, resulting in an imbalance between pro- (IL-20) and anti-inflammatory IL-10 cytokine family members. Subsequently enhanced NLRP3 inflammasome activation results in IL-1β secretion which may in turn contribute to inflammatory bone-loss. A complete understanding of the molecular pathophysiology of CNO will aid in developing new disease biomarkers and therapeutic targets.

O29

Neutralization of Interferon-gamma is efficacious in a mouse model of HLH secondary to chronic inflammation

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Introduction: Macrophage activation syndrome is a term used to identify hemophagocytic lymphohistiocytosis secondary to rheumatic diseases (rheuHLH). It is a severe, potentially fatal condition that occurs in the context of rheumatic diseases, particularly systemic juvenile idiopathic arthritis. It is part of secondary HLH forms, that are clinically and biochemically similar to primary HLH (pHLH), with generalized hypercytokinemia as a major feature. While the triggering mechanism behind pHLH is the defect in cytotoxicity, caused by mutations in genes encoding proteins required for lymphocyte and natural killer cell activity, the rheuHLH pathogenesis is not clearly understood.
Objectives: Based on data obtained in animal models of pHLH, showing that interferon-gamma (IFN-γ) neutralization reverts the disease, we aim to demonstrate, in a murine model of rheuHLH, the role of IFN-γ and the efficacy of an anti-IFN-γ antibody.
Materials and methods: A mouse model of rheuHLH (Strippoli R, Arthritis Rheum 2012), recently developed in our laboratory, relying on an exaggerated response to toll-like receptor ligands in mice transgenic for the pro-inflammatory cytokine interleukin-6 (IL6TG), has been used to evaluate levels of IFN-γ and the therapeutic potential of a rat neutralizing IFN-γ antibody (XMG1.2, BioXcell, USA).
Results: LPS-treated IL-6TG mice showed an exaggerated inflammatory response, with significantly higher IFN-γ mRNA expression levels in liver and spleen, compared to LPS-treated WT mice. Moreover, we observed a significant increase in the expression of genes known to be induced by IFN-γ, such as CXCL9, CXCL10 and H2-Aa (class II antigen A, alpha), in the spleen and in the liver from LPS-treated IL-6TG mice compared to WT mice. IFN-γ neutralization studies have revealed that, in LPS-injected IL-6TG mice, anti-IFN-γ treatment significantly improves survival and body weight recovery, compared to control antibody-treated animals. Furthermore, a significant reduction in ferritin levels was observed in mice treated with anti-IFN-γ Ab, compared to control antibody-treated animals. Finally, a significant reduction in the mRNA expression of IFN-γ-induced genes was observed in spleen and liver from mice treated with anti-IFN-γ Ab.
Conclusion: These data demonstrate that the IFN-γ pathway is up-regulated in LPS-treated IL-6TG mice, compared to the wild type mice. Neutralization of IFN-γ significantly improves survival and clinical hematological parameters. These results provide insights into the pathophysiology of rheu-HLH, further support the hypothesis that IFN-γ is the common mediator of all HLH forms and provide the rational for the therapeutic use of a monoclonal anti- IFN-γ antibody in this fatal disorder.

O30

Hexameric S100A12 is required for pro-inflammatory TLR4-signalling

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Introduction: The human granulocyte-specific Ca^{2+}-binding protein S100A12 is particularily over-expressed in autoinflammatory diseases such as juvenile idiopathic arthritis (JIA) as well as other inflammatory conditions (i.e. infections, vasculitides) and has been ascribed to the group of pro-inflammatory damage associated molecular pattern molecules (DAMPs). In order to operate as DAMP, S100A12 requires binding to cellular receptors. Although the protein was originally found to bind the receptor of advanced glycation endproducts (RAGE), we recently demonstrated S100A12 to stimulate proinflammatory cytokine production in monocytes via TLR4 instead of RAGE.
Objectives: DAMP:TLR4 signalling is often discussed controversial. Mechanistic insights into the protein: receptor interaction as available for HMGB1, for example, can help to explain the powerful pro-inflammatory potential of these proteins. A peculiarity of granulocytic S100A12 is it's oligomerization into di-, tetra- or hexamers upon Ca^{2+} and Zn^{2+}-binding. In this study we assessed the mechanism of the S100A12:TLR4 interaction for these individual protein complexes.
Methods: We performed extensive chemical crosslinking studies to assess S100A12 oligomerisation of both recombinant as well as native protein in autoinflammatory patients' sera as well as protein directly isolated from granulocytic cytosol. For receptor-interaction studies, defined LPS-free chemically crosslinked S100A12-complexes were isolated via combined HPLC and gel filtration. TLR4-binding and signalling was tested on receptor-expressing cell lines as well as primary human cells. Cytokine expression in response to stimulation was quantified on mRNA and protein level.

Results: In our assays, only combined presence of Ca^{2+} and Zn^{2+} concentrations in extracellular ionic-strength could induce S100A12 hexamer-formation. Ca^{2+}/Zn^{2+}-levels within physiological intracellular range could only induce oligomerisation up to the tetrameric complex. Correspondingly, we could detect hexameric S100A12 in patients' serum, while we did not find this particular protein complex inside granulocytes. In vitro binding assays as well as cell stimulation experiments using chemically crosslinked HPLC-separated S100A12-oligomers revealed the S100A12-hexamer as the paramount TLR4-targeting pro- inflammatory active complex. Stimulation could be abrogated by interfering with TLR4-binding and, in particular, by blocking access to CD14.
Conclusion: We have identified the S100A12-complex, which is responsible for pro-inflammatory signalling through TLR4. This is of great interest for designing improved diagnostics as well as precisely targeted therapeutic approaches, as currently tested with us.

O31

Preliminary response to Janus kinase inhibition with baricitinib in chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE)

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Background: CANDLE is a novel autoinflammatory syndrome presenting early in infancy with attacks of fever, panniculitis, arthritis, myositis, lipodystrophy, cytopenias, dyslipidemia and growth retardation. CANDLE is caused by mutations in a number of proteasome-associated genes including *PSMA3*, *PSMB4*, *PSMB8* and *PSMB9*. Elevated serum IP-10 (CXCL10) levels and gene expression studies show a prominent "interferon (IFN) signature". These findings and the successful *in vitro* blocking studies with Janus kinases (JAKs) inhibitors, raised the question whether IFN could be a therapeutic target in CANDLE.
Objectives: The objective of this compassionate use study (NCT01724580) is to determine whether the treatment with baricitinib (JAK1/JAK2 inhibitor) results in the reduction of the mean Autoinflammatory Diary Score (ADS) to below 0.5 (ranges 0-4) and to determine whether treatment with baricitinib allows reduction of steroid doses by at least 50% in patients receiving steroids at baseline.
Methods: Patients with CANDLE are eligible to participate in the study. Statistical analyses were performed using a paired t-test to compare baseline to last clinic visit data. Baricitinib dosing is based on a defined dose-escalation scheme. Patients who are unresponsive to treatment may have their dose escalated. Demographics, vital signs, safety laboratories, ADS, adverse events (AEs) and prednisone doses are captured at each visit.
Results: To date, 12 CANDLE patients have been enrolled. One patient discontinued from the study due to lack of efficacy and development of debilitating avascular necrosis. All patients have been followed for at least 6 months (mean 1.7 years). The mean ADS decreased significantly (1.3 (SD ± 0.8) at baseline, 0.3 (SD± 0.3) at the time of their last visit, $p < 0.005$). The mean total prednisone dose of 14 mg/day (SD± 8.5) decreased by 73% to 3.8 mg/day (SD± 3.6). Four patients discontinued prednisone completely. Myositis has improved in 5 out of 6 patients and signs of bone marrow immunosuppression have improved in all but 1 patient (subject#1008), with increases of platelets, absolute lymphocyte counts ($p < 0.05$) and hemoglobin. Seventeen serious adverse events (SAEs) were reported in 4 patients (presumed Pneumocystis jiroveci pneumonia, avascular necrosis, urinary tract, port cath-related, rotavirus, and C. difficile infections). The most common adverse events were upper respiratory infections, two patients have developed anemia. One patient has been treated successfully with IV iron.
The mean dose of baricitinib at last patient visit was 8.5 mg/day (SD± 2.1).
Conclusion: Preliminary data in 11 CANDLE patients treated with baricitinib are encouraging and suggest that targeting IFN signaling with a JAK1/JAK2 inhibitor may be a successful therapeutic strategy for CANDLE patients, and possibly other IFN mediated autoinflammatory disorders.

O32

Interstitial lung disease in STING-associated vasculopathy with onset in infancy (SAVI): preliminary genotype-phenotype correlation

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Background: Some monogenic interferonopathies are caused by innate immune dysregulation and form a subclass of autoinflammatory disorders characterized by systemic inflammation due to chronic Type I interferon stimulation. STING-Associated Vasculopathy with Onset in Infancy (SAVI) is an IFN-mediated disease caused by gain-of-function mutations in *TMEM173*, the gene encoding the stimulator of interferon genes (STING).
Objectives: This study was undertaken to understand the variable disease severity of the interstitial lung disease (ILD) in SAVI patients. We hypothesized that the severity of the interstitial lung disease may be modulated by a common SNP (R232H, rs1131769) that is functionally associated with decreased *IFNB1* transcription.
Methods: We studied nine SAVI patients with N154S, V155M, or V147L mutations. Lung involvement was assessed by chest computed tomography (CT) and pulmonary function tests (PFTs) for all patients, a lung biopsy was available for five patients. Peripheral blood genomic DNA samples were obtained and *TMEM173* (NM_198282.3) was sequenced by Sanger technique. STING function was evaluated in the different *TMEM173* haplotypes by *IFNB1* Luciferase Reporter assays performed with cells transfected with wildtype or mutant *TMEM173* on the R232 and the H232 backgrounds.
Results: We described the clinical features of ILD in nine SAVI patients. Six patients had evidence of severe ILD characterized by moderate to severe abnormalities on chest CT, PFTs and/or lung biopsy. Two patients presented with mild ILD and one did not have any evidence of ILD. Four out of the six patients with severe ILD succumbed to pulmonary complications. Five patients with severe ILD were homozygous for R232 (R232/R232) and one was heterozygous for the SNP. Conversely, the two patients with mild ILD were heterozygous (R232/H232) and the patient without ILD was homozygous for the H232 allele (H232/H232). Thus, the severity of interstitial lung disease seems to correlate with the STING haplotype. Transfection of HEK293T cells with the H232 *TMEM173* haplotype with or without SAVI causing mutations results in decreased *IFNB1* expression in the presence of both low affinity and high affinity STING stimulator cGAMP in comparison with cells transfected with the R232 haplotype. These findings suggest that the H232 haplotype background may be protective from the development of ILD.
Conclusion: The variable presentation and severity of ILD in SAVI patients seems to correlate with the *TMEM173* haplotype at position 232 and possibly with the local induction of an IFN response. Our data

suggest that common variants can modify disease expression specific to one organ and provide a model to assess the variable disease phenotype in other interferonopathies.

O33

Spontaneous Type I IFN response in SAMHD1-deficient mice requires both, functional intracellular RNA and DNA sensing pathways

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Introduction: Nucleic acids are potent inducers of the antiviral type I interferons (IFN) and therefore represent prototypic PAMPs in viral infections. Innate nucleic acids sensors patrol the cytoplasm for the presence of RNA and DNA but have only a limited capacity to discriminate between endogenous and exogenous nucleic acids. Therefore mechanisms evolved that prevent the accumulation of endogenously derived nucleic acids. In Aicardi-Goutières syndrome (AGS), which represents a rare monogenic variant of the prototypic autoimmune disease systemic lupus erythematosus, genetic defects of these mechanisms result in production of large amounts of type I IFN. SAMHD1 is an intracellular nuclease that degrades RNA and DNA and cleaves deoxynucleotides (dNTP) into nucleosides and inorganic triphosphate, mutation of which cause AGS. The nucleotide triphosphohydrolase also confers antiretroviral activity to SAMHD1, as dNTP degradation was shown to represent a major block to reverse transcription of retroviruses. How and why SAMHD1-deficent cells activate the type I IFN system is not known so far. Furthermore, a comprehensive analysis of the antiretroviral potential of mouse SAMHD1 is still lacking.
Objectives: We aim to identify essential pathways that mediate the spontaneous type I IFN response in SAMHD1 knockout mice.
Materials and methods: We generated SAMHD1 knock out mice and in parallel inactivated crucial molecules of the type I IFN systems in these mice. Activation of the type I IFN system was quantified by global transcriptome sequencing. Antiretroviral activity of murine SAMHD1 was assessed by infection of mutant mice with GFP-Reporter retroviruses.
Results: SAMHD1-deficient mice do not develop any signs of inflammation or systemic autoimmunity. However, they spontaneously produce IFNb that subsequently activates transcription of interferon-stimulated genes (ISG). This response was abolished in SAMHD1 IFNb and in SAMHD1 IFNAR1 double deficient mice. Surprisingly, additional inactivation of both, the intracellular RNA and DNA sensing machinery, by knocking out MAVS or STING in SAMHD1-deficient mice, respectively, suppressed the spontaneous IFN production. In vivo infection experiments showed that single deficiencies of IFNAR1 and SAMHD1 only slightly or moderately increased reverse transcription, respectively. Interestingly, in SAMHD1 IFNAR1 double deficient mice we found reverse transcription increased by an order of magnitude compared to wild type and the single deficient mice.
Conclusion: In contrast to the situation in other AGS-mouse models, in SAMHD1-deficient mice both, a functional intracellular RNA and DNA sensing machinery are required to spontaneously activate the type IFN system. Understanding the mechanisms that establish the chronic IFN response in this model will be instrumental to elucidate whether there is a unifying concept underlying the pathogenesis of AGS.

O34

Tracing cellular sources of pathogenic type I-interferon in the TREX1$^{-/-}$ mouse model of lupus like-disease

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Introduction: Loss of function mutations of the intracellular enzyme 3' repair exonuclease (TREX) 1 cause Aicardi-Goutières syndrome (AGS). As AGS clinically overlaps with systemic lupus erythematosus (SLE) and, like SLE, features a spontaneous activation of the antiviral type I-interferon (IFN) system as well as production of antinuclear autoantibodies, this condition may be considered a monogenic variant of SLE. TREX1-deficient mice spontaneously develop multiorgan autoimmune disease that is fully dependent on a functional type I-IFN system. This phenotype suggested a new concept of systemic autoimmunity arising from intracellular accumulations of (so far enigmatic) nucleic acid substrates of TREX1, which trigger chronic antiviral IFN responses and thereby autoimmunity. Nonhematopoietic cells were proposed to be the cellular source of the pathogenic IFN. Uncontrolled activity of endogenous retroelements was suspected to induce the chronic antiviral response.
Objectives: We sought to identify cell types responsible for the loss of self tolerance and to clarify whether there is a role for endogenous retroelements in the disease of TREX1-deficient mice.
Materials and methods: In order to elucidate the role of Trex1 in the hematopoietic system, lethally irradiated mice were reconstituted with TREX$^{-/-}$ fetal liver cells. Furthermore we generated a Trex1 flox mouse line which was used to specifically delete TREX1 in CD11c-expressing cells (CD11c-Cre), in DCs (Clec9a-Cre), and in B cells (CD19-Cre). To test a potential role of retroelements, TREX1$^{-/-}$ were crossed to transgenic retrotransposition reporter mice and were treated with reverse transcriptase inhibitors.
Results: While Cre/loxP-mediated conditional inactivation of *Trex1* in various non-hematopoietic compartments did not result in detectable inflammatory disease, selective knock out in the hematopoietic system largely reproduced the TREX1$^{-/-}$ phenotype. Mice with inactivation of the *Trex1* gene in DCs developed only mild autoimmunity, but featured massive upregulation of interferon stimulated genes suggesting a critical pathogenic role of DCs. Interestingly, selective loss of TREX1 in CD19+ B cells did not result in a detectable activation of the type I-interferon system.
Treatment of TREX1$^{-/-}$ mice with the reverse transcriptase inhibitor Truvada® did not ameliorate the phenotype. Retrotransposition events were not increased in frequency in a TREX1$^{-/-}$ compared to a wildtype background.
Conclusion: We demonstrate that specific cell types differentially respond to the loss of TREX1 and show that DCs are an important source of type I IFN in the TREX1$^{-/-}$ model. Our findings that loss of TREX1 had no impact on Line1 retrotransposition and that pharmacological inhibition of retrotransposition in vivo did not rescue TREX1$^{-/-}$mice from lethality challenges the concept of a pathogenic role of endogenous retroelements in this model.

O35

Stratification of patients with autoinflammatory phenotypes by interferon (IFN) score suggests a new group of IFN mediated autoinflammatory diseases with overlapping clinical phenotypes

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Background: We have identified mutations in proteasome components as the cause of CANDLE syndrome and in *TMEM173/STING* as the cause for a severe vasculopathy and lung disease, SAVI. CANDLE and SAVI patients do not respond to IL-1 inhibition and consistently demonstrate marked up-regulation of IFN-inducible genes. Our data suggest innate

immune dysregulation caused by chronic Type I IFN signaling in both conditions.
Objective: We hypothesize that the presence of IFN signature may identify patients with autoinflammatory disease (AID) who have genetic mutations in other IFN regulating genes.
Methods: To identify patients with IFN signatures, RNA sequencing (RNA-seq) from whole blood RNA was performed using HiSeq 2000 Illumina® platform. Heatmaps with 64 IFN response genes were assessed. Whole exome sequencing (WES) was performed from whole blood DNA.
Results: We identified 19 patients with marked upregulation of IFN inducible genes. WES was performed in 14 patients and parents (trios) and in 5 individual patients. Of the probands, 9/19 were female, 8/19 were Caucasian, 3 Asian, 2 Hispanic, 2 Norwegian and 4 had other ethnicities. All patients presented with immunodysregulatory phenotypes with clinical similarities to the previously described interferonopathies, including skin vasculitis/vasculopathy (9/19), panniculitis (12/19), myositis (5/19) and basal ganglion calcifications (5/19), but had no genetic diagnosis prior to NIH evaluation. The bioinformatics variant annotation, analysis and filtering workflow successfully identified mutations in IFN-regulating genes in 7 of the 19 probands. In one patient, we found a disease causing *de novo* and somatic mutation in *TREX1*. This patient also presented with an in-frame deletion in *DHX9* inherited from her mother and a missense mutation in *MAVS* inherited from her father. In one patient, we identified a *de novo* mutation in *DHX9* and this patient is also a compound heterozygous for mutations in *IFIH1/MDA5*. In a third patient, we found a missense mutation in *TREX1* inherited from the mother and a heterozygous variant in *MB21D1* (gene encoding cGAS) inherited from the father. A fourth patient with a clinical phenotype of CANDLE had two novel compound heterozygous mutations in *PSMG2*. Additionally, a male patient with lupus-like clinical and laboratory findings was found to have an X-linked mutation in *TREX2* gene. All mutations described were confirmed by Sanger sequencing.
Conclusion: RNA-seq can be a tool for the identification of patients with an IFN signature and guide the search for disease causing variants in IFN-regulating genes by WES. However, disease causality of these mutations needs to be assessed in functional assays. Moreover the identification of patients with a type I interferon signature and a set of clinical features that are not seen in IL-1- mediated-AIDs allow stratification of a subset of AIDs that are typically "poor IL-1 responsive". Whether the IFN signature identifies a subset of patients that respond to the blockade of Type I IFN signaling needs to be further validated.

O36

Neurology of the Cryopyrin-Associated Periodic Feversyndrome

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Introduction: There are little data on the multiple neurological manifestations of CAPS in adult patients.
Objectives: To retrospectively analyse the neurological features of CAPS in a cohort of adult patients treated at a single UK centre.
Patients and methods: 38 patients (aged 16-69 years) with confirmed CAPS and on long term treatment with anti IL-1 agents were included. All patients were reviewed annually by a neurologist, an ophthalmologist and had audiometry. 35 patients had cranial MRIs, 4 patients had lumbar punctures.
Results: Ninety-five percent of our patients had neurological features, with 84% describing some form of headache, 66% having sensorineural hearing loss on audiometry, 60% reporting myalgia, 34% having papilloedema during the course of their illness and 26% having evidence of optic atrophy. Twenty-five patients had normal MRI brain scans. Six scans demonstrated T2 hyperintensities in the subcortical white matter (in one case from prior ischaemic insult). Incidental findings in 4 cases included: an arachnoid cyst, an acoustic neuroma, a pineal cyst and a meningioma. All lumbar punctures were consistent with chronic meningitis. There was a marked response to pharmacological interleukin-one-beta (IL-1β) inhibition, (29/32 patients with headache and 22/23 with myalgia responding). Patients with the T348M mutation (N= 8) tended to have a more severe neurological phenotype with an earlier age of onset, more hearing loss, papilloedema and optic disc atrophy, the R260W mutation (N=8) was associated with an intermediate severity phenotype, and A439V (N=11) a relatively milder neurological phenotype.
Conclusion: This case series demonstrates a much higher prevalence of neurological symptoms in CAPS than reported from EUROFEVERS (Levy 2014) and highlights the importance of increased awareness of CAPS amongst neurologists, to aid diagnosis and allow implementation of the highly targeted and effective therapies available.

O37

Novel mutation in NLRP3 Exon 7 results in sensorineural hearing loss without chronic inflammation

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Introduction: Cryopyrin-associated periodic syndromes (CAPS) are a spectrum of autoinflammatory disesase with spontaneous activation of the NLRP3 inflammasome, leading to hypersecretion of IL-1b. To date, more than 90 mutations have been described in *NLRP3*,primarily in exon 3, leading to the autoinflammatory symptoms observed in CAPS. Here, we describe a family with autosomal dominantly inherited, unilateral sensorineural hearing loss, found to have a novel mutation in exon 7 of *NLRP3*.
Objective: To evaluate the clinical and genetic features of a 2-generation pedigree with unilateral sensorineural hearing loss.
Methods: Patient data, including audiologic studies, and detailed family history was obtained. DNA was isolated using saliva samples from all family members and Sanger sequencing of all 9 exons of *NLRP3* was performed.
Results: The proband was first identified during an evaluation for recurrent fevers and aphthous stomatitis, occurring 2-3 times per month, beginning at 4 months of age, without infectious etiology. Episodes were not associated with cold exposures or rash. Physical exam was notable for an otherwise well appearing child, without evidence for inflammation or autoantibodies. At the age of 5, he was noted to have unilateral sensorineural hearing loss in the range of 4000-8000 Hz, similar to his father. Two younger siblings were noted to have similar symptoms, with similar age of onset, including unilateral hearing loss. Between flares, ESR and high sensitivity CRP were normal in all three children suggesting an absence of chronic inflammation. We identified a novel variant in *NLRP3* exon 7: c.2753 G>A, R918Q in all 4 affected members of this 5-person pedigree. Modeling of the mutation localizes the substituted amino acid residue to the inner surface of the LRR domain and suggests an alteration in charge distribution predicted to affect inter or intraprotein protein-protein interactions.
Conclusions: While bilateral sensorineural hearing loss has been described in association with Muckle Wells Syndrome and Neonatal onset multi-inflammatory disease (NOMID), both on the CAPS spectrum, in this family, a novel NLRP3 variant was associated with unilateral hearing loss in the absence of serologic evidence for chronic inflammation. This is the first potentially disease associated variant to be described in exon 7 of *NLRP3*.

O38

Pharmacokinetics of Canakinumab in children younger than 2 years old with CAPS

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Background: Canakinumab (CAN) is indicated for the treatment of cryopyrin-associated periodic syndrome (CAPS) in patients ≥2 years of age [1]. However, information on the pharmacokinetics (PK) of CAN in patients <2 years of age is not available. Here, we present preliminary PK data from a phase III study in CAPS patients.
Objectives: To assess the efficacy of CAN with respect to the treatment response in CAPS patients ≤4 years of age and to evaluate PK and pharmacodynamics (PD) profiling of CAN.
Methods: CAN-naïve patients with confirmed CAPS aged 44 days to 4 years received open-label CAN 2 mg/kg every 8 weeks for 56 weeks. For NOMID patients, an initial dose of 4 mg/kg was administered. Patients who did not achieve complete response following CAN injection, or experienced a flare before the next planned administration, were eligible for dose up-titration with possible maintenance and step wise up-titration regimens of 4, 6, or 8 mg/kg s.c.
Results: Seventeen patients, 6 patients <24 months old (44 days to 14 months; mean age = 7 months), were enrolled and administered body weight-based (2 mg/kg up to 12 mg/kg) doses of CAN s.c. every 8 weeks, with the exception of one patient who received doses of 4-6 mg/kg once weekly. Of the 6 patients <24 months old, 5 were dosed with 2 mg/kg at each dose while 1 NOMID patient started with 4 mg/kg and up-titrated to 8 mg/kg at last dose. Sixteen patients achieved a complete response, with 7 patients requiring dose escalation to achieve and/or maintain their responses. Mean dose-normalized CAN trough concentrations at steady-state in the patients <24 months old were similar across the 6 patients from 44 days to 15 months, while the range of exposures as represented by the dose normalized trough levels overlapped with the remaining 11 study patients >2 years old who received CAN doses ranging from 2 mg/kg up to12 mg/kg.
Conclusions: Canakinumab is an effective treatment for patients with CAPS aged as young as 44 days old. The preliminary PK results demonstrated that dose-normalized canakinumab exposure in patients <2 years old was similar to patients >2 years supporting the utilization of weight-based dosing in the CAPS infantile population.

Reference

1. ILARIS [summary of product characteristics]: Novartis Europharm Limited 2014.

O40

The deficiency of adenosine deaminase type 2-results of therapeutic intervention

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Introduction: The deficiency of adenosine deaminase type 2 (DADA2) is a recessively inherited condition caused by mutations in *CECR1*. Patients present with recurrent fevers and evidence of vasculitis/vasculopathy, including livedo racemosa, lacunar strokes, polyarteritis nodosa, endothelialization of the hepatic sinusoids with portal hypertension, and active colitis. There is no recombinant form of ADA2 and thus we attempted a) exogenous replacement of ADA2 via fresh frozen plasma (FFP) and b) suppression of the inflammatory response using anti-tumor necrosis factor (anti-TNF) therapy. This abstract documents 22 months of clinical treatment in the NIH DADA2 cohort.
Objectives: a) To determine if FFP infusion is safe and if it would result in sustainable increased ADA2 levels; b) assess clinical and laboratory response to anti-TNF agents.
Patients and methods: ***FFP safety and pharmacokinetics:*** To assess the safety of FFP infusion, 3 DADA2 patients were admitted to the NIH Clinical Center for 5 consecutive days of FFP infusions. Serial ADA2 levels were drawn and FFP volumes were increased, barring adverse effects, each day. Subsequently, the patients were re-admitted and administered a single 100 mL FFP dose. Blood samples were drawn for ADA2 analysis.
Anti-TNF therapy: After comprehensive evaluation, patients were started on anti-TNF therapy. Patients underwent follow-up evaluation after approximately one year on treatment. Follow-up procedures were completed as needed.
Results: ***Pharmacokinetic results:*** Utilizing the observed increase in ADA2 levels in patients from the pre- and post-infusion samples over the 5 days of FFP infusions, a volume of distribution for ADA2 of approximately 1100 ml (range: 605-2207 ml) was calculated. When serial ADA2 sampling was conducted in the subsequent admission, the median terminal half-life was approximately 6.4 hours (range: 4.95-8.95 hours).
Anti-TNF results: 12 patients were treated with anti-TNF agents. The median time on therapy was 10 months (IQR: 8.5-19). There were no significant new disease complications (strokes, GI ischemia, worsening portal hypertension). Inflammatory markers stabilized in all cases and anemia improved. One patient with documented portal hypertension and esophageal varices had variceal resolution after 12 months of therapy. The cutaneous PAN lesions improved but there was persistent livedo racemosa.
Conclusion: Although the administration of FFP caused a transient increase in serum ADA2 levels, the short half-life would necessitate that large volume infusions (>200-300 ml) be given at least daily, rendering this treatment unfeasible. There has been dramatic clinical and laboratory improvement on anti-TNF agents.

O41

Pulmonary hypertension in familial Mediterranean fever: consequence or coincidence?

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Objectives: FMF is the most common autoinflammatory disease characterized by recurrent febrile polyserositis. The gravest consequence of FMF is nephropatic amyloidosis of AA type, which may progress to affect other organs, including the lungs[1]. Pulmonary hypertension (PH) in FMF related amyloidosis is rare; only a few cases have been reported so far[2,3]. We aim to elucidate development of PH in FMF in Armenian patients group.
Methods: 80 FMF patients without amyloidosis (mean age 33.6±11.8, male/female 45/35) and 75 FMF-amyloidosis patients (37.8±7.4, 42/33) were included in the study and followed prospectively for 3 years. All patients had recurrent pleuritis except of three phenotype II patients. Selected patients were homo/heterozygous for the M694V(n=122), M680I (28), V726A(4) and E148Q(1) mutations. Chest X-ray, pulmonary function test, ECG, transthoracic Doppler echocardiography (TTE) and CT-scan were carried out. Hb, ESR, leucocytes, fibrinogen, CRP, SAA, creatinine and capillary blood gases were measured. All patients were attack-free under colchicine treatment at the time of the study except of two hemodialysis patients. We considered patients to have PH if their estimated pulmonary artery systolic pressure (PASP) was >35 mm Hg as measured by TTE.
Results: (6%) FMF patients without amyloidosis and 9 (12%) with amyloidosis were diagnosed having PH (male/female 5/9). The median age at the diagnosis of PH was 48 years (range, 36-72). The median FMF duration at the time of PH diagnosis was 36 (1-60) years. All patients had symptoms related to PH: exertional dyspnea and fatigue (14 patients), chest pain (10), hepatomegaly (7), anorexia and weight loss (7), peripheral edema (6), ascites (5), cough (3), palpitation (3) and syncope (1). 4 patients had palpable right ventricular lift and 6 had increased intensity of P2 or splitting of S2. TTE data were as following: the median ejection fraction was 50% (20-60), the median PASP was 40 mm Hg (36-64). 9 patients had right ventricular dilatation and/or hypertrophy, tricuspid regurgitation of different degree, and 3 patients had pericardial effusion (90, 120, 150ml). Chest X-ray findings were abnormal in 11 patients (9 FMF-amyloidosis and 2 without amyloidosis) and showed opacities, hilar adenopathy, pleural thickening and pleural effusion. The other 3 patients had pleural adhesions. Chest CT scans findings were suggestive for cardiac and pulmonary amyloidosis (interstitial reticulonodular infiltrates) in 8 patients. PFT revealed restrictive pattern in 10 patients,

restriction with mild obstruction in 2 patients and lung volumes were normal in 1 patient without amyloidosis.
Patients were not treated for their PH. Three patients died of cardiac complications (congestive heart failure), they had impaired kidney function as well, and one amyloidosis patient died because of uremia. On autopsy amyloid deposits in kidney, spleen, liver, heart and lungs were found in three cases. The median time to death after the diagnosis of PH was 470 days (range 135-1095).
Lab tests were as following in FMF-amyloidosis patients group vs group without it (mean±SD): CRP 17.74±13.74 mg/L vs 11,88±13.79 mg/L and SAA 33±66.6 mg/L vs 5.25±4,45mg/L (p<0,0001). $PO_2$83.6±8.95 mmHg, $PCO_2$39.4±3.6 mmHg, O_2Sat 94.6±3.38% vs. $PO_2$74±11.36, $PCO_2$35.3±4.5, O_2Sat 90.1±10.26% (P<0.0001).
Conclusions: Patients with FMF may develop PH late in the disease process with resultant right-sided heart failure. The prognosis of AA amyloidosis depends on the degree of renal dysfunction at presentation and whether the underlying inflammatory disease can be effectively suppressed[4]. We speculate that PH in FMF is a consequence of ongoing inflammation and amyloidosis with subsequent early vascular alteration. This may explain PH development in the absence of severe intravascular amyloid deposits. PH develops in most untreated patients and in those who are on hemodialysis. In published case reports[2,3] the prognosis of FMF patients with PH and amyloidosis was poor. Our data are in accordance with them; PH in FMF may have a fatal course. We conclude that PH should be considered in FMF patients with dyspnea, fatigue or fluid overload, especially in individuals with amyloidosis. If present, PH should be monitored and treated.

References

1. Livneh A, Langevitz P, Pras M: **Pulmonary associations in FMF.** *Curr Opin Pulm Med* 1999, **5**:326-331.
2. Johnson WJ, Lie JT: **Pulmonary hypertension and FMF: a previously unrecognized association.** *Mayo Clin Proc* 1991, **66**:919-925.
3. Dingli D, *et al*: **Pulmonary hypertension in patients with amyloidosis.** *Chest* 2001, **120**:1735-9.
4. Lachmann HJ, Hawkins PN: **Amyloidosis and the lung.** *Chron Respir Dis* 2006, **3**:203-14.

O42

Deletion in *MEFV* resulting in the loss of p.M694 residue as the cause of autosomal dominant familial Mediterranean fever in North Western European Caucasians - a case series and genetic exploration

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Introduction: Familial Mediterranean fever (FMF) is the commonest hereditary autoinflammatory disease and it has mostly been reported in populations of Mediterranean ancestry, especially Armenians, Arabs, Turks, non-Ashkenazi and Sephardic Jews. FMF has a very low prevalence amongst Western Europeans. Its hallmarks are autosomal recessive inheritance and short bursts of illness lasting up to 3 days comprising fever, serositis and arthritis, and which can largely be prevented by daily colchicine treatment. The FMF gene *MEFV* is located on chromosome 16p andconsists of 10 exons. Although the disease is usually inherited in an autosomal recessive fashion, deletion of methionine at position 694 has been associated with dominant inheritance.
Objective: This study characterised the phenotype and response to treatment in patients with p.M694del.
All of the patients were of Northern European heritage, prompting us to performed haplotype reconstruction to investigate the possibility of common ancestry.
Methods: *MEFV* gene was analysed in 3500 subjects with suspected FMF referred to a single UK centre between 2002 and 2014. Patients with p.M694del underwent additional screening of the *SAA1* gene as well as haplotype reconstruction of the *MEFV* locus.
Results: The p.M694del variant was identified in 21 patients, sharing an identical disease haplotype that appears to have arisen about 550 years ago. The clinical features comprised typical FMF symptoms with median age at onset of 18 years; three patients presented with AA amyloidosis, of whom two had had symptoms of FMF in retrospect. Fifteen patients had received colchicine treatment, all with excellent responses. The *SAA1.1* allele was found in four patients, including two with AA amyloidosis.
Conclusion: The p.M694del variant is associated with autosomal dominantly inherited FMF in Northern European Caucasians. Symptoms may develop later in life than in classical recessive FMF but are otherwise similar as is the response to colchicine treatment. The 14% incidence of AA amyloidosis may reflect delay in diagnosis associated with extreme rarity of FMF in this population. The common haplotype suggests a single founder living in about 1460.

O43

Unified modeling of Familial Mediterranean Fever and Cryopyrin Associated Periodic Syndromes

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Background: Familial Mediterranean Fever (FMF) and Cryopyrin Associated Periodic Syndromes (CAPS) are two prototypical hereditary autoinflammatory diseases, characterized by recurrent episodes of fever and inflammation as a result of mutations in MEFV and NLRP3 genes encoding Pyrin and Cryopyrin proteins, respectively. Pyrin and Cryopyrin play key roles in the multiprotein inflammasome complex assembly, which regulates activity of an enzyme, Caspase 1, and its target cytokine, IL-1 beta. Overproduction of IL-1 beta by Caspase 1 is the main cause of episodic fever and inflammatory findings in FMF and CAPS.
Objectives: Quantitative models have been used in order to understand how the immune cells and key molecular players interact with each other to constitute the total activity of the immune system. This study aimed to develop a mathematical model to describe the quantitative behavior of all immune cells and inflammatory molecules involved.
Methods: Critical components involved in the pathogenesis of FMF and CAPS were determined by extensive literature review. A unified dynamical model that captures key aspects of FMF and CAPS was introduced in the form of coupled nonlinear ordinary differential equations. Detailed bifurcation analyses were performed on the model, and simulation results were obtained.
Results: The model is composed of two subsystems. One of the subsystems, which contains a coupled positive-negative feedback motif, captures the dynamics of inflammation formation and regulation. A comprehensive bifurcation analysis of the model shows that it exhibits three modes, capturing the Healthy, FMF and CAPS cases. The mutations in Pyrin and Cryopyrin are reflected in the values of three parameters in the model. According to the bifurcation analyses performed and simulation results obtained from the model, Caspase 1 level is the most critical parameter in determining the three modes that the model exhibits, “Healthy”, “FMF” and “CAPS”. In accordance with the clinical literature, FMF comes out as trigger-dependent condition, while CAPS is mostly due to autonomous events, mainly associated with self dynamics of the immune response-related inflammasome and associated proteins’ concentrations. In the presence of a trigger, there was a normal increase in inflammation initiators in the Healthy mode. In FMF, the response to trigger introduction is more intense and a severe inflammatory cascade is activated. In CAPS, on the other hand, even in the absence of a trigger, periodically recurring, severe inflammatory episodes are observed. The proposed model also explains why a proCaspase 1 inhibitors can be effective in treating FMF, but not CAPS, for which drugs that directly inhibit IL-1 are used.
Conclusions: This provisional mathematical model for FMF and CAPS may help explain their pathogenesis and observed clinical behavior as well as designing custom drug therapies for better control of disease activity. Further studies are necessary to link and fine tune all model variables and parameter values to clinical observations and measurements from in vitro experiments, as well as to assess this models potential in the drug development process.

O44

Colchicine therapy in children with FMF

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Introduction: Colchicine treatment is the standard therapy for prophylaxis of attacks and amyloid deposition in familial Mediterranean fever. In the guidelines of 2007 an initial dosage in regard of the age of the patient is recommended (0.5 mg/day for children <5 years of age, 1.0 mg/day for children 5-10 years of age, 1.5 mg/day for children >10 years of age).
Objectives: The following objective were raised in the study: To analyze factors, which influences the individual colchicine dose in children with FMF. To analyze the impact of dose adjustment on the clinical course and the degree of subclinical inflammation. To analyze parameters, which predict dose increase in the upcoming 12 month including levels of SAA and S100A12-molecules.
Patients and methods: Data of the FMF-patients were extracted from the AID-Net-register and analysed with SPSS (Patient number n=409, Visit number n= 4750). Correlation and regression analyses were performed with the aim of an multivariant analyses. The extraction of the data was in January 2015.
Results: Colchicine dose correlates with the genotype. It further increases linearly with an increase of age (1 - 18 years), body weight and body surface area. The correlation of colchicine dose with body surface revealed an average of 1 mg colchicine / 1 m^2.
A high response rate in the CRP and the attack rate is shown both at time of therapy introduction (70% response CRP: 45.5% / attack frequency: 61.4%), as well as dose increases during the course of the disease (with an increase of 1.0 mg to 1 5 mg: 70% Response CRP: 32.1% / attack frequency: 42.3%).
The severity of disease at diagnosis increases the likelihood of a dose raise in the first 12 months and 24 months after therapy introduction.
An increase of classical inflammatory markers (CRP, SAA) and S100 proteins show a significant correlation to a dose increase in the following 12 or 24 months ($p < 0.05$).
Conclusion: The mutation, the age, the body weight, the body surface area and the initial disease severity affect the colchicine dose.
Dose increase is effective to control disease activity and subclinical inflammation. This effect was also observed when increasing the dose from 1.5 mg to 2.0 mg a day.
An increase in the classical inflammation markers and S100A12- or S100A8 / A9-value are predictors for an upcoming increase in the dose in the next 12 or 24 months, None of these factors showed an advantage in predicting a higher dose.

O45

Canakinumab therapy in patients with Familial Mediterranean Fever

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Background: It has been reported that canakinumab, a monoclonal anti IL-1 antagonist, was effective in patients with FMF who are either non-responsive or intolerant to colchicine.
Objectives: To share our experience with canakinumab in a larger group of FMF patients treated for diverse indications and with a longer follow-up.
Methods: The data of the patients on canakinumab who are examined physically and checked for laboratory parameters before each injection is evaluated with regard to response and safety.
Results: Data of 31 (17F/14M) patients who had received more than 3 injections of canakinumab (150mg/mo) were included. The indications were insufficient response to colchicine in 22 (>1 attack/month), amyloidosis in 6, injection site reaction with anakinra in 5, and adverse effects of colchicine in 4 patients (azoospermia and neuropathy in one each, myopathy in 2). Six patients had concomitant diseases like ankylosing spondylitis and polyarteritis nodosa. The mean age of the patients was 35,90±13 years, the mean disease duration was 15±9,30 years, the mean injection number was 9,80±6 and the mean duration of canakinumab therapy was 16±9,26 months. Twenty four patients had no attacks after canakinumab. The attack frequency was reduced more than %50 in 5 patients, did not change in 2. In 6 cases with FMF amyloidosis, proteinuria decreased in 2 (from 15020 to 1098mg/dl; from 6135 to 300mg/dl), increased in the other 2 (from 1700 to 4700mg/dl; from 5001 to 7061 mg/dl) and was stable in the remaining 2 patients. Fourteen patients with myalgia and calf pain unresponsive to colchicine, improved significantly as the mean patient global assessment score decreased from 8,25 ± 2,48 to 2,06±2,63 (p.
Conclusion: Canakinumab is effective in controlling the febrile attacks and calf pain in FMF patients with inadequate response or intolerant to colchicine, yet its effect in amyloidosis is variable. It seems to have an acceptable safety profile. Further controlled studies are needed to better assess the safety and efficacy of canakinumab in FMF.

O46

Semaphorin 3A, a potential immune regulator in Familial Mediterranean Fever

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Introduction: Semaphorin 3A (sema3A) plays a regulatory role in immune responses, mainly affecting the activation of regulatory T cells. It has been found to correlate with disease activity in rheumatoid arthritis and systemic lupus erythematosus. Familial Mediterrenean Fevere (FMF) is an autoinflammatory disease; yet a possible role for regulatory T cells has been described.
Aim: To evaluate the expression of sema3A in peripheral blood, on B cells and on regulatory T cells, of FMF patients during attack, in remission and with smoldering disease, in comparison with healthy controls.
Methods: 17 FMF patients in attack and in remission, 8 FMF patients with smoldering disease and 12 healthy controls were enrolled. Smoldering disease was defined in FMF patients with high CRP level (above 20mg/dL) without concurrent symptoms of serositis, arthritis nor concomitant known infectious disease. Sema3A in peripheral blood was measured by ELISA and expression of sema3A on regulatory T cells and regulatory B cells was evaluated by FACS analysis. FMF patients were evaluated for demographics and disease severity by the Mor severity score.
Results: The 3 groups of patients: a. FMF in attack and remission, b. FMF with smoldering disease and c. healthy controls were similar with regard to age (38.1±10.2 vs.48±15 vs.43.1±3.4) and female gender (8 [47%] vs. 4 [50%] vs.5 [41%]). The age of onset was 10 years ±7.9 in group a and 15 years ±6.3 in group b. The mean colchicine dose was similar between groups a and b (1.9±0.48 vs. 2.25±0.5) and so was the Mor severity score (3.2± 1.9 and 2.8± 1.6).
Semaphorin 3A expression on regulatory T cells in FMF patients during an attack was lower than in remission and in healthy controls (57.2%± 8.3 vs. 77.2%±10.3 vs. 88.7%± 3.6%, $p<0.01$) but similar to patients with smoldering disease.
Semaphorin 3A expression on regulatory B cells were lower in FMF patients during an attack than FMF in remission and in healthy controls (72.9%±8.5 vs. 83.4%±5.8 vs. 82.6%±6.4 ng/ml, $p<0.05$), but was similar to patients with smoldering disease.
Semaphorin 3A concentration in peripheral blood as evaluated by ELISA was lower in FMF patients during an attack, in smoldering disease and in remission than in healthy controls (242.3±9.8 ng/ml vs. 258.9±11.5 ng/ml vs. 232.5±22.7 ng/ml vs. 323.3±160.2 ng/ml, $p<0.05$).
Conclusion: Sema3A expression on regulatory T cells and regulatory B cells is low in FMF patients during an attack and in smoldering disease compared to the expression in FMF remission and in healthy controls. Regulatory T cells have been described to increase one week after an attack and were speculated to have a role in the termination of FMF attacks. A decrease in sema3A in an attack and normalization at remission may help explain the decrease and subsequent normalization

of regulatory T cells. The role of regulatory T cells and semaphorin 3A in termination of FMF attacks needs further evaluation on a wider scale.

O47

Factors affecting cardiovascular morbidity in young FMF patients. A comparative analysis in colchicine treated FMF patients with and without cardiovascular disease

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Background: Familial Mediterranean fever (FMF) is the prototype of chronic auto-inflammatory diseases. During the FMF attacks there is an uncontrolled activation of an inflammatory cascade with a consequent release of many pro-inflammatory molecules, which subsides or slows down in between the attacks. Chronic inflammation has been found to be associated with higher incidence of atherosclerotic cardiovascular disease (CVD). Thus, being a chronic inflammatory disorder, it is speculated that FMF may be considered as an independent risk factor for CVDs. Most studies looking at the association between FMF and CVDs have focused on markers, suggesting increased atherosclerosis in FMF as compared to the general population. Yet, these studies yielded conflicting results. In the present study we analyze atherosclerosis morbidity in FMF, by comparing affected to unaffected FMF patients.
Objective: To determine FMF related and other underlying factors leading to cardiovascular disease in FMF.
Methods: All files of colchicine treated FMF patients, 50 years old or less, cared for in Sheba Medical Center (the largest FMF Center in Israel, with registered FMF population larger than 10000 patients), as in patients or FMF clinic outpatients, over the last 10 years, bearing a diagnosis of cerebral, cardiac or peripheral vascular disease were pulled out and reviewed. For each studied patient 2 FMF control subjects were adjusted from patients arriving to FMF clinic for their periodic follow up visit. Our endpoints were: 1. Incidence of FMF related (without additional risk factors) CVD in this population compared to the general population 2. Elucidation of FMF related and unrelated risk factors for CVD in FMF. FMF severity, one of the FMF related factors studied, was assessed using the severity score-2 (SS-2) by Mor et. Al.
Results: There was only one FMF patient younger than 50 year old, who suffered of CVD and had none of the traditional risk factors. All other FMF patients with CV morbidity (23 cases) had other risk factors for CV disease. Compared with the control FMF subjects, none of the assessed FMF related parameters, including increased disease severity, was more common in FMF-CVD. However, in the FMF-CVD cohort, the rate of other inflammatory diseases was higher.
Conclusion: These findings suggest that in colchicine treated FMF population younger than 50 years of age, FMF per se is not a risk factor for CVD.

O48

Altered cytokine pattern and inflammatory pathways in monogenic and complex autoinflammatory diseases

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Introduction: Autoinflammatory disorders (AIDs) are a group of innate immunity-related diseases, characterized by seemingly unprovoked inflammatory episodes mainly involving skin, eyes and joints. They can be categorized into hereditary monogenic and multifactorial complex disorders. The inflammatory mechanisms underlying both monogenic and complex AIDs are not completely understood.
Objectives: In order to clarify them, we started to evaluate the *ex vivo* cytokine profile and the activation of the principal inflammatory pathways in Familial Mediterranean Fever (FMF), TNF-receptor associated periodic syndrome (TRAPS), Blau syndrome (BS) and Adult Onset Still's Disease (AOSD) patients during attack-free periods, and compare the results with those from healthy controls.
Patients and methods: The study included 7 FMF, 12 TRAPS, 2 BS, and 8 AOSD patients and 27 healthy controls. Cytokine levels were evaluated by Antibody microarray in serum, whereas pathway activation through Reverse phase protein array (RPPA) in peripheral blood mononuclear cells (PBMCs).
Results: Interleukin (IL)-17, IL-22, and IL-23 were all significantly raised in our cohort of AIDs compared to controls, whereas IL-1β, IL-6, IL-8, and TNF-α levels were differently heighten among the diseases. Thus, the cytokine pattern may help to distinguish the AIDs in term of number of enhanced cytokines, as follow BS<TRAPS<FMF<AOSD. Moreover, the upregulation of Th17-related cytokines may suggest an important role for Th17 or Th17-like cells in AIDs, that are directly involved in inflammatory processes.
All diseases patients presented a general constitutive activation of inflammatory pathways compared to the control group. In particular, NF-κB, MAPK, PI3K/AKT, JAK/STAT and NLRP1 inflammasome pathways were all upregulated in AOSD, whereas TRAPS, FMF and BS show different specific activation. So, the variance among the results suggested a more complicated relationship between individual patients and pathways compounds, associated with their peculiar clinic and genetic conditions.
Conclusion: Our results suggested an ongoing subclinical inflammation related with the abnormal and constitutive signalling pathways and defined an elevated inflammatory cytokine signature.
Thus, if confirmed, the evaluation of the number of raised cytokines could be a new way to stratify autoinflammatory diseases patients. Furthermore, critical in AIDs may be the modulation of the Th17 cytokine network pathway.

O49

Two types of human Th17 cells with pro- and anti-inflammatory properties and distinct roles in autoinflammation

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Introduction: Th17 cells are known to be crucial mediators of autoimmune inflammation. However, two distinct types of Th17 cells have recently been described, which differed in their ability to coproduce IL-10 or IFN-g due to differential polarizations requirements for IL-1b. Whether these distinct Th17 phenotypes translate into distinct Th17 cell functions and whether this has implications for human health or disease has not been addressed yet.
Objectives: We hypothesized that IL-1b independent IL-10$^+$Th17 cells have anti-inflammatory functions whereas IL-1b dependent IL-10$^-$ Th17 cells are pro-inflammatory. Considering the crucial role of IL-1b in the pathogenesis of autoinflammatory syndromes, we hypothesized an IL-1b mediated loss of anti-inflammatory Th17 cell functions in Schnitzler Syndrome, an autoinflammatory disease.
Methods: To assess pro- versus anti-inflammatory Th17 cell functions we performed suppression assays and tested the effects of IL-1b dependent and independent Th17 subsets on modulating pro-inflammatory cytokine secretion by monocytes. Schnitzler Syndrome patients were analyzed for changes in Th17 cell functions before and after therapy with IL-1b depleting drugs.
Results: IL-10$^+$ Th17 cells, which differentiated independently of IL-1b, have regulatory functions similar to Treg cells while IL-1b dependent IL-10$^-$ Th17 cells have not. Both Th17 cell subsets differ in their ability to suppress T cell proliferation as well as in their ability modulate pro-inflammatory cytokine production by antigen presenting cells. In Schnitzler Syndrome, an autoinflammatory syndrome, overproduction of IL-1b translates into pro-inflammatory Th17 cell functions, which can be reversed by anti-IL-1b treatment.
Conclusion: Th17 cells are not *per se* pro-inflammatory but can also have anti-inflammatory IL-10 mediated functions if generated independently of IL-1b. Our data introduce Th17 cell subsets as novel players in autoinflammation and thus novel therapeutic targets in autoinflammatory

syndromes including other IL-1b mediated diseases. This demonstrates for the first time alterations in the adaptive immune system in autoinflammatory syndromes.

O50

A unifying molecular mechanism underlying the association of *CARD14* alleles with autoinflammatory and T-cell mediated skin disorders

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Introduction: The *CARD14* (Caspase Recruitment Family Member 14) locus encodes a scaffold protein that mediates NF-kB signalling in keratinocytes and is therefore crucial to the maintenance of skin immune homeostasis. In keeping with this notion, gain-of-function *CARD14* mutations have been observed in patients with plaque psoriasis and pityriasis rubra pilaris, two skin disorders mediated by abnormal T cell activation. More recently, a *CARD14* missense variant has been tentatively associated with generalised pustular psoriasis (GPP), an auto-inflammatory condition characterised by acute episodes of skin pustulation and systemic upset.
Objectives: The aim of this study was to establish whether *CARD14* alleles are genuinely associated with GPP and to investigate the molecular mechanism underlying any effect on disease risk.
Patients and methods: We investigated an extended case cohort (n=100) ascertained in Europe and East Asia. As all disease alleles described to date cluster to exons 3 and 4, we screened this mutation hotspot in all patients. We also sequenced the entire *CARD14* coding region in a subset of 16 individuals. We analysed population matched, control genotypes (n= 997) that were generated in-house or had been previously released by the 1000 Genomes Consortium. Finally, we investigated the accumulation of CARD14 oligomers by western blotting, following the transfection of HEK293 cells with wild-type or mutant cDNA constructs.
Results: We found that a non-conservative p.Asp176His substitution was significantly associated with GPP in the Chinese and Japanese populations (combined P=0.0001; OR:5.3). Bioinformatics showed that this change had pathogenic potential and was likely to disrupt the coiled coil of CARD14. Importantly, our analysis predicted a similar effect for p.Glu138Ala and p.Leu156Pro, two disease alleles previously associated with psoriasis and pityriasis rubra pilaris. Since the coiled coil domain of CARD14 mediates protein oligomerization, we investigated the effects of the above mutations on the accumulation of CARD14 aggregates. We found that all three disease alleles caused spontaneous protein oligomerization.
Conclusion: Given that CARD14 oligomerization is a pre-requisite for downstream signal transduction, our results indicate that disease alleles promote abnormal NF-kB signalling by causing constitutive protein aggregation. Thus, our work points to a unifying pathogenic mechanism underlying the effects of *CARD14* mutations on auto-inflammatory and T-cell mediated disorders.

O51

An optimized whole blood assay measuring expression and activity of NLRP3-, NLRC4 and AIM2-inflammasomes

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Introduction: Caspase-1 activates proIL-1β and proIL-18 and plays a fundamental role in innate immunity. This pro-inflammatory immune response is required for the initiation of pathogen clearance. Caspase-1 itself is activated in so-called "inflammasomes" which are assembled in response to distinct pathogen associated molecular patterns (PAMP) or danger associated molecular patterns (DAMP). Most studies analyzing the inflammasome/caspase-1 activity of patients *ex vivo* use PBMC-based assays. Beside monocytes and macrophages, caspase-1 is also activated in PMNs representing the major leukocyte subset in peripheral blood. Thus, analyzing purified PBMCs seems to be highly artificial by excluding caspase-1 activity in PMNs and ignoring cellular interactions. Furthermore, PBMC purification requires large amounts of blood, thereby rendering the assay impractical for the use in small children or neonates.
Objective: To establish and validate a small-volume based human whole blood assay facilitating the measurement of inflammasome-related gene expression and caspase-1 activation upon stimulation of the NLRP3, NLRC4 or AIM2 inflammasome.
Methods: We set up a whole-blood assay in 96-well format using 140µl blood per well. Following stimulation with LPS/ATP, Salmonella typhimurium or LPS/polydA:dT (activation of NLRP3, NLRC4 or AIM2) IL-1β was measured in the supernatant and used as surrogate of caspase-1 activation. The inflammasome-specificity was studied for each stimulus by inflammasome inhibition using high potassium buffer, methylsulfonylmethane or MCC950. Furthermore, gene-expression analysis following red cell lysis was performed.
Results: Using our optimized whole-blood assay, we are able to measure IL-1β secretion as well as gene-expression of *IL-1β, NLRP3, NLRC4, AIM2, PYCARD*, and *CASP1* following differential activation the NLRP3, NLRC4 or AIM2 inflammasome. Priming of cells with ultra pure LPS directly induced IL-1β secretion. This constitutive caspase-1 activity is already known for monocytes and depends on NLRP3. Further increase in NLRP3 activity was achieved using additional ATP stimulation, but this effect was dependent on continuous agitation of the probes. Secretion of IL-1β after stimulation with *S. typhimurium* was shown to be dependent on NLRP3 and NLRC4. In the early phase, *S. typhimurium* primed the cells and IL-1β secretion was mainly dependent on NLRP3 activity. Later on, IL-1β secretion was less susceptible to inhibition of NLRP3 inflammasome activity. Transfection of polydA:dT using Lipofectamine 2000 led to activation of the AIM2 inflammasome mainly. Interestingly, Lipofectamine without polydA:dT led to exclusive activation of NLRP3.
Conclusion: It is possible to analyze caspase-1 activity and inflammasome-related gene expression in whole-blood samples following distinct inflammasome activation. Based on our data we assume that there is a superposition of NLRP3 and NLRC4 or AIM2 inflammasome activities in human whole blood following stimulation with *S. typhimurium* or polydA:dT.
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O52

Cofilin-1 is an essential redox sensor for NLRP3 inflammasome activation

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Introduction: NLRP3 has a pivotal role in nucleating the inflammasome, a cytoplasmic multiprotein complex that mediates the maturation of the proinflammatory cytokine interleukin-1β (IL-1β) by activating caspase-1. Mutations in the gene encoding NLRP3 cause a spectrum of autoinflammatory disease, the cryopyrin-associated periodic syndromes (CAPS). It has been reported that generation of reactive oxygen species (ROS) is a major NLRP3 inflammasome-activating factor. However, the molecular mechanism by which a change in cellular redox state leads to NLRP3 inflammasome activation has not been elucidated. Here we show that cofilin-1, a redox sensitive actin binding protein, is involved in NLRP3 inflammasome activation.
Objectives: To investigate how ROS activates the NLRP3 inflammasome.
Methods: Cell culture supernatants from bone marrow derived macrophages (BMDMs) of wild-type or NLRP3-KO mice were analyzed by mass spectrometry. Inflammasome activation was analyzed by Western blotting of secreted IL-1β or ASC oligomerization. The interaction of NLRP3 with cofilin-1 was examined by co-immunoprecipitation from BMDMs or transfected cells.

Results: Cofilin-1 is highly secreted along with IL-1β from LPS-primed BMDMs in response to the known NLRP3 activator, ATP, whereas knockdown of cofilin-1 reduces NLRP3 inflammasome activation. Cofilin-1 directly interacts to the nucleotide-binding domain (NBD) of the NLRP3 protein in LPS-primed BMDMs. However, cofilin-1 is dissociated from NLRP3 in a ROS-sensitive manner when the cells are stimulated with the NLRP3 inflammasome activators, ATP or nigericin, which induce oxidation of cofilin-1. Indeed, the interaction of cofilin-1 with NLRP3 is increased significantly when the oxidation site of cofilin-1 is substituted from cysteine to alanine. Moreover, the assembly of inflammasome components is impaired in cells expressing oxidation-resistant mutant cofilin-1.
Conclusion: Taken together, these findings suggest that cofilin-1 is a key component in regulating the NLRP3 inflammasome in response to ROS. In addition, our data suggest a potential target for the inflammatory conditions involving the NLRP3 inflammasome, including gout, type 2 diabetes mellitus, atherosclerosis, and Alzheimer's disease.

O53

Enzymatically inactive caspase-1 mediates a proinflammatory phenotype in mice

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Introduction: The proinflammatory enzyme caspase-1 belongs to the family of cystein proteases and plays a pivotal role in innate immunity. Classically, caspase-1 mediated proinflammatory signaling is thought to be dependent on its enzymatic activity. Interestingly, a number of patients carrying variants of the *CASP1* gene show proinflammatory symptoms like febrile episodes despite of reduced enzymatic activity of the caspase-1 protein resulting in decreased IL-1β production. *In vitro* investigations of this contradictory phenomenon revealed receptor interacting protein kinase 2 (RIP2) as a potential mediator between decreased caspase-1 activity and increased inflammation. In order to investigate the role of enzymatically inactive caspase-1 in an *in vivo* situation, we generated a mouse model expressing enzymatically inactive caspase-1.
Objectives: The main objective of the study is to uncover the effects and mechanisms of enzymatically inactive caspase-1 in inflammatory signaling *in vivo*.
Materials and methods: We generated two different mouse models of enzymatically inactive caspase-1 C284A using a BAC transgene and a conditional gene targeting approach. Successful integration was verified by PCR for the BAC transgene or by PCR and Southern Blot analyses for the gene targeting approach. Transgene expression was proven by qRT-PCR and Western Blot analyses of different tissues. In order to specifically investigate the effect of inactive caspase-1, the transgenic mice were crossed back on a caspase-1 knockout background. The influence of enzymatically inactive caspase-1 on inflammatory signaling was analyzed using a model of LPS-induced endotoxin-shock. Readout was change of body temperature and cytokine levels in the serum.
Results: Following LPS-induced endotoxin shock, mice expressing enzymatically inactive caspase-1 ($Casp1^{-/-}$/$R26Casp1C284A^{+/+}$) showed a pronounced decrease of body temperature and increased levels of the proinflammatory cytokines TNF-α and IL-6 compared to caspase-1 knockout mice ($Casp1^{-/-}$). However, the strongest decrease of body temperature was found in wildtype controls ($Caspase\text{-}1^{+/+}$).
Conclusion: Taken together, we show for the first time that enzymatically inactive caspase-1 is able to initiate proinflammatory signaling *in vivo*. This data is in line with several publications assuming a scaffold-function of caspase-1 independent of its enzymatic activity. However, the proinflammatory signaling mediated by enzymatically active caspase-1 seems to be stronger in the context of LPS-induced endotoxin shock. In order to uncover potential mediators and mechanisms of proinflammatory signaling initiated by enzymatically inactive caspase-1, gene expression profiling and secretome analysis are needed in the future.
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O54

Monocytes and neutrophils in the inflammatory cascade of systemic onset Juvenile Idiopathic Arthritis

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Background: Systemic onset Juvenile Idiopathic Artritis (sJIA), also known as Still's disease, is characterized by arthritis with symptoms of systemic inflammation such as spiking fever, rash and serositis. It is considered an autoinflammatory disease with a major role for the innate immune system, reflected by extremely high serum levels of S100 proteins and interleukin (IL)-18. How the number of monocytes and neutrophils relate to the increased levels of S100-proteins and IL-18 and to sJIA disease progression is still unknown.
Objective: To study the role of monocytes and neutrophils in the inflammatory cascade of sJIA.
Methods: We determined *ex vivo* cell frequencies and cell surface activation markers of sJIA patients at disease onset, in remission and healthy controls by flow cytometric analysis. For the *in vitro* assessment of neutrophils, we stimulated whole lysed blood or isolated neutrophils with S100-proteins, IL-18, platelet-activating factor (PAF) with or without Formyl-Methionyl-Leucyl-Phenylalanine (fMLP) or phorbol 12-myristate 13-acetate (PMA) and determined intracellular ROS production, degranulation and apoptosis. To investigate the role of monocytes, we stimulated peripheral blood mononuclear cells (PBMCs) from sJIA patients and healthy controls with S100-proteins (+/- ATP) or other TLR-ligands and determined the concentration of cytokines in the supernatant by multiplex immunoassays.
Results: Patients with new onset sJIA had significantly elevated neutrophil counts compared to healthy controls and sJIA patients with clinically inactive disease, while the amount of monocytes was not significantly different between the groups. Neutrophils from new onset sJIA patients showed an activated phenotype, reflected by higher *ex vivo* expression of Fc-gamma receptors (CD32 and CD64), markers of secretory vesicles (CD35) and specific granules (CD66b) compared to healthy controls. Neutrophils from new onset sJIA showed enhanced ROS production and degranulation and appeared to be more resistant to apoptosis. In contrast to the hyperactivated status of neutrophils in active sJIA,, PBMCs from these patients produced less IL-18 upon S100 stimulation compared to PBMCs from the same patient in remission or healthy controls. The same trend was observed when PBMCs from sJIA patients were stimulated with LPS, TLR2- or TLR7/8 ligands, suggesting cross-tolerance in these patient cells.
Conclusions: Although monocytes from sJIA patients with active disease are less responsive towards stimulation, neutrophil counts, ROS production and degranulation are clearly elevated. The exact role of each cell type and activity and their interaction in sJIA pathology is currently under investigation.

O55

NextGen sequencing (NGS) panel for hereditary recurrent fevers: mutation spectrum, novel mutations, and evidence for re-classification of common variants based on analysis of >3000 cases from North America

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Aim: Hereditary recurrent fevers (HRF) are genetically heterogeneous and often present a diagnostic challenge. To aid in molecular diagnosis, we developed and utilized a 7-gene NGS panel for HRF.
Methods: The HRF panel includes *MEFV*, *MVK*, *NLRP3*, *TNFRSF1A*, *PSTPIP1*, *LPIN2* and *ELANE*, which are associated with familial Mediterranean fever (FMF), hyper-IgD syndrome, cryopyrin-associated periodic syndrome, tumor necrosis factor receptor-associated periodic syndrome (TRAPS), pyogenic

sterile arthritis-pyoderma gangrenosum and acne syndrome, Majeed syndrome, and cyclic/severe congenital neutropenia, respectively. It utilizes a multiplex PCR approach, followed by NGS on HiSeq 2000/2500 instruments, and variant analysis using a custom-developed analysis pipeline.

Results: Using this NGS panel, 3,248 individuals with suspected HRF were tested in our diagnostic laboratory. In this largely North American population, the majority of pathogenic/ likely pathogenic variants (PV/LPV) were identified in the *MEFV* gene (66.6%; n=187), followed by *MVK* (14.6%; n=41), *NLRP3* (8.9%; n=25), *TNFRSF1A* (3.6%; n=10), *PSTPIP1* (2.5%; n=7), *ELANE* (2.1%; n=6), and *LPIN2* (1.7%; n=5). Sixty percent of PV/LPV were recurrent mutations in *MEFV* (M680I, M694V, K695R, V726A), *MVK* (I268T and V377I) and *NLRP3* (R490K). The remainder were low-frequency or unique PV/LPV. Co-occurrence of pathogenic variants in 2 different genes was only observed in 2 families. Novel PV/LPV were identified in 5 genes, including *LPIN* associated with Majeed syndrome. Originally reported as pathogenic, the genetic contribution of several common variants to HRF remains unclear, including *MEFV*-E148Q and *TNFRSF1A*-P75L. In our cohort, both variants co-occurred with definite pathogenic mutations in *MEFV* or another fever-associated gene. While their minor allele frequency (MAF) in affected individuals was higher than in our exome sequencing controls (*MEFV*-E148Q: 2.8% vs. 1.9%; *TNFRSF1A*-P75L: 0.6% vs. 0.3%), numerous healthy individuals were homozygous for either variant. Newly available population data (1000 Genomes, ESP and ExAC) revealed a MAF for p.E148Q as high as 30% in Asians, and 10% for p.P75L in African individuals, including a large number of homozygotes (p.E148Q: 701/7768; p.P75L: 20/2775), which exceeds by far the prevalence of FMF and TRAPS in these populations.

Conclusions: Our 7-gene NGS results represent the largest molecular-diagnostic dataset for HRF in the North American population, revealing mutation distribution and novel PV/LPV. We provide new evidence to reconsider the clinical significance of *MEFV*-E148Q and *TNFRSF1A*-P75L and propose these are population-specific polymorphisms that are unlikely to contribute to FMF or TRAPS. Our study underscores the utility of large datasets from diverse ethnic populations in clarifying the clinical significance of common HRF variants.

O56

Mevalonate kinase deficiency: an early onset inflammatory bowel disease?

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Introduction: Mevalonate kinase deficiency (MKD) is a rare autoinflammatory, autosomal-recessive defect on MVK gene. Clinical spectrum ranges from recurring febrile attacks to malformations and neurologic disorders. Gastrointestinal symptoms are cardinal. Severe gastrointestinal involvement has been described at the onset.

Objective: To analyse severe gastrointestinal events (SGE) complicating MKD.

Patients and methods: Retrospective observational French cohort of MKD patients. SGE were defined as complicated inflammatory involvement, requiring an abdominal surgery and/or enteral/parenteral nutrition. Data were collected from clinical charts provided by the members of the Francophone Society for Paediatric Rheumatism and Inflammatory Diseases (SOFREMIP).

Results: From a 53-patient cohort, nine presented a SGE (17%). From these, disease onset median age was 1.0 months (0-12); one patient deceased (22 months) from a non-gastrointestinal event. Compound heterozygote mutations were found in 7/8, being Val377Ile the commonest (6/8). The main symptoms during febrile attacks were: diarrhoea (100%, 7/7), lymphadenopathy (89.9%, 8/9), skin lesions, joint pain (85.7%, 6/7 each), aphtous ulcers, abdominal pain (83.3%, 5/6 each), splenomegaly (66.7%, 6/9), hepatomegaly (62.5%, 5/8) and vomiting (57.1%, 4/7). Median mevalonic aciduria: 23.05 mmol/mol of creatinine (P_{25}=13.7; P_{75}=55.5); median MK activity: 2.2% (P_{25}=1.0; P_{75}=24.0). The significant co-morbidities found in SGE patients in comparison with the global cohort were: failure-to-thrive in 85.7% (6/7), pulmonary diseases in 37.5% (3/8) and feeding disorders in 28.6% (2/7) ($p<0.05$).

Severe gastrointestinal involvement was the first event in 6% (3/50), representing 43% (3/7) of patients with severe gastrointestinal disease: abdominal adhesions (66.6%, 6/9) and colitis/enterocolitis (4/9, 44.4%) were mainly found. 87.5% (7/8) needed surgery and 44.4% (4/9) required enteral/parenteral nutrition. Despite digestive resection, disease progression remained; two patients needed re-intervention due to surgical complications. Aphtous/ulcerative damage was the main endoscopic feature (4/9, 44.4%). The most consistent microscopic finding was lymphocytic infiltrates. IL-1 antagonists were the most used/effective treatment (4/9), resulting in with complete remission in all three patients with data available.

Conclusion: MKD severe gastrointestinal involvement presentation has a non-negligible frequency. It usually appears as an aphtous/ulcerative disease involving any part of the digestive tract or as abdominal adhesions, frequently requiring surgery. The treatment with IL-1 antagonists resulted in complete remission in a majority of treated patients. Thus, MKD should be added to the list of monogenic early-onset inflammatory bowel disease.

O57

Diagnostic value of urinary mevalonic acid excretion in patietns with a clinical suspicion of mevalonate kinase deficiency (MKD)

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Introduction: Mevalonate kinase deficiency (MKD) is a rare hereditary autoinflammatory syndrome, characterized by recurrent fever episodes with gastrointestinal complaints, rash and arthralgia. In patients suffering from MKD, the reduced enzyme activity leads to an accumulation of mevalonic acid which is excreted in the urine. Therefore, an elevated mevalonic acid excretion is suggestive of MKD. However, the diagnostic value of this analysis has not been investigated yet and remains unclear.

Objectives: To investigate the diagnostic value of urinary mevalonic acid excretion in patients with suspected MKD.

Patients and methods: In this retrospective analysis, all patients in whom both measurement of mevalonic acid and genetic testing had been performed in the preceding 17 years have been included. Samples were analyzed by using gas chromatography-mass spectrometry (GC-MS) and concentrations were expressed as mmol/mol creatinine. The excretion of mevalonic acid was compared with age dependent reference values, validated at our hospital. The presence of two pathogenic *MVK* mutations was considered to be the gold standard for the diagnosis of MKD.

Results: This study included 62 patients (33 male, 29 female, aged: 0-36 year) with clinical features suggestive of MKD.

Thirteen patients harboured two *MVK* mutations, twelve of them excreted elevated amounts of mevalonic acid. In one patient mevalonic acid could not be detected, despite the fact that urine was collected during a febrile episode. Six patients had an elevated mevalonic acid excretion, but harboured no *MVK* mutations. However, repeated measurements in all six patients were ultimately normal.

This resulted in a sensitivity of 92%, a specificity of 88%, a positive predictive value of 68% and a negative predictive value of 98%. The positive likelihood ratio is 7.7 and the negative likelihood ratio is 0.09.
Conclusion: MKD seems very unlikely in patients with a normal mevalonic acid excretion, but it cannot be excluded completely. Furthermore, a positive urinary mevalonic acid excretion requires *MVK* analysis to confirm the diagnosis MKD. However, as long as genetic testing is not widely available and affordable, measurement of urinary mevalonic acid is a fair way to select patients for *MVK*-gene analysis.

O58

Long-term efficacy and safety of Canakinumab in active Hyper-IgD syndrome (HIDS): results from an open-label study

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Introduction: Hyper-IgD with periodic fever syndrome (HIDS) is an autoinflammatory disease characterized by periodic episodes of fever, abdominal distress, joint pain, and skin rashes. IL-1 blockade was previously reported as effective in reducing the frequency of episodes and improving clinical symptoms.[1,2] We report the results of the study assessing the efficacy and safety of canakinumab, an anti-IL-1β human monoclonal antibody, in patients with active HIDS.
Objectives: The primary objective was to assess the reduction of frequency of flares during the 6-month treatment period compared to a historical period (HP). Secondarily, assessments of reduction in frequency of flares during 24-month long-term follow-up and adverse events (AE) were conducted.
Patients and methods: This was an open-label, single treatment arm study to assess the efficacy and safety of canakinumab in HIDS patients aged ≥2 years with biallelic *MVK* mutations. The study included a 6-month treatment period (6TP) with up to 6-month withdrawal period (WP) and 24-month long-term treatment period (24TP).
Results: All enrolled patients (n=9) completed the 6TP and the WP, with 8 completing the 24TP. The median number of flares decreased from 5 (3-12) during HP to 0 (0-2) during 6TP. The median remained at 0 (0-3) until the end of study. During the 24TP, the median flare duration was 3.5 days (2-8, first year) and 8.5 days (6-11, second year). Flare severity remained 'mild' to 'moderate' at baseline and decreased to 'mild' or 'minimal' signs/symptoms" and to 'mild' or without signs/symptoms" at the first and second year of the 24TP, respectively. Physician's global assessment scores for HIDS disease control changed from either 'no control' or 'poor control' at baseline to 'good' or 'excellent control' by Day 4 and were maintained until the end of study. CRP and SAA plasma levels normalized by Day 15 and remained normal thereafter. The most frequent AEs were infections, with four patients experienced 14 mild to moderate SAEs. No AEs were drug-related nor led to discontinuation of study treatment.
Conclusions: Canakinumab markedly reduced the frequency of flares, rapidly alleviated signs and symptoms of acute episodes and normalized the serological inflammatory markers. The safety profile is consistent with other canakinumab studies. These data support a safe and maintained disease control and reinforce the ongoing development of canakinumab in this therapeutic area.

References

1. Drenth JP, *et al*: *J Pharmacol Exp Ther* 2001, **298**:1221-6.
2. Bodar EJ, *et al*: *Neth J Med* 2005, **63**:260-4.

O59

Canakinumab treatment in patients with active recurrent or chronic TNF-receptor associated syndrome (TRAPS): Efficacy and safety results from a proof of concept study

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Introduction: Tumor necrosis factor receptor-1 associated periodic syndrome (TRAPS) is a periodic fever syndrome, characterized by recurrent fever attacks associated with rashes, musculoskeletal and abdominal pain, and periorbital edema. In few patients TNF inhibitors have been shown to be effective, however, their efficacy tends to decrease over time [1-4]. Anti-IL-1 treatments have also been used in an effort to provide long term control of the inflammatory manifestations.
Objectives: The main objective was to determine whether canakinumab (CAN) induced complete or almost complete response in patients with active TRAPS at Day 15, as defined by Physician's Global Assessment (PGA). Evolution of C-reactive protein (CRP) and serum amyloid A (SAA), relapse rate and time to remission were additional measures. An additional objective was to determine the appropriate dosing for further development of CAN treatment in TRAPS patients.
Patients and methods: This was an open-label, single treatment arm, efficacy and safety study of monthly CAN 150 mg (2 mg/kg for patient ≤40 kg) SC in patients with active recurrent or chronic TRAPS [NCT01242813]. Patients were treated for 4-months with a maximum 5-month follow-up period. The initial follow-up period was followed by a maximum 24-month long-term treatment period. Here we report the efficacy and safety results of the completed study.
Results: A total of 20 patients were exposed to study medication, out of which 18 (90%) patients completed the study. At Day 15, complete response or almost complete response was achieved in 18 patients [90%; 95% CI: 75.1, 99.9], while 20 (100%) and 12 (60%) patients had clinical and serological remission, respectively. Already at Day 8, 16 patients (80%) achieved a complete or almost complete response, while 18 (90%) and 7 (35%) patients had clinical and serological remission, respectively. A total of 60% of patients experienced study drug-related AEs, most commonly upper respiratory tract infections. Seven patients (35%) experienced SAEs, none of which were related to study drug. Furthermore, there were no deaths during the study.
Conclusions: Canakinumab was effective in rapidly improving clinical signs and symptoms of TRAPS, whilst normalizing serological inflammatory markers and providing sustained disease control. The safety profile was consistent with previous canakinumab studies in other indications. These data support the ongoing development of canakinumab in this therapeutic area.

References

1. Simon A, et al : *Am.J.Med* 2004, **117**:208-210.
2. Weyhreter H, et al : *J Pediatr* 2003, **142**:191-193.
3. Jacobelli S, et al : *Rheumatology* 2007, **46(7)**:1211-1212.
4. Arostegui JI, et al : *Eur.J.Pediatr* 2005, **164**:13-16.

O60

First case of somatic mosaicism in TRAPS caused by a novel 24 nucleotides deletion in the TNFRSF1A gene

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Introduction: TNF receptor associated periodic syndrome (TRAPS) is an autosomal dominant disease caused by gain-of-function mutations in the TNF superfamily receptor 1A (*TNFRSF1A*) gene encoding 55 kDa TNF receptor type I (TNFR1). TRAPS is characterized by episodes of fever accompanied by severe abdominal pain, arthralgia, myalgia, rash, chest pain, enlarged glands and red, swollen eyes. Duration of the attacks can range from few days to several weeks, with the onset from early childhood to adulthood.
Objective: To identify the cause of fever in a 41 year old man who suffered with symptoms of severe abdominal pain, headache, arthralgia, myalgia, night sweats, generalised erythema and unilateral non-painful cervical lymphadenopathy which started from early adolescence, but became more severe after he recently returned from working overseas. He had 10 and 12 attacks per year each lasting almost exactly two weeks.

Methods: The patient underwent screening of the four genes: *MEFV* (the gene associated with FMF); *TNFRSF1A* (the gene associated with TRAPS) *NLRP3* (the gene associated with CAPS) and *MVK* (the gene associated with MKD).
DNA was extracted from whole blood, saliva, buccal epithelial cells, hair root and from isolated monocytes, T and B lymphocytes and neutrophils.
Result: A novel in-frame deletion of 24 nucleotides (c.255_278del) in exon 3 of the *TNFRSF1A* gene was identified by PCR and Sanger sequencing in DNA extracted from whole blood, but the size of mutated nucleotide peaks on the chromatogram were notably smaller than wild-type, raising the possibility of mosaicism. The latter was confirmed by targeted sequencing, which established the frequency of the mutant allele in the DNA isolated from whole blood as 7.36% and the virtual absence of variant sequence in purified epithelial cells (buccal swab), but substantial representation of the mutation in T lymphocytes and neutrophils. Analysis of parental DNA showed only wild-type *TNFRSF1A* gene sequence, corroborating that the deletion identified in our patient had occurred *de novo*.
Conclusion: We report first mosaic patient with TRAPS caused by a novel *TNFRSF1A* deletion of 24 nucleotides found in 7.36% of cells. The patient responded extremely well to treatment with anakinra, including a complete remission of symptoms and normalization in SAA and CRP.
The deletion of highly conserved residues, including the F60 amino acid, which is crucial for proper protein folding, is likely to introduce profound changes to the function and three dimensional shape of the TNFR1 potentially impairing its folding and binding with TNF.

O61

Influence of the naturally occurring human *CASP1* variant L265S on subcellular distribution and pyroptosis

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Introduction: Patients with unexplained recurrent febrile episodes and *CASP1* variants suffer from systemic sterile inflammation despite altered enzymatic activity of procaspase-1 and reduced IL-1β release. Most recent findings from our group indicate that the proinflammatory effects of *CASP1* variants with reduced or abrogated enzymatic activity could be due to receptor interacting protein kinase 2 (RIP2) mediated increase of NF-kB activation. These findings are additionally supported by a trend to elevated IL-6 and TNF-α expression in patients with *CASP1* variants.
Objectives: The objective of this project is the identification of possible subcellular mechanisms how the *CASP1*-L265S variant influences proinflammatory cell death (pyroptosis) and IL-1β secretion.
Methods: We established an in vitro model of a virally transduced human monocytic cell line (THP-1 with shRNA knock-down of endogenous procaspase-1), expressing either wild type or enzymatically inactive (L265S) procaspase-1 fusion-reporter proteins and characterized them after NLRP3-inflammasome stimulation. Using confocal microscopy and in vivo live cell imaging we analyzed the subcellular distribution of fluorescently labeled procaspase-1 wildtype and variant as well as the interaction with ASC.
Results: First results revealed a disturbed nuclear localization of the *CASP1*-L265S variant compared to procaspase-1 wildtype. Furthermore, *CASP1*-L265S variant revealed a strongly decreased pyroptosome formation and a less intense interaction with ASC (apoptosis-associated speck-like protein containing a CARD) after NLRP3-stimulation. Variant procaspase-1 L265S and ASC formed smaller pyroptosomes than wildtype procaspase-1and ASC.
Conclusion: Those findings suggest a model, in which variant procaspase-1 L265S impairs nuclear localization, pyroptosome formation and ASC-interaction, leading all together to reduced IL-1β production and secretion.

O62

Familial chilblain lupus caused by an activating mutation in STING

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Familial chilblain lupus is a monogenic form of cutaneous lupus erythematosus characterized by cold-induced cutaneous lesions at acral location. It is caused by loss-of-function mutations in the nucleic acid metabolizing enzymes TREX1 or SAMHD1. Gain-of-function mutations in STING (stimulator of Interferon genes) have been described in an infancy-onset autoinflammatory syndrome with fever, inflammatory cutaneous lesions and interstitial lung disease.
Here we report on a family with dominant chilblain lupus over 4 generations. Affected family members presented with acral inflammatory and partially necrotizing lesions beginning in early childhood. In some cases, low-titered ANAs and immune complexes were detectable. The family tested negative for TREX1 or SAMHD1 mutations. Exome sequencing revealed a heterozygous STING mutation segregating with chilblain lupus in the family. The mutation affects a highly conserved residue within the STING dimer interface and is predicted to be pathogenic. Quantitative RT-PCR analysis showed an increased expression of IFN-stimulated genes in blood cells of affected family members suggesting that the identified mutation has an activating effect on type I IFN signaling. Taken together, our findings demonstrate that gain-of-function mutations in STING can cause familial chilblain lupus and expand the spectrum of type I interferonopathies.

O63

Interferon gamma (IFNg) drives disease in the TLR9-mediated cytokine storm syndrome in mice

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The Cytokine Storm Syndrome (CSS) is characterized by an overwhelming activation of immune cells observed in life-threatening disorders such as familial hemophagocytic lymphohistiocytosis (fHLH) and secondary (s) HLH/macrophage activation syndrome (MAS) as well as during serious infection. However, it is not known if the CSS can be attributed to a single cytokine. Increased blood levels of interferon gamma (IFNg) in HLH and sHLH/MAS patients potentially indicate a central role for this cytokine in the CSS. Using a mouse model that mimics an infection-driven CSS (i.e., CpG-ODN), our study showed that total IFNg levels originating within organs are 500 to 2,000-fold higher than those measured in peripheral blood as CSS develops. Ablation of IFNg activity in tissues led to the amelioration of the plethora of associated CSS clinical and laboratory parameters. Furthermore, the IFNg signature gene products, CXCL9 and CXCL10, correlated with disease severity in the mouse model of CSS and patients with sHLH. Thus, anti-IFNg targeted therapy should control diseases associated with the cytokine storm and we propose the use of CXCL9 or CXCL10 as a means to monitor total IFNg activity in patients.

O64

S100A12 as diagnostic tool in the differential diagnosis of sJIA associated MAS vs. hereditary or acquired HLH

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Question: Macrophage activation syndrome is a severe complication of autoimmune and autoinflammatory disease. MAS is most strongly associated with systemic juvenile idiopathic arthritis (sJIA) but can also be seen in Kawasaki disease, SLE or IBD. Clinically, MAS is strikingly similar to hemophagocytic lymphohistiocytosis (HLH) and the initial differentiation between sJIA-associated MAS and hereditary HLH or acquired HLH is very difficult. Due to recent advances in the description of HLH-related gene defects, most patients with hereditary HLH can be identified through genetic or functional analysis (intracellular perforin, SLAM-associated protein analysis, x-linked inhibitor of apoptosis, CD107 degranulation assay). Unfortunately these investigations are not always easily available. Since viral infections such as EBV and CMV can trigger both hereditary and acquired forms of HLH, the presence of a viral trigger in a patient with HLH does not necessarily allow classification of the disease as acquired HLH.
S100A12 is an endogenous TLR4 ligand that induces monocyte activation, thereby acting as an amplifier of innate immunity during early inflammation. S100A12 is highly overexpressed in sJIA, and the assessment of S100A12 serum levels helps distinguish sJIA from other febrile illnesses. The main goal of this study was to determine whether S100A12 might help distinguish sJIA-associated MAS from HLH.
Methods: S100A12 serum levels were assessed in 177 samples obtained from 114 unique patients using the in-house ELISA kit. Of 177 samples, 152 samples were also available for a multiplex immunoassay including 53 cytokines and chemokines. Serum samples were obtained from 9 healthy controls, sJIA patients without MAS (17 active/ 19 remission), sJIA patients with MAS (33 active/ 33 remission), acquired HLH (22 active/ 20 remission) and 33 patients with hereditary HLH at disease onset. Additional data obtained at the time of serum collection included clinical features, conventional laboratory markers (CRP, ESR, differential blood count, fibrinogen, ferritin, triglycerides) and when available, NK cell function test results and sCD25 levels.
Results: Patients with hereditary and acquired HLH could be differentiated by S100A12 serum levels from patients with sJIA-associated MAS. S100A12 levels >1400 ng/ml were seen only in patients with active sJIA (with MAS (mean±SD 5470±3042 ng/m)l or without MAS (4150±3251 ng/ml), but not in patients with acquired (451±351 ng/ml) or hereditary HLH (216±170 ng/ml). Healthy controls were in the range of 85±44 ng/ml. Although S100A12 levels correlated closely with disease activity in sJIA patients as determined by JIA core set criteria, there was no significant difference between sJIA patients with or without MAS.
Conclusions: S100A12 serum levels are useful to differentiate between sJIA-associated MAS and inherited or acquired HLH. The combination with conventional laboratory markers, serum cytokine profiles and clinical characteristics might allow creating a diagnostic panel for the differentiation of MAS vs. HLH. Since at the onset of disease sJIA MAS and acquired HLH are difficult to discriminate this might be a helpful diagnostic tool.

O65

A decade of anti-IL-1 therapy in CAPS - a spectrum of efficacy in this spectrum of diseases

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Pediatric Rheumatology 2015, **13(Suppl 1):**O65

Introduction: Discovery of the role of the IL-1 inflammasome in CAPS has revolutionised treatment, and anti-IL-1 therapies have successfully switched off disease activity in many patients. More than 110 CAPS patients (including 24 children) in the UK have been treated with drugs targeting IL-1, over 90% of these have had complete resolution of disease.
Objectives: We describe a cohort of 10 patients who had sub-optimal response to canakinumab and/or anakinra.
Patients and methods: Patients were diagnosed by genetic sequencing and clinical assessment. Serial SAA concentrations were analysed, along with patient reported symptom scores. Partial response was defined as improved but incomplete resolution of patient-reported symptoms and/or reduction, but not normalisation, of SAA concentration.
Results: Patients 1 (male; age at treatment: 40; mutation in *NLRP3*: T436I; phenotype: CINCA) and 2 (female; 43; R260W; MWS) experienced modest and transient reductions of SAA and symptoms when treated with canakinumab. Both patients developed marked inflammation with morphea-like rash shortly afterwards. Both were switched to anakinra with excellent effect.
Patient 3 (male; 47; A439V; FCAS) weighed 130kg, had a partial response to both 300mg canakinumab q8w and 200mg anakinra daily. Patients 4 (female; 1; Y570F; CINCA), 5 (male; 16; S547C heterozygote; CINCA) and 6 (female; 59; Y547C mosaic; atypical) had severe disease. Patient 4, experienced partial response to canakinumab and anakinra, although feels better on 300 mg canakinumab (10mg/kg q8w). Patient 5 has not responded to 10mg/kg q8w canakinumab started 12 weeks ago. Patient 6 has been treated with both canakinumab and anakinra over 10 years, at times concurrently. She experienced a severe flare with aseptic meningitis after attempted conversion to canakinumab. She is now on anakinra 300mg daily and inflammation and headaches have remained consistent.
Patients 7 to 10 had CNS inflammation symptomatically, and on lumbar puncture/MRI. Patients 7 (male; 41; T348M; CINCA) and 8 (female; 20; A352T; CINCA) had resolution of most CAPS symptoms and SAA normalised on canakinumab 300mg q8w, however headaches and fatigue continued. Both had previous strokes attributed to CNS inflammation. Patient 7 had some improvement on anakinra. Patient 9 (male; 48; E567K mosaic; atypical), weighing 102kg, had complete resolution of most CAPS symptoms and SAA normalised, but headaches, poor balance and fatigue continued on both treatments. Patient 10 (male; 24; T348M; MWS) had a complete normalisation of inflammatory markers and peripheral symptoms on 300mg canakinumab. However, headaches and fatigue remained. Encouragingly, in patients 9 and 10 fatigue and mood have improved after over 5 years of treatment.
Conclusion: Patients 1 and 2 raise the possibility of IL-1α mediated inflammation. Patients 3-6 suggest incomplete blockade of IL-1 activity with the maximum doses available on the NHS. Finally in patients 7-10 who had decades of CNS inflammation prior to treatment, headache and fatigue linger.

O66

Effectiveness of canakinumab treatment in Schnitzler's syndrome: a multi-center randomized placebo-controlled study

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Background: Schnitzler's syndrome (SchS) is an adult-onset autoinflammatory disease characterized by urticarial exanthema and monoclonal gammopathy in combination with episodes of fever, arthralgia, fatigue, and bone and muscle pain. Anti-IL-1 targeting therapies in small patient numbers showed to be effective in reducing the clinical symptoms of SchS.
Methods: The current placebo-controlled multi-center study was designed to assess the effects of the anti-IL-1ß monoclonal antibody canakinumab (CAN) on the clinical signs and symptoms of SchS. We randomly assigned 20 patients with active disease to receive CAN 150 mg or placebo s.c. injections (day 0). Following the evaluation of treatment responses on day 7 the study was continued by a 16-week open label phase with CAN injections upon confirmed relapse of clinical symptoms. Efficacy was determined by changes in the physician's global assessment (PGA; range 0-20), a combined symptom score which includes 5 key symptoms of SchS

(urticarial rash, fever, fatigue, myalgia and arthralgia/bone pain), measurement of the inflammation markers C-reactive protein (CRP) and serum amyloid A (SAA) as well as changes in quality of life assessment (DLQI, SF-36).
Results: CAN was highly effective in reducing median PGA total scores (14.0 to 2.0) as compared to placebo treatment (15.0 to 13.0) within 7 days after first administration (changes between treatment groups $p < 0.0001$). Median CRP reduced from 9.3mg/dL at baseline to 0.6mg/dL at day 7 in the CAN group vs. increase from 3.0mg/dL to 5.0mg/dL for the placebo group. Similarly, median SAA levels reduced from 428mg/L to 13mg/L for the CAN group vs. increase from 160mg/L to 205mg/L for the placebo group. The median changes from baseline to day 7 between treatment groups for CRP ($p = 0.002$) and SAA ($p = 0.032$) were significant. In addition, quality of life markedly improved. Changes in both physical component SF-36 scores and in DLQI sum scores were significantly greater ($p < 0.0001$) in the CAN vs. placebo group. The clinical and laboratory improvements were maintained during the open label phase of the study. Also, all placebo-treated patients responded well to CAN therapy during the open-label phase. Adverse events were manageable and included respiratory tract infections, gastrointestinal symptoms and hypertension.
Conclusion: In this placebo-controlled study, CAN s.c. injections significantly improved the clinical signs and symptoms of SchS, reduced inflammation markers, and enhanced quality of life. CAN treatment may be considered a promising therapeutic option in these patients.

O67

International experience of pregnancy outcomes in auto-inflammatory syndromes treated with Interleukin-1 inhibitors

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Introduction: Many patients on anti-IL-1 therapy are unable to stop treatment prior to conception or during pregnancy but little data exist regarding safety. We report the outcomes of 20 IL-1 inhibitor exposed pregnancies in 18 women from 7 countries (including the first data on canakinumab exposed pregnancy) and paternal exposure to anakinra or canakinumab at conception.
Objectives: To collate data on outcomes of IL-1 inhibitor exposed pregnancies.
Patients and methods: A standardised data collection sheet was sent to clinicians known to treat SAID. Responses were collated and analyzed retrospectively.
Results: We received data on 20 pregnancies born to 18 women (Diagnoses: CAPS (11), AOSD (2), TRAPS (2), FMF (2), and Idiopathic Pericarditis (1)).
4 pregnancies were exposed to canakinumab with theoretical exposure up to 18 weeks; 18 were exposed to anakinra (2 were exposed to both agents).
All pregnancies reported were completed. The median gestation at delivery was 38+4 weeks (range 35+1 to 41). APGAR score was >9 in all recorded cases, birth weight range 2.02-3.94 kilos.
Two congenital abnormalities were reported in a single infant; growth hormone deficiency due to ectopic neurohypophysis and unilateral renal agenesis in a boy born to a mother with active AOSD at conception treated with anakinra from 9 weeks gestation.
7 babies were breast fed by mothers receiving anakinra (range 3 - 40 weeks). None have reported infections or developmental abnormalities.
A total of 13 babies were born to 10 fathers treated with either anakinra (5) or canakinumab (8) at conception with no reported abnormalities.
Conclusion: This is the largest study to date of pregnancy outcomes in parents receiving anti-IL1 therapies. Overall the data are reassuring but numbers remain small.
Based on available data we currently advise preconception planning, with the risks and benefits of therapy individually discussed with potential parents. The data regarding canakinumab are limited and our advice is to switch to the more physiological anakinra, ideally prior to conception. Whilst anakinra is detectable in breast milk the data from 7 infants suggests it has no deleterious effects.
The significance of unilateral renal agenesis in a foetus exposed to anakinra is uncertain given a previous report of fatal bilateral renal agenesis *in utero* of a twin foetus exposed to anakinra (Chang *et al*., Arthritis & Rheumatology 2014). This requires further investigation, and there is a need for the SAIDs community to develop a registry in order to provide the best information for patients.

O68

Anti interferon-gamma (IFNγ) monoclonal antibody treatment in a patient carrying an *NLRC4* mutation and severe hemophagocytic lymphohistiocytosis

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Background: A growing body of evidence, in animals and humans, suggest that IFNγ plays a pathogenic role in primary HLH (pHLH) and in secondary HLH, including macrophage activation syndrome. A pilot study in pHLH with NI-0501, an anti-IFNγ monoclonal antibody, is ongoing. Mutations in *NLRC4* have been recently reported to cause recurrent MAS and an increased production of IL-18, a cytokine known to induce IFNγ
Objectives: To report safety and efficacy of NI-0501 in a patient, carrying a de novo *NLRC4* mutation and presenting with severe recalcitrant HLH.
Results: A 4.5 month-old Caucasian child presented at 20 days of age with fever, rash hepatosplenomegaly, pancytopenia, hypofibrinogenemia, hypertriglyceridemia, marked ferritin and sCD25 increase. Severe liver insufficiency, followed by multiorgan failure, required ICU admission. HLH diagnosis was based on 6 HLH-2004 criteria. Analysis of genes involved in familial-HLH and functional tests (perforin expression, degranulation and cytotoxicity) were negative. Subsequent analysis of the *NLRC4* gene showed a *de novo* missense mutation (c.1010 C>A, encoding p.T337N), absent in his parents. Elevated serum levels of IL-18 and spontaneous IL-18 production were documented, confirming the relevance of the *NLRC4* mutation. Treatment with high-dose glucocorticoids and cyclosporine-A led to progressive improvement. Development of sepsis triggered an HLH reactivation with ICU admission. Treatment with etoposide and/or ATG was not considered because of active infections in an immunocompromised child. Measurement of IFNγ and of the IFNγ-inducible chemokines showed measurable serum levels of IFNγ and high serum levels of CXCL9 (5670 pg/ml) and CXCL10 (4400 pg/ml). Compassionate use treatment with NI-0501 was started on background dexamethasone (13.6 mg/m2) and cyclosporine-A. He received NI-0501 for 3 months, initially every 3 days and subsequently every 7 days according to NI-0501 pharmacokinetics. No infusion reaction was observed. HLH clinical and laboratory features progressively improved. Glucocorticoid tapering was rapidly initiated. After 3 months, the child is in excellent conditions; all HLH clinical and laboratory parameters have normalized. CRP occasionally increases. Serum IL-18 levels remain elevated. High circulating levels of IFNγ complexed with NI-0501, reflecting the high IFNγ production are detectable, but fully neutralized as shown by undetectable levels of IFNγ-inducible chemokines. He is receiving oral cyclosporine-A (6 mg/kg) and prednisone (0.3 mg/kg equivalent to 0.9 mg/m^2 of dexamethasone).
Conclusions: In a patient, carrying a de novo pathogenic *NLRC4* mutation and presenting with severe recalcitrant HLH, NI-0501 administration was well tolerated allowing control of all HLH features, while enabling glucocorticoid tapering. No safety concern emerged.

O70

Empirical use of anakinra in AA amyloidosis of uncertain aetiology

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Pediatric Rheumatology 2015, **13(Suppl 1):**O70

Introduction: AA amyloidosis is a serious complication of uncontrolled inflammation, which if left untreated will progress to renal failure and death. Effective suppression of the underlying inflammatory condition can halt organ damage or even lead to improved organ function. However, in 7% of our cohort the underlying inflammatory disease remains uncharacterised, creating a dilemma as to the choice of empirical treatment.
Objectives: We empirically treated a small cohort of seven patients with AA amyloidosis of uncertain cause with the IL-1 receptor antagonist anakinra.
Patients and Methods: All seven patients were under the care of the UK National Amyloidosis Centre. Each patient underwent extensive investigation without diagnosing of the underlying inflammatory condition. Each patient subsequently underwent a trial of treatment with anakinra. Serum SAA and renal function as well as urine protein excretion were monitored closely, and all patients underwent serial SAP scintigraphy to monitor organ amyloid load.
Results: Six of seven patients experienced suppression of inflammatory disease activity with the median pooled pre-anakinra SAA level falling from 63 mg/L (interquartile range, IQR, 42 - 119) to 5 mg/L (IQR 4 - 7). In these six patients this effect lasted for a median of 5.6 years, the duration of therapy, (IQR 2.4 - 7.6). In 2 patients proteinuria improved from 10.5 to 1.9 g/24 hr and 2 to 0.6 g/24 hr. Four patients showed regression of amyloid deposits on SAP scintigraphy. Five patients reported improvement in symptoms and one had been asymptomatic. One patient experienced no improvement in either inflammatory markers or in symptoms, and treatment with anakinra was discontinued.
Conclusion: AA amyloidosis is a potentially reversible cause of renal failure. A therapeutic trial of anakinra is worth trying as it is potentially completely effective and has a better safety profile than high dose corticosteroids, other anti-cytokine or immunosuppressive drugs.

O71

A dominantly-inherited Behcet-like disorder caused by haploinsufficiency of the TNFAIP3/A20 protein

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Introduction: *TNFAIP3* encodes the anti-inflammatory A20 protein that functions as a potent negative regulator of NFκB signaling and the NLRP3 inflammasome. Low penetrance common variants of *TNFAIP3* have been associated with a number of autoimmune diseases. Here we report 5 high penetrance dominantly-inherited frameshift and nonsense *TNFAIP3* mutations in 11 patients with early-onset systemic inflammation, arthralgia/arthritis, oral and genital ulcers, and ocular inflammation.
Objectives: To identify a possible genetic cause of dominantly-inherited early-onset systemic inflammatory disease.
Patients and methods: We performed exome sequencing in 3 families, candidate gene screening in 2 families, and targeted sequencing of 384 Turkish and 384 Japanese patients. We utilized immunoblotting, cytokine profiling, immunostaining, immunofluorescence, real-time PCR, and flow cytometry to study abnormalities in patients' immune cells.
Results: Four *TNFAIP3* mutations were located in the N-terminal OTU domain of A20 and generated truncated proteins of similar length, while the fifth mutation was a truncating frameshift in the more C-terminal ZnF4 domain. Targeted sequencing of *TNFAIP3* in Turkish and Japanese GWAS cohorts with Behcet' disease identified one patient with a novel frameshift mutation in the OTU domain. None of the mutations were found in any public database. Expression of A20 was reduced in patients' PBMCs and fibroblasts relative to healthy controls, and the mutant truncated proteins were not detectable by Western blots. Overexpression of wild type (wt) A20 inhibited TNF-α-induced NF-κB activity and removed K63 ubiquitin chains from RIP1, NEMO, and TRAF6, whereas A20 mutants did not. Co-expression of wt and mutant A20 demonstrated haploinsufficiency of the mutant protein rather than a dominant negative effect. In leukocytes from patients possessing A20 mutations, enhanced IκB degradation and NF-κB translocation were observed upon TNF-a stimulation. This was accompanied by reduced A20 binding to TRAF2 and RIP1 in the TNFR complex. Immunoprofiling of patients' cells confirmed enhanced gene expression of NF-κB target genes and overproduction of the proinflammatory cytokines IL-1β, TNF-α, IL-9, IL-17 and IP-10. Patients' PBMCs also exhibited constitutive NLRP3 inflammasome activation, skewed subsets of pro-inflammatory monocytes, increased gene expression of IL-1β and TNFα in M1 macrophages, and increased Th9 and Th17 differentiation.
Conclusion: Truncating *TNFAIP3* mutations cause haploinsufficiency of the A20 protein, with upregulation of the NFκB signaling pathway, NLRP3 inflammasome activation, and overproduction of proinflammatory cytokines. Targeted therapies with biologics that inhibit these cytokines may be effective in these patients. This is the first report of a human disease caused by high penetrance germline mutations in *TNFAIP3*.

O74

A functional inflammasome activation assaydifferentiates patients with pathogenic NLRP3mutations and symptomatic patients with lowpenetrance variants

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Question: The cryopyrin-associated periodic syndromes (CAPS) are characterized by recurrent episodes of systemic inflammation. CAPS is caused by mutations in the *NLRP3* gene encoding cryopyrin, an important component of the NLRP3 inflammasome that activates caspase-1 resulting in inflammation by excessive production of IL-1β and others. Besides confirmed pathogenic NLRP3 mutations, patients with CAPS-like symptoms frequently show low penetrance variants in NLRP3. The disease relevance of these variants is inconsistent. The analysis of IL-1β in the serum did not prove to be a valid diagnostic test in these individuals.
Methods: In this study, we investigated if an inflammasome activation assay differentiates between patients with confirmed pathogenic CAPS mutations, patients with low penetrance NLRP3 variants (V198M and Q703K) and healthy controls. The study population consisted of 17 patients with genetically proven Muckle-Wells syndrome, 11 patients with low penetrance *NLRP3* variants and 15 healthy controls. Concentrations of IL-1β, IL-18, Caspase-1, TNF-α and IL-6 were quantified in cell culture supernatants after inflammasome stimulation with LPS and LPS + ATP for several time intervals.
Results: The release of mature IL-1β, IL-18, and caspase-1 into cell culture supernatants after 4h of inflammasome stimulation was significantly increased in patients with confirmed pathogenic CAPS mutations compared to low penetrance NLRP3 variants and controls. IL-1β secretion in CAPS patients correlated with disease severity. TNF-α secretion was significantly reduced in CAPS patients and NLRP3 variants when compared to healthy controls after 4h of stimulation.
Conclusion: This inflammasome activation assay differentiates between autoinflammation patients with confirmed pathogenic CAPS mutations and patients with low penetrance NLRP3 variants, and points towards alternative pathophysiological mechanisms in low penetrance NLRP3 variants.

O75

*HLA-DRB1*11* and variants of the MHC class II locus are strong risk factors for systemic juvenile idiopathic arthritis

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Introduction: Systemic juvenile idiopathic arthritis (sJIA) is a severe, potentially life-threatening childhood inflammatory disease whose pathophysiology is poorly understood.
Objective: To determine whether genetic variation of the major histocompatibility complex (MHC) locus influences sJIA susceptibility.
Patients and methods: Single nucleotide polymorphism (SNP) genotypes were determined in 982 children with sJIA and 431 healthy subjects, and were combined with *in silico* SNP data from 7579 additional healthy subjects. The collection was divided into 9 strata by country of origin and subjected to stringent quality control procedures. MHC region SNPs and classical human leukocyte antigen (HLA) alleles and amino acids were determined by imputation and were tested for association with sJIA in the individual strata and by meta-analysis. To assess whether disease-associated MHC variants influenced sJIA through regulatory mechanisms, RegulomeDB was used to cross reference sJIA-associated SNPs with regulatory information from over 100 tissues and cell lines, including data from the ENCODE and NIH Roadmap Epigenome Projects.
Results: Association testing and meta-analysis of MHC region SNPs in 9 strata found a strong, dominant association between sJIA and a 400 kb region of the MHC locus that included most of the class II HLA region. The strongest sJIA-associated SNP was rs151043342 (p=2.8E-17, OR 2.6 [2.1, 3.3]), and conditional analysis controlling for its effect revealed that a second SNP, rs12722051, independently influenced sJIA risk (p=1.0E-5, OR 0.7 [0.5, 0.8]). Meta-analysis of classical HLA type associations in the 6 strata of Western European ancestry revealed a strong association between sJIA and *HLA-DRB1*11* (p=2.7E-16, OR 2.3 [1.9, 2.9]) and the *HLA-DRB1*11-HLA-DQA1*05-HLA-DQB1*03* haplotype (p=6.4E-17, OR 2.3 [1.9, 2.9]). Examination of sJIA-associated SNPs (p<1.0E-5) with RegulomeDB identified 18 SNPs that were linked to expression of one or more class II HLA genes AND had strong evidence supporting an effect on transcription factor binding (RegulomeDB scores 1a - 1f). Importantly, the majority of sJIA-associated SNPs in the class II HLA locus intersected with super-enhancers that were cell-type specific for B cells and monocytes (as reported by Hnisz *et al.*, Cell 2013).
Conclusions: Using meta-analysis of directly observed and imputed SNP genotypes and imputed classical HLA types, we identified the MHC locus as a *bona fide* sJIA susceptibility locus that influences disease risk in multiple independent populations. These data suggest that the class II HLA locus influences sJIA susceptibility through both protein coding variation and noncoding variation that alters gene expression.

O76

A family carrying a homozygous *LACC1* truncated mutation expands the clinical phenotype of this disease beyond systemic-onset juvenile idiopathic arthritis

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Introduction: We identified a consanguineous Moroccan family with three affected siblings diagnosed with rheumatoid factor-negative polyarticular juvenile idiopathic arthritis. They all suffered from an early-onset (2-4 years-old) chronic and symmetric polyarthritis affecting both large and small joints. The joint involvement was markedly erosive in two siblings, with the older sister requiring hip prosthetic replacement at the age of 18 years. None of the patients had fever, skin rash, uveitis or other extra-articular manifestations. Laboratory analyses revealed leukocytosis, thrombocytosis, severe anaemia, marked increase of inflammatory markers and negative results for rheumatoid factor, anti-nuclear antibodies and HLA-B27.
Objective: We suspected a genetic cause for the disease in this family on the basis of the atypical phenotype, the presence of consanguinity and the recurrence of the disease following a recessive mode of inheritance. The aim of this study was to identify the causal genetic defect underlying this family by means the use of novel genetic technologies.
Patients and methods: We performed genome-wide SNP analyses and whole-exome sequencing in both affected and unaffected siblings to identify those homozygosity regions that were exclusively shared by patients, and the candidate causal gene variants inside these regions, respectively.
Results: Four homozygosity regions were identified in chromosomes 3, 6 (n: 2) and 13, containing over 330 genes. Whole-exome sequencing identified three potential candidate variants within these regions, located in the *TATDN2, FARS2*, and *LACC1* genes, respectively. Bioinformatics and genetic studies performed in a group of healthy Moroccan individuals (n: 352) finally supported the frameshift c.128_129delGT variant in the *LACC1* gene, leading to a truncated protein (p.Cys43Tyrfs*6), as the most probable causal gene defect.
Conclusions: Our findings show homozygous *LACC1* mutations as the genetic defect underlying a severe inflammatory joint disease with a recessive mode of inheritance. These evidences expand the phenotype of this rare genetic disorder to other forms of juvenile idiopathic arthritis in addition to the previously described systemic-onset juvenile idiopathic arthritis [1].

Reference

1. Wakil SM, Monies DM, Abouelhoda M, Al-Tassan N, Al-Dusery H, Naim EA, *et al*: Association of a Mutation in LACC1 With a Monogenic Form of Systemic Juvenile Idiopathic Arthritis. *Arthritis Rheum* 2015, **67(1)**:288-295.

O77

Serum biomarkers for the diagnosis of chronic recurrent multifocal osteomyelitis (CRMO)

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Introduction: Chronic nonbacterial osteomyelitis (CNO) is an autoinflammatory bone disorder mostly affecting children and adolescents. Chronic recurrent multifocal osteomyelitis (CRMO) is the most severe form of CNO. It is characterized by recurring episodes of bone inflammation that can last for years and may cause chronic pain, pathological fractures, and disability. Despite recent advances in targeting disease mechanisms, the exact pathophysiology of CNO/CRMO remains unknown. Diagnosis of CNO can be challenging, because symptoms tend to be mild and highly variable, and is further complicated by the absence of widely accepted diagnostic criteria and disease biomarkers.
Objectives: The aim of our study was to determine serum biomarkers for the diagnosis of CRMO, discriminating CRMO patients from healthy individuals and patients with other inflammatory conditions (Crohn's disease and JIA).
Methods: Serum of treatment-naïve CRMO patients was collected at the time of diagnosis (N=56). As controls, sera from treatment-naïve age matched patients with Crohn's disease (N=62) or JIA (N=27), as well as healthy individuals (N=62) were collected. Sera were subjected to proteomic analysis, using the Human Cytokine 25-plex Assay (Life Technologies) on the Luminex® 200™ platform. Standard inflammation markers from our routine clinical chemistry laboratory (CrP) were included in our analysis. Statistical analysis was performed using non-parametric Kruskal-Wallis tests, Mann-Whitney-U tests, and canonical discriminant analysis to test between disease and control groups.

Results: The following (9 out of 25) serum proteins were detectable and significantly differed between groups: IL-1RA, IL-2R, IL-6, IL-12, Eotaxin, MCP-1, MIG, MIP-1b, RANTES. Kruskal-Wallis and Mann-Whitney U tests confirmed significant differences between three groups: CRMO, Crohn's disease, and healthy controls. Biosamples from CRMO and JIA patients were less clearly distinguishable. Multi-component canonical discriminant analysis allowed for the definition of algorithms differentiating between CRMO, Crohn's disease, and healthy controls. We failed to differentiate sera from patients with JIA from CRMO samples. However, JIA and CRMO can usually be differentiated by their clinical presentation.
Conclusion: Our serum marker based discrimination algorithm can discriminate CRMO patients from patients with Crohn's disease and healthy individuals. Though confirmation of our findings in larger, multi-ethnical cohorts is currently lacking, in a clinical setting this may prove useful and valuable to differentiate between individuals with "bone pain" and CRMO.

O78

Long-term outcomes of tonsillectomy in children with periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) syndrome

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Introduction: Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome is an inflammatory disorder of childhood classically characterized by recurrent fevers, pharyngitis, stomatitis, cervical adenitis and leukocytosis. Little is known about the true incidence, natural course, pathogenesis, and appropriate therapy in this recently described syndrome. While the mechanism is unclear, previous studies have shown that tonsillectomy can be a therapeutic option with improvement in quality of life in many patients with PFAPA, but long-term clinical follow up is lacking.
Objective: To evaluate the long-term response of tonsillectomy in patients with PFAPA syndrome treated at a tertiary care center in San Diego, CA.
Methods: We initiated a prospective cohort study in 2008 to better understand the natural history of PFAPA in children treated at a tertiary care center in San Diego, CA. Any patient aged 1-17 years, seen in the Rady Children's Hospital-San Diego Allergy/Immunology clinics, diagnosed clinically with PFAPA syndrome and undergoing tonsillectomy (with or without adenoidectomy) were eligible for inclusion. Patient data was collected on over 200 children with recurrent fevers including 94 patients with PFAPA under an IRB-approved protocol. Using patient charts and a standardized questionnaire, demographic data, including age, gender, and ethnicity, clinical profiles (presence of symptoms, fever profile, treatments) and detailed family histories were obtained from patients seen in a children's hospital-based clinic over a 7-year period. A subset of samples (10 PFAPA and 10 control) underwent 16S rDNA profiling.
Results: To date, 63 patients with PFAPA and 11 patients with other non-infectious recurrent fevers have undergone tonsillectomy. Forty-four patients with PFAPA syndrome have had complete resolution of symptoms after surgery, with the time to resolution of symptoms post-tonsillectomy approximately 2 months (range 1-11 months). The average length of follow up is 31.6 months (range 1-58 months). In 3 patients, there has been a relapse of symptoms, defined as fevers persisting for more than 6 months, which remain responsive to medical therapy. Post-operatively, tonsils from patients with PFAPA are notably smaller and grossly friable. No granulomas or abscesses were noted on histological examination. While small differences exist in the tonsillar microbiome of PFAPA patients compared to controls, these taxonomic groups were only very low abundance, and were not outside the range of normal flora observed in the Human Microbiome Project.
Conclusions: Our cohort of patients demonstrates clinical characteristics consistent with PFAPA. This study demonstrates that tonsillectomy is an effective surgical treatment option for management of children with PFAPA syndrome.

O79

Enzymatically inactive procaspase-1 stabilizes the ASC-pyroptosome

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Introduction: Caspase-1 (or interleukin-1 converting enzyme, ICE) plays an important role in mediating proinflammatory innate immune responses, especially by activation of pro-IL-1ß within inflammasomes. Some patients with recurrent febrile episodes and systemic inflammation of yet unknown origin harbor *CASP1*-mutations with incomplete penetrance. These *CASP1*-variants cause reduced enzymatic activity of procaspase-1 and less IL-1ß secretion.
Objectives: The paradox of reduced IL-1β secretion but increased inflammation led to the hypothesis, that *CASP1*-variants have different protein interaction clusters and thus enhance alternative signaling pathways.
Material and methods: We established an in vitro model of transduced immortalized murine macrophages, expressing either wild type (WT) or enzymatically inactive (C284A) procaspase-1 fusion-reporter proteins and characterized them after NLRP3-inflammasome stimulation.
Results: As expected, variant procaspase-1 (C284A) macrophages did not secret IL-1ß and pyroptosis was reduced. In addition, the usage of fluorophore-tagged fusion proteins revealed a longer and more intense interaction of the enzymatically inactive procaspase-1 (C284A) with ASC (apoptosis-associated speck-like protein containing a CARD) compared to WT. Variant procaspase-1 (C284A) and ASC formed macromolecular complexes in the cytosol (so called pyroptosomes), that were significantly larger than those formed in cells expressing fluorophore-tagged WT procaspase-1. We could confirm our results by adding the caspase-1 inhibitor YVAD-CMK to Casp1-WT macrophages: the pyroptosomes became larger, more intense and more stable over time. Furthermore, life-cell-imaging detected for the first time, that pyroptosomes of enzymatically inactive procaspase-1 were spread by cell division.
Conclusion: Variant procaspase-1 stabilizes inflammasome/pyroptosome formation. This may enhance inflammation via two IL-1ß-independent mechanisms: The pyroptosome causes a proinflammatory stimulus through increased recruitment and interaction of further proinflammatory proteins (e.g. RIP2, receptor interacting protein 2). Moreover, this stimulus might be amplified via pyroptosome- and cell division.

O80

Rapid and sustained effect of anti-TNF treatment in patients with ADA2 deficiency

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Introduction: Mutations of CERC1 have been recently reported as causative of an inflammatory condition characterized by polyarteritis, cerebral stroke and immunodeficiency; the response to immunosuppressors and biological drugs is not univocal.
Aim of the study: To describe a series of patients with DADA2, focusing on the response to treatment and outcome.
Patients and methods: Patients with a clinical history consistent with a possible DADA2 were retrospectively analyzed; molecular analysis of the CECR1 gene was performed. Detailed analysis of the clinical presentation, disease course and response to treatment was retrospectively performed in patients with confirmed diagnosis.
Results: The retrospective analysis of patients allowed to identify 5 patients presenting with a strong clinical suspicion of DADA2. The mean age of disease onset was 12 months (range 6-36). The disease course was chronic

in two patients and recurrent in three. All patients presented skin involvement and elevation of acute phase reactants; three patients presented multiple strokes, one patient acute invagination of the small bowel. Skin biopsy was consistent with PAN in three patients.
The molecular analysis of CECR1 gene identified homozygosis or compound heterozygosis for deleterious mutations (G47R, G47A, P251L, R312X, E328D, T360A) in all patients.
All patients required high doses of steroids to control the skin manifestations and the systemic inflammatory features but a clinical relapse was observed at the time of steroid tapering. In two patients thalidomide was able to completely control the disease manifestations while one patient presented a partial response. Other immunosuppressant (oral cyclophosphamide and cyclosporine) were not able to control the disease activity; treatment with anakinra was tempted in one patient, without clinical improvement. After the suspension of thalidomide and the failure of cyclophosphamide, etanercept was started in one patient on may 2008 at the dose of 0.8 mg/kg/daily. The patients presented a rapid and complete control of the skin manifestations with a rapid normalization of acute phase reactants, despite the withdrawal of steroidal treatment. The same brilliant response to etanercept was also observed in the brother and, more recently, in other three patients. The median duration of treatment with anti-TNF agents is now 34 months (range 6 months-7 years). All 5 patients display a complete control of clinical manifestations and laboratory parameters and are off from any steroid treatment. No severe infectious or other complications have been described so far.
Conclusion: This series of 5 patients with DADA2 enlightens the long-term efficacy of anti-TNF agents.

O81

Development of a workflow to analyze autoinflammatory-associated genes using AccessArray™ system and next generation sequencing

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Introduction: Monogenic autoinflammatory diseases are a group of genetic conditions characterized by a dysregulation of inflammatory response that include more than 20 diseases. Despite the fact that they are different clinical entities, several signs and symptoms are shared, making their differential diagnosis difficult. Moreover, in most cases, a definitive diagnosis is exclusively achieved by a positive genetic study. The need to analyze different genes makes this process costly in terms of both time and money. The arrival of next generation sequencing into clinics can overcome these problems.
Objective: To develop a complete workflow to analyze simultaneously the most common autoinflammatory-associated genes.
Materials and methods: A panel of 80 different primer pairs was designed according to AccessArray™ and PGM Ion Torrent platform specifications. This panel covers *MEFV* (all exons except 2), *TNFRSF1A* (exons 2-to-7), *MVK* (all exons), *NLRP3* (all exons), *NOD2* (exons 4, 8, 11) and *PSTPIP1* (exons 10-11) genes. Libraries were prepared using 48.48 AccessArray™ chips and sequenced in the PGM platform using 400 bp kits. Quality control, mapping and variant calling were performed using Torrent Server and variant annotation using Ion Reporter Suite. Coverage information was analyzed with BEDtools software.
A set of 25 control samples was used to validate our sequencing and data analysis workflow. Then, a total of 288 patient samples were analyzed. Any possible disease-causing mutation detected was confirmed by Sanger sequencing.
Results: The results obtained in control samples were concordant with the expected known genotypes. All germline mutations (100%) and 4 out of 5 somatic mutations (80%) were detected. Only a sample harboring extremely-low somatic *NLRP3* mosaicism (less than 4%) was not initially called by our pipeline. In the analysis of 288 samples, all mutations detected were confirmed by Sanger sequencing. Moreover, in one of the analyzed patients, a somatic *NLRP3* mutation (12%) was identified in the routine screening.
Conclusion: We have designed a complete workflow to simultaneously analyze the most common autoinflammatory-associated genes in a clinical setting. This workflow allowed us to identify all of the germline mutations and most of the somatic mutations previously detected in our control group. All the detected mutations in new samples where confirmed by Sanger sequencing. This NGS-based approach also enabled us to detect a novel case of somatic *NLRP3* mosaicism in a patient with disease symptoms compatible with CAPS.

O82

Recruitment of abundant NK cells to the PFAPA tonsils support the crucial role of innate immunity in pathogenesis of PFAPA syndrome

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Introduction: Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome is a more frequent cause of recurrent fever in children. The exact etiology of this pediatric disorder is still unknown. Palatine tonsils are sites where innate immunity leads to the onset of adaptive immunity, mediated by B and T lymphocytes.
Objective: Our purpose is to understand if infiltrating inflammatory cells in PFAPA tonsils contribute to evolving disease.
Methods: We have collected tonsil samples from 2 groups of pediatric patients undergoing tonsillectomy: PFAPA patients (n=20) and children who had indication of bacterial tonsillitis (control group, CG) (n=20). We have performed a precise phenotypic analysis of subpopulations on tonsil cells and tissues using flow cytometry and immunohistochemistry.
Results: During the asymptomatic phase the number of monocytes did not differ between the PFAPA and control tonsils. We observed a considerable recruitment of NK cells in tonsils of PFAPA patients with respect to CG. Surprisingly, we have detected a significant expansion of both $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$NK cell subsets when compared to CG. Strict characterization of activating and inhibitory NK receptors revealed a crucial role of $CD56^{dim}CD16^{+}$. Specially, activating receptors, such as natural cytotoxicity receptors (NCRs) and NKG2D, are higher in NK of PFAPA patients. Furthermore, FACS analysis displayed a higher number of naïve and a significantly lower percentage of effector memory $CD4^{+}$ and $CD8^{+}$ T cells in PFAPA patients compared to CG. Additionally, PFAPA patients presented a significant decrease of functional follicular helper T cells (Tfh) and regulatory T cells.
Conclusions: These results confirm a relevant involvement of NK cells in pathogenesis of PFAPA supporting the crucial role of the innate immunity. Nonetheless, the abundant and activated NK cell subsets (particularly $CD56^{dim}CD16^{+}$) might influence adaptive immune responses demonstrated impaired in the tonsils of PFAPA patients.

O83

Long term efficacy and safety of canakinumab in children with systemic juvenile idiopathic arthritis with and without fever

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Introduction: Rapid and sustained efficacy of canakinumab (CAN) were previously demonstrated in patients with systemic juvenile idiopathic arthritis (SJIA) [1]. However, little is known about potential differences in response to CAN treatment between patients with vs. without SJIA-associated fever at the time of the first CAN administration.

Objectives: To evaluate the long-term efficacy and safety profile of CAN-naïve SJIA patients with and without SJIA-associated fever.
Patients and methods: patients aged 2-20 years with SJIA with and without SJIA associated fever at enrollment received open-label CAN 4mg/kg s.c. every 4 wks. Every 3 months, response to CAN was measured by adapted JIA ACR response criteria (aACR/JIA); juvenile arthritis disease activity score (JADAS); clinical inactive disease; clinical remission on medication (CRM, 6 months continuous clinical inactive disease). Safety was assessed monthly.
Results: Data on 122/267 patients, 53 (43%) with and 69 (57%) without SJIA associated fever, were available for analysis with a median 94 wk study duration. At Wk4, ~75% of both subgroups had responded (≥ aACR/JIA30), increasing to 90% at Wk12. At Wk2, ~21% of both subgroups had inactive disease; 44% at Wk8; 60% at Wk20 and then 60-70% for the remainder of the trial. CRM was achieved in about 29% of patients in both subgroups with ~22% maintaining it for ≥12 consecutive months. At baseline, the median JADAS score was 21.5 with 8 (7.5%) and 99 (92.5%) patients meeting the criteria for moderate (JADAS >3.8 and ≤10.5) and high disease activity (JADAS >10.5), respectively. At Day 15, the median JADAS was 6.8 and 1.5 at the last assessment. At the last assessment, 53 (48%) patients had inactive disease (JADAS ≤ 1); 10 (9%) with low active disease activity (JADAS >1 and ≤3.8); while 14 (13%) had moderate and 31 (28%) with high disease activity. Infection (0.56 infections/100 patient-days), typically involving upper respiratory tract was the most common type of adverse event. Fifteen patients discontinued due to an AE and 40 had >1 SAE (mostly infections, macrophage activation syndrome (MAS), or flare-associated) and no deaths. Eight cases of MAS (0.013 events/100 patient-days) were reported.
Conclusion: Canakinumab provides similar efficacy in SJIA patients with and without SJIA-associated fever at treatment onset. The long-term safety profile was acceptable and similar to the pivotal program in SJIA children with fever at enrollment.
Reference

1. Groom JR, Luster AD: **CXCR3 ligands: redundant, collaborative and antagonistic functions.** *Immunol Cell Biol* 2011, **89(2)**:207-15.

O84

High levels of interferon-gamma (IFNγ) in macrophage activation syndrome (MAS) and CXCL9 levels as a biomarker for IFNγ production in MAS

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Background: A vast body of evidence in animal models points to a pivotal pathogenic role of IFNγ in primary hemophagocytic lymphohistiocytoses (HLH). High levels of IFNγ are also found in humans with HLH.
Objectives: Given the similarities between primary and secondary HLH (sec-HLH), including MAS, we measured levels of IFNγ, IFNγ-related chemokines (CXCL9, CXCL10, CXLC11), and IL-6 in patients with sec-HLH, and in patients with systemic Juvenile Idiopathic Arthritis (sJIA) with or without MAS at sampling and evaluated their relation to disease activity. In addition, we evaluated the correlation between serum levels of IFNγ and of the three IFNγ related chemokines with themselves and with laboratory parameters of disease activity in patients with active MAS.
Methods: We measured circulating levels of IFNγ, CXCL9, CXCL10, CXCL11 and IL-6 in patients with sJIA (n=54) of whom 20 had MAS at time of sampling using the Luminex multiplexing assay.
Results: Levels of IFNγ and of IFNγ-related chemokines (median pg/ml (IQR)) were markedly elevated in active MAS and active sec-HLH, with no significant differences between active sec-HLH (IFNγ 34.7 (23.9-170.1); CXCL9 33598 (3083-127687); CXCL10 4420 (799.7-8226); CXCL11 1327 (189-2000)) and active MAS (IFNγ 15.4 (5.1-52.6); CXCL9 13392 (2163-35452); CXCL10 1612 (424.8-4309); CXCL11 564.8 (197.5-1007)). Levels in active sJIA without MAS at sampling were lower (all p values 2=0.47; p=0.001), to a lesser extent of CXCL10 (r=0.53; r^2=0.28; p=0.015), and not of CXCL11 (r=-0.04;p=0.886). In active MAS ferritin, neutrophils, platelets, alanine aminotransferase and lactate dehydrogenase were significantly correlated with IFNγ and CXCL9, and to a lesser extent with CXCL10 and CXCL11; no correlation with IL-6 levels was found. In patients with active sJIA without MAS there was no significant correlation between laboratory parameters and cytokine levels (Table 1).
Conclusions: IFNγ, and IFNγ-related chemokines, levels were increased in patients with MAS compared to patients with active sJIA without MAS. The high levels of IFNγ and of CXCL9 present in patients with active MAS were significantly correlated with laboratory parameters of disease severity. In patients with active MAS IFNγ and CXCL9 are tightly correlated. Since CXCL9 has been shown to be induced only by IFNγ and not by other interferons [1], our findings support the conclusion that CXCL9 is a potential biomarker of IFNγ production in MAS.
Reference

1. Groom JR, Luster AD: **CXCR3 ligands: redundant, collaborative and antagonistic functions.** *Immunol Cell Biol* 2011, **89(2)**:207-215.

Table 1(abstract O84) Correlation of laboratory parameters of disease activity with IFN-g, CXCL9, CXCL10, CXCL11 and IL-6 in patients with MAS and in patients with active sJIA

	Macrophage Activation Syndrome	IFNg		CXCL9		CXCL10		CXCL11		IL-6	
		r*	p	r*	p	r*	p	r*	p	r*	p
Ferritin	8000 (3158 - 13174) [1]	0.57	0.014	0.49	0.041	0.66	0.002	0.62	0.023	0.17	>0.1
N	6.9 (3.4 - 13.9) [1]	-0.64	0.005	-0.61	0.010	-0.37	>0.1	-0.08	>0.1	0.09	>0.1
PLT	197 (114 - 392) [1]	-0.53	0.017	-0.52	0.022	-0.58	0.008	-0.22	>0.1	-0.02	>0.1
ALT	46 (18 - 164) [1]	0.49	0.045	0.49	0.044	0.51	0.038	0.06	>0.1	-0.44	0.080
LDH	1152 (722 - 2135) [1]	0.45	0.095	0.62	0.013	0.64	0.001	0.64	0.048	0.08	>0.1
	Sistemic Juvenile Idiopathic Arthritis	r*	p	r*	p	r*	p	r*	P	r*	p
Ferritin	214 (37 - 1669) [1]	-0.27	>0.1	0.28	>0.1	0.27	>0.1	0.29	>0.1	-0.12	>0.1
N	8.4 (5.2 - 14.5) [1]	0.30	>0.1	0.40	0.061	0.32	>0.1	0.40	0.067	0.28	>0.1
PLT	444 (353 - 544) [1]	0.21	>0.1	-0.14	>0.1	-0.13	>0.1	0.27	>0.1	0.35	0.064
ALT	16 (11 - 24) [1]	0.29	>0.1	0.42	0.049	0.50	0.011	0.44	0.039	0.04	>0.1
LDH	506 (455 - 851) [1]	0.07	>0.1	0.49	>0.1	0	>0.1	0.26	>0.1	0	>0.1

N=neutrophil count; PLT=platelet count; ALT=alanine aminotransferase; LDH=lactate dehydrogenase; [1]= Median (IQR); r*= Spearman r

O85

STING-associated vasculopathy with onset in infancy: new clinical findings and mutation in three Turkish children

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Pediatric Rheumatology 2015, **13(Suppl 1):**O85

Objective: STING-associated vasculopathy with onset in infancy (SAVI) is a recently identified autoinflammatory disease caused by gain-of-function mutations in *TMEM173*. This syndrome is a new interferonopathy characterized by neonatal-onset systemic inflammation with a severe cutaneous vasculopathy leading to extensive tissue loss and interstitial lung disease.
Patients: We clinically evaluated three patients with acral necrosis and systemic inflammation from three unrelated nonconsangineous Turkish families. Genetic analysis of TMEM173 was performed by direct sequencing.
Results: Case 1 was a 17 year old boy who presented with tachypnea in infancy and had cold-induced acral necrosis of fingers, toes and ear. His skin biopsy revealed *Periodic acid-Schiff* (*PAS*) positive fibrin thrombi in the lumen of ectatic vessels beneath the epidermis. The vessel walls also had PAS-positive thickening, immunofluorescence staining showed fibrinogen and C3. He was initially diagnosed with probable cryofibrinogenemia due to the presence of serum cryofibrinogen in one test. On follow up he had features of interstitial lung disease. His mutation analysis revealed an N154S mutation. Case 2 was a 14 year old girl presenting with joint contracture since infancy, cold induced gangrene of fingers, toes and recurrent sinusitis and cellulitis. Because of high transaminase levels, a liver biopsy was done revealing inflammation, hepatosteatosis and focal fibrosis. Paranasal sinus tomography showed extensive opacification of sinuses and left maxillary sinus medial wall defect. Case 3 was a 17 year old boy presented with cold induced ischemic acral lesions since 3 years of age and progressive spasticity of lower limbs since 12 years of age. Brain CT had showed basal ganglia calcification. Mutation analysis of case 2 and 3 revealed a novel compound heterozygous mutation of V155E/L170Q in TMEM173.
Conclusion: These three cases widen genetic and clinical features of SAVI syndrome. Besides the vascular ischemic lesions, cranial involvement resembling Aicardi-Goutiers syndrome is a new feature of SAVI syndrome.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

O86

Defective removal of ribonucleotides from DNA promotes systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease in which environmental exposures like virus infection and UV-irradiation trigger activation of the innate and adaptive immune system in genetically predisposed individuals. Heterozygous mutations of the 3' repair exonuclease 1 (TREX1) are associated with SLE. Biallelic mutations in TREX1 and the three subunits of ribonuclease H2 (RNASEH2A-C) cause Aicardi-Goutières syndrome, an inflammatory encephalopathy with clinical overlap with SLE. We therefore investigated the role of RNase H2 in SLE pathogenesis. RNase H2 is responsible for the removal of misincorporated ribonucleotides from DNA and is indispensable for genome integrity. We demonstrated a genetic association for rare RNase H2 sequence variants with SLE. RNase H2-deficient fibroblasts of AGS and SLE patients accumulated ribonucleotides in genomic DNA resulting in chronic low-level DNA damage, constitutive p53 phosphorylation and senescence. Patient fibroblasts proliferated slower than fibroblasts from healthy individuals and showed impairment of cell cycle progression. In addition, patient fibroblasts exhibited constitutive up-regulation of interferon-stimulated genes and an enhanced type I interferon response to the nucleic acid poly(I:C) and UV-irradiation. UV-irradiation induced enhanced cyclobutane pyrimidine dimer formation in ribonucleotide-containing DNA. This suggests that innate immune activation may be caused by immune recognition of DNA metabolites of DNA damage repair and may also explain photosensitivity in SLE patients with RNase H2 mutation. In summary, our findings implicate RNase H2 in the pathogenesis of SLE, and suggest a role of DNA damage-associated pathways in the initiation of autoimmunity.

O87

Prevalence of CECR1 mutations in pediatric patients with polyarteritis nodosa, livedo reticularis and/or stroke

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Background: Mutations of CECR1 have been recently reported as causative of an inflammatory condition characterized by polyarteritis nodosa, cerebral stroke and immunodeficiency; the clinical manifestations of the disease are heterogeneous with a wide range of severity.
Objectives: To analyze the prevalence of CECR1 mutations in pediatric patients with polyarteritis nodosa, livedo reticularis and/or stroke.
Methods: Pediatric patients of Caucasian Italian origin with the following diseases/manifestations were included in the study: i) histologically confirmed polyartiritis nodosa (PAN) or cutaneous polyartiritis nodosa (cPAN), ii) persistent livedo reticularis with elevation of acute phase reactants, iii) ischemic or hemorrhagic strokes with systemic inflammation.

Direct sequencing of CECR1 gene (exons 1-9) was performed with Sanger analysis.
Results: Up to January 2015, 33 patients from 30 families were included in the study. Homozygous or compound heterozygous *CECR1* mutations with deleterious effects (G47R, G47A, P251L, R312X, E328D, T360A, L249P) were detected in 7 patients. A heterozygous causative mutation (G47V) was observed in 2 affected brothers, their father and the unaffected brother; another patient with clinical manifestations consistent with the disease was found to be heterozygous for the Y453C mutation. In the remaining patients common polymorphisms (L46L, N53N, H335R, Y453Y) were detected.
The mean age of onset of the disease in genetically confirmed patients was 24 months (range 6 months - 5 years); all of them presented fever, elevation of acute phase reactants, livedo reticularis and a skin biopsy suggestive for vasculitis; two of them presented subcutaneous nodules while one of them presented ulcerations at extremities. Hypertension was detected in four patients, while one presented miocarditis. 3 patients presented one or more cerebral stroke during their disease course, while in 3 patients peripheral neuropathy was detected. 4 patients presented intestinal involvement (ranging from recurrent abdominal pain to intestinal perforation) and 2 patients presented growth delay, independent from steroidal treatment. Low immunoglobulin levels were detected in two patients.
The clinical characteristics of the heterozygous patients were similar: fever, livedo reticularis, increased acute phase reactants and hypogammaglobulinemia; cerebral stroke occurred in one of them.
Conclusions: CECR1 mutations are present in the Italian population and associated with severe cases of ADA2 deficiency. A clinical heterogeneity has been detected in genetically confirmed patients. In a few patients a typical phenotype was associated to incomplete or negative genotype, thus supporting the hypothesis of a genetic heterogeneity of this condition.

O88

Immune dysregulation in patients with TRNT1 deficiency

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Introduction: Next-generation sequencing has led to the discovery of a number of disease-associated genes in patients with uncharacterized autoinflammatory diseases and has pointed to new pathways regulating immune function.
Objective: To investigate the pathogenesis of disease in patients with the deficiency of the CCA-adding enzyme tRNA nucleotidyltransferase 1 (TRNT1). The TRNT1 enzyme catalyzes an essential step for tRNA maturation and protein synthesis, however it is largely unknown how abnormalities in this pathway lead to inflammation and immunodeficiency.
Patients and Methods: Whole exome sequencing (WES) was performed in a consanguineous Saudi family with 2 affected siblings and in a parent-child trio; candidate gene screening was subsequently performed in 3 sporadic Caucasian patients. Cytokine profiling, tissue immunohistochemistry, and deep RNA and tRNA sequencing were performed in patients' primary cells. Protein function was studied in zebrafish embryos.
Results: We have identified 7 patients with biallelic homozygous or compound heterozygous mutations in the *TRNT1* gene. Three patients died due to multiorgan failure. We identified 6 disease-associated missense mutations that affect evolutionarily conserved amino acid residues. These variants are either novel or found at an allele frequency less than 0.0001 in public databases, consistent with recessive disease inheritance. Two apparently unrelated patients shared the same genotype. Recently, loss-of-function mutations in the same gene were reported in patients with a syndrome termed SIFD (sideroblastic anemia, immunodeficiency, periodic fevers and developmental delay). Four mutations from our study have not been reported in patients with SIFD. Knockdown of the zebrafish *TRNT1* homologue caused abnormalities resembling the human phenotype. Preliminary results of next-generation tRNA sequencing showed a significant down-regulation of mature tRNAs in patient's fibroblasts compared to healthy control. RNA-sequencing of patients' whole blood showed an up-regulation in the expression of neutrophil-related genes. Consistent with these data, analysis of lesional biopsies from one patient's colon showed cryptitis with neutrophilic infiltration. Cytokine profiling of stimulated patients' leukocytes and serum samples suggest that the inflammatory phenotype is likely driven by IL-6 and interferon. Treatment with TNF inhibitors has shown promising results in attenuating the systemic inflammation and stabilizing the anemia in 3 patients who experienced recurrent fevers and required transfusions.
Conclusions: Hypomorphic mutations in *TRNT1* are associated with a new autoinflammatory disease manifesting a variable phenotype of fevers, congenital sideroblastic anemia, immunodeficiency, and developmental delay. Study of the underlying disease mechanisms might lead to the discovery of a new pathway regulating immune function and inflammation.

POSTER PRESENTATIONS

P1

Efficacy, safety, and post-vaccination antibody titer data in children with CAPS treated with Canakinumab

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Background: Canakinumab (CAN) is indicated for the treatment of cryopyrin-associated periodic syndrome (CAPS) in patients ≥2 years of age.[1] However, patients may require treatment in infancy where CAN has not yet been studied. IL-1 inhibition has not affected antibody production after vaccination in healthy volunteers[2], but no data in patients receiving standard childhood vaccines are available.
Objectives: To evaluate the efficacy and safety of CAN, including post-vaccination antibody production, in children with CAPS <4 years of age.
Methods: CAN-naïve patients aged 28 days to 4 years with CAPS received open-label CAN dosed 2-12 mg/kg every 4 or 8 weeks for 56 weeks. Efficacy was evaluated by complete response (clinical response and normal C-reactive protein [CRP]) and subsequent relapse. Safety was assessed by adverse event (AE) reporting and vaccination response evaluated by post-vaccine antibody titers measured at 28 and 57 days post vaccination. Vaccines evaluated included DTP; H. Flu; N. Men.; influenza; Hep B; and Strep. Pneum.
Results: Of 17 patients enrolled, 6 were less than 24 months old (44 days-5 months). The phenotypic distribution was: FCAS (n=1), MWS (n=12), and NOMID (n=4). All 17 patients achieved a clinical response and 16 achieved a complete response. Seven patients required dose escalation to achieve and/or maintain their responses. The patient who did not achieve a complete response was a 1 year old with persistently elevated CRP. Of the 16 patients with a complete response, 4 (2 with MWS and 2 with NOMID) subsequently relapsed, but all regained complete response; 2 (1 MWS; 1 NOMID) with and 2 (1 MWS; 1 NOMID) without dose escalation. No CAPS flares were reported with vaccination and a rise in post-vaccination antibody titers was observed for all vaccines evaluated. The most common type of AE reported was an infection, typically involving the upper respiratory tract. Four patients experienced a serious AE (SAE), with no SAE occurring more than once. No patient discontinued due to an AE.
Conclusions: Canakinumab is an effective treatment for patients with CAPS aged as young as 44 days old. Canakinumab appears to have no effect on the ability to produce antibodies against standard childhood non-live vaccines. The safety profile of canakinumab was acceptable and similar to that observed for older patients.

References

1. ILARIS [summary of product characteristics]: Novartis Europharm Limited 2014.
2. Chioato A, *et al*: *Clin Vaccine Immunol* 2010, **17**:1952-57.

P2

A case with IGG4-related retroperitoneal fibrosis-periaortitis rapidly diagnosed and dramatically responded to steroid treatment

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Background: Retroperitoneal fibrosis is a rare disease characterized by development of fibro-inflammatory tissue, which surrounds and causes compression of the retroperitoneal structures such as abdominal aorta, iliac vessels, vena cava and ureters[1]. It's prevalence was reported as 1.4 /100,000 [2]. It has been recently shown that it is one of the IgG4-related disorders[3]. Herein, we report a male patient who admitted to emergency department with acute abdominal pain, diagnosed with IgG4-related retroperitoneal fibrosis-periaortitis in a very short time, and both high baseline serum creatinine level as well as abdominal pain requiring opioids on admission improved with steroids.
Case: Forth-one-year-old male admitted to emergency department with the complaint of abdominal pain. Wall of abdominal aorta seemed thickened on ultrasonography (USG). Abdominal computerized tomographic angiography showed soft tissues surrounding aorta, beginning 9 cmproximal to iliac bifurcation and continuing up to the level of common iliac artery (aortitis? retroperitoneal fibrosis?), and the patient was referred to rheumatology clinic. The patient was hospitalized for further investigations. He did not complain of fever, fatigue, or urinary symptoms. The blood tests revealed the following: Erythrocyte sedimentation rate (ESR) 57 mm/h, CRP 43 mg/L, HGB 14.1 g/dL, WBC count 9800/μL, PLT count 306000/μL, MCV 82.3 fL, creatinine 1.15 mg/dL and ALT 18 U/L. The urinalysis of the patients was normal, and he was negative for ANA, ANCA and ENA. His IgM was 128 mg/dL (46-304), IgG was 2060 mg/dL (751-1560), IgA was 415 mg/dL (82-453), IgG_1 was 10700 mg/L (3824-9286), IgG_2 was 9070 mg/L (2418-7003), IgG_3 was 1400 mg/L (218-1761) and IgG_4 was 3550 mg/L (39.2-864). IgG_4/total IgG ratio was 17.2%. Aortoiliac arterial Doppler USG showed that the soft tissue surrounded the left ureter completely and the right ureter partially, and it caused grade 1 hydronephrosis and obstructive volume increase in the left kidney. The patient was diagnosed with IgG4 related disease and retroperitoneal fibrosis, and administered 60 mg methyl prednisolone as well as amlodipine due to high blood pressure. Venous and colored arterial Doppler examinations of the lower extremities were normal. The patient needed narcotic analgesics due to severe abdominal pain at the time of hospitalization. His abdominal pain improved with steroids in one week, and his ESR, CRP and creatinine levels decreased. ESR was 7 mm/h, and serum creatinine was 0.84 mg/dL at the second week of the treatment. The patient was discharged from the hospital with steroids, amlodipine, and PPI, and called for follow up visit 2 weeks later.
We did not biopsy the lesion since history, radiological findings and blood tests were characteristic for IgG_4 mediated retroperitoneal fibrosis-periaortitis. Most of the cases with IgG_4 mediated retroperitoneal fibrosis reported in the literature had a long diagnostic delay. Our patient is interesting since he was diagnosed with the disease shortly after beginning of his symptoms, steroids were administered immediately, and he responded steroids dramatically.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. Vaglio A, Salvarani C, Buzio C: **Retroperitoneal fibrosis.** *Lancet* 2006, **367(9506)**:241-51.
2. Thongprayoon C, Spanuchart I, Cheungpasitporn W, *et al*: **Idiopathic retroperitoneal fibrosis: a challenging case in a rare disease.** *N Am J Med Sci* 2014, **6(5)**:237-8, doi: 10.4103/1947- 2714.132945.
3. Chiba K, Kamisawa T, Tabata T, *et al*: **Clinical features of 10 patients with IgG4-related retroperitoneal fibrosis.** *Intern Med* 2013, **52(14)**:1545-51, Epub 2013 Jul 15.

P3

Long-term safety and efficacy of Canakinumab in cryopyrin-associated periodic syndrome (CAPS) patients: results from beta-confident registry

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Background: CAPS encompasses a spectrum of three phenotypes: familial cold auto-inflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurologic cutaneous and articular syndrome/neonatal onset multisystem inflammatory disease (CINCA/NOMID)[[1]]. The β-Confident Registry, the largest CAPS cohort documented in a registry, enrolled the last patient in December 2014. Here, we report interim data for the complete cohort of enrolled patients.
Objectives: To monitor the overall safety of canakinumab (CAN) focusing on SAEs including serious infections, vertigo, malignancies, and hypersensitivity reactions.
Patients and methods: The registry protocol does not mandate any visits or procedures, but records all observed and reported AEs and SAEs or AEs potentially CAN-related. Cumulative safety data are reported as incidence rate per 100 patient-years (IR/100 pyr). Data is partial for 11 patients due to the cut-off date for the analysis and will be updated at a later date. Efficacy was measured using physician global assessments (PGA).
Results: 288 patients were enrolled with a mean±SD duration of 193±72 weeks. Of these, 21 (7.3%) patients discontinued CAN: 5 each due to AE, poor efficacy and patient preference; and 6 due to unknown reasons. The IR/100 pyr for overall AEs was 100.0. FCAS patients had the lowest AE IR/100 pyr (60.9) compared with MWS (IR/100 pyr 107.2) and NOMID (IR/100 pyr 120.3) patients. The most common types of AEs were infections and infestations (IR/100 pyr 36.7). Vertigo was reported by 19 patients (IR/100 pyr 3.7). 117 SAEs were reported by 62 patients (IR/100 pyr 15.0), with infection being the most common (IR/100 pyr 4.1). One death (metastatic rectal adenocarcinoma in 76 yr old MWS patient) was reported. Of 18 patients receiving pneumococcal vaccinations (PPV), 13 (72%) reported a local post-PPV injection site reaction, of which 5 were considered as serious. Based on PGA, nearly half the patients had no disease activity while most others had mild/moderate disease activity. Similarly, disease activity was mostly absent in *NLRP3* mutation negative CAPS patients (n=14) treated with CAN. There was no evidence of loss of effect with time. Further analyses of this cohort are ongoing.
Conclusions: Canakinumab demonstrated a safety profile consistent with that observed in the clinical trial program and provided continued effectiveness in CAPS patients for up to 5 years. Canakinumab therapy was also effective in *NLRP3* mutation negative CAPS patients.

Reference

1. Kuemmerle-Deschner JB, *et al*: *Arthritis Res Ther* 2011, **13(1)**:R34.

P4

Genes responding to Canakinumab therapy in SJIA are -inversely - disregulated in adult onset Still's disease

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Introduction: Adult-onset Still's disease (AOSD) is a rare auto-inflammatory disorder resembling a similar pediatric syndrome known as systemic juvenile idiopathic arthritis (SJIA).[[1]] The superimposable systemic and clinical features in SJIA and AOSD suggest that both clinical

phenotypes represent a disease continuum with a pediatric (SJIA) and more adult-onset (AOSD).[2] Analyses of gene expression profiles may be useful not only for disease classification, diagnosis, and prognosis, but also to identify disease specific treatment effects that counteract the underlying pathological mechanisms. Here, we address the question: How do genes that respond to canakinumab treatment in SJIA patients[3] behave in AOSD patients with active disease relative to healthy controls and prior to IL-1 targeting therapy?

