Phenotypic Associations of PSTPIP1 Sequence Variants in PSTPIP1-Associated Autoinflammatory Diseases

Guilaine Boursier, Maryam Piram, Cécile Rittore, Guillaume Sarrabay, Isabelle Touitou

PHENOTYPIC ASSOCIATIONS OF *PSTPIP1* SEQUENCE VARIANTS IN *PSTPIP1*-ASSOCIATED AUTOINFLAMMATORY DISEASES (PAIDS)

Guilaine Boursier[1] 0000-0002-2903-3135, Maryam Piram[2,3] 0000-0002-8634-5530, Cécile Rittore[1] 0000-0001-5018-5101, Guillaume Sarrabay[4] 0000-0002-9943-9368 and Isabelle Touitou[4] 0000-0002-5171-1000.

[1]Department of Medical Genetics, Rare diseases and Personalized medicine, Rare and Autoinflammatory diseases unit, CHU Montpellier, Univ Montpellier, Montpellier, France

[2]CEREMAIA, AP-HP, CHU de Bicêtre, Univ. Paris Sud Paris Saclay, Le Kremlin-Bicêtre, France.

[3]Department of Paediatric Dermatology, CHU Sainte Justine, Montréal, Canada.

[4]Cellules souches, plasticité cellulaire, médecine régénératrice et immunothérapies, INSERM, Univ Montpellier, CEREMAIA, Department of Medical Genetics, Rare Diseases and Personalized Medicine, Rare and Autoinflammatory diseases unit, CHU Montpellier, Montpellier, France

Corresponding author:

Dr Guilaine Boursier
CHU Montpellier
371 Av. du Doyen Gaston Giraud
34295 Montpellier, France
Email: g-boursier@chu-montpellier.fr / Tel: +33467335857

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Abbreviations:
AIDs: autoinflammatory diseases
ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain
CD2BP1: CD2-binding protein 1
CRP: C-reactive protein
IL: interleukin
PAIDs: *PSTPIP1*-associated inflammatory diseases
PAC: pyoderma with acne and ulcerative colitis
PAPA: pyogenic sterile arthritis, pyoderma gangrenosum, and acne syndrome
PAPASH: pyoderma gangrenosum, acne, suppurative hidradenitis and pyogenic arthritis syndrome
PAMI: *PSTPIP1*-associated myeloid-related proteinemia inflammatory syndrome
PASH: pyoderma gangrenosum, acne and suppurative hidradenitis syndrome
PTP-PESTs: proline-, glutamic acid-, serine- and threonine-rich family of protein tyrosine phosphatases
SH: suppurative hidradenitis
VUS: variant of uncertain significance

ABSTRACT

Pathogenic variants in the proline-serine-threonine phosphatase interacting protein 1 (*PSTPIP1*) cause pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome. They were also identified in a broad spectrum of phenotypes. As their interpretation is sometimes challenging, we discuss the genotype–phenotype association in *PSTPIP1*-associated autoinflammatory diseases (PAIDs) in light of a recent consensus classification of variant pathogenicity. Only 7/39 (18%) of the *PSTPIP1* variants found in all reported cases and our national reference center [161 patients (114 probands)] were pathogenic. They were clearly associated with PAPA and *PSTPIP1*-associated myeloid-related proteinemia inflammatory syndrome (PAMI), reflecting a variable clinical expression of PAIDs.

INTRODUCTION

PSTPIP1 (proline-serine-threonine phosphatase-interacting protein 1) was identified in 1997 in mice (Spencer et al. 1997). In the same year, Lindor et al. described the first family of patients with pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome [MIM:604416] over 3 generations, highlighting an autosomal dominant transmission (Lindor et al. 1997). A few years later, p.(Ala230Thr) and p.(Glu250Gln) variants in *PSTPIP1* on chromosome 15, formerly called *CD2BP1* (CD2-binding protein 1), were identified as causing PAPA syndrome in this family and another with a similar disorder (Wise et al. 2002; Wise et al. 2000).

PAPA syndrome is clinically characterized by recurrent episodes of fever, severe acne, pyoderma gangrenosum (PG), and arthritis in non-axial joints (knees, ankles, and elbows) (Marzano et al. 2016). Standard laboratory findings reflect systemic inflammation with leukocytosis. Recurrent, sterile pauciarticular arthritis with a prominent neutrophilic infiltrate usually occurs in childhood and may be the presenting sign of the disease (Martinez-Rios et al. 2019; Marzano et al. 2016). The joint symptoms tend to decrease around adulthood and cutaneous symptoms become more prominent (Marzano et al. 2016). Skin involvement includes nodulocystic acne that usually occurs during puberty and may worsen with it, and PG lesions characterized by recurrent unique or multiple rapidly enlarging painful ulcer with violaceous and raised borders, usually on the lower extremities that may occur before or after puberty (Vinkel and Thomsen 2017). Pathergy manifesting by pustule formation followed by ulceration upon minimal trauma may be noticed early in life (Cugno et al. 2017).

