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Excess Serum Interleukin-18 Distinguishes Patients with Pathogenic Mutations in *PSTPIP1*

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Abstract

Objective: Dominantly-inherited *PSTPIP1* mutations cause a spectrum of autoinflammatory manifestations epitomized by Pyogenic Arthritis, Pyoderma gangrenosum, and Acne (PAPA) syndrome. The connections between PSTPIP1 and PAPA are poorly understood, although evidence suggests pyrin-inflammasome activation. Interleukin (IL)-18 is an inflammasome-activated cytokine associated with susceptibility to Macrophage Activation Syndrome (MAS), but its association with PAPA is unclear.

Methods: Clinical and genetic data, and serum samples were obtained from patients referred with symptoms concerning for PAPA syndrome. Serum Interleukin-18 (IL-18), IL-18 Binding Protein (IL-18BP), and CXCL9 were assessed by bead-based assay, and free IL-18 was assessed by ELISA.

Results: *PSTPIP1*-positive PAPA patients' symptoms overlapped with those of mutation-negative PAPA-like patients, but mutation-positive patients had earlier onset and more arthritis. We found uniform elevation of total serum IL-18 in treated PAPA patients at levels nearly as high as NLRC4-associated autoinflammation with infantile enterocolitis (AIFEC) patients and well above levels in most Familial Mediterranean Fever patients. IL-18 elevation in PAPA patients' serum persisted despite fluctuations in disease activity. The soluble IL-18 antagonist IL-18BP was modestly elevated, and PAPA patients had detectable free IL-18. PAPA syndrome was rarely

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associated with elevation of CXCL9, an indicator of Interferon-gamma activity, but no PAPA patients had histories of MAS.

Conclusion: PAPA syndrome is a refractory and often disabling monogenic autoinflammatory disease associated with chronic and unopposed elevation of serum IL-18 but not risk for MAS. These findings affect our understanding of the diseases in which IL-18 is over-produced and suggest a link between pyrin-inflammasome activation, IL-18, and autoinflammation without susceptibility to MAS.

INTRODUCTION

Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome was first associated with dominant mutations in *PSTPIP1* in 2002[1], but since that time the links between such mutations and the protean clinical manifestations have been challenging to unravel. *PSTPIP1* mutations are now known to cause an array of manifestations that includes systemic features, early onset (sterile) neutrophilic arthritis, and variable cutaneous involvement. Skin manifestations include simple ulceration, pyoderma gangrenosum, cystic acne, and hidradenitis suppurativa[2]. Other clinical findings can include cytopenias, lymphadenopathy, and hepatosplenomegaly, particularly in the context of high serum zinc and extremely elevated S100 proteins[3]. This spectrum has been collectively termed *PSTPIP1*-associated inflammatory diseases (PAID)[2]. Causative mutations operate in a dominant inheritance pattern and include both missense mutations and potentially promotor microsatellite expansions[4].

Mechanistically, several *PSTPIP1* mutations have been shown to increase binding to pyrin (the protein mutated in Familial Mediterranean Fever, or FMF), and activate both the pyrin- and NLRP3-inflammasomes. PSTPIP1 also interacts with the actin cytoskeleton and regulates the Wiskott-Aldrich Syndrome protein, and *PSTPIP1* mutants potentially alter actin dynamics, pyrin-inflammasome activation, and innate immune cell motility[5, 6].

Interleukin-18 (IL-18) is expressed by macrophages and by epithelia of the skin and intestine. It requires proteolytic activation, usually via inflammasome-dependent caspase-1 activation, and is thought to escape the cytosol through Gasdermin-D-mediated pyroptosis. Active IL-18 has a high affinity, soluble inhibitor called IL-18 Binding Protein (IL-18BP), which is itself induced by interferon-gamma (IFNg) signaling[7]. IL-18 canonically acts on NK- and activated T-cells to promote production of type 1 cytokines and granule-mediated cytotoxicity. It usually acts in concert with cytokines of the Jak-STAT pathway like IL-12 or IL-15. However, it may serve homeostatic roles at tissue sites. Reports of serum IL-18 elevation have been published across a wide variety of infectious, malignant, and rheumatic diseases. However, the extraordinarily high serum levels necessary to overcome inhibition by IL-18BP and generate “free IL-18” appear restricted to diseases at highest risk of Macrophage Activation Syndrome (MAS), including Systemic Juvenile Idiopathic Arthritis (SJIA), Adult-Onset Still’s Disease (AOSD), and monogenic disorders like the NLRC4 inflammasomopathy AIFEC (OMIM 616050) and C-terminal mutations in the Rho GTPase CDC42[7-10].

METHODS

Patients were recruited and evaluated as part of Natural History protocols ongoing at the intramural programs of NHGRI and NIAID, as well as the University of Pittsburgh and Hospital Clinic, Barcelona (see Supplemental Methods). All patients referred for evaluation of autoinflammatory disease concerning for PAPA syndrome (arthritis with suggestive skin findings, idiopathic pyoderma gangrenosum, severe acne/hidradenitis suppurativa, or known pathogenic mutation in *PSTPIP1*) and serum available for study were included. PAPA patients (n=20) were defined as those with a known mutation in *PSTPIP1*. "PAPA-like" patients (n=11) had refractory, idiopathic pyoderma gangrenosum and often other symptoms (see Table 1) but no pathogenic mutation (in *PSTPIP1* or other genes) was detected. Samples from treated Familial Mediterranean Fever (FMF, n=18) and NLRC4-associated AIFEC (two with distant enterocolitis n=3) were included as pyrin-inflammasome and high IL-18 disease controls, respectively. Serum total IL-18, IL-18BP, and CXCL9 were measured as in Weiss et al.[7]. Briefly, serum was diluted 25-fold and assayed on a Magpix or FLEXMAP 3D multiplex instrument per the manufacturer's instructions (Luminex). Recombinant IL-18 and CXCL9 were used as standard (MBL International and Peprotech, respectively). Human IL-18BPa-Fc (R&D Systems) was run separately given its interaction with recombinant IL-18[7]. IL-18 and IL-18BPa beads were generated by conjugating capture antibody to magnetic beads per the manufacturer's instructions (Bio-Rad), whereas CXCL9 beads were purchased (Bio-Rad). Free IL-18 was measured in some samples by ELISA as previously described[10]. Minimal variation between plates and runs was verified using bridging controls.

RESULTS

This study arose, while assessing AID controls for a MAS study[7], from the observation of highly elevated serum total IL-18 levels in two patients with PAPA syndrome. Subsequently, a total of thirty-one patients were identified as having been referred for symptoms suggestive of PAPA. Twenty patients were found to bear heterozygous mutations in *PSTPIP1* (PAPA patients), with the p.Ala230Thr (11/20) and p.Glu250Gln (6/20) as the most prevalent (Supplemental Table 1). By contrast, 11 PAPA-like patients did not carry mutations in *PSTPIP1* (PAPA-like patients). One PAPA-like patient (pyoderma gangrenosum) carried a heterozygous Arg405Cys variant, whereas a patient with an undifferentiated AID (recurrent fevers) carried a heterozygous p.Gly258Ala variant, both of which we classified as benign.

Clinical features overlapped substantially between patients with and without *PSTPIP1* mutations, consistent with their pattern of referral. However, PAPA patients harboring *PSTPIP1* mutations were younger at disease onset, more had a history of arthritis, and fewer had a history of pyoderma gangrenosum (Table 1). Both groups' treatment history reflected the often-recalcitrant nature of these symptoms, with many patients having been treated with glucocorticoids and more than one biologic medicine (Supplemental Table 1). Two patients had clinical features of PAPA but also cytopenias, organomegaly, and mutations associated with the Hyperzincemia/hypercalprotectinemia (Hz/Hc) syndrome[11]. No PAPA or PAPA-like patients had a history of MAS.

As part of an effort to determine the distribution of serum IL-18 across autoinflammatory diseases[7], stored serum from enrolled patients were assayed retrospectively and compared with relevant disease controls. These controls included patients with activating mutations causing NLRC4 inflammasome-induced AIFEC as well as patients with FMF, all of whom were undergoing anti-inflammatory treatment (Supplemental Table 1). Previous work demonstrated that dramatically elevated serum IL-18 levels and detectable free IL-18 were unique to patients at significant risk for MAS and not for other inflammasomopathies, type I IFN-mediated diseases, or other autoinflammatory diseases[7]. Contradicting this, we observed highly elevated serum IL-18 levels in the group of patients with mutations in *PSTPIP1* (Figure 1A). PAPA-like patients' serum IL-18 was largely in the normal range. As expected, AIFEC serum IL-18 levels were highly elevated. About half the FMF patients' sera were above-normal, but only a few samples showed elevations in the range routinely observed in PAPA. This did not appear to correlate with genotype, even in patients bearing homozygous p.Met694Val mutations in *MEFV*[12] (Figure 1A, open circles, Supplemental Table 1). CXCL9 levels were not consistently elevated in any group. Though IL-18BP levels were significantly higher in PAPA than PAPA-like patients, we were nevertheless able to detect free IL-18 in samples from PAPA patients.