Objectives: To determine how genes that respond to IL-1β blockade with canakinumab in SJIA patients behave in AOSD patients relative to healthy controls.

Patients and methods: SJIA gene expression profiles pre- and post canakinumab treatment were compared with AOSD patients relative to healthy subjects using Affymetrix U133Plus2 DNA microarrays.

Results: Consistently, all genes down-regulated in SJIA following canakinumab treatment were upregulated in a majority of AOSD patients with active disease relative to healthy subjects and prior to canakinumab treatment. A few of the AOSD patients resembled healthy subjects. Comparison of the gene expression patterns to neutrophil counts suggested that elevated neutrophil numbers were closely correlated to the up-regulation of IL-1 associated gene expression.

Conclusions: Results are consistent with and further support the concept of a Still's disease continuum that presents as pediatric/juvenile SJIA or adult-onset Still's disease. Moreover, they suggest that AOSD is an IL-1 driven condition that is also mechanistically similar to SJIA and that the observed canakinumab response signature is likely to show a comparable treatment response to IL-1β blockade in AOSD.

References

1. Martini A: *Ann Rheum Dis* 2012, **71(9)**:1437-39.
2. Jamilloux Y, *et al*: *Immunol Res* 2015, **61(1-2)**:53-62.
3. Brachat A, *et al*: *Ann Rheum Dis* 2014, **73**:62.

P5

Monogenetic autoinflammatory syndromes and nephrology - therapy is usefull even in advanced kidney failure

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Objectives: The identification of genes involved in the modulation of inflammatory processes has allowed the delineation of a new group of diseases called "Monogenetic Autoinflammatory Syndromes - MAISs".

At the moment, 25 syndromes and their gene-disorders are known. Some of them are well known (eg Familial Mediterranean Fever), most of them are rare diseases (eg FCAS-Familial Cold-Autoinflammatory Syndrome, Blau Syndrome, HIDS-Hyperimmuno-globulinemiaD with Periodic Fever Syndrome, MA-Mevalonate Aciduria).

These disorders of innate immunity characterized by episodes of fever and systemic inflammatory symptoms affect for instance the serosal surfaces and bear the risk of developing reactive systemic (AA) amyloidosis due to excessive production of serum amyloid-A (SAA). SAA is deposited in various organs, particularly the kidneys, with the consequent progressive development of kidney failure[1,2].

Case 1 and 2: Blau Syndrome: We report a now 26-year old patient, who was first admitted aged 5 months due to periodic fever. Additional patient's history included polyarthritis, uveitis and a worsening of renal function over the years due to histological signs of sarcoidosis in the kidney. Different immunosuppressive treatments with steroids, azathioprin, methotrexat and cyclosporin did not prevent renal failure requiring dialysis in 2005. In 2010 our patient underwent renal transplantation. Despite standard triple immunosuppression patient's symptoms, especially uveitis, occurred even after transplantation whenever steroids were reduced. The initial diagnosis "juvenile sarcoidosis" was corrected to "Blau Syndrome" due to mutation analysis carried out in April 2012 (mutation on NOD2 gene). Thanks to treatment with adalimumab since 2012, uveitis and polyarthritis are under control. Kidney function has remained stable with creatinin values within normal range up to this day.

Familial Mediterranean Fever: A 54-year old dialysis patient with atrophic kidneys (reason unknown) underwent renal transplant in October 2012. A few days after discharge a fever of unknown origin occurred with an increase of c-reactive protein (CRP), creatinin, severe abdominal pain and diarrhoea without detection of pathogens. As Familial Mediterranean Fever (FMF) was suspected patient underwent a colchicin therapy. The creatinin, CRP and SAA value decreased at normal range within 6 weeks of treatment. To confirm the diagnosed FMF, a mutation analysis was carried out. We found a heterocygous mutation in the MEFV gene, which confirms the FMF diagnosis.

Case 3: Familial Cold-Autoinflammatory Syndrome: We report a women born in 1971 who was diagnosed with reduced kidney function in 2008. In addition the patient has been suffering from cold-dependent urticaria and joint pain without known reason since childhood. There was a rapid worsening of renal function over the next months. The kidney biopsy shows signs of amyloidosis (picture a-f). Mutation analysis in June 2013 confirms the diagnose "Familial Cold-Autoinflammatory Syndrome - FCAS". Due to treatment with anakinra, the cold-dependenturticaria and joint pain are eliminated, SAA is normalized and serumkreatinin and proteinuria are decreased.

Conclusion: Monogenetic Autoinflammatory Syndromes are rare causes of kidney failure due to excessive production of serum amyloid-A (SAA). The detection by means of mutation analysis allowed a disease-specific therapy with stabilization of serumcreatinin and SAA in addition to reduced disease-specific symptomes even in advanced kidney failure and after renal transplantation.

Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. Caso F, *et al*: *Int J Rheumatol* 2013, **2013**:513782, doi:10.1155/2013/513782. Epub2013Oct24.
2. Hedrich CM, *et al*: *Rheumatol Int* 2012, **32(9)**:2629-36.

P6

Correlation between serum amyloid-A and serum levels of proinflammatory cytokines in patients with Behçet's disease

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Introduction: Behçet's disease (BD) is an inflammatory disorder of unknown aetiology, unanimously recognized as both autoimmune and autoinflammatory disease. Indeed many of its classical manifestations overlap with those of monogenic autoinflammatory disorders. Clinically disease is characterized by multiple organ involvement, in particular by the "triple symptom complex", consisting of recurrent oral aphthosis, genital ulcers and recurrent bilateral uveitis. The abnormal activation of either innate and adaptive immunity, triggered by some microbial agents in genetically predisposed individuals, with consequent interaction of both T lymphocytes and activated neutrophils would seem to be involved in the disease onset. Therefore multiple cytokines may contribute to the pathological scenario of BD playing a pivotal role in the occurrence of the clinical manifestations.

Objectives: To determine serum levels of IL-8, IL-18, IFN-α2a, IL-6, IFN-γ, CXCL10, CXCL11, CXCL9 and serum amyloid-A (SAA) concentration in patients with BD, in comparison to healthy controls (HC), and to correlate their concentration with the status of disease activity.

Materials and methods: 78 serum samples were collected from 58 BD patients (28 males, 30 females, mean age 44.7±12.2 years). Serum cytokine levels of IL-8, IL-18, IFN-α2a, IL-6, IFN-γ, CXCL10, CXCL11 and CXCL9 were determined using a multiplex bead analysis as well as SAA was assessed by Enzyme linked-immunosorbent assay.

Results: In BD patients serum concentrations of IL-8 (p=0.0001), IL-18 (p=0.0058), IFN-α2a (p=0.0181) and IL-6 (p=0.0233) were significantly higher than in HC. When BD patients were divided into active and inactive group, IL-8 and IL-18 resulted higher in both active- (p=0.0001 and p=0.012 respectively) and inactive-BD (p=0.0001 and p=0.0128 respectively) than in HC, while IFN-α2a (p=0.0141) and IL-6 (p=0.0332) serum levels were significantly higher in active-BD than HC. Moreover, CXCL11 (p=0.0154) serum concentrations were significantly lower in inactive-BD than HC. We also compared serum cytokine profiles between

BD patients with SAA serum levels ≤20 mg/L, >20 mg/L and HC. Interestingly, we observed that BD patients with SAA >20 mg/L showed higher levels of inflammatory markers than HC. Among these cytokines, IL-18, IFN-α2a and IL-6 were higher in BD group with SAA >20 mg/L than HC, whereas IL-8 and CXCL9 levels were higher than in patients with SAA ≤20 mg/L and HC.
Conclusions: BD patients exhibit elevated levels of specific inflammatory mediators, especially during active disease periods and in those patients with SAA serum levels >20 mg/L, thus suggesting a possible role of SAA in the induction of BD inflammatory manifestations.

P8

Monogenic polyarteritis nodosa caused by ADA2 Deficiency: the GOSH experience

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Introduction: Recessive mutations in Cat Eye syndrome Critical Region 1 (*CECR1*), the gene encoding adenosine deaminase 2 (ADA2) have been recently reported to cause polyarteritis nodosa (PAN) with highly varied clinical expression.
Objectives: The aim of this study was to identify the relevance of this mutation in children with PAN at Great Ormond Street Hospital (GOSH).
Patients and methods: We used next generation (NGS) and Sanger sequencing to study select paediatric cases of PAN. Inclusion criteria were: 1. Onset of PAN <age-10-years; 2. Suspected familial PAN; 3. Sporadic PAN particularly with neurological involvement; and 4. Clinical features resembling the recent description of deficiency of ADA2 (DADA2). Whole exome sequencing was performed using a commercially available kit (Illumina) and a NextSeq500 sequencer. We used the Infinium HumanCytoSNP-12 v2.1 DNA Analysis BeadChip Kit for homozygosity mapping. Sanger sequencing was performed using the Applied Biosystems 3730 DNA Analyzer. ADA2 enzymatic assay was performed using Diazyme ADA Test Kit.
Results: Fourteen patients with PAN and one patient with unclassified vasculitis were included. Mutations in *CECR1* were identified in 8 patients: 4 homozygous mutations and 4 compound heterozygous mutations. Of the 8 patients with *CECR1* mutation, all had the evidence of systemic inflammation; 8/8 had cutaneous vasculitis; 3/8 had arterial ischaemic stroke; 3/8 had vasculitic polyneuropathy; 1/8 had long-tract signs, cause undetermined; and 3/8 had B cell immunodeficiency. Four/8 ultimately required treatment with anti-TNF-alpha having failed other treatments. We also screened 25 subjects from 7 families related to the index cases with DADA2. Homozygous (n=4) or compound heterozygous mutations (n=1) were identified in 5/25 of these asymptomatic subjects. ADA2 enzyme activity in both affected and asymptomatic subjects with *CECR1* mutations was significantly lower compared to healthy paediatric controls (p value = 0.0022) and sporadic PAN patients without *CECR1* mutation (p value = 0.0189).
Conclusions: We identified DADA2 as the cause of vasculitis in 8/15 (53%) of cases with childhood PAN; and 5 currently asymptomatic cases in their relatives. The clinical severity of DADA2 is heterogeneous ranging from asymptomatic to full-blown systemic PAN. It is currently unknown how DADA2 causes vasculitis. Possibilities include alteration of macrophage biology; endothelial activation; effect of chronic high extracellular adenosine; and/or other as yet poorly defined immunodysregulation (with or without immunodeficiency). Irrespective of the mechanism, TNF blockade seems to be effective therapeutically. Homozygous symptomatic patients may first present in middle age hence asymptomatic DADA2 requires close monitoring.

P9

A case with febrile attacks and vasculopathy associated with ADA2 and MEFV gene mutations

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Background: Decreased activity of ADA2, caused by recessive mutations in CECR1 gene (Cat Eye Syndrome Chromosome Region, Candidate 1 also known as ADA2), results in cutaneous or systemic vasculitis with variable clinical manifestations.
Case: A patient with juvenile onset recurrent febrile attacks associated with familial polyarteritis nodosa (PAN) and who carries CECR1 and MEFVgene mutations is described. The index case, a 23 year old male patient with recurrent attacks of fever and arthritis since the age of 7 was diagnosed initially as Familial Mediterranean Fever (FMF). A beneficial response to treatment with colchicine was observed. A year later he developed livedo reticularis and nodular dermal lesions compatible with cutaneous PAN. He was treated with prednisolone and azathioprine. Arthralgia, fever and dermal lesions regressed and he was in remission until he developed anemia and macrocytosis a year later and azathioprine was stopped. Due to the activation of his skin vasculitis anakinra 100mg/day was instituted. The beneficial response obtained with anakinra was lost when he discontinued the treatment. A family history revealed a brother two years older than himself who also had livedo reticularis, Raynaud's phenomena, fever and arthritis since the age of 8, diagnosed as PAN before the index case and died at age 22 because of gut perforation secondary to acute mesenteric ischemia. With the probable diagnosis of ADA2, the index patient was analyzed for CECR1 gene mutations on chromosome 22q11.1. After amplification of the exons 2, 4, 5, 6 and 9 on PCR, DNA sequencing analysis was performed. A homozygous c.139G-A transition in exon 2, resulting in a gly47-to-arg (G47R) substitution at a highly conserved residue in the dimerization domain was identified in this patient who was known to be heterozygous for M694V and R202Q mutations of the MEFV gene. His vasculitic lesions responded to Infliximab after the fourth infusion together with daily colchicine treatment.
Conclusion: CECR1 gene mutation should be considered in cases presenting especially with early onset PAN. Infliximab maybe an effective therapy in these cases which are related with increased mortality. This is the first case that is reported to carry both CECR1 and MEFV gene mutations presenting with characteristic phenotypes of FMF and ADA2. The role of CECR1 mutations in the well documented association of FMF and PAN will be an interesting field of investigation in near future.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P10

Serum IL-18 is a specific biomarker for Macrophage Activation Syndrome across several autoinflammatory diseases

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Question: IL-18 is a pro-inflammatory cytokine produced by a variety of myeloid and non-hematopoietic cells. It is canonically associated with enhancing interferon gamma (IFNg) and cytotoxicity in collaboration with IL-12p70, IL-15, or type I IFN. However, in other contexts IL-18 can promote IL-17, IL-22, or allergic responses. Macrophage Activation Syndrome (MAS) is a sepsis-like syndrome that has been associated with elevated serum IL-18 in systemic Juvenile Idiopathic Arthritis, Stills disease, and XIAP-deficiency. We sought to characterize IL-18 and associated cytokines in a cohort of patients with a variety of monogenic or complex autoinflammatory syndromes.
Methods: Serum IL-18 was measured across several platforms and normalized to healthy controls run in the same batch. For many patients, IL-18 binding protein (IL-18BP) and IL-37 were measured from the same sample. Results were correlated with clinical laboratory findings, most notably acute phase reactants like C-reactive protein and erythrocyte sedimentation rate obtained on the same date.

Results: We found three patterns of serum IL-18: 1) normal IL-18 in healthy controls, patients with STING mutations, patients with chronic non-bacterial osteomyelitis (CNO), and patients with deficiency of IL-1 receptor antagonist (DIRA); 2) Mild elevation (less than 10-fold above normal) of serum IL-18 in patients with defects in NLRP3 (Cyropyrin Associated Periodic Syndromes, CAPS) or proteasomal defects (Chronic Atypical Neutrophilic Dermatosis Lipodystrophy Elevated Temperature, CANDLE); and 3) extraordinary elevation (100 to 500 fold above normal) in patients with a history of MAS regardless of disease activity. Multiple serial IL-18 measurements were made in a patient harboring an *NLRC4* mutation, as well as a patient with clinical NOMID (including severe epiphyseal overgrowth) with no detectable germ-line or somatic gene defect who had multiple severe MAS episodes. There are two endogenous antagonists of IL-18: IL-18BP and IL-37. These cytokines correlated moderately with CRP, but not with serum IL-18.
Conclusions: Our data suggest that extreme elevation of serum IL-18, particularly in the absence of acute inflammation, is a unique biomarker for MAS risk across many autoinflammatory phenotypes. The mechanisms by which chronic elevation of IL-18 may promote the MAS phenotype need to be further investigated.

P11

Interleukin-1 receptor antagonist treatment revealed active hepatitis B infection in a boy with PAPA syndrome

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Background: PAPA syndrome (pyogenic sterile arthritis, pyoderma gangrenosum and acne) is a rare autosomal-dominant autoinflammatory disease caused by mutations in PSTPIP1gene. Typically presents with recurrent sterile, erosive arthritis in childhood, occurring spontaneously or after minor trauma, occasionally resulting in significant joint destruction. By puberty, joint symptoms tend to subside and cutaneous symptoms increase. Cutaneous manifestations include pathergy, frequently with abscesses at the sites of injections, severe cystic acne, and recurrent nonhealing sterile ulcers, often diagnosed as PG.
Objective: To report a case of hepatitis B infection revealed with interleukin-1 receptor antagonist for PAPA syndrome.
Patient and method: 16,5y boy with PAPA syndrome presenting at age 2 with pyogenic sterile arthritis requiring multiple surgeries (shoulders, elbows, knees, ankles, wrists). He was treated as JIA for 14y (NSAID, steroids (11 years, had growth retardation due to steroids), immunosupressants (metotrexate) and for short time biologicals (infliximab, adalimumab). Several years ago developed severe acne and nonhealing skin ulcers. In august 2013, the boy was referred to our Department and completely reevaluated. He had raised inflammatory markers, microcytic anaemia, normal transaminases. Skin biopsy: pyoderma gangrenosum. Kidney biopsy excluded amyloidosis. Started with adalimumab for 1 year. Joint disease was under control, but not skin disease. Clinical diagnosis of PAPA syndrome was confimed by genetic analysis in March 2014. He was heterozygous for the substitution 748G>C in exon 11 that predicts the E250Q aminoacid substitution. When available, adalimumab was switched to anakinra in November 2014 (after washout period of 8 weeks). One month later, the patient showed raised transaminases (10 times above upper limit of normal). Active hepatitis B infection was proved by PCR. His mother had hepatitis B and boy had several surgeries with blood transfusions. How did he harbour the infection? Anakinra was stopped. On symptomatic therapy, transaminasemia soon normalised, but PAPA worsened. At present, he is on antiviral therapy and the issue if and when anakinra should be restarted.
Conclusion: We reported a boy with genetically confirmed PAPA syndrome. Treatment with adalimumab controlled joint but not skin disease. Interleukin-1 receptor antagonist was associated with activation of previously unknown hepatitis B infection, requiring antiviral therapy and withdrawal of treatment with anakinra. This case raises the question of the possible role of anakinra in activating the viral infection and the usefullness routine srceening for hepatitis B prior biological.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. Dierselhuis MP, Frenkel J, Wulfraat NM, Boelens JJ: **Anakinra for Flares of Pyogenic arthritis in PAPA Syndrome.** *Rheumatology* British Society for Rheumatology 2005, **44(3)**.
2. Brener M, Ruzicka T, Plewig G, Thomas P, Herzer P: **Targeted treatment of pyoderma gangrenosum in PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome with the recombinant human interleukin -1 receptor antagonist anakinra.** *British Journal of Dermatology* 2009, **161**:1199-1201.
3. Caorsi R, Insalaco A, Marotto D, Frenkel J, Martini A, De Benedetti F, Gattorno M: *pediatric Rheumatology* 2013, **11(2)**:P228.
4. Caorsi R, Federici S, Gattorno M: **Biologic drugs and autoinflammatory syndromes.** *Autoimmunity Reviews* 2012, **12**:81-86.
5. Touitou I: **Pyogenic artheritis - pyoderma gangrenosum - acne. Th portal for Rare disease and orphan drugs, october 2006.**, dostupno na : http://orpha.net/consor/cgi-bin/OC_Exp.php?Lng=EN&Expert=69126.
6. Demidowich AP, Freeman AF, Kuhns DB, Aksentijevich I, Gallin JI, Turner ML, Kastner DL, Holland SM: **Genotype, phenotype and Clinical Course in Five Patients With PAPA Syndrome (pyogenic sterile arthritis, pyoderma gangrenosum and Acne).** *Arthritis 6 Rheumatism* 2012, **64(6)**:2022-2027.

P12

Monogenic interferonopathy presenting as CMV infection in infancy

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The patient is the 4th child of a consanguineous Turkish couple. She was diagnosed with CMV- pneumonitis at 7 months of age. In addition she presented with pernio-like skin lesions of cheeks and ear lobes. Immunologically she was hypergammaglobulinemic for her age (IgG 11,5 g/l), her T-cell subpopulations, T-cell-proliferation to mitogens, number of B- and NK-cells were normal. IL2 and INFg production was diminished upon stimulation. Histology of lung tissue revealed alveolitis with infiltration by macrophages and histiocytes as well as lymphofollicular hyperplasia with activation of germinal centres.
At the age of 8 y/o the patient was diagnosed with pulmonary hypertension. She is being treated with sildenafil since the age of 12 y/o in addition to continuous oxygen. At the age of 15 years the patient presented for the first time with a vasculitic rash of arms, upper legs and feet. Immunofluorescence showed a positive lupus band. Immunologically she is persistently hypergammaglobulinemic (IgG 18,4 g/l) and has an upregulated interferon signature.
Although the presumed diagnosis in infancy was a functional T-cell deficiency, the disease course points towards a lupus-like disease with chronic pneumonitis and skin vasculitis as well as development of an antibody-profile compatible with SLE. Known genes for monogenic SLE including TMEM173 were excluded. Results of whole exome sequencing are pending.

P13

Various inflammatory phenotypes in V200M NLRP3 carriers

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Background: Cryopyrin-associated periodic syndrome (CAPS) is a very rare auto-inflammatory syndrome. CAPS is caused by mutations of the

NLRP3 gene that encodes crypopyrin protein that is a part of inflammasome complex. CAPS patients can present with different phenotypes of the disease - Familial cold urticaria, Muckle-Wells syndrome, and the most severe phenotype - neonatal onset multisystem inflammatory disease (NOMID). CAPS patients experience symptoms of systemic inflammation, intense fatigue and have poor quality of life. In the most severe forms, they may develop serious organ damage such as visual and hearing impairment, arthritis, neurological deterioration and renal insufficiency. However, V200M mutation has been associated with mild inflammatory phenotype and its pathogenic role is even questioned by some. We report two families of V200M mutation carriers, with variable inflammatory phenotypes. In all cases mutations of MEFV, MVK, TNFRSF1A genes were excluded.

Family 1: Index case is a 1,5 years old female, that has been suffering from symptoms similar to Muckle-Wells syndrome: weekly episodes of fever and urticarial rash since infancy, accompanied by high laboratory inflammatory parameters (elevated ESR, CRP, hypergammaglobulinemia). Focal and diffuse changes were found on brain MRI, mild developmental delay was noted. Cerebrospinal fluid, audiogram were normal. She was started on IL1 inhibitor therapy (anakinra) with complete resolution of her symptoms. Sister, mother and grandmother of girl have the same mutation. The sister and mother have no symptoms of the disease. Yet, grandmother started having symptoms of recurrent fever, arthritis since the age of 3 years, and currently, at the age of 60 years, has a significant a hearing loss.

Family 2: The index case, 4 year old female, has been suffering from 2 years of age from monthly episodes that could be classified as PFAPA (periodic fever-pharyngitis-polyadenitis- aphtous stomatitis): 1-2 days long episodes of fever, pharyngitis, stomatitis and high laboratory activity. Brain MRI, cerebrospinal fluid, audiogram, vision were normal. Girl's father carries the same mutation, but he has no clinical symptoms of PFAPA or CAPS.

Conclusions: The NLRP3 V200M variant carriers show variable expressivity of the disease, the pathogenic role of this mutation and indications for therapy require further investigation. It will be interesting to screen typical PFAPA patients for V200M carrier status.

Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P14

Identification of ERAP1 protein allotypes in the Turkish population and evaluation of their contributions to Behçet's disease risk

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Introduction: Ankylosing spondylitis, psoriasis, and Behçet's disease are seronegative genetically complex diseases that are also considered complex autoinflammatory diseases. These diseases are characterized by strong association of class I human leukocyte antigen (HLA) alleles and also have a strong contribution to disease-risk by variants of the endoplasmic reticulum-associated amino-peptidase 1 (*ERAP1*) gene that is limited to individuals who carry the disease-specific HLA class I allele. The ERAP1 protein is responsible for trimming peptides for loading onto HLA class I molecules, which are displayed on the surface of nearly all cells, where they play important roles in immune surveillance and in innate and adaptive immune functions. *ERAP1* is highly polymorphic with several SNPs encoding variant amino acids that are likely to influence the nature of peptides bound as well as their ability to be trimmed. These non-synonymous coding variants are not found in isolation, but in combinations or allotypes that act in concert to influence the peptidome available for HLA binding and presentation.

Objective: To determine the common protein allotypes of ERAP1 in the Turkish population and evaluate their contributions to risk of Behçet's disease.

Materials and methods: Dense genotyping of the *ERAP1* gene region was performed in 1900 patients with Behçet's disease and 1799 healthy controls from previously reported Turkish GWAS and replication studies using the Immunochip. Additional marker genotypes were imputed. Haplotypes of non-synonymous coding SNPs were determined with SVS (Golden Helix) and disease association was evaluated by comparison of haplotype frequencies in cases and controls.

Results: SNPs encoding ten amino acid variants of the ERAP1 protein were found with minor allele frequency greater than 1% in 1000 Genomes EUR super-population (ancestral amino acid, position, derived amino acid: T12I, E56K, P127R, I276M, G346D, M349V, K528R, D575N, R725Q, Q730E). These SNPs defined 8 haplotypes with frequency from 2.4 to 23.7%. A single haplotype (0.17 frequency) bearing 5 non-ancestral amino acids (V349, R528, N575, Q725, and E730) was associated with Behçet's disease susceptibility (recessive model, OR = 2.55, 95% CI = 1.70-3.82, P=3.13 x 10^{-6}).

Conclusion: The ERAP1 allotype, T12, E56, P127, I276, G346, V349, R528, N575, Q725, E730, was associated with Behçet's disease. In previously reported studies this ERAP1 variant has been associated with poor peptide trimming. This study supports the hypothesis that a functionally distinct coding allotype of the ERAP1 protein contributes to Behçet's disease risk by altering the production and/or destruction of peptides available for binding class I HLA.

P15

The frequency of MEFV gene variations in Adult-onset Still's disease and Gout

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Objectives: Adult onset-Still's disease (AOSD) and gout are considered as auto-inflammatory disorders. Both diseases run recurrent episodic course and respond to anti-IL 1 treatment. Additionally, increased frequency of MEFV variations in other inflammatory diseases other than FMF such as Behçet's disease, ulcerative colitis and rheumatoid arthritis and raises the possibility that MEFV gene may play a general role in the inflammatory pathway. Therefore in this study, we explored the MEFV exon 2 and 10 variations in a group of AOSD and gout patients and compared the frequencies between disease groups and healthy controls.

Patients and methods: We studied all consecutive 42 patients with FMF (mean age: 33.3 ± 15.2), 28 patients with adult onset Still's disease (mean age: 37.9±8.4), 29 patients with gout (mean age: 50.3 ± 9.7), and 44 healthy controls (mean age: 33.6±10.2).

Genomic DNA was isolated from venous blood, using basic salting-out technique. PCR amplifications were done in three sets of primers covering exon 2 and exon 10 regions. Gel purified products were Sanger sequenced and chromatograms were analysed using Genious Software by two independent researchers. MEFV variation frequencies were calculated using chi-square analysis.

Results: The frequency of common exon 2 variation E148Q was found to be similar between the study groups (FMF: 5%, AOSD: 4%, gout: 3% and healthy controls: 3%). In exon 2, only R202Q variation was significantly more frequent in FMF group (43%) compared to other groups (18-25%) (*P=0.004*).

There was also significant difference in pathogenic exon 10 variations between FMF and other groups. The most prominent of these variations, M694V, was significantly more common in FMF group (49%), compared to AOSD (2%), gout (7 %) and healthy controls (1 %) (P<0.0001). The frequency of non-synonymous variations such as D102D-G138G-A165A, the common haplotype, was more likely to be more common in FMF group (66%) compared to AOSD (22%), gout (30%) and healthy controls (38%) (p< 0.05).

Conclusions: AOSD and gout do not seem to be associated with MEFV gene mutations.

P16

Challenging the opinion that SAPHO syndrome is associated with low intracellular ROS production in neutrophils

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Background: SAPHO syndrome, characterized by synovitis, acne, pustulosis, hyperostosis, and osteitis, belongs to the autoinflammatory bone disorders, in which dysregulation of innate immunity typically causes inflammation in sterile bone. The mechanisms underlying SAPHO syndrome are unknown, but neutrophil activation is suggested as part of disease pathophysiology. Previously, a patient with SAPHO syndrome-like phenotype was shown to lack production of intracellular NADPH-oxidase-derived reactive oxygen species (ROS) in response to phorbol myristate acetate (PMA; Ferguson et al, Arthritis and Rheumatism, 2008). In absence of phagosome-formation, such ROS are produced in intracellular granules, and are suggested to be part of regulatory signaling associated with hyperinflammatory disease.
Objective: To investigate if aberrant neutrophil intracellular production of NADPH-oxidase-derived reactive oxygen species is a general feature and disease mechanism in SAPHO syndrome.
Patients and methods: Neutrophil function was explored in a cohort of four patients with SAPHO syndrome, two of which were sampled during both inflammatory and non-inflammatory phase. Intracellular neutrophil reactive oxygen species production was determined by luminol-amplified chemiluminescence in response to PMA.
Results: Cells from all patients produced normal amounts of reactive oxygen species, both intra- and extracellularly, when compared to internal controls as well as to a large compilation of healthy controls assayed in the laboratory over time (showing an extensive inter-personal variability in a normal population). Further, intracellular production of reactive oxygen species increased during the inflammatory phase. Neutrophil activation markers were comparable between patients and controls.
Conclusion: Dysfunctional generation of intracellular ROS in neutrophils is not a general feature of SAPHO syndrome. Secondly, serum amyloid A appears to be a more sensitive inflammatory marker than C-reactive protein during improvement and relapses in SAPHO syndrome.

P17

Expression of Caspase-1 variants induced ER stress

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Pediatric Rheumatology 2015, **13(Suppl 1):**P17

Introduction: Caspase-1 is a proinflammatory enzyme that is activated by the NLRP3 inflammasome in response to endoplasmic reticulum (ER) stress independent of the classical unfolded protein response. This finding linked ER stress to chronic inflammatory diseases. In patients suffering from unexplained recurrent febrile episodes we detected several genetic variants of *CASP1* leading to reduced enzymatic activity due to destabilization of the caspase-1 dimer interface.
Objective: We investigated a possible association of expression level and reduced enzymatic activity of variant caspase-1 with impaired ER-stress responses.
Methods: We analysed ER stress markers in THP-1 cells and lymphoblastoid cells lines (LCL, EBV transformed B cells) from individuals harbouring *CASP1* variants and healthy donors. Additionally, we knocked-down endogenous caspase-1 in THP-1 cells and in LCL to determine caspase-1 involvement in ER stress responses. Knock-down of caspase-1 was functionally verified by FLICA-assay. We used Western blot analyses and quantitative real time RT-PCR to examine mRNA expression of genes involved in ER stress. In a next step, we infected LCL with microorganism, such as *Salmonella typhimurium*, to investigate the inflammation dependent cell death regulated by the proteolytic activity of caspase-1.
Results: Activation of NLRP3 inflammasome and also induction of ER stress by tunicamycin lead to secretion of IL-1β, IL-8, and TNF-α. In an analogous manner, LPS induced activation of the NLRP3 inflammasome or treatment of cells with tunicamycin resulted in an increased expression of ER stress related genes in native THP-1 cells. Expression of ER stress marker genes was also increased in native patients' LCLs with reduced enzymatic activity of caspase-1. After infection with microorganisms the amount of death cell was significantly increased in LCL expressing wildtype procaspase-1 compared to LCL with variant procaspase-1. In patients' LCLs this cell death could be prevented more efficiently by YVAD (caspase-1 inhibitor) and zVAD (general caspase inhibitor) than in wildtype LCLs. Cell death during inflammation was dependent on enzymatic activity of caspase-1.
Conclusion: This data indicate that ER stress induces activation of wildtype caspase-1 and that vice versa inflammasome activation induces ER stress in different cell types. In LCL of patients harbouring caspase-1 variants, knock-down of pro-caspase-1 reduces ER stress. This indicates a role of variant caspase-1 in dealing with intrinsic danger signals.
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P18

A transgenic in vitro cell model for the analysis of proinflammatory effects of naturally occurring genetic variants of caspase-1

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Pediatric Rheumatology 2015, **13(Suppl 1):**P18

Introduction: Caspase-1 (Interleukin-1 Converting Enzyme, ICE) is a proinflammatory enzyme mediating cleavage and secretion of the proinflammatory cytokines IL-1β and IL-18. Caspase-1 plays pivotal roles in the innate immune system and in inflammatory diseases like periodic fever syndromes, arthritis, or type-II-diabetes. In previous studies published by our group, genetic variants of procaspase-1 had been detected in patients suffering from autoinflammatory symptoms. Analyses of the procaspase-1 variants in HEK293T cells revealed reduced enzymatic activity of caspase-1 but enhanced ability to activate NF-κB signaling. The latter was mediated by enhanced CARD/CARD interactions of procaspase-1 with RIP2.
Objectives: The primary objective of this study was to analyze the effects of procaspase-1 variants in a monocyte/macrophage cell model. Furthermore, protein expression and enzymatic activity of procaspase-1 with or without a C-terminal FLAG-tag was analyzed.
Materials and methods: Genetically engineered THP-1 monocytes were used for the *in vitro* study. First, THP-1 cells were transduced with lentiviral vectors expressing shRNA against procaspase-1 mRNA. Subsequently, procaspase-1 wildtype (wt) or variants with or without a C-terminal FLAG-tag were reconstituted using a second lentiviral transduction. THP-1 cells were differentiated into macrophages and stimulated with different inflammasome activators. Activation and release of caspase-1 and IL-1β was assessed using immunoblotting (caspase-1) or immunoblotting and cytometric bead arrays (IL-1β). The activation of NF-κB was estimated by measuring the NF-κB regulated cytokines IL-6 and IL-8. Cell death following inflammasome activation was analyzed by measuring LDH in the cell culture supernatant.
Results: The protein expression level of reconstituted FLAG-tagged procaspase-1 variants was significantly reduced compared to expression of endogenous procaspase-1 or reconstituted procaspase-1 variants without FLAG-tag. In line with this data, the release of IL-1β after inflammasome stimulation was reduced in cells expressing FLAG-tagged procaspase-1 compared to cells expressing procaspase-1 without FLAG-tag. Interestingly, the mRNA expression of the FLAG-tagged procaspase-1 variants was not reduced compared to reconstituted procaspase-1 without FLAG-tag. Furthermore, cells expressing the procaspase-1 variants released reduced amounts of IL-1β and showed a reduced frequency of cell death following inflammasome stimulation. No differences in IL-6 or IL-8 secretion were detected when cells expressing wt or enzymatically inactive procaspase-1 were compared.

Conclusion: This study shows that even short protein-tags can influence protein expression significantly. Therefore, phenotypic rescue in knockdown studies can be complicated when using tagged proteins. Using the genetically engineered THP-1 cells we were able to show a reduced IL-1β release and reduced frequency of pyroptosis following inflammasome stimulation without detecting any differential regulation of the proinflammatory cytokines IL-6 or IL-8.
This study was supported by the German Research Foundation (DFG, KFO 249) and by a MeDDrive project (University of Technology, Medical Faculty) to SW.

P19

The intracellular signalling pathway signature (the signalome) in PBMCs in the presence of a common TRAPS-associated genetic variant, TNFRSF1A p.(Arg121Gln) (legacy p.R92Q) is distinct from normal PBMCs and from other pathogenic variants

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Question: Like other disorders with a strong genetic association, a growing sequence dataset from various autoinflammatory syndrome patients continues to identify many variants of uncertain significance (VUS), i.e. missense and intronic variants or small insertions/deletions, for which the impact on protein function and pathways, and therefore the clinical significance, is unknown. Since it is unclear how these variants are associated with an increased risk of autoinflammatory disease, the clinical management of carriers of VUS is complicated. Therefore, there is a strong demand for reliable tests to rapidly assess the clinical significance of VUS, providing carriers of these variants with the necessary information to make an informed clinical decision and refining treatment by stratified therapy strategies.
In many cases, a novel VUS is not common enough to evaluate its significance. Common variants with apparently variable penetrance can be more accessible as a model to test functional aspects. A missense variant in TNFRSF1A rs41495584 ("p.R92Q") is the most commonly identified variant associated with TRAPS within our mainly Caucasian UK patient population. The aim of this study was to comprehensively examine the intracellular signalling pathways that are affected by the presence of p.R92Q, in comparison to normal cells and to well-known symptomatic variants such as p.C33Y.
Methods: PBMCs were collected from patients and healthy volunteers with informed consent. Reverse-phase protein microarray was applied to examine a large number of signalling molecules and inflammatory cytokines, using a feature subset selection process to identify distinctive subsets.
Results: The resulting p.R92Q patient signatures demonstrated that a particular range of inflammation-associated pathways were dysregulated in p.R92Q variant carriers, grouping carriers together and readily distinguishable from normal PBMCs and other known pathogenic variants.
Conclusions: The inflammatory signaling pathways activated by the TRAPS-associated variant p.R92Q are distinctive and provide an opportunity to identify a strategy for correlation of genetic findings and functional analysis. This is applicable to straightforward or subtle phenotypes, and common or rare genetic variants not only in TRAPS, but also in other autoinflammatory diseases such as CAPS, FMF, MKD, Blau syndrome and others. This represents an important step towards genetic-bioinformatic disease portraits with statistical and clinical relevance.

P20

Severe macrophage activation syndrome. Is there a causative role for a homozygous A91V mutation in the perforin gene?

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A 16 year old lebanese girl with consanguinous parents presented with a severe "abdominal" sepsis supposedly resulting from an infected vaginal tampon (ESBL E.coli). She had been healthy before. She developed severe hepatic functional disorder, infarction of the spleen, cardiovascular and renal insufficiency, as well as anemia and thrombocytopenia. Macrophage activation syndrome was diagnosed subsequently and systemic glucocorticoid treatment initiated. The girl recovered clinically. Of note, she developed severe cushingoid syndrome. Due to limited compliance she discontinued all anti-inflammatory medication (nsaids, gc and ciclosporin) 4 weeks later. The following 18 months inflammatory parameters were persistantly elevated (ESR>100mm/h). Familial mediteranean fever was excluded. Genetic analysis revealed a homozygous perforin I gene mutation 91 (GCG) -> Valin (GTG)/.pAla91Val-/A91V in exon 2. Familial hemophagocytic lymphohistiocytosis type II was discussed as a potential diagnosis. Perforin expression was diminshed to about 50% in NK-cells, however functional NK-cell cytotoxicity was in the lower normal range, considered not impaired.
On the basis of these findings, we want to discuss the role of the homozygous A91V perforin mutation for the initiation or perpetuation of a life-threatening macrophage activation syndrome, in addition to the further management of the patient.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P21

In search of human proteins and infectious triggers involved in periodic fever, aphthous stomatitis, pharyngitis and adenitis syndrome

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Pediatric Rheumatology 2015, **13(Suppl 1):**P21

Introduction: Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome is the most prevalent pediatric autoinflammatory syndrome. For unexplained reasons tonsillectomy induces remission. The etiology of PFAPA is unknown; however, mutations of TNF receptor superfamily 1A (*TNFRSF1A*) and elevated circulating TNF-a have been described in some patients.
Objectives: 1) To identify transcriptomic or microbial signatures specific for PFAPA tonsils vs. controls; 2) to determine the presence and distribution of TNFRSF1A in tonsils of patients with PFAPA and the control population.
Methods: Using 3 age-matched groups (6 PFAPA, 4 chronic tonsillitis and 4 obstructive sleep apnea, OSA), total RNA was extracted from tonsil punch biopsies and were subjected to massively-parallel, paired-end sequencing (~50,000,000 reads per sample) on the Illumina HiSeq platform. Resultant sequences were aligned to human and microbial reference transcriptomes in order to quantify human transcript, bacterial, and viral sequences. Tonsils from 16 children with PFAPA and 8 with streptococcus/chronic tonsillitis or OSA were stained immunohistochemically for TNFRSF1A.
Results: Transcriptome analyses identified several genes involved in innate immune response, including TNFRSF1A, to be statistically significantly overexpressed in PFAPA tonsils. TNFRSF1A immunohistochemistry highlighted a network of dendritic cell processes extending from the basal layer of the squamous epithelium to the mantle zone of subjacent follicles. Additionally, follicles away from the squamous epithelium were lined by a similar dendritic cell network along the thin side of the mantle zone. The pattern and intensity of staining were not appreciably different between cases and controls. Computational analysis of bacterial and viral species present in PFAPA tonsils did not reveal a candidate pathogen. Unsupervised machine learning methods did not support the presence of a conserved microbial signature specific for PFAPA.
Conclusion: Differential expression of innate immunity-related genes in PFAPA samples strengthens the hypothesis that PFAPA is mechanistically similar to other periodic fever syndromes. In this small cohort, the pattern

and intensity of TNFRSF1A immunohistochemical staining were not appreciably different between cases and controls. The PFAPA tonsillar microbiome did not reveal candidate pathogens, although the study is limited by the small sample size. To our knowledge, this is the first study defining the anatomic distribution of TNFRSF1A in pediatric tonsils. TNFRSF1A is expressed in dendritic cell processes. Their localization in the interface between lymphocyte-rich squamous epithelium and subjacent germinal centers suggests that TNFRSF1A may have a role in lymphocyte trafficking to and/or from the mucosal surface.

P22

The patient experience of Colchicine Resistant-Familial Mediterranean Fever (cr-FMF)

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Pediatric Rheumatology 2015, **13(Suppl 1):**P22

Introduction: Familial Mediterranean Fever (FMF) is a genetic disorder characterized by recurrent attacks of fever and pain, which is most common in those of Sephardic Jewish, Armenian, Turkish or Arabic descent. Although colchicine is the mainstay of treatment for FMF, an estimated 5 to 15% of patients have an incomplete response to colchicine (e.g. colchicine-resistance FMF [cr-FMF]).[1,2].
Objectives: To determine the impact of cr-FMF on patients' or caregivers' lives, to describe patient's journey from first onset of symptoms to present, and to identify areas for improvement in cr-FMF patient care.
Patients and methods: Patients with cr-FMF or their caregivers (paediatric patients) (N=16) were recruited through rare disease experts and patient support groups. Patients completed a 20 page pre-interview questionnaire and an in-depth 90 minute interview. Complete data (n=14) were quantified with topics focused on symptoms, diagnosis process, treatment experience, treatment needs and impact on wellbeing.
Results: The majority of patients were adults (n=14) with a family history of FMF (n=14). The disease generally starts in childhood, with 65% of patients experiencing symptoms before 10 years. Attacks occurred with variable frequency, ranged from weekly to every 3-4 months, lasting between 12-72 hours. Reported triggers included physical or emotional stress, or menstruation or occured spontaneously. Commonly reported symptoms were stomach, fever, joint pain, difficulty breathing and chest and back pain. Diagnosis delays were also variable (4 months-44 yrs), with half of patients experiencing delays of ~5 years. Flares were extremely debilitating and patients were often bedridden, leading to missed work and school. Entire families were impacted, especially caregivers. Patients/caregivers were often dependant on family for support and financial assistance. Most patients continue to be treated with colchicine despite only partial response and distressing side-effects. They expressed the need for treatments that provided not only rapid relief during flares, but also prevented or reduced attacks.
Conclusion: Patients with cr-FMF reported a significant impact of the disease on physical, social, emotional, and practical/financial aspects of their lives. In their journey with cr-FMF, they commonly experienced diagnostic delays and misdiagnoses. Therapeutic options with improved efficacy and fewer side-effects are needed for the treatment of cr-FMF.

References

1. Jacobs Z, *et al*: *Curr Allergy Asthma Rep* 2010, **10(6)**:398-404.
2. Ben-Chetrit E, *et al*: *Clin Exp Rheumatol* 2008, **26(4)**:S49-S51.

P23

Living with Tumour necrosis factor receptor-associated periodic fever syndrome (TRAPS)

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Pediatric Rheumatology 2015, **13(Suppl 1):**P23

Introduction: TRAPS is a genetic disorder characterised by recurrent attacks of fever, abdominal and muscle pain and rash, most common in patients of northern European origin.[1] Very little is known about the patient experience with TRAPS.
Objectives: To qualitatively assess the burden of disease on patients and caregivers and describe patient's journey.
Patients and methods: TRAPS patients or caregivers (paediatric patients) (N=16) were recruited via rare disease experts and patient groups. A 20 page pre-interview questionnaire and in-depth 90 minute interview were completed. Complete data (n=15) were quantified with topics focused on symptoms, diagnosis, burden, treatment experience and unmet needs.
Results: Most patients were female (n=13) and had a family history of TRAPS (n=9). Physical functioning was greatly impaired during an attack with most common symptoms including fever, abdominal pain, joint pain, rash, vomiting and diarrhoea. Fever was the most prominent symptom in children and muscle and joint pain in adults. Paediatric patients reported missing school due to attacks or attending medical appointments were common. Adults frequently missed work for similar reasons, resulting in anxiety over work due to frequent absences. Damage to education or career had a negative financial impact as did travelling long distances to medical appointments. Social activities were also restricted, creating a sense of isolation, embarrassment and uncertainty. Delay in diagnosis was frequently reported with time to diagnosis greater than five years in more than 50% of patients. Steroids are typically the first treatment, while providing temporary relief may lead to uncertainly of long-term side effects, Biologics, typically IL-1 or TNF-alphas, are initiated subsequently and majority of patients reported a decrease in severity and frequency of attacks. Common concerns include, injection site reactions, increased susceptibility to infection, and maintenance of efficacy. Patients expressed interest in alternative administration and storage conditions as potential ways to ease the burden of management. Finally, an increase in disease awareness was important, as many patients receive little information about their condition and are often required to do their own research, perhaps due to lack of physician awareness.
Conclusions: The study demonstrated that the burden of TRAPS is considerable, impacting physical, social, emotional and practical/financial aspects of patients' and caregivers lives. Greater physician disease awareness may lead to improvements in diagnosis. Finally, improved therapeutic options with alternative modes of administration are needed for the treatment of TRAPS.

Reference

1. Masson C, *et al*: *Joint Bone Spine* 2004, **71(4)**:284-290.

P24

Hyper Immunoglobulin D syndrome (HIDS): understanding what it is like to live with this rare condition

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Pediatric Rheumatology 2015, **13(Suppl 1):**P24

Introduction: Hyper Immunoglobulin D syndrome (HIDS) is a genetic disorder characterized by recurrent attacks of fever and inflammatory symptoms and is most common in those of French or Dutch descent.[1,2].
Objectives: To understand the impact of HIDS on the lives of patients/caregivers, to describe patient's journey from first symptoms and to learn what patients hope to emerge in terms of future therapy, support and information.
Patients and methods: Fifteen patients with HIDS were recruited across US, Europe and Australia. They completed a 20 page pre-interview questionnaire and an in-depth 90 minute in-home interview. Patient responses were recorded and summarized; for patients with complete data available (n=13), responses were quantified. The topics covered in the interview were symptoms, patient journey, and unmet needs.
Results: Patients reported periods of wellness and symptomatic flares, where flares were characterised by high fevers and nausea (especially in children), as well as pain. In many cases flares were so severe that patients were bedridden. In children, flares led to severely disrupted education. In caregivers and adult patients, attacks and medical appointments resulted in missed work and limited career choices, leading to financial dependency. HIDS also had an impact on patients' relationships and social lives by limiting their activities. Severity of symptoms, the duration and frequency of flares decreased with age and treatment. Patients frequently experienced a delay in diagnosis (15 mos-

20 yrs), where they were subjected to a variety of diagnostic tests for other conditions. While most patients were initially treated with non-steroidal anti-inflammatories, colchicine, or steroids; they transitioned to biological treatment. Responses to biologic agents varied, although many reported shorter attack duration and frequency, all respondents continued to experience attacks. Around half of patients have switched biologics due to lack of efficacy. Patients and parents seek out information independently at diagnosis, usually via the internet. Patients identified improved treatment efficacy and reduced side effects as the areas in greatest unmet need.

Conclusions: HIDS significantly impacts the physical, social, emotional and practical/financial aspects of patients' and caregivers' lives. Greater awareness of HIDS among HCPs may improve diagnostic delays. Patients expressed interests in gaining a greater understanding of their disease and treatment options. There is an unmet need for therapy that prevents or reduces the number of attacks and which has patient-friendly administration.

References

1. van der Hilst JC, *et al*: *Curr Rheumatol Rep* 2010, **12(2)**:101-107.
2. Lainka E, *et al*: *Rheumatol Int* 2012, **32(10)**:3253-3260.

P25

Genetic and phenotypic characteristics of 114 patients with mevalonate kinase deficiency

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Pediatric Rheumatology 2015, **13(Suppl 1):**P25

Introduction: Mevalonate kinase deficiency (MKD) is a rare autoinflammatory syndrome, characterized by febrile episodes and generalized inflammation.

Objectives: This study aims to describe the genetic and phenotypic characteristics of MKD in a large international patient cohort.

Methods: Patients were enrolled in the Eurofever registry (EAHC Project No. 2007332), a registry that retrospectively collects information on patients suffering from periodic fever. An expert on MKD validated all patients on clinical and genetic criteria.

Results: One hundred and fourteen patients (53 male, 61 female) with two *MVK* mutations were included in this study. The median age of onset was 0.5 years. The median follow up period was 11.5 years. The majority of the patients were Caucasian (90%). Ninety-six patients harboured at least one V377I mutation, fourteen of them had a homozygous V377I mutation. The second most frequent mutation was I268T occurring in 29 patients.

Ninety-nine of 114 patients had recurrent inflammatory episodes, while six patients suffered from a chronic course and nine patients had a chronic course with exacerbations. The median disease duration was five days. The median frequency was 12 per year. Triggers inducing febrile episodes were mentioned in 108 patients, the most important ones were vaccination (n=39), infection (n=18) and stress (n=26).

One hundred and twelve patients suffered from gastrointestinal complaints, most of them suffering from vomiting (69%), diarrhoea (84%) and abdominal pain (88%). Ninety-nine patients also experienced mucocutaneous symptoms, mainly pharyngitis (28%), stomatitis (60%) and maculopapular rash (39%). Seventy-one percent of all patients had arthralgia and 57 percent had myalgia. Arthritis was less common and occurred in 28%. Neurological complaints occurred in 46 patients, most of them suffering from headache (38%). Cerebellar syndrome (3%), mental retardation (4%) and seizures (5%) were noted in some patients. Many patients had constitutional symptoms, such as malaise (65%), weight loss (66%), fatigue (63%) and mood disorders (24%). AA-amyloidosis was noted in six patients. One patient suffered from macrophage activation syndrome, a life-threatening complication characterized by high fever, pancytopenia and liver damage.

Abnormal IgD levels were observed in 55 of 76 tested patients, while 37 of 40 tested patients showed elevated urinary mevalonic acid excretions. Inflammatory parameters, such as erythrocyte sedimentation rates (98%), C-reactive protein (94%) and white blood count (66%), were abnormal in many patients.

Conclusion: This study describes the clinical and genetic characteristics of 114 MKD patients, which is the largest cohort so far.

P26

Genetic analysis of MEFV mutation negative familial Mediterranean fever for non-MEFV mutations is rarely effective

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Pediatric Rheumatology 2015, **13(Suppl 1):**P26

Background: Systemic autoinflammatory diseases (SAIDs) are a group of diseases characterized by episodes of inflammation, usually manifested with fever and a variety of symptoms, including skin-rash, arthritis and abdominal pain. A clinical overlap between different SAIDs, may cause diagnosis uncertainty. Familial Mediterranean fever (FMF), the prototype of the autoinflammatory syndrome, is manifested with recurrent attacks of fever and serositis. Although most FMF patients present with a typical picture, approximately 10% of them, present with atypical phenotype, and may harbor no mutations in their MEFV gene. In these patients further genetic analysis may be advocated.

Objectives: In this study we aimed to study the frequency of gene mutations of 3 SAIDs in a population of atypical FMF patients.

Patients and methods: By reviewing our records of FMF patients at Tel-Hashomer, we identified 10 patients with atypical FMF phenotype, who were non-responsive to colchicine, and carried no MEFV mutations. In these patients, we tested genetic mutations for TNF-receptor associated periodic syndrome (TRAPS), Hyper IgD Syndrome (HIDS), caused by Mevalonate kinase (MVK) deficiency and Cryopyrin associated periodic syndrome (CAPS).

Results: Of the 10 patients who were recruited, 9 patients were found not to carry TRAPS, CAPS or HIDS mutations and 1 was heterozygous for the NLRP3 mutation K705Q, considered a non-pathogenic polymorphism. This patient had recurrent attacks of fever and skin-rash, without attacks of serositis. Since some patients with this polymorphism and SAID's symptoms were reported to respond to anti IL-1 treatment we offered our patients treatment with canakinumab, but she declined our advice.

Conclusion: In FMF endemic area, screening of atypical FMF patients only rarely lead to a detection of another non-FMF SAID.

P27

Variant CAPS in an adult- the use of genetics

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Pediatric Rheumatology 2015, **13(Suppl 1):**P27

Introduction: Cryopyrin-associated periodic syndrome (CAPS) includes three overlapping disorders: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and neonatal onset multisystem inflammatory disorder (NOMID). Once considerate separate entities, these hereditary autoinflammatory disorders have been found to share a common genetic basis, pathogenesis and treatment and are therefore now considered a continuous clinical spectrum of a single entity. CAPS is caused by dominantly inherited or de novo NRLP3 mutations.

Objectives: The use of genetic methods for detecting somatic NLRP3 mosaicism in adult onset CAPS phenotype.

Patients & methods: A 53 year old male, Christian Arab from North Israel, suffered for 2 years from an unclassified multi-system inflammatory disorder. Its chief features were weight loss, fever, maculopapular skin eruption, arthritis, lymph node enlargement, hepatosplenomegaly, bone periosteal reaction, all associated with a major acute phase response. The arthritis was chronic, with disease flares lasting weeks to months. He responded moderately well to high dose corticosteroids but not to immunosuppressive agents including DMARDs, anti TNF agents, rituximab, nor tocilizumab. Subsequently, he developed a moto-sensory peripheral neuropathy of the legs and sensorineural deafness. There was no evidence of amyloidosis nor a relevant family history.

Results: No mutations in MEFV, nor for TRAPS and MVK were found.

A variant in NRLP3 Tyr 570 Cys (Y750C) was found in peripheral blood cells and buccal mucosa.

The patient was treated with anti-IL-1 drugs. Anakinra was poorly tolerated and then switched to canakinumab 300 mg / 4 weeks sc with complete remission of all symptoms except for the sensorineural deafness and peripheral neuropathy.

Conclusion: The detection of somatic mosaicism can have major clinical implications for patients including access to efficacious treatment duly recognized by regulators, and more availability of needed frequent monitoring. Taking into account the patient's excellent response to IL -1 blockade, it is reasonable to hypothesize that its earlier institution might have prevented the appearance of the severe complications of deafness and neuropathy.

P28

IL-1 inhibition in Muckle-Wells-Syndrome: withdrawal resulting in rapid deterioration of hearing loss

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Introduction: Muckle-Wells syndrome (MWS), a phenotype within the spectrum of cryopyrine-associated periodic syndrome (CAPS) is characterized by excessive IL-1 release resulting in chronic systemic and organ-specific inflammation including sensorineural hearing loss. During continuous anti-IL-1-therapy clinical symptoms are controlled and hearing loss remains stable. Limited data exists about discontinuation of IL-1-inhibition during the course of disease.
Objective: To report the case of a MWS patient, with sudden deterioration of hearing loss due to discontinuation of anti-IL-1-therapy and improvement of hearing after re-therapy.
Case report: A 29 year old female patient was diagnosed with MWS at age 17. Her left ear had been deaf since early childhood. The patient experienced arthralgia, exanthema, fatigue and progressive hearing loss of the right ear during childhood. Anti-IL-1-therapy with Anakinra was started 4 years after diagnosis. In 2007, she was switched to Canakinumab resulting in complete resolution of exanthema and arthralgia and improved fatigue. Hearing loss was stable during therapy as documented by frequent high frequency pure tone assessments (HFPTA).
When she became pregnant, MWS treatment was changed from Canakinumab to Anakinra as suggested by the safety profile. The patient gave birth to a healthy boy, who has a CAPS mutation.
The patient decided to discontinue IL-1-inhibition while breast-feeding. After four months off anti-IL-1-therapy, her hearing had markedly deteriorated: HFPTA demonstrated a decrease of 20-30 dB in frequencies most relevant for speech discrimination, a substantially impairment for a unilateral deaf patient. The patient decided to stop breast-feeding and Canakinumab therapy was immediately re-initiated. Two months later improved hearing was documented with 5-10 dB but still 10-25 dB less than before discontinuation.
Conclusion: Long-term IL-1-inhibition prevents decline of hearing ability in CAPS. Withdrawal of treatment may result in rapid and marked hearing loss. An early restart of anti-IL-1-therapy may partially reverse hearing loss. This indicates a window of opportunity for reversal of hearing loss by IL-1-inhibition. To our knowledge, this is the first case in which such a close connection between IL-1-inhibition and hearing ability in MWS has been documented. This case report shows the importance of continuation of anti-IL-1-therapy during pregnancy and breast-feeding to reduce the risk of sequel.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P29

Development of the autoinflammatory disease damage index (ADDI)

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Introduction: Autoinflammatory diseases cause systemic inflammation which can result in damage to multiple organs. Organ damage can occur before the start of therapy, or when patients experience ongoing inflammation. A validated instrument to measure damage is essential to quantify damage in individual patients, and to compare disease outcomes in clinical studies. At this moment, there is no such instrument. In the context of the RaDiCEA project, a common damage index for Familial Mediterranean Fever (FMF), Cryopyrin Associated Periodic Syndromes (CAPS), Tumor Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS) and Mevalonate Kinase Deficiency (MKD) will be developed.
Objectives: To develop an autoinflammatory disease damage index (ADDI).
Methods: The ADDI will be developed by consensus building based on the Delphi method. The top 40 enrollers of autoinflammatory disease patients in the Eurofever registry, were invited to take part in the expert group, as well as nine important experts from the Americas. A systematic literature search for damage in autoinflammatory diseases was performed to establish possible damage items. These items were rated by the experts in an online survey. Experts could also provide new damage items that were not found in the literature. Furthermore, in close collaboration with the Autoinflammatory Alliance, twenty-two patients and parents of patients were asked to rate damage items from the literature and to suggest other damage items. Based on the comments and suggestions of experts and patients, the damage items will be adapted; multiple rounds of online surveys will be performed to reach consensus. The final damage index will be determined in a face-to-face consensus meeting.
Results: Fifty-seven possible damage items from the literature were selected for the online survey. Forty of forty-nine experts completed the first round of the online survey. Based on the first round, five items were excluded and seventeen new items were suggested by experts. Twenty-one patients completed the online survey. Patients suggested nineteen new items, for example learning disabilities, chronic fatigue and speech development delay. The second round will be adapted based on these results.
Conclusion: An instrument to measure damage caused by autoinflammatory diseases (ADDI) will be developed based on the Delphi consensus procedure, including online surveys and a consensus meeting. Patients fulfil a significant role in this process.

P30

A novel mutation in *NLRC4* in a large pedigree with an anakinra responsive autoinflammatory disease

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Introduction: Autoinflammatory disorders (AID) are characterized by chronic or recurrent systemic inflammation associated with various clinical presentations. It is a genetically heterogeneous group of diseases. Recently, gain of function mutations in *NLRC4* have been described to be associated with autoinflammatory disease. Here, we report a novel NLRC4 mutation in a large pedigree with an anakinra responsive autoinflammaotry disease.

Objective: To identify the genetic defect causing an anakinra responsive autoinflammatory syndrome in a large pedigree.
Patients and methods: We performed whole exome sequencing in the members of a large 6 generation pedigree with an autoinflammatory disease, characterized by recurrent episodes of urticarial skin rash, joint pain and/or swelling, irritation of the eyes, and fatigue. Data with regards to the clinical phenotype were collected retrospectively from the medical charts. Functional studies in monocytes, and histology and immunohistochemical staining in skin biopsies obtained from lesional and uninvolved skin of three patients are currently being performed.
Results: No mutations were detected in the 20 autoinflammatory associated genes known at the time. Exome sequencing revealed a novel p.Ser445Pro variant in *NLRC4*.The p.Ser445Pro variant segregated with the 13 affected family members. Prediction software programs (Sift, Polyphen) indicate the variant has pathogenic properties. Moreover the variant is located next to the recently described p.His443Pro pathogenic mutation. In all affected family members, the clinical phenotype was influenced by weather conditions, stress, and infection. Severity of the clinical phenotype varied considerably. Moreover, in a subset of the patients, the clinical symptoms resolved promptly after anakinra treatment, indicating involvement of interleukin-1 while other other patients only partially responded to anakinra. Biopsies of lesional skin showed a neutrophilic infiltrate in the dermis. Functional studies in monocytes, and immunohistochemical staining in skin biopsies obtained from lesional and uninvolved skin of patients will provide more insight in the functional effects of the identified *NLRC4* variant.
Conclusions: In this study we identify and describe a novel variant in *NLRC4* in a large pedigree with an partially anakinra responsive autoinflammatory syndrome, and expanded the clinical phenotype associated with NLRC4-inflammasome mediated autoinflammatory disease.

P31

A large family having Muckle Wells Syndrome

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Introduction: CAPS is a rare autoinflammatory disease associated with mutations in the NLRP3 gene that result in overactivation of the inflammasome, increased secretion of IL-1beta and IL-18, and systemic inflammation. Mutations in the *NLRP3* gene on chromosome 1q44 causes cryopyrinopathies. These are autosomal dominant disorders with varying penetrance, which may also present *de novo*. MWS is an intermediate phenotype characterized by chronic or intermittent episodes of fever, headache, urticarial rash, arthralgias or arthritis, CNS involvement, ocular disorders and progressive deafness. Treatment is based on IL-1 antagonism, which usually results in prompt clinical response and may prevent amyloidosis.
Methods: Here we present a family whose 11 members have similar symptoms. Clinical data is collected during the course of ongoing patient care.
Results: We evaluated the clinical features of 11 patients who were referred to our center. The median age of the patients was 25 years (range: 9-65 years). The ratio of females /males was 1.2 (6/5). All patients had arthritis with exacerbation on exposure to cold and ocular involvement, mostly in the form of conjunctivitis and far less commonly uveitis, irideal synechiae, band keratopathy, cataract, and impaired vision. The median age of onset of arthritis was 7 years (2-30 y), the median age of onset of ocular involvement was 8 years (2-45 y). Hearing loss in 73.6% of patients was detected. The median age of onset of hearing loss was 15 years (12-63 y). All patient except one had urticarial rash. The median age of onset of urticarial rash was 8 years (7-30 y). Genetic testing for mutations of NLRP3 gene has not done yet.
Two patients were treated with canakunimab (Ilaris, Novartis, Switzerland) which is a human anti-IL-1 beta monoclonal antibody given by subcutaneous injections every 8 weeks (2mg/kg). Following canakunimab treatment, attacks of arthritis and urticaria are getting fully under control, advances in keratopathy and hearing loss could be partially controlled.
Discussion: MWS is characterized by recurrent fever and urticarial rash, progressive sensorineural deafness and the development of secondary amyloidosis. Treatment is based on IL-1 antagonism, which usually results in prompt clinical response and may prevent amyloidosis.

P32

French Amyloidosis CAPS study: AA Amyloidosis complicating cryopyrin-associated periodic syndrome: a study on 14 cases and review of 53 cases from literature

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Background: The cryopyrin-associated periodic syndrome (CAPS) is a rare but treatable inherited autoinflammatory condition including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurologic cutaneous articular syndrome (CINCA). Without treatment, some patients develop AA amyloidosis with consequent renal failure and death.
Objective: To describe the main features of CAPS-associated AA amyloidosis and the efficacy of interleukin-1 inhibitors in this complication.
Methods: We retrospectively analysed all current French CAPS-associated amyloidosis cases through the French network for rare diseases, and performed a systematic literature review of such cases published since 1950.
Results: Fourteen French patients were identified (6 women/8 men) including MWS (n=9), FCAS (n=3), CINCA (n=2) and having received interleukin-1 inhibitors in 7 cases. Mean age at the diagnosis of amyloidosis was 22.6 years and five (35.7 %) patients died. We found 53 patients in the literature, with a sex ratio of 1. They included MWS (n=34), FCAS/MWS (n=12) and FCAS (n=7). Among 67 patients (French and literature), the median age at amyloidosis diagnosis was 30 years, ranging from 12 to 61 years. The *NLRP3* gene was sequenced in 30 patients (45.5%), and the distribution of amino acids changes was as follows: R262W (n=16), T348M (n=5), A439V (n=4), D303N (n=3), T436N (n=2) and L353P (n=1). 23 patients had died (35%), but none of them had received interleukin-1 inhibitors. Since 2002, 24/67 (36%) patients with CAPS-associated amyloidosis have received interleukin-1 inhibitors, with at least a decrease of proteinuria and creatininemia in 9 of them (37.5%).
Discussion: AA amyloidosis can occur in all CAPS phenotypes, even if it was more frequent in MWS. This study underlines that even if FCAS is considered as a milder clinical phenotype compared to MWS or CINCA, it can also lead to amyloidosis. Thus, if FCAS patients display continuous subclinical inflammation, they should receive Interleukin-1 inhibitors as well, in order to prevent AA amyloidosis. Interleukin-1 inhibitors were introduced since a few years (anakinra and canakinumab), and it is still unclear if they can cure secondary amyloidosis. However, in 39% of cases they could allow a decrease of both proteinuria and creatininemia. In addition, in our experience and in the literature, anti IL1 treatments were able to prevent amyloidosis-related fatality.
Conclusion: AA amyloidosis can occur in all types of CAPS. IL-1 inhibitors prevent the occurrence of AA amyloidosis and should be started as soon as possible, even in FCAS patients in case of subclinical inflammation.

P33

Characterisation of human iPS cells harbouring the p.A329T variant of caspase-1

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Introduction: Naturally occurring genetic variants of the *CASP1* gene are associated with autoinflammation in patients suffering from recurrent febrile episodes and generalized inflammation. The resulting caspase-1 variants have reduced enzymatic activity due to destabilization of the caspase-1 tetramer. In addition, autoprocessing of the pro-caspase-1 variants is reduced and hence, CARD-CARD interactions with RIP2, enabling NFkB activation, is enhanced in a cell culture model.
Objectives: In order to characterize the effects of the p.A329T caspase-1 variant detected in a child suffering from severe autoinflammatory symptoms, we tried to establish a reliable cell model based on patient derived induced pluripotent stem cells (iPSCs).
Methods: We generated iPSCs by reprogramming fibroblasts of the patient's tympanic membrane (and wild type fibroblasts) using a replication deficient retrovirus (pRRL.PPT.SF.hOKSMco.idTomato.preFRT pMD.G (VSVG) psPAX2). We were able to verify the pluripotency of these iPSCs in different tests (quantitative real time PCR analysis of pluripotency markers, Immunocytochemistry of three germ layer markers and pluripotency markers). Subsequently the resulting iPSCs formed embryoid bodies (EB) which were then cultured in X-Vivo 15 media containing M-CSF and IL-3. After a few weeks, the adherent cells spreading from the settled EBs began to release monocytes (CD14+, CD45+, CD105+, CD192 +). These were harvested from the supernatant and differentiated for 7 days in the presence of M-CSF (without IL-3). Thereafter, they were detached, counted and plated as $1x10^5$ cells per 96-well. After allowing them to rest for one day, we primed the cells with ultrapure LPS and stimulated them with ATP or Nigericin. The supernatant was analyzed for cytokine concentrations of IL-1β, IL-6, IL-10 and TNFα using BD™ Cytometric Bead Array.
Results: The generated monocytes expressed the commonly established cell surface markers (CD14, CD16, CD45, CD163, and CD192) and could be differentiated into functional macrophages that seemed to react to stimulation as expected. Furthermore, first results indicated a different reaction of the cells generated from the patient (p.A329T) in comparison to the wild type control.
Conclusion: Human induced pluripotent stem cells are a useful option to gain monocytes and macrophages in order to study defects of the immune system without the necessity to repeatedly draw large amounts of blood.
Acknowledgements: This study was supported by German Network on Primary Immunodeficiency Diseases (pid.net).

P34

Detection of low frequency variants of the *NLRP3* gene in "mutation-negative" CAPS patients using massive parallel sequencing

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Introduction: Recent studies showed a notable frequency of somatic mosaicism of the *NLRP3* gene in chronic, infantile, neurological, cutaneous and articular (CINCA) syndrome (35%) and Muckle-Wells syndrome (MWS) (12.5%) patients that were not detectable by common Sanger sequencing.
Objectives: We are currently trying to detect and quantify low frequency *NLRP3* variants in mutation-negative patients, who suffer from a CINCA Syndrome, MWS or Familial Cold Autoinflammatory Syndrome (FCAS) or show cryopyrin-associated periodic syndrome (CAPS)-like symptoms without a classical phenotype.
Methods: The exons of the *NLRP3* gene were amplified via PCR from genomic PBMC DNA. The obtained PCR products were sequenced with an Illumina HiSeq platform. For SNP calling we used the GATK pipeline of the 1000 Genome Project, if the coverage attained 40,000 fold. In order to prove the accuracy of the method, we quantified dilutions of a known heterozygous mutation (T348M) mixed with wildtype DNA. For the correlation between the test results and the phenotype of the patients we developed a survey including symptoms and medical treatment.
Results: In one CINCA patient we detected a new *NLRP3* variant (L359S) in 30% of the sequence. Using a cut-off of 5% mutated DNA sequences, we did not detect any other mutation of the *NLRP3* gene in the other 47 samples we tested so far. We tried to increase the sensitivity by establishing a new statistic method (M+2SD), setting the cut-off at 0.5%. This led to a drastic reduction of specificity with irreproducible results.
Conclusion: Massive parallel sequencing is a reliable method for the quantification of low frequency variants of the *NLRP3* gene. Increasing the sensitivity (<5% cut-off) results in detecting PCR artifacts and a dramatic loss of specificity. The probability of somatic mosaicism in mutation-negative CAPS patients is higher if the symptoms are more severe. Although 35% of mutation-negative CINCA patients and 12.5% of mutation-negative MWS patients harbor somatic mutations, this seems to be extremely rare in patients with CAPS-like symptoms without classical CAPS phenotype.
Acknowledgements: This study was supported by Novartis Pharma GmbH, Nürnberg (ARW); BMBF Projekt NGSgoesHPC (ML) and SFB655 (DFG) (AD, AK).

P35

Diagnostics of CAPS in Quebec thanks to teaching program

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Introduction: We describe here the first 16 patients diagnosed with Cryopyrin-Associated Periodic Syndromes (CAPS) in Quebec for the past ten years. These disorders are unfortunately often left undiagnosed and untreated because of lack of knowledge.
Objectives: To inform the medical community on these diagnosis to avoid misdiagnoses and to reduce the time to reference to the appropriate specialist.
Methods: We gave lectures on an auto-inflammatory learning program dedicated to medical care practitioners from different specialities to educate them on these diagnosis. This program was given to pediatricians, adult rheumatologists, allergists, dermatologists, infectious disease specialists and internists.
Results: 16 patients were diagnosed with CAPS. The adult and pediatric rheumatologist who developped and gave the learning program diagnosed 11 cases, directly or referred by allergists, pediatricians and dermatologists. 4 adult rheumatologists diagnosed one or two cases of CAPS each. 8 diagnostics were made at pediatric ages, 8 at adult ages. 2 presented as chronic infantile neurological cutaneous and articular syndrome (CINCA), 9 as Muckle-Wells syndrome (MWS), and 5 as familial cold autoinflammatory syndrome (FCAS). Diagnosis was supported in 7 cases by genetic mutation of NLRP3 (44%). Delay in diagnosis was between 2 weeks and 50 years, mean 15,8 years.
Conclusion: Developping teaching tools permitted to educate physicians on these underdiagnosed diseases. This population of 16 CAPS represents the first cases diagnosed in Quebec thanks to interaction between specialities. Improvement can still be made to reduce time to diagnosis.

P36

Clinical and genetic features of Spanish patients with Mevalonate kinase deficiency

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Introduction: Mevalonate kinase deficiency (MKD) is a recessively-inherited autoinflammatory condition caused by *loss-of-functionMVK* mutations. This gene encodes for the enzyme mevalonate kinase (MVK), which catalyzes a crucial step of the biosynthetic pathway of cholesterol and isoprenoids. The partial deficiency of enzymatic activity causes the Hyper-IgD and periodic fever syndrome (HIDS), whereas it complete deficiency provokes the Mevalonic Aciduria (MA).
Objectives: The aim of this study was to describe the clinical and genetic features of Spanish patients with MKD diagnosed during the past 15 years.
Methods: The patients' data as well as the outcome of the administered treatments were collected from charts reviews. *MVK* analysis was performed by Sanger-based sequencing.
Results: Forty-one patients from different Spanish hospitals were included. Thirty-eight patients (92.7%) suffered from HIDS and three patients (7.3%) from MA. The MKD diagnosis was established in all of them by the detection of biallelic *MVK* mutations. Eighteen different *MVK* mutations were detected, with the p.[(V377I)] and p.[(I268T)] mutations as the most prevalent, accounting for 54.9% and 26.8% of mutated alleles, respectively. The majority of these mutations (96.4%) were missense mutations. The remainder mutations included premature stop (1.2%), frameshift (1.2%), and splice site mutations (1.2%). In the group of patients with HIDS (n: 38), twelve patients (31.6%) carried homozygous genotypes and twenty-six patients (68.4%) compound heterozygous genotypes. In the group of HIDS patients with homozygous genotypes (n= 12), ten patients (83.3%) carried the p.[(V377I)];[(V377I)] genotype. By contrast, in the group of patients with MA only one patient (33.3%) carried a homozygous genotype (the p.[(I268T)];[(I268T)]).
From a clinical point of view, the median age at the disease onset was 6 months (range 0-408), and the median duration of flares was 4.8 days (range 2-17.5). Mandatory vaccinations were identified as triggering factors for acute episodes in eleven patients (26.8%). The most prevalent manifestations during inflammatory episodes were fever (80.5%), lymphadenopathies (70.7%), abdominal pain (63.4%), diarrhea (58.5%), aphthous ulcers (53.7%) and arthralgia (51.2%). AA amyloidosis was only detected in one patient (2.4%), but had a severe course.
Conclusion: We herein provide a detailed description of the clinical and genetic features of a Spanish cohort of MKD patients carrying biallelic *MVK* mutations. Most of patients suffered from the mild MKD phenotype, the HIDS syndrome. Two prevalent *MVK* mutations, p.[(V377I)] and p.[(I268T)], were found in our cohort, and fever and lymphadenopathies were the most common features in enrolled patients.

P37

Late onset of the cryopyrin-associated periodic syndrome (CAPS) associated with low level of somatic mosaicism in six patients

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Introduction: CAPS is caused by mutations in the *NLRP3* gene and is inherited in an autosomal dominant fashion. About 40% of children with CINCA are mutation negative by conventional Sanger sequencing, but *NLRP3* somatic mosaicism can be identified by sensitive multi-parallel sequencing (MPS) in a significant proportion of such patients.
Objectives: To analyse the *NLRP3* gene in six patients with typical CAPS other than onset in mid-late adult life. All patients responded to IL-1 blockade and none had a family history.
Methods: DNA was extracted from whole blood, saliva, buccal epithelial cells and from isolated monocytes, T and B lymphocytes and neutrophils. *NLRP3* gene was analysed using Sanger sequencing and MPS.
Results: MPS detected a variable degree of somatic *NLRP3* mosaicism in all patients: two carried previously described variants p.E567K and p.A352T in 5.4% and 14.6% of alleles respectively; four had novel mutations: p.G569V, p.G564D and p.Y563C (found in two unrelated patients) in 21.1%, 5%, 5.1% and 11.1% of alleles respectively. Analysis of purified B and T lymphocytes, neutrophils and monocytes revealed a greater proportion of mutant cells among myeloid lineage; only a small fraction of T lymphocytes and buccal cells carried the *NLRP3* mutation. In a single adult patient who was heterozygous for germline *NLRP3* substitutions p.A439V and p.S434S, the mutation was present in all lymphoid and myeloid cells. We re-analysed the *NLRP3* gene in one subject who had been healthy until age 45, but had had relentlessly worsening CAPS and steadily increasing IL-1 inhibitor requirement, using a fresh sample obtained nine years after her initial assessment; this demonstrated an increase in the frequency of the mutant allele from 5.4% to 28.6% in DNA isolated from whole blood.
Conclusion: These studies identified post-zygotic mutational events as the aetiology of late onset CAPS. All patients had excellent response to IL-1 blockade, including stabilisation of the amyloid load in the two subjects diagnosed with AA amyloidosis. AA amyloidosis is a severe complication of CAPS and hitherto has only been reported in patients with germline *NLRP3* mutations. Interestingly, the population of *NLRP3* mutant granulocytes and monocytes increased substantially in the single patient in whom a time course study was possible, by definition representing clonal expansion. Whilst further studies at the bone marrow level are planned, the current findings suggest that acquired *NLRP3* mutations may confer affected cells with a selective advantage.

P38

Clinical symptoms and molecular investigations in 13 patients with Schnitzler syndrome identified at the single UK centre

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Introduction: Schnitzler syndrome (SchS) was first described in 1972 and to date 281 cases have been reported. SchS is an adult onset, apparently acquired disease, which clinically closely resembles CAPS including the response to IL-1 blockade. A hallmark of the disease is the IgM kappa paraprotein, identified in 85% of the patients; recently variant IgG have been reported in 7% of cases.
Objective: To characterise clinical symptoms in the 13 patients with SchS identified at the single UK centre. Recently, mosaicism in the *NLRP3* gene was described in two cases with SchS, which prompted us to search for mosaic variants in our cohort.
Methods: 13 patients underwent detailed clinical investigations and analysis of the *NLRP3* and *NLRP12* genes by Sanger and multiparallel sequencing (MPS). In addition *MYD88* gene was sequenced in the DNA extracted from whole blood.
Results: The median age at disease onset was 55 years (range 35-78). All patients presented with uricarial rash, other manifestations included fever (77%), arthralgia (69%), weight loss (46%), fatigue (38%), bone pain (38%) and lymphadenopathy (23%). One patient was diagnosed with AA amyloidosis. In all subjects low grade IgM kappa paraprotein had been detected.
Genetic testing revealed two patients had V198M and F402L variants in the *NLRP3* and *NLRP12* genes respectively. No additional nucleotide alternations, including somatic mosaicism, in the *NLRP3* exons: 3, 4 and 6 have been identified by MPS. In addition no mutation was found in *MYD88* gene by PCR/Sanger sequencing.
Conclusion: Despite of the recent report of *NLRP3* somatic mosaicism in two cases, in the current study, except for the two variants of unknown significance: V198M and F402L identified in the *NLRP3* and *NLRP12* genes respectively, no other genetic alternation was found by either Sanger or MPL sequencing in the 13 cases with SchS. We failed to identify variation in the *MYD88* gene, looking specifically for the L265P variant, which is a known risk factor for the development of Waldenstorm macroglobulinemia. The

limitation of this study is that the analysis was performed on the DNA isolated from peripheral blood rather than the bone marrow (BM) and we plan to repeat this experiment on the BM samples.

P39

Characterization of the TNFR1-d2 protein: Implication in TNF receptor associated periodic syndrome (TRAPS)?

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Introduction: Binding of TNF to TNF receptor 1 (TNFR1) induces both the survival pathway by activation of the NF-kB transcription factor, and the death pathway by apoptosis. Mutations in the TNFR1 gene (*TNFSFR1A*) are responsible for the auto-inflammatory disease TRAPS, a dominantly inherited hereditary recurrent fever. Various defects such as defective TNFR1 receptor shedding, protein misfolding, NF-kB activation, or apoptosis have been associated with the pathogenesis of TRAPS.
Previously, we have identified TNFR1-d2, an exon2-spliced transcript of *TNFRSF1A*. TNFR1-d2 is expressed in a tissue-specific manner in contrast to ubiquitous expression of the full-length TNFR1 transcript.
Objectives: This study aimed to analyze the TNFR1-d2 protein expression and its function in NF-kB signalling pathways and to investigate the possible role of TNFR1-d2 in TRAPS physiopathology.
Materials and methods: Translation analyses of TNFR1-d2 were performed in HEK293T by over-expression of different TNFR1-d2 cDNA constructs fused to the Flag tag. HEK293T transfected cells were used to measure internal ribosome entry site (IRES) activity and NF-κB-activation by luciferase assays. Subcellular localization of the TNFR1-d2 fused to the GFP protein was studied in MCF7 cells, followed by staining of different cellular compartments and confocal fluorescence microscopy analysis.
Results: We showed that TNFR1-d2 is translated from an alternative start codon due to an IRES activity created by the exons 1 and 3 junction. The methionine 109 located in exon 4 in-frame with TNFR1 was used, resulting in a putative new protein isoform lacking its N-terminal region. Subcellular localization showed that the full-length and TNFR1-d2 proteins shared the same intracellular localization to the Golgi complex. Since the c.224C>T (p.Pro75Leu, P46L) and c.236C>T (p.Thr79Met, T50M) mutations in exon 3 lie in close vicinity to a strong Kozak consensus sequence, we hypothesized that these 2 sequence variants could affect TNFR1-d2 translation. Interestingly, we found that only TNFR1-d2 carrying the severe T50M mutation was translated through the mutated codon which induced a decrease of the IRES activity. Moreover, whereas overexpression of wild type TNFR1-d2 was not associated with increased NF-κB transcriptional activity, TNFR1-d2-T50M seemed to increase NF-κB activity as compared to the empty vector.
Conclusion: Our results support that the TNFR1-d2-T50M translation defect could lead to a gain-of-function of TNFR1-d2, suggesting that TNFR1-d2 may account for the physiopathology of TRAPS in patients carrying the T50M mutation, which is associated with a severe TRAPS phenotype.

P42

The clinical phenotype of Israeli patients with Q703K mutation in the NLRP3 gene

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Background: Cryopyrin associated periodic syndromes (CAPS) comprise a spectrum of autoinflammatory disorders of varying severity caused by mutations in the NLRP3 gene. The Q703K allele, reaching 5% of the total allele count in the general population, is considered either functional polymorphism or a low penetrance mutation.
Aim: To describe the clinical phenotype of the Israeli patients in whom the Q703K allele was found.
Methods: Ten patients carrying the Q703K mutation were identified among 70 patients in whom the diagnosis of CAPS was suspected on clinical grounds.
Results: Seven female and 3 male patients with a mean age of 22.5±17.8 years and a mean diagnosis delay of 12.4 years were identified. Their clinical characteristics ranged from self resolving attacks of fever, urticaria and arthralgia to a chronic, debilitating steroid-dependent inflammatory disease. Splenomegaly, transfusion-dependent anemia, sensory neuropathy and pericarditis, manifestations which are not included in the traditional CAPS-spectrum, were detected in some of the patients. The majority of patients responded to high dose steroid therapy. DMARDS such as methotrexate, azathioprine and colchicine were generally ineffective at reducing steroid dose or attack rate. Therapy with TNF inhibitors or anti IL-1 agents was instituted in 4 patients with a favorable response. All but one patient needed chronic anti-inflammatory therapy to prevent attacks and reduce steroid dose.
Conclusions: Our cohort of patients with the Q703K mutation, the largest reported to date, show a heterogeneous inflammatory phenotype, in which a CAPS component may be appreciated. Both IL-1 and TNF inhibitors seem to be effective in treating steroid resistant manifestations of this subgroup of CAPS patients.

P43

Atypical clinical presentation of a severe Tumor Necrosis Factor Receptor-associated Periodic Syndrome (TRAPS) without mutation in the TNFRSF1A gene and good response to anakinra. Case report of a ten year old girl with fever, skin edema and abdominal pain (AID-registry)

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Pediatric Rheumatology 2015, **13(Suppl 1):**P43

Background: Tumor Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS) is a hereditary autoinflammatory syndrome characterized by reccurrent episodes of fever and localized inflammation. It is characterized by recurrent fever accompanied by abdominal pain, pleuritis, migratory skin rashes, fasciitis, headache, conjunctivitis and periorbital edema.
Case report: We report about a 10 year old girl from Romania who suffered from fever, skin edema and abdominal pain. She was no longer able to walk because of pain and took NSAIDs and tramadol. The right arm was swollen. In the short past she suffered from pneumonia and took cefuroxime oral. Five years ago tuberculosis was diagnosed and completely healed up by a triple therapy for 6 months. A few months ago periorbital edema were described in Romania. She spent 2 months in hospital. Infectiological, oncological and other causes for fever were excluded and no diagnosis was found but prednisolone improved edema and fever. Blood test showed leukocytosis, thrombocytosis and high elevated CrP and SAA. S 100 A12 Protein was normal. Genetic analysis for hereditary recurrent fever syndromes (HRFs) showed no mutations in the four commonest genes. There is a negative family history for periodic fever syndromes or rheumatic diseases. After reduction of prednisolone symptoms immediately recurred. Etanercept was unsuccessful. After literature research [1] we diagnosed TRAPS without mutation and started a therapy with anakinra (IL-1 inhibitor). The response was prompt and dramatic. Now the patient gets anakinra daily s.c. and she had no longer fever or edema. Because of problems with the health insurance and because of the off-label indication for TRAPS she had to interrupt the medication for a few days and the symptoms came back again. Now she takes anakinra since 3 months.
Conclusions: TRAPS should be considered in cases like this without mutation. Anakinra is a good therapeutic option; actually the use is off-label.
Acknowledgements: The AID-Registry is funded by the BMBF since 2009 (01GM08104, 01GM1112D).

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

Reference

1. *Dermatol Online J* 2013, **19(11)**:20405.

P44

A 56 year old woman with clinically significant p.Arg121Gln-/R92Q TNFRSF1A mutation

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Background: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is a rare autoinflammatory disorder caused by TNFRSF1A mutations. The symptoms of TRAPS are various and often unspecific. The R92Q variant is considered to be a low penetration mutation with mild phenotype.
Objective: To analyze the clinical course of a woman carrying the R92Q Mutation and presenting with rheumatic disease and some symptoms consistent with autoinflammatory syndrome.
Methods: A woman was identified as carrying the p.Arg121Gln-/R92Q mutation in the TNFRSF1A gene and presenting with autoinflammatory symptoms.
Results: The patient was hospitalized 3 times with abdominal pain elevated CRP and fever. She was presumed to have recurrent abdominal infections and treated with antibiotics. The laboratory tests showed no increase on leukocytes counts but elevated CRP (to198mg/l), which normalized after a few days. CT-scans and 2 ultrasounds were performed, which showed signs of panniculitis of the abdominal fat, but no other pathology. Even a diagnostic laparoscopy was performed with no further results. The patient had a history of recurrent fever (39°C), arthralgia, myalgia, abdominal pain, diarrhoe, backpain, hyperkeratotic skin lesions and constantly elevated ESR and CRP. Interestingly, ANA and dsDNS antibodies had been showed to be elevated over many years. The patient was first diagnosed an overlap connective tissue disease/undifferentiated SpA. Later on, periodic fever syndrome was suspected and the genetic tests performed. The genetic analysis showed the presence of p.Arg121Gln-/R92Q mutation. When treated with methorexate and etanercept the patient showed improvement of most symptoms including arthralgia, back pain and skin disease. ANA and dsDNS antibodies turned negative but CRP and ESR remained elevated. The fever episodes were persisting so that etanercept was stopped and the treatment with IL-1 antagonist startet. Under the treatment with anakinra 100 mg every second day, we observed an improvement of fever and abdominal symptoms although no remission. The skin lesions, the arthralgia and back pain returned. Anakinra dosis was enhanced to daily applications of 100 mg with further improvement of the autoinflammatory symptoms. Back pain, arthralgia and skin lesions persist.
Conclusion: Our findings suggest that the R92Q mutation is influencing the phenotype of the rheumatic disease in this patient and seams to be responsible for part of the presenting symptoms.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

Reference

1. Lachmann HJ, *et al*: **The phenotype of TNF receptor-assotiated autoinflammatory syndrome (TRAPS) at presentation: a series of 158 cases from the Eurofever/EUROTRAPS international registry.** Paediatric Rheumatology International Trials Organisation (PRINTO), the EUROTRAPS and the Eurofever Projekt.

P45

Whole Exome Sequencing reveals a NLRP3 mutation in exon 5 in a patient with CINCA

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Introduction: Cryopyrin-associated periodic Syndromes (CAPS) are caused by heterozygous mutations in the NLRP3 gene. More than 80 disease causing mutations have been identified, mostly clustered in NLRP3 exon 3, but also described in exons 4 and 6. However, up to 50% of clinically diagnosed CAPS patients (with identical clinical features and response to anti-IL-1 treatment) show no mutation in NLRP3 detected by conventional DNA sequencing analysis of exons 3, 4 and 6. Recently somatic NLRP3 mosaicism has been shown to account for up to 70% of these mutation negative CAPS patients.
Objective: To ascertain a genetic cause in a patient with a CINCA phenotype labeled as NLRP3 mutation negative.
Methods: Massively Parallel Sequencing (MPS) and Whole Exome Sequencing (WES) were performed on DNA extracted from peripheral blood. WES results were confirmed by conventional Sanger sequencing.
Case report: Twenty month old boy who presented with an urticarial-like rash since birth. At 6 months of age he was noted to have frontal bossing with increasing head circumference, hepatosplenomegaly, and papilledema with no signs of uveitis. A mild conductive hearing impairment was detected, but no sensorineural hearing loss. Inflammatory markers were raised with a CRP of 40mg/L.
Brain MRI showed signs of hydrocephalus and a ventriculo-peritoneal shun was inserted.
A clinical diagnosis of CAPS was made at this point. Sanger sequencing of NLRP3 exons 3, 4 and 6 was consistent with wild-type.
Over the following months his clinical status deteriorated with slowing development and significant failure to thrive with growth below the 0.4th centile. Inflammatory marks were persistently raised.
Anakinra treatment was started at 12 months of age, resulting in a marked clinical and serological improvement, with normalization of CAPS disease activity score and inflammatory response at a dose of 3mg/kg/day.
MPS ofNLRP3 exons 3, 4 and 6 didn't reveal any somatic mutations. WES was then performed for the proband and his parents revealing a potentially damaging heterozygous variant in NLRP3 exon 5 (c.G2336T; p.G779V), which segregated with disease. Sanger sequencing of exon 5 confirmed these findings.
Conclusion: To the best of our knowledge this is the first potentially disease associated mutation in NLRP3 exon 5. Although we have not yet performed functional studies, in silico prediction is consistent with a damaging role.
Our work shows that other NLRP3 exons apart from 3, 4 and 6 should be screened for germline and/or somatic mutations in patients with a clinical diagnosis of CAPS.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P46

Impact of hereditary periodic fever syndromes and its change over time

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Introduction: Hereditary periodic fever syndromes (HPF) with their clinical inflammation and associated symptoms impair many aspects of affected children's and adolescents' lives. Patients experience fever, pain and fatigue; they are restricted in their overall-wellbeing, functioning and participation. Little is known about the extent of the perceived burden of illness and its change over time.
Objectives: To study the perceived burden and treatment of the HPF FMF, TRAPS and CAPS over 10 years.
Patients and methods: Data from patients with genetically proven FMF, TRAPS and CAPS, who were recorded in the National Paediatric Rheumatologic Database (NPRD) in the years from 2004 to 2013 were used for this analysis. Temporal changes in patient-reported outcomes (e.g.,

overall wellbeing [NRS 0-10], pain [NRS 0-10], functional capacity [CHAQ], school attendance) and anti-inflammatory medication were evaluated.
Results: Altogether, 819 cases with HPF were recorded between 2004 and 2013: 703 with FMF, 47 with TRAPS and 69 with CAPS. The number of HPF patients recorded per year increased from 98 in 2004 to 346 in 2013. Treatment of HPF patients changed over time. While in 2004 5% of HPF patients were on biologics, this applied to 13% 10 years later. In 2013, 2% of FMF, 22% of TRAPS and 72% of CAPS patients were treated with IL-1 blockers and 3% of FMF, 56% of TRAPS, and 6% of CAPS patients with TNF-blockers. On these drugs, 51% of all patients had an active disease (physician global >0) at documentation in 2013 in comparison to 48% in 2004.
Considering the whole HPF group, patients perceived health did not change over time. In 2013, 42% of patients had restrictions in overall wellbeing (NRS>0) in comparison to 38% in 2004. However, CAPS patients reported better health in 2013, with lower mean values for overall wellbeing (1.35), pain (1.6) and functional limitations (CHAQ 0.32) than in 2004 (mean values 3.25, 1.9 and 0.47, respectively). CAPS and TRAPS patients as well reported a higher burden of illness in comparison to FMF patients still in 2013. One in four patients of the whole HPF group had missed school or kindergarten within the 4 weeks prior to documentation, without change over time. However, the average number of days missed at school decreased from 10.2 in 2004 to 5.1 in 2013.
Conclusion: HPF place a significant burden on the affected individuals. Measures beyond drug treatment seem necessary to ensure that patients can live a full life.
Acknowledgements: Supported by a grant from the German Child Arthritis Foundation (Deutsche Kinderrheuma Stiftung).

P47

NLRP3 Q703K and TNFRSF1A R92Q mutations in a patient with auto inflammatory disease

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Background: Cryopyrin-associated periodic syndrome (CAPS) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS) are rare autoinflammatory disorders caused by NLRP3 and TNFRSF1A mutations. The Q703K (CAPS) and the R92Q (TRAPS) variants however, are considered to be low-penetrance variants with little or no clinical significance [1,2].
Objective: To describe the clinical presentation and disease course in a young man with the Q703K and the R92Q mutations.
Methods: Thorough clinical and laboratory examination and genetic testing were carried out in a patient presenting with autoinflammatory symptoms.
Results: At the age of 2 the patient was hospitalized for recurrent fever (39 - 41°C) during several months. Recurrent infections were suspected, although presence of bacteria or virus could not be confirmed. Clinical examination revealed multiple lymph nodes and enlarged tonsils. At the age of 4, the boy was hospitalized because of chronic abdominal pain. At the age of 5, neurological development became abnormal and psychomotor development deficiency was diagnosed. By the age of 8 symptoms included ataxia, abdominal pain, headache, fatigue, arthralgia and myalgia. Neurological symptoms were rapidly progressive. Within few months the patient was unable to walk. In addition, pyoderma grangrenosum-like skin lesions appeared. After several months, slow improvement was noticed. He was 15 years old when he presented with an episode of periorbital edema and fever. At the age of 18 recurrent fever, headache and abdominal pain persisted. The laboratory tests showed elevated CRP and IgD, but no signs of infection. Periodic fever syndrome was suspected and genetic analysis confirmed the presence of a Q703K and R92Q Mutation.
Conclusion: The course of disease in this patient suggests, that the presence of a Q703K and R92Q mutation, although each known as low-penetration variants, may be clinically significant and consistent with an autoinflammatory syndrome.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References
1. Vitale A, *et al*: *Clin Exp Rheumatol* 2012, **30(6)**:943-6.
2. Lachmann HJ, *et al*: *Ann Rheum Dis* 2014, **73(12)**:2160-7.

P48

Mevalonate kinase somatic mosaicism and bigenic genotypes may explain heterogeneity in mevalonate kinase deficiency

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Question: The diagnosis of mevalonate kinase deficiency is often delayed, due to the rarity and phenotypic heterogeneity of the disease. We evaluated the autoinflammatory genetic makeup of 4 referrals suspected to have MKD.
Methods: Exons 2-11 of the mevalonate kinase gene, exon 10 of MEFV, exons 2-4 of TNFRSF1A and exon 3 of NLRP3 were Sanger sequenced in referrals with an autoinflammaory disease. Targeted massive parallel sequencing by the PGM Ion Torrent platform was performed on one peripheral blood DNA sample.
Results: Case 1 A pediatric case with splenomegaly, cervical lymphoadenopathy, failure to thrive and anemia, was found to have two pathogenic MVK variants, p.V250I and p.L315G*51, and the Q705K variant on the NLRP3 gene, considered a functional polymorphism. Typical symptoms and a high level of urinal mevalonic acid allowed closure on MKD diagnosis this case.
Case 2 A pediatric case with recurrent and vaccination triggered attacks of high fever, sore throat, cervical lymphadenopathy and abdominal pain since infancy was a carrier of the MEFV p.V726A variant. The patient was diagnosed with PFAPA and treated with steroids. In the last, 10 days long attack the patient developed arthritis, a maculopapular rash and high blood pressure. MKD genetic testing revealed two pathogenic MVK variants, p.V377I and p.G202R, confirming the diagnosis of MKD.
Case 3 A patient with adult onset Still's disease symptoms including fever attacks, arthralgia, urticaria, pericarditis, and partial response to NSAIDs was shown to have a rare MVK variant, p.R121W, and the NLRP3 p.Q705K functional polymorphism. The diagnosis is yet unresolved.
Case 4 An adult patient developed high fever and abdominal pain lasting 2-3 and HLA-B51 positive aphthous stomatitis. We identified somatic mosaicism (22%) for a novel, p.Ala161Thr MVK variant. The patient's attacks resolved with anti TNF treatment and the current diagnosis is Behcet's disease.
Conclusion: Four referrals suspected to have MKD had in common autoinflammatory variants in two autoinflammatory genes. The MVK genotypes of two pediatric patients met the recessive mode of MKD inheritance. By contrast, two late onset patients had somatic/heterozygous MVK variants of unknown clinical significance but may be placed at the milder clinical range of MKD**.**
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P49

Looking back at the diagnosis of PFAPA: a retrospecitve analysis of a prospective cohort study

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Pediatric Rheumatology 2015, **13(Suppl 1):**P49

Introduction: Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) is the most common periodic fever syndrome of childhood. The original diagnostic criteria were introduced in 1989. However, published studies show large heterogeneity in PFAPA patients, promting attempts at refining the criteria.

Objectives: To describe the clinical and laboratory findings in a large, prospective cohort of patients with PFAPA, to discuss the criteria used for diagnosis, and to revisit their original diagnosis.
Methods: Children diagnosed with PFAPA were prospectively recruited from a large, tertiary hospital in Boston. Diagnosis was made by pediatric rheumatologists, otolaryngologists, or infectious diseases specialists with expertise in PFAPA. Clinical history was gathered at diagnosis, and laboratory testing was performed during flares. Years after diagnosis, we performed a retrospective chart review and reconsidered the original diagnosis of PFAPA.
Results: 76 patients were recruited; 70% were male. The ethnicity of the group was mixed, usually with more than one ancestry: 61% were Irish, 36% Italian, 26% English, 25% German, 22% Scottish, 20% French, 18% French Canadian, 11% Polish, 11% Portuguese, 9% Swedish, 7% Native American.
The average disease onset was 3 years of age. The frequency of cardinal features was as follows: 71% had pharyngitis, 67% had adenitis, 33% had aphthous stomatitis. 16% of childen had three cardinal features during flares, 50% had two features, 22% had one feature, and 12% had no features. In 66/68 (98%) patients, fevers occured at regular intervals. 14% of patients had disease onset at 5 years of age or older.
Prednisone was used at the onset of symptoms in 44 patients; 91% had complete response, 4% had incomplete response, and 1% did not respond.
In 46/48 (96%) of patients, inflammatory markers were elevated during flares. 26/44 (59%) had leukocytosis. 5/41 (12%) had lymphopenia,10/34 (29%) had eosinopenia, 17/37 (46%) had monocytosis.
10 patients had genetic testing for periodic fevers; 7 were negative, and one patient each had the following mutations: E148Q in MEFV, R92Q in TNFRSF1A, and PSTPIP1.
A retrospective chart review of these patients questioned the diagnosis of PFAPA in 27 (36%) patients due to lack of clear documentation of regular episodes of fevers (9), lack of any cardinal features (8), poor response to prednisone (1), follow-up notes revealed alternative diagnoses (eg hypogammaglobulinemia), or because the story did not seem consistent with PFAPA.
Conclusions: Our study showed that physicians that frequently diagnose PFAPA do not use a common criteria for their patients. Only 16% of patients had the three cardinal features of PFAPA, while 12% had none. Leukocytosis was common during flares. Monocytosis, noted in prior studies, was seen in almost half of our patients.
However, almost all had evidence of systemic inflammation and showed resolution of the episodes with administration of prednisone. These features were not part of the original diagnostic criteria, but they may be useful for diagnosis.

P51

Targeted NGS based hereditary autoinflammatory disorder screening in routine diagnostics, two year experience in the Netherlands

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Introduction: Hereditary autoinflammatory diseases (AID) are characterized by recurrent bouts of systemic inflammation caused by dysregulation of the innate immunity system. The genotype-phenotype correlation can be highly variable which makes a genetic diagnosis in AID patients complex and laborious. A clear and definitive diagnosis cannot be provided for up to 80% of AID patients, which can be important for treatment options. To date, over 20 causal genes have been identified for monogenic AIDs.
Patients and methods: We have developed a diagnostic method to facilitate genetic evaluation of the 23 known AID related genes at once using Next Generation Sequencing (NGS). We performed targeted *in-solution* enrichment followed by sequencing using the SOLID 5500 platform. An *in-house* developed bioinformatic pipeline was used to detect DNA variants in the selected AID genes. Cartagenia Bench lab NGS was used for filtering and classifying detected variants. Variants were confirmed by Sanger sequencing. Since May 2013, 120 patients have been tested with NGS.
Results: We identified a genetic diagnosis in 17 patients (14%). Mutations were detected in classic autoinflammatory genes like *MVK, NLRP3* and *TNFRSF1A.* Moreover, we detected mutations in recently discovered genes, like *CECR1*, thereby demonstrating that regular updating of the targeted gene panel is warranted. Interestingly, we detected mosaicism for a pathogenic *NLRP3* mutation which had been missed by Sanger sequencing. Moreover in a patient with the phenotype of PAPA-syndrome, without a mutation in the PSTPIP1-gene, pathogenic compound heterozygous mutations in the MVK-gene were detected with NGS. This further supports the application of NGS based testing in these patients. In 20 additional patients (17%) only one disease causing allele was detected in genes involved in autosomal recessive disorders or the genetic results need further testing because the detected variants were of unknown clinical significance. The NGS method has been mainly applied to autoinflammatory patients other than FMF patients. In these patients diagnostic yield improved from 4% using Sanger sequencing single genes to 14% using the NGS based testing.
Conclusions: Our results indicate that this NGS-based approach is an efficient strategy for rapid mutation detection in AID. Moreover, the increased diagnostic yield will give more insight in the genotype phenotype relationship for the different AID disorders enabling earlier diagnosis and better treatment.

P52

Single center experience in Next Generation Sequencing for genetic diagnosis of Autoinflammatory Disorders (AIDs)

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Pediatric Rheumatology 2015, **13(Suppl 1):**P52

Introduction: Autoinflammatory disorders (AIDs) represent an expanding group of complex diseases characterized by periodic or chronic systemic inflammations. Mutations in more than 15 geneshave been associated with autoinflammatory recessive or dominant syndromes. Next Generation Sequencing (NGS) has emerged in the last year as new diagnostic tool in this field.
Objectives: To share data obtained by the use of NGS in a cohort of patients affected by an autoinflammatory disease of undefined origin evaluated at our center.
Materials and methods: In this study we enrolled 158 patients from 2010 to 2014. We developed NGS starting with 11 genes already known to be involved in AID (Panel 1: MVK, MEFV, NRLP12, NRLP3, NOD2, TNFRSF1A and PSTPIP1 and Panel 2: IL1RN, LPIN2, IL36RN, PSMB8). Targeted resequencing was performed using customized panel and analyzed with the MiSeq® sequencing platform (Illumina, San Diego, CA). All variants identified have been confirmed by Sanger sequencing.
Results: 48,(73%) of the patients present variants in genes of Panel 1. 32,46% have variants in NLRP3 gene: the most frequent variants are Q705K (56%) and V200M (32%). About 26% have variants in NOD2, the most frequent variant is R702W (25%). 30% have variants in NLRP12, the most frequent variant is F402L (65%), in two cases in homozygosity. 23,4% have variants in MEFV, the most frequent variant is E148Q (22%). 4% have variants in MVK, V377I (100%). 10,38% have variants in TNFRSF1A, the most frequent variant is R121Q (75%). 9% have variants in PSTPIP1. 69% of the patients present variant in only one gene; 28,57% present variants in two different genes and two patients in three genes. We performed 15 familial study to unravel the segregation of some variants.
Conclusion: NGS leads to the identification of many genetic variants that could be associated with disease susceptibility. The major challenge is in the interpretation of the clinical relevance of identified variants. Some patients show variants in multiple analyzed genes: it can be assumed that different variants in different genes may cooperate to determine a pathological phenotype. This will necessitate large-scale population studies, in vitro functional assay and careful correlation of genetic information with phenotypic data. Therefore, close collaboration with clinicians is crucial.

P53

Chronic recurrent multifocal osteomyelitis (CRMO): typical patterns of bone involvement on MRI with particular emphasis on Whole Body MRI (WBMRI)

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Introduction: Chronic recurrent multifocal osteomyelitis (CRMO) is an autoinflammatory bone disorder of unknown ethiology. The clinical manifestations of CRMO are highly variable. One to 20 sites can be affected at one time. Since CNO is a systemic disorder that can affect multiple skeletal sites, whole-body imaging techniques (Tc-99 bone scintigraphy or MRI) provide major contribution to the initial diagnostic approach, as well as during follow-up.
Objectives: To evaluate typical patterns of bone involvement on MRI in paediatric patients with CRMO.
Materials and methods: We retrospectively reviewed 112 MRI performed at the diagnosis and during follow-up from 2010 to 2014 of 40 children with CRMO. Thirty-two patients underwent bone biopsy that confirmed the diagnosis. 28/40 (70%) underwent one or more WBMRI. Coronal STIR images were obtained in all children. Additional sequences were performed in doubtful cases.
Results: A total of 360 lesions were detected. Lesions were multifocal in 36/40 patients (90%) and were symmetric in at least one localization in 24/40 patients (60%). In 30/40 patients (75%) lesions were located on the metaphyses of long bones (especially femur and tibia) close to one or both sides of an epiphyseal or apophyseal growth plate and/or on pelvic bones (particularly on the sacro-iliac joints and close to the triradiate cartilage) and/or on clavicle/sternum. The spine was involved in 14/40 (34.1%) patients, in all but 2 in combination to the submentioned locations. No patients had carpal or head bone involvement, 16/40 had tarsal involvement.
Conclusion: MRI, and particularly WBMRI, should be considered the diagnostic modality of choice in patients with clinical multifocal pain. Simmetricity, multifocality and particularly specific patterns of lesions appear highly suggestive of CRMO.

P54

A case of systemic juvenile idiopathic arthritis with pulmonary hemosiderosis secondary to recurrent macrophage activation syndrome or a new autoinflammatory syndrome?

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Introduction: Macrophage activation syndrome (MAS), a severe complication of systemic juvenile idiopathic arthritis (JIA) and other inflammatory diseases, represents a one of the most important rheumatological emergencies. Delayed diagnosis could lead to life-threatening complications. Pulmonary hemosiderosis (PH), possible seen at all ages but most commonly among children, usually appears as an idiopathic PH.
Objective: In this case report of a child with systemic JIA diagnosed at infancy, we aimed to analyze the results of recurrent MAS attacks and to revise the patient's clinical course. The answer being sought in this presentation: does the high ferritin level, being increased secondary to recurrent MAS attacks at a patient with systemic JIA diagnosed at infancy, represent a reason for the PH? Is there a need for further investigations of MAS secondary hemosiderosis?
Case report: A 13 months old previously healthy infant, admitted to our clinics because of high fever and rash. The fever showed an intermittent course, lasting for two weeks. A physical examination revealed a remarkable hepato-splenomegaly, bilateral wrist and ankle arthritis and a maculopapular, pink coloured rash being prominent especially during high fever. In order to exclude the infectious diseases, viral and bacterial infectious markers were tested and it was found to be negative. A bone marrow biopsy was performed for differential diagnosis of malignant or storage disease: no depot cells or malignant cells were found in the bone marrow.
A persistence of high fever was highly suggestive for systemic JIA and MAS as its secondary complication. The pulse steroid, cyclosporine (CYC) and anakinra were induced in therapy. Diagnosis of systemic JIA secondary MAS was a reason for hospitalization of patient about five times during the one year follow up. During the hospitalization of patient, a ferritin level was found to be as high as 120.990 ng/ml. Due to recurrent MAS attacks, a genetically investigation for familiar hemophagocytic lymphohystiocytosis was performed. The result was negative.
During the last hospitalization (2,5 years old patient), a respiratory difficulties and diffuse infiltrations on chest radiography, accompanied with a high ferritin level and anemia were a reasons to consider a pulmonary hemosiderosis in a differential diagnosis of patient. Thoracic tomography revealed a diffuse fibrous changes and a reticulo-nodular image in lung. Histochemical investigation of broncho-alveolary lavage fluid by the ferrous stain showed iron bearing macrophages. Thereby, a diagnosis was pulmonary hemosiderosis secondary to MAS, one of the most severe complications of systemic JIA.
The pulse steroid therapy was given in order to keep the MAS attacks under control. Prednisone 15 mg/day, methotrexate 5mg/week/oral route, CYC 50mg/day, anakinra 60 mg/day were used as a maintenance treatment.
Since the dyspnea became prominent during the clinical course of the disease, patient was admitted to the intensive care unit. At the time of discharge from the hospital, patient remained dependent on oxygen therapy. Mentioned therapy resulted with patient's good general status with no high fever but the need for permanent oxygen therapy continues.
Conclusion: Pulmonary hemosiderosis is being divided in a two main grup: idiopathic (primary) and secondary PH. IPH is considered to be more common than secondary, which is thought to be very rare. In the case of MAS, the most important and the most destructive complication of SJIA, ferritin could reach a very high level. Recently conducted a multi-centric study among SJIA secondary MAS cases showed a beginning ferritin level to be high as 8,325 ng/ml (2,048-22,977 ng/ml). None of those cases had a clinical presentation of pulmonary hemosiderosis.
Our patient with SJIA secondary MAS had a ferritin level of 120.990 ng/ml. Prominent dyspnea, reticular image on the chest radiography and anemia were suggestive for diagnosis of pulmonary hemosiderosis. Histochemical investigation of broncho-alveolary lavage fluid by the ferrous stain showing an iron bearing macrophages confirmed the diagnosis of pulmonary hemosiderosis.
For the best of our knowledge, this is the first case of pulmonary hemosiderosis secondary to MAS. In MAS patients with high ferritin level, PH should be considering as a possible complication.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P55

Development and validation of juvenile autoinflammatory disease multidimensional assessment report (JAIMAR)

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Introduction: There are lots of effects of auto-inflammatory diseases (e.g. pain, fatigue, fear of attack, lifelong drug use, being nervous and angry, problems at school) and those are quite important to patients but have not been measured with the outcome instruments currently included in clinical trials of auto-inflammatory diseases.

Objectives: The aim of this study is to develop and validate a new multidimensional questionnaire for assessment of children with auto-inflammatory disease (AID) in standard clinical care.
Methods: JAIMAR includes 16 parent or patient-centered measures and four dimensions that assess functional status, pain, therapeutic compliance and health-related quality of life (physical, social, school, emotional status) with disease outcome. The JAIMAR is proposed for use as both a proxy-report and a patient self-report, with the suggested age range of 8-18 years for use as a self-report. The study was conducted both children with FMF and their parents in seven different paediatric rheumatology centers from Turkey. To validate the JAIMAR, the Outcome Measures in Rheumatology Clinical Trials (OMERACT) filter for outcome measures in rheumatology was applied.
Results: The analysis data set was collected between December 2012 - April 2013 from the parents of 250 children with FMF in 351 visits and from 179 children in 187 visits. The median age of the children was 10.64 ± 4.38. The JAIMAR was found to be feasible and to possess face, content, criterion and construct validity. Completing and scoring of the JAIMAR is quick and can be finished approximately in 15 minutes. The Cronbach's alpha coefficient for internal consistency for the JAIMAR dimensions was between 0.507-0.998. Between the test-retest scale scores, there is a significant and a positive correlation from medium level to high level (0.607-0.966). For construct validity all the factor loadings are above 0.30. When the criterion validity is considered, we would say that the correlation level between the each subscale and the related scale spanned from medium ($r = 0.329$, $p<0.0001$) to large ($r=0.894$, $p<0.0001$). Parents' proxy-reported and children's self-reported data were outstandingly concordant. Cronbach's alpha values were between 0.770-0.989.
Conclusion: The development of the JAIMAR introduces a new and a multi-dimensional approach in pediatric rheumatology practice. It is a valid tool for children with autoinflammatory disease and will help enhance the quality of care in this group of patients.

P56

Role of polymorphonucleates in the pathogenesis of systemic juvenile idiopathic arthritis and Still's disease: a proof of concept study

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Background: Systemic juvenile idiopathic arthritis (sJIA) is an autoinflammatory disorder, characterized by neutrophilia and abnormal innate immunity response. Its counterpart in adult patients is the adult-onset Still's disease (AOSD). It has been hypothesized a pathogenic role of neutrophils in both conditions, because of patients neutrophilia, maybe related to the typical higher production of pro-inflammatory cytokines, like IL-1β, whose role in these disorders should explain the efficacy of IL-1 blockers. IL-1β is synthesized as inactive form and its activation is mediated by the NLRP3 inflammasome. Increased release of this cytokine in the extracellular environment lead to a positive feedback loop that perpetuates and amplifies itself stimulation. This mechanism as well as neutrophils' activation state could be modified in sJIA and AOSD.
Objectives: Our aim was to verify possible differences between sJIA and Still's patients compared to healthy donors, in term of PMNs responsiveness to the extracellular environment and activation state.
Methods: PMNs were obtained from heparinised venous blood of sJIA patients (n=6), AOSD patients (n=8) and healthy controls (HC, n=14). All patients' samples were collected during stages of active or non-active disease, according to international disease activity criteria used for the assessment of each disease. After lipopolysaccharide (LPS) treatment, IL-1β content in PMNs supernatants was measured by ELISA. CD11b and CD66b expression levels were measured by flow cytometry, together with intracellular ROS levels through the H_2DCFDA ROS-indicator.
Results: In comparison with HC, sJIA and AOSD PMNs showed an increased IL-1β secretion after LPS or LPS+ATP stimulation ($p<0.05$). Reduced IL-1β secretion was observed through caspase-1 inhibitor pre-treatment. About neutrophils activation state, higher intracellular ROS levels were detected in PMNs of sJIA patients ($p<0.05$) than HC, at basal condition. Moreover, also CD11b and CD66b surface marker expression levels, were higher at baseline in sJIA respect HC ($p<0.01$ and $p<0.05$ respectively). Instead, no differences were obtained between AOSD and HC PMNs.
Conclusions: Data from our proof of concepts study suggest a possible involvement of PMNs in the pathogenesis of sJIA and AOSD, since they seem more sensitive to pro-inflammatory stimuli respect healthy controls. An important difference interests PMNs' activation state, that seems higher in sJIA but comparable to HC in AOSD. Further studies are necessary to confirm and validate these results, but the trend observed may have a potential role in the direction of future therapeutic studies.

P57

Severe erytrodermic psoriasis and arthritis as clinical presentation of a CARD14-mediated psoriasis (CAMPS)

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Introduction: Autosomal dominant gain of function mutations in caspase recruitment domain family member 14 (CARD14) were found to cause plaque psoriasis in two families and severe generalized pustular psoriasis as a monogenic form of childhood (CARD14-mediated psoriasis, CAMPS) [1]. CARD14 mutations have also been implicated in plaque-type psoriasis and pityriasis rubra pilaris [2].
Objectives: Describing a family with an unusual clinical phenotype characterized by some members with childhood-onset erytrodermic psoriasis first localized and then diffuse over all the skin surface; in some family members is also reported psoriatic arthritis.
Patients and methods: We assessed for the first time the family in december 2013, because of their skin lesions and family recurrence for erytrodermic psoriasis associated to arthritis in some cases. There are three pairs of twins, five of them presenting psoriasis and two of them presenting psoriatic arthritis. The children presented poor clinical response to topic and systemic therapy with antihistamine, steroid, retinoids, cyclosporine and etanercept. After exclusion of the most common genes associated to autoinflammatory diseases (IL36RN, IL1RN, MVK, TNFRSF1A, NLRP3, NLRP12, MEFV, IL1RN, NOD2, PSMB8, PSTPIP1, LPIN2) we approached the new gene search by subjecting to Whole Exome Sequencing (WES) analysis five members of the family. Samples were processed in outsourcing and raw data were transferred to our lab for the bio-informatic analysis.
Results: Among variants shared by the four affected individuals and not present in the unaffected subject, a missense mutation of the CARD14 gene resulted worth of further investigation. In particular, it was the case of an exon4 heterozygous nucleotide change, c.446T>G, leading to the missense amino acid substitution p.L149R. The presence of this variant was validated by Sanger sequencing not only in the affected members undergone WES, but also assessed in all the rest of the available family members. This allowed us to confirm the expected segregation of the CARD14 mutation with the disease phenotype.
Conclusions: CARD14 gain of function mutations can give rise to unusual clinical phenotype like diffuse erytrodermic psoriasis and psoriatic arthritis. WES appears to be a powerful, suitable and proper approach to identify new SAID genes, thus also disclosing new molecular pathogenic mechanisms.

References

1. Jordan CT, Cao L, Roberson ED, Pierson KC, Yang CF, Joyce CE, *et al*: PSORS2 is due to mutations in CARD14. *American journal of human genetics* 2012, **90(5)**:784-95, Epub 2012/04/24.
2. Fuchs-Telem D, Sarig O, van Steensel MA, Isakov O, Israeli S, Nousbeck J, *et al*: Familial pityriasis rubra pilaris is caused by mutations in CARD14. *American journal of human genetics.n* 2012, **91(1)**:163-70, Epub 2012/06/19.

P58

Whole-Body MRI versus bone scintigraphy: which is the best diagnostic tool in patients with chronic recurrent multifocal osteomyelitis (CRMO)?

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Introduction: Chronic recurrent multifocal osteomyelitis (CRMO) is an autoinflammatory bone disorder of unknown etiology with a wide range of clinical manifestations. Since CRMO is a systemic disorder that can affect multiple skeletal sites, whole-body imaging techniques (whole body bone scintigraphy -WBBS- or whole body magnetic risonance -WBMRI-) provide major contribution to the initial diagnostic approach, as well as during follow-up.
Objective: To compare WBMRI with WBBS in the assessment of bone lesions in patients with CRMO.
Materials and methods: We retrospectively evaluated all WBMRI examinations performed between January 2010 and December 2014 in 18 patients with clinical, laboratory and histology findings suggestive for CRMO.WBMRI were evaluated independently by two experienced paediatric radiologists, who eventually reached consensus. All patients also underwent WBBS within four weeks; WBMRI and WBBS findings were compared. Signal hyperintensity compared with normal bone on STIR images and increased tracer activity in bone delayed scans on WBBS were considered indicative of disease involvement.
Results: WBMRI and WBBS showed 225 and 132 lesions, respectively. In the appendicular skeleton, WBMRI demonstrated 143 lesions and WBBS 66 lesions; in the axial skeleton, WBMRI demonstrated 63 lesions and WBBS 18 lesions. WBMRI demonstrated joint involvement in 19 sites and WBBS in 48. The higher concordance between the two methods was observed in the sacroiliac joint (13 lesions for both methods); the higher discordance was observed in spine (WBMRI showed 55 lesions and WBBS 5) and in sterno-clavicular joint (WBMRI showed 2 lesions while WBBS 12).
Conclusion: Both WBBS and WBMRI confirmed to be useful tools for the detection of CRMO lesions, as it is reported in literature. Discordance between WBBS and WBMRI is probably due to several factors. In the evaluation of axial skeleton (in particular of the spine), WBMRI shows higher spatial resolution than planar WBBS, but tomographic acquisition (SPECT) enhances the sensitivity of WBBS. Moreover, age-related conversion of hematopoietic marrow to fatty marrow in children may create a confusing appearance on MRI and may be misleading. Finally, in particular in WBMRI performed during treatment and follow-up, clinical relevance of the WBMRI-positive but WBBS-negative lesions is unclear, as still debated in current literature.

P59

***NOD2* mosaicism in Blau syndrome**

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Introduction: Gene mosaicism describes an individual who has developed from a single zygote and has two or more cell types with distinct genotypes. Three major types of mosaicism have been described and are referred as gonadal, somatic and gonosomic mosaicism. They mainly differ on the body distribution of the somatic mutation and in their clinical consequences. The recent use of next-generation sequencing technologies is revealing the relevant role of gene mosaicism in various diseases other than cancer, including monogenic autoinflammatory diseases such as cryopyrinopathies and STING-associated vasculopathy with onset in infancy [1-3].
Objectives: To describe the first known cases of somatic and gonosomic *NOD2* mosaicism in Blau syndrome (BS).
Patients and methods: Genomic DNA was extracted from hematopoietic and non-hematopoietic cell types. *NOD2* analyses were performed by both Sanger sequencing and targeted deep sequencing (TDS).
Results: The first patient is a 17-year-old Moroccan girl who presented with bilateral red eye at the age of 3 years. Severe panuveitis, erythematous rash and oligoarthritis were observed during the following years. Sanger sequencing did not clearly detect any disease-causing *NOD2* mutation. However, a careful analysis of Sanger chromatograms revealed a potential c.1001G>A transition that might cause the appearance of the well-known p.Arg334Gln *NOD2* mutation. TDS confirmed the presence of somatic *NOD2* mosaicism by means of the detection of the somatic p.Arg334Gln mutation in hematopoietic and non-hematopoietic tissues with variable frequencies (4.9-11.0%).
The second patient is a 37-year-old Malaysian male suffering from skin rash and bilateral uveitis since the age of 22 years. He has two affected daughters, aged 4- and 2-year-old, with the classical BS triad (skin rash, polyarthritis and uveitis). Sanger *NOD2* sequencing revealed the germline p.Arg334Gln mutation in both daughters, and unexpectedly its absence in the father. Further analysis of father's Sanger chromatograms suggested the presence of the p.Arg334Gln mutation as a potential somatic mutation. TDS confirmed the presence of gonosomic *NOD2* mosaicism in the father by means of the detection of the somatic p.Arg334Gln mutation in different tissues with different allele frequencies (0.9-12.9%).
Conclusions: We describe for the first time the involvement of *NOD2* mosaicism in BS pathogenesis by using the novel TDS technology. Our findings clearly support that gene mosaicism might have a relevant role in either "mutation-negative" BS patients or in parents of an affected child with an apparent *de novo* germline mutation.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. *Arthritis Rheum* 2011, **63**:3625-3632.
2. *Ann Rheum Dis* 2015, **74**:603-10.
3. *N Engl J Med* 2014, **371**:507-518.

P60

Description of a case of late-onset cryopyrin-associated periodic syndrome due to low-level somatic *NLRP3* mosaicism

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Introduction: Cryopyrin-associated periodic syndromes (CAPS) usually present in early childhood as an urticaria-like skin rash associated with an increased inflammatory response, with additional manifestations (i.e. arthropathy, AA amyloidosis or deafness) typically restricted to certain phenotypes. CAPS are caused by dominantly inherited or *de novogain-of-functionNLRP3* mutations. The introduction of next-generation sequencing (NGS) into clinics has revealed the important role of somatic *NLRP3* mosaicism in these syndromes by means of its detection in a high proportion of patients who were apparently mutation-negative by Sanger sequencing. Thus, NGS technologies are becoming essential for routinely identifying the genetic cause of the suspected autoinflammatory disease.
Objective: To describe a Spanish patient with CAPS that start in adulthood in whom molecular analyses detected a novel *NLRP3* mutation as a somatic mutation.
Patients and methods: Genomic DNA was extracted from peripheral blood. The analysis of the six most common genes associated with autoinflammatory diseases (*MEFV*, *TNFRSF1A*, *MVK*, *NLRP3*, *NOD2* and *PSTPIP1*) was performed by NGS. Additional molecular studies of somatic *NLRP3* mosaicism were performed by targeted deep sequencing (TDS).
Results: The patient is a 63 year-old Spanish male who presented with a generalized urticarial rash, a gradually worsening oligoarthritis at wrists, elbows and knees, and a moderate bilateral sensorineural hypoacusia

starting in his 50s. Laboratory results showed a marked leucocytosis, neutrophilia and increased inflammatory markers without evidence of circulating autoantibodies. Multiple therapeutic approaches including NSAID, corticosteroids, methotrexate and colchicine result in poor or partial responses. Screening of autoinflammatory-associated genes identified a novel *NLRP3* variant (c.1906C>G; p.Gln636Glu) with an allele frequency of 12.2% (coverage: 738x). We hypothesized that this variant could be a somatic *NLRP3* mutation. TDS confirmed the somatic *NLRP3* mosaicism at 18.4 % (mean of triplicates; mean coverage: 6225x). Additional analyses showed that this variant has never been reported in public databases, that is located on a highly evolutionary conserved amino acid residue and that is predicted to be possibly damaging by PolyPhen-2 algorithm. Additional functional and genetic studies are currently ongoing.

Conclusions: We herein describe the case of a patient with a clinical picture compatible with MWS, with the exception of a late onset of the disease, who carries a somatic *NLRP3* mosaicism. Our findings highlight the diagnostic utility of NGS technologies in detecting low-level somatic gene mosaicism and support its use as a routine genetic screening tool.

Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P61

Somatic *NLRP3* mosaicism in Muckle-Wells syndrome

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Introduction: Familial cold autoinflammatory syndrome, Muckle-Wells syndrome (MWS), and chronic, infantile, neurological, cutaneous and articular (CINCA) syndrome are dominantly inherited autoinflammatory diseases associated to gain-of-function *NLRP3* mutations. All these diseases are currently considered as different phenotypes of the cryopyrin-associated periodic syndromes (CAPS). A variable degree of somatic *NLRP3* mosaicism has been recently detected in ≈35% of patients with CINCA. However, no data are currently available regarding the relevance of this genetic mechanism in other CAPS phenotypes.

Objective: To evaluate somatic *NLRP3* mosaicism as the disease-causing mechanism in patients with CAPS phenotypes other than CINCA and *NLRP3* mutation-negative by conventional, Sanger-based genetic studies.

Materials and methods: *NLRP3* analyses were performed by Sanger sequencing and by targeted deep sequencing. Apoptosis-associated Speck-like protein containing a CARD (ASC)-dependent nuclear factor kappa-light chain enhancer of activated B cells (NF-κB) activation and transfection-induced THP-1 cell death assays determined the functional consequences of the detected variants.

Results: 32 Spanish patients fulfilling clinical inclusion criteria were enrolled. A variable degree (9.4-34.9%) of somatic *NLRP3* mosaicism was detected in 9.3% of enrolled patients (3/32). Their clinical phenotypes were identical to that seen in MWS. Three different missense variants (p.D303A, p.L411F and p.F523L) were identified, being two novels (p.D303A and p.L411F). Bioinformatic and functional analyses confirmed that they were disease-causing, gain-of-function *NLRP3* mutations. Treatment with anti-IL-1 drugs showed long-lasting and positive clinical and biochemical responses.

Conclusion: We herein show novel evidence about the role of somatic *NLRP3* mosaicism in MWS pathogenesis, which probably represents a shared genetic mechanism in CAPS not restricted to CINCA syndrome. The data here described allowed us to achieve the definitive diagnoses of these patients, which have had serious clinical implications such as gaining access to anti-IL-1 treatments under legal indication and genetic counseling. The detection of somatic gene mosaicism is difficult when using conventional methods. Potential candidates should benefit from the use of novel technologies such as targeted deep sequencing.

P62

A case series of adenosine deaminase 2 deficient patients emphasizing treatment and genotype-phenotype correlations

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Introduction: Deficiency of adenosine deaminase 2 (DADA2) causes a vasculopathy with autoinflammatory features associated with mutations in *CECR1*. The phenotype of DADA2 varies from only cutaneous lesions to full-blown systemic disease with central nervous system (CNS) involvement and aneurysms in visceral arteries which may overlap with the spectrum of polyarteritis nodosa (PAN).

Objective: Our aim was to assess the characteristics of our patients with DADA2.

Patients and methods: This is a descriptive case series of Turkish patients diagnosed with DADA2 at Hacettepe University. We performed Sanger sequencing in order to sequence 10 exons of *CECR1*.

Results: We report six DADA2 patients with homozygous p.G47R mutation in *CECR1*. All were initially diagnosed as PAN (one cutaneous, others systemic) fulfilling the classification criteria for the disease and all but one having necrotizing arteritis lesions at skin biopsy and two had arterial aneurysms. All patients had a childhood onset of disease (median age 7.2 years). All had skin lesions varying from livedo racemosa to necrotic ulcers on fingers. There were recurrent fever and abdominal pain attacks in our patients. Four had CNS involvement; three in the form of strokes and one had borderline intelligence. Two patients had strabismus and one had optic neuritis. Two of the patients were sibs and these patients had low IgM. There was autoantibody positivity in two patients. Two patients had hematological involvement, one in the form of macrophage activation syndrome and one myelofibrosis. One of our patients had focal segmental glomerulosclerosis while another patient had renal AA type amyloidosis. All patients were refractory to corticosteroid treatment. One patient with extensive systemic amyloidosis was resistant to immunosuppressive and plasma treatments and died due to necrotizing pneumonia. One had prolonged remission on colchicine. Two responded to etanercept (one partially), one to mycophenolate mofetil (the patient with a previous diagnosis of cutaneous PAN), and one plasma treatment (temporarily). Literature review revealed that patients with homozygous p.G47R mutation have fewer strokes and predominantly PAN-like phenotypes compared to the patients with other mutations.

Conclusion: DADA2 may be classified as a secondary vasculitis due to probable cause. Genotype-phenotype correlation may exist in DADA2 and etanercept may be a promising treatment; however, longer follow-up and prospective studies are needed.

P63

Interleukin (IL)- 6 inhibition - Follow-up data of the German AID-registry[1]

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Pediatric Rheumatology 2015, **13(Suppl 1):**P63

Introduction: Systemic juvenile idiopathic arthritis (SJIA) is regarded as an autoinflammatory disease (AID) of unknown etiology related to abnormalities of the innate immune system. A major role in the pathogenesis has been ascribed to proinflammatory cytokines as interleukin (IL)-6 and IL-1.
Objectives: Analysis of treatment results with the IL-6 inhibitor tocilizumab.
Patients and methods: From 7/2009 to 4/2014 200 patients with SJIA were documented in the AID-registry. 46 of 200 patients (19 m, 27 f) at the age of 1-18 years (median 9) received therapy with tocilizumab (median 13 months, range 1-48). 24 of 46 patients received long term treatment (median 23 months, range 12-48) and were evaluated concerning Wallace criteria [1]. Different clinical courses were continuous (C) n=12, polycyclic (PC) n=16, arthritic (A) n=18. Besides we estimated a response rate (definition: no clinical manifestation and no inflammation parameters) in the first 12 weeks of treatment. Data are based on the AID-Registry (http://www.aid-register.de).
Results: According to Kaplan-Meier analysis 30% reached inactive disease or remission after the first 12 weeks of treatment. A rapid response to tocilizumab seems to be related to long term inactivity of SJIA. Comparison of the three disease courses (PC, C, A) revealed significant differences in the outcome; polycyclic courses show the fastest response followed by continuous courses. Worst outcome was evaluated in arthritic courses. Wallace criteria measured after at least 12 months: remission 54%, active disease 25%, inactive disease 21%. 4 (9%) patients were non-responders over the whole time. 60% of the patients showed no measurable CRP within the first 4 weeks and during tocilizumab therapy. Adverse events were reported in 11 (24%) patients: most leukopenia, infections and elevated transaminases, one Hodgkin's lymphoma, one gut perforation.
Conclusion: A significant proportion of patients documented with SJIA in der German AID-registry is treated with tocilizumab (23%). We estimated a good response in the first 12 weeks of therapy of 30% and also by Wallace of 76% (inactive disease or remission). The response appears to depend on different disease phenotypes.
[1]The AID-Registry is funded by the BMBF (01GM08104, 01GM1112D).
Reference
1. Wallace CA, *et al*: *J Rheumatol* 2004, **31**:2290-4.

P64

CECR1 p.Gly47Arg mutations are not increased in frequency in Turkish Behçet's disease patients compared with healthy controls

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Question: In Behçet's disease (BD), vasculitis involving blood vessels of nearly all sizes and types may underlie the diverse tissue and organ involvement. Loss of function mutations in the *CECR1* gene (encoding adenosine deaminase 2) have recently been shown to cause a recessive genetic disease, deficiency of adenine deaminase 2 (DADA2). Patients with DADA2 exhibit systemic vasculopathy characterized by intermittent fevers, skin rash, and neurovascular manifestations along with other features that can lead to a diagnosis of polyarteritis nodosa. Patients homozygous for the *CECR1* p.Gly47Arg mutation are reported in two non-consanguineous Turkish families and this mutation is found at low frequency in the Turkish population. We therefore attempted to determine whether some BD cases may be explained by adenosine deaminase 2 deficiency and whether this mutation contributes to BD risk in patients of Turkish ancestry.
Methods: Turkish BD patients (n = 1,609) and controls (n = 1,519) were genotyped for p.Gly47Arg mutations in the *CECR1* gene using a Sequenom assay. The assay interrogated two mutant alleles of the first nucleotide of the Gly47 codon that both encode the glycine to arginine missense change.
Results: We found p.Gly47Arg mutations in 4 BD patients and 3 healthy controls. No individuals (neither cases or controls) carried two mutant alleles. The carrier frequency for p.Gly47Arg mutations was 0.002 in cases and in controls.
Conclusions: These data show that the carrier rate of *CECR1* p.Gly47Arg mutations is very low in Turkish Behcet's patients and not different from controls, suggesting no contribution to Behcet's disease.

P65

B cells characterization in ADA2 Deficiency patients

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Introduction: ADA2 deficiency, a recently described disease, is characterized by systemic vasculopathy and episodes of strokes. The defect is due to a loss of function mutation of *CECR1* gene, codifying for Adenosine Deaminase 2 protein. This protein regulates the catabolism of extracellular adenosine, which we have recently shown is an important regulator of Class Switch Recombination in B lymphocytes. Accordingly DADA2 patients can present hypogammaglobulinemia.
Objectives: Therefore we decided to characterize peripheral B and T lymphocytes of DADA patients to directly address if ADA2 mutation affects B-cell function and in particular we focused on B cell- T cell interaction.
Patients and methods: 3 patients carrying mutations in *CECR1* were examined. They showed clinical history with livedo reticularis, fever, vasculitis and neurological symptoms. Two patients presented hypogammaglobulinemia requiring intravenous immunoglobulin replacement therapy. We analyzed peripheral B and T cell phenotype by flow cytometry, *in vitro* B-cell proliferation and differentiation to Immunoglobulin secreting cells in response to CpG and T cell help.
Results: Flow cytometer analysis showed a reduction of total B cells compared with age matched controls. Intriguingly a decrease in the percentage of memory B cell compartment (CD19+CD27+) was observed. Moreover we noted that the rate of B cells proliferation and differentiation to Immunoglobulin Secreting Cells of DADA2 patients with autologous T cell help are impaired. In fact in vitro IgM, IgG and IgA secretion is significantly reduced with respect to HD B lymphocytes in presence of mutated CD4 helper T cells.
Conclusions: Our findings suggest that ADA2 defect could lead to a defect in B cell function and to a reduced T cell dependent B cell response.

P66

Atypical manifestations in CAPS sydrome: not so unfrequent?

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Clinical picture of CAPS syndrome includes periodic fever, skin rash, arthritis / arthralgias, conjuntivitis, and neurisensorial deafness with hereditary pattern and early onset (<1y in 50% of cases). In last decade, atypical manifestations have emerged expanding the clinical spectrum of CAPS.
Question: To evaluate the clinical, laboratory and genetic profile of 26 patients of 4 spanish unrelated pedigrees with special attention to the atypical manifestations of the disease.
Methods: Review of clinical, analytical and genetical data of a cohort of 26 patients from 4 unrelated non-consanguineous Spanish pedigrees.

Results: Rash (14/26), arthritis (14/26), and deafness (12/26) were the most common features. Episodic fever accounted in 8/26 patients. 14/26 patients did not present episodic course and disease onset was over 10 years in 17/26 patients. 2/26 patients developed amyloidotic hemorrhagic cystitis and other 2/26 patients explained olfactive disfunction. Amyloidosis was confirmed in 2/26 patients and considered as probable in other 4/26 patients. Acute phase reactants were normal in 7/26 patients. CIAS1 mutations were identified in 23/26 patients (Ala439Thr in 5, Arg488Lys in 6 and Arg260Trp in 12). 5/6 patients carriers of an heterozigous Arg488Lys mutation were asymptomatic while it failed to isolate any CIAS1 germinal, somatic or mosaic mutation in 3 members of the same pedigree although their clinical profiles were consistent with Muckle-Wells syndrome (1 case) and CINCA / NOMID (2 cases).
Conclusions: Despite the traditional clinical picture of CAPS includes periodic fever, rash, arthritis and deafness with onset usually <1 year, in its absence CAPS cannot be ruled out. The clinical spectre may vary from the chronic afebrile course, to atypical amyloidosis, or olfactive disfunction. The profile of CIAS1 mutations carriers may include asymptomatic individuals or absence of mutations in pedigrees with several members with a highly suggestive clinical profile of severe variants of CAPS syndrome.

P68

A heterozygous variant in *MEFV* in a familial autoinflammatory syndrome with PAPA-like features

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Introduction: Autoinflammatory disorders are a group of diseases whose nosology and etiology are only partly understood. Among Mendelian forms, familial Mediterranean fever (FMF), due to mutations in *MEFV*, is one of the most frequent. Most *MEFV* mutations are located in exon 10 and are usually associated with an autosomal recessive mode of inheritance. *MEFV* encodes pyrin, which interacts with PSTPIP1, a protein involved in the rare autosomal dominant pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome.
Objectives: We aimed to identify the underlying genetic defect in a very large family of Belgian ancestry with an autosomal dominant autoinflammatory syndrome showing PAPA-like features.
Patients and methods: 12 family members out of 22 spanning three generations presented with a PAPA-like syndrome. All patients suffered from childhood-onset recurrent episodes of fever, highly increased levels of acute-phase reactants, arthralgia, myalgia/myositis and neutrophilic dermatosis with variable manifestations (severe acne, skin abscesses, pyoderma gangrenosum, leucocytoclastic small vessel vasculitis). In between attacks low-grade systemic inflammation remained present. One patient also presented with cardiac failure which led to cardiac transplantation at the age of 18 years.
Linkage study was performed using 6K DNA chips. Whole-exome sequencing was carried out on two trios each consisting of an affected 'child' with an affected and unaffected 'parent'. High throughput sequencing of the whole candidate region was performed in two patients using the SureSelect Custom Agilent library.
Results: Through linkage analysis we identified a single 6.3Mb region on chromosome 16 segregating with the disease with a lod score of 3.6 and containing *MEFV*. Exome and targeted sequencing identified a single rare potentially functional sequence variantion in the linkage region. This variant is located in *MEFV* exon 2: c.726C>G; p.Ser242Arg, and was confirmed by Sanger sequencing. Whole-exome sequencing and high throughput sequencing of the target region did not reveal anyNo additional molecular defects, which might explain this autoinflammatory syndrome, were found by exome-sequencing of the target region.
Conclusion: Our data reveal that a heterozygous variant in the exon 2 of *MEFV* could underlie an autosomal dominant autoinflammatory syndrome with neutrophilic dermatosis, as seen in PAPA syndrome. Consistent with this idea, heterozygous mutations in *MEFV* exon 2 were recently found in two patients who developed acute febrile neutrophilic dermatosis (Sweet syndrome) in the context of a myelodysplastic syndrome. Also, several autosomal dominant forms of FMF of smaller size have been reported previously, though the mutations were not located in exon 2. The present study underlines the close links between the nature of mutations in a given gene, the mode of inheritance, and the disease phenotype.

P69

Differences in disease activity in cryopyrin-associated periodic syndrome in mutation-positive and mutation-negative patients

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Introduction: Cryopyrin-associated periodic syndrome (CAPS) is an inherited disease which is caused by gain-of function mutations in CIAS1 gene function resulting in increased secretion of active IL-1β. As known more than 40% of patients with CAPS are genetic negative in CIAS1 gene. One of theories explains this is a fact is a somatic mozaicism.
Objective: The aim of our study was compare activity of CAPS patients depends on presence or absence of mutations in CIAS1 gene.
Materials: 9 patients with CAPS (6 CINCA and 3 Muckle-Wells syndromes-MWS) were included in our study. In all patients genetic tests in CIAS1 gene was performed. 4 patients have positive mutations (3 CINCA and 1 MWS) and 5 have not any mutations in CIAS1 gene (3 CINCA and 2 MWS). Disease activity was measured with applying simplified auto-inflammatory disease activity index (sAIDAI, M.Piram et al, 2013), MDVAS, levels of erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), hemoglobin (Hb), white blood cells (WBC), platelets (PLT), and fibrinogen. All patients were treated with canakinumab. MDVAS and sAIDAI were evaluated at twice: before canakinumab and during the treatment.
Results: We have not detected differences in laboratorial markers, such as ESR, CRP, Hb, WBC, PLT and fibrinogen depends on the presence of the CIAS1 mutation, but in mutation positive patients WBC was 2 times higher (n.s.), PLT and fibrinogen was +25% higher than in genetic negative patients. No differences in MDVAS before and on canakinumab, but we have detected differences in sAIDAI before canakinumab: 88.5 (78.5 - 106.5) vs 51.0 (48.0 - 55.0), p=0.014. On canakinumab no differences in sAIDAI depends on the mutation presence. Also mutation-positive patients required higher dosis of canakinumab and shorter intervals between injections.
Conclusion: Patient with CAPS with mutation in CIAS1 gene have higher inflammatory activity and required more intensive treatment with canakinumab.

P70

Enlarging the clinical spectrum of SAVI syndrome

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Background: SAVI syndrome is a recently identified condition associated to mutations of TMEM173. Up to know only few cases of this disease have been described.
Aim of the study: To describe the clinical manifestation of an Italian patient affected by SAVI syndrome.

Results: The girl, first born from healthy, not relatives parents, at the age of 8 months started to present erythematosus-infiltrated skin lesions with pustular evolution and finally hesitating in scars in 15-20 days. From the age of three years chilblains and severe nail dystrophy appeared.
A CT scan performed at the age of 8 years revealed the presence of diffuse interstitial thickening with ground-glass appearance. A restrictive framework was detected at spirometry (FVC 51%).
The autoantibodies detection revealed positive ANA (1: 160), ANCA (1:80) and Coombs test.
The skin biopsy revealed a predominantly granulomatous nodular dermatitis, with folliculitis and secondary fibrosis.
The lung biopsy revealed focal hemorrhage, edema and predominantly lymphocytic inflammatory aggregates in the peribronchial interstitial areas with aspects of capillaritis and contiguous focal subatelettasia with alveolar cavity filled of macrophages.
Steroidal treatment (prednisone 1 mg/kg/day) was started with improvement of clinical manifestation, anemia and normalization of inflammatory markers. However attempts to reduce such therapy were followed by an exacerbation of the clinical picture.
Treatment with both immunosuppressive (azathioprine) and biologic (etanercept) drugs was tempted, without clear improvement. Unsatisfactory growth was also detected.
In the following months the child started to present a mild renal involvement with microscopic hematuria and hypertension, requiring anti-hypertensive treatment.
Given the evocative framework, interferon gene signature was performed, revealing a significant activation; the molecular analysis of TMEM173 gene showed the presence of the de novo Val155Met mutation, already described as causative of SAVI syndrome.
The child continued to present persistent severe microcytic anemia, requiring erythrocytes' transfusions, despite high levels of erythropoietin. Bone marrow aspiration revealed dysmaturative signs in the in erythroid progenitors.
Treatment with jak1/2 inhibitor (Ruxolitinib, 5 mg day) was just started at the time of abstract presentation.
Conclusion: This report of the first Italian patient with SAVI syndrome confirms the presence of the previously described clinical manifestations. Persistent hematuria and hypertension are reasonably signs of an underlying renal involvement, not previously described in this condition.

P71

Familial Mediterranean Fever and Human autoinflammatory diseases

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Introduction: Human Autoinflammatory Diseases (HADs) is a heterogeneous group of rare genetic diseases, which are characterized by unprovoked onsets of inflammation, fever and clinical symptoms analogous with rheumatic diseases with absence of immunological indicators. Familial Mediterranean Diseases (FMF) is one of the popular forms in the group of syndromes which are called HPFS.
Clinical characterization is presented in symptoms which are common for all hereditary fevers like: relapsing onsets of inflammation (serous membrane) of muscular-joint syndrome, various skin rash, high rates of inflammatory processes (ESR, leucocytosis, c-reactive protein, SAA), secondary amyloidosis complications, absence of autoantibodies. Absence of specific symptoms of manifestation hampersdifferential diagnosis. The used diagnostic criteria are insufficient for timely diagnosis of Familial Mediterranean Diseases, especially in case of atypical debut of disease.
It is actualforthe group of Hereditary Periodic Fever Syndromes and Human Autoinflammatory Diseases, such as Still's disease, Crohn's disease, Bekhchet's disease and Juvenile Idiopathic Arthritis (JIA) that are characterized by the multifactor types of inheritance.
Aim: In spite of the existing data onthe pathogenic relationship between FMF and the above mentioned diseases, it is still unclear whether they are concomitant with FMR or are associated with FMR.
Methods: Via retrospective analysis we examined medical history of 24 patients for a period of 2011-2013 (medical histories taken from the archive of "Muratsan" clinical hospital).
Results: Below isthe description of two cases of patients diagnosed with FMF, which started with Crohn's disease and JIA.
Case 1. Patient A., 22 years old. The disease started up in childhood with symptoms of arthritis, fever, hepatosplenomegaly and skin rash. The patient was diagnosed with JIA and steroid treatment was prescribed. At the age of 14 attack-like pain appeared in the abdomen and chest, accompanied with fever. Periodicity of symptoms was twice a month. Based on the clinical picture (serositis, synovitis, fever) and genetic investigation (M694V), the FMR was diagnosed in 2012. The colchicines therapy was prescribed.
Case 2. Patient S., 60 years old. The disease started upin childhood with acute symptoms of abdominal pain which was not always accompanied with fever. 15 years later symptoms of Crohn's disease developed, such as diarrhea, intestinal bleeding, joint syndrome (sacroileitis and talocrural arthritis). The patient was hospitalized with the nephritic syndrome. Based on the clinical picture and genetic investigation (M694V in homozygote condition) and biopsy of rectum (amyloidal deposits were discovered), the abdominal form of FMR, systemic amyloidosis with affection of kidneys and intestine were diagnosed.
Conclusion: Summarizing the above mentioned, we have come to the conclusion that there is need to do genetic investigation of MEFV in the population ethnically significant for FMR, when inflammatory intestinal diseases and clinical pictures of JIA are registered.
Consent to publish: Written informed consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P72

The IL-1 inhibitor Canakinumab for Familial Mediterranean Fever: the Greek experience in 12 patients

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Background: IL-1 is a major mediator of the inflammatory cascade in Familial Mediterranean Fever (FMF) and an established therapeutic target [1].
Objective: To retrospectively assess the efficacy and safety of the IL-1 inhibitor Canakinumab in adult and adolescent FMF patients, including cases resistant to the IL-1 receptor antagonist anakinra.
Methods: Twelve patients (7 men) with genetically confirmed FMF, fulfilling the Tel Hashomer criteria, aged 32.5 years (median, range 13-70), with median disease duration of 168 months and active disease refractory to colchicine (n=8) and/or anakinra (n=4), received Canakinumab 150mg subcutaneously every 4 (n=7) or 6 (n=3) or 8 weeks (n=2) for a median of 12 months (range 4-46). Canakinumab was given as monotherapy in 9; 3 patients received concomitant treatment with colchicine and/or corticosteroids. Clinical and laboratory parameters during follow-up were recorded.
Results: Seven out of 12 patients (58%) achieved complete clinical remission within a median time of one month. Normalization of all laboratory parameters associated with inflammation occurred in 70% of patients within a median time of 2 months and in all, but one, of these patients complete clinical remission was also reached. Response was maintained until the last visit in all patients with clinical and/or serological complete remission. The remaining patients achieved partial responses, with persisting, albeit milder, arthralgias and abdominal pain, and lower, but abnormal CRP levels. Overall, the concomitant corticosteroid dose was

significantly reduced during follow up. The recently proposed FMF50 score for assessing outcome in FMF (2) was achieved by 67% and 88% of patients at one month and 12 months, respectively. Canakinumab was well tolerated; one patient experienced an urinary tract infection, which resolved with antibiotics.

Conclusion: The rapid and sustained response to Canakinumab in the majority of our patients, together with the favorable safety profile, encourages its further use in FMF.

References

1. Ter Haar N, *et al*: *Ann Rheum Dis* 2013, **72(5)**:678-85.
2. Ozen S, *et al*: *Ann Rheum Dis* 2014, **73(5)**:897-901.

P74

Toll like receptor 2 is overexpressed in FMF patients during attacks and inhibited by colchicine treatment

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Background: FMF is a systemic auto-inflammatory disorder, characterized by recurrent episodes of fever and serosal inflammation. The *MEF* gene, which is associated with FMF, encodes for the protein pyrin. FMF associated mutations, interrupt with pyrin normal function, leading to activation of the innate immune system and overexpression of IL-1β, and consequently to a systemic inflammatory response. Toll-like receptors (TLRs) play an essential role in the innate immune responses, by recognition of pathogen-associated molecular patterns and endogenous peptides. TLRs trigger a cascade of signaling events, leading to cytokine production. TLR2 is implicated in several inflammatory conditions, but its role in the pathogenesis of FMF is not completely clear.

Objectives: To study the role of TLR2 in the inflammatory process of FMF.

Materials and methods: We tested TLR2 naïve expression on monocytes of FMF attack-free patients (n=20) by FACS. We further tested the effect of sera from FMF patients in acute attack (n=6) on TLR2 expression by monocytes of healthy controls. The role of TLR2 was studied in respect to *MEFV* mutation, performed in THP-1 cells. TLR2 downstream signaling was studied by ELISA to measure IL-1β secretion, or by Western-blot to measure NF-κB.

Results: FMF attack-free patients have increased $CD14^{+}TLR2^{+}$ cell-count, as compared to healthy donors. High dose of colchicine treatment (≥2mg/d) inhibited this increased expression of TLR2 in FMF patients. Colchicine *in vitro* also inhibited the levels of TLR2 expression on THP-1 cells. Sera from FMF patients in acute attack induced TLR2 expression by both monocytes of healthy donors and THP-1 cells, and IL-1β secretion in healthy monocytes, and colchicine inhibited this induction. Furthermore, TLR2 agonist (Pam2CSK4) increased the secretion of IL-1β by PBMCs of healthy donors, and this activation was inhibited by colchicine. In *MEFV* -mutated THP-1 cells, TLR2 expression was spontaneously up-regulated by 3.8 folds, while TLR4 expression was elevated by 2 folds as compared to wild-type. Wild-type THP-1 cells presented elevated NF-κB expression when cultured with Pam2CSK4, whereas colchicine treatment abolished this expression. *MEFV*-mutated THP-1 cells expressed elevated levels of NF-κB, as compared to their wild-type counterparts.

Conclusion: TLR2 activation is up-regulated in monocytes of FMF patients, and colchicine inhibits this up-regulation *in-vitro* and *in-vivo*. Elevated expression of TLR2 promotes IL-1β production, and thus contribute to the uncontrolled inflammation manifested in FMF.

P75

Does the age at disease onset cause a delay in diagnosis and affect disease severity in the children with familial Mediterranean fever?

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Introduction: Familial Mediterranean fever (FMF) is a systemic autoinflammatory disease characterized by recurrent attacks of fever and sterile peritonitis, pleuritis, arthritis or erysipelas-like erythema. Symptoms related with FMF can begin at very early ages of life. However, since most symptoms related to FMF are often well-known only when children become more verbal, and fever in children in the first years of life are common, attacks of fever alone may cause or contribute to diagnosis delay.

Objectives: In the present study we aimed to investigate whether the age at onset of the symptoms cause a diagnosis delay in the children with FMF.

Materials and methods: Ninety FMF subjects (M/F: 43/47) who fulfilled the Livneh criteria for the diagnosis of FMF were enrolled into the study. Subjects were between 8-18 years age and having the symptoms of FMF for at least one year. Demographic data including age, age at onset of the symptoms, duration of symptoms and family history were recorded. The subjects were questioned about periodic fever, abdominal and chest pain, arthritis, and erysipelas-like erythema. The severity score of the disease was calculated based on the Tel-Hashomer severity score. 6 Minute Walk Test (6MWT) was used to evaluate functional capacity.

Results: The mean age of the FMF subjects was 12.21±2.75 years. Abdominal pain attacks were the most common symptom, occurring in 77 (85.6%) of our patients; periodic fever was in 74 (82.2%), chest pain in 36 (40%), arthritis in 41 (45.6%), and erysipelas-like erythema in 12 (13.3%). A positive family history for FMF was detected in 51 (56.6%) of the patients. Onset age and diagnosis delay duration did not significantly differ between the groups regarding family history ($p>0.05$). Twenty-one children (23.3%) had the first FMF symptoms at the age of <5 years. While the mean onset age was 6.91± 3.44 years, delay in diagnosis was 2.12±2.70 years. In the children with FMF, while there was a negative correlation between the age at onset of the symptoms and duration of delay and disease severity score, there was no correlation between 6MWT.

Conclusion: We determined that FMF beginning at early ages causes a delay in diagnosis and also a worse disease severity score. Physicians practice in the regions that FMF is prevalent should be aware of different features of the disease in childhood in order to reduce a possible delay in diagnosis and prevent the development of complications.

P76

MEFV mutation carriage as possible predisposition factor for the development of Post Pericardiotomy Syndrome (PPS)

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Background: PPS is a syndrome, which manifests with pleuropericardial inflammation and occurs in about 15-20% of patients undergoing surgery, involving the pleura, pericard or both. The pathogenesis of the syndrome is not yet fully understood. Carriage of MEFV mutations may explain the occurrence of this syndrome, which largely overlaps with FMF, in only part of the operated population.

Goal: To determine whether MEFV mutation carriage may precipitate PPS or affect its phenotype.

Methods: 86 patients who underwent cardiac surgery were studied, 45 of whom developed PPS (study group) and 41 have not (control group). Demographic data (gender, age, region of residence, ethnic origin) and type of surgery were collected. The severity of PPS was evaluated, based on a predefined scale. Genetic analysis determining carriage of one of the three most common MEFV gene mutations (M694V, V726A, E148Q) was performed.

Results: The rate of women was higher in the PPS group ($p=0.001$). No significant differences were found between the 2 groups with regards to the rate of mutation carriage. Subgroup analysis for age, ethnic origin and gender also failed to yield significant results. The severity of the PPS in carriers was lower compared to non carriers.

Conclusions: Carriage of MEFV mutations does not predispose for the development of PPS. However carriage of MEFV mutations does affect PPS phenotype ($P<0.05$).

P77

Screening of free carnitine and acyl-carnitine status in patients with Familial Mediterranean Fever

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Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurring self-limited fever, abdominal pain and chest pain caused by serositis. FMF mainly affects Middle-East populations with a high prevalence in Sephardic Jews, Turkish, Arabs and Armenians. Carnitine is an important molecule in cellular energy metabolism. Secondary carnitine deficiency can be detected in chronic diseases by either renal loss or increased needs.
Objectives: Our hypothesis was that FMF patients would have lower free carnitine levels than their healthy age and gender matched controls due to increased need of carnitine because of recurrent auto-inflammation. The present study was conducted to determine the patterns of free carnitine and acyl-carnitine esters in FMF patients.
Methods: This is a cross-sectional study of 205 FMF patients who were attending the outpatient Pediatric Rheumatology clinic of Cerrahpasa Medical Faculty Children's Hospital. The patients were selected by random sampling and FMF diagnosis was confirmed by a pediatric rheumatologist according to Yalcınkaya criteria. 50 healthy subsects were enrolled to the present study. A fasting died blood sample was taken for studying free carnitine and acyl-carnitine esters with tandem mass spectrometry from children in both groups.
Results: Acyl-carnitine analyses in spot dried blood samples with ESI-MS/MS were performed in all patients and control group. Screening of acyl-carnitine profile revealed free carnitine, C16-OH and C18:2 carnitine levels were higher (p<0,0001, p<0,0001 and p-0,003 respectively), while C4-OH and C4DC carnitine levels were lower (p<0,0001) in FMF patients than the control group.
Conclusions: In the present study we were not able to define secondary carnitine deficiency in FMF patients, therefore usage of carnitine in all patients with FMF is not recommended.

P78

Featuring the phenotype of the FMF prototype

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Pediatric Rheumatology 2015, **13(Suppl 1):**P78

Background: The presentation of FMF is extremely variable, ranging from a quiescent to severe disabling disease. The M694V mutation is one of approximately 300 published genetic variations of MEFV and is thought to be associated with a typical clinical picture of the disease, but studies featuring the phenotype of homozygous M694V phenotype are meager.
Objectives: To describe the clinical trait of M694V homozygous FMF as compared to the phenotype of FMF with mixed MEFV genotypes.
Patients and methods: Fifty seven FMF patients homozygous for the M694V genotype were compared to 56 patients carrying other mutations. A questionnaire, including items related to demographic and clinical features was completed for each patient based on interview, physical examination and file notes.
Results: Compared with the control group, more patients, homozygous for the M694 mutation, suffered from a severe disease (p=0.001), had higher frequency of attacks before and during colchicine treatment (p=0.0001 and 0.0007, respectively), had more related diseases (p=0.0373) and needed higher dose of colchicine to control their disease (p=0.0001). Most other

Table 1(abstract P78)

Parameter	694 Homozygous (N=57)	Other mutations (N=56)	P
Average length of attack (days)	2.66±1.5	3.03±1.2	0.073
Abdominal attacks	50 (87.7%)	48 (85.7%)	0.788
Arthritis attacks	52 (91.3%)	28 (50%)	**<0.0001**
Pleuritis attacks	36 (46.2%)	18 (38.2%)	**0.0013**
Exertional leg-pain	47 (82.5%)	36 (64.3%)	**0.034**
ELE attacks	10 (17.5%)	3 (5.4%)	0.073
Attacks of fever alone	20 (35.1%)	12 (21.4%)	0.143
Average colchicine dose (mg/day)	1.9±0.48	1.48±0.54	**0.0001**
IV colchicine treatment	5 (8.8%)	0 (0%)	0.057
Proteinuria or amyloidosis	6 (10.5%)	1 (1.8%)	0.113
Anemia of chronic disease	14/53 (26.4%)	7/52 (13.5%)	0.142
Elevated acute phase reactants	10/18 (55.6%)	4/16 (25%)	0.092
Chronic renal failure	6 (10.5%)	0 (0%)	**0.027**
Chronic arthritis	11 (19.3%)	2 (3.6%)	**0.015**
Work days lost each month	4.4±7.2	2.6±4.6	0.718
Harm to quality of life	(1-10) 5.6±3.3	4.1±3	**0.013**
Number of attacks per year w colchicine	7.2±7.8	3.5±5.5	**0.0007**
Number of attacks per year w/o colchicine	23.6±9.3	15.6±11.7	**0.0001**
Crohn's disease	4 (7%)	2 (3.6%)	0.679
Ankylosing Spondylitis	3 (5.3%)	1 (1.8%)	0.619
Behcet's Disease	7 (12.3%)	1 (1.8%)	0.061
Henoch Schonlein Purpura	1 (1.8%)	0	1
All FMF associated diseases	17 (29.8%)	7 (12.5%)	**0.0373**

features tested (Table 1) appeared to be more pronounced in M694V homozygous patients (either with or without statistical significance).
Conclusion: The phenotype of FMF, as manifested in M694V homozygous patients, is the gold standard, to which other FMF presentations should be compared.

P79

Neonatal onset Familial Mediterranean Fever

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Pediatric Rheumatology 2015, **13(Suppl 1):**P79

Question: Familial Mediterranean fever (FMF) is an autosomal recessive disease, characterised by recurrent, self limited attacks of fever with serositis. Recently it was shown that FMF patients with early disease onset have more severe disease. The aim of our study was to describe the demographic, clinical and genetic features of FMF patients who had disease onset at the neonatal period.
Methods: Files of patients who had been seen in our department (during routine follow-up visits) between January 2013 and January 2014 were retrospectively evaluated. Patients with disease onset during the neonatal period were included to the study.
Results: Among 317 patients; 18 (7 females, 11 males) were enrolled. Consanguinity and family history of FMF were present in 28% and 56% of the patients, respectively. Clinical features seemed to be similar to general FMF patients; however, 50% of the patients were fussy children. The diagnosis of FMF was significantly delayed; the mean age at onset of therapy was 65.44 + 43.75 months. 38% of the patients had homozygous M694V mutation.
Conclusions: Patients with FMF could have complaints even in the neonatal period. The smaller the age of disease onset, the more likely their diagnoses are delayed. Homozygous M694V mutation is a prominent mutation in this group of patients.

P80

IL-18 serum concentration is continuously elevated in typical familial Mediterranean fever with M694I mutation and can distinguish atypical type

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Objectives: Familial Mediterranean fever (FMF) can be classified into typical and incomplete/atypical types based on clinical findings and gene analysis, although biomarkers that distinguish typical from atypical FMF have not been unclear.
Methods: We here investigated the serum cytokine profiles of IL-1β, IL-6, IL-8, TNF-α, IFN-γ, and IL-18 in FMF compared with those in Kawasaki disease.
Results: IL-1β, IL-6, IL-8, TNF-α, and IFN-γ were not increased in either type of FMF in the remission state and in controls, and IL-6 was elevated during attack periods among patients. Serum IL-18 levels were significantly higher in typical FMF patients with M694I *MEFV* mutation in remission than in controls at the same level as flared Kawasaki disease, which further increased during attack periods. In contrast, IL-18 levels in atypical FMF with P369S-R408Q mutation or in typical FMF without M694I mutation was not increased, in either disease states.
Conclusion: Thus, serum IL-18 levels at attack increase more than in remission, and that are an excellent marker to distinguish between the two types of FMF.

P81

Compliance to colchicine treatment and disease activity in Familial Mediterranean Fever (FMF) patients in Middle/Black Sea Region of Turkey (in Çorum region)

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Background and question: Colchicine is the gold standard treatment for prevention of inflammatory attacks and prevention of reactive amyloidosis in FMF. However, noncompliance to colchicine treatment is common among FMF patients. On the other hand, the disease may not be controlled in some patients despite use of full dose colchicine. In this study, we aimed to investigate the rates of disease control, compliance to colchicine treatment, and need for an additional treatment despite use of full dose colchicine in patients with FMF in Çorum region where FMF is common in Turkey.
Methods: The patients with FMF who admitted to Rheumatology Clinic established 1 year ago in a tertiary medical center located in Corum province, and followed up with the diagnosis of FMF for at least 6 months were analyzed. A total of 96 consecutive patients who admitted to our center in last 3 months, fulfilled Tel-Hashomer FMF diagnostic criteria, and on colchicine treatment were included in the study.
Results: The mean age of the patients was 33.1±10.8 years, female/male ratio was 65/31, and the mean disease duration was 20.6±14.4 years. 39.6% of the patients included in the study had at least three or more attack in last 12 months (2.2% of the patients had 25 or more attack, 4.2% patients had 12-24 attack, 18.8% patients had 5-11 attack and 11.5% patients had 3-4 attack in last 12 months).
The mean attack frequency in last 12 months was determined 3.2. The levels of acute phase reactants were high in 27.3% of the patients examined in attack free period. 4.2% patients had markedly proteinuria.
Use of colchicine was very regular (>90%) in 38.5%, predominantly regular (75-90%) in 26%, and moderately regular (50-74%) in 21.9% of the patients while 8.3% of the patients reported that they used colchicine sometimes, or only during attacks (90%) use of colchicine, 10.8% of the patients reported at least 3 or more attack in last 12 months, and 16.2% of the patients had high acute phase reactant levels in attack free period. 1 patients were on anti-IL-1 antagonist treatment.
Conclusions: In real life, the disease was not under control in 39.6% of FMF patients who used colchicine. 35.5% of FMF patients did not use the drug regularly, and an additional treatment was needed in 10.8% of the patients despite regular use of the agent in a region of Turkey where FMF is common (in Çorum region).

P82

How long is the diagnosis of Familial Mediterranean Fever (FMF) delayed in a region where FMF is common in Turkey?

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Pediatric Rheumatology 2015, **13(Suppl 1):**P82

Background and question: Similar to other autoinflammatory diseases, diagnosis of FMF is often missed and markedly delayed, particularly when its prevalance is very low in a community. It's reported that diagnosis of FMF might be delayed more than 20 years. Although Turkey is one of the countries with a high prevalence of FMF, the diagnosis of the disease is markedly delayed in clinical practice.
In this study, we aimed to investigate the delay of diagnosis in patients followed up due to, or new diagnosed with FMF in the newly established Rheumatology Unit of a big tertiary medical center located in Çorum region. Çorum province is one of the region where FMF is very common in Turkey.
Methods: Consecutive 112 patients (38 male, 74 female) who admitted for follow up in the previous 3 months, fulfilling Tel-Hashomer FMF criteria were included in this study.

Results: The mean age of the patients was 32.9 ± 11.7 years (range 17-59, median 30), mean age at the onset of disease was 13.8 ± 8.8 years (range 2-40, median 10), and the mean age at diagnosis was 25.7 ± 12.8 years (range 3-57, median 23). The mean delay for diagnosis was found as 12 ± 11.8 years (range 0-49, median 8). There was a delay of 10 years or more in 53.6% of the patients, and the delay for diagnosis was 20 years or longer in 11.6% of them.
Among the patients with a delay of diagnosis more than 10 years, before diagnosis of FMF, 11.7 % of the patients were diagnosed with acute rheumatic fever, 5.0% of the patients were diagnosed with rheumatoid arthritis or juvenile chronic arthritis, 8.3% of the patients were diagnosed with spondylarthritis, and 10.0% of the patients were diagnosed with infection and 13.3% of the patients were diagnosed with other disease. 51.6% of the patients did not have any diagnosis.
There was abdominal pain in 92%, fever in 87.5%, arthritis in 35.7%, chest pain in 21.4%,, erysipelas-like rash in 12.5% and history of an increase in acute phase reactants during attack in 97% of our FMF patients. Family history was positive in 65.4% of the patients, and 43.9% of them had history of surgery.
Conclusions: It is not difficult to diagnose FMF after onset of its clinical symptoms. But the diagnosis of FMF delayed for more than ten years above 50% of patients in Çorum region in Turkey. The most important reason for delay of FMF diagnosis in this region may be lack of "clinical suspicion" for the FMF among primary care and other physicians. Therefore measures must be taken to increase awareness of FMF in physicians and in community in this region.

P83

Serum uric acid levels in patients with Familial Mediterranean Fever and healthy controls

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Introduction: Familial Mediterranean Fever (FMF) is one of the best described auto-inflammatory diseases. It has also been suggested recently that gout is an autoinflammatory disease. Monosodium urate crystals are known to induce inflammation by complex cellular mechanisms, mainly involving inflammasome and toll-like receptors which are also involved in the pathogenesis of inflammation in FMF. Uric acid itself has been reported to influence inflammatory responses. Hyperuricemia is defined as serum uric acid levels > 6.8 mg/dl. In this study, it was aimed to investigate whether uric acid, which is a well-known risk factor for gout, is also a contributory risk factor for FMF.
Methods: A retrospective review was made of the charts of a total of 40 patients (23 female, 17 male; mean age: 31± 9.7 years) with FMF and 43 age and gender-matched healthy controls. The patient demographics, clinical findings and serum levels of creatinine, glucose, CRP, uric acid and erythrocyte sedimentation rate were recorded. Patients with creatinine levels > 1.2 mg/dL, renal amyloidosis or diabetes mellitus were excluded from the study.
Results: The mean serum uric acid levels were 4.5 ± 1.3 mg/dL in patients with FMF and 4.05 ± 1.04 mg/dL in healthy control subjects, and the difference was statistically significant (p = 0.04) [Table 1]. Peritonitis followed by arthritis was the dominant symptom during FMF attacks. Blood tests were applied during an FMF attack in 20 patients. There was no statistically significant difference in respect of serum uric acid between FMF patients with or without an attack (4.3 vs 4.6, respectively, p =0.7). The serum uric acid levels were determined as not significantly different between FMF patients with or without arthritis (4.5 vs 4.4, respectively, p=0.7).
Conclusion: In this study, serum uric acid levels were found to be higher in FMF patients than in the healthy control subjects. Further prospective studies are needed to reveal the role of uric acid in the pathogenesis of FMF.

References

1. Punzi L, Scanu A, Ramonda R, Oliviero F: **Gout as autoinflammatory disease: new mechanisms for more appropriated treatment targets.** *Autoimmun Rev* 2012, **12(1)**:66-71, doi: 10.1016/j.autrev.2012.07.024. Epub 2012 Aug 2.
2. Pétrilli V, Martinon F: **The inflammasome, autoinflammatory diseases, and gout.** *Joint Bone Spine* 2007, **74(6)**:571-6, Epub 2007 Aug 21.
3. Crişan TO, Cleophas MC, Oosting M, *et al*: **Soluble uric acid primes TLR-induced proinflammatory cytokine production by human primary cells via inhibition of IL-1Ra.** *Ann Rheum Dis* 2015, ii, annrheumdis-2014-206564. doi: 10.1136/annrheumdis-2014-206564. [Epub ahead of print].

P84

Familial Mediterranean Fever associated diseases in children

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Question: Familial Mediterranean fever (FMF) is an autosomal recessive disease, characterised by recurrent, self limited attacks of fever with serositis. Certain diseases were more commonly detected in patients with FMF. The aim of our study was to investigate the frequency of FMF-associated diseases in children.
Methods: Files of FMF patients who had been seen in two reference hospitals in Ankara, in the last two years, were retrospectively evaluated. Patients with FMF and concomitant diseases were incuded to the study.
Results: Among 600 FMF patients 30 (18 females, 12 males; mean age 14,72 ± 5.47 years) of them (5%) were found to have a concomitant disease. Fourteen patients had juvenile idiopathic artritis; 5 had sacroilitis (3 of them had HLA B27 positivity); 6 had inflammatory bowel disease and 5 had other diseases including a patient with Behçet's disease and one with systemic lupus erythematosus. Mean age at FMF onset and associated disease onset were 54,00 ± 46,35 months and 90,46±51,65 months, respectively. 52% of the patients had homozygous M694V mutation. Classical FMF attacks were present in 26 patients; remaining 4 patients had atypical symptoms but had 2 mutations.
Conclusions: Certain inflammatory diseases were more frequently detected in patients with FMF during childhood. In countries where FMF is prevalent clinicians dealing with FMF and other inflammatory diseases should be aware of these associations.

P85

Musculoskeletal complaints in patients with Familial Mediterranean Fever

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Question: Familial Mediterranean fever (FMF) is an autosomal recessive disease, characterised by recurrent, self limited attacks of fever with serositis. The aim of our study was to describe the frequency of musculoskeletal complaints in children with FMF and investigate the effect of genotype on these findings.
Methods: Files of patients who had been seen in our department (during routine follow-up visits) between January 2013 and January 2014 were retrospectively evaluated. Patients with two mutations were divided into 3 groups; M694V/M694V, M694V/Other mutation and patients carrying two mutations other than M694V. Patients with one mutation were divided into 2 groups; M694V and non M694V carriers.
Results: The study group comprised 317 FMF patients (170 females, 147 males) with a mean age of 12.2 ± 5.7 years. The frequency of musculoskeletal complaints were as follows; arthritis 18%, arthralgia 43%, leg pain 43%, heel pain 36%, myalgia 8%, protracted arthritis 2%, protracted febrile myalgia 2%. Leg pain and heel pain were more frequently detected in patients with homozygous M694V ($p<0.05$). Among patients with heterozygous mutations; children with M694V mutation had more frequently artralgia, leg pain and heel pain ($p<0.05$).
Conclusions: Musculoskeletal problems were common complaints in patients with FMF. Genotype seems to effect the frequency of these problems and M694V mutation is a predisposing factor for musculoskeletal complaints.

P86

Zytokin profiles in familial Mediterranean fever patients: IL-18 as a major cytokine and potential biomarker or target

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Introduction: Familial Mediterranean fever (FMF) is an autoinflammatory disease which is characterized by recurrent episodes of typical attacks with fever and abdominal pain. The pathophysiology shows an ineffective pyrin which leads to brakeless inflammation. It is not exactly known which factors lead to initiation of the attacks. Furthermore there seems to be sustained inflammation between the attacks.
IL-18 is acytokinethat belongs to theIL-1superfamily and is produced bymacrophagesand other cells. It plays an important role in the initiation and maturation of the inflammasome and is therefore able to induce severeinflammatory reactions.
Objectives: In our study we aimed to clarify the cytokine profiles of patients with genetically confirmed FMF in mainly attack free periods and compare it with healthy controls.
We evaluated type 1 cytokines: IFN-g, TNF-b, type 2 cytokines: IL-5, IL-10, IL-13 and other inflammatory cytokines: IL-1, IL-6, IL-17, IL-18, TNF-a and GMCSF.
Patients and methods: We examined 42 samples of 36 FMF patients between 07/2008 and 03/2015. Six patients were evaluated at two time-points. Ten healthy subjects were examined as control group. The levels of the cytokines were determined by commercial ELISA kits.
Results: The mean age of the FMF patients was 34,1 years, 43 % with homozygotic and 57 % with combined heterozygotic mutations.
Serum levels of IL-18 (normal range up to 400pg/ml) were 1946 pg/ml in mean for FMF patients. None of the controls showed an increased level. Serum levels of homozygotic patients were with 3654 pg/ml (mean value) higher than in patients with combined heterozygotic mutations (1290 pg/ml).
Patients with a complicated form of FMF (no colchicine response, therapy with IL-1 antibody) have the highest values of IL-18 (4233 pg/ml mean). It remains unclear if this is a consequence of IL-1 antibody treatment or active disease. All of the eight FMF patients which showed no increase in IL-18, had only a mild disease-activity and heterozygotic mutations.
All other cytokines showed no significant differences between the FMF patients and the control group with a tendency of higher IL-10 and IL-13 levels. IL-1 and IL-6 levels were not elevated. The results of the 6 patients which were evaluated at two time-points showed repetitive and comparable results.
Conclusion: Our results suggest a major role of Interleukin 18 in pathogenesis and disease course of familial Mediterranean fever. Further studies need to clarify the specific role of IL-18 and the option as a potential marker for severe disease and a target for therapy in FMF patients.

P87

Over representation of the A allele in the IL23R rs1004819 polymorphism in M694V homozygote non-responsive FMF patients

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Objectives: Recent studies have shown that interleukin-23 receptor (IL23R) polymorphisms confer susceptibility to ankylosing spondylitis, psoriasis, psoriatic arthritis and Crohn's disease. The A allele of rs1004819 was found in a significantly higher frequency among patients suffering from these diseases compared to controls. We aimed to determine the affect of rs1004819 in M694V homozygote FMF patients.
Methods: We typed 59 M694V homozygote FMF patients for the rs1004819 polymorphism, 27 of whom were defined as non responders to colchicine treatment. In addition we typed 57 ethnically matched controls.
Results: We found an over representation of the A allele in the non responders (A-29, G-25) as compared to the controls (A-33, G-77) (p=0.01), while a similar frequency was found between the responders (A-24, G-40) and the controls (p= 0.3).
Conclusions: These results suggest that IL23 and its pathways are involved in the FMF inflammatory response. This association may provide new insights and treatment possibilities for FMF patients who do not respond to colchicine.

P88

Renal biopsy findings in children with FMF in Armenia: trends over the study period

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Amyloidosis is not the only renal involvement in FMF pediatric patients.
The purpose of this study is to evaluate the renal biopsy results and look for the trends over the time in FMF children.
Methods: From 1993 to 2014 renal biopsies were done in 83 FMF patients with renal involvement (mean age 12±4 year; range 2.2-18; 45 males) at "Arabkir" MC -ICAH. The diagnosis of FMF based on international clinical criteria and genetic analysis.
Results: Renal amyloidosis (RA) revealed in 56 patients out of 83 (67%), who actually had never been treated by colchicine and FMF as well as amyloidosis were diagnosed initially on admission.
32.5% of FMF patients had other nephropathies: minimal change nephrotic syndrome (7); focal segmental glomerulosclerosis (7); acute postinfection glomerulonephritis (6); IgA nephropathy/Henoch-Schönlein nephritis (2/2); membranoproliferative glomerulonephritis type I (1); thin basement membrane disease and tubulointerstitial nephritis (1 of each).
Since 2003 the long-term program on "Early diagnosis and treatment of FMF in children in Armenia" has been implemented at National Pediatric Centre of FMF (NPC FMF) and more than 2700 children with FMF got regular colchicine therapy up to now. This resulted in *dramatic* decline of frequency of RA from 2003 to 2014: only two cases of amyloidosis have been registered in NPC FMF, while, prior to the regular colchicine treatment 16.2 % of FMF patients developed amyloidosis.
In the past 10 years a tendency of lowering the number of amyloidosis in children has been observed throughout Armenia as well (42% in 1993-2003 vs 25% in 2004-2014). Moreover during last 5 years there were only 6 pediatric cases of renal amyloidosis. Most of the patients had never received colchicine therapy.
Conclusion: • In the last 10 years RA frequency has markedly decreased at NPC FMF due to the long-term program on improving of early diagnosis and regular colchicine treatment in FMF children. This program should be continued as the number of FMF patients is constantly increasing and amyloidosis is still a problem in children in Armenia.
• Amyloidosis is not the only type of renal involvement in FMF.
• The biopsy is mandatory in any case of renal involvement.

P89

Quality of life changes with canakinumab therapy in adults with colchicine resistant FMF

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Introduction: Familial Mediterranean Fever (FMF), the most common form of the hereditary autoinflammatory disorders, is characterized by recurrent attacks of fever along with serosal or synovial inflammation lasting usually

12 to 72 hours. FMF is associated with impaired functional ability, and the persistent disabling features and chronic pain, emotional and physical limitations can have a negative impact on the health-related quality of life (QoL) of the patients.
There is no established treatment available for those resistant or intolerant to standard of care colchicine treatment. Interleukin-1 (IL-1) plays a pivotal role in the pathogenesis of crFMF. Canakinumab, a fully human, selective, anti-IL-1β monoclonal antibody, binds to IL-1β and inactivates its signalling activity. Gul et al. have described the efficacy and safety of canakinumab in adults with colchicine resistant (cr) FMF in a local pivotal phase II trial. Here, we report the effect of canakinumab treatment on QoL measured by SF-36 Questionnaire.
Objectives: This study aimed to show the effects of canakinumab treatment on quality of life by 8 sub-items of SF-36 as well as to see the correlation between the Physician Global Assessments (PGA) and SF-36 scores.
Methods: 9 crFMF patients with ≥1 attack/month in the preceding 3-months despite the highest tolerated colchicine dose entered the study. Canakinumab injections were administered at Day 1, Day 29 and Day 57. Changes in the quality of life was recorded in 9 subjects by using SF-36 at Day 1, 8, 29, 57, 86, 115 and the end of the study.
Results: In all 8 items (physical functioning, role limitations due to emotional/physical health, energy, emotional-well being, body pain, social functioning and general health) of SF-36 scores improved dramatically with canakinumab treatment, starting from 1st day. However the differences in emotional well-being and role limitations due to emotional problems scores couldn't reach the statistical significance. All scores are showed in tables below. Also there was a strong negative correlation between Physician Global Assessment (PGA) and Physical Component Score (R-sq: -0,793). A weaker correlation observed between Mental Component Score and Physical Global Assessment (R-sq: -0,540).
Conclusion: Canakinumab treatment in cr-FMF patients resulted in a rapid improvement in QoL measures. Also these improvements sustained during the withdrawal period. PGA scores appear to be in compliance with the Physical and Mental Component scores of the SF-36 questionnaires. A stronger correlation was observed for the Physical Component Score.

P90

Epidemiology of colchicine resistant Familial Mediterranean Fever disease (CrFMF) in Turkey

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Introduction: Familial Mediterranean fever disease (FMF) is an autosomal recessively inherited disease characterized by recurrent, self-limited febrile episodes (attacks) with serositis, synovitis, and occasionally skin involvement. The disease primarily affects people of eastern Mediterranean descent, typically presenting at age <20. AA amyloidosis is the most serious complication of FMF and can be life-threatening. Daily colchicine is considered standard of care, and is expected to prevent attacks and amyloidosis in most patients. Turkey has the highest prevalence with 0.1% in general population.
Objectives: This study aimed at exploring the potential number of FMF patients with colchicine resistance in Turkey.
Methods: The study was based on expert opinions, as there is currently no defined diagnosis criteria or available data to estimate the patient number. In the first stage of the study, a questionnaire inquiring the type, frequency and duration of the health care resources used for diagnosis and treatment of the disease was prepared. Specific questions were also asked about the epidemiology of the disease and colchicine resistant patients. Ten medical experts were involved in the study. The questionnaire was e-mailed to the experts and median numbers of their answers were calculated. In the next stage, combined answers were discussed in a face-to-face meeting with the experts. All items were discussed one by one until a consensus is reached about the type, frequency and duration of health care resources used during the diagnosis and treatment of FMF patients.
Results: According to the expert views the prevalence of the disease is 0.1%. Among these patients, 65% are responders to colchicine treatment, 30% are partial responders and 2-5% are colchicine resistant. Colchicine resistant FMF (crFMF) patients are defined by consensus as *patients who have ≥ 1 attacks per month despite maximum tolerable dose of colchicine treatment for 6 months period.*
Conclusion: Since FMF is highly prevalent in Turkey, there is an unmet need for CrFMF patients. It is estimated that approximately 2.300 CrFMF patients are present in Turkey. This study has also shown that IL-1 agents are used in treatment of CrFMF patients in Turkey.

P91

Clinical and genetic peculiarities of vasculitis associated with Familial Mediterranean fever in Armenian children

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Introduction: Familial Mediterranean fever (FMF) is the most common hereditary disorder among Armenians. It manifests mainly in childhood and represents a significant health care pediatric problem. The clinical picture of FMF and vasculitis have much in common: fever, abdominal pain, arthritis, myalgia, skin lesions. Numerous data indicate a higher incidence of vasculitis in FMF patients, compared with healthy ethnically matched populations.
Objective: To investigate clinical and genetic peculiarities of vasculitis associated with FMF in children in Armenia.
Methods: A group of 715 children with FMF was observed at the National Pediatric Centre for FMF (438 boys, 277 girls, mean age 8.64 ±0.17). The diagnosis of FMF was confirmed based on the Tel-Hashomer criteria and molecular genetic detection of MEFV mutations. For statistical analysis standard statistical Epi-Info 2000 Program was performed.
Results: Frequency of vasculitis in Armenian children with FMF was rather high - 4.3% (31 children). Henoch-Shonlein Purpura (HSP) was diagnosed in 1.5% (11) patients, Protracted Febrile Myalgia (PFM) - in 2.7% (20). FMF in these patients characterized by early onset (mean age 3 years), high (4 fold) risk of PFM [RR = 3.90 (1.32 ÷ 11.35); $\chi 2 = 5.94$; $p = 0.015$], as well as late diagnosis of FMF (9.42 ± 0.72) and late onset of colchicine treatment. They had also high frequency of severe FMF attacks, prevalence of acute recurrent arthritis and HSP and PFM manifestation after 5-6 year of FMF onset. The risk of HSP was 5-fold increased in children with severe FMF compared with moderate activity of disease. The development of vasculitis was associated with M694V-homozygous and compound-heterozygous genotypes. Particularly, HSP and PFM were observed respectively at 2.9% and 4.6% M694V-homozygous patients ($\chi 2 = 8.27$; $p < 0.02$), which confirms the influence of *MEFV* genotype on the development of vasculitis.
Conclusion: Armenian children with FMF had higher than expected frequency of vasculitis (4.3%). We suppose, that in children with FMF: 1) HSP and PFM vasculitis might be considered as markers of severe FMF and early disease onset 2) M694V homozygous genotype is a risk factor for the development of PFM. These results are consistent with data on the susceptibility of FMF patients in ethnically matched populations in the development of HSP and PFM *[Lange-Sperandio B. et al., 2004]*.MEFV mutation genetic screening is recommended for Armenian children with HSP, PFM vasculitis for early diagnosis of FMF, treatment and prevention of complications.

P92

Clinical and subclinical features and MEFV mutation distribution in of FMF patients' siblings

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Objective: Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever and peritonitis, pleuritis, arthritis or an erysipelas-like skin disorder. The disease may present at any age, more than 80% of patients being symptomatic by the age of 20 yr. Its main long-term complication is amyloid A (AA) amyloidosis, a severe manifestation with poor prognosis. Mutations in the MEFV gene, on chromosome 16th, is encoding a protein named as pyrin. We aim to analyze clinical characteristics, subclinical inflammation, and carried MEFV mutation in siblings with FMF.
Methods: We obtain FMF patients who were followed in Erciyes University Faculty of Medicine, department of pediatric rheumatology and their siblings. All children were evaluated with a questionnaire containing 21 questions which symptoms may be signs of FMF. All subjects were invstigated for laboratory disease features, genetic analysis of MEFV mutations.
Results: The study included 53 pediatric patients and their 76 sibling total 129 children from 50 different families. In patients, the most frequent mutations were homozygous pM694V (60%), pM694V/pM680I (9.5%), pM694V/pV726A (3.6%) and heterozygous pM694V mutation (3.6%). Therefore, in siblings of the patients had mostly homozygous pM694V (12%), pE148Q/P369S and heterozygous pM694V mutation (35.5%) while 11 (14.5%) sibling had no mutation in MEFV gene. We also completed a questionnaire form included findings of FMF in all subject. We obtained the typical history of FMF (the presence of fever, recurrent typical attacks of FMF (including peritonitis, pleuritis and arthritis); and transient inflammatory response) in 9 of 76 siblings (12%) from the questionnaire. In addition, the presence of rare but important manifestations such as erysipelas like erythema and leg pain of FMF were determine in patients' siblings. The exertional leg pain had found in 21 siblings (28%) who carried at least one mutation in the MEFV gene. Also we evaluated acute phase reactants (ESR, CRP, SAA and S100A protein) in all subjects. The siblings with homozygous mutation had elevated levels of SAA and S100A protein than others.
Conclusion: Our findings showed that siblings with FMF had different clinical findings each other. In these children should be questioned in terms of FMF findings before screen mutation in MEFV. Fever, serositis symptoms and musculoskeletal symptoms in children, especially presence of homozygous mutation, even if there has no typical attacks, we believe that the colchicine treatment should be considered.

P93

Arterial stiffness as a model to dissect chronic inflammation in FMF

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Background: Familial Mediterranean fever (FMF) is an autoinflammatory disorder, characterized by short attacks of sterile serositis, successfully suppressed by continuous colchicine treatment. Subclinical chronic inflammation however, is a frequent finding in FMF, manifested with elevated levels of inflammatory markers. (CRP, SAA). While chronic inflammation is considered an important cardiovascular (CV) risk factor in most inflammatory disorders, findings on the impact of chronic inflammation in FMF are conflicting. Chronic inflammation is an important cause of arterial stiffness, an early sign and independent risk factor of cardiovascular atherosclerosis. Pulse wave velocity (PWV) measurement is a useful marker of arterial stiffness.
Objectives: To study arterial stiffness in FMF and evaluate its predisposing factors.
Methods: Eighty consecutive FMF patients without known CV risk factors, were enrolled to the study, during their scheduled visit to the clinic. Demographic, genetic, clinical, and laboratory data were obtained from their file and examination. Arterial stiffness was determined through pulse wave velocity (PWV), measured by Sphygmocor system algorithm (AtCor Medical, Sydney, Australia). The recorded PWV values were standardized per age and blood pressure and coded as PWV_Z, relating to the reference general population.
Results: Compared with the adjusted general population, FMF patients displayed normal PWV values, even with a significantly reduced dispersion around the mean (5% vs 10%, p=0.02). In a trial to identify pro- or counteracting driving factors no statistically significant variables were found, yet the dose of the colchicine demonstrated the strongest trend for normalizing PWV.
Conclusion: Arterial stiffness is not increased in FMF. Our data support a role for colchicine prophylaxis in this finding.

P94

Prevalence of Mediterranean fever gene mutations in clinically suspected FMF patients in Algeria

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Introduction: The familial Mediterranean fever (FMF, OMIM 249100) is an autosomal recessive auto-inflammatory disease primarily occuring in Armenian, Turkish, Jewish and Arabic populations. The first clinical symptoms of FMF usually appear in childhood. The chronic relapsing inflammation of the serous membranes leads to febrile attacks often associated with abdominal, joint and/or chest pains. The most common mutations associated with FMF were identified in exon 10 of *MEFV* located on the short arm of chromosome 16p13.3. *MEFV* consists of 10 exons and encodes for pyrin/marenostrin involved in the regulation of NLRP3-inflammasome activity.
Objectives: In Algeria, FMF is clinically well diagnosed but the disease-causing variations in *MEFV* remain poorly explored. This study aimed to explore the most recurrent mutations in exon 10 in suspected Algerian FMF patients.
Patients and methods: This study included 84 unrelated Algerian patients (42 males and 42 females) aged between 2 to 56 years. All the patients were recruited from Algerian hospitals and were clinically suspected to have FMF. Genomic DNA was extracted from peripheral blood samples using a standard protocol. Three mutations p.M694I, p.M694V and p.M680I were initially analyzed by PCR-ARMS. In the 84 patients, resequencing the entire coding region of exon 10 was performed on the amplified products on an ABI Genetic Analyzer for confirmation of the identified mutations and identification of others.
Results: Genetic analysis showed that 33/84 (39.28%) of patients carried at least one mutation in exon 10. The most recurrent mutation was p.M694I which accounted for 18.45% of the total alleles (n= 31/168), followed by p.M680I (8.33%; 14/168), p.M694V (2.38%; 4/168) and p.A744S (2.38%; 4/168). The allele p.M694I accounted for 58.49% of the mutant alleles. Among the patients with mutations, 9 patients were homozygous, of them 7 were p.M694I/p.M694I, 11 were compound heterozygous, of them 9 were p.M694I/p.M680I and 13 were heterozygous. In the rest of the patients, no mutation could be identified.
Conclusion: The current study shows clearly the predominance of the p.M694I mutation among the Algerian FMF patients which confirms our precedent results. The mutational profile identified here offers a tool for guiding the molecular diagnosis of FMF in Algeria.

P95

Development of focal segmental glomerulosclerosis in a patient with Familial Mediterranean Fever resistant to colchicine therapy under treatment with Canakinumab

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Introduction: Familial Mediterranean Fever (FMF) represents an autoinflammatory disease caused be MEFV gene mutation characterized with attacks of polyserositis, commonly seen among Turks, Arabs, Armenians and Jews. Therapy with colchicine was proven to be effective in treatment of FMF polyserositis and in prevention of amyloidosis development. Colchicine resistant FMF is defined as 6 or more attacks of polyserositis in a year, despite the regular and sufficient usage of colchicine. The percentage of resistant FMF has been reported as a 5-10% in the literature. Although colchicine remains the gold standard for the FMF treatment, anti-IL-1 drugs are used in the cases of FMF resistant to colchicine therapy. Particularly carriers of the M694V homozygous mutation prone to be resistant to standard therapy and show a risk for development of additional diseases.
Objective: In this case report, we are presenting a M694V homozygous, colchicine resistant FMF patients with the diagnosis of inflammatory bowel disease, who developed proteinuria, and who was consequently diagnosed with focal segmental glomerulosclerosis (FSGS).
Case report: A two years old female patient, admitted to our hospital with high fever and severe abdominal pain. She was complaining of high fever (until 39^0 C) lasting for 3 days accompanied with severe abdominal pain, being repeated every week for the last 6 months. The patient was hospitalized at Gastroenterology department where she was diagnosed as an inflammatory bowel disease and treated with sulphasalazine and low dose prednisone. No clinical response to the therapy was a reason for rheumatologic consultation. Clinical findings of the patient and her higher acute phase reactant levels are compatible with FMF. Colchicine 1 mg/day was started with 1 mg/day. MEFV gene analysis was revealed homozygote M694V mutations. In spite of initial clinical improvement, attacks of disease started to repeat so the patient was consider to be a resistant to standard therapy so the therapy with Anakinra 50mg/day was initiated. Because of partial clinical response to Anakinra, canakinumab 5 mg/kg/dose once in every two months was added. As a result, no disease attack was registered during the time period of one with decreased acute phase reactants to normal value. In further follow up of patient, disease attacks restarted so the canakinumab dose interval was decreased to be given once per month. Although the initial analysis of urine was found to be normal, control urine tests revealed a proteinuria and hematuria. The renal ultrasound examination was normal. Serological tests: ANA, Anti-ds-DNA, C3, C4 levels were normal. Daily protein excretion was measured as a 625 mg/day. Persisting proteinuria and hematuria were a reason to perform a renal biopsy's which suspected amyloidosis. Pathological examination showed mesengial cell proliferation and segmental sclerosis by PAS stain. Congo stain was negative for amyloidosis. Proteinuria together with mentioned pathological findings was compatible for FSGS. Since the pulse prednisone therapy showed no clinical improvement, cyclosporine A was added in therapy. FMF attacks (fever, abdominal pain) restarted so the canakinumab dose was increased to a 65 mg/day. Attacks were disappeared.
Conclusion: Amyloidosis and other renal pathological conditions should be suspected in the case of proteinuria developed at FMF patient being treated regularly with colchicine and other treatment modalities. It is important to consider a FSGS in differential diagnosis of FMF patients with renal involvement.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P96

Articular involvement in childhood Familial Mediterranean Fever

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Introduction: Familial Mediterranean fever is an autosomal recessively inherited autoinflammatory disease which is clinically manifested with periodic episodes of fever, serositis and arthritis. Articular involvement is also a frequent presentation after fever and peritonitis in childhood FMF.
Objective: The aim of this study is to evaluate the demographic and clinical features of the common articular findings in the course of FMF and compare these with the relatively rare occasions of chronic arthritis and FMF comorbidity.
Patients and results: Among the 708 patients diagnosed with FMF; arthritis was defined in 288 (40,7%) (female/male 150/138) cases. In 192 (66%) children the affected joints were observed primarily as the ankles, subsequently followed by knees in 148 (51%) cases. The incidence of the rare articular involvements were respectively, the elbows in 14 (4,8%) children, the wrists in 11 (3,8%) children and the hip joint with the rate of % 2,7 of all cases. The shoulder girdle was affected only in one patient. Exertional leg pain was reported by 467 (66%) patients. Erysipelas like erythema coincidentally found in 213 (73%) cases and in 26 (3,7%) patients enthesitis was detected. Articular findings were mainly acute monoarthritis without leading to sequelae.
When the children presented with arthritis were considered, the mean age at disease onset was estimated to be 5,2 ± 3,7 years and the mean age at diagnosis was 8,1±4 years.
The analysis of MEFV genetic mutation of the patients with arthritis also resulted mainly as homozygote M694V mutations with the rate of 104 (36,1%); heterozygote M694V mutations in 52 (18%) and M694V/M680I compound heterozygote in 26(9%) counting for the total of M694V mutations in 192 (66,6%) of cases. The rate of the rest of the mutations in exon 10 region was corresponded to 20(6,9%) and E148Q mutations, which is a common mutation in exon 2 region were found in 11(3,8%) of the all cases.
The mean duration of the episodes of FMF mainly manifested by articular symptoms was yielded as 97 ± 53,6 hours (with the median of 72 hours, ranging between 24-168 hours) and when compared with the cases primarily manifested with abdominal pain and fever that was declared to be 59,4±34,3 hours in a former study, a statistically significant difference was revealed ($p<0,0001$).
In 23 (8%) cases with the diagnosis of FMF and articular manifestations, a coincidental diagnosis of chronic arthritis was considered to be more consistent as the disease progressed. While 9 of the patients were turned out to have juvenile spondyloarthropathies (JSpA), 13 of them were identified as having oligoarticular juvenile idiopathic arthritis (JIA) and the remaining one was also diagnosed with seropositive polyarticular JIA. As 20 (%86) of those cases were presented with arthritis at the disease onset, with respect to the main clinical symptoms 6 (%26) cases of all showed episodes of abdominal pain and periodic fever at the same time. Among the ones with oligoarticular JIA, 7 (%30,4) of them were ANA positive and 5 of the 9 JSpA cases (%55,5) were found to be HLA B27 positive. MEFV genetic mutations of the children with the comorbidity of FMF and chronic arthritis were detected to be homozygote M694V in 5 (21,7%), M694V/M680I in 4(17,4%) and heterozygote M694V in 7(30,4%) of patients.
Conclusion: Articular involvement in FMF is acute monoarthritis typically affecting the lower extremities in a periodic manner with short duration and without sequelae formation. The clinical entity of chronic arthritis and FMF coincidence can emerge as a rare occasion. Especially with the cases of oligoarticular JIA that show recurrence and quickly respond to treatment, the diagnosis of FMF should also be kept in mind.

P97

Screening for inherited metabolic disorders in patients with Familial Mediterranean Fever

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Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disease, presenting with recurrent episodes of fever and polyserositis. Diagnosis of FMF is may be challenging especially in pediatric population. Mitochondrial fatty acid oxidation disorders and porphyrias can present with periodic abdominal and muscle pain. Incidence of both FMF and inherited metabolic disorders (IMD) are increased in Turkish patients partially due to high consanguinity rates.
Objectives: The aim of the present study is determine the inherited metabolic disorders in differential diagnosis of Turkish pediatric FMF patients.
Methods: 174 patients who were diagnosed as FMF enrolled the study. In all patients, a fasting dry spot blood sample was taken for acyl-carnitine analyses by tandem mass spectrometry. Fresh, light-protected spot urine test was performed for porphobilinogen screening. Second-tier test with urine organic acid analysis and urine porphyrin metabolites were performed if pathologic findings were detected in acyl-carnitine profile or in porphobilinogen screening, for confirmation. An age matched healthy 50 children served as control group.
Results: Of the 174 patients diagnosed with FMF, none of our patients was diagnosed with porphyria; two patients with fatty acid oxidation defect, one with multiple acyl-CoA dehydrogenase deficiency and one with possible medium-chain acyl-CoA dehydrogenase deficiency were detected during the study.
Conclusion: Our data revealed that screening for porphobilinogen for pediatric FMF patients is unnecessary, but an investigation of tandem mass based acyl-carnitine analyses can be helpful for the differential or additional diagnosis of FMF in developing countries that does not have nationwide expanded newborn screening programme.

P98

Could pentraxin-3 be a new marker for subclinical inflammation in familial Mediterranean fever?

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Introduction: Pentraxin-3 (PTX-3) is a long pentraxin that is structurally related to the short pentraxins as C-reactive protein (CRP). It is known to play an important role in innate immunity and inflammatory regulation. CRP and serum amyloid A (SAA) are sensitive and reliable markers of inflammation in FMF attack as well as chronic and subclinical inflammation during attack-free period. To date, there is no information about PTX-3 in FMF inflammation.
Aim: The aim of the study was to investigate the progress of serum PTX-3 levels together with traditional acute phase reactants in FMF patients during attack and attack free period (two weeks after the attack) and also assess whether PTX-3 could be related with subclinical inflammation.
Material and method: A prospective cross-sectional study was conducted between June 2013 and July 2014. A total of 45 consecutive children with FMF who were diagnosed according to the Tel-Hashomer and Yalçınkaya criteria were enrolled during the attack period. Blood samples were obtained from the patients during attack and attack free period (two weeks after the attack) and healthy children who were matched in terms of age and sex.
Results: The study group consisted of 45 children with FMF (24 boys, 21 girls, mean age 9.5±3.8 years) and 40 healthy children. In FMF patients attack white blood cell (WBC) count, CRP, erythrocyte sedimentation rate (ESR), fibrinogen, SAA and PTX-3 levels were significantly higher than attack-free period and healthy subjects. In attack-free period, there were no significant differences between patients and healthy children in terms of WBC, CRP levels. Although mean attack-free period ESR, fibrinogen and SAA levels were higher than the controls, those markers were within the normal range. Whereas, mean attack-free PTX-3 level was still significantly higher than controls.
Conclusion: Serum PTX-3 levels increased during the attacks of FMF and decreased during the attack free period however mean level of it was still higher than healthy subjects. We suggest that PTX-3 might be a new marker for both attack period and subclinical inflammation in FMF patients.

P99

Adrenomedullin levels in patients with Familial Mediterranean Fever: a long term follow-up

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Introduction: Familial Mediterranean Fever (FMF) is the most common periodic fever syndrome, characterized by recurrent fever and serositis attacks. It has been shown that there might be an ongoing subclinical inflammation between attacks. Adrenomedullin (ADM) is synthesized in endothelium, and has been shown to have high levels in patients with inflammation such as FMF. Colchicine is the treatment of choice and given once or twice daily depending on expert opinion.
Objectives: In this study, it was aimed to investigate ADM as a marker for inflammation in pediatric patients with FMF who are using colchicine in different dosage schema.
Methods: Pediatric patients diagnosed with clinically and genetically confirmed FMF diagnosis were included in the study. The Colchicine was started in one or two doses randomly. The clinical and laboratory parameters were assessed on six clinical visits made every two months. After

Table 1(abstract P98)

	During Attack (Mean±SD)	After Attack (Mean±SD)	Controls (Mean±SD)	P_1	P_2	P_3
WBC (x10^9/L)	12.1±4.6	8.1±2.5	8.2±2.5	***<0.001***	***<0.001***	*>0.05*
MPV (fL)	7.5±0.70	7.6±0.58	7.3±0.57	*>0.05*	*>0.05*	*>0.05*
CRP (mg/dL) (normal: 0-1)	4.7±4.2	0.15±0.19	0.11±0.15	***<0.001***	***<0.001***	*>0.05*
ESR (mm/hour) (normal < 20)	44.1±20.8	13.9±7.24	6.6±4.5	***<0.001***	***<0.001***	***<0.001***
Fibrinogen (mg/dL) (normal: 180-350)	371.0±70.9	257.0±55.3	220±45	***<0.001***	***<0.001***	***<0.001***
SAA (mg/L) (normal: 0-7)	306.5±283.1	5.5±3.8	4.1±1.2	***<0.001***	***<0.001***	***0.032***
Pentraxin-3 (ng/mL)	3.2±0.6	1.89±0.14	0.87±0.38	***<0.001***	***<0.001***	***<0.001***

P_1: During attack vs after attack, P_2: During attack vs controls, P_3: After attack vs controls

the third visit the dosing schema was changed to twice or once depending on the schema at the beginning.

Results: A total of 37 patients were included in the study. Mean age of patients was 7.78±2.00 years, mean age at disease onset was 5.05 ± 3.04 years and mean age at diagnosis was 7.51 ± 2.66 years. Twenty patients received colchicine in once daily dosage while 17 patients had in twice daily dosage at the beginning of the study. There were 10 patients with heterozygote and 27 with homozygote MEFV mutations. After the treatment was started all patients demonstrated improvement in clinical and laboratory findings such as erythrocyte sedimentation rate and C-reactive protein. However, ADM levels did not show any correlation with ESR and CRP levels. Mean ADM levels in six consequtive visits were as follows, first 322.19±161.92 ng/L; second 330.50±189.63 ng/L; third 339.54±168.03 ng/L; forth 378.11±177.63 ng/L; fifth 328.91±172.30 ng/L and sixth 326.25±165.87 ng/L. ADM levels were similar in all visits (p=0.954) and did not show any difference between the first and second three visits i.e. before and after changing the dosage schema (p=0.593).

Conclusion: The results indicated that patients using colchicine in once or twice daily doses did not demonstrate any difference considering clinical and laboratory findings and had similar effects in controlling disease manifestations. ADM levels did not demonstrate any alterations in all visits which may suggest the continuation of subclinical inflammation in these patients.

P100

Genotype -phenotype correlation of *MEFV* mutations in Eastern / Central European population

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Introduction: Familial Mediterranean fever (FMF) is a rare disease in Central and Eastern European countries and phenotype -genotype correlation in this population is not well established.

Objectives: To study phenotype of patients with *MEFV* mutations and periodic fevers or any other periodic complaints in Eastern / Central European population.

Methods: Patients who tested positive for *MEFV* mutations were included. Age at disease onset, delay to diagnosis, clinical picture and *MEFV* mutations were collected. Genetic testing was performed in the Genetic laboratory of University Children's Hospital Ljubljana. All 10 exons and exon/intron regions of *MEFV* gene were directly sequenced with ABI Prism 310 Genetic analyzer.

Results: Fifteen patients (8 male, 7 female) from Eastern / Central European population with median age at disease onset 4 years (range 1.5-17) and median age at *MEFV* testing 8 years (range 3-17) were included; 9/15 patients with mutations in exon 10 (M694V, K695R, A744S, S730F), 3/15 with mutations in exon 3 (P369S/R408E, P369S/R408Q) and 3/15 with mutations in exon 2 (E148Q, A289E). One patient had 2 mutations; in exon 9 (I591T) and exon 10 (K695R).

Variant K695R, found in 6 patients, was associated with a very heterogeneous clinical manifestations; 3 patients presented as PFAPA, 1 patient had clinical features of systemic juvenile idiopathic arthritis and macrophage activation syndrome, 1 patient presented with myelodysplastic syndrome and periodic fevers and later developed acute lymphoblastic lymphoma and one patient who was compound heterozygote for K659R and I591T presented with recurrent pericardial effusions, fever and at first attack at the age of 5 years appendicitis and hemophagocytic lymphohistiocytosis.

Variant M694V was found in 1 patient with typical FMF picture. Variant A744S was found in 1 patient with recurrent pericardial effusions and fever. Variant S730F, a novel variant, was found in one patient with severe recurrent abdominal pains, vomiting and arthralgia without fever.

Variant P369S/R408Q, found in 2 patients, variant A289E, found in 1 patient and variant E148Q, found in 1 patient, were associated with clinical features of PFAPA. One patient with variant E148Q had persistent abdominal pain. Variant P369S/R408E was found in 1 patient with recurrent abdominal pain without fever.

Conclusion: Patients from Eastern / Central European population with MEFV mutations presented with very heterogeneous clinical phenotypes. To better define clinical presentation of patients with *MEFV* mutations genetic testing should be performed also in cases with inconclusive FMF clinical presentation.

P101

L-ficolin and H-ficolin in patients with Familial Mediterranean fever

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Pediatric Rheumatology 2015, **13(Suppl 1):**P101

In the present study we performed comparative determination of the blood levels of L-ficolin and H-ficolin, opsonins and the initial components of the complement lectin pathway, in patients with Familial Mediterranean fever (FMF) in attack and attack-free periods and healthy subjects. In addition, in all study subjects serum levels of IL-1b and and total leukocyte count (TLC) were determined. Fifty FMF-affected subjects (female/male: 25/25; mean age ± SD: 31.3 ± 11; in attack/attack free: 23/27) and 81 age- and sex-matched healthy subjects (control group) without family history of FMF or other autoinflammatory diseases were enrolled in this study. Elevated H-ficolin level and TLC were detected in patients during attack period, whereas increased levels of L-ficolin and IL-1b were found in both attack and attack free patients with higher values during attack. Positive correlation between H-ficolin and L-ficolin levels in patients and healthy subjects was detected. Our results suggest excess production of L- and H-ficolins and increased apoptosis rate in FMF and indicate that H-ficolin is operating during development of acute autoinflammatory reactions, whereas L-ficolin is operating in both acute and subclinical autoinflammatory responses associated with this disease.

P102

Coexistence of PFAPA syndrome and FMF in Armenian children

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Introduction: Familial Mediterranean Fever (FMF) is an ethnic disease for Armenians. Recurrent attacks of fever with tonsillitis are also clinical symptom of FMF, especially in early childhood. They are also characteristic of the PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, adenopathy), which can be confused with FMF.

Objective: To investigate the peculiarities of the coexistence of PFAPA syndrome and FMF in Armenian children.

Methods: 28 children with recurrent episodes of fever and tonsillitis was observed at the National Pediatric Centre for FMF (19 boys, 9 girls, aged from 1 to 8 year). The diagnosis of FMF was confirmed by Tel-Hashomer criteria and molecular genetic detection of MEFV mutations. PFAPA syndrome was diagnosed based on recognized clinical criteria and excluding other causes of tonsillitis.

Results: Coexistence of FMF and PFAPA was in 10 out of 28 patients (36%). They had earlier FMF onset (mean age of 1y. 3 mo.), frequent febrile attacks of abdominal pain and/or pleuritis, pericarditis (7 out of 10 patients), aphthous stomatitis (6 patients), tonsillitis with cervical lymphadenitis (9 children), as well as marked increase of acute inflammatory markers. An average age of PFAPA manifestation was 2 years 2 mo. MEFV gene M694V mutation was found in 8 patients out of 10 with the following distribution of genotypes:: one homozygous genotype (V726A/V726A), 5 compound heterozygotes: (M694V/V726A (2), M694V/F479L (2), M694V/E148Q (1), 4 heterozygotes (M694V/N (3 children), V726A/N (1).

Isolated PFAPA syndrome was diagnosed in 18 out of 29 children (mean age of onset - 2 years 4 mo.). They had repeated attacks of fever and tonsillitis, rare - pleuritis or pericarditis (4 children), occasionally - mild abdominal pain and a moderately increased acute phase reactants. 13 out of 18 patients were carriers of one mutation, mostly M694V/N (9 out of 13). The frequency of PFAPA attacks in both groups of patients was similar (1-2 times a month).
Conclusion: The coexistence of PFAPA syndrome and FMF was noticed in 36% of investigated children. They were characterized by earlier FMF onset, severe course of disease with frequent febrile attacks and the prevalence of MEFV compound-heterozygous genotype. In contrast, among patients with isolated PFAPA syndrome prevailed heterozygous genotype. M694V mutation was the most frequent.
MEFV mutation genetic screening is recommended for Armenian children, especially under the age of 5, with recurrent fever and tonsillitis for early diagnosis of FMF, differential diagnosis with PFAPA syndrome, treatment and prevention of complications.

P103

Sweet's syndrome in a patient with compound heterozygous mutations in the Mediterranean fever gene (MEFV)

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Sweet's syndrome (SS) or acute febrile neutrophilic dermatosis is a rare disorder that often occurs in association with other systemic diseases.
The disorder is characterized by development of nonpruritic, painful erythematous plaques with pseudovesicles, occasional pustules and rare bullae. SS consists of a triad of erythematous plaques infiltrated by neutrophils in association with fever and leukocytosis. The pathological features of SS involve the dermis.
The condition presents in three clinical settings: classic (or idiopathic) SS, malignancy-associated SS and drug-induced SS syndrome.
The treatment of choice for SS are systemic corticosteroids, although colchicine and potassium iodide are also considered to be effective for SS.
We present an unusual recurrent course of SS in a 38 year old man who carries compound heterogous mutations in the MEFV gene.
A 38 year old, generally healthy man from Sephardic Jewish ancestry, had suddenly developed fever, malaise, artralgia and painful erythematous plaques with pustules and bullae on the anterior aspects of the upper extremities. Diagnostic evaluation included moderate leucocytosis, elevated erythrocyte sedimentation and C-reactive protein rate and normal liver functions. antistreptolysin O-titer. Blood cultures and tuberculosis test were negative. Chest radiography was normal.
The symptoms exacerbated despite treatment with systemic corticosterids. Clinical improvement appeared after administration of colchicine.
Mutational analysis of the MEFV gene revealed compound heterozygous M694V and V726A mutations.
Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever with serosal inflammation. The FMF gene (MEFV) encodes the protein pyrin that plays an important role in modulating the innate immune response.
MEFV mutations have been identified primarily in patients from Mediterranean populations and in Israel the carrier state is as high as1: 5.
Sweet's syndrome has been described in a patient with classical FMF as a possible new cutaneous feature and has never been described as a presenting sign of FMF.
Although various skin lesions have been described with FMF, erysipelas-like erythema (ELE) has been reported the only pathognomonic cutaneous manifestation.
Our patient, carrying compound heterozygous mutations in MEFV presented with Sweet's syndrome.
We suggest, that SS skin lesions might be an only cutaneous presentation of FMF.
Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P104

Chronic recurrent multifocal osteomyelitis in a patient with Familial Mediterranean Fever

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Introduction: Patients with more than one autoinflammatory disorder are rarely reported in the literature [1]. Additionally, rare reports suggest that *MEFV* mutations might be associated with atypical manifestations for familial Mediterranean fever (FMF), such as isolated recurrent muscle pain in one patient [2] and chronic recurrent multifocal osteomyelitis (CRMO) responsive to colchicine in another patient heterozygote for E148Q-P369S-R408Q *MEFV* complex allele [3]. We report a unique case of a patient with classic FMF developing CRMO later in the disease and discuss genetic test results, imaging features, and response to treatment.
Methods: A retrospective review of medical records was conducted and one radiologist reviewed all imaging studies. The molecular genetic testing for *MEFV*, *PSTPIP1*, *IL1RN*, *PSTPIP2*, and *LPIN2* was done by direct resequencing of the entire coding sequence and the splice sites.
Case report: A 13 years old Arabic female diagnosed with FMF at the age of 10 years based on recurrent 3-days episodes of abdominal pain every 2-3 weeks occasionally associated with fever but without chest pain, rash, or arthralgia for at least one year. Genetic testing revealed heterozygote p.M694V *MEFV* mutation. She was responsive to daily colchicine at 0.5 mg. At the age of 12 years she developed severe acute dorsal and lower back pain, and arthralgia of the lower extremities. MRI imaging demonstrated both epiphyseal and metaphyseal involvement of left femoral head, right proximal tibial metaphysis, right distal tibial metaphysis and epiphysis, proximal epiphysis of first metatarsal bone, bilateral knee synovitis with effusion, and D-11 vertebra and left sacroiliac disease. She received one cycle of monthly pamidronate for three months in addition to naproxen with clinical and radiological improvement as demonstrated in repeated MRI at 6 months intervals. Imaging showed full resolution of bone lesions without recurrences or new lesions but continue to demonstrate active knees arthritis at 24 months follow-up. Spine MRI showed resolution of inflammatory changes noted at D-11 with shortening of vertebral body height and end plate erosions. She developed pustulosis palmoplantaris at the age of 13 years. She is currently on colchicine 1 mg twice daily due to elevated serum amyloid A level despite being asymptomatic. Methotrexate and infliximab will be started due to persistent arthritis. HLA-B27 test was negative and testing for *LPIN2*, *IL1RN*, *PSTPIP 1* and *PSTPIP 2* did not detect any mutation in the coding region or splice sites.
Conclusion: The unique clinical course of our patient demonstrates the importance of considering a coexisting autoinflammatory disorder when patients present with atypical features of an already established and diagnosed autoinflammatory disease.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. Moussa T, *et al*: Overlap of Familial Mediterranean Fever and Hyper-IgD Syndrome in an Arabic Kindred. *J Clin Immunol* 2015, **35**:249-253.
2. Ben-Chetrit E, *et al*: The spectrum of MEFV clinical presentations-is it familial Mediterranean fever only? *Rheumatology* 2009, **48**:1455-1459.
3. Shimizu M, *et al*: Colchicine-responsive chronic recurrent multifocal osteomyelitis with MEFV mutations: a variant of familial Mediterranean fever? *Rheumatology (Oxford)* 2010, **49**:2221-2223.

P105

Hidradenitis suppurativa and familial Mediterranean fever: a report of 6 cases and literature review

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Introduction: Familial Mediterranean fever (FMF) is the most frequent monogenic autoinflammatory disease (AID). Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by recurrent abscesses and scarring in apocrine gland-bearing areas. HS shares common features with AID including a positive correlation between the increase of acute phase reactants (APR) and the severity of the disease. HS can be associated with other AID.
Objectives: To describe a series of patients who had both HS and FMF.
Patients and methods: Descriptive observational retrospective study relating the main characteristics of patients who had HS among the FMF cohort followed in the French adult reference center.
Results: Among 151 FMF adult patients, we identified 6 patients with HS, 2 women, with a median age of 36 years [range 27-70]. Median age for first symptoms of FMF was 11.5 years [3-30], and for colchicine administration 20.5 years [4-34] with a mean dosage at the time of the study of 1.25mg/day [1-2]. HS was diagnosed at a median age of 31.5 years [13-63]. Treatment consisted in consecutive antibiotics (5 patients), surgery (3 patients), and no treatment in one recent diagnosed patient. There was no concurrence between HS and FMF flares. APR were normal in 3 patients. For the 3 remaining patients: one took irregularly 1mg/d of colchicine and had no FMF flares but an active HS; the second one had 1.5mg/d of colchicine and the third patient 2mg/d, both had active FMF and HS. For the older patient of the series, FMF occurred at the age of 10 years and HS at 15 years; AA amyloidosis was diagnosed when he was 69 years old because of proteinuria and increased APR whereas he took 1mg/d of colchicine, thus he received interleukin (IL)-1 inhibitor in association with colchicine.
In the literature, IL-1 inhibitor demonstrated an efficacy in 9 out of 12 patients with HS and no AID. Three cases of AA amyloidosis associated with HS were reported.
Conclusion: FMF and HS are suspected to share common pathways to the activation of inflammasome and the secretion of IL-1 beta. The association of FMF and HS could induce a greater and protracted secretion of IL-1 beta and enhance the risk of earlier occurrence of AA amyloidosis. Thus, APR should be carefully monitored in patients with FMF and HS and treatment should be intensified if needed in order to prevent AA amyloidosis.

P106

The portrait of Familial Mediterranean Fever in N. Greek pediatric patients: a 30-year experience

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Introduction: Familial Mediterranean Fever (FMF), is the second commonest autoinflammatory disease in pediatric Greek patients (pts) after PFAPA. So far, long-term follow-up case series in Greek FMF pts have not been emerged.
Objectives: To depict: a) the clinical phenotype, the genotype-phenotype interplay and b) the response to treatment and the long-term outcome in a large series of Greek FMF pts.
Patients and methods: Seventy children (46 females) with established FMF according to Tel Hashomer classification criteria were enrolled in this retrospective study. Pts were followed-up over a 30-year period (1986-2015) and most of them underwent a genetic molecular analysis using either FMF strip assay (Vienna Lab) or NIRCA or recently NGS.
Results: The mean age at FMF onset was 3.54 years (range 0.83-18.5), the mean lag time 36.72 months (range 91-185) and the cumulative follow-up time 731.53 years (9.88/pt). A positive family history was recorded in 27/70 pts (38.6%).
In respect to phenotype, a typical phenotype was recorded in 75.7%; the commonest manifestations at onset were periodic fevers (100%), abdominal pain (84.3%), rheumatic attacks (arthritis or arthralgias in 52.85%) and chest pain (40%), while monosymptomatic were only 5.7%. Genotyping studies traced ≥ 1mutation in 63/67 pts; compound heterozygotes or homozygotes were 22 and 13 pts respectively. The commonest mutations were M694V (51.6%) and M680I (38.7%), the combination M694V/M680I, the most frequent one (19%) and M694V was significantly correlated with rheumatic manifestations (p=0.003). A complete or partial response to colchicine was recorded in 51.5% and 42.4% respectively, whereas unresponsiveness was observed in 6%. One refractory patient responded to anti-IL1β administration and no patient ever developed amyloidosis.
Conclusions: In this case series, the FMF phenotype in N. Greece was a typical one and not differential from other Caucasian ethnicities, although it was milder and absent of amyloidosis. The genotype distribution was in line with previously described mutations in other Mediterranean Countries. The systemic follow-up and contemporary management in an organized Center hinders non-compliance and contributes to an optimal disease outcome.

P107

The role of oxidative stress in determining prognosis in children with FMF and the relationship between markers of oxidative stress and gene mutation

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Background: Uncontrollable neutrophil activation and its migration to serous tissues that result in oxidative stress responsible for the tissue damage due to pyrin mutation in FMF patients. Reactive oxygen radicals are released by active neutrophils and the basic cell compounds such as nucleic acids, proteins and lipids during the inflammation. The aim of our study was to measure plasma advanced oxidation protein product (AOPP) and serum amyloid A (SAA) in children with FMF and to investigate the relationship with clinical findings and gene mutations as well.
Material and methods: One-hundred nineteen patients with FMF patients aged between 7 and 15 years and 29 age-and sex-matched healthy controls were enrolled in the study. Patients were classified based on response to colchicine. Briefly, group 1; 29 children taking colchicine without any attack, group 2; 30 children with frequent attack despite taking colchicine, group3; 30 children taking colchicine with unchanged frequency of attack, group 4; 30 children newly diagnosed as FMF and not taking colchicine, group 5; control. Acute phase reactants including ESR, CRP and SAA and were measured at the beginning of study and plasma AOPP levels were as well. The patients were also evaluated according to the presence or absence of attacks and the correlation of clinical and genetic features with oxidative stress markers were investigated.
Results: AOPP levels of group 2, 3 and 4 were significantly higher than group 1 and control. There was statically significant different between patients and control in terms of the SAA levels. 80 % of the patients in group 3 and 43.3 % of the patients in group 4 had SAA level >6.4 mg/L. The patients having FMF attacks at the time of study had significantly higher SAA and AOPP levels than patients being attack free and control. Regardless of FMF attack, patients with FMF had higher level of AOPP and SAA than control. Patients bearing the M694V/M694V genotype had high level of SAA. There was no significant difference between patients with and without M694V/M694V genotype in terms of AOPP level.
Conclusion: The present study demonstrates that children with FMF have high level of AOPP that reflects the oxidative damage of proteins and level of SAA that thought to be a marker of both a chronic inflammation and the activation in FMF.

P108

The effect of Anakinra on Quality-of-Life in a family with colchicine-resistant FMF

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Introduction: About 5-10% of patients with FMF do not to colchicine treatment to control inflammation. In these patients anti-IL1 therapy seems to be effective in controlling inflammation, although clinical trials are lacking. However, many patients on Anakinra experience side effects including headache and injection-site reactions. How Anakinra treatment influences quality-of-life (QoL) in these patients is unknown.
Objectives: To investigate the effect of Anakinra on inflammation and QoL in a family with a severe phenotype colchicine-resistant FMF.
Patients and methods: Four sisters of Italian descent with homozygote M694V-positive FMF, had an extraordinary phenotype with continuous inflammation, daily fevers, erysipelas-like skin lesions and serositis. Colchicine was initiated in all patient, but showed ineffective in controlling inflammation. Eventually, Anakinra was initiated. Before and during treatment with Anakinra inflammatory parameters (CRP, leukocyte-count, Serum amyloid A) were measured. One patient only started Anakinra after type AA amyloidosis had developed leading to a renal replacement therapy and renal transplantation. In the other three patients QoL questionnaire (RAND-36) was obtained, before and 4 weeks after start of Anakinra-therapy. The study was approved by the local ethical committee. Because of reimbursement was initially declined, Anakinra was discontinued. Later, it could be re-initiated. QoL questionnaires were obtained after stopping and after re-initiation of Anakinra.
Results: During Anakinra treatment the CRP values were significantly lower than without Anakinra (mean 3.7 mg/L vs 30 mg/L, $p<0.001$). The QoL improved significantly after initiation of Anakinra in 5 out of 6 scales of the RAND-36 ($p<0.05$). The QoL deteriorated after withdrawal of Anakinra to pre-treatment level and improved again after re-initiation of Anakinra.
Conclusion: Here we show that Anakinra can effectively suppress inflammation in a family with severe colchicine-resistant FMF. This lead to a significant improvement in quality of life.

P109

Developing of a new scale for assessing the adherence to colchicine treatment in pediatric patients with FMF

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Introduction: Familial Mediterranean Fever (FMF) is a disease characterized by attacks and colchicine is the medication considered most effective in reducing the intensity and frequency of attacks. Adherence to the medication regiment is important not only to manage FMF symptoms, but also to prevent amyloidosis.
Objective: In this study, it is aimed to develop and assess the validity and reliability of the adherence scale for colchicine treatment in pediatric FMF patients.
Methods: This study was planned as a methodological study to development of scale for assessment of adherence to treatment of pediatric patients with FMF using colchicine treatment. Pediatric patients (2-18 ages) with FMF using colchicine at least 6 months and accepted to participate in the study constitute the sample of the study. "Data collection forms about the sociodemographic and medical information (demographic, clinical and laboratory findings) of patients", "adherence scale for colchicine in pediatric FMF patients" and "Morisky Medication Adherence Scale" were used as data collection instruments. If the patient was under 7 years old, his parents filled the forms.
Results: There were 150 patients with FMF enrolled for the validation of the study. The median age of the patients was 11.11±4.02 (min.2.74-max.17.99) and 48.7% of them were male. The median of the attack frequency was 11,00±10,74(min. 0-max 52) and 60.7% of the participants had irregular attacks.
For internal consistency, Cronbach's alpha was 0,728 for "adherence scale for colchicine in pediatric FMF patients". Also, there was a positive and significant correlation (r: 0.843, p.
Conclusion: Based on these results, using this scale for the purpose of the assessment and follow up of adherence to treatment of pediatric patients with FMF who use colchicine is recommended.

P110

Anti-IL1 therapy in patients with refractory FMF living inGermany

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Introduction: About 10-20% of patients with familial Mediterranean fever (FMF) show an inadequate response to colchicine. Patients with colchicine-resistant FMF with or without AA-Amyloidosis can be treated with Interleukin-1 (IL-1)-inhibiting drugs.
Objective: We report our experience in adult patients with colchicine-resistant FMF who were treated with anakinra or canakinumab.
Patients and methods: Demographic data, clinical and laboratory parameters, MEFV mutations, patient reported outcomes and physician global health were analyzed in 15 patients treated with anakinra or canakinumab.
Results: Within our cohort of 160 adult patients with FMF, we identified 15 patients (4 female and 11 male) who were treated with anakinra (n=13) or canakinumab (n=2). Twelve of 15 patients (80%) were of turkish-armenian ancestry. The median FMF severity score was 8 (range 5-14). Patients carrying two high-penetrance MEFV mutations (M694V or M680I) had a severity score of 9 (8/15=53%). Patients with a single high penetrance mutation had a severity score of 11 (3/15=20%). Four patients (4/15=27%) had no MEFV mutations and the FMF severity score was 7.5 ($p=0.2$). FMF-related AA amyloidosis was diagnosed in 6 patients (40%) and the median FMF severity score was 10 compared to a severity score of 7 in 9 patients without amyloidosis (60%) ($p=0.3$). Anakinra was used continuously in 13 patients and in 2 patients only during attacks. The number of FMF attacks was significantly reduced by anti-IL1 treatment ($p=0.0024$). The patient reported health and the physician reported global health were both improved significantly (p.
Conclusion: IL-1-blocking therapies are well tolerated and effective in patients with colchicine-resistant FMF. Blocking IL-1 reduced the number and severity of FMF attacks.

P111

Evaluation of familial mediterranean fever patients: a single center experience

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Introduction: Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disease due to mutations in MEFV, and characterized by recurrent acute attacks of fever and serosal inflamation. The disease mainly affects populations from the Mediterranean basin, especially Arabs, Turks, Jews, and Armenians. The diagnosis of the disease relies on clinical criteria, family history, and ethnic considerations, and genetic analysis of known mutations. Standart therapy for the prevention of acute attacks and also disease-related amiloidosis is colchicine. Valid therapeutic alternatives are anti-IL-1 agents in unresponder or noncompliant patients.

Objectives: The aim of the study to evaluate familial mediterranean patients and to determine clinical characteristics, efficacy of colchicine drug and type of MEFV mutations.
Patients and methods: In this study we evaluated FMF patients diagnosed with the clinical criteria and genetic analysis. Their family history, consanguine, symptom-onset age, and age at the diagnosis, their complaints, the course of the disease, genetic analysis, therapy, complications, were recorded from their hospital records.
Results: Total 355 FMF patients included the study. Their madian symptom-onset age was 6,0 year, and age at the diagnosis was 8,3 years. Almost a half of them had family history, their parents of 17,2% patients were relative. The most complaints were abdominal pain (72,1%), fever (70,4%), and artralgias (48,2%). 47,9% of patients had have M694V mutation, 69% of patients treated with colchicine, but 6 (2,5%) of them were resistance of colchicine, and anti-IL-1 agents used for therapy. In only one patient (0,3%) renal amiloidosis was developed. There was no complication observed due to therapies.
Conclusion: We found the period from the disease onset to diagnosis is shorter, the responce to colchicine therapy is found more favorable than other studies. We also found unresponsiveness to colchicine therapy is smilarly with literature.

P112

Validity and reliability of medication adherence scale in FMF

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Objective: The optimal level of adherence necessary to achieve acceptable disease and quality-of-life outcomes for patients is not known. In order to identify these optimal levels, we need reliable and valid measures of adherence. Medication Adherence Scale in FMF (MASIF) is an instrument designed to measure adherence to treatment in children with Familial Mediterranean Fever (FMF). We have developed this scale for children with FMF and found valid and reliable. In this study, it was aimed to assess the validity and reliability of this adherence scale for medical treatment in adult FMF patients.
Methods: This study is multicentre and 14 centers participated to the study. Patients with FMF using medication at least for 6 months and accepted to participate constituted the sample of the study. Besides "Medication Adherence Scale in FMF Patients (MASIF)", "Data collection forms about the sociodemographic and medical information (demographic, clinical and laboratory findings) of patients", and "Morisky Medication Adherence Scale (MMAS)" were used as data collection instruments. Morisky medication adherence scale was used as a gold standard in order to evaluate the criterion validity of MASIF. We assessed the validity of the adult version of the MASIF using the Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) filter.
Results: There were 133 patients with FMF enrolled for the validation of the study. The median age of the patients (n=133) was 28.60 years (min.18.12-max.71.34) and 52.6% of them were female. The median number of the attack frequency was 13.50 (min. 0-max 99) in a year and 57.9% of the participants had irregular attacks. For internal consistency, Cronbach's alpha was 0,764 for MASIF adult version. Also, there was a positive and significant correlation between test and retest score (t=0.971; p=0.340). For the "criterion validity" the correlation with Morisky and MASIF was evaluated (r=0.530, p=0.000) and for the "structure" validity, factor analyzes and Kaiser-Meyer-Olkin tests were performed. After these tests, MASIF was found as a valid and reliable instrument. MASIF consists of 18 items and 4 sub-dimensions: knowledge about the medication, adherence to the treatment, barriers to drug use, factors that may increase compliance. The participants answered each item on a Likert scale (1=strongly agree, 2=agree, 3=no idea, 4=disagree, 5=strongly disagree). The total score ranged from 18 to 90. A high score showed a good adherence to treatment. The cut-off point was determined as 55 points. A point over 55 was accepted as "good medication adherence" and a point less than 55 was considered as "bad medication adherence".
Conclusion: Approximately 10-15% of patients with FMF are non-responders but it was claimed that in fact they are non-compliers that causes these patients receive unnecessary biologic agent treatment procedures, which are expensive and have some serious adverse effects. This scale will provide assessment and follow up of adherence to treatment patients and determine whether the patient is non-responders or non-compliers. It may help to determine the non-compliance and prevent unnecessary and expensive biologic agents.

P113

MEFV gene methylation pattern analysis in familial Mediterranean fever patients with altered expression levels

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Introduction: Familial Mediterranean Fever (FMF) is caused by mutations in the MEFV (Mediterranean FeVer) gene, which encodes pyrin. Phenotypic heterogeneity is very common in FMF patients and may partly rely on genetic heterogeneity. However, many cases having weak phenotypic-genotypic correlation, different clinical findings and therapeutic approaches with the same genotype show that FMF is not a simple monogenic disorder. Thus we hypothesized that possible epigenetic factors such as; DNA methylation, post-transcriptional histone modifications and microRNAs may contribute to disease phenotype in FMF patients and decided to analyze MEFV gene expression and compare the levels according to DNA methylation status in neutrophils.
Objectives: To better understand the complexity underlying disease phenotype in FMF by the analysis of DNA methylation patterns in neutrophils and to find possible mutations in MEFV mRNA that can be related with the phenotypic heterogeneity in patients.
Patients and methods: Blood samples were collected from 6 controls, 6 M694V/M694V patients, 6 M694V/- patients, and 6 M694V/- carriers. Neutrophil cells were isolated with Lympholyte-poly solution, Cedarlane. RNA and DNA molecules were extracted from neutrophil cells. qPCR was performed to measure MEFV gene expression levels in different individuals. For methylation analysis, DNA was treated with bisulfite by using EZ DNA Methylation-Lightning Kit, D5030, Zymo Research. Bisulfite treated DNA was amplified with special designed bisulphite primers that were specific to promoter region of the MEFV gene. DNA sequencing was performed in order to calculate the percentage of the methylation.
Results: According to qPCR analysis, decrease in MEFV gene expression was more in homozygote group (0.12, pC and L57L were found in MEFV promoter region and exon 1, respectively (published in Infevers database). Then MEFV gene promoter region is analyzed by bisulfite sequencing in different individuals. There were no difference between patients and healthy group by means of the methylation pattern at the promoter region that we analyzed.
Conclusion: Based on the results of this study, methylation pattern at the promoter region may not be the cause of heterogeneity for our patients with different clinical phenotype. Therefore other epigenetic mechanisms that may control MEFV expression remain to be studied.
This project is supported by Hacettepe University, Scientific Research Project Coordination Unit, Grant number: 013D05101005.

P114

Magnetic resonance imaging features of Familial Mediterranean Fever associated spondyloarthritis

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Background: Inflammatory back pain is a frequent complaint among Familial Mediterranean Fever (FMF) patients and spondyloarthritis is a

well-known chronic manifestation of disease affecting about 10% of patients. However, literature is lacking for a systematic study investigating radiologic features of this particular patient group.
Objectives: To determine Magnetic Resonance Imaging (MRI) characteristics of patients with FMF associated spondyloarthritis (FMF-SPA).
Methods: Twenty-nine patients followed up in our clinic with FMF-SPA who fulfilled ASAS classification criteria for axial spondyloarthritis. To figure out only characteristics of FMF-SPA, we excluded those patients with psoriasis, Crohn disease/ulcerative colitis or positive HLA-B27 tests. Patient demographics, clinical features and MEFV mutation analyzes were recorded. All patients underwent sacroiliac and spinal contrast enhanced MR examination. T1, T2 weighted images (WI), STIR sequence and post-contrast fat saturated T1 WI were used to define MRI features.
Results: The mean (min-max) age of patients was 32.8±7.7 (19-53) years and 55.2% were female. Age at the onset of the inflammatory back pain was 20.5±5.5 (10-30) years and age at the diagnosis was 29±5.4 (22-35). The duration of symptoms was 11.9±8.6 (1-28) years at the time of MR examination. M694V mutation was the most commonly observed MEFV mutation in FMF-SPA patients (75.9%). In sacroiliac joint, active lesions were evident in 19 patients and sacroiliac joint involvement was bilateral in 20 of them. Spinal lesions were quite rare. The most common finding in the axial skeleton was facet joint arthritis.
Conclusions: Our results confirmed the role of M694V on risk of spondyloarthritis development in patients with FMF. Unlike ankylosing spondylitis, spinal chronic lesions were quite uncommon, even those patients with substantial disease duration and severe sacroiliac joint involvement. Therefore, these results suggest that FMF-SPA might be a distinct type in spondyloarthropathy spectrum of diseases.

P115

Acute phase reactants in the follow-up of patients with FMF

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Introduction: Familial Mediterranean Fever (FMF) is the most common periodic fever syndrome. FMF characterized by recurrent fever, serositis attacks and chronic subclinical inflammation in attack-free periods.
Objective: The aim of this study was to evaluate the relevance of acute phase reactants (APR) in FMF and to determine their correlation with each other during attacks and attack-free periods.
Methods: Twenty-three children diagnosed as FMF according to the previously published criteria and followed-up at the Pediatric Rheumatology Clinic of Hacettepe Children's Hospital were enrolled in the study. The erythrocyte sedimentation rate (ESR), C reactive protein (CRP), white blood cell (WBC) count, platelet count, and serum amyloid a (SAA) were tested in the patients during an attack and in-between attacks.
Results: There were 9 male and 14 female patients. Tests were performed in 11 patients with an attack and in 12 without attacks. All patient had homozygous or compound heterozygous FMF-associated mutations. ESR, CRP, WBC, SAA were statistically significantly higher in patients with an attack (respectively $p < 0,001$, $p < 0,001$, $p=0.03$, $p=0,003$). Highly significant and perfect correlation was found between SAA and CRP in patients with attack ($r= 0,939$, $p<0,001$).
There was a significant correlation between the number of attacks in the last 6 months and SAA in the patient with attack-free periods ($r=0,746$, $p=0,005$). There was no other significant correlation.
Conclusion: CRP and SAA levels correlated with each other during the FMF attacks. SAA is important to identify subclinical inflammation in FMF patients when other APRs were normal.

P116

Comorbidities in patients with Familial Mediterranean Fever

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Introduction: Familial Mediterranean Fever (FMF) is the most common periodic fever syndrome, characterized by recurrent fever, serositis attacks. There are limited data on comorbidities seen in patients with FMF.
Objective: Our objective was to evaluate comorbidities among individuals with FMF in a large cohort.
Methods: We used Hacettepe University- Department of Medical Biology's genetic database records of 5636 FMF patients for MEFV mutation. 1998 patients among this group who are followed by rheumatologists in our hospital, with homozygous and compound heterozygous MEFV mutations were included in the study. We analyzed, Hacettepe University clinical records.
Results: The mean age was 27,5±16 (1-86) years. Our hospital mean follow-up period was 48,5±48 (1-352) months. 1343 patients (67,2%) had no comorbidities. 655 patients (32.8%) had comorbidities. Comorbidities were as follow: Appendectomy 30 (4,6%), cholecystectomy 20 (3,1%), acute rheumatic fever (2,4%), ankylosing spondylitis 155 (23,7%), juvenile idiopathic arthritis 31 (4,6%), rheumatoid arthritis 10 (1,5%), renal amyloidosis 54 (8,2%), intestinal amyloidosis 4 (0,6%), chronic renal failure 33 (5%), Behçet's disease 1 (0,2%), Henoch-Schönlein purpura 25 (3,8%), osteoporosis 77 (11,8%), celiac disease 5 (0,8%), inflammatory bowel disease 16 (2,4%), hepatosplenomegaly 33 (5%), hepatosteatosis 20 (3,1%), hypertension 53 (8,1%), polyarteritis nodosa 9 (1,4%), epilepsy 7 (1,1%), multiple sclerosis 5 (0,8%), PFAPA 4 (0,6%), uveitis 1 (0,2%), asthma 6 (0,9%), IgA deficiency 4 (0,6%), thalassemia trait 8 (1,2 %), thalassemi major 1 (0,2 %), systemic lupus erythematosus 5 (0,8 %), sjogren syndrome 2 (0,4%), autoimmune hepatitis 2 (0,4%), autoimmune hemolytic anemia 2 (0,4%) ….
Homozygous M694V frequency in patients with comorbidities was 42,7% while it was 34,4% in those without comorbidities. M694V/M694V mutation in patients with comorbidities was significantly more frequent.
Conclusion: These comorbidities can be classified in 3 groups: those comorbidities directly related to FMF such as amyloidosis, the second being comorbidities that may be incidental such as IgA deficiency. The last group of comorbidities were those associated with FMF due to increased innate inflammation such as PAN, PFAPA.
It is important to establish the relationship between different diseases' phenotypes and pathologies underlying cellular functions. Thus the results of our study would lead to improved clinical care and epidemiology in FMF.

P117

The effect of anti-inflammatory drugs on ASC gene level and cellular viability

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Introduction: FMF is the most common of the autoinflammatory diseases and is characterized by recurrent attacks of fever and painful inflammation. Treatment of colchicine reduces the frequency and severity of FMF attacks. The FMF gene, MEFV, encodes a protein called pyrin, appears to be a regulator of inflammation through its interactions with several proteins that are related to regulation of cytokine secretion, cytoskeletal signaling and cell death. ASC (Apoptosis-associated Speck-like protein containing a Caspase recruitment domain) is a pyrin-interacting protein, which is a key adaptor component of the inflammasome. In this study, we hypothesized that many anti-inflammatory drugs with different mechanisms of action may have an effect on ASC gene expression level and cellular viability.
Objectives: To determine the effect of colchicine, naproxen, prednol-l, acetylsalicylic acid, and azathioprine on ASC gene expression level and cellular viability using a monocytic cell line.
Materials and methods: We used a differentiated monocytic cell line, THP-1 cells, which naturally express Pyrin interacting proteins. Cells were differentiated with PMA and treated with 100 ng/ml, 5 uM, 50 nmol/L, 600 uM, and 10 uM of colchicine, naproxen, prednol-l, acetylsalicylic acid, and azathioprine containing medium respectively, for 24 h. After qPCR was performed to measure ASC gene expression level in differentially treated cells. Student's t test was used for comparison of the means among

groups. We used impedance-based xCELLigence Real-Time Cell Analysis detection platform, for real-time monitoring of cell viability of treated THP-1 cells. THP-1 cells were monitored for 24 h after treatment and electrical impedance, which is recorded as Cell Index (CI) values, reflected the biological statue of monitored cells' viability.
Results: According to qPCR analysis; ASC gene expression was down regulated in colchicine ($p<0.01$), naproxen, prednol-L ($p<0.01$) and acetylsalicylic acid ($p<0.001$) treated cells. There was no change in gene expression in azathioprine treated cells. According to cell viability assay; CI values indicating the cells' viability were increased in naproxen ($p<0.05$), prednol-L and acetylsalicylic acid ($P<0.05$) treated cells. We observed non-specific increase in CI values of colchicine and azathioprine treated cells.
Conclusion: These anti-inflammatory drugs are known to have different mechanisms of action however they are all used to treat pain or inflammation. In order to understand whether these drugs' therapeutic mechanism is related with ASC or not, we tested ASC gene expression level and cellular viability. Since ASC is a very well known proapoptotic protein, our results showing a decrease in gene expression and increase in cell viability suggested that naproxen, prednol-L and acetylsalicylic acid may have a therapeutic effect through ASC-inflammasome platform.

P118

Colchicine measurement using LC-MS/MS with ESI in serum with liquid liquid extraction

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Introduction: Familial Mediterranean fever (FMF) is an inherited autoinflammatory disorder characterized by recurring attacks of fever accompanied by intense pain in the abdomen, chest, or joints. Colchicine, is an important drug which is used in diseases such as FMF and acute gout attacks. However, it has significant side effects such as diarrhea, nausea, vomiting and fatigue. Some of the patients does not respond to treatment due to resistance. Therefore it is important to measure the serum levels of colchicine. The expected serum levels of colchicine is about 5-15 ng/ml. Correct measurement of this level with the classical analytical methods are not easy. Phase extraction and sample concentration of colchicine in routine laboratory conditions is time-consuming, so advanced analytical instruments such as LC-MS/MS and easy sample preparation methods are needed.
Method: We used Agilent model 6420 LC-MS / MS to measure the serum level of colchicine. Pimozide was used as an internal standard and 100 microliters of IS (7.5 ng/ml) was added into 200 microliters serum. Then serum proteins was precipitated by 900 microliters of methanol and 20 microliters of the supernatant was injected into LC-MS/MS. LC flow rate of 0.5 ml, the phase gradient of 10 mM ammonium acetate + 0.1% formic acid for phase A and the mixture consisted of 0.1% formic acid in methanol for phase B were used.
Results: Calibration curve for colchicine was constructed with five levels (1.56, 3.1, 6.25, 12.5, 25 ng/ml). r2 value was found to be 0.999. Peak arrival time of 1.9 minutes, the accuracy value of 100.44, the recovery value was found to be 82%. LOD (limit of detection) value was 0.05 ng/ml and the LOQ was 0.1 ng/mL. We tried different substances as an internal standard such as caffeine, cotinine, amlodipine, nifedipine and diltiazem but pimozide was the more stable and consistent, in addition to the least possibility of endogenous existince and reproducibility was found to be the best amongst the others.
Conclusion: We have developed a method for the measurement of colchicine using LC-MS/MS facilitated the extraction step, and can be applied in routine practice. Dose adjustment may become possible for rational drug use by comparing the serum concentration in response to different doses of colchicine. Thus, to avoid the side effects of medication can be possible with the use of the lowest dose producing responses to the treatment.

P119

Hypohidrotic ectodermal dysplasia and Familial Mediterranean Fever in a child with recurrent episodes of hyperthermia

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Introduction: Ectodermal dysplasia (ED) is a clinically heterogeneous condition characterized by the abnormal development of two or more ectoderm-derived structures. Mutations in ED1 gene, (Xq12-13.1), are the most frequent cause. X-linked Hypohidrotic Ectodermal Dysplasia (XL-HED) is characterized by association of sparse hair, abnormal or missing teeth and variable inability to sweat that may cause recurrent episodes of hypertermia in the first years of age.
Familial Mediterranean Fever (FMF) is an autoinflammatory autosomal recessive disorder characterized by recurrent attacks of fever and serosal inflammation caused by mutations in the Mediterranean fever gene (MEFV), on chromosome 16p13.3.
An Italian child with clinical and molecular diagnosis of both conditions is described.
Clinical report: The patient is the third child of unrelated parents. Dry skin with scaly lesions on trunk and limbs were noted at birth. At eight months of age they showed recurrent episodes of hyperthermia with normal inflammatory indices (ESR, CRP). At the age of 12 months, he showed the highly typical phenotype of HED and the molecular analysis of the ED1 gene was performed.
At the age of 32 months the child started presenting recurrent episodes of hyperthermia, colicky abdominal pain and abdominal rigidity at palpation with alterations of inflammatory indices and spontaneous resolution in 24 hours. Serum amyloid-A was: 808 mg/L (r.v.: < 6.40mg/L). Suspecting FMF, molecular analysis of the MEFV gene was performed.
Methods and results: XL-HED was confirmed in the proband by direct sequencing of the entire coding region of the *EDA* gene and multiplex ligation-dependent probe amplification analysis to exclude genomic rearrangements, showing a missense mutation on exon 3 R156H (Arg156His), absent in the mother. FMF was confirmed in the proband by direct sequencing of the exons 2,5,10 showing the presence of three missense mutations: on exon 2 E148Q (p.Glu148Gln), on exon 10 A744S (p.Ala744Ser) and R761H (p.Arg761Cys). The proband inherited from his mother the mutations E148Q and R761H, and from his father the mutation A744S.
Conclusions: ED are seldom associated to immunedysregulation. In our patient XL-HED and the disorder of the innate immune system co-exist for the association of two different gene mutations. The diagnosis of FMF is usually unproblematic in the presence of typical symptoms, however in our patient, the diagnosis was more challenging because of the overlapping symptoms of the two diseases. Molecular analysis played an essential role and allowed to start specific therapy with resolution of symptoms.

P120

Calprotectin (S100A8/A9) in Familial Mediterranean Fever

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Introduction: Calprotectin (S100A8/A9) is an example of a damage associated molecular pattern and a TLR4 ligand. It is expressed in neutrophils, monocytes and early infiltrating macrophages. Calprotectin, once secreted, has a number of pro-inflammatory effects on activated endothelium and on phagocytes and reflects activation of the innate immune system. Calprotectin has been demonstrated to be secreted during inflammation and play a role in a number of inflammatory diseases. In view of calprotectin reflecting monocyte activation, we aimed

to investigate calprotectin in patients with Familial Mediterranean Fever (FMF).
Patients and methods: All patients were genotyped with detailed well characterised mutations. Serial levels of serum of calprotectin were measured by ELISA. Patients and healthy controls (HC) cell surface calprotectin on monocytes and neutrophils as well as intracellular peripheral blood mononuclear cells (PBMC) calprotectin expression were measured by flow cytometry (FACS). CD14 cells were isolated and following overnight incubation with or without LPS, calprotectin was measured in the supernatants by ELISA. Patient and HC CD14 cells apoptosis was compared.
Results: Patients with FMF have greatly elevated levels of calprotectin. Median levels (range) in homozygous patients (n=87) 9039ng/ml (500-33544ng/ml), heterozygous with symptoms (n=81) median 9062ng/ml (1744-38119ng/ml), heterozygous without symptoms (n=56) 9736ng/ml (5205-19205), homozygous/compound without FMF (n=16) 34033ng/ml (range 2547-40000ng/ml). All groups were significantly greater than HCs (n=15)($p<0.001$). There was no difference between the different mutations. Patients on anakinra (n=4) had persistently high levels despite controlled disease. There was a correlation between cell surface expression of calprotectin on monocytes and neutrophils (n=22) with CRP ($r=0.62$). Following stimulation of CD14 cells overnight with LPS, there was significantly more calprotectin detected in the supernatants in patients (n=7) than healthy control CD14 cells (n=3)(patients median 157.9ng/ml [range 90.8-313.4], HC median 90.62ng/ml [range 76.0-122.3]) ($p<0.05$ Mann Whitney U-test). There was a trend to an increased mean florescent intensity (MFI) in intracellular monocyte calprotectin following PBMC isolation (patients n=8, HC n=4), but this didn't reach significance.
Conclusion: Patients with homozygous mutations both with and without disease have elevated serum calprotectin which doesn't correspond to disease activity unlike some autoimmune diseases. Cell surface expression on monocytes and neutrophils is low and correlates with the CRP response. The results suggest a trend to increased intracellular calprotectin expression, and more secretion by CD14 cells following stimulation with LPS. Lastly, apoptosis of monocytes is similar between patients and HC, suggesting that increased apoptosis does not have a role in the high serum levels observed. The exact mechanism by which these patients, especially those with mutations but no clinical disease, demonstrate elevated serum calprotectin remains to be elucidated.

P121

Description of a case of daily fever, developmental abnormalities, and a heterozygous M694I *MEFV* mutation

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Introduction: As a referral center for children with unexplained autoinflammatory disease, the NIH sees a spectrum of patients with previously unrecognized clinical syndromes. Here we present one such case.
Objective: To describe a patient with unexplained fevers and a novel clinical presentation.
Patients and methods: The patient is a 6 year-old boy referred from a small town in Mexico. The patient was screened for mutations in the known periodic fever syndrome genes by Sanger sequencing. Whole genome SNP genotyping and whole exome sequencing are under way.
Results: The patient was referred to our clinic for evaluation of febrile episodes that started when he was 3 months old. Fever was present every day, and was associated with a rash that started in his feet and spread to his legs, with irritability, abdominal pain, diarrhea, arthralgia, and arthritis. During his first year of age, he was treated empirically for multiple infections, but continued to have daily fevers. As an infant, he underwent diagnostic testing for malignancy and infectious diseases that were negative. At 18 months of age he was diagnosed with Still's Disease. He underwent multiple treatments, including oral steroids, methotrexate, mycophenolate mofetil, etanercept and intravenous cyclophosphamide, none of which relieved his symptoms. When the patient was 3 years old he was started on tocilizumab; the fevers evolved from daily to episodic, occuring every 4 to 6 weeks and lasting 3 to 5 days. Genetic testing revealed a heterozygous M694I mutation in the *MEFV* gene. At age 5 the patient developed seizures. MRI of the brain showed hypo-intense lesions and for this reason tocilizumab was discontinued with an almost immediate return of his daily fevers and resolution of the seizures. At NIH, he was found to have growth and developmental delay, striking dysmorphic features including microcephalus, coarse facies, muscle hypotonia, and impressive joint hypermobility. He also displayed abnormal behavior. He was febrile, irritable, and had a papular rash in his feet and ankles; inflammatory markers were elevated. After he started anakinra 4mg/Kg once a day, his fevers, arthritis, and rash disappeared and his general status improved dramatically. Further genetic analysis is pending.
Conclusions: At present it is unclear whether the patient's fevers and developmental abnormalities represent manifestations of a common genetic lesion, or are independent phenotypes. Although responsive to IL-1 inhibition, the fever syndrome appears atypical for FMF. Genomic analysis may help to resolve these issues.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P122

Assessment of the pathogenicity of the p.K695R and p.A744S Mediterranean fever gene variants

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Question: How strong is the association of the NM_000243:c.2230G>, p. Ala744Ser variant and the c.2084A>G, p.Lys695Arg, with an FMF phenotype? The worldwide population frequency of these variants is 0.001-0.005, and they are uncommon among Israeli referrals for genetic diagnosis of FMF.
Methods: Pathogenicity is indicated by the degree of positive association between the variant in its hetero- and monozygous forms, and an FMF phenotype. Subjects genotyped with p.K695R or p.A744S from 1500 referrals for FMF genetic testing between 2010-14 performed at the Sheba Medical Center, were clinically characterized using medical records or phone interview, by an expert blinded to the genotype. FMF diagnosis, and severity assessed by the Mor score were related to the genotype.
Results: The p.K695R variant was found in 10 referrals (0.003 frequency) one of whom had a second, p. E148Q variant. All but 2 patients with IBD comorbidity had mild autoinflammatory symptoms. FMF criteria were met in one patient with IBD genotyped p.[R695K(;)E148Q] and 3 heterozygotes (40%). A North African Jewish or Arab ancestry was associated to this variant. The p.A744S variant was found in 17 referrals (0.006 frequency) 7 of whom, 5 heterozygotes and two compound heterozygotes met FMF clinical criteria (41%) with mild severity. However, a homozygous subject and two sibs with a second, p.M694V clearly pathogenic variant were not diagnosed with FMF. p.A744S referrals had in common a Mediterranean ancestry.
Conclusion: The high frequency of FMF among patients carrying the p. A744SS and the p.K695R variants favors the inclusion of these variants within the spectrum of mild pathogenic mutations.

P123

Anakinra treatment in patients with Familial Mediterranean Fever: a single-center experience

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Background: Approximately 5 to10% of FMF patients do not respond and/or intolerant to to colchicine treatment. Several case reports and case series have pointed out the efficacy of IL-1 blockade in colchicine resistant FMF subgroup.

Objectives: To review the patients followed in our center with FMF who received anakinra, an anti IL-1 receptor antagonist, because of insufficient colchicine response.
Methods: FMF patients who were treated with anakinra were retrospectively reviewed with regard to indication, efficacy and adverse events. Patient global assessment was recorded before and throughout anakinra treatment.
Results: There were 36 FMF patients with FMF who were treated with anakinra for various indications (amyloidosis in 11, colchicine resistant recurrent febrile attacks in 21, colchicine related side effects in 4). Two patients were excluded since they have been on anakinra for less than one month (one with amyloidosis, one pregnant). The mean age of the group was 34.8±10.8 years. The mean duration of the disease was 22.8 ±10,7 years. There were various co-existing pathologies among this study group like multiple sclerosis (1), ankylosing spondylitis (1), SLE (1), Behçet's disease (1), low grade lymphoma (1) and PAN (2). Five patients received anakinra during pregnancy. The mean colchicine dose was 2,09±0,49 mg/d. The mean duration of anakinra treatment was 13,34± 13,26 months. Twenty seven patients reported no attacks after the initiation of anakinra treatment whereas 5 patients reported at least 50% decrease in the attack frequency. Mean patient global assessment decreased from 8,74±2,2 to 1,74±2,6 under anakinra treatment (p=0.001).
Among the 9 patients with amyloidosis, anakinra was stopped in 2 patients because of increased proteinuria. However, a significant decrease in proteinuria was detected in 4 patients. Overall, 6 of our patients with amyloidosis are still on Anakinra treatment.
The treatment was stopped due to severe allergic reactions in 3 patients (severe disseminated rash in 1 and severe injection site reaction in 2) and because of infections in 2 patients (genital warts and urinary tract infection in 1 and sinusitis and folliculitis in 1). One of our patients reported that her psoriatic lesions got worse on anakinra. Twenty six patients reported no adverse events.
Conclusions: Anakinra seems to be an effective and safe alternative in patients who have insufficient response to colchicine as well as in FMF related amyloidosis. The major cause of treatment termination is injection site reactions.

P124

How safe it is to treat pregnant FMF patients with Anakinra?

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Background: It has been reported that anakinra, an anti-IL-1R antagonist, may be a safe alternative during pregnancy in patients with various autoinflammatory syndromes (1,2).
Objectives: To assess the safety and efficacy of anakinra in pregnant FMF patients.
Methods: Five FMF patients, treated with anakinra during pregnancy were monitored for side effects, fetal and maternal outcomes.
Results: We present five FMF cases treated with Anakinra during pregnancy due to severe protracted febrile myalgia in 3, thrombocytopenia in 1 and amyloidosis in 1. One of these cases is among the 5 patients that have been previously reported (1). Throughout pregnancy no anakinra-related adverse event was observed in any of the patients. During the postpartum period one patient had an incision-site infection and the baby of the patient with thrombocytopenia also developed low platelet count which resolved with IVIG therapy. Otherwise all patients delivered normal babies. One of the patients is still pregnant and expecting twins. All of the patients, except one with colchicine intolerance continued with daily prophylactic colchicine treatment. Anakinra was terminated shortly after birth with success in all. Pregnancy-related features are listed in Table 1.
Conclusions: Anakinra promises to be a safe alternative in pregnant FMF patients who are unresponsive or intolerant to colchicine. It can be administered transiently only during pregnancy and stopped after delivery.
References

1. Lachman HJ, *et al*: **Pediatric Rheumatology.** 2013, **11(Suppl1):**A269.
2. Chang Z, *et al*: **Arthritis Rheumatol.** 2014.

Table 1(abstract P124) pregnancy-related features

Case	Maternal Age	Anakinra Relation to pregnancy	USGs	Weeks at delivery or currrent gestational age	Gender of the baby/ fetus	Mode of the delivery	1st minute APGAR	Follow-up duration after birth (months)	Complications after birth
1	33	started at 21st GW and used continiously until birth	normal	Birth at 36th GW	boy	C/S	8	32	No
2	28	started at 12th GW and used until birth	normal	Birth at 40th GW	girl	vaginal	10	20	No
3	31	started at 12th GW and used until birth	normal	Birth at 38th GW	boy	C/S	6	2	Methicillin-Sensitive Staphylococcus Aureus incision-site infection (treated with Tygecycline) in mother
4	24	started at 15th GW and used until birth	normal	Birth at 38th GW	boy	Vaginal	8	2	Low thrombocyte count in the baby at birth (23,000/mm3); resolved after 3 infusions of IVIG (269,000/mm3)
5	33	started at 16th GW, still using	normal	At 20th GW	Expecting 2 girls				

P125

Identification of pyrin targets by CHiP-Seq

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Introduction: Familial Mediterranean fever (FMF) is a recessive disorder characterized by episodes of fever and neutrophil-mediated serosal inflammation. The gene causing FMF, *MEFV*, encodes a protein, pyrin. Pyrin is expressed predominantly in innate immune cells such as neutrophils, monocytes, and dendritic cells, but not in lymphocytes. Studies of pyrin localization show a cell-type dependency. Recent studies have demonstrated that the N-terminal fragment of cleaved pyrin binds to p65 and enhances its entrance into the nucleus. Also, we have previously shown by chromatin immunoprecipitation coupled with PCR

(ChIP-qPCR) that in THP1 cells, pyrin can bind to the promoter of the transcription factor, IRF2.
Objective: To further examine the hypothesis that pyrin binds to DNA and acts as a nuclear factor.
Methods: THP1 cells were formaldehyde cross-linked and sonicated. Chromatin was immunoprecipitated with antibodies against pyrin or normal IgG as control. After immunoprecipitation, the DNA was purified and then sequenced on an Illumina HiSeq 2000. Sequencing reads were mapped to human genome hg18 and significant peaks were then called with the Model-based Alignment of ChIP-Seq (MACS) program. Peaks were assigned to genes if they are within promoters (TSS -/+ 5000 bases) or gene bodies. For ChIP-PCR assays, purified ChIP DNA samples were used for amplifications of specific regions of genomic DNA.
Results: The initial ChIP-Seq was performed on two biological replicas. A total of 25,576,964 (IgG) and 22,124,860 (pyrin) nonredundant reads from replica-1 and 12,382,375 (IgG) and 10,612,526 (pyrin) nonredundant reads from replica-2 were aligned onto the human genome (hg18). We identified a total of 211 and 226 pyrin-occupied peaks, respectively. Peaks that hit in the same gene were further evaluated with the UCSC Genome Browser. Only one peak was shown to overlap in the two replicas. This peak corresponded to a gene involved in the nuclear pore complex, *NUP98*.With so few hits, another ChIP-Seq was done. We identified a total of 1343 peaks. We compared peaks from replica 1 and replica 2 with the new ChIP seq peaks to see if any of these peaks overlapped. This identified an additional 3 genes, *ATF6*, *IRF4,* and *TLR6*. Preliminary results by ChIP-PCR show enrichment of TLR6 after pyrin pull down.
Conclusion: Our findings suggest that in THP1 cells pyrin binds to DNA and might act as nuclear factor. Further research is needed to validate the genes identified from ChIP-Seq and examine the downstream effect of pyrin-DNA binding.

P126

Is Familial Mediterranean Fever a clinical diagnosis? Results of a field survey

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Background: It has been reported that FMF was more prevalent among Turkish people whose parental origin came from central Anatolia.
Objectives: To determine the prevalence of clinically diagnosed FMF blind to the results of mutation analysis and to assess the frequency and distribution of MEFV mutations, in Sivas, Zara.
Methods: 15906 people live in Zara, a small town of Sivas. To detect a prevalence of 0.6% with an error of 0.35% and 95% CI, 1673 people were to be screened. One parent from each house (index person) was directly interviewed and also questioned for the family members (FM) of the household with two standard questionnaires. Subjects were revisited by a group of rheumatologists for 3 times. Finally they were classified clinically as definite, probable and possible FMF, blind to genetic analysis. Blood was drawn from every fifth index case (n=336) to analyze MEFV-mutations by sequencing and from the definite and suspected cases of FMF (104/121, 86%).
Results: 1727 inhabitants were interviewed directly (index population). (M:F=176:1551, mean age: 40.2±11.4). Information concerning the 4591 family members was obtained via the index person (M:F=2939:1652, mean age: 33.5±19.6). According to the results of the first visit, 384/6318 (165 index, 219 FM) had suspected FMF. In the second visit 368/384 (95.8%) were contacted. A final diagnosis based on clinical criteria, was obtained after the third visit (121/368, 47 index, 74 family members). Among 121 patients 68 were considered as definite (68/6318;1.07%), 21 as probable (21/6318;0.34%) and 32 as possible FMF (32/6318;0.50%). The results of the mutation analysis were unblinded only after this classification was finalized. The distribution of patients carrying at least one exon 10 mutation were 32 of the 60 tested (53.3%) in the definite, 5 out of 19 (26.3%) in the probable, and 11 of the 25 (44.0%) in the possible group. In the general Zara population, 60 of the 336 tested (17.8%) carried at least one exon 10 mutation.
Conclusions: The minimal prevalence of definite clinical FMF in Zara was estimated as 1.07%. Only half of the patient group, as a whole, carried at least one exon 10 mutation. This was significantly different from the carrier rate of exon 10 mutations in the general population of Zara (1:6). However, even in a small population like this, MEFV gene mutations do not seem to be the only explanation why clinical FMF is so prevalent among people of Zara.

P127

MEFV gene mutation distrubution in Azerbaijan population

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Introduction: Familial Mediterranean fever (FMF)(MIM 249100) is a hereditary autoinflammatory disorder characterized by episodes of inflammation in the absence of high-titer autoantibodies or antigen-specific T cells. The Mediterranean fever (MEFV) gene(OMIM 608107) located on chromosome 16p13.3, which encodes the 781-amino-acid protein pyrin, is the causative gene for this monogenic Mendelian disease. This study presents the molecular analysis of an MEFV gene mutation screen of 268 patients from Azerbaijan Republic, with clinical diagnoses of FMF.
Materials and methods: Genomic DNA was obtained from peripheral blood. 4 exons of MEFV gene were analysed by PCR and direct DNA sequencing method. Furthermore the obtained nucleotid sequence compaired with reference sequence published in NCBI (NM_000243.2).
Results: %56 of 268 patients were found to have mutations. Allele frequency of common five major mutation of MEFV gene E148Q,M680I, M694V, V726A and R761H were 8.2%;1.3%;,3.1%, 9.8%; and %4.2 respectively. it is revealed one novel MEFV mutation E148D in 3 patients wich updated to INFEVERS. The mutation carrier frequency of majör common alleles was 15% in healthy individuals from Azerbaijan population. In addition, the frequency of R202Q polymorphism in patients group was 45%. and 32% in control group in this population.
Conclusion: The mutation carrier frequency and disease causative common major MEFV gene mutations in Azerbaijan population are similar of certain ethnic groups. Moreover the mutation of R761H more frequently, compared to other ethnic groups, may be a founder effect.

P128

Vasculitis associated with familial Mediterranean fever: a study on 16 french adult cases

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Background: Familial Mediterranean Fever (FMF) has been described in association with various vasculitis, especially Henoch-Schonlein purpura (HSP), polyarteritis nodosa (PAN), protracted febrile myalgia (PFM) and Behcet disease (BD).
Objectives: To describe the features of French patients with FMF displaying a vasculitis.
Materials and methods: A multicentric retrospective study was performed thru the French national center of FMF to identify patients displaying a vasculitis and FMF.
Results: 16 patients (12H, 4F) with a median age of 41 years [29-61] were included. Patients were Sefarad Jews (n=9), Turkish (n=2) and Arabic (n=5). Seven of them had a familial history of FMF, none had a familial history of auto-immune diseases. Their FMF was symptomatic during childhood except for two patients; most of them had colchicine. They displayed various type of vasculitis such as: HSP (n=7), PAN (n=4; two of them with viral B hepatitis), Granulomatosis with Polyangitis (GPA) (n=1),

Microscopic Polyangitis (MPA) (n=1) and unclassified (n=3). Their vasculitis was diagnosed at a median age of 32 [5-43]. For one patient the diagnosis of vasculitis was made before the diagnosis of FMF. Patients with PAN mostly displayed weight loss, fever, myalgia, abdominal pain, arthralgia, with evidence of vasculitis of small or medium-sized blood vessels on a skin biopsy (n=3) or renal arterial aneurysms on an angiography (n=1). *MEFV* sequencing showed: homozygous M694V/M694V mutations (n=2), homozygous E148Q/E148Q mutation (n=1) and double heterozygous M694V/V726A mutation (n=1).
The patient with GPA displayed an intra-alveolar hemorrhage with ORL involvement. He had a M694V/M694V mutation and was the only patient which FMF was diagnosed after the vasculitis. All of 5 patients with HSP displayed a M694V/M694V *MEFV* mutation.
The patient with MPA displayed erythema nodosum, arthro-myalgia, neuropathy, constrictive pericarditis, and cutaneous vasculitis. He displayed a M694V/E148Q *MEFV* mutation.
Conclusion: FMF can be associated with various vasculitis, especially PAN and HSP. The most common *MEFV* mutation in our patients is M694V/M694V. This cohort of 16 patients includes various origins such as jewish, Arabic and Turkish. Interestingly, the diagnosis of vasculitis can either precede the diagnosis of FMF, or occur in the course of a previously known FMF. Thus, persistence of an inflammatory syndrome after resolution of vasculitis among a mediterranean patient should lead to look for autoinflammatory disease such as FMF. Actually the link between these two conditions is not known and would need further physiopathological studies.

P129

MEFV mutations - therapeutic guides or red herrings?

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Background/question: Familial Mediterranean Fever (FMF) is a hereditary inflammatory disorder characterised by self-resolving attacks of fever and serositis common in populations from Mediterranean ancestry[1]. Mutations affecting MEFV gene is believed to be responsible for the disease phenotype[1]. The correlation between the genotype and phenotype is not very strong, indicating the presence of other modifying factors which alter clinical manifestation. We describe 2 children with autoinflammtory symptoms, who had MEFV mutations, the significance of which we are unsure of.
Methods: A 13-year-old Polish girl of Romani descent of non-consanguineous parents presented with one-week history of fever, chest pain and vomiting. She was alert; but pale, tachycardic and hypotensive with normal capillary filling time and warm peripheries, despite saline boluses and broad spectrum antibiotics. She had no rash, mouth ulcers, lymphadenopathy or hepatosplenomegaly. Her investigations showed leucocytosis (22.2 x 10^9/L) with neutrophilia (20.2), hypoalbuminaemia (19g/L) and high inflammatory markers (CRP 222mg/L/ ESR 142mm/hour). She had past history of 3 -4 similar episodes every year since 5 years of age, one of which needed a lengthy admission to the Intensive Care Unit. She was then advised a 3-day course of oral Prednisolone at the onset of each episode, to which she did not respond this time. She continued to spike temperature in excess of 39°C daily for the next five weeks. Her inflammatory markers went up further, along with high ferritin (3729 microg/L) and LDH (1003 U/L) with persistent hypoalbuminaemia, leucocytosis with neutrophilia and thrombocytosis (platelets 597 x 10^9/L) and worsening anaemia (Haemoglobin 63 g/L). Investigations for an underlying immunodeficiency, infection, autoimmune disorder, macrophage activation and inflammatory bowel disease were negative. Her serum Amyloid A (SAA) was 798 mg/L. A diagnosis of an unclassified autoinflammatory disorder was made by exclusion, for which she was commenced on Anakinra at 100mg daily. Her symptoms settled and investigations normalised dramatically, including SAA. Her autoinflammtory genetics screen later revealed she is positive for MEFV E148Q mutation. Because her symptoms were not typical of FMF and were well controlled on Anakinra, treatment was not altered.
A 2-year-old boy of non-consanguineous parents of Indian origin was referred with recurrent episodes of high fevers lasting 4 days, since nine months of age with frequency increasing from 8 weeks to every 3 weeks. A typical episode starts with him rubbing ears and nose, followed by fever up to 40°c, with little response to Paracetamol and Ibuprofen. He had no other symptoms during or between these episodes. Two maternal nieces had recurrent fevers when young, which improved with tonsillectomy. His examination was normal with no rashes, mouth ulcers, lymphadenopathy or hepatosplenomegaly, although he had large tonsils with small cervical lymph nodes. He had raised inflammatory markers during the episodes. With a possible diagnosis of PFAPA syndrome, he was advised a single dose of Prednisolone at 2mg per kg at the start of an attack. He responded to Prednisolone during subsequent attacks, but parents were concerned about worsening frequency. His autoinflammtory genetics screen revealed a mutation in E148Q in exon 2 of the MEFV gene. His clinical features were not typical of FMF without any evidence of serositis and good response to Prednisolone, which prompted us to withhold Colchicine for the time being.
Results and conclusion: Although E148Q is one of the one of the five most frequent MEFV mutations in the classically affected ethnic groups, it is considered to be the mildest and least penetrant with no reported incidence of amyloidosis[1]. Some studies[3] report a high frequency of E148Q mutation (21%) in Indian population used as control, an ancestry both these patients may well share. Although it may cause FMF when associated with certain other MEFV mutations, homozygosity for E148Q alone must be insufficient to produce FMF[3]. That leaves us with the question of the significance of the mutations in these children, who do not have classical symptoms of FMF but are significantly symptomatic, especially with regards to switching therapy when there is a response to the existing agent.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

References

1. Touitou I: **The spectrum of familial Mediterranean fever (FMF) mutations.** *Eur J Hum Genet* 2001, **9**:473-483.
2. Yepiskoposyan L, Harutyunyan A: **Population genetics of FMF.** *European Journal of Human Genetics* 2007, **15**:911-916, doi:10.1038/sj.ejhg.
3. Booth DR, *et al*: **Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148.** *Q JMed* 2001, **94**:527-531.

P130

Quality of life in children with familial Mediterranean fever

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Objective: Familial Mediterranean fever (FMF) is a lifelong disorder, characterized by self-limited and recurrent attacks of fever and polyserositis. It is known that many chronic diseases have a negative effect on quality of life (QoL) multidimensionally. In our study, we aimed to assess the quality of life and psychological factors (anxiety and depression) in children with FMF.
Method: A prospective cross-sectional study was conducted between September 2013 and September 2014. A total of 70 consecutive children with FMF who were diagnosed according to the Tel-Hashomer and Yalçınkaya criteria during the attack free period and 70 healthy children who were matched in terms of age and sex were enrolled. The Pediatric Quality of Life Inventory 4.0 (PedsQLTM 4.0), Child Depression Inventory (CDI) and Screen for Child Anxiety and Related Disorders (SCARED) were used for the psychosocial assessment.
Results: Mean age of the patients (27 girls and 43 boys) was 11±3 years. The physical health, psychosocial health and total summary scores of the children with FMF were significantly lower than healthy children. In terms of sub dimension of psychosocial health, in the children with FMF, emotional functioning and school functioning domains' scores were significantly lower

Table 1(abstract P130)

		Children with FMF	Healthy Children	p
		Mean ±SD	Mean ±SD	
Physical Health		77,7 ± 13,19	88,93 ± 10,17	**<0.001***
Psychosocial Health		77,43 ± 13,04	87,26 ± 5,18	**<0.001***
	Social Functioning	90,14 ± 12,94	89,93 ± 7,54	**0.093**
	Emotional Functioning	73,29 ± 19,85	88,43 ± 6,23	**<0.001***
	School Functioning	68,86 ± 16,07	83,43 ± 13,47	**<0.001***
Total Summary Score		77,5 ± 11,26	87,68 ± 4,68	**<0.001***
Depression Scores		15,43 ± 5,75	9,87 ± 2,83	**<0.001***
Anxiety Scores		22,9 ± 12,63	17,21 ± 7	**0.004***

Independent samples t test, *statistical significance

than healthy children. Depression and anxiety scores were higher in the children with FMF than in healthy children.
Conclusion: We found that the children with FMF have high level of depression and poorer QoL. FMF is a life-long disorder that has not only physical but also psychosocial impairments for the affected children. Therefore a biopsychosocial approach should be essential to treatment of the FMF.

P131

Increased prevalence of attention deficit hyperactivity disorder in children with Familial Mediterranean Fever

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Introduction: Attention deficit hyperactivity disorder (ADHD) is a developmental neuropsychiatric disorder characterized by inappropriate levels of inattention, impulsivity and hyperactivity. The cause of ADHD is unknown, but may involve both genetic and environmental factors. It has been suggested that exposure to inflammation in infancy may increase the risk for ADHD in later life. Familial Mediterranean Fever (FMF) is the most common inherited autoinflammatory disorder. In many FMF patients the inflammation persists in attack-free periods. The prevalence of ADHD among FMF patients has not been studied previously.
Objectives: To explore the prevalence of ADHD among FMF patients and to examine the relationship between FMF characteristics and ADHD.
Patients and methods: The cohort consisted of 103 consecutive children with FMF, followed in a single referral center. Clinical manifestations, demographic and genetic data were abstracted from the patients' medical records, supplemented by information obtained by interviews conducted during routine follow up visits. The presence of ADHD was assessed using the Diagnostic and Statistical Manual of Mental Disorders questionnaire (4th ed.; DSM-IV).
Results: ADHD was diagnosed in 33 (32.4%) FMF patients, a rate significantly higher that known in our local unselected population (about 8%). The distribution of ADHD subtypes in our patients was similar to the general population: 10 children had predominantly inattentive type (9.8%), 6 hyperactive-impulsive (5.9%) and 17 combined type (16.7%). FMF patients diagnosed with ADHD had a higher rate of arthritis and family history of FMF than patients without ADHD.
Conclusion: The high prevalence of ADHD in children with FMF may support the neuroimmune hypothesis, in which inflammatory conditions increase the risk for ADHD. Furthermore, our findings suggest that physicians should be alert to the possible presence of ADHD among FMF patients.

P132

Challenging case of Familial Mediterranean Fever and its management

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive disease which is characterized by recurrent, self-limiting, short attacks of serositis (peritonitis, pleuritis or arthritis), fever and erysipelas-like skin lesions along with a marked increase in acute phase reactants. Although FMF is not associated with immune-mediated tissue damage, however these individuals are prone to develop type AA amyloidosis and associated renal impairment progressing eventually to renal failure as the most severe long term complications. Colchicine is the best treatment option for the time being, however 10 to 15% of patient with FMF are unresponsive or intolerant to colchicine. for those cases recent literature review have shown successful use of biologic agents in management.
The case: We present a challenging case of FMF in a 14 yrs old syrian girl who was diagnosed to have FMF at age of 6 years, she was started on colchicine at the age of 8 years, family history was significant for FMF and two of the family members developed renal amyloidosis requiering renal transplant. She presented to the clinic at 12 yrs old with bilateral ankle swelling. On examination she was found to have bilateral lower limb edema, her lab tests were significant for albumin of 18, urinalysis was positive for +4 proteins, renal biopsy was done which confirmed renal amyloidosis. The girl was on colchicine, we maximised the dose but the proteinuria persisted, she was also started on multiple courses of steroids and methotrexate with short lived improvement only. After reviewing the literature we found case reports that describe the successful use of biological agents especially anakinra (interleukin 1 receptor antagonist) in treatment of resistant cases of FMF with success. It was decided then to place her on anakinra, 2 months after being on anakinra we witnessed a significant improvement the edema totally resolved and amyloid protein has decreased from 203 to less than 5 (normal value).
Discussion: The major cause of mortality in FMF is the insidious development of secondary (AA) amyloidosis with eventual renal failure. Treatment of FMF is centered on prevention of painful attacks and the development of amyloidosis. Colchicine has been the main stay for treatment of FMF since 1974. Since then colchicine has been used to prevent and treat the acute attacks of FMF but in 10-15 % of pt, the response to colchicine may be minimal or absent. These patients also have an increased risk of developing amyloidosis. For such cases some of the newly discovered biological agent like: anakinra (IL-1 receptor antagonist) and etanercept have been tried with promising results, however the the clinical efficacy and safety of these agents are yet to be determined.
Conclusion: The new discovery of biological agents has changes the outcome of many rheumatological diseases especially in paediatric

population. A few colchicine resistant FMF patients have shown responsiveness to anakinra (an interleukin-1 receptor antagonist). However, the true efficacy and safety of treatment with biological agents remains uncertain because of the paucity of reports and absence of controlled trials.

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P133

Recommendations for the management of autoinflammatory diseases

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Background: Autoinflammatory diseases are rare disorders that lead to significant morbidity and mortality. Due to the low patient numbers, evidence-based guidelines are lacking and management is mostly based on physician's experience. In 2012, a European initiative called SHARE was launched to optimize and disseminate diagnostic and management regimens in Europe for children and young adults with rheumatic diseases. One of the aims of SHARE was to provide evidence-based recommendations for the management of the autoinflammatory diseases Cryopyrin-Associated Periodic Syndromes (CAPS), Tumor necrosis factor Receptor Associated Periodic Syndrome (TRAPS) and Mevalonate Kinase Deficiency (MKD).

Methods: Evidence-based recommendations were developed using the European League Against Rheumatism (EULAR) standard operating procedure. An expert committee of paediatric and adult rheumatologists was convened. Recommendations derived from the systematic literature review were evaluated by an online survey and subsequently discussed at a consensus meeting using Nominal Group Technique. Recommendations were accepted if more than 80% agreement was reached.

Results: In total, four overarching principles, six recommendations on diagnosis, twenty recommendations on therapy and twelve recommendations on monitoring were accepted with ≥80% agreement among the experts. Topics include (but are not limited to) the use of validated scores for diagnosis and disease activity, therapy with biologicals, NSAIDs and corticosteroids, and items to assess in monitoring of a patient.

Conclusion: The SHARE initiative provides recommendations for the management of the autoinflammatory diseases CAPS, TRAPS and MKD.

P134

Turkish Pediatric Rheumatology Society consensus statements on systemic onset juvenile idiopathic arthritis in Turkey

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Objective: The aim of this study was to describe the current practice and to reach consensus of management in patients with systemic juvenile idiopathic arthritis (sJIA) in the Turkey.

Methods: Recommendations were established via consensus by a panel of experts in pediatric rheumatology, based on analysis of available scientific evidence obtained from the clinical experience of panelists. The Delphi method solicits the opinion of an expert panel through a carefully designed questionnaire which in this case included questions on: epidemiology, diagnosis, treatment of symptoms, drug choice, adverse events, follow-up visits, hospital and emergency service admissions. The responses were analyzed and discussed in a face to face meeting followed by consensus building steps.

Results: Profiles of sJIA in Turkey were evaluated based on clinical experience of panelist. The percentage of patients diagnosed with JIA was found 0,004% in the general population in Turkey. Estimated number of JIA patients was reported 342 per year. Twenty of them were diagnosed sJIA. Estimated sJIA patients were 55 per year. The median age of diagnosis was 5.5 years. Consensus was achieved on diagnosis of the patients which established by detail investigation inpatients clinics. Both infectious and malignancy must be excluded in the patients before diagnosis.

The all sJIA patients was distributed according to subtypes; monocyclic 23%, polycyclic 30% and persistent/ polyarticular 47%. The mortality rate of sJIA patients was found 1%. The most used drugs were reported as glucocorticoid (100%), methotrexate (58%) and cyclosporine (10%), respectively. Etoposide and IVIG were used only selected patients. In the treatment of disease was determined to be the most preferred drug anakinra after failure DMARDs therapies. Also, we determine that canakinumab and tosilizumab were equally preferred (15-20%). The efficacy of the three drugs was expressed as the same (80-90%). The selection criteria of biological agents used in the treatment were reported: joint involvement was evident, the presence of MAS, characteristics of the patient, effective availability and easy accessibility. The total duration of the treatment was reported two years. The remission rates have been reported to according to the course of the disease; monophasic disease recovered as 100% while polycyclic and poliarticular forms' remission rate were reported 80% and 60%. The morbidity of polyarticular JIA has been reported as high such as joint deformities, growth retardation, delayed puberty, and osteoporosis.

Conclusions: This consensus, produced through a modified Delphi process, reflects our current recommendations for the diagnosis and management of sJIA in Turkey. The consensus statements are intended to serve as a reference point for teaching, clinical practice, and research in Turkey.

P135

Anti-IL1ß-monoclonal antibody in two adult patients with PFAPA Syndrome - a single centre experience

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Background: The periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome belongs to the rare nonhereditary autoinflammatory diseases and normally onsetting in childhood, but despite of tonsillectomy persisting or newly occurring in adulthood. The exact pathogenesis is not clear, but to our understanding it is an acquired autoinflammatory disease due to an unregulated production of cytokines. The common treatment varies from NSAIDS, colchicine, corticosteroids up to blocking IL1- receptor.

Objectives: The anti-IL1ß-monoclonal antibody canakinumab has been introduced as a specific therapy in patient with cryopyrin-associated

periodic syndromes (CAPS). There is only very few data about the effect of canakinumab in patients with PFAPA syndrome in off label use.
Methods: Here we report about two unrelated female patients, 20 and 49 years old, with PFAPA syndrome diagnosed in 2014 respectively 2012 with no treatment response to NSAIDS, colchicine, corticosteroids and severe side effects or no reduction of disease activity under the therapy with anakinra. Therefore, we initiated a subcutaneous therapy with canakinumab using standard dosage of 150 mg every eight weeks while monitoring clinical response and inflammation markers C-reactive protein (CRP) plus serumamyloid A (SAA).
Results: Immediately after first injection both patients showed a good response to canakinumab with reduced activity of inflammation markers, reduction of the typical clinical symptoms and ultimately with an improved quality of life. Even over the period of 6 months respectively two years we have seen a lasting effect of canakinumab with reduction of disease activity.
Conclusions: In our experience, treatment with the anti-IL1ß-monoclonal antibody canakinumab might be an effective, safe and feasible treatment showing no severe side effects and should be considered as a new therapeutic option in off label use in patients with PFAPA syndrome.

P136

The analysis of inflammatory cell migration using primary neutrophils

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Introduction: Neutrophils are the primary defense of the host against pathogens. They remain in resting state in the circulation of the healthy individuals. When encountering cytokine or chemokine signals caused by pathogen products, neutrophils are activated and mobilized to the infected sites. The life span of neutrophils which have an essential role in terms of pathogenesis of auto-inflammatory diseases are very short. In consideration of this issue, it is restricted to study neutrophil migration *in vitro*. Hence, we developed a new approach on cell migration assays and neutrophil priming for long lasting research.
Objectives: The aim of this study is to constitute a model for the analysis of inflammatory cell migration using primary neutrophils.
Methods: Neutrophil cells were isolated from peripheral blood samples by using Lympholyte-poly solution. The purity and viability of the isolated neutrophil samples were assessed by May-Grundwald Giemsa staining and trypan blue exclusion, respectively. Following that, freshly isolated blood neutrophils were placed into glass-bottom chamber slides. Some of slides were treated with collagen in order to see the effect of extracellular matrix. Then, fMLP (N-formyl- Met-Leu-Phe), a classical chemoattractant for neutrophil was used to stimulate the cells for cell migration. The optimal chemoattractant dose was determined by imaging the polarization state of cells. Lastly, a modified Boyden chamber assay was used to quantitate chemotaxis rate of the neutrophils.
Results: Neutrophil cells showed higher affinity to glass surface than plastic. The cells attached to the bottom of the slides were primed for long duration. We observed more cells in non-collagen slides when compared to collagen coated slides. Neutrophil cells were cultured for maximum 4 days under these conditions. The optimal fMLP dose was determined as 50μM for 30 minutes with 60% efficiency of neutrophil polarization visualized by actin staining. Quantitation of migrating cells using calcein-AM staining after Boyden chamber assay showed that 100nM fMLP for 24 hours was resulted in a high rate of migration.
Conclusion: Most of *in vitro* experiments using primary neutrophils often fail to accomplish as a consequence of their short life span. We managed to keep neutrophil alive for 4 days. Furthermore, our results suggest a method for studying cell migration using primary neutrophils. The ability of longer neutrophil priming would enhance outputs of the researches in terms of studying molecular pathophysiology of auto-inflammatory diseases. Hereby, this study provides a novel approach to expand our knowledge of disease pathogenesis associated with neutrophil cells.

P137

Comparison of the colchicine concentration between different matrix; plasma, leucocytes, ficoll solution measured by ESI LC-MS/MS

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Introduction: Colchicine is the main therapeutic drug which is used for Familial Mediterranean Fever (FMF) and acute gout attacks. In a limited study, the leucocyte colchicine level has been implicated to be important for monitoring of drug level. It will be important especially in the drug resistant patients. Therefore, we aimed to investigate the usability of different matrix for the monitoring of colchicine level.
Methods: We used Agilent model 6420 LC-MS/MS using electrospray ionization (ESI) to measure colchicine level. We used whole blood as a specimen, first we obtained plasma by centrifugation, second we collected leucocytes using Ficoll-Hypaque, and the last we separated Ficoll during leucocyte isolation. We added colchicine into whole blood at a concentration that to obtain 10 ng/ml final concentration. Tegafur was used as an internal standard. We lysed leucocytes by TritonX (1/1000: v/v). Colchicine was extracted from the all three matrix with 3 fold volume of the matrix of a dichloromethane/isopropanol/ethyl acetate mixture (1:1:3;v/v). After phase separation by centrifugation, the organic layer was transferred and evaporated to dryness. The residue was reconstituted with mobile phase and injected into LC-MS/MS. LC flow rate of 0.5 ml, the phase gradient of 10 mM ammonium acetate + 0.1% formic acid+water for phase A and the mixture consisted of 0.1% formic acid in methanol for phase B were used.
Results: We found that colchicine levels of the three matrix were correlated each other. However, the necessary volume of plasma samples, 200 microlitre, was lower than Ficoll-Hypaque solution (1 mL) which was used for leucocyte separation. Plasma specimens seem more clear and suitable than serum specimens. Colchicine concentration of leucocyte obtained from 1 mL of whole blood is nearly half of plasma level. In addition, tegafur, IS, was correlated with colchicine level well in all matrix.
Conclusion: We showed that plasma, Ficoll-Hypaque, and leucocyte could be used for the monitoring of colchicine level in patients. However, we do not know which matrix is the best suited for the monitoring of colchicine level in resistant patients. Therefore, further studies are needed to clarify this point in a large patient population comprising resistant patients as well.

P138

Use of TNF inhibitors in the treatment of PAPA syndrome

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Introduction: PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum and acne syndrome) is a rare autoinflammatory disease caused by mutations in the *PSTPIP1* gene. This disease is difficult to treat, but the combination of prednisone, an IL-1 inhibitor and a TNF-inhibitor has, in our experience, helped even the most severely affected patients. Treatment with anakinra appears to prevent most of the severe joint manifestations, and treatment with a TNF-inhibitor is most effective for treating and preventing the pyoderma gangrenosum lesions.
Objectives: We report our observations on the treatment of 4 severely affected PAPA syndrome patients with extensive pyoderma gangrenosum lesions.
Patients and methods: Medical records from 4 patients with PAPA syndrome and severe pyoderma gangrenosum skin lesions were reviewed. Three of these patients had remained at our institution for extended visits while different therapeutic regimens were tried. Photos of lesions were taken periodically. CRP and ESR were followed.
Results: One patient, an 8 year old girl with extensive lesions on her extremities requiring sedation for dressing changes, had been treated with adalimumab, 20 mg every 14 days, and pulses of methylprednisolone

without effect. She showed some improvement after being treated with infliximab. However, she developed generalized urticaria after the second infusion. Despite etanercept, 2 mg/kg/week, and frequent infusions of methylprednisolone, she worsened and did not improve until she was started on golimumab, 50 mg every 10 days. She eventually healed completely.
A second teenage patient had extensive pyoderma gangrenosum lesions, severe acne, and elevated inflammatory markers. Etanercept was stopped when he developed elevated transaminases. Adalimumab, 40 mg every week, led to a minimal improvement. Infliximab caused an anaphylactic reaction on the second infusion. Golimumab, 100 mg every 7 to 14 days, and a course of isotretinoin led to the greatest improvement in this patient.
Two other patients with difficult-to-treat PAPA disease improved, one on golimumab after an anaphylactic reaction to infliximab and the other on high doses of infliximab. Both had shown minimal improvement on etanercept and adalimumab.
Conclusion: Our observations indicate that treatment with infliximab, if tolerated, or high dose golimumab may be more effective than high doses of etanercept or adalimumab in those patients with PAPA syndrome and severe pyoderma gangrenosum lesions.

P139

Underlying causes of persistently elevated acute phase reactants in patiens with Familial Mediterranean Fever

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Background: Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are most commonly employed acute phase reactants in follow up of patients with Familial Mediterranean Fever (FMF). As a rule CRP increases during FMF attacks but it returns to normal values in attack free periods. Persistently elevated acute phase reactants in attack free periods can be occasionally observed in patients with FMF and is suggested to be a risk for the development of amyloidosis. Some authors suggested the use of IL-1 antagonists in such patients to prevent from amyloidosis. However there is no data regarding causes of elevated acute phase reactants in patients with FMF.
Objectives: In this study we aimed to investigate causes of persistently high ESR and CRP in patients with FMF.
Methods: Electronic medical records of our well-defined FMF cohort were analyzed. Persistently elevated CRP was defined as more than 2-fold increased values and elevated ESR was defined as > 40 mm/h arbitrarily and these must be evident in all consecutive visits in a year period. There were at least four visits in a year for each patient. Measurements performed during attacks or apparent infections were ignored. A detailed history and physical examination was performed in each patient. All patients were underwent relevant tests according to their clinical evaluation.
Results: There were 310 patients in cohort and 83 (26.8%) of them was found to have elevated CRP and ESR. Twenty six (31%) patients had spondyloarthritis who fulfilled ASAS criteria for axial spondyloarthritis. In 34 patients (41%) either attacks were very frequent or patients had chronic manifestations of disease (chronic arthritis, myalgia, ascites etc) indicating active FMF. Four patients had inflammatory bowel disease. One patient had Sjögren's syndrome, 1 had scleroderma and 1 had vasculitis. In 16 patients (19.3) any cause couldn't be identified.
Conclusions: The most common causes of persistently elevated acute phase reactants were found to be active FMF disease, spondyloarthritis and inflammatory bowel disease which could be easily identified with history and simple tests. However in a substantial number of patients any cause couldn't be identified. Therefore, not all patients with increased CRP and ESR are good candidates for IL-1 antagonists. A through history and simple tests can identify most causes of increased acute phase reactants in these individuals.

P140

Mosaic tetrasomy 9p: a mendelian interferonopathy associated with pediatric-onset overlap myositis

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Background and objectives: Pediatric-onset inflammatory myositis (IM) and systemic lupus erythematosus (SLE) are rare inflammatory diseases. They result from the complex interaction between genetic and environmental factors. An increasing number of Mendelian conditions predisposing to the development of SLE have been recently identified. They mostly include monogenic conditions, especially type I interferonopathies, associated with an up-regulation of type I interferon (IFN), a key cytokine in SLE and some IM pathogenesis.
Methods: Report on a pediatric-onset overlap myositis in a 6-year-old girl who carries mosaic tetrasomy 9p.
Results: The patient presented with myositis overlapping with lupus-like features. Myositis was characterized by a proximal muscular weakness and HLA class I antigens myofiber overexpression on muscle biopsy. Lupus-like manifestations consisted in pericarditis, pleuritis, and positive ANA and anti-SSA antibodies. Complete remission was achieved with three pulses of methylprednisolone, followed by an oral course of steroids in combination with mycophenolate mofetyl. Analysis of tetrasomy 9p showed mosaic tetrasomy in 9p24.3p12 region, including the type I IFN cluster, and increased expression of interferon (IFN)-regulated genes was found.
Conclusion: These data suggest that mosaic tetrasomy 9p isa new monogenic interferonopathy predisposing to inflammatory myositis and lupus-like features. Therefore, unexplained inflammatory muscle or other organ involvement in patients carrying mosaic tetrasomy of the type IFN cluster of chromosome 9p should lead to the search for IM and/or lupus-like disease, and karyotype should be performed in SLE or IM patients with mental retardation.

P141

Severe immune dysregulation with neurological impairment and minor bone changes in a child with spondyloenchondrodysplasia due to two novel mutations in the ACP5 gene

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Spondyloenchondrodysplasia (SPENCD) is a rare skeletal dysplasia, characterized by metaphyseal lesions, neurological impairment and immune dysregulation associated with lupus-like features. SPENCD is caused by biallelic mutations in the *ACP5* gene encoding tartrate-resistant phosphatase. We report on a child, who presented with spasticity, multisystem inflammation, autoimmunity and immunodeficiency with minimal metaphyseal changes due to compound heterozygosity for two novel *ACP5* mutations. These findings extend the phenotypic spectrum of SPENCD and indicate that *ACP5* mutations can cause severe immune dysregulation and neurological impairment even in the absence of metaphyseal dysplasia.

P142

Identification of type I interferonopathies using blood interferon signature: the experience of a pediatric rheumatology center

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Question: To test blood interferon signature as a screening tool for type I interferonopathies in children with early-onset SLE and vasculopathy.