Over the years, *PSTPIP1* variants have been identified in a broad spectrum of phenotypes such as isolated PG (Nesterovitch et al. 2011), PG with acne and ulcerative colitis (PAC) (Zeeli et al.

2015), PG, acne and suppurative hidradenitis with or without pyogenic arthritis (PAPASH and PASH, respectively) (Calderón-Castrat et al. 2016; Cugno et al. 2017; Marzano et al. 2013) and *PSTPIP1*-associated myeloid-related proteinemia inflammatory (PAMI) syndrome (Belelli E 2017; Hashmi et al. 2019; Holzinger et al. 2015; Klötgen et al. 2018; Takagi et al. 2018). PAMI syndrome, also called hyperzincemia/hypercalprotectinemia, is characterized by early-onset chronic systemic inflammation, skin inflammation (i.e., skin ulcerations, abscesses, vesiculo-bullous or pustular lesions), arthralgia/arthritis, hepatosplenomegaly, pancytopenia and failure to thrive (Holzinger et al. 2015). A hallmark of the disease is the extreme concomitant increase in serum concentrations of calprotectin and zinc (Holzinger et al. 2015).

All these conditions share common pathophysiological mechanisms consisting of over-activation of the innate immune system leading to increased production of interleukin 1 beta (IL-1β) and sterile neutrophil-rich cutaneous inflammation (Cugno et al. 2017). The PAPA-associated PSTPIP1 mutants p.(Ala230Thr) and p.(Glu250Gln) were shown to increase IL-1β secretion *in vitro* compared to wild-type PSTPIP1 (Shoham et al. 2003). One mechanism that may explain this overproduction is decreased affinity for the proline-, glutamic acid-, serine- and threonine-rich family of protein tyrosine phosphatases (PTP-PEST), which results in increased PSTPIP1 tyrosine phosphorylation and increased affinity for pyrin that would bind to the same region of PSTPIP1 near the coiled-coil domain (Shoham et al. 2003). Hyperphosphorylated PSTPIP1-pyrin interaction triggers activation of the inflammasome and induces formation of the ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) pyroptosome, which by activating caspase-1, induces the subsequent secretion of pro-inflammatory IL-1β and IL-18 (J. Smith et al. 2010; Veillette et al. 2009; Yu et al. 2007). In contrast, the mechanisms of the pathogenesis of PAMI syndrome, associated with the p.(Glu250Lys) and p.(Glu257Lys) substitutions are more elusive. Structural models of the mutant proteins suggest alterations of

electrostatic potential in regions of PSTPIP1 that are critical for protein-protein interactions, enhancing pyrin binding through phosphorylation processes (Holzinger et al. 2015). This might explain the inappropriate inflammation process and the significantly increased calprotectin and zinc serum levels observed *in vitro* with the p.(Glu250Lys) variant. Indeed, calprotectin secretion and its zinc-capturing property secretion is hypothesized to be regulated by the PSTPIP1–pyrin axis (Holzinger et al. 2015).

The *PSTPIP1* variants reported in patients with *PSTPIP1*-associated autoinflammatory diseases (PAIDs) have sometimes generated various interpretations ranging from pathogenic to uncertain clinical significance. In this review, we discuss the association of such variants with patient phenotypes in light of a recent consensus classification of variant pathogenicity by experts of the International Society for Systemic AutoInflammatory Diseases (ISSAID) consortium based on published and unpublished experience (Shinar et al. 2020; Van Gijn et al. 2018). This article will help clinicians understand how the evolution of knowledge has led to a better understanding of genotype–phenotype associations in PAIDs.

PATIENTS AND METHODS

Identification of patients

In order to identify patients with *PSTPIP1* variants, we conducted a systematic literature review. Articles published up to April 25, 2020 were searched by querying MEDLINE via PubMed. Key words were *PSTPIP1* OR *CD2BP1* OR "PAPA syndrome". Eligibility criteria for inclusion were English language and at least one patient with a sequence variant in *PSTPIP1* without restriction on age, sex or ethnicity. We also screened the references in the selected articles. The search strategy for patients with *PSTPIP1* variants is illustrated in Figure 1.

We moreover added information for 7 patients with variants identified from the Infevers database (the reference database for variants in AID-associated genes available at https://infevers.umai-montpellier.fr/web/ Accessed 04/25/2020*)* (Milhavet et al. 2008) and 23 patients with a molecular diagnosis from our routine laboratory. Blood samples were collected after obtaining informed consent.

Clinical data collection

We collected the following information: phenotype, age at onset, published clinical diagnosis (i.e. PAPA syndrome, PAMI syndrome, Behçet's disease etc.), presence of fever, mention of a biological inflammatory syndrome (and elevated C-reactive protein [CRP] level if available), and presence of sterile arthritis, PG, cystic acne or suppurative hidradenitis (SH). When PAMI syndrome or the p.(Glu250Lys) or p.(Glu257Lