vs inactive BD, 118 for active BD vs healthy controls, 129 for inactive BD vs healthy controls. Among them, the strongest 45 spots were further analyzed through MALDI-TOF-MS. Following differentially expressed proteins were determined as the final candidates: calreticulin, WD repeat-containing protein-1 (WDR1), Fructose-bisphosphate aldolase-C, ficolin-1, fibrinogen alpha chain, fibrinogen beta chain, filamin-A, FUSE-binding protein-1, phosphoglycerate kinase-1 (PGK1), stathmin, vinculin, hnRNP-M, HSPA8, myosin light polypeptide-6, talin-1, and tropomyosin alpha-3 chain. Western blot analyses of WDR1 and PGK1 confirmed that WDR1 is decreased (1.3 fold), whereas PGK1 is increased (1.5 fold) in BD patients, although the differences were not statistically significant. However, although decreased calreticulin level was observed in BD patients compared to healthy controls by 2D-DIGE analysis, we detected increased calreticulin level in BD patients by western blot analysis.
Conclusion: We identified proteins related to glycolysis, coagulation, ER stress, and cytoskeleton by 2D-DIGE analysis. Although none of these proteins have been previously implicated in BD, lower levels of stathmin, WDR1, and calreticulin having roles in inflammation was revealed in BD patients. Also increased PGK1 level was observed in BD patients, which is a glycolytic enzyme and previously suggested to have a role in rheumatoid arthritis. The expression of proteins involved in ER protein processing (calreticulin, HSPA8, GRP78-BiP) were down-regulated in BD patients compared to healthy controls, which hints the involvement of ER stress in BD and will be investigated in more detail.

Disclosure of Interest
None Declared

P2061
IL1RAP as a candidate gene for autosomal dominant Behçet's disease

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Introduction: Behçet's Disease (BD) is a chronic inflammatory disorder with unknown aetiology. Higher prevalence along the Silk Route and familial cases suggest a genetic contribution to its pathogenesis. We present a Turkish family diagnosed with BD involving the father and three of the four children, all with disease onset before age 16. All affected individuals meet the International Study Group criteria for BD. Oral/ genital ulcers and ostiofolliculitis were shared by all probands. Posterior uveitis and deep vein thrombosis were diagnosed in the father while recurrent arthritis was prominent in the affected children.
Objectives: This study aimed to identify the putative gene responsible for autosomal dominant BD.
Methods: Exome sequencing was performed for unaffected mother and the four affected family members. Heterozygous, rare (MAF <0.005) and exonic/splicing variants were selected from among the shared variants, and those shared also with mother were excluded. Candidate variants that were chosen after analysis via bioinformatics tools were tested by Sanger sequencing of aforementioned members along with an unaffected and an undiagnosed sibling. Those variants were also screened in 152 healthy controls with high resolution melting (HRM) method. To evaluate the effect of the strongest candidate gene to autoinflammation, levels of IL1β isolated from PBMC cells in four healthy controls and an affected member in exacerbation period were compared by ELISA method.
Results: Exome analysis showed that patients did not carry variants in *TNFAIP3* which is the known gene for autosomal dominant BD. Six rare, heterozygous variants which were shared among four affected members but absent in unaffected mother were selected as initial candidates. Three of those variants were eliminated because they were present in a healthy sib. The remaining three prominent candidate variantsc.35A>T (p.D12V) *in NAGK* (rs150821125), c.1490C>G (p.A497G) in *SLC9A2*and c.11T>G (p.L4R) in *IL1RAP* (rs200782803) were absent in control samples. Furthermore, variants in *SLC9A2*and *IL1RAP* were not reported in gnomAD, 1000G, ExAC and ESP6500 databases. They are predicted as deleterious by computational prediction tool such as Mutationtaster, SIFT. Due to its involvement in IL1 signaling, *IL1RAP* stood out as the most prominentcandidate.No significant difference in IL1β levels were observed between the affected member and the healthy controls (165.4 μg/ml vs 165.4 μg/ml respectively).
Conclusion: Our results suggest that in this family the most relevant candidate isc.11T>G (p.L4R) in IL1RAP.This is the first report suggesting a possible association of *IL1RAP* with autosomal dominant BD. Further studies are needed to better define *IL1RAP* effect in BD pathogenesis.