Methods: We collected blood samples from a cohort of pediatric rheumatologic patients and scored them according to a qPCR based IFN gene signature assay. Expression of 6 type I IFN-related genes (*IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1*) was quantified by standard RTPCR techniques. An IFN score was calculated for each patient using the median fold change of gene expression related to a healthy control. Patients were selected based on the presence of the following features: i) atypical or incomplete SLE-like symptoms occurring in infancy or in preprepubertal age; ii) vasculopathy (skin ulcers, chilblains, strokes) iii) panniculitis with or without lypodystrophy iv) interstitial lung disease in the context of systemic inflammation.
Results: We screened 24 patients from the rheumatology unit of the Gaslini Children's Hospital, Genova (Italy). 14 out of 24 patients had a positive signature with an IFN score ranging from 3.5 to 23,7. Based on the clinical presentation and the result of the IFN score we further analyzed two patients for mutations affecting *TMEM173* gene: in one patient we identified the previously reported V155M variant and in the second patient we identified a new variant whose pathogenic significance is currently under study. Molecular screening for genes associated to type I Interferonopathies and whole exome sequencing is ongoing for selected patients.
Conclusions: Several studies have shown how mutation in genes involved in type I interferon (IFN) pathway can be responsible of severe inflammatory syndromes such as Aicardi-Goutières syndrome (AGS) and spondyloenchondrodysplasia with immune disregulation (SPENCD). STING associated vasculopathy with onset in infancy (SAVI) and a familial inflammatory syndrome with systemic lupus erythematosus (SLE)-like manifestations are among the last clinical phenotypes that have been linked to an increase of type I IFN - in both cases as a result of *TMEM173* gain of function dominant mutations. With the present ongoing study we anticipate that testing blood for an interferon signature is a valuable screening tool for the identification of type I interferonopathies in selected children with rheumatic diseases.

P143

Deficiency of Interleukin-1 Receptor Antagonist (DIRA): Report of the First Indian Patient and a Novel Deletion Affecting *IL1RN*

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Introduction: Deficiency of interleukin-1-receptor antagonist (DIRA) is a rare autoinflammatory disease clinically characterized by early-onset generalized pustulosis, multifocal osteomyelitis and elevation of acute-phase reactants. DIRA is caused by autosomal recessive loss of function mutations in *IL1RN*. Seven DIRA causing mutations have been described, including one 175Kb genomic deletion, 4 premature stopcodons, 1 inframe deletion, and 1 missense mutation.
Objective: To report a novel disease-causing deletion affecting *IL1RN* gene in an Indian patient with DIRA.
Methods: The patient was enrolled into a NIH natural history protocol. Peripheral blood genomic DNA was obtained and patient was genotyped for the detection of copy number variations (CNVs) by a SNP array technique. As determined by SNP array, primers flanking the deletion ends were designed and the breakpoint area was amplified by polymerase chain reaction (PCR) and sequenced by Sanger technique.
Results: Clinical description: A 5 month-old Indian girl, born to healthy non-consanguineous parents presented with pain on manipulation and irritability at the 3rd week of life and developed a mild pustular rash limited to the back of neck and upper forehead. Bone scintigraphy suggested osteomyelitis of multiple bones, including ribs, clavicles and long bones and cultures from left wrist bone biopsy material were negative. Primary immunodeficiency work up was negative. There was no clinical response to a 5-week course of broad-spectrum antibiotics, and thus an autoinflammatory condition was suspected. Prednisolone was initiated and the patient had significant clinical improvement. Due to a clinical suspicion of DIRA, the patient was enrolled in a NIH protocol and started on recombinant interleukin-1 receptor antagonist (anakinra). Anakinra initiation resulted in marked and sustained clinical and laboratory improvement.
Genetic analysis: SNP array analysis of patient's genomic DNA showed loss of heterozygosity for all SNPs in a region of approximately 21.4 to 23.7kb, indicating a homozygous deletion. PCR and sequencing of the breakpoint area allowed us to identify the breakpoints of the deletion, at chr2_hg19_113,865,011 and chr2_hg19_113,887,227, confirming a homozygous 22,216bp deletion that spans the first four exons of *IL1RN* (NM_173843). This deletion has not been previously reported in patients with DIRA.
Conclusion: We describe the first Indian patient with DIRA and a novel homozygous 22Kb deletion spanning 2 coding exons of IL1RN. The primers designed to detect the novel deletion will be useful to screen Indian patients with a clinical suspicion of DIRA.

P144

Identification of rare genetic variants in Juvenile Idiopathic Arthritis using whole exome sequencing

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Introduction: Juvenile Idiopathic Arthritis (JIA) is the most common form of chronic arthritis in children. JIA is characterized by onset of disease before the age of 16, with arthritis lasting >6 weeks, and with an unknown cause. Among JIA, seven sub-groups based on clinical and biological features have been individualized namely: systemic arthritis (sJIA) with autoinflammatory conditions, persistent and extended oligoarthritis (per-oJIA and ext-oJIA, respectively), rheumatoid factor-positive polyarthritis (RFpos-pJIA), enthesitis-related arthritis (ERA), psoriatic arthritis (PsA), and undifferentiated arthritis. Physiopathology of JIA is complex and JIA is considered to be a multifactorial disease due to the combination of genetic and environmental factors. Searching for genetic factors in JIA during the last decade, the introduction of genome-wide association studies (GWAS) and whole-exome sequencing have discovered several new loci associated with JIA susceptibility and have identified the disease-associated gene monogenic form of sJIA, respectively.
However, despite these novel knowledge, our understanding of JIA pathogenesis still remains poorly elusive and accumulating evidence supports genetic variability as playing a key role in JIA development.
Objectives: The aim of our study is to identify novel genes involved in AJI in order to identify novel signaling pathways.
Patients and methods: Thanks to a French National Hospital Research program (PHRC) including five french recruitment centers (Montpellier, Nîmes, Nantes, Marseille and Bordeaux), we have collected blood samples from 30 AJI patients and their unaffected parents. Based on clinical and biological data, the patients were classified in 5 groups: 1) oligoarticular form and negative autoantibodies (n=5), 2) oligoarticular form and positive antibodies (n=5), 3) polyarticular form and negative rheumatoid factor (n=10), 4) polyarticular form and positive rheumatoid factor (n=5) and 5) systemic form (n=5).
Results: Using a whole exome sequencing trio strategy (i.e. patients and their unaffected parents) considering each family separately, we identified sequence variants in several candidate genes. Functional studies are ongoing in an attempt to demonstrate the pathological roles of the identified genetic variations.

Conclusion: Our results could contribute to development of diagnostic tests, orientation of drug therapies as well as development of novel therapeutic targets. In addition, this study could demonstrate the ability to use whole exome sequencing in the context of the development of personalized medicine.

P145

How experts on Autoinflammatory diseases classify Periodic Fever, Aphthous stomatitis, Pharyngitis and Cervical Adenitis (PFAPA): preliminary results of the Eurofever Delphi survey

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Introduction: The diagnosis of PFAPA is currently based on set of criteria proposed in 1999 and known as the modified Marshall's criteria. Pediatricians rely on their own clinical experience to diagnose PFAPA, but no validated set of classification criteria has been established up to now.
Objectives: To understand how physicians involved in the clinical care of patients with Autoinflammatory diseases (AIDs) classify patients with PFAPA in daily practice.
Methods: by using the Delphi and Nominal Group Technique, we started an initial phase of three following e-mail surveys. In the first survey, clinicians/biologists or other health professionals working in the field of autoinflammation were asked to identify the variables that they consider as important, in the current clinical practice, for the diagnosis of patients with PFAPA. This survey was open to not influence the experts.
Results: We sent the first survey to 124 experts. The overall rate of response was 107 (86%): 101 experts responded to be interested in the survey, 76 completed and confirmed it, 6 responded not to be interested. There was any clinical variable chosen by all the participants. The five most cited clinical variables were periodic fever (80% of experts), cervical adenitis (79% of experts), aphtous stomatitis (76% of experts), pharingotonsillitis (71% of experts), abdominal pain (24% of experts).
The increase of acute phase reactants during episodes and the response to steroids were proposed as interesting variable by 44% and 43% of experts respectively. The exclusion of the inherited genetic periodic fever by genetic test is important for a little part of experts (24%). Response to tonsillectomy was cited by only one expert.
Conclusions: The preliminary results of the first Eurofever Delphi Survey show a high rate of response, underlying the interest of the scientific community in this topic. At the end of the Delphi Survey rounds, we will obtain different set of clinical criteria and we will verify their performance in comparison to already existing criteria in a cohort of patients with PFAPA enrolled in the Eurofever Registry. The final step will be a Consensus among experts (geneticists and clinicians) in order to define the best combination of clinical and genetic data for the definitive classification of patients with PFAPA.

P146

How experts on autoinflammatory diseases classify inherited periodic fevers: preliminary results of the Eurofever Delphi Survey

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Background: Provisional evidence-based classification criteria for Familial Mediterranean Fever (FMF), Cryopyrin Associated Periodic Syndrome (CAPS), Tumor Necrosis factor Receptor Associated Periodic Syndrome (TRAPS) and Mevalonate Kinase Deficiency (MKD) have been recently developed based on data coming from the Eurofever registry. However, no consensus on how to combine clinical criteria with results of molecular analysis has been reached so far.
Objectives: To understand how physicians involved in the clinical care of patients with Autoinflammatory diseases (AIDs) classify patients with inherited periodic fever in daily practice.
Methods: By using the Delphi and Nominal Group Technique, we started a process made of three consecutive e-mail surveys. In the first survey, clinicians/biologists and other health professionals working in the field of autoinflammation were asked to identify the variables that they consider as important, in their clinical practice, for the diagnosis of patients with inherited periodic fever. This survey was open not to influence the experts.
Results: We sent the first survey to 124 experts. The overall rate of response was 107 (86%): 101 experts responded to be interested in the survey and 88 completed and confirmed it for at least one disease; 6 experts responded not to be interested. No clinical variable was chosen by all the experts for any disease the five most cited clinical variables for FMF were recurrent fever (80% of experts), abdominal pain (67%), arthritis (53%), thoracic pain (47%) and arthralgia (36%). The five most cited clinical variables for CAPS were fever (75%), urticarial rash (71%), hearing loss (49%), ocular involvement (40%) and arthralgia (35%). The five most cited clinical variables for TRAPS were long lasting fever (92%), rash (84%), periorbital edema (59%), myalgia/myositis (57%), and abdominal pain (55%) while the five most cited clinical variables for MKD were abdominal pain (61%), fever (59%), skin rash (41%), diarrhea (43%) and arthralgia (39%). A confirmatory genetic test resulted a relevant element for the diagnosis of FMF, TRAPS, CAPS and MKD while the response to treatment for FMF and CAPS.
Conclusions: The preliminary results of the first Eurofever Delphi Survey show a high rate of response by Expert, underlying the interest of the scientific community in this topic. A wide heterogeneity in their response was observed. At the end of the Delphi process, we will obtain different set of clinical criteria whose performance will be tested in comparison to already existing criteria in a cohort of patients affected by AIDs. The final step will be a Consensus among experts (geneticists and clinicians) in order to define the best combination of clinical and genetic data for the definitive classification of patients with inherited periodic fevers.

P147

Anti-IL1ß-monoclonal antibody in a patient with Muckle-Wells Syndrome and renal transplantation - five years experience

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Pediatric Rheumatology 2015, **13(Suppl 1):**P147

Background: The Muckle-Wells syndrome (MWS) is a rare inherited disease and belongs to the group of cryopyrin-associated periodic syndromes (CAPS). Recurrent fever attacks, myalgia, arthralgia, urticarial rash, headache, conjunctivitis, sensorineural deafness and a severe fatigue syndrome are the typical symptoms of MWS. Due to an unregulated production of IL1 a continuous formation of serum amyloid leads ultimately to the development of AA-amyloidosis, which is life-threatening and in some cases the fatal complication of MWS.
Objectives: The anti-IL1ß-monoclonal antibody canakinumab has been introduced as a specific therapy in patient with MWS with normal and impaired renal function. There are no long term data about the effect from canakinumab in patients with renal transplantation.
Methods: Here we report the five years follow up on a 36-year old female patient with Muckle-Wells syndrome and biopsy proven systemic AA amyloidosis and end stage renal disease. After renal transplantation therapy with canakinumab subcutaneously in a dosage of 150 mg every eight weeks was continued in combination with the immunosuppressive therapy.
Results: Before and after renal transplantation the patient had a very good response to canakinumab with low activity in inflammation markers

with an improved quality of life. Over the period of five years the triple immunosuppressive therapy (CSA, MMF, Prednisone) in combination with canakinumab has had no negative effect on activity of MWS and no pharmacological interactions between medications were observed. Even five years after renal transplantation, the patient remains an excellent kidney function without proteinuria. There are no signs of recurrence of AA-amyloidosis in the transplanted kidney.
Conclusions: According to our data, treatment with different immunomodulators in patients with Muckle-Wells syndrome and renal transplantation is safe, feasible and without severe side effects also over a longer time.

P148

Clinical and genetic analyses in a patient with PAPA syndrome complicated with inflammatory bowel disease

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Pediatric Rheumatology 2015, **13(Suppl 1):**P148

Introduction: PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome is an autoinflammatory disease linked to mutations in the *PSTPIP1* gene. These mutations produce a hyper-phosphorylated PSTPIP1 protein and alter its participation in the activation of the "inflammasome".
Objectives: To elucidate the pathogenesis of PAPA syndrome, we examined the clinical status and complications and also analyzed the *PSTPIP1* gene.
Patients and methods: We herein report a 23-year-old Japanese male who suffered from recurrent arthritis in his knee and ankle joints, pyoderma gangrenosum, and acne. Recently, he had experienced melena and multiple colonic ulcers had been detected by colonfiberscopy. His ulcerations resembled ulcers associated with Crohn's disease. A histological examination was then performed for the synovium of this knee joints, skin lesions of pyoderma gangrenosum, and the colon. The genomic DNA of *PSTPIP1* were analysed in both the patient and his family.
Results: 1) A histological analysis revealed that a large number of neutrophils had accumulated in the skin lesions; however, very few neutrophils were detected in the pathological lesions of the knee joints and colon. 2) According to a gene analysis, we detected a novel heterozygous mutation in the *PSTPIP1* gene; however, his healthy father also had the same mutation, thus suggesting that this mutation of *PSTPIP1* might not be related to his phenotype. We are searching for other affected genes besides the *PSTPIP1* gene for PAPA syndrome in this case.
Conclusion: We herein reported a Japanese PAPA syndrome patient who was complicated with inflammatory bowel disease. A genetic analysis suggested that this particular phenotype might not have been affected by a mutation of the *PSTPIP1* gene.

P149

Anti-interleukin 1 treatment in secondary renal amyloidosis associated with autoinflammatory diseases

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Introduction: Amyloidosis represents a heterogeneous group of disorders characterized by extracellular deposition of autologous fibrillary proteins which impair normal organ function. Reactive AA type amyloidosis may complicate autoinflammatory diseases (AID).
Objective: To evaluate and compare the renal biopsy findings and clinical and laboratory parameters in patients with amyloidosis secondary to AID who have responded to the anti-interleukin 1 (IL1) treatment.
Patients and methods: Two children with systemic juvenile idiopathic arthritis and one with cryopyrin-associated periodic syndrome diagnosed as AA type amyloidosis were treated with anti-IL1 drugs and we have evaluated the course and management of these patients for a follow-up of median 56 (41-56) months. The renal biopsies at the time of diagnosis of amyloidosis and after the onset of anti-IL1 treatment were evaluated and compared according to the amyloid scoring and grading system based on the histopathological findings.
Results: The median age of AID onset was three years, while the patients were diagnosed to have amyloidosis at a median of 12 years of age. The patients previously used nonsteroidal anti-inflammatory drugs, corticosteroid, methotrexate, azathioprine, infliximab, and intravenous immunoglobulin treatments. After the diagnosis of amyloidosis, anakinra was started. All three responded to anakinra treatment; however, canakinumab was commenced in patient 3 since anakinra caused local cutaneous reaction at the site of drug administration. Proteinuria was improved in patients after anti-IL 1 treatment. Control renal biopsies were performed a median of three years later than the diagnosis of amyloidosis. At the renal biopsy level, we have seen that the renal amyloid prognostic score did not improve in patient 1 and progressed in patient 2 and 3. The renal amyloid grade has also progressed in patient 2.
Conclusion: To the best of our knowledge, this is the first series showing progression of renal tissue damage after the improvement of proteinuria with anti-IL 1 treatment in AID-associated amyloidosis. After the development of amyloidosis, it is crucial to control inflammation effectively and prevent further amyloid accumulation in patients with anti-inflammatory treatments such as anti-IL1 drugs. However, new treatment strategies are needed to target the amyloid deposits for patients with severe organ involvement.

P150

Neurological manifestations in autoinflammatory syndromes: a series of 131 patients from our neuroimmunology departement

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Pediatric Rheumatology 2015, **13(Suppl 1):**P150

Introduction: Cryopyrin-associated periodic syndrome (CAPS), familial mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndromes (TRAPS) are rare systemic, monogenetic inherited autoinflammatory diseases. The clinical significance of low penetrance mutations in TRAPS and CAPS as well as heterozygosity in FMF is still under debate. The frequency and clinical pattern of peripheral and central nervous system (CNS) manifestation in those patients remain unclear.
Objective/methods: To describe the neurological phenotype of patients with a low penetrance variant in the *TNFRSF1A* gene, the *NLRP3* gene, or with a heterozygous mutation in the *MEFV* gene seen in our neuroimmunology department between 2006 and 2015.
Results: 131 patients (f=88, m=43; mean age 41 ± 10 years) have been identified in our outpatient clinic: 65 with a low penetrance mutation in the *TNFRSF1A* gene, 23 with a low penetrance variants in the *NLRP3* gene and 43 with a heterozygous mutation in the *MEFV* gene. 90 patients had an additional diagnosis of multiple sclerosis (MS). Headache and chronic pain syndromes were observed among TRAPS, CAPS and FMF patients. Inflammation predominately affected the eye and optic nerve. Otherwise the neurological phenotype was heterogeneous among groups and included mostly dysesthesia in TRAPS patients, cranial nerve (CN) affection in CAPS patients and CNS vasculitis in the FMF non-MS cohort. Other systemic symptoms included abdominal pain, arthralgias and myalgias, urticarial rash and severe fatigue. So far none of the patients had developed AA amyloidosis.
Conclusion: In this large mono-centric cohort of patients with autoinflammatory syndromes concomitant MS was frequently observed indicating that mutations in the *TNFRSF1A, NLRP3* and *MEFV* gene may contribute to MS susceptibility. Besides MS a variety of neurological

manifestations can occur and TRAPS, CAPS and FMF should be included as differential diagnosis in patients with unusual CNS inflammation including CN affection, severe headache syndromes and additional systemic symptoms such as abdominal pain, arthralgia and urticarial rash suggestive for an autoinflammatory disease.

P151

Refractory epilepsy responsive to nonspecific immunossupression: autoimmune or auto inflammatory disease? A case report and review of literature

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Introduction: Over the past few years, some epilepsies have been found to be associated with immunomediated disorders. The presence of antibodies against intracellular and extracellular proteins especially in refractory epilepsy provides additional evidence of immunomediated diseases in the brain. In refractory epilepsy, autoantibodies against NMDAR, GAD and the VGKC-complex can be found in 2 to 16% of patients. The definition of autoinflammatory diseases was first introduced by Kastner et al in 2009 after the discovery of a homozygous mutation in the gene MEV1, responsible for Mediterranean Fever. After that, the recognition of inflammassome and interleukin 1 as the main actors in the innate immune system responsible for inflammation in these diseases has changed the treatment for some rare disorders.
Objective: To present the case of a patient that had persistent pleocytosis in the cerebrospinal fluid and seizures refractory to current drugs for epilepsy, with clinical improvement with nonspecific imunossupression. To discuss the aetiology of inflammation in the brain with a review of the literature.
Methods: Case report and review of the literature with the terms: "refractory epilepsy"; "chronic encephalitis"; "autoimmune epilepsy"; "autoinflammation of the brain"; "immunomediated brain diseases"; "autoinflammatory disease".
Results: Case report: The patient, A.C.S. female, 19 years old, is a Brazilian girl, that grew up in São Paulo, from non-consanguineous and healthy parents. Seizures started at 2 years old characterized as motor arrest during seconds followed by crying. When she was 13, she dropped after a spell, seeked medical attention and started medication for epilepsy. Seizures are controlled and usually happened during sleep. At 16 years old, seizures increased and began to happen while she was awake. At this point, seizures are characterized as motor arrest, right arm elevation and cephalic version to the left. Despite the use of innumerous antiepileptic drugs in elevated dosages, she maintained refractory seizures (weekly). Extensive complementary epilepsy investigation was performed. Unexpectedly, CSF studies revealed persistent pleocitosis and/or elevated protein. PET showed marked hypometabolism in left cerebral hemisphere, specially in frontal and temporal regions. Head MRI showed inespecific white matter hyperintensities in FLAIR sequences. A panel of known neural auto-antibodies (NMDA, GABA, AMPA, VGKC) and anti GAD were negative. Considering the CSF alterations, the hypothesis of an immunomediated epilepsy was made and we decided empirically treat with intravenous high-dose corticosteroids (metylprednisolone 5 g monthly for 6 months). After the second month, she improved dramatically and had no more seizures. After 6 months, we decided to keep imunosuppresion with azatioprine with excellent response (2 focal seizures in 18 months). CSF results were normal after treatment. Her quality of life and cognition also improved.
Review of literature: Immunomediated epilepsy is a controversial issue that is gaining more relevance since the discovery of many neural autoantibodies related to paraneoplasic/immunomediated encephalitis. Frequently, immunomediated encephalitis presents with seizures, including status epilepticus, sometimes refractory. In these disorders, seizures are only controlled after treatment with immunotherapy (high dose corticosteroids, gammaglobulin, etc). A considerable portion of chronic refractory epilepsies are of unknown causes. The role of neural autoantibodies in these cases are under investigation. Besides that, to what extent the damage is caused by the antibodies itself or by the inflamatory reaction in the brain is also debatable. In this case, despite negative antibodies, the patient had CSF alterations that could point to a inflammatory process in the CNS. Based on this hypothesis, we treated with high doses corticosteroids and the patient had an excellent response. Until this moment, the only well recognized monogenic autoinflammatory disease with central nervous involvement are cryopirin-asociated periodic syndromes (FCAS, Mucke-Wells and NOMID), mevalonate kinase deficiency (Hyper IgD and mevalonate aciduria) and mutations in the proteasome (CANDLE disease). Salsano et al, 2013, reported an adult patient with chronic meningitis and progressive hearing loss without evidence of mutations in the IL-1 pathway. This patient also demonstrated only IL-6 hypersecretion and clinical improvement with tocilizumab (interleukin 6 blocker). Dutra et al, 2013 also described an adult patient with CNS involvment and clinical manifestation of CAPS that were responsive to interleukin 1 blocker (anankira) with extensive genetic analysis negative.
Conclusion: We propose that this patient, with refractory epilepsy and CSF chronic inflamation might be a prototype of a novel category of autoinflammatory disease.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P152

Remarkable improvement of articular pain by biologics in a Multicentric carpotarsal osteolysis patient with a mutation of *MAFB* gene

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Introduction: Multicentric carpotarsal osteolysis syndrome (MCTO) is a rare autosomal dominant disorder, characterized by aggressive osteolysis of the carpal and tarsal bone, and progressive nephropathy leading to end-stage renal disease. Recently, heterozygous mutations in *MAFB* gene within a short region of the amino-terminal transcriptional activation domain had been reported to cause MCTO. Although affected patients suffer from early childhood with a clinical appearance mimicking juvenile idiopathic arthritis, most of anti-rheumatic agents are ineffective to control their pain.
Patients and methods: A fifteen year old boy was born to healthy parents. There was no family history of consanguinity, skeletal disorders, and rheumatic disorders. He was well until 26 months of age, when he showed claudication symptoms. He was referred for painful and swollen feet, wrists and pes cavus. He also had craniofacial abnormalities of micrognathia, hypotelorism, chubby cheeks and flat face. He showed gradual progression of osteolysis predominantly in the carpal and tarsal bones, and progressive nephropathy with hematoproteinuria. Whole exome sequencing analysis detected a de novo heterozygous mutation in *MAFB* gene which was confirmed by Sanger sequencing. Because this mutation had been reported as a responsible mutation of MCTO, we diagnosed the patient as MCTO caused by the mutation. Until this point, he was treated as a relative disease of juvenile idiopathic arthritis with non-steroidal anti-inflammatory agents, methotrexate, which was not effective to relieve not only osteolysis but articular pain of the patient. At the age of five, he was started to treat with intravenous infliximab by which pain decreased and eventually disappeared in a mean time. Proggressive osteolysis of the carpal and tarsal bone continued and the deformity of fingers was evolved. Therefore, at the age of eight, his biologics was changed to intravenous tocilizumab every 6 weeks. After 3 months treatment, the pain and tenderness in his wrists and fingers disappeared.
Results: Intravenous tocilizumab every 6 weeks, resulted in remarkable improvement of his articular pain, although osteolysis of the patient

remained progressing. The patient became free to pain and could do personal care without difficulty.
Conclusion: Tocilizumab could be an effective therapy for relief of articular pain of MCTO.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P153

Identification of three ADA2 deficiency families with novel *CECR1* mutations

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Introduction: Adenosine desaminase 2 deficiency (DADA2) is a rare monogenic autoinflammatory disease, presenting with systemic inflammation, vascular pathology with possible strokes, and mild immunodeficiency. Recently, the *CECR1* gene was found to be responsible for this disease with an autosomal recessive inheritance.
Objective: We report new mutations identified in 3 families referred to our laboratories for genetic diagnosis.
Patients and methods: In family 1 (French), the proband, a 9 year-old girl, exhibited fever, pseudo periarteritis nodosa (PAN) and several strokes. Her 7-year old sister presented with fever, anemia and neurological impairments (headaches, drowsiness and third cranial nerve palsy). In family 2 (Italian), the index-case had recurrent fever, arthromyalgia, and livedo reticularis of trunk and limbs. In family 3 (Tunisian), consanguineous brother and sister exhibited pleiomorphic vascularitis features (PAN, livedo, renal microaneurysms) with inflammation and anemia. Parents in all families were asymptomatic.
Using double-strand Sanger sequencing (ABI3130x, Life Technologies), we searched for point mutations in the *CECR1* coding regions. Quantitative polymerase chain reaction (qPCR) analysis was subsequently performed (LightCycler, Roche) when only one point mutation was identified (family 1).
Results: The two sisters of family 1 harbored two compound heterozygous mutations: p.Tyr453Cys (published) and deletion of the entire exon 7 (novel). We identified a new variant in family 2: p.Arg49Glyfs*4 (c.144del), and p.Thr360Ala, a published mutation. In family 3, we found a homozygous substitution, p.Gly25Cys in both siblings. This new variant was predicted to be benign by different algorithms but was absent from general databases (allele frequency $<10^{-4}$ in ExAC). Exome analysis was performed and this variant was the only one to be retained after the various filters. However, this variant remains controversial until extensive family investigation is performed. We confirmed that each parental allele carried a mutation in the 3 families.
Conclusion: We report here 3 new variants in the *CECR1* gene. Since DADA2 is an auto-inflammatory disease with original neurological involvement, *CECR1* testing should be widely performed in patients with such a phenotype. Both point mutations and deletions have to be searched.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P154

Histological and Immunohistochemical Features of the Skin Lesions in CANDLE Syndrome

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Question: Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome is caused by mutations in *PSMB8*. It occurs with early-onset fevers, accompanied by a widespread, violaceous and often annular, cutaneous eruption. It is postulated that the inflammatory disease manifestations stem from excess secretion of interferons, mostly type I interferons, which are proposed to lead to the recruitment of immature myeloid cells into the dermis and subcutis.
Methods: We systematically analyzed skin biopsies from 6 CANDLE syndrome patients by routine histopathology and immunohistochemistry methods.
Results: In all cases, skin lesions showed the presence of extensive mixed dermal and subcutaneous inflammatory infiltrate, composed of mononuclear cells, atypical myeloid cells, neutrophils, eosinophils and some mature lymphocytes. Positive LEDER and myeloperoxidase staining supported the presence of myeloid cells. Positive CD68/PMG1 and CD163 staining confirmed the existence of histiocytes and monocytic macrophages in the inflammatory infiltrate. CD123 staining was positive, demonstrating the presence of plasmacytoid dendritic cells.
Conclusion: The histopathology and IHC panel in the skin lesions of CANDLE syndrome is highly specific and should lead to a promto and specific diagnosis of this disorder. Both histopathology and IHC provide further insight into the pathogenesis of CANDLE syndrome.

P155

A case of corticosteroid-dependent recurrent pericarditis with different response to two IL-1 blocking agents

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Introduction: Recurrent pericarditis (RP) has a controversial pathogenesis that crosses infectious, auto-immune and auto-inflammatory pathways. It has been suggested that in some cases it might be an unrecognized auto-inflammatory disease. Recent studies have demonstrated that anakinra, an interleukin-1 receptor antagonist (IL-1RA), represents an effective treatment for the control of corticosteroid-dependent cases.
Objectives: Here we describe a case of cortico-dependent recurrent pericarditis with a different response to two IL-1 blocking agents, anakinra and canakinumab.
Materials and methods: Case report.
Results: A 11-year-old boy was admitted to our hospital with acute precordial pain, orthopnea, fever and increased levels of acute phase reactants. Acute pericarditis was confirmed by echocardiography and a treatment with prednisone was started with prompt clinical improvement. Pericarditis recurred twice during steroid tapering (at 1mg/kg/day and 0.5mg/kg/day respectively). After exclusion of infectious origin, therapy with anakinra (2mg/kg/day) was established (to avoid long term steroid side effects) followed by dramatic clinical response and normalisation of laboratory findings despite tapering and discontinuation of prednisone. Treatment with anakinra was discontinued after 5 months with recurrence of pericarditis one week later. Anakinra was resumed with an excellent response. Five months later, while being in complete remission, anakinra was replaced with canakinumab (2mg/kg/dose) due to patient's intolerance of daily injections. One week later, the patient experienced a new episode of pericarditis requiring corticotherapy. Two more relapses occured during steroid tapering, after 6 weeks and 2 months, in spite of the uptitration of canakinumab to 4mg/kg/dose. Anakinra was restarted with prompt clinical and biological remission and prednisone was discontinuated without recurrence of pericarditis. Four weeks later, anakinra was spaced out every 2 days and a treatment of colchicine was added. After further 12 weeks follow-up under anakinra and colchicine, the pericarditis is still in remission.
Conclusion: We describe a case of steroid-dependent RP with a dramatic therapeutic response to IL-1RA (anakinra) but without response to IL-1β monoclonal antibody (canakinumab). This unexpected observation could suggest that Il-1α might have a role in the pathogenesis of RP. A more precise usefulness of each IL-1 blocking agent requires confirmation in prospective controlled trials.

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P156

MAGIC- is it for real?

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Question: MAGIC syndrome, an acronym for mouth and genital ulcers with inflammed cartilage, is a rare condition described in 1985 [1]. About 20 cases have been reported [2], and its existence is challenged, as it could be just a mere association of Bechet's disease (BD) with relapsing polychondritis (RP) [3]. Other authors, however, consider it a distinct entity with higher risk of aortic aneurysms [4]. We tried to find out whether this syndrome is a true nosologic entity.
Methods: We retrospectively reviewed our tertiary referral centre's database from 2000 to 2015 in order to identify the cases of RP and BD. All patients fulfilled the International Criteria for BD [5] and the Damiani-Levine criteria for RP [6].
Results: Three cases have been identified, all diagnosed with MAGIC's syndrome since the first presentation. No other case evolved into MAGIC after an initial diagnosis of RP or BD. Hematological screening was negative in all patients; one had gastrointestinal vasculitis and another one panniculitis. No one in our series had eye or CNS involvement. Aortic aneurysms were absent (as yet) in the 2 patients searched for. Azathioprine, colchicine and corticotherapy were employed effectively in all patients.
Conclusion: RP and BD have overlapping features and may share pathogenetic mechanisms. The same time of onset of the main MAGIC clinical features could favour the classification of the disease as distinct from RP and BD alone. However, in our small and incompletely followed-up series aortic aneurysms were not seen, like in other MAGIC cases reported. An aortic follow-up is nevertheless advisable, as in any RP.

References

1. Firestein GS, Gruber E, Weisman MH, Zvaifler NJ, Barber J, O'Duffy JD: **Mouth and genital ulcers with inflamed cartilage: MAGIC syndrome.** *Am J Med* 1985, **79(1)**:65-72.
2. Wajed J, Kiely P: **19. Could it be Magic?** *Rheumatology* 2011, **50(Suppl 3)**: iii43-iii50.
3. Kotter J, Deuter C, Gunaydin I, Zierhut M: **MAGIC or not MAGIC (mouth and genital ulcers with inflamed cartilage) syndrome really exists? A case report and review of the literature.** *Clin Exp Rheumatol* 2006, **24(5 Suppl 42)**:S108-S112.
4. Hidalgo-Tenorio C, Sabio-Sanchez JM, Linares PJ, Salmeron LM, Ros-die E, Jimenez-Alonso J: **Magic syndrome and true aortic aneurysm.** *Clin Rheumatol* 2008, **27(1)**:115-117.
5. Kronborg C, Mahar PD, Kelly R: **Should we keep changing the diagnostic criteria for Behcet's disease?** *Dermatology* 2014, **228(1)**:1-4.
6. Damiani JM, Levine HL: **Relapsing polychondritis- report of ten cases.** *Laryngoscope* 1979, **89(6 Pt 1)**:929-946.

P157

Distinct cerebrovascular features in patients with ADA2 deficiency

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Background: Mutations of CECR1 have been recently reported as causative of an inflammatory condition characterized by an early onset vasculopathy resembling polyarteritis nodosa. The clinical manifestations of the disease are heterogeneous with a wide range of severity. Patients with a more serve phenotype present early onset cerebral stroke, which can be either ischemic or hemorrhagic.
Objectives: To describe the neuroradiologic features of patients affected by ADA2 deficiency with central nervous system (CNS) involvement.
Methods: We reviewed the contrast-enhanced brain MR, MR angiography (MRA), and digital subtraction angiography (DSA) examinations of 3 male patients with a confirmed molecular diagnosis of ADA2 deficiency and CNS involvement: two brothers (R312X and E328D mutations in compound heterozygosis) and a third unrelated patient (T360A homozygosis). Age at first MR examination was 6 years, 1 year 5 months, and 6 years 2 months, respectively.
Results: All patients presented multiple acute and/or chronic small ischemic infarcts involving the basal ganglia and the midbrain, in keeping with small-vessel occlusions (lacunar strokes). One patient additionally presented a large hemorrhagic infarct in the temporal lobe. Areas of focal accumulation of hemosiderin due to intraparenchymal bleeding were also noted. Interestingly, two patients presented an abnormal contrast-enhancing soft tissue in the interpeduncular cistern, encasing the midbrain perforating arteries. These neuroradiological findings regressed after the treatment with anti-TNF agents. The brain MRA and DSA showed no relevant vascular stenosis of the major arteries of the circle of Willis.
Conclusions: Typical lacunar strokes in patients with ADA2 deficiency are due to occlusion of small perforating arteries, which can be missed on conventional arterial imaging focusing on the vessel lumen of relatively large arteries. We hypothesize that the abnormal soft tissue detected in the interpeduncular cistern in the present patients may represent an abnormal inflammatory perivascular response, leading to small vessel stenosis. Wider use of contrast-enhanced high-resolution MR examinations may better demonstrate inflammatory vascular manifestations in patients with ADA2 deficiency.

P158

The familial Mediterranean fever (FMF) 50 score: does it work in a controlled clinical trial? Re-analysis of the trial of rilonacept for patients with colchicine resistant or intolerant FMF

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Background: The familial Mediterranean fever 50 score (FMF50) was recently devised to define response to treatment and as an outcome measure for clinical trials of FMF.

Table 1(abstract P156) Clinical features of MAGIC patients in our series

Case	Sex, age	Clinical features	Therapy	Aortic involvement	Outcome
1	F, 63	oral aphtae since youth; genital aphtae, deep vein thrombosis, migratory seronegative polyarthritis, reccurent bilateral auricular chondritis, wheesing	CS, AZA,Col	NK	Lost to follow-up (after 2 years)
2	F, 35	bipolar aphtae, asymmetric sacroiliitis, acneiform rash, intermittent seronegative polyarthritis, gastrointestinal involvement, bilateral auricular and nasal chondritisANA positive, dsDNA negative	CS, AZA,Col	No/NK	Lost to follow-up (after 3 years-emigrated)
3	M, 2	bipolar aphtae, pseudofolliculitis, erythema nodosum, arthritis, panniculitis, recurrent auricular chondritis, nasal chondritis, positive cartilage biopsy	CS, AZA,Col	No	Rare chondritis flares

CS=corticosteroids, Col=colchicine, AZA=azathioprine

Objectives: To examine the performance of the FMF50 score in a previously published trial of rilonacept [1] for patients whose FMF was resistant or intolerant to colchicine.
Methods: We reanalyzed the data from the controlled trial of rilonacept vs. placebo in 14 patients with colchicine-resistant or intolerant FMF using the FMF50 score as the primary outcome. The FMF50 score required improvement by ≥50% in five of six criteria (attack frequency, attack duration, global patient assessment, global physician assessment, frequency of attacks with arthritis, and levels of acute-phase reactants) without worsening of the sixth criterion.
Results: In the original trial rilonacept was considered effective according to the primary outcome measure (differences in the attack frequency) with eight analyzable patients considered responders and four as non-responders. According to the FMF50 score, only two participants would have been considered responders to rilonacept, and one to placebo. Only two participants had ≥50% differences between rilonacept and placebo in five criteria. The major explanation for non-response to treatment was that with rilonacept the duration of attack decreased by ≥50% in only 2 participants and 5 participants had no attacks of arthritis either during screening (before randomization) or during treatment with rilonacept.
Conclusions: The proposed FMF50 score did not differentiate well between responders and non-responders compared to the a priori defined primary outcome measure in this successful controlled study and should be revisited prior to adoption as a primary outcome measure in multinational FMF trials.

Reference

1. Hashkes PJ, Spalding SJ, Giannini EH, Huang B, Johnson A, Park G: Rilonacept for colchicine-resistant or -intolerant familial Mediterranean fever: a randomized trial. *Ann Intern Med* 2012, **157(8)**:533-541.

P159

Implementation of home measurement of CRP levels in diagnosis and monitoring of children with autoinflammatory diseases

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Introduction: Recognition of systemic autoinflammatory diseases (SAIDs) in the last decade is growing rapidly. About 250 children is under care with suspicion or diagnosis of SAIDs in our centre and monitoring of inflammatory markers is fundamental in their diagnosis and monitoring of treatment.
Objective: To check the practical usefulness of home CRP monitoring in patients during diagnosis or monitoring of autoinflammatory diseases.
Material: Ten patients with genetically confirmed or suspicion of AIDs (2 FMF, 2 TRAPS, 1 MWS, 2 PFAPA, 3 suspicion of SAIDs) were monitored at home with both use of diary of symptoms/treatment and regular CRP measurement.
CRP levels were checked by parents at home using ready-to-use system on a finger-prick blood sample of their child.
Results: Ready-to-use system provided patients with very fast, easy to perform and safe CRP measurement, having obtained reliable results.
Children and their parents found avoidance of frequent outpatient clinic visits and painful collection of blood from veins extremely important. Parents were able to use the system properly just after 2-3 hours of practical training.
Regular, up to twice a day measurement of CRP greatly facilitated the diagnosis and monitoring of patients' treatment. It proved to be extremely useful in an appropriate modification of treatment with the use of steroids in PFAPA or IL1-blockers in TRAPS.
Conclusions: The results of introductory pilot study of home measurement of CRP levels in children with autoinflammatory diseases are encouraging. The opportunity of fast, regular monitoring of inflammatory marker in home conditions improved both diagnostic-therapeuthical process as well as quality of children's life.

P160

Monogenic and multifactorial autoinflammatory diseases: Clinical and laboratory characterization in a pediatric Saudi population

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Objective: To report on the clinical and laboratory features of both monogenic and multifactorial autoinflammatory diseases in Saudi children.
Methods: This retrospective report comprised all children with autoinflammatory diseases treated at the Pediatric Rheumatology Clinic at King Faisal Specialist Hospital and Research Center, Riyadh, between January 2000 and December 2014. Demographic characteristics, diagnosis, age at onset, disease duration, follow-up duration, clinical features and laboratory variables including genetic results if available, and treatment were collected.
Results: A total of 75 patients (43 females) with various autoinflammatory diseases were included; consanguinity was present in 45%. The mean age was 11.6 years with mean age at onset of 2.7 years and mean disease duration was 8 years. Patients were diagnosed as follows: familial Mediterranean fever (FMF) 19; chronic recurrent multifocal osteomyelitis (CRMO) 18; monogenic form of systemic onset juvenile idiopathic arthritis (So-JIA) 14, early onset sarcoidosis 7, familial Behect's disease 5, periodic fever, aphthosis, pharyngitis and adenitis (PFAPA) 4, CINCA 3. Five patients had periodic fever but without definite diagnosis. Most of the cases were referred with inaccurate diagnosis. FMF patients had the usual manifestations but one patient had sacroiliitis. *MEFV* genetic testing showed pathogenic mutations of *M694V* gene in 12 patients while 7 patients had heterogonous sequence variants. All FMF patients had favorable response to colchicine. All CRMO patients presented with bone pain and fever with elevated inflammatory markers and abnormal radiographic findings. Biopsy results were consistent with osteomyelitis, but cultures were negative. All CRMO patients had favorable response to treatment (16 treated with pamidronate and 3 patients required infliximab). Patients with So-JIA had autosomal-recessive pattern on inheritance and whole-exome sequencing identified a homoallelic missense mutation in LACC1. Patients with early onset sarcoidosis had multi-organ involvement, diagnosis was proven by histopathology with negative cultures and treatment included prednisone, methotrexate and biologic agents. Three PFAPA patients responded well to corticosteroid and one patient underwent tonsillectomy. All CINCA patients had a good response to IL-1 blocker.
Conclusion: Autoinflammatory diseases other than FMF may be overlooked in our region. Increased awareness among pediatricians is needed for timely and accurate diagnosis and proper management. Association of LACC1 with monogenic So-JIA justifies investigation of its role in autoinflammatory disorders.

P161

Juvenile eosinophilic fasciitis: report of three cases with a review of the literature

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Introduction: Eosinophilic fasciitis (EF), also called Shulman's syndrome or fasciitis-panniculitis syndrome, is an uncommon scleroderma-like disorder of unknown etiology, characterized by induration and thickening of skin and soft tissue, usually associated with peripheral eosinophilia. In childhood, EF is poorly characterized.
Methods: A case series of all patients diagnosed with EF at our Department between 2011-2014 is reported. All cases with onset of EF before 18 year-old in PubMed-MEDLINE were reviewed: complete data were available for 16 pediatric cases only. These cases are compared to the present series and the adult form.
Results: We report three cases of females (age range: 3-4 years) who developed a rapidly progressive motility impairment started from fingers,

without cutaneous signs. They presented joint contractures, hepatosplenomegaly and generalized lymphadenopathy. Two of them developed acute complications (pharyngo-laryngeal incoordination and pericarditis). Laboratory investigations showed eosinophilia (range 1850-11670/mmc), increased erythrocytes sedimentation rate and hypergammaglobulinemia. The whole-body magnetic resonance imaging revealed thickening and hyperintensity of muscular fascia. A full-thickness biopsy showed inflammatory infiltration of the muscular fascia by mononuclear cells, confirming the diagnosis of EF. All patients were treated with oral prednisolone, plus methotrexate (1 patient) and cyclosporine (two patients), with partial benefit. Two years later, two of them show disabling outcomes despite intensive physiotherapy. In contrast with EF commonly described in adult patients, jEF is characterized by a very early onset. An association with trauma is missing, while history of infection was reported. The clinical presentation is dominated by a severe articular involvement that is prevalent in respect to the typical skin changes observed in adults. A systemic involvement (hepatosplenomegaly, lymph nodes enlargement) is more frequently present. Moreover, the disease course in children appears to be more severe.
Conclusions: Juvenile EF may have onset with progressive motility impairment at upper extremities, without skin abnormalities, and may show systemic inflammatory involvement. Juvenile EF requires early recognition in order to start appropiate treatments aimed to prevent acute complications and long-term disabling outcomes.

P162

"Daily life of CAPS patients treated with canakinumab (Ilaris®) : data from the French observational study - ENVOL Study"

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Background: The long-term efficacy of canakinumab has not been thoroughly evaluated since its first use in France in 2007 and its approval for CAPS in 2010. In addition, changes in the daily lives of patients (and their caregivers) since they have received this drug have never been reported.
Objectives: A multicentre retrospective, observational study set up to assess the "real life" use of canakinumab in all French CAPS patients ever treated since 2007, to describe their clinical course on a long-term, and to analyse changes in their (and their caregiver's) quality of life.
Methods: We targeted the 70-80 patients ever treated for CAPS in France, at least once and even during a clinical trial. Patients' (parents end caregivers) gave their informed consent to enter the study. Investigators were known experts in the field of CAPS in France who accepted to take part in the study. Data were collected through questionnaires by phone interviews and medical chart reviews, at treatment initiation, 6 months, 12 months and at the last medical visit. They included: clinical data, canakinumab use in real life conditions, impact on patients' (and caregivers') quality of life, and care consumption. The significance limit was set at 5% for all of the statistical tests.
Results: 68 CAPS patients, >90% of the target number, were enrolled (23 children, 45 adults). Sixteen patients (24%) had FCAS, 43 (63%) had MWS and 9 (13%) had NOMID-CINCA. The median duration of treatment was 5 years (from July 2007 to July 2014). >95% of patients remained on treatment. Doses were not modified in nearly half cases (31/68). For 37 patients, dosage adjustments (more often increase) were required (102 in total), especially in younger patients and those with the most severe phenotypes. All clinical symptoms monitored during the study got better under canakinumab. The global activity of the disease, the skin disorders and most of the symptoms were significantly better ($p<0.001$) at the different study timelines. The evolution of the quality of life score showed a significant improvement (median was at 8 before canakinumab versus 2 after; $p<0.0001$). Canakinumab treatment allowed also improvement in patient's daily activities, mood, and social life. Patients reported less school absences (79% versus 36%), and less sick leaves (48% versus 6%) after the initiation of canakinumab. The effect was weaker in heavily handicapped CINCA patients; due in part to the late initiation of anti IL1 treatment. Caregivers (49) were mostly family members and 35% of them had CAPS. They dedicated a mean of 7 hours/week to the CAPS patients before treatment and 4 hours during the last year. They spent on average 11.1 days per year of their job before canakinumab treatment versus 3 days after. The trend was less pronounced for caregivers of NOMID-CINCA patients.
Conclusion: Even retrospectively fashioned, the ENVOL study showed real-life results similar to those obtained during the phase III clinical trials with canakinumab, reinforcing its sustained activity. The maintenance of more than 95% of patients on therapy confirmed its major benefit to CAPS patients, which was demonstrated herein by the positive impact of canakinumab in patients (and their caregivers) social, emotional, educational and professional lives.

P163

Prevalence of polymorphisms of the genes responsible for auto-inflammatory diseases among 236 patients with recurrent fever in a rheumatology institute in Japan

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Introduction: Auto-inflammatory syndromes are defined as conditions caused by an exaggerated innate immune system response, resulting in episodes of spontaneous inflammation affecting multiple organs. The prototypical auto-inflammatory disorders are associated with periodic febrile episodes. *Auto-inflammatory syndromes* now include polygenic diseases, such as Behcet's syndrome and Still's disease; however, the best characterized auto-inflammatory diseases are relatively rare, but florid conditions arising from mutations in single genes. The prevalence of each auto-inflammatory disease varies depending on ethnic background.
Objectives: To analyze the prevalence of polymorphisms of the genes responsible for auto-inflammatory diseases managed in a single rheumatology institute in Japan.
Methods: A total of 202 individuals < 40 years of age with recurrent febrile episodes were enrolled in this study. Recurrent fever was defined as > 2 episodes of fever > 38.5 degrees Celsius lasting > 3 days in a year. Infections, autoimmune disorders, and malignancies were excluded as causes of fever prior to enrollment. Genomic DNA was isolated from the patients' peripheral blood and a polymerase chain reaction (PCR) was used to amplify the indicated exons of 10 genes [MEFV (exons 1-10), TNFRSF1A (exons 2-4), MVK (exons 9-11), NLRP3 (exon 3), NOD2 (exon 4), LI1RN (exons 2-4), IL36RN (exons 2-5), PSMB8 (exons 2, 3, and 5), NALP12 (exons 3 and 9), and PSTPIP1 (exons 10 and 11)], which have been reported as the genes responsible for auto-inflammatory diseases. After cleaning the PCR products, cycle sequencing was carried out using the Big Dye® Terminator v3.1 kit and analyzed with an ABI 3130xl Prism Genetic Analyzer. For the most frequently reported 4 genes, genetic polymorphisms within MEFV (exons 1-10), TNFRSF1A (exons 2-4), MVK (exons 9-11), and NLRP3 (exon 3) were examined. With respect to the other 6 genes, the existence of polymorphisms was also determined within NOD2 (from L248R to P727L), LI1RN (from N52KfsX25 to C91F), IL36RN (from R10X to G141Mfs*29), PSMB8 (from T75M to G201V), NALP12 (from T260M to F402L and R1016X), and PSTPIP1 (from A230T to E277D), with reference to the INFEVERS database, an evolving mutation database for auto-inflammatory syndromes (http://fmf.igh.cnrs.fr/ISSAID/infevers/index.php).
Results: Gene polymorphisms in the targeted genes were identified in 137 of the 236 patients (58.1%) based on INFEVERS. One hundred thirty-five of the 137 (98.5%) were associated with MEFV genes. Other polymorphisms were identified in TNFRSF1A (n=7), NLRP3 (n=5), NOD2 (n=4), MVK (n=2), and PSTPIP1 (n=1).
Conclusion: Polymorphisms in MEFV were most frequently identified among Japanese patients with recurrent fevers. Further evaluation with clinical features is warranted.

P164

The case of Schnitzler syndrome in one single rheumatologic center

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Background: Schnitzler syndrome is characterized by chronic, nonpruritic urticaria in association with recurrent fever, bone pain, arthralgia or arthritis, and a monoclonal immunoglobulin M (IgM) gammopathy. Pathogenesis of Schnitzler syndrome is unclear. Some hypothesize that the deposition of the IgM paraprotein, leading to the formation of immune complexes and the activation of the complement cascade, is responsible for the cutaneous manifestations. Another proposed mechanism involves the uncontrolled activation of interleukin 1-alpha (IL-1 α).
Aim: To report the case of Schnitzler syndrome in our clinic, that to be considered like rare entities.
Case report: A 44-year-old Caucasian man had had symptoms beginning at the age of 40 years, including fever, fatigue, recurrent urticaria, conjunctivitis, swelling of the eyelids, angioedema sometimes. Urticaria lasted 2-3 days and then disappeared without sequelae. These episodes recurred once per month. The laboratory findings included an ESR of up to 36 mm/h, neutrophil leukocytosis - 12.4-18.7 x 109, C-reactive proteine rise -107 mg/l (normal 0-5.0 mg/l), ferritin - 415 mkg/l (normal 20-150 mkg/l)„ SAA-127 ng/ml (normal 0-6, 4 ng/ml), ANA and RF were negative, a monoclonal immunoglobulin M (IgM) gammopathy detected with serum immunoelectrophoresis in a concentration 5 g/L. A histopathologic examination was not done. The patient was treated with nonsteroidal anti-inflammatory drugs (NSAIDs), systemic steroids intravenously (120-90-60 mg) and orally (20 mg), methotrexate 20 mg/week, that were somewhat effective at controlling the urticaria and fever. Differential diagnoses was made with Systemic Lupus Erythematosus, Still's disease in adult, Acute Urticarial Vasculitis, also hereditary autoinflammatory diseases - cryopyrin-associated periodic syndromes (CAPS) - Muckle-Wells syndrome (MWS).
A genetic analysis on the patient did not show a mutation in the NLRP3 (CIAS1), MVK, TNFRSF1A genes.
Conclusions: We report the rare case of Schnitzler syndrome. It was very difficult to diagnose and confirm this diagnosis. The clinical and laboratory signs are wery similar with MWS. But later then in pts with MWS adult age (40 years old) of onset of disease and also a monoclonal immunoglobulin M (IgM) gammopathy put the correct diagnosis.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P165

Safety and efficacy of tocilizumab in children with systemic juvenile idiopathic arthritis

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Background: Since its approval in 2011 for treatment of systemic juvenile idiopathic arthritis (JIA), tocilizumab treatments are followed by the German biologics register (BiKeR). The aim of this interim analysis is to evaluate the efficacy and safety of tocilizumab under practical conditions in childhood.
Methods: Demographics, clinical characteristics, previous and concomitant therapy, parameters of disease activity and adverse events were documented prospectively. The efficacy was based on the PedACR 30/50/70/90 criteria and the JADAS10. Tolerability was captured by the primary treating physician to the adverse event reports.
Results: Until 31.12.2014 60 sJIA patients were included in BiKeR in which treatment with tocilizumab was started. The mean age at onset was 4.6 (median 3.3) years and the mean age at baseline was 9.4 (median 9.8) years and mean disease duration was 4.7 (median 3.6) years. Only 20.3% of patients were treated with tocilizumab in the first two years of their disease. Pre-treatment with NSAIDs was carried out at 52 (86.7%), with steroids in 58 (96.7%), with methotrexate in 53 (88.3%). 26 treatment attempts with other DMARDs, most commonly with azathioprine (9; 15%) or CSA (9; 15%) and 44 therapeutic trials with other biologics were made. Etanercept was used most frequently (25; 41.7%) followed by anakinra (23.3%; 14). Concomitant therapy consisted of NSAIDs (38; 63%), steroids (42; 70%) and methotrexate (38; 63.3%) with other DMARDs only in individual cases.
Most patients showed a significant response to treatment. At last documentation 62% / 58% / 50% reached a JIA ACR30/50/70 response. The mean JADAS10 showed a decrease from 17.5 to 6.0 / 3.0 / 4.0 / 3.0 / 4.0 after 3/6/12/18/24 months. The proportion of patients in remission (JADAS10≤1) at month 6, 12, 24 was 42% / 25% / 27%, and the proportion in JADAS minimal disease actvity, MDA (JADAS10≤3,8) 55%, 35% and 55%.
Until 31.12.2014 a total of 74 adverse events (AE) were reported (101.7 / 100 patient-years (CI 81.0 to 127.8)), of which 10 (13.7/100 patient-years (CI 7.4 to 25, 5)) were serious (SAE). Infections were reported most frequently with 33 events. 5 AEs related cytopenias (without MAS), 4 intolerance reactions. 3 infections were SAE (appendicitis, pneumonia, herpes zoster). 4 patients developed a macrophage activation syndrome. Further SAE occurred once, anaphylaxis, seizure, fracture. Opportunistic infections, including tuberculosis, malignancies or deaths were not reported. In 32 patients (53.3%) the treatment was stopped. Reasons were (several simultaneously possible) remission in 15 (25%), ineffectiveness 7 (11.7%), patient request 5 (8.3%), intolerance 4 (6.7%) other 1 (1.7%).
Summary: Upon therapy with tocilizumab a high ACR response was achieved by many sJIA patients, as well as a JADAS MDA. A JADAS remission was documented in up to 40% of patients. In many patients, tocilizumab was used late and as second biologic. The tolerability was good overall and comparable to those of other biologics in JIA. Only a few patients discontinued therapy because of intolerance or side effects.

P166

Development of anti-infliximab antibody is associated with reduced efficacy and infusion reaction in Behçet's disease with uveitis

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Backgrounds: Infliximab (IFX) potently suppresses ocular attacks in Behcet's disease (BD) with uveitis, resulting in favorable long-term visual prognosis. However, about one third of the patients had ocular attacks one or two weeks before the next IFX infusion, suggesting that the efficacy of IFX depends on the concentration. This study investigates IFX trough levels and antibody toward IFX (ATI) in BD patients receiving IFX and analyzes the relationship of the pharmacokinetics with clinical efficacy.
Objective: To investigate effects of infliximab (IFX) trough levels and antibody toward IFX (ATI) on efficacy and adverse events in Behçet's disease (BD) patients receiving IFX.
Patients and methods: A total of 160 BD patients who were or had been receiving IFX were enrolled (male 115, age 43.2+ 12.1 y.o.) from 10 institutes in Japan. The primary target was eye in 135 patients including 8 patients who previously discontinued IFX, intestine in 18, and CNS in 7. After initial boosting, IFX was given every 8 weeks but the interval was shortened when clinical efficacy was insufficient. The trough level was measured in sera from 122 patients with uveitis when 5mg/kg of IFX was administered with 5 to 10 week interval, whereas ATI was determined by ELISA in all patients. The patient conditions at sampling were divided into the symptomatic phase when the patients had any of

symptoms due to BD except oral aphthae, and the asymptomatic phase when the patients were asymptomatic. Patients were classified into 5 groups according to physicians' global assessment of IFX efficacy for uveitis; Group A: very effective, B: effective, C: insufficient, D; ineffective, and E: discontinued the IFX.
Results: Mean serum IFX trough level was 4.4+ 4.7mg/ml in 430 samples from 122 patients. The level was significantly lower in symptomatic phase (n=73, 2.5±5.3 μg/ml) than in the asymptomatic phase (n=357, 4.9 ± 4.6μg/ml). ROC analysis determined the cut-off level was 0.93μg/ml. Patient-based analysis revealed that the trough level was lower in Group C+D (1.3±3.3μg/ml) than Group A (4.2±4.4μg/ml) and B (5.4±5.7μg/ml), whereas administration interval was significantly shorter in Group B (7.2 ±1.1 wk) and C+D (6.8±1.5 wk) than Group A (8.2±0.9 wk). ATI(+) was found in 18 (11.3%) of all patients. The frequency was significantly higher in Group C+D (5/10, 50%) and E (4/8, 50%) than Group A (3/75, 4%) and B (6/38, 16%). Thus, unfavorable clinical responses were associated with low trough level and positive ATI. Muitivariate analysis using logistic regression model revealed association of therapeutic failure (Group C+D and E) with female, positive ATI, and infusion reaction (IR). Moreover, ATI was strongly associated with IR. We found that shortening administration intervals restored IFX level with clinical efficacy in 2 ATI positive patients, whereas switching to adalimumab was successful in 2 patients having serious IR.
Conclusion: ATI is associated with reduced efficacy due to decreased IFX trough level, and IR. When IFX efficacy is reduced, shortening IFX administration intervals restores clinical efficacy in most of patients but other therapeutic option such as adalimumab is necessary in a part of the patients.

P167

A decision tree based on procalcitonin and C-reactive protein levels as a potential diagnostic tool to distinguish PFAPA flares from acute bacterial and viral infections

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Introduction: Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) is a disease of unknown etiology and unclear pathophysiology. Considering the inexistence of specific laboratory test for PFAPA, it remains a diagnosis of exclusion.
Objective: We searched for practical use of procalcitonin (PCT) and C-reactive protein (CRP) in differentiating PFAPA attacks from acute bacterial and viral infections.
Methods: Levels of PCT and CRP were measured in 35 PFAPA patients during 67 PFAPA febrile episodes and in 86 children diagnosed with acute bacterial (n=47) or viral (n=39) infection. We used the C4.5 algorithm (statistical classifier) to construct a decision tree.
Results: Statistical analysis with the use of C4.5 algorithm resulted in the following decision tree: viral infection if CRP≤19.1 mg/L; otherwise for cases with CRP>19.1 mg/L: PFAPA if PCT≤0.65 ng/mL, bacterial infection if PCT>0.65ng/mL. The rule was effective in 83.7% of the cases. Febrile episodes during PFAPA flares, bacterial and viral infections were classified with the sensitivity of 76.1%, 93.6% and 84.6% and specificity of 89.5%, 88.7% and 96.5% respectively.
Conclusion: Differences in PCT and CRP levels during PFAPA attacks, bacterial and viral diseases may be used to build a simple decision tree. When interpreted cautiously and with reference to the clinical context, it might present a potential diagnostic tool for distinguishing PFAPA flares from acute infections.

P168

Cronic non-bacterial osteomyelitis (CNO) in a cohort of pediatric patients: clinical, biological and radiological response to treatment with Anakinra

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Introduction: Chronic nonbacterial osteomyelitis (CNO) is the most common autoinflammatory bone disorder in childhood (1). Diagnostic information is provided by TC-99 bone scintigraphy (BS) and/or whole body MRI. Non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, bisphosphonates and tumour necrosis factor inhibitors have been used until now with variable response (2).
Objectives: To describe clinical, biological and radiological response to treatment with anakinra in patients with CNO refractory to NSAIDs and bisphosphonates.
Materials and methods: Seven patients (4 females and 3 males) with refractory CNO were treated with anakinra for at least 6 months in our institution. Response to treatment was evaluated assessing clinical manifestations (pain, local swelling, functional impairment), laboratory findings (C-reactive protein (CRP)), erythrocyte sedimentation rate (ESR)) and serum amyloid A level (SAA))) and number of bone lesions on TC-99 BS at the start of treatment and at 6 months.
Results: The median age at diagnosis and before starting anakinra was 9.7 years (IQR 7.8-14.7) and 13.3 years (IQR 8.0-15.9) respectively. All were treated with NSAIDs and bisphosphonates as first-line therapy. Glucocorticoid therapy was required in one patients with concomitant recurrent fever and pleural effusion. These patients did not respond satisfactorily and anakinra (2 mg/kg/day) was started. At the start of treatment 7/7 patients (100%) had pain, 3/7 (43%) local swelling and 5/7 (71%) functional impairment; at 6 months of follow up 6/7 patients (86%) were completely asymptomatic, with one patient complaining of arthralgia. Before starting anakinra the median CRP, ESR and SAA were 2.7 mg/dl (IQR 1.7-4.9) 26 mm/h (IQR 12-46) and 53 mg/dl (IQR 27-112); at 6 months 5/7 patients (71%) normalized CRP, ESR and SAA; 2/7 had a decrease in inflammatory markers. Before anakinra 59 bone lesions were detected on TC-99 BS. After 6 months of therapy 24/59 lesions (40%) had completely resolved, 1/59 lesions (2%) had partially improved and 29/59 lesions (49%) remained stable. In two patients with persistent high biological inflammatory markers, new lesions (14) developed during treatment.
Conclusion: Our data suggest that anakinra appears effective in CNO in controlling symptoms and laboratory findings; subclinical bone inflammation was still detectable by BS after 6 months of treatment. Long-term follow-up studies with a larger number of patients are needed.

P169

Temporal changes of serum cytokine/chemokine levels in patients of Nakajo-Nishimra syndrome treated with tocilizumab

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In Nakajo-Nishimura syndrome (NNS), proteasome disability due to a loss-of-function *PSMB8* mutation induces storage of ubiquitinated proteins and overproduction of inflammatory cytokines and chemokines. However, the precise mechanisms causing complex phenotypes of the disease, including pernio-like eruptions, lipodystrophy and calcification of basal ganglia, is mostly unclear. As IL-6 overproduction in association with p38 hyperactivation was supposed to have a role in NNS (Arima *et al*, PNAS 2011), tocilizumab, a monoclonal antibody for IL-6 receptor, has recently been applied for two patients with NNS after informed consents were obtained. Decreased serum CRP and CPK levels in both patients and improved myalgia and arthralgia in one patient have been observed, whereas none of decrease in serum LDH level or improvement of fever and eruptions have been achieved. By analysis of serum cytokine/chemokine levels, IL-6, G-CSF and MCP-1 levels have changed in accordance to the CRP level, whereas IP-10 has shown constantly high levels independent of the CRP level. Furthermore, both the patients-derived peripheral blood monocytes and monocytes differentiated from a patient-derived iPS cells produced higher level of IP-10 than control cells after IFNgamma stimulation. These findings suggest that monocyte-derived IP-10 has a major role in pathogenesis of the sustained/progressing phenotypes in NNS.

P170

Behcet Disease in pediatric Argentinian patients

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Behcet's Disease (BD) is a multisystem inflammatory disorder of chronic course, highly frequent in certain ethnic groups (Turks, Israelis, Oriental). Although the disease is considered of low prevalence in children, recent registries indicates an increase in disease recognition. It characterizes by a broad spectrum of clinical manifestations, including ocular, musculoskeletal, CNS, gastrointestinal, vascular and mucocutaneous involvement. Its pathogenesis is very complex to be classified exclusively as an autoimmune or autoinflammatory entity. Objective: To describe the clinical and therapeutical characteristics of a series of patients with BD of onset in childhood. Patients and Methods: We reviewed the clinical charts of patients with BD age of onset ≤ 16 years, evaluated in our Rheumatology Section since 1994. The disease diagnosis was based on the International Study Group (ISG) criteria. Different variables were analyzed: demographic (including ancestry, age at presentation and delay time to diagnosis), clinical symptoms and affected organs, laboratory and therapeutic management (corticosteroids, immunosuppressive and biological agents) considering the response achieved. Results: 6 patients with BD were included, 4 females (66.7%) with mean age at onset of symptoms 9.05 years (range 4-12) and mean age at diagnosis 12.2 years (r 9-16). The average time of delay in diagnosis was 3.12 years. Turkish, Israeli and Armenian ancestry was found in 4 patients. No patient had positive family history of BD. Half of pts met criteria for classification of ISG. All patients had recurrent oral ulcers and 66.6% genital ulcers (n = 4) with no gender prevalence. Cutaneous manifestations were observed in 4 patients (66.6%) and consisted in erythema nodosum. The pathergy test was performed on 2 patients being negative. Four children presented uveitis (2 with hypopyon), and 2 of them received Infliximab as treatment due to a severe course (visual acuity 1/10). Clinical onset related to gastrointestinal symptoms (abdominal pain and enterorrhagia) was observed in 2 girls, associate in one case with neurological manifestations (pseudotumor cerebri). Molecular analysis was performed only in 1patient being HLA-B51 positive. All patients received steroids. Conclusion: In our series of patients with BD, the prevalence of clinical manifestations was similar to other series. Suspected symptoms of BD in the pediatric group are crucial for an early diagnosis and appropriate treatment.

P171

Recurrent Inflammatory Panniculitis with Partial Lipoatrophy and Elevated temperature: a possible new autoinflammatory disorder

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Question: We report a series of cases with recurrent episodes of inflammatory subcutaneous nodules followed by fat atrophy in the affected area in children with fever, malaise, abdominal pain, hepatosplenomegaly, and some laboratory abnormalities that often persist beyond the febrile attacks of panniculitis.
Patients: Five children with the above mentioned clinical features are presented. Skin histopathology with a panel of immunohistochemistry markers was obtained from all patients.
Results: Histopathology showed a mostly lobular panniculitis, without vasculitis, with a mixed inflammatory infiltrate with evolving prominent cellularity with neutrophils, then lymphocytes and finally histiocytes. Lipophagia was considered the cause of lipoatrophy. On immunohistochemistry, T-lymphocytes, both CD4 and CD8, were strongly represented, with a higher proportion of CD8. Myeloperoxidase (MPO) staining was positive in the infiltrate in all cases. MPO-positive cells were mainly located surrounding individual adipocytes. CD68/PGM1 stain was positive in all cases, and the infiltrate was more prominent in late lesions, showing prominent lipophagia. CD68/PGM1 cells were located within the infiltrate, but were also strikingly distributed around individual adipocytes. Double staining with MPO and CD68/PGM1 showed that MPO-positive and CD68/PGM1 cells were different, thus indicating that in early stages MPO-positive cells induce damage to adipocytes and later, CD68/PGM1 macrophages will phagocyte adipocytes, leading to lipophagia and lipoatrophy. We found intensely positive stain for STAT1 in all cases, whereas STAT2 was negative in all samples. Only the cells in the inflammatory infiltrate in the fatty lobules were stained for STAT1.
Conclusion: We speculate that this allegedly new disease is possibly one autoinflammatory disease with enhanced IFN-I signaling.

P172

Chronic recurrent multifocal osteomyelitis in N. Greece: disease burden in pediatric patients

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Introduction: Chronic Recurrent Multifocal Osteomyelitis (CRMO) is classified among the rare auto-inflammatory diseases. It has a genetic predisposition and a pathogenesis largely under investigation. Greek publications regarding CRMO in pediatric patients are currently absent.
Objectives: The aim of the study was to describe the disease phenotype, course and outcome of CRMO Northern Greek pediatric patients, and assess the disease activity at diagnosis by the recently proposed Jansson clinical score, which has a cut-off value of 39.
Patients and methods: Pediatric patients with a diagnosis of CRMO diagnosed and followed-up a tertiary University Hospital during the last decade (2005-2014) were included in this retrospective study.
Results: Nine patients (female:8, mean age: 10 yrs) were diagnosed with CRMO. The main presenting symptoms in all patients were inflammatory pain in multiple sites, mainly in the metaphyses and epiphyses of long bones in lower limbs (mostly in proximal femur (4/9)) and swelling (7/9). 6/9 patients reported hip pain and only 1, clavicular pain. At diagnosis, fever

Table 1(abstract P170)

Pt	Age Dx / Gender	Oral/genital Ulcers	Compromised systems	Treatments	Response
1	16 / M	+/+	Eyes, skin, epididimitis	Methotrexate, Cyclosporine, Infliximab	Partial
2	12/F	+/+	Skin, CNS	Azathioprine, Mesalazine, Thalidomide	Complete
3	9/F	+ / +	Skin, eyes, Gastrointestinal	Azathioprine	Complete
4	10/F	+ / -	Eyes	Azathioprine, Infliximab	Partial
5	14/M	+ / +	Skin, eyes, arthritis	Colchicine, MTX, Azathioprine	Complete
6	12/F	+ / -	Eyes	Cyclosporine, Azathioprin	Complete

was noted in 5/9 patients, a raised ESR (mean: 52.5mm/h) in 6/9 and CRP >1mg/dl (≤ 0.5mg/dl) in 5/9. Location of MRI lesions were vertebral (3/9), thoracic rib (1/9), femur (5/9), fibula (5/9) and tibia (3/9). The predominantly affected joints were the hip (5/9) and the ankle (5/9). The calculated clinical score at diagnosis was >39 in 8/9 patients and 36 in 1 pt.
In respect to treatment, the applied NSAIDs provided partial pain relief without preventing the disease course in 8/9 patients; notably, indomethacin had no effect in 1/9 pt. Steroids combined with methotrexate were administered in 5/9 patients, but 4/5 required an anti-TNF to achieve remission. In 2/9 patients antibiotics did not alter the progress of the disease.
Conclusions: This is the first Greek study that ranks the severity of this rare multi-faceted disease by Jansson clinical score recording a rather severe phenotype, despite the insidious presenting symptoms at onset. As the disease burden was high, half of the patients required biologics for disease taming. Jansson score proved an easy tool that can primary rank the disease severity and identify refractory cases in need for early aggressive treatment.

P173

Canakinumab treat-to target strategies increase complete response rate in CAPS

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Objective: Cryopyrin-associated periodic syndrome (CAPS) is a heterogeneous group of diseases characterized by excessive Interleukin-1β (IL-1 β) release resulting in severe systemic and organ inflammation. Canakinumab targets IL-1β and is approved at standard dose for children and adults with all CAPS phenotypes. Limited data are available regarding real-life effectiveness of canakinumab in patients living with CAPS. Therefore the aim of the study was to evaluate the real-life dosing practice and effectiveness of canakinumab in CAPS.
Methods: A multi-center study of consecutive children and adults with CAPS treated with canakinumab was performed. Demographics, CAPS phenotype and disease activity, inflammatory markers and canakinumab treatment strategy were recorded. Treatment response was assessed using CAPS disease activity scores, CRP and/or SAA levels. Comparisons between age groups, CAPS phenotypes and centers were conducted.
Results: A total of 68 CAPS patients at nine centers were included, these were 31 males and 37 females; median age was 25 years and 27 (40%) were children. All CAPS phenotypes were represented. Median follow up was 28 months. Overall, complete response (CR) was seen in 72% of CAPS patients, significantly less often in severe (14%) than in mild CAPS phenotypes (79%). Only 53% attained CR on standard dose. Dose increase was more commonly required in children (56%) than in adults (22%). Centers with treat-to-target approach achieved significantly higher CR rates (94% vs 50%).
Conclusion: Real-life effectiveness of canakinumab in CAPS was significantly lower than in controlled trials. Treat-to-target strategies may improve the outcome of children and adults living with CAPS.

P174

Determinants of health-related quality of life in children and adults with autoinflammatory diseases

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Pediatric Rheumatology 2015, **13(Suppl 1):**P174

Background: Familial Mediterranean fever (FMF) and cryopyrin-associated periodic syndrome (CAPS) are rare inherited autoinflammatory diseases (AID). Chronic inflammation may result in severe organ damage. Beyond the physical burden of the disease, its impact on psychosocial well-being affects all areas of life. Patients may encounter rejection resulting in isolation and the risk of psychological disorders. While the positive effects of IL-1 inhibition on the physical aspects of AID are well documented little is known about the psychosocial condition of these patients. Therefore, the aim of the study was to evaluate HRQL in patients with FMF and CAPS.
Patients and methods: A single centre study of consecutive patients diagnosed with autoinflammatory diseases age ≥ 4 years was performed. Semi-structured interviews focussing on domains of burden of disease, activities of daily life, family, school and job, participation in social life and self-management were conducted. In addition, patients completed validated HRQL questionnaires including KINDL-R (children) and SF-36 (adults). Questionnaires were analyzed using descriptive statistics. Results were correlated with patient-related variables.
Results: Interviews were conducted with 55 patients, 24 males and 31 females. Age distribution: 10 children age 4-7 years, 30 adolescents age 8-18 years and 15 adults. Diagnoses: FMF in 21, CAPS in 30 and unclassified AID in four. A total of 80 questionnaires were completed by affected children (7, 9%), adolescents (24, 30%), and adults (21, 26%) in addition to 28 unaffected parents (35%).
Overall, the patients' social well-being was impaired. The experience of not being believed was rated worst by almost all patients. Lack of understanding by doctors on their odyssey to diagnosis was experienced by 70%. Challenges in school and job were: above average times of absence (67%), impaired ability to concentrate (80%) and limited productivity (65%). The discrepancy between self-perception (feeling ill very often) and perception of others (not noticing the patients' disease) causes self-doubt (30%).
Conclusion: Children and adults with autoinflammatory diseases report significantly impaired HRQL. Patients identified challenges in school and job as the key concern. Targeted interventions like school visits by trained social workers may address this area of need.