In several patients we were also able to analyze serial samples. Though we observed some variation in total and free IL-18 levels, the degree of this variation was minor in comparison to the degree of C-reactive protein variation (Figures 1B, C, and D). All patients followed longitudinally had dramatic elevation of total IL-18, and detectable free IL-18, at all timepoints.

DISCUSSION

Many infectious, oncologic, or rheumatic causes of systemic inflammation have been associated with elevated peripheral levels of IL-18, and even very small differences have been independently associated with worse outcomes in chronic inflammatory diseases like atherosclerosis[13]. However, IL-18 has an extraordinary dynamic range of over 4 logs in human serum, and extremely high total IL-18 levels have heretofore been observed almost exclusively in diseases associated with MAS, including SJIA, AOSD, and a few rare monogenic immune dysregulation disorders such as AIFEC/NLRC4-MAS[7]. This has spurred investigation of IL-18 as a fundamental cause of MAS and clinical trials of IL-18 blockade in genetically-mediated MAS are in progress (NCT03113760, NCT04641442).

Though PAPA patients do not appear to be at risk for MAS, we found highly and chronically elevated total serum IL-18 levels, and detectable free IL-18, in mutation-positive PAPA patients. Disease activity can be challenging to quantitate in PAPA, but IL-18 levels did not clearly correlate with acute phase reactants or with arthritis-, pyoderma-, or acne-predominant patients (Supplemental Table 1). This suggests a direct pathogenic link between *PSTPIP1* and increased IL-18, likely through pyrin-inflammasome activation. Supporting this, we corroborate others' work that IL-18 can be significantly elevated in some FMF patients' serum, possibly related to disease activity and/or p.Met694Val homozygosity[12]. Likewise, mutations in *WDR1* causing periodic fever, immunodeficiency, thrombocytopenia (PFIT) syndrome may also cause unopposed IL-18 elevation[14]. However, most FMF

patients' IL-18 levels are not significantly elevated, nor are those of Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND)[7], The specific genetic, cellular, and environmental circumstances driving IL-18 through the pyrin inflammasome remain unclear.

IL-18 may be significantly elevated in PAPA syndrome without increasing MAS risk for a variety of reasons. First, although ample animal work and preliminary case reports suggest otherwise[7, 15], it is possible that IL-18 elevation is associated with AIDs like PAPA, PFIT, and MAS, but not contributory to the pathology. Second, IL-18 typically functions by amplifying the effects of other cytokines; the inflammatory milieu of SJIA/MAS and NLRC4-MAS/AIFEC may be substantively different than PAPA. Third, though both are associated with detectable free IL-18, it may be that only the highest levels of IL-18 activity are sufficient to promote MAS. Finally, the source of IL-18 may dramatically alter its effects. The sources of extreme and chronic IL-18 elevation remain unclear. Macrophages are the canonical sites of inflammasome activation and IL-18 production, and *PSTPIP1* mutations have been shown to activate the pyrin-inflammasome in macrophages *in vitro*[16]. However, recent work in *Nlrc4*-hyperactive mice suggests (intestinal) epithelial cells have both inflammasome machinery and abundant pro-IL-18 as a substrate[7]. Notably, skin epithelium is also a substantial source of *Il18* transcript[7, 13].

Measurement of peripheral IL-18 may be diagnostically useful in the evaluation for PAPA syndrome regardless of its pathogenic role. The difference in IL-18 between *PSTPIP1* mutation-positive and -negative patients appeared binary, and helped confirm the p.Arg405Cys and p.Gly258Ala variants as likely non-pathogenic. Serum was available from only one patient bearing a PAMI-associated mutation and was elevated similarly to other PAPA patients. Some patients in our cohort had almost exclusively cutaneous or articular disease, suggesting that IL-18 elevation correlates with *PSTPIP1* mutations rather than specific phenotypic features. Thus, serum IL-18 appears to reliably distinguish patients carrying true *PSTPIP1* mutations from patients with suspicious clinical findings or rare variants. Unraveling what connects specific autoinflammatory genes with dramatic elevations of S100 proteins, IL-18, and possibly zinc remains an important area of future research[2, 3].