Disclosure of Interest
None Declared

Systemic-onset JIA and AOSD

P2062
Canakinumab in systemic juvenile idiopathic arthritis: clinical inactive disease rate and safety initalian patients

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Introduction: Systemic juvenile idiopathic arthritis (sJIA) is a polygenic autoinflammatory disease. The pathophysiology is still unclear, it is now well known that innate immune mechanisms play a central role with overproduction of inflammatory cytokines. The increased knowledge on the role of these cytokines has provided a change in the natural history of the disease with the introduction of the targeted treatments. Remarkable results has been observed with canakinumab, an anti-interleukin-1β monoclonal antibody, in two clinical trials but little information are available in real life.
Objectives: To evaluate clinical inactive disease rate and safety of canakinumab in Italian patients with sJIA.
Methods: We have collected retrospectively clinical and laboratory data of patients with sJIA treated with canakinumab in 9 Italian Paediatric Rheumatology centers. Clinically inactive disease (CID) at 6 months was defined according to Wallace criteria.We analyzed the effect of canakinumab on fever, rash, number of actives joints, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and physician's global assessment of disease activity score. Clinical and laboratory data were obtained using a standard data collection form.
Results: Forty seven patients (26 F) were included in the analyses. The median age (range) at the diagnosis and at the beginning of treatment with canakinumab was 7.6 (1-14.7) and 10.2 (1.7-22.2) years, respectively. Twenty seven patients (57.4%) had been previously

treated with other biologic agents (18 with anakinra, 1 with tocilizumab, 6 with both and 2 with etanercept), withdrawn for inefficacy in 15/27 (55.5%). Thirty patients (63.8%) were receiving concomitant treatment with glucocorticoids at the median dose (range) of 0.69 (0.02-2.75) mg/kg/die. Thirty nine out of 47 patients had > 6 months of follow-up. Among these 39 patients, 27 (69.2%) achieved CID at 6 months and 5/27 (18.5%) were still on glucocorticoids. Of the 30 patients who received concomitant glucocorticoids at baseline, 24 achieved 6 months of follow-up and 12 (50%) of these were able to withdraw glucocorticoids. Minor adverse events were reported in 5/30 (16.6%) patients:upper respiratory tract infections in 4 and transient injection site reaction in 1. No cases of macrophage activation syndrome was reported.

Conclusion: Our results provide initial real world evidence of the efficacy of treatment with canakinumab in patients with sJIA. In our study the percentage of patients who reached CID at 6 months is slightly higher (69.2%) than reported at the end (from 3 months to one year) of the 2 published randomized trials (60%) (1). No serious adverse events were recorded in our population.

Reference

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Disclosure of Interest

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P2063

Whole blood phosphorylated STAT1 levels in patients with active macrophage activation syndrome and secondary hemophagocytic lymphohistiocytosis

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Introduction: A large body of evidence demonstrates the pivotal role of interferon gamma (IFNγ) in the pathogenesis of secondary hemophagocytic lymphohistiocytosis (sHLH) and macrophage activation syndrome (MAS). IFNγ is a key endogenous activator of macrophages and exerts its biological activities by phosphorylation of the transcription factor Signal transducer and activator of transcription 1 (STAT1).

Objectives: In this study, we aimed to investigate whether the phosphorylation status of STAT1 in whole blood cells represents a good biomarker for the identification of patients at early stages of MAS/sHLH.

Methods: Whole blood samples from patients with suspected untreated MAS/sHLH (n=7) and suspected treated (glucocorticoids) MAS/sHLH (n=9) were collected prospectively. As controls, whole blood samples from patients with active systemic Juvenile Idiopathic Arthritis (sJIA) without MAS at sampling (n=6) and healthy subjects (HS, n=7) were used. Fresh whole blood cells were left unstimulated or stimulated with different concentrations of IFNγ (0.01, 0.1, 1, 10 ng/ml) for 10 minutes. The intracellular phosphorylation levels of Tyrosine (701) STAT1 (pSTAT1) were evaluated by flow cytometry. Results have been expressed as Delta mean fluorescence intensity (MFI), calculated by subtracting the MFI of cells stained with isotype control antibody (Ab) from that stained with anti-pSTAT1 Ab. Anti CD3, CD14 and CD16 staining was performed to discriminate the monocyte, neutrophil, natural killer- and T- cell subpopulations.

Results: In both treated and untreated MAS/sHLH patients, flow cytometric analyses showed no significant differences in pSTAT1 levels in unstimulated monocyte, neutrophil, natural killer and T cell subpopulations, compared to sJIA and healthy subjects. Interestingly, we found that, compared to sJIA and healthy subjects, in patients with untreated MAS/sHLH, pSTAT1 levels were significantly higher in monocytes ($p<0,01$ Vs HS, $p<0,05$ Vs sJIA and $p<0,01$ Vs HS, $p<0,05$ Vs sJIA, for stimulation with 1 and 10 ng/ml of IFNg respectively) and neutrophils ($p<0,05$ Vs HS, $p<0,05$ Vs sJIA and $p<0,01$ Vs HS, $p<0,01$ Vs sJIA) stimulated with the higher concentrations of IFNγ (1 and 10 ng/ml). In contrast, we did not find differences in the levels of pSTAT1 observed in stimulated monocytes and neutrophils from treated MAS/sHLH patients or those observed in cells from active sJIA and healthy subjects.

Conclusion: Our results demonstrate that the combined evaluation of pSTAT1 levels by flow cytometry in monocytes and neutrophils stimulated with high doses of IFNγ show high levels of pSTAT1 and might contribute to the identification of patients at early stages of MAS/sHLH. In addition, our results further support the involvement of IFNγ in the development of the diseases, as suggested by the increased phosphorylated STAT1 levels exclusively in patients with active MAS/sHLH and not in patients with active sJIA.

Disclosure of Interest

None Declared

P2064

Whole blood cells from patients with systemic juvenile idiopathic arthritis (SJIA) in clinical inactive disease displayed a dysregulated response to TLR-4 stimulation

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Introduction: Systemic juvenile idiopathic arthritis (sJIA) is a polygenic autoinflammatory disease. Innate immune mechanisms appear to play a central role in the pathogenesis of the disease. Nevertheless, a better understanding of the pathophysiology of sJIA is still needed to identify patients responsive to IL-1 or IL-6 targeted therapies.

Objectives: In this study, we evaluated the production of IL-1β, IL-6 and TNF-α by fresh whole blood cells isolated from sJIA patients in disease remission, after stimulation with the TLR-4 ligand lipopolysaccharide (LPS), and we investigated whether sJIA patients that respond or not-respond to treatment with the IL-1 receptor antagonistanakinra show a different response.

Methods: We collected fresh whole blood samples from sJIA patients during clinical inactive disease (inactive sJIA, n=19) and sJIA patients during active disease (active sJIA n=4). Active and inactive disease was defined at time of sampling. As controls, fresh whole blood samples from healthy subjects (HS, n=10) were used. Whole blood cells were left unstimulated or stimulated with 10ug/mL of LPS for 24 hours. Cytokine levels (IL-1β, IL-6 and TNF-α) released in the supernatants were measured by ELISA. Response to anakinra was defined as achievement of clinical inactive disease off glucocorticoids at 6 months after initiation of anakinra treatment.

Results: We found that LPS-stimulated cells from inactive sJIA patients released significantly higher amounts of all the inflammatory cytokines tested, compared to HS ($p<0.01$). In addition, cells from inactive sJIA patients produced significantly higher levels of IL-1β also compared to active sJIA patients ($p<0.05$). When we divided inactive sJIA patients in two groups (responders and non-responders), depending on the clinical response to anakinra treatment, we observed