P175

Unmet psychosocial needs in CAPS

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Introduction: Cryopyrin-associated periodic syndrome (CAPS) is a rare autoinflammatory disease. Generalized manifestations like recurrent fever and fatigue and organ disease like reduced vision, hearing loss, bone deformities and aseptic meningitis impair patients' well-being. While most physical complaints are well defined and managed by effective IL-1 inhibition, areas of psychosocial needs are much less explored and often unsatisfied even in treated patients.
Objective: To identify unmet needs in the psychosocial support of children and adults with CAPS.
Patients and methods: A qualitative study of children and adults diagnosed with CAPS cared for at the autoinflammation reference center Tuebingen was performed. Patients and their families were invited to participate in structured focus group interviews grouped according to age and involvement with the disease: children <14 years, adolescents and young adults 14-21 years, adults >21 years; parents; other family

members). Open questions were asked to the group. The group discussion was recorded, transcribed to text and analysed for mentioning of certain topics. Frequency of naming and relevance indicated by discussion participants was calculated and graded.
Results: The five focus groups comprised of 42 individuals including 25 CAPS patients; 10 females, 15 males, including five children, eight adolescents/young adults and 12 adults. In addition unaffected individuals included 14 parents and three other family members. Key domains of unmet needs identified included information about the disease, understanding of patients' needs, intervention in social network and exchange of experiences. The area of need identified in all focus groups and named most often was school. Specifically lack of appreciation by teachers (13) and fellow students (21) was named. In adolescent and adults groups other frequently named areas were employment agency, health insurance organizations and general practitioners.
Conclusion: Major unmet needs of children and adults with CAPS were identified as various displays of ignorance by the patients' environment. The need for psychosocial support exists particularly in school.

P176

Adult PFAPA - a single centre experience

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Introduction: Variant PFAPA affecting adults has been previously reported but appears to be either extremely rare or systematically underdiagnosed.
Objectives: To retrospectively analyse patients with symptoms resembling PFAPA seen in an adult fevers clinic in the UK.
Patients and methods: Patients were sought from the UK National Amyloidosis Centre database using PFAPA, fever, pharyngitis, lymphadenopathy, and aphthous ulcers as search terms. Data were collected on demographics, symptoms, investigations and treatment.
Results: 15 patients were identified. 13 were male and all of white European origin. None gave a family history of similar symptoms. Current median age is 28.3 years with median symptom duration of 15 years. 6 patients presented after the age of 16, and 5 before the age 5. 3 patients reported precipitants for their attacks, in all cases stress and fatigue. 13 patients reported regular attacks every 4-6 weeks. Fever was present in 100%; cervical lymphadenopathy in 93%, pharyngitis in 73%; oral aphthous ulceration in 40%; abdominal pain in one third, rash and red eyes in 13%. 13 of 15 patients reported at least 3 of fever, lymphadenopathy, pharyngitis or aphthous ulceration with attacks.
Sequencing of MEFV, MVK, TNFRSF1A was normal in all cases. 7 patients provided samples during attacks with a median CRP 27 mg/L and SAA 205 mg/L. All 15 had normal inflammatory markers when well.
47% underwent tonsillectomy without lasting benefit in any case. Corticosteroids had been used by 60% with 4 good responses and 4 partial responses; 4 patients continue on intermittent prednisolone. 14 (93%) have tried colchicine with 2 complete, 4 good and 6 partial responses and 12 (86% of exposed) remain on long term prophylaxis. One patient received anakinra and and 3 tried cimetidine with little effect.
All patients achieved heights and weights within the normal adult range. 13 of 15 are either in full time education or employment. No patients have developed AA amyloidosis.
Conclusion: Variant PFAPA is seen in adults. In our series 40% presented after the age of 16 and 33% presented in the typical age range of less than 5 years with persistent symptoms into adulthood. Compared to typical childhood PFAPA symptoms seem very similar but more patients are refractory to conventional treatment with corticosteroids or tonsillectomy. Colchicine given as long term prophylaxis is the most effective treatment although complete responses are rare. Despite ongoing symptoms and elevated CRP and SAA with attacks no patients have severe social or physical consequences of their disease.

P177

Chronic myelomonocytic leukemia as a cause of fatal uncontrolled inflammation in familial Mediterranean fever

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Introduction: Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder caused by mutations (mainly p. M694V in exon 10) in the *MEFV* gene. It is the most common hereditary fever syndrome. Daily and lifelong colchicine administration can prevent both fever attacks and occurrence of inflammatory amyloidosis. Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder classified as a myelodysplastic/myeloproliferative neoplasm. The median age of CMML diagnosis is 70 years and current treatment includes hydroxyurea and/or 5-azacitidine.
Objective: Circulating monocytes express the *MEFV* gene responsible for FMF. Monocytes are also the target cells of CMML. We aimed to test the inflammatory status of monocytes in a patient with a severe clinical phenotype combining FMF and CMML.
Patients and methods: We report on an FMF patient who developed CMML leading to an uncontrolled and fatal inflammatory syndrome. Nine FMF patients, including the CMML patient, were included in this study. IL-1β, IL-6 and IL-18 cytokine levels were measured by ELISA in the plasma from patients and apparently healthy donors.
Results: The patient, who was homozygous for the p.M694V mutation, was explored at the age of 83 for a profound anemia revealing a myelodysplastic syndrome. Despite colchicine therapy, an important inflammatory syndrome persisted. His status deteriorated quickly with severe uncontrolled inflammation and occurrence of peripheral monocytosis revealing the transformation of his myelodysplastic syndrome into CMML, with fatal outcome within a few months. Plasma levels of IL-6 and IL-18 were found to be very high, as compared to healthy controls and other CMML-free FMF patients.
Conclusions: Our study unveils the interplay between two different disorders involving the same target cells, suggesting that in myelodysplasia with inflammatory manifestations, mutations in genes causing autoinflammatory syndromes, like *MEFV*, can be present and thus could be sought. Early chemotherapy with interleukin inhibitors could be proposed in such unusual situations.

P178

Juvenile chronic non-bacterial osteomyelitis (CNO): Long term course of disease and response to treatment in a large institutional cohort

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Introduction: Chronic non-bacterial osteomyelitis (CNO) is an inflammatory disorder of the skeletal system of unknown etiology. Long-term follow-up and response to treatment data have rarely been reported. The aim of the study was to characterize the clinical, radiological, histological and laboratory data at juvenile CNO onset, and to analyze the long term treatment response.
Methods: The course of disease of 93 juvenile patients (58% female) with non-bacterial inflammatory bone lesions was evaluated retrospectively. 1098 patient visits and 558 MRI findings were reviewed. Clinical,

radiological, histological and laboratory data were assessed at disease onset and for a median time of disease of 46 months.
Results: The mean age at disease onset was 10.9 years, the mean time between the first symptoms and the diagnosis of CNO was 11 months. 84% of the patients had multifocal bone lesions. Biopsy was performed in 80 patients. Only when bone biopsy was taken within 12 months of symptom onset, cellular infiltrates could be observed. At later time points, fibrosis, hyperostosis and bone edema predominated. 25% of all patients developed peripheral arthritis, 8% inflammatory bowel disease. The initial treatment consisted of non-steroidal anti-inflammatory drugs (NSAIDs). 37% of the patients required second line therapy consisting of sulfasalazine and short term oral corticosteroids, 8% of the patients required bisphosphonates or TNF-blocking agents. Median time to first clinical remission was 6.0 months and 94% of patients achieved clinical remission within the period under review. Median time to first radiological remission, however, was 27.4 months and only 67% of all patients achieved complete radiological remission. Time to remission was independent of the CNO being unifocal or multifocal. In detail analysis of the treatment response revealed that initiation of sulfasalazine treatment in NSAID non-responders led to a significant and sustained decline of the clinical, as well as the radiological number of lesions.
Conclusion: The rapid clinical improvement in CNO, following initiation of therapy with NSAIDs, is not accompanied by a likewise decrease of the number of radiological lesions. Treatment with sulfasalazine is effective in childhood CNO.

P180

Pediatric chronic non-bacterial osteomyelitis in Goteborg, Sweden

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Introduction: Chronic non-bacterial osteomyelitis (CNO) is today included among the autoinflammatory bone diseases. An alternative term in the literature is chronic recurrent multifocal osteomyelitis (CRMO). The etiology of the autoinflammatory bone diseases is unknown except for a few extremely rare monogenic diseases.
Objectives: To describe a pediatric CNO cohort with respect to age at onset, age at diagnosis, number and location of lesions, imaging and treatment.
Patients and methods: Patients with CNO treated at the Queen Silvia Children's Hospital, Goteborg from 2000 to 2015. It is a retrospective file review of patients diagnosed before the age of 18 years.
Results: Twenty-seven patients with CNO were identified. The majority of the patients were females (22/27, 81%). The median age at onset of symptoms were 8.6 years (range 4.3 to 16 years) and the median age at diagnosis were 11.2 years (range 5.3 to 17.8). In total 80 lesions were found in the 27 patients. In 5 patients (19%) only one lesion was found. Most frequently lesions were found in proximal tibia (n=14), distal femur (n=11), clavicle (n=11), distal tibia (n=10) and vertebral column (n=5).
A biopsy was performed in 24 patients and it confirmed osteomyelitis in 22 (92%). A microorganism was identified in 6 cases, but was considered a non-significant finding and not the etiology of the osteomyelitis.
Plain x-rays were performed in all patients. In addition, 21 (78%) patients had a bone scan, 16 (59%) a MRT and 3 (11%) a whole body MRT.
Twelve patients (44%) were treated with antibiotics in an early phase of the disease.
Almost all patients were treated with NSAID (n=25, 93%) and many with a short course of corticosteroids (n=15, 59%). DMARDS were used in 14 patients including methotrexate that was used in 13 patients (48%) and sulfasalazine in 1 patient.
TNF inhibition was used in 6 patients and IL-1 blockade in 2 patients. Bisphosphonate was used in 3 patients.
Conclusions: In our cohort of patients with CNO there is a predominance of females. The median age of onset is 8.6 years. There is a large diagnostic delay. About 1/5 of patients had only one lesion which, makes the term CRMO problematic. The severity of CNO ranges from only one mild lesion to a severe phenotype with many lesions. Some patients only needed treatment with NSAIDs while others required intense treatments with biologics or bisphosphonates.

P181

Turkish DIRA patient with novel IL1RN gene mutation

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Pediatric Rheumatology 2015, **13(Suppl 1):**P181

Introduction: Deficiency of the interleukin-1 receptor antagonist (DIRA) (OMIM 612852) is a recently described rare autoinflammatory and autosomal recessive disease, caused by loss of function mutations in interleukin-1-receptor antagonist gene (IL1RN) leading to the unopposed activation of the IL-1 pathway. The human IL1RN gene is localized to the long arm of chromosome 2 at band 2q13 (OMIM 147679). Until now, only thirteen cases resembling this have beeen reported in the world.
In this study, we described clinical and molecular date male child who had clinical signs of DIRA syndrome firstly analysed in Turkey.
Materials and methods: Genomic DNA was obtained from peripheral blood. All exon and intron of IL1RN gene were analysed by PCR and direct DNA sequencing method. Furthermore the obtained nucleotid sequence compaired with reference sequence published in NCBI (NM_173841.2).
Results: The patient is a boy child, was born in 2007 and he is 7 years old now. Since birth he has had skin lesions like erythema and pustules, and was put different clinical diagnosis in different dermotology clinics. He was admitted to our center with the same recurrent skin lesions, erythema covered his entire body, scaling and crusting manifestations in 2013.
Molecular analysis of IL1RN gene revealed a single homozygous C nucleotide deletion at nucleotide position 396 (c.396delC). The novel c.396delC mutation that was found in our study caused frameshift mutation and as a result, stop codon of IL1RN at c.534* position disappeared and the respective protein became non-functional.
Conclusion: In our laboratory, it is the first case that the accurate genetic diagnosis of a case considered as DIRA has been confirmed with whole sequence analysis of IL1RN gene. Also the patient has been provided with appropriate biological targeted therapy with Anakinra, that is specific molecule for IL1B inhibition.

P182

The Eurofever Project: towards the longitudinal stage

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Background: In 2008 the Paediatric Rheumathology European Society (PReS) promoted an International Project for the study of Autoinflammatory Diseases (AID) named Eurofever whose main purpose is to create a web-based registry for the collection of clinical, laboratory and response to treatment information in patients with AID.
Objectives: To implement the Registry with the new recently described AID and genes and modify the web-system to make it suitable for the collection of longitudinal data.
Methods: With the technical help of Paediatric Rheumatology INternational Trial Organization (PRINTO) web-masters, we were able to revise the electronic case report forms bringing 2 main novelties: i) inclusion of the more recently described AID (DADA2, CAMPS, SAVI) and the relative clinical

manifestation ii) modification of drug layout. In this process information on safety and efficacy has been included. Very soon Centres will be asked to update on a yearly base the information regarding each patient with particular focus on modification of the clinical picture, onset of complication/sequelae, treatment, adverse events if present, laboratory and instrumental findings.

Results: At present baseline demographic information from 3089 (M:F=1513:1576) patients from 101 centers in 38 countries are available. In 77% complete clinical information from disease onset to diagnosis and response to treatment is also available. For each disease the number of enrolled patients is: FMF 894 pts (708 with complete clinical data); TRAPS 268 pts (226 complete); CAPS 284 pts (208 complete); MKD 189 pts (165 complete); Blau's disease 71 pts (22 complete); PAPA 27 pts (22 complete); NLRP-12 mediated periodic fever 14 pts (9 complete); DIRA 3 pts (all complete); CANDLE 2 pts (1 complete), Schnitzler 1 pt and Majeed 2 pts (both complete). Among multifactorial autoinflammatory diseases: PFAPA 612 pts (380 complete); CRMO 417 pts (394 complete); pediatric Bechet disease 92 pts (72 complete) and 217 patients with undefined periodic fever (182 complete). Longitudinal electronic data capture is now ready and online for authorized PRINTO members.

Conclusions: A common registry for collection of patients with AID is available and the enrollment is ongoing. This project represents the first attempt of a common registry for different autoinflammatory syndromes. The longitudinal collection and analysis of data coming from the Registry will improve our knowledge in the field of autoinflammation both on the natural history of the single disease and the efficacy and safety of the treatment commonly used in the clinical practice with particular regards to biologics.

P183

A case of neonatal-onset autoinflammatory syndrome with a *de novo PSMB9* mutation resembling Nakajo-Nishimura syndrome

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We report a case of neonatal-onset autoinflammatory syndrome that resembles Nakajo-Nishimura syndrome (NNS), a rare autoinflammatory syndrome caused by a homozygous *PSMB8* mutation. The patient is a 7-year-old boy who demonstrated clinical findings that differ from those of typical NNS.

Exudative erythemas on his face, trunk, and extremities developed 2 weeks after birth. At 1 month of age, the patient developed fever, elevated serum C-reactive protein levels, and elevated hepatic amino transferase levels. A febrile convulsion caused basal ganglia calcification at 4 months of age. Severe pulmonary arterial hypertension of unknown origin (NT-proBNP 33,445 pg/mL) was diagnosed at 7 months of age. These symptoms improved after administration of bozentan and ambrisentan, which are endothelin receptor antagonists. At 9 months of age, he developed heliotrope rash, edematous erythema on his extremities, and myositis with elevated serum creatine kinase levels (16,000 IU/L). Antinuclear antibodies, as well as anti-Jo-1 and anti-DNA antibodies, were negative. Skin and muscle biopsies revealed massive cellular inflammation, consisting primarily of lymphocytes and monocytic cells, located in the epidermis and between muscle fibers with fat tissue necrosis in the subcutis. Concomitant degeneration and regeneration of muscle fibers occurred and resulted in moderate variations in the fiber size.

These results initially led to a diagnosis of juvenile dermatomyositis. His symptoms temporarily improved with corticosteroid treatment but recurred periodically. Administration of systemic agents such as azathioprine, methotrexate, cyclosporine, cyclophosphamide, mycophenolate mofetil, and intravenous immunoglobulins did not lead to remission.

Hepatosplenomegaly and liver cirrhosis due to pulmonary arterial hypertension induced portal hypertension beginning at age 6. The serum levels of interleukin (IL)-1β (28 pg/mL), IL-6 (84.3 pg/mL), TNFα (21.6 pg/mL), IL-18 (595 pg/mL), and IFN-γ (7.0 IU/mL) were all increased during the periodic recurrences. These periodic symptoms were refractory to several immunosuppressive agents, which suggested an autoinflammatory syndrome. Although presence of fever, erythemas, basal ganglia calcification and hepatosplenomegaly suggested NNS, some of the characteristic symptoms of NNS, such as lipomuscular atrophy in the upper body and long clubbed fingers with joint contractures, did not occur in this patient.

Since no significant mutations had been detected in the *NLRP3, NOD2, MEFV, MVK, TNFRSF1A*, and *PSMB8* genes, whole exome sequencing of the patient and his parents was performed and a novel *de novo* heterozygous mutation has been identified in *PSMB9,* encoding the immunoproteasome subunit beta1i, in the patient. Our case might represent a novel proteasome-associated autoinflammatory syndrome similar to but distinct from NNS, in which a heterozygous mutation of the *PSMB9* gene is responsible.

Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P184

Chronic non-infectious osteitis: single centre case series

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Background: Chronic non-infectious osteitis (CNO), also known as Chronic Recurrent Multifocal Osteomyelitis, is a non-infectious inflammatory osteopathy predominantly affecting children and adolescents. Diagnosis is often difficult as initial symptoms and clinical course can vary widely. CNO can result in significant morbidity. Treatment regimes are varied but bisphosphonate therapy with pamidronate is proving effective at reducing pain and improving bone remodelling in some patients.

Objective: To review the presentation, diagnosis, management and clinical course of patients with CRMO in one paediatric centre.

Method: Case-notes of forty seven patients undergoing treatment for CNO from 2003 to 2015 at Bristol Children's Hospital were analysed and details entered into a custom-made spreadsheet containing eighty two parameters. In order to establish the criteria that had been used for diagnosis, plain films, CT, MRI, bone scans and biopsy results were recorded alongside presenting characteristics.

Results: Median age at presentation was 10 years (range 1.5 to 14). 33 were female, 14 male. The initial differential diagnoses included Langerhans Cell histiocytosis (5), malignancy (4), infectious osteomyelitis (12), arthritis (4), musculoskeletal (5), other viral illness (11) and CNO (6). All patients had x-ray and MRI studies. Whole body STIR MRI was used to detect silent areas of osteitis and monitor treatment. The predominant sites of bony involvement on MRI included femur (12), clavicle (9), tibia (13) and spine (14). 25 patients underwent bone biopsy and all had extensive microbiological investigations, with no organisms identified. Mean time from initial presentation to medical services to diagnosis was 26 months (range 1-84). 24 of patients had been initially treated with at least one course of antibiotics for a presumed infectious osteomyelitis. Patients were also treated with NSAIDs (42), steroids (5) and DMARDs (8), although only a small proportion had pain cessation. 24 patients received pamidronate, of which 12 completed the course. Of those who completed the course 4 had persistent pain and 8 had pain cessation.

Conclusions: This cohort is one of the largest series in the literature. It is important to increase awareness of CRMO as a diagnostic differential when a child presents with insidious onset bone pain. MRI STIR provides important evidence in the diagnosis of CRMO. A central database would facilitate a greater understanding of the diagnostic criteria and treatment options. Pamidronate has thus far appered effective in the management of 8 patients.

P185

Cost-effectiveness analysis and prevention effects of ultra-orphan drugs for rare diseases: an *in silico* model applied to Cryopyrin Associated Periodic Syndromes (CAPS)

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Objectives: This three-year, international, multicentre, longitudinal, observational, cost-effectiveness study named RaDiCEA (RareDisease &Cost-EffectivenessAnalysis) will assess the economic evaluation (cost of illness - COI and cost-effectiveness analysis - CEA) of innovative therapies (i.e., anti IL-1 agents), quality of life (QoL) and effects of the prevention of otherwise irreversible central nervous system, eye, ear, kidney, and cartilage damages of different treatment strategies for cryopyrin-associated periodic syndromes (CAPS) of adults and children.
Methods: A virtual time-cohort approach and a Markov model simulating health states corresponding to different CAPS severity will be developed to assess the cost-effectiveness of two different treatment strategies: i.e., either anti IL-1 agents or other than anti IL-1. Due to the lack of a CAPS-specific severity index/damage score, a linear combination of existing indexes and damage scores will be used to rank patient's health status with respect to damages involving specific organs and systems. Coefficients of the resulting function will be assigned following both a top-down (Delphi) and an interim-ex post-bottom-up approach (principal component analysis) considering covariances of all the variables adopted to describe the disease evolution or response to therapies. The model uses relevant economic measures to quantify resource utilization for patients' care in the National Health Systems' perspectives and a broader societal perspective to take into account direct nonmedical costs and indirect costs, in addition to direct costs. QoL will be evaluated using EQ-5D questionnaires. To assess how the model reacts to changes in singular and multiple disease parameters, univariate and probabilistic sensitivity analyses will be performed.
Expected results: The RaDiCEA project will assess the long-term effectiveness of different potentially life-long treatment strategies and COI, while exploring the feasibility of a new CEA model to be generated from a rare disease (CAPS) observational study. The economic outcomes will be given as the number of years spent in each health state, the related yearly costs and QoL.
Conclusions: The importance and novelty of the model is twofold: i) in its application, adopting the cost-effectiveness approach for assessing the impact of CAPS therapies, and ii) in the methods, extending the analyses of the impact of CAPS therapies in reducing the speed of disease progression.

P186

The RaDiCEA Project: cost of illness (COI) analysis applied to Cryopyrin Associated Periodic Syndromes (CAPS)

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Introduction: When a disease affects only a few individuals in each country (ultra-orphan disease), it can be very hard to establish the costs of illness (COI) and the cost-effectiveness (CE) of treatments (ultra-orphan drugs). Conventional methods for COI and CE of drugs for common conditions do not apply, and additional factors need to be considered. As expensive medications (biologicals) show promising results, it becomes crucial to have detailed information on as many patients as possible.
Objectives: This international, multicenter, longitudinal observational COI and CE study will evaluate the burden of CAPS in terms of direct and indirect costs and quality of life (QoL). The COI analysis implies the identification, quantification and evaluation of resources from a societal perspective.
Materials and methods: In the frame of the EuroFever registry (http://www.printo.it/eurofever/), 8 Centers of reference for autoinflammatory diseases in Italy and France (sentinel countries) are currently enrolling all consenting/assenting CAPS adult patients and children with the indication for either the use of an anti IL-1 agent or other than anti IL-1 therapies (NSAIDs, systemic corticosteroids, immunosuppressive drugs), and compliant to the study. To perform the COI analysis, clinical and economic data are collected at country-specific- and general levels, both retrospectively (since disease onset) and prospectively (from study enrollment to the last visit) by mean of general questionnaires administered to patients or their proxy on direct medical and non-medical costs, and indirect costs of productivity loss. QoL is prospectively measured using EQ5D to estimate utility score and calculate QALY.
Results: COI analysis results based on the Italian and French Healthcare Systems will test the robustness of the proposed methodology, assumptions and source of data before a generalization to other countries. To properly value the used resources, country-specific questionnaire administered to the budget and management control officer at each center will collect information about the healthcare system type, hospitalization reimbursement system, out of hospitalization drug payment, payment of special schools and devices, application of exemption, disability pension and/or allowance for caregivers. All costs will be referred to a common base year and adjusted to eliminate the effect of inflation. The differences in price levels between countries will be eliminated applying the Purchase Power Parities using Euros as the reference base.
Conclusion: The ERANET-PRIOMEDCHILD RaDiCEA Project (No. 40-41800-98-007) will develop a model to evaluate costs and long-term benefits in an ultra-orphan group of diseases such as CAPS. The same model may be used in other very rare disorders.

P187

Studying patients with autoinflammatory diseases: the past, present, and a perspective for the future

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Introduction: Autoinflammatory diseases (AID) are rare disorders characterized by recurrent episodes of systemic and organ-specific inflammation. Studying AID has been limited by the difficulty in finding and enrolling large numbers of patients with these rare illnesses. We used a traditional retrospective chart review to describe patients with AID at a single academic medical center, and compared the results with those of participants within the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry, a multicenter observational pediatric rheumatic disease registry in North America. We discuss the benefits and limitations of these types of studies, and suggest new ways to conduct future research.
Methods: We included patients with a clinical or genetic diagnosis of AID. We conducted a retrospective chart review at Boston Children's Hospital (BCH) from 2002-2012. Charts were identified by keywords and billing codes related to AID, and patients with CRMO were excluded. We also conducted a cross-sectional study of children with AID enrolled in the CARRA registry from May 2011 to December 2013.
Results: At BCH, we identified 169 subjects with AID. In the CARRA registry, of 9,523 subjects enrolled, 87 patients had AID. The distribution of diagnoses from patients at BCH included PFAPA (48%), FMF (22%), TRAPS (2%), MKD (2%), NOMID (2%), FCAS (2%), and undefined (23%). In the CARRA registry, diagnoses included CRMO (39%), FMF (17%), PFAPA (10%), TRAPS (7%), NOMID (3%), MWS (3%), SAPHO (2%), PAPA (1%), FCAS (1%), MKD (1%), and undefined (14%).

Conclusions: The past: Using the traditional method of a single-center retrospective chart review, we described the patients with AID seen at BCH over a 10-year period. This research required little cost and relatively little time. Limitations included incomplete documentation of some patients, and the variety of AID which were identified.
The present: The CARRA registry was a multicenter effort where patients were enrolled at a faster rate, and with a greater variety of diagnoses. However, this registry required significant financial investments in technology and operational costs. PFAPA, the most common pediatric AID, represented only a minority of subjects within the CARRA registry, suggesting an enrolment bias, perhaps due to the time required for consent, enrolment, and data-uploading process.
The future: Future registries will likely be integrated into the patient's electronic health records, avoiding many of the current barriers to research. In addition, we believe that online patient communities can contribute valuable information regarding patient-reported outcomes. In future studies, we plan to empower and engage patients with AID through social media to collaborate in design and research implementation. Our efforts will exponentially expand the number of patients available to participate in research, and will provide more complete and meaningful data.

P188

Chronic recurrent multifocal osteomyelitis: follow up Longitudinal case series study for five years with radiographic and scintigraphic imaging perspective and health outcome analysis with EQ5D-5L

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Introduction: Chronic recurrent mutifocal osteomyelitis (CRMO) (OMIM-number-259680), a rare form of autoinflammatory disease of the bone marrow of unknown aetiology, which takes a sub-acute course. First described in 1972 by Gideon et al, CRMO is primarly a diagnosis of exclusion as suggested by negative bone biopsy for neoplasms and negative bone culture to rule out infectious causes. In our case, we have a case cohort of a female child from 2011 until the present. During each episode of the disease, NSAIDs, empirical antibiotics and a course of steroids were given.
Objectives: Primary objective: To identify the radiographic imaging perspective of the recurrent inflammatory attacks on the bone, considering time period of each attack, a follow up case cohort for five years.
Secondary objective: Elucidation of existing proposed diagnostic (King et al and Iyer et al) criteria & utility in aiding diagnosis as to the imaging features and validation of generic health outcome of a CRMO patient with EQ-5D 5L.
Methods: Study design: A longitudinal case series study.
Study setting: Trivandrum Medical College.
Study period: 18 May 2010 – Ongoing.
Study subjects: Patient and relevent family members with genotypic variance.
Study tool: EQ-5D-5L, Descriptive system of health related quality of life states in 5 dimensions by EuroQoL with permission.
Results: The first presentation was at 9 years of age with a clinical lesion on the right 2nd metatarsal. X-ray revealed hypolucent lytic lesion. A bone biopsy for histopathology revealed chronic inflammatory infiltrate with predominant monocytes & lymphocytes suggestive of osteomyelitis, bacteriological culture of the sample was sterile and mycobacterial-RT-PCR was negative.
The second episode was after two weeks at the right-shoulder and X-ray revealed a permeative sclerotic lesion at humerus. PDFS, T1WI and T2WI MRI shows hypointense rim was suggestive of sclerosis with contrast enhancement suggestive of subperiosteal infection. Investigations included negative HLA-B27, negative ANA-profile for screening of autoimmune disorders, negative CRP and ESR showed elevation. Abdominal-ultrasound to rule out IBD and was normal. MRI was taken & provisional diagnosis of CRMO was made based on Iyer et al criteria.
In 2015, recurrence of pain occurred in the right arm. 3-phase-Tc99MDP whole-body-scintigraphy did not show any asymptomatic foci of infection other than the humerus. A follow up MRI showed that the lesion has extended distally towards the diaphysis of humerus 23cm down towards elbow, when compared to the previous year imaging. EQ5D-5L index value as generic health outcome assessment is 0.722, with Zimbabwe taken arbitarily as standard. (max value is 1 assigned for positive health), EQ-VAS scale coded response value in is 72. (max value for best possible state of health is 100).
Discussion: The radiographic pattern, shows that TIRM, STIR sequence is helpful in assessing marrow edema, diffusion restriction is not seen usually, CT scan aids in assessing cortical thickening of bone and post contrast enhancement of lesion present indicative of a severe disease. A 3-phase-bone-scintigraphy, help to pick asymptomatic sites(sensitivity 73% to 100%) usually shows uptake in all three phases.
As per Angela et al, the effect of attenuated TLR4/MAPK signalling & IL-10 polymorphism with reduced Sp1 recruitment & attenuated H3S10 phosphorylation contributes to central pathophysiology of CNO. Hence satisfying Iyer et al or King et al criteria alone does not serve as a platform to start on a patient with immunomodulatory therapy, cytogenetic profiling is advised prior to its commencement. The serum level of calcium shows hypocalcaemia, hypophosphatasia was not attributed in this case. TPMT assay is withheld considering financial condition of patient. Treatment protocol with anti-TNFα inhibitors & other immunomodulatory therapy is initiated for severe disease with low heath outcome. immunomapping of chromosome 18q21.3, an assessment of 1L10, TNFα, IL-1β, and LPIN2 mutation in (Majeed Syndrome), TNSALP gene mutation (metabolic defect with hypophosphatasia involving the NLRP3 inflammasome) are evidence based optional cytogentic tests. Dysregulation in sex hormones in CRMO are being investigated.
Conclusion: Radiographic imaging (MRI) & whole-body-bone scintigraphy should be preferably used as initial diagnostic modalities for screening and follow up. Standardised imaging analysis as compared to existing proposed clinical criteria (King et al, Iyer et al etc) can only aid in establishing diagnosis. Health outcome assessment using EQ5D-5L with crossover with EQ5D-3L index value which shows moderate satisfactory outcome.

P189

Anakinra as a diagnostic challenge and treatment option for systemic autoinflammatory disorders of undefined genetic cause

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Background: Diverse monogenic autoinflammatory diseases share responsiveness to interleukin (IL)-1 blockade. This study explored the utility of anakinra (an IL-1 receptor antagonist) as a treatment option for clinically heterogeneous systemic inflammatory disease with autoinflammatory presentations where a genetic cause was not defined.
Methods: A total of ten adult cases with ongoing inflammatory episodes, where alternative diagnoses, including malignancy and infection, were evaluated. Genetic screening was also performed to exclude known genetic causes of autoinflammatory disorders (e.g. cryopyrin-associated periodic syndromes (CAPS), tumour necrosis factor receptor-associated periodic syndrome (TRAPS), etc.).
Results: All patients had presentations that were atypical of recognised autoinflammatory disorders and all were negative on genetic screening. Eight of ten cases showed rapid responsiveness to anakinra with the ability to subsequently taper alternative immunosuppression. Good responses to anakinra were maintained with inadvertent drug discontinuation being linked to disease flares.
Conclusions: The spectrum of poorly defined clinical and genetic autoinflammatory disorders that show responsiveness to anakinra is considerable. In fact, responsiveness to anakinra appears to be useful in diagnosis given the characteristically rapid onset of efficacy and symptomatic improvement.

P190

Choosing the right treatment for patients with a severe course of chronic non-bacterial osteomyelitis (CNO) - pamidronate or TNF-α blockade?

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Chronic non-bacterial osteomyelitis (CNO) is an inflammatory, non-infectious disorder of the skeletal system with unknown aetiology. Therapeutic options are NSAIDs, steroids and DMARDs (MTX or sulfasalazine). However, a considerable number of patients have a severe disease course and bisphosphonates or TNF-α blockade might be a therapeutic option.

We performed a multicentre, retrospective chart review of all patients diagnosed with CNO in two paediatric rheumatology centres in the last 10 years and treated with pamidronate and/or TNF-α blockade. 17 patients were treated with pamidronate and/or TNF-α blockade. Out of these 17 patients, 10 were treated with pamidronate alone and showed clinical improvement. Three of the 17 patients were initially treated with TNF-α blockade; two had a positive response, one patient stopped therapy due to minor side effects. Two or one patients were initially treated with pamidonate or TNF-α blockade, respectively, but did not show clinical improvement. Interestingly, switching from pamidronate to TNF-α blockade or vice versa was associated with clinical improvement in these patients. One patient was treated with a combination therapy using pamidronate and TNF-α blockade and showed a good clinical response.

Pamidronate and TNF-α blockade showed a good response in therapy refractory CNO patients. Severe adverse effects were not observed. However, some patients seem to benefit from one of the two therapeutic options in particular. Future studies are needed aiming to identify clinical and/or radiological parameters that might guide the decision to the appropriate treatment in an individual patient.

P191

MPO deficiency confers impaired processing of neutrophil reactive oxygen species in a patient with severe CRMO

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Introduction: We report a severe case of chronic recurrent multifocal osteomyelitis (CRMO) associated with total myeloperoxidase (MPO) deficiency. The etiology of CRMO is in most cases unknown, and this is to our knowledge the first case associated with MPO-deficiency. Leukocyte MPO-deficiency renders neutrophils unable to process superoxide to secondary reactive oxygen species (ROS). Partial MPO deficiency is seldom associated with pathology but little is known about the effects of total MPO deficiency.

Objectives: To increase our understanding of disease mechanisms in CRMO by describing symptoms, treatment outcomes, basic inflammatory parameters, and innate immune cell function in this patient.

Patient and methods: The patient, a girl of 18 years, was healthy until the age of 10 when she developed bilateral swollen and painful thighs. After a four-year period with numerous clinical investigations she was diagnosed with CRMO. At the time, she suffered from severe weight loss (BMI 10.5) and showed elevated inflammatory markers (ESR 80 mm/h and CRP 54 mg/L). Radiology showed bilateral inflammation of femur and biopsy showed unspecific inflammation. The patient displayed complete MPO deficiency (genetically confirmed).

Blood counts, inflammatory markers and neutrophil function were assessed by standard laboratory techniques. Samples were analyzed both during a flare, induced by withdrawal of TNF-blockade, and after treatment reintroduction.

Results: The patient responded extremely well to TNF-blockade, but not to IL-1 blockade, both clinically and in terms of inflammatory markers. The treatment did however not abrogate the underlying inflammatory trigger as symptoms returned after treatment withdrawal.

Comparing neutrophil function during a flare and under treatment, no substantial differences was detected, neither in primary ROS production, nor in surface marker exposure. The inability to process ROS due to MPO deficiency resulted in impaired formation of neutrophil extracellular traps (NETs), is suggested to be of importance for bacterial clearance.

Conclusion: Our clinical data indicate that inflammation was driven by TNFa rather than IL-1 in the patient. The laboratory results confirmed the inability of MPO-deficient neutrophils to form NETs, but further studies are needed to elucidate the possible causal relationship between CRMO and MPO/NET formation.

P192

Periodic fevers associated with celiac disease and marked increase of NK cells

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Pediatric Rheumatology 2015, **13(Suppl 1):**P192

Case report: A five year old boy was referred to our department for a history of repeated fevers. Family history was uneventful, however his father suffered from repeated episodes of tonsilitis in childhood.

Our patient presented with a history of repeated fevers with a clinical signs of tonsilitis since he was 2.5 years old. Fever attacks recur approximately every 6 weeks and are associated with lymphadenitis, sometimes accompanied by aphthous stomatitis. His inflammatory markers were elevated in a time of fever episode, but did not reach very high values typical for periodic fever syndromes. Chronic fatigue and progressive anemia also formed a persistent part of his clinical presentation.

Immunological investigation revealed significant decrease of T lymphocytes (CD3+ cells between 25-40%, normal values above 60%, absolute number between 0.5-0.9x10/9/l, normal values above 1x10/9/l), and a surprising increase of NK cells reaching over 50% of lymphocyte population. Further detailed search for underlying cause led to an unexpected diagnosis of celiac disease. Appropriate diet was introduced and led promptly to a decrease of celiac disease associated antibodies and to an improvement of anemia and fatigue.

His fever attacks, however, did not resolve, and his highly pathological findings of low T cells and extremely high NK cells were still detectable, not responding to antiinflammatory and corticosteroid treatment. PET scan was performed and showed an accumulation of activity in a pharyngeal arch and tonsil area. Tonsilectomy was performed at his 6 years of age and led to a prompt improvement in his clinical presentation, with a complete resolution of febrile episodes. His abnormal laboratory findings in cellular immune parameters very slowly improved within the next 6 months, with an increase of CD3+ T cells and drop of NK cells to their normal values.

Conclusion: Here we present an unusual combination of periodic fevers with celiac disease, accompanied by peculiar and highly significant changes in cellular immunity, with low T cells and high NK cells. Combination of celiac diet and tonsillectomy led to a resolution of all clinical problems. Slow and gradual improvement of deep immune disbalance in lymphocyte subpopulations took further 6 months after tonsillectomy before it reached almost normal values. Association of celiac disease and periodic fevers is extremely rarely mentioned in a literature, but might be considered in some cases. Aphthous stomatitis is a common feature of both celiac disease and PFAPA syndrome.

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P193

Successful kidney transplantation during anakinra treatment without complications

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Although the incidence of end stage renal failure in patients with autoinflammatory diseases has decreased since the introduction of the interleukin-1 (IL-1)-receptor antagonist anakinra, it is still relatively common. Cessation of anti-IL-1 therapy during and after kidney transplantation will not be possible in most patients because of the risk of recurrence of inflammation. The effect of continued anti-IL-1 therapy on transplantation-related complications is currently unknown.
Recently, we have performed kidney transplantation in two patients while on anakinra therapy. The first patient is a 70 year old male, who suffered from febrile episodes since 2004 and was diagnosed with adult onset Still's disease in 2010. He had been using anakinra ever since. Chronic renal insufficiency due to thrombotic microangiopathy had been present since 2005. In September 2014 he received a kidney transplant of a living related donor, under standard immunosuppression (tacrolimus, prednisone and mycophenolate mofetil (MMF)), while anakinra was continued. Kidney function improved rapidly and he could be discharged from the hospital 7 days post transplantation. Until March 2015, he has been readmitted two times: once for a single day because of drainage of a wound abscess, and for 5 days 1 year post-transplantation because of sepsis with unknown cause. He now has adequate and stable transplant function.
The other patient is a 20 year old woman with mutation-negative Chronic Infantile Neurologic Cutaneous Articular (CINCA) syndrome. She had been using anakinra since 2004, which was switched to canakinumab in 2012. She was known with chronic renal insufficiency with proteinuria, probably due to recurrent urinary tract infections, vesico-urethral reflux and chronic use of NSAIDs since 2009. Kidney biopsy was contra-indicated. Because of rapidly progressive renal function loss, she received a pre-emptive kidney transplant of a healthy related donor in September 2014, under standard immunosuppression. Canakinumab was switched to anakinra just before transplantation, because of shorter half-life, making it easier to cease in case of complications. Kidney function increased rapidly and she could be discharged 6 days post-transplantation. She had been readmitted a single time for two days because of a primo-EBV and influenza infection in December 2014. She now has normal kidney function.
In these two cases we did not find an increase of transplantation-related complications or post-transplantation infectious complications, when kidney transplantation with a standard immunosuppresive regimen was performed during anakinra therapy. It is important to adapt the dosing of anakinra to the kidney function before and after transplantation.
Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P194

Diet and diet combined with chronic aerobic exercise decreases body fat mass and alters plasma and adipose tissue inflammatory markers in obese women

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The purpose of this study was to investigate the effect of 6 months aerobic exercise and diet alone or in combination on markers of inflammation (MOI) in circulation and in adipose abdominal tissue (AT) in obese women. Thirty obese subjects were randomized into a 24 weeks intervention: 1) exercise (EX), 2) diet (DI) and *3*) exercise and diet (EXD). Blood samples were collected at baseline, after 12 wk and 24 wk. AT biopsies were obtained only at baseline and after 24 wk. In the EXD and DI groups the fat loss was after 12 wk -13.74% and -7.8% ($P<0.01$) and after 24 wk -21.82% and -17% ($P<0.01$) with no changes in the EX group. After 12 and 24 wk, VO_2 max was increased by 21.81-39.54% ($P<0.05$) in the EXD group and 18.09-40.95% in the EX group with no changes in the DI group. In the EXD and DI groups, circulating levels of TNF-a and IL-6 were decreased after 24 wk for both groups ($P<0.01$). No changes in the EX group. HOMA-R decreased ($P<0.05$) only after 24 wk in the EXD group. In AT biopsies, subjects in the EXD and DI groups exhibited a significant decrease in MO ($P<0.01$ for all). No changes in AT biopsies were found in the EX group. In conclusion, chronic aerobic exercise was found to have no effects on circulating and AT MOI despite an increased VO_2 max. Rather important body composition modifications were found to have beneficial effects on circulating and AT MOI in these obese women.

P195

Understanding the pathophysiology of NOMID arthropathy for drug discovery by iPSCs technology

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Introduction and objectives: NOMID, also known as CINCA syndrome, is a dominantly inherited autoinflammatory disease caused by *NLRP3* mutations. The pathophysiology of NOMID is explained by gain of function mutation of NLRP3, which activates NLRP3 inflammasome and produce an excess of IL-1β. This mechanism is supported by clinical observation that anti-IL-1 therapy is effective on its systemic inflammation. However, one of its characteristic features, epiphyseal overgrowth, is considered to be resistant to anti-IL-1 therapy, which raises a question that other mechanism than NLRP3 inflammasome may play a role in the epiphyseal overgrowth. In this study, we investigated the effect of mutated NLRP3 on chondrocytes using induced pluripotent stem cells (iPSCs) derived from NOMID patients, and tried to identify drugs to treat the abnormal chondrocytes overgrowth.
Methods: We established isogenic iPSCs with wild-type or mutant NLRP3 from 2 NOMID patients with NLRP3 somatic mosaicism. We differentiated the iPSCs into chondrocytes, and the phenotypes of chondrocytes derived from iPSCs with wild-type NLRP3 and mutant ones were compared, particularly the size of the chondrocyte tissue produced.
Results: Mutant iPSCs produced larger chondrocyte masses than wild-type iPSCs owing to glycosaminoglycan overproduction. We also observed increased expression of SOX9, which is a chondrocyte master-regulator, on chondrocyte masses derived from mutant iPSCs. In addition, in vivo transplantation of mutant cartilaginous pellets into immunodeficient mice NOG caused disorganized endochondral ossification. Enhanced chondrogenesis observed in chondrocyte masses derived from mutant iPSCs was independent of caspase-1 and IL-1, and thus probably the

NLRP3 inflammasome. Reporter assays using the human *SOX9* promoter in chondroprogenitor cells revealed that the proximal CREB/ATF-binding site was critical for SOX9 overexpression caused by mutated NLRP3. These data was correlated with increased levels of cAMP and phosphorylated CREB in mutant chondroprogenitor cells. We are now developing high throughput screening system to identify compounds to inhibit the abnormal chondrocytes overgrowth.
Conclusion: Our findings indicate that the intrinsic hyperplastic capacity of NOMID chondrocytes is dependent on the cAMP/PKA/CREB pathway, independent of the NLRP3 inflammasome.

P196

Immune dysregulation in Periodic Fever, Aphthous atomatitis, Pharyngitis, Adenitis (PFAPA) syndrome

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Introduction: Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome is an autoinflammatory disorder of childhood and little is known about the underlying etiology, pathogenesis or the reason behind the success of tonsillectomy. Numerous immune cell types are present in the tonsillar microenvironment, each of which may contribute to the pattern of symptoms observed in PFAPA patients.
Objectives: To identify differences in cellular populations in patients with PFAPA syndrome compared to controls with recurrent pharyngitis and obstructive sleep apnea, and analyze if these cells have a proinflammatory phenotype.
Methods: Patient data and detailed family histories were collected for over 200 children with recurrent fevers including 94 patients with PFAPA to create a prospective cohort of children treated at a tertiary care center in San Diego, CA, USA. Patient data was collected under an IRB-approved protocol using patient charts and a standardized questionnaire, demographic data, including age, gender, and ethnicity, clinical profiles (presence of symptoms, fever profile, treatments) and detailed family histories over a 7-year period.
For patients electing to undergo tonsillectomy (n = 63), whole tonsillar tissue was obtained post-operatively under an IRB-approved protocol. Control tonsils (n = 22) were obtained from children of similar age with either recurrent streptococcal pharyngitis or obstructive sleep apnea. Single cell suspensions were derived and fluorescently stained for evaluation by eight-color flow cytometry. In some cases, cells were cultured with LPS or CpG *in vitro*, prior to flow cytometric analysis.
Results: Flow cytometry of isolated cellular constituents reveals that tonsils from patients with PFAPA have a significant memory B cell population, defined as CD27+, CD19+, CD3 negative cells ($p<0.01$), with similar T cell, NK cell and monocyte/macrophage populations. PFAPA patient tonsillar memory B cells express higher levels of the survival markers BAFF-R and TACI and significantly more intracellular IL-1b, compared to memory B cells from controls ($p<0.05$). Activation of tonsillar cells in culture with lipopolysaccharide (LPS) significantly increases the percent of IL-1b positive cells in control cultures ($p<0.01$), but has no effect on cells derived from PFAPA patients. Similarly, stimulation with CpG failed to further upregulate BAFF-R and TACI expression on PFAPA tonsillar memory B cells to the extent observed in stimulated control cultures.
Conclusions: Taken together, these findings suggest that PFAPA patient tonsillar cells are constitutively activated or primed for hyperresponsiveness even during afebrile periods.

P197

Interleukin-1-related cytokines as potential biomarkers in autoinflammatory skin diseases

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Introduction: Urticarial rash is a hallmark symptom of autoinflammatory diseases such as Cryopyrin-associated periodic syndrome (CAPS) and Schnitzler's syndrome (SchS). Clinically, the urticarial rash may not be distinguished from the skin symptoms in chronic urticaria patients. As interleukin-1ß (IL-1ß) has been shown to play a pivotal role in the pathogenesis of CAPS and SchS we here aim at investigating IL-1ß and related cytokines for their potential as diagnostic skin biomarkers in patients with urticarial autoinflammatory syndromes.
Materials and methods: Immunohistochemical stainings (neutrophil marker myeloperoxidase (MPO), IL-1ß, IL-6, IL-18) from lesional skin of patients with CAPS (n=3), SchS (n=9) and chronic spontaneous urticaria (csU) (n=10) as well as healthy control skin samples (n=10) were analyzed by quantitative histomorphometry and compared with cytokine protein concentrations assessed by ELISA.
Results: Quantitative histomorphometry revealed a higher percentage of neutrophil-dominated dermal cell infiltrate in autoinflammatory diseases that was significant for SchS skin samples as compared with csU samples and healthy controls ($p \leq 0.05$). Analysis of IL-1ß, IL-6 and IL-18 positive cells in CAPS and SchS skin showed higher cell numbers which were much less pronounced in csU and healthy control samples. In addition, protein concentrations of all three cytokines were significantly higher in autoinflammatory diseases as compared with csU patients and healthy controls ($p \leq 0.05$).
Conclusion: Our study confirms the predominance of neutrophil-dominated cell infiltrates and demonstrates an upregulation of IL-1-related cytokines in the skin of urticarial autoinflammatory diseases. We suggest to further explore these cytokines as diagnostic biomarkers in larger patient samples.

P198

PFAPA syndrome as an hereditary autoinflamatory disorder

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Introduction: PFAPA syndrome (periodic fever, aphtous stomatitis, pharyngitis, adenitis) is an autoinflammatory disease, for which no genetic marker has been identified yet, and its etiology remains unknown. However, the clinical and biochemical similarities to other autoinflammatory conditions, including Familial Mediterranean Fever (FMF), suggest that a genetic impairment might constitute the underlying cause of the disease. FMF is the most widespread monogenic autoinflamatory disorder. In 60% of patients affected by FMF two concurrent mutations of MEFV gene have been demonstrated, whereas in 30% one mutation of the same gene has been shown. In only 10% of patients, no genetic marker has been identified.
Objectives: Our study stems from the hypothesis that PFAPA and FMF MEFV-negative (MEFVneg) patients might share a genetic marker accounting for the development of signs and symptoms of the disease. In these patients, a careful familiar history and the presence of accompanying symptoms throughout the flares were investigated.
Materials and methods: We have been performing a cohort study, involving 67 MEFVneg patients and 51 PFAPA patients. These populations have been compared in terms of clinical manifestations and evidence of periodic fever and surgical tonsillectomy in parents.
Results: A substantial overlap of clinical manifestations has been observed in the two cohorts. Patients affected by PFAPA frequently presented with abdominal (49%), articular (64%), thoracic pain (14%). On the other hand, MEFVneg patients showed aphthosis (58%), pharyngitis (55%) and adenopathies (49%). Moreover in 58% of PFAPA patients a history of periodic fever in one or both parents during childhood was demonstrated. Tonsillectomy was performed in 51% of the parents of PFAPA patients. In MEFVneg patients, on the other hand, the parents with a history of periodic fever during childhood were 32%, whereas the amount of tonsillectomies reached up to 28%.
Conclusion: These findings unveil the possibility that PFAPA might be a genetic disease, whose pathogenesis recapitulates the hereditary transmission pattern already observed in MEFV-positive FMF or other

autoinflammatory disorders. This hypothesis clearly sheds the light on the need to identify the gene(s) involved in the activation of the inflammasome and, hence, in the development of the disease. Furthermore, due to high clinical affinity to FMF, such a genetic signature for PFAPA might potentially result as useful to account for MEFV-negative FMF.

P199

Beneficial effect of methotrexate on a case of Nakajo-Nishimura syndrome

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Nakajo-Nishimura syndrome (NNS) is a very rare autosomal recessively-inherited autoinflammatory disorder that onsets in infancy with pernio-like rashes and gradually develops into partial lipodystrophy, accompanied with remittent fever and nodular skin eruptions. This disease is caused by a unique mutation of the *PSMB8* gene, which not only impairs an enzymatic activity of the encoding beta5i subunit, but also disturbs formation of the immunoproteasome complex. As the pathogenesis for NNS, cellular accumulation of ubiquitinated and oxidized proteins due to immunoproteasome deficiency is considered to cause MAP kinase activation with nuclear accumulation of phosphorylated p38 and following IL-6 production.
The treatment for Nakajo-Nishimura syndrome has not been established. Inflammatory attacks can temporarily respond to the oral administration of high-dose corticosteroid, but they easily recur by tapering the dose of corticosteroid. Furthermore, the high-dose corticosteroid therapy has various side effects such as growth failure in infancy. In our child case of NNS (Kunimoto *et al*, Dermatology 2013), additional administration of methotrexate (MTX) significantly decreased a frequency of febrile attacks, in comparison to the treatment with oral corticosteroid alone. Notably, effectiveness of MTX was previously described on some infant cases of CANDLE syndrome, another *PSMB8*-mutated proteasome-associated autoinflammatory syndrome (PRAAS). MTX is known to execute anti-inflammatory effects through inhibition of folic acid-dependent enzymes, including dihydrofolate reductase (DHFR) and aminoimidazole carboxamide ribonucleotide transformylase (ATIC). ATIC inhibition causes accumulation of intracellular aminoimidazole carboxamide ribonucleotide and inhibits AMP deaminase, to increase the production of adenosine. Adenosine can inhibit superoxide production of neutrophils and their attachment to endothelial cells. As preliminary results, increased ROS production has been observed in primary neutrophils of NNS patients, suggesting one of the points that MTX affects.

P200

Cryopyrin associated periodic syndromes (CAPS): immunological characterization of knock-in mouse model to exploit novel approaches for the modulation of the NLRP3 inflammasome

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Question: CAPS are autoinflammatory diseases characterized by recurrent episodes of fever and systemic inflammation, subdivides into three different severity phenotypes (FCAS, MWS, CINCA). These syndromes are caused by mutations of NLRP3 gene coding for an intracellular multiprotein complex that mediates IL-1β processing and secretion. These mutations are gain-of-function, resulting in an inflammasome hyperactivity and IL-1β hypersecretion. We aimed to: increase the knowledge on pathologic consequences of NLRP3 mutations in CAPS patients; understand the molecular and regulatory mechanisms of CAPS disease; identify novel molecular targets for the treatment of cryopyrin/NLRP3 related disorders.
Methods: We generated a Knock-in (KI) mouse carrying the N475K mutation into the murine NLRP3 gene. This mutation corresponds to the N477K human mutation, associated with a severe CINCA phenotype with neurological complications; phenotypical and immunological characterization of KI has been performed by flow cytometry; IL1β secretion from bone marrow derived dendritic cells (BMDCs) and peritoneal macrophages (PMs) of KI has been evaluated by ELISA.
Results: NLRP3 KI mice show hair loss, skin rash and reduced survival time compared to wild type mice (WT). Autopsy of KI mice, prematurely dead, revealed splenomegaly and a relevant inflammatory status. We compared IL-1β secretion of inflammatory cells from WT and KI mice. PMs and BMDCs from mutant mice did not secrete mature IL-1β spontaneously. When stimulated with 100 ng/ml of LPS KI cells secreted higher levels of IL-1β than WT cells. The kinetics of IL-1β secretion was much faster in KI cells, reaching the plateau at 3h from exposure to LPS, reproducing the results obtained from monocytes of CAPS patients. As in CAPS monocytes, brief exposure to ATP strongly induced the secretion of IL-1β by LPS-activated WT cells while failed to stimulate further IL-1β secretion by KI mice inflammatory cells. Finally, PMs and BMDCs from KI are more responsive to agonists of TLRs compared to WT cells: LPS at 0.01 ng/ml triggered high levels of IL-1β secretion in KI cells indicating that the presence of the mutation lowers the threshold of activation. Immunological and functional studies of peritoneal cavity are in progress, interestingly we noticed a reduction of B lymphochytes especially in innate-like B1 cells. We are also evaluating neurological aspects of CINCA disease in NLRP3 KI mice.
Conclusions: The NLRP3 KI mice recapitulates phenotype and functional characteristics of CAPS patients. Thus, this model will provide elucidations in the mechanisms underlying CAPS as well as other inflammasomepathies.

P201

Differential response to anakinra and adalimumab in a patient with DADA2 syndrome

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Deficiency of adenosine deiminase 2 (DADA2) syndrome is a recently described autosomal recessively inherited autoinflammatory disorder associated with missense mutations in CECR1 gene. Clinical manifestations include early onset stroke, livedoid vascular changes, and a vasculopathy mimicking classical polyarteritis nodosa (cPAN) characterized by microaneurysms and associated inflammatory findings. We herein describe a male patient with homozygous G47R mutation in the CECR1 gene, whose inflammatory findings did not respond to immunosuppressive treatments and recombinant IL-1 receptor antagonist, anakinra injections, but controlled with adalimumab.
Case report: A 20-year-old male patient presented to our clinic two years ago with findings of cPAN. He had a consanguineous family from South East Turkey without any other affected individuals. He started to experience a skin rash at the age of 5, and he had a history of polypectomy following a rectal bleeding and then an explorative laparotomy due to skin rash, abdominal pain, and fever, with the diagnosis of Henoch Schönlein purpura at the age of 7. He also had hepatosplenomegaly, and he continued to experience 2-3 day lasting abdominal pain, especially after cold exposure until the age of 16. A skin biopsy revealed livedoid vasculitis. He also had a history of facial paralysis 3 years ago, and a sudden vision loss due to right retinal artery occlusion and hypertension 2 year ago. He was admitted to our hospital because of microaneurysms in cranial and renal arteries, with signs of renal infarcts as well as findings of mononeuritis multiplex and systemic inflammation. He was first diagnosed with familial Mediterranean fever (FMF) associated cPAN, and started to receive corticosteroids along with 3 courses of 500mg cyclophosphamide pulses. He then continued to receive azathioprine. MEFV gene screening revealed no exon 10 variations compatible with FMF diagnosis, and after the description of DADA2 syndrome, he was screened for CECR1 mutations, which revealed

homozygous G47R mutation. During follow-up, he experienced transient ischemic attacks, and his acute phase response could not be controlled with cortiocoteroids and 2mg/kg azathioprine. After addition of 100 mg/day anakinra to his treatment, his acute phase response partially reduced and clinical findings remained stable for nearly 3 months, and increased doses of 200 mg/day did not provide additional help. However, switching his treatment to adalimumab dramatically reduced his CRP and ESR levels to normal limits.

Conclusion: The pathogenesis and inflammatory characteristics of CECR1 mutation associated DADA2 syndrome and its vascular findings have not been elucidated yet, and a favorable response of our patient with DADA2 to adalimumab but not to anakinra treatment may provide insights to its inflammatory mechanisms.

Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P202

Clinical experiences with canakinumab as a treatment for autoinflammatory disorders

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Rationale: Patients seeking clinical treatment for urticaria, while presenting with elevated inflammatory markers, were found to be suffering from auto-inflammatory disorders, such as Cryopyrin Associated Periodic Syndromes (CAPS), Familial Mediterranean Fever (FMF) and Schnitzler's syndrome. Patients were screened for, and/or began treatment with canakinumab.

Methods: The 32 patients identified for investigation presented with cases of chronic urticaria along with some of the following symptoms: joint and bone pain, chills, conjunctivitis, periodic fever, fatigue, weight loss, and hearing loss. 11 patients (34%) presented with Muckle-Wells syndrome. Seven patients (22%) presented with Familial Cold Autoinflammatory Syndrome and a history of treatment with rilanocept. Three patients (9.5%) presented with FMF. Three patients (9.5%) presented with Schnitzler's syndrome. Eight patients (25%) presented with undiagnosed forms of CAPS. All patients showed elevated inflammatory markers, including Serum Amyloid A (SAA) and C-reactive protein (CRP). Nine patients with Muckle-Wells syndrome and a confirmed R260W mutation at the NLRP3 gene, and one patient with an undiagnosed form of CAPS began treatment with canakinumab. Patients received a 150 mg treatment via sub-cutaneous injection every eight weeks. Total injections to date for individual patients range from two to seven. The remaining 22 patients are currently being investigated for treatment with canakinumab.

Results: All ten patients treated with canakinumab experienced remission within one week of their first injection. Remissions were maintained by subsequent injections. Seven patients with SAA levels >16,000 ng/mL (normal range 1000-5000 ng/mL) prior to treatment showed normal SAA levels within one week of their first injection.

Conclusions: Canakinumab treatment for patients with Muckle-Wells syndrome was associated with complete spontaneous remissions within one week of commencing treatment, and sustained clinical improvements over time. Patients presenting with chronic urticaria should be carefully assessed for disguised auto-inflammatory disorders, through genetic testing and examination for elevated inflammatory markers.

P203

Comparison of different treatment approaches in chronic non-bacterial osteomyelitis

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Chronic non-bacterial osteomyelitis (CNO) is a heterogenous group of immune-mediated inflammatory bone diseases, which often co-exist with other rheumatic diseases. There are no approved treatments for CNO, except non-steroid anti-inflammatory drugs (NSAID). The efficacy of methotrexate (MTX), sulfasalazine, pamidronate (PAM), anti-IL1 and TNFα-inhibitors was shown in different reports, but there are some concerns about safety of pamidronate due to long-term accumulation and persistation in bone.

The aim of our study was to compare the efficacy of non-randomized different treatment approaches in pediatric patient cohort with CNO.

Materials: 37 children (16 M and 21 F) with CNO from medical centers in Saint Petersburg. The average age at the onset of disease was 8.5 years (5.9÷10.5), the number of foci - 3.0 (2.0÷6.5, incl. multifocal cases in 78.4%), fever at the onset -37.8%, spine involvement - 32.4%, positive family autoimmune diseases (AID) history - 8.1%, concomitant AID - 64.9%. NSAID was the first-line treatment for non-vertebral cases, as well as PAM for vertebral involvement. Second-line treatment includes MTX, PAM and TNFα-inh. Dynamics of pain, patient's (PVAS) and physician's (MDVAS) assessment of CNO activity we evaluated.

Results: According to the NSAID, MTX, PAM and TNFα-inh groups next data were registered:

PVAS: -26.2% (p=0.05), -14.6% (p=0.06), -84.7% (p=0.0002),-75.6% (p=0.012);

pain: -36.4% (p=0.028), -15.6% (p=0.31), -84.8% (p=0.0002), -82.6%, p=0.012);

MDVAS: -33.8% (p=0.08); +2.4% (p=0.24),-81.4% (p=0.0002), -75.8%, (p=0.012), respectively.

The therapy was effective in 38.9%, 57.1%, 83.3% and 88.8% respectively (log-rank test, p=0.012). TNFα-inh usually used as second-third line treatment in cases where other options, especially PAM were fall.

Conclusions: The most effective treatment approaches for CNO were PAM and TNFα-inh. The randomized controlled trials for assessment efficacy and safety of these medications is mandatory to confirm these results.

P206

Interleukin 1 blockade withcanakinumab for Hyper IGD syndrome (HIDS)

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Introduction and question: Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS; MIM# 260920) is a rare autosomal recessive autoinflammatory condition caused by mutations in the *MVK* gene, which encodes for mevalonate kinase. There is no standard treatment for HIDS. Therefore new therapeutic options might be developed.

Methods and results of this case report: We report on a 2 year-old Austrian boy with recurrent episodes of fever, febrile seizures, arthralgias, and splenomegaly. Rash and abdominal pain were also seen occasionally. During attacks an acute-phase response was detected. Clinical and laboratory improvement was seen between attacks. These findings led to the tentative diagnosis of HIDS. Sequencing of the *MVK* gene showed a homozygous c.1129G>A (p.Val377Ile, also known as V377I) mutation in the child, while the healthy non-consanguineous parents were heterozygous. The mutation is known to be associated with HIDS.

Therapy with nonsteroidal anti-inflammatory drugs during attacks had poor benefit. A further febrile episode resulted in a status epilepticus. Treatment with canakinumab was initiated and a final dose of 4 mg/kg every 4 weeks resulted in the disappearance of febrile attacks and a considerable improvement of patient's quality of life during a 6-month follow-up period. The drug has been well tolerated, and no side effects were observed.

Conclusion: Treatment with canakinumab is a therapeutical option for patients with HIDS.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P207

Long-term efficacy of IL-1 blockers in PAPA patients

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Introduction: PAPA syndrome (Pyogenic Arthritis, Pyoderma gangrenosum, and Acne) is an ultra-rare autosomal dominant, auto-inflammatory disease associated to mutations in the PSTPIP1/CD2BP1 gene. The therapeutic approach during recurrences consists of steroids, while no agreement exists on the chronic management. Evidences on the use of biologics are anecdotal and variable results have been reported.
Objectives: To evaluate the long-term response to treatment with IL1 antagonist in six patients affected by PAPA syndrome.
Methods: Six patients (M:F=3:3; 4 pediatric, 1 young adult and 1 adult, mean age 18 years, range 3-50) affected by PAPA syndrome were enrolled and treated with IL1 blockers (5 patients Anakinra, 1 patient Anakinra followed by Canakinumab). Three patients were already treated with anti-TNFα monoclonal antibodies without benefit. Data were collected retrospectively (mean follow-up 26 months, range 4-38). The frequency of articular and cutaneous flares in the 24 months before starting therapy where compared to those occurred during anti-IL1 regimen. Acute phase reactants (ESR, CRP, SAA) were assessed at the last visit before the study enrolment and at last follow-up.
Results: All the patients displayed a significant decrease in frequency of disease flares (Table 1) and normalization of acute phase reactants. Three patients were asymptomatic during whole follow-up. Patient #5, with a severe and persistent pyoderma gangrenosum, displayed a partial response to Anakinra partially due to a poor compliance to daily s.c. administration. The shift to Canakinumab lead to a fast and complete resolution of the skin manifestations.
Conclusions: The long-term use of IL1 blockers is associated to satisfactory and persistent control of clinical manifestations and laboratory findings in PAPA syndrome.

P208

Differential diagnosis of CRMO

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Introduction: Chronic recurrent multifocal osteomyelitis (CRMO) is a term, referred to a group of several autoinflammatory disorders (some of unknown genetic background) of children and young adults that is characterized by non-infectious osteomyelitis with or without high inflammatory activity and occasionally involvement of other organs. Patients typically present with bone pain secondary to multifocal osseous lesions, the disease has a remitting course. To specialists who care for patients with autoinflammatory disorders the clinical presentation of CRMO is very recognizable. Yet in the settings of multi-specialty clinic, a newly referred patient with bone lesions poses a

Table 1(abstract P207)

Pt	Sex	Mutation	Main manifestations	Manifestations in the 24 months before treatment	N± of flares during follow-up	Treatment (dose, duration)
1	F	E256G	Pyogenic arthritis Pyoderma gangrenosum Cystic acne/ foruncolosis	2 flares of pyogenic arthritis 5 pyoderma gangrenosum Cystic acne	0	Anakinra 100 mg/day (36 months)
2	F	E250Q	Pyogenic arthritis Sterile osteomyelitis Palpebral edema	3 flares of pyogenic arthritis 1 sterile osteomyelitis 1 palpebral edema	0	Anakinra 2 mg/kg/day (21 months)
3	M	E250Q	Pyogenic arthritis	7 flares of pyogenic arthritis (polyarticular)	3 (mild) articular flares	Anakinra 1.5 mg/kg/day (38 months) and low dose steroid
4	M	WT	Cutaneous abscesses Pyoderma gangrenosum Pyogenic muscular abscess	1 persistent muscular abscess	0	Anakinra 100 mg/day (26 months)
5	M	E250K	Pyogenic arthritis Pyoderma gangrenosum Severe anemia Splenomegaly Growth delay	1 cutaneous abscess 3 pyoderma gangrenosum Anemia	1 pyoderma gangrenosum (resolved after Canakinumab)	Anakinra 2 mg/kg/day (31 months) - Canakinumab (4 months)
6	F	E250Q	Dactylitis/tendinitis Pyogenic arthritis Acne and furunculosis	6 articular flares	0	Anakinra 100 mg/day (4 months)

certain diagnostic challenge and thorough differential diagnosis is required.
Objectives: We conducted a study analyzing diagnosis and outcomes of children referred to tertiary center with bone lesions in 2014. Children with bacterial osteomyelitis were not included in the study.
Methods: Laboratory, radiological tests, bone lesion biopsies were performed in all cases, other types of tests - as were required by clinical situation.
Results: Most of the patients were diagnosed with various oncological/ oncohematological disease: Ewing's sarcoma was found in 22% of cases, osteosarcoma - 29%, histiocytosis X - 9%, bone metastases - 9%. In 24% of cases the disease was not associated with bone tissue: chondroblastoma, bone cyst, synovial sarcoma, osteochondroma etc. Only 5% of patients we were able to confidently diagnose chronic multifocal osteomyelitis.
Conclusion: In conclusion, newly diagnosed bone lesions in children require joint diagnostic efforts of various specialist. Our study showed, that in most cases swift and correct diagnosis and pathogenic treatment was only possible upon biopsy.

P209

Safe and effective canakinumab-treatment of neonatal onset multisystem inflammatory disease (NOMID)/ chronic infantile neurologic cutaneous and articular (CINCA)

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Introduction: NOMID/CINCA is the most severe phenotype of cryopyrin-associated periodic syndrome (CAPS), characterized by persistence of inflammation-mediated symptoms and overproduction of interleukin (IL)-1β, associated with significant morbidity, if untreated. In CAPS-patients early initiation of anti-IL1β-treatment appears to prevent severe disease sequelae. However, canakinumab as a 1st-line treatment in young infants suffering from NOMID has been scarcely reported.
Objectives: To report the effects of early-onset canakinumab-treatment in NOMID/CINCA.
Patients and methods: Case presentation.
Results: A late-preterm (37-weeks- gestational-age) girl presented fever, urticarial-like rash, perilimbal redness, meningitis, elevation of WBC/ neutrophils, ESR, CRP on 20 hours of life and severe anemia necessitating RBC-transfusion in the 5th day of life. NOMID/CINCA was suspected on the basis of persistence of elevated inflammatory (including SAA) markers in the absence of infection-causative organisms in blood, CSF and urine, of non-responsiveness to antibiotics, of persistent CNS inflammation (CSF pleiocytosis and elevated protein) and of neutrophilic infiltration (revealed by skin biopsy) in the areas of urticarial-like rash. The detection of the c.1792A>T (p.Ile598Phe) mutation (de-novo as it was no detected in parents) in exon 3 of the *NLRP3*-gene, causative for NOMID/CINCA according to the infevers-database, confirmed the diagnosis. Brain-MRI was normal despite persistent CNS-inflammation represented by pleiocytosis and elevated protein and IL-6 and IL-8-levels in CSF (lumbar puncture performed on the 1st-day and 3rd-month of life). In peripheral blood IL-1β, IL-6 and IL-8 were undetectable. Opthalmoscopy/fundoscopy, and auditory-evoked-potentials were normal. After providing immunizations anti-IL1β-treatment with canakinumab 4mg/kg/8 weeks was initiated in the age of 4-months. Fever and rash remitted in 24h. Inflammatory markers normalized after 5-days. On 16-months of age the disease remains into remission. The only sign that persists is perilimbal redness. Mental and motor development is normal. No sensorineural or skeletal manifestations developed. Self-limited, 24h-duration, scarce urticarial-rash appeared in the age of 6 and 15 months with concomitant mild elevation of WBC/ neutrophils and SAA but normal CRP and ESR levels. Repeat MRI revealed absence of CNS involvement and lumbar puncture was not repeated. No adverse reactions presented apart from 1 URI after 12-months of canakinumab treatment.
Conclusion: Early initiation of canakinumab-treatment in CINCA leads to disease-remission and appears to prevent the development severe disease-sequelae such as CNS, sensorineural and skeletal manifestations. The patient presented no severe adverse reactions.
Consent to publish: Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

P210

Our experience of anti-interleukin 1 therapy

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Introduction: Cryopyrin-associated periodic syndromes (CAPS) are characterized by apparently unprovoked attacks of fever, rashes, and musculoskeletal and sensorineural inflammation accompanied by high acute-phase reactants. Excessive interleukin-1 (IL-1) signaling appears to be a constant feature in the pathomechanism of the disease, driven by a gain-of-function mutation in the NLRP3 gene. Familial Mediterranean fever (FMF) is a auto-inflammatory disorder characterised by recurring, self-limited episodes of fever and serositis resulting in abdominal, chest, joint and muscular pain due to mutation of MEFV. Colchicine is generally safe, nevertheless, 5-10% patients do not respond to treatment. IL-1blockade is the treatment of choice of CAPS and colchicine resistant FMF. IL-1 receptor antagonist-anakinra, human dimeric protein that incorporates the extra-cellular domain of IL-1 receptor and IL-receptor accessory protein-rilonacept, and a human anti-IL-1b monoclonal antibody-canakinumab are biologic agents used for this aim.
We report the effect of anti-interleukin 1 treatment (canakinumab, anakinra) in 12 patients (2 CAPS cases and 10 FMF cases of resistant to colchicine therapy).
Patients and methods: We evaluated 12 anti-IL1 used patients diagnosed CAPS and FMF. Their demographic findings, the course of the disease, genetic analysis, therapy, were recorded from their hospital records.
Results: Two patients (1 male, 1 female) were diagnosed with Systemic Onset Poliarticular Juvenile Idiopathic Arthritis when they were 2 years old. They were treated with this diagnose up to two years. After the reevaluation of patients according to the new literature, they diagnosed CAPS and canakinumab were used for therapy. Both of them has been followed for 1.5 year with clinical and laboratory remission. Ten FMF patients were evaluated for therapy in this study, and we observed that 9 of them have a homozygous M694V mutation and one of them has got M694V/M680I coumpound heterozygous mutation. Their age, symptoms onset age, and age at the diagnosis were 12.8±5, 3.2±1.9, 6.7±3.4 years respectively. The duration of colchicine was 5.9±3.5 year. We used canacinumab (5 patients) and anakinra (5 patients) because their disease was resistant to colchicine therapy. The median duration of IL1 inhibitors was 7 months. All of them has been followed with clinical and laboratory remission.
Conclusions: Interleukin-1 inhibitors should be selected for treatment of CAPS and they may be good candidates when looking for an alternative or supplementary treatment to colchicine in FMF. These observations highlight the need for controlled trials to further evaluate the safety and efficacy of interleukin-1 antagonists in FMF patients.

P211

CIAS1-associated autoinflammatory syndrome first diagnosed at age 48 years: a case report

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Cryopyrin-associated periodic syndromes are a group of autoinflammatory syndromes caused by mutations of the *CIAS1*gene (currently named *NLRP3*), and are characterized by periodic attacks of an urticaria-like rash, fever, headache, conjunctivitis and arthralgia. Often patients present in

early childhood, but the great diversity of manifestations and the difficulties in genetic analyses make the diagnosis of these diseases a challenge. The authors describe the clinical features of a male patient who presented first symptoms approximately at age 23 years. with recurrent fevers up to 40°C, urticaria-like rash, orbital swelling, headache and fatigue. *CIAS1*-associated autoinflammatory syndrome was diagnosed at age 48 years. and confirmed genetically (mutation: c.598G>A (p. Val200Met) in Exon 3 of *NLRP3* (*CIAS1*)-gene.

Written informated consent for publication of their clinical details was obtained from the patient/parent/guardian/relative of the patient.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Fasshauer *et al.*: *CIAS1*-associated autoinflammatory syndrome first diagnosed at age 48 years: a case report. *Pediatric Rheumatology* 2015, 13(Suppl 1):P211