tested in HEK293T cells stably expressing the adaptor protein apoptosis-asso-
ciated speck-like (ASC) which were analysed by flow cytometry to visualise inflammasome formation, with and without stimulation by Closstridium difficile toxin B (TcdB). Inflammasome dependent cytokine secretion was also quantified by ELISA of supernatants from THP-1 cells transduced with lentiviral expression vectors.

Results: In AADRY, we observed the p.E148Q allele in individuals with autoin-
flammatory diseases alone or in conjunction with other pyrin variants. Two FMF
families harbored the allele p.M694I-E148Q in cis with dominant heritability. In vitro, p.E148Q pyrin could spontaneously potentiate inflammasome formation, with increased IL-1β and IL-18 secretion. p.E148Q in cis to classical FMF muta-
tions provided significant potentiation of inflammasome formation.

Conclusion: The p.E148Q variant in pyrin potentiates inflammasome activation in vitro. In cis, this effect is additive to known pathogenic FMF mutations. These observations may help to explain the association of p.E148Q with FMF and other inflammatory diseases.

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Methods: Sera from 103 AOSD patients (treatment naïve on treatment, and patients who previously failed several bDMARDs and were included in a CONSIDER clinical trial (n=30)) were analysed. These were compared to Systemic Juvenile Idiopathic Arthritis (SjIA, n=14) and Familial Mediterranean Fever (FMF, n=27) patients. Extracellular NLRP3-derived ASC specks were identified using flow cytometry (double positivity for ASC-Pe and NLRP3-APC), gating for events around 1μm in size, in sera. To determine if ASC/NLRP3 speck levels provide additional biological information, the correlation between the specks and known biomarkers (such as IL-1β and C-reactive protein (CRP)) were analysed in sera. This involved cytokine profiling, using 13-plex Inflammatory LEGENDplex assay and high-sensitivity CRP ELISAs. Deep whole exome sequencing (WES x100) (analysis restricted to autophosphorylated panel) was carried out on DNA from the 30 CONSIDER trial patients.

Results: In serological analyses, extracellular ASC/NLRP3 speck levels were increased in AOSD patients compared to 32 healthy control (HC) sera (p<0.01, Figure 1A). ASC/NLRP3 levels defined three subgroups of AOSD patients (low, moderate and high). High ASC/NLRP3 levels were present in all pre-treatment sera from AOSD patients (p<0.001), compared to HC, suggesting their role may be dependent on the stage of the disease process. Interestingly, these patients were still responsive to canakinumab, showing significant reduction in extracellular ASC/NLRP3 levels in CONSIDER clinical trial cohort (baseline to week 12), compared to placebo (p<0.01) (Figure 1B) [1]. There was no correlation between ASC/NLRP3 specks and CRP levels (Figure 1C) or ASC/NLRP3 and total IL-18. No germline or somatic variants in NLRP3 were identified from patients in the CONSIDER cohort despite very high levels of ASC/NLRP3 specks being detected in their serum.

Conclusion: This study involved development of an assay that quantifies extracellular ASC specks as a biomarker of NLRP3 activation, to improve classification and classification of SAIAs, particularly those with sporadic or unknown causes, such as AOSD. Increased levels of extracellular NLRP3-derived ASC specks were found in sera of AOSD compared to HC and autoinflammatory disease controls. Our findings also demonstrate heterogeneity within AOSD cohorts, with high-ASC speck levels in therapy-resistant CONSIDER trial patients suggesting the role of ASC specks may be dependent on the particular stage of the disease. Further analysis of rare somatic variants in genes associated with myelodysplastic syndrome, another condition associated with elevated ASC/NLRP3 specks and systemic inflammation, is currently underway, since this may provide an alternative explanation for our findings.

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