Although it includes 20 PAPA patients from various institutions, our study was limited by its relatively small size and retrospective nature. Future studies would benefit from multi-center prospective enrollment and concomitant measurement of other PAPA-associated biomarkers (e.g., aldolase, zinc, S100 proteins). Nevertheless, our observations add a puzzling diversity to the group of disorders characterized by chronic elevation of total and free IL-18, and outline a path for studying the pathogenic effects of IL-18 beyond MAS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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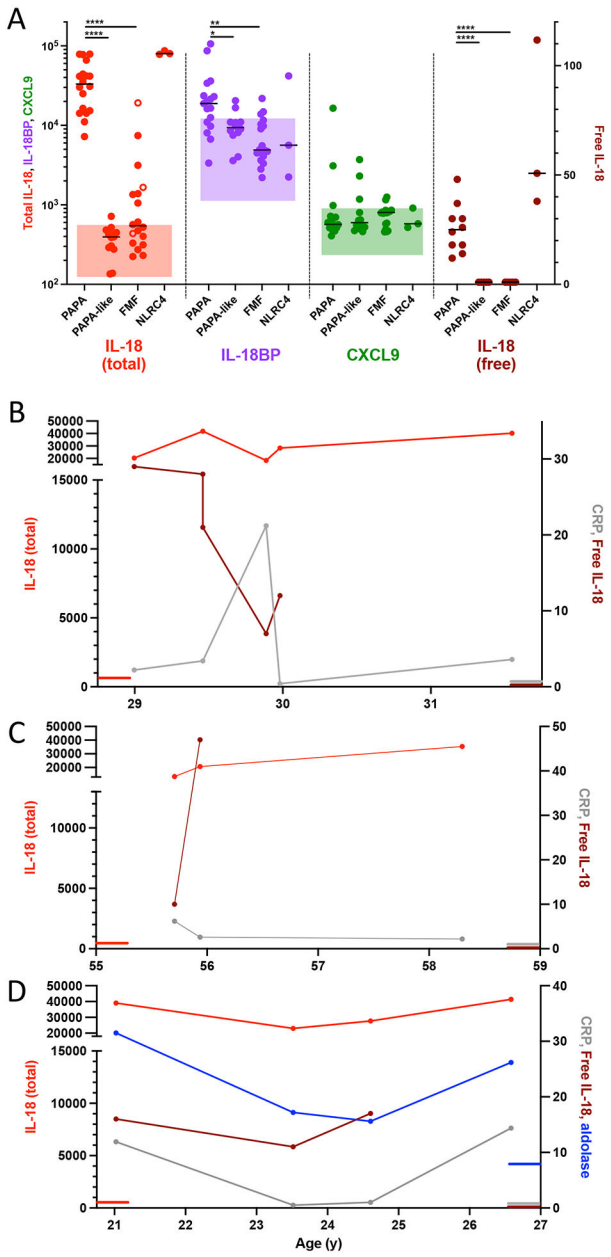

Figure 1: PAPA Syndrome is associated with elevated Total and Free IL-18.

A) Serum was assayed for the indicated cytokines. Each point represents the first available sample from each patient. Shaded bars indicate normal range. PAPA, PAPA-like, and FMF groups were compared by one-way ANOVA with Tukey's post-test. Adjusted p-values are displayed as *p<0.05, **p<0.01, ****p<0.0001. Open circles in Total IL-18 indicate Met694Val homozygous FMF patients. B-D) Longitudinal measurements of IL-18, free IL-18 and C-reactive protein (CRP) from three PAPA patients bearing *PSTPIP1* mutations.

Table 1:

Clinical Characteristics of PAPA & PAPA-like patients

Feature (n)	PAPA (20)	PAPA-like (11)	Univariate p
Sex (F;M)	9;11	7;4	n.s.[a]
Age at onset [*]	2.3 (0.2 - 45)	8.5 (3 - 19)	p=0.04[b]
Arthritis (y;n)	16;4	1;10	p=0.0004[a]
Cystic Acne (y;n) [#]	11;9 (4,1,6)	6;5 (1,0,5)	n.s.[a]
Pyoderma Gangrenosum (y;n) [#]	13;7 (3,3,7)	11;0 (1,2,8)	n.s.[a]
Max CRP [*] **(mg/dL)**	2.4 (0.2 - 21.2)	1.15 (0.4 - 7.2)	p=0.03[b]

[*] Median (range) of records available for timepoints associated with biomarker measurements

[#] (Mild, Moderate, Severe)

[a] =Fisher's exact

[b] =Welch's t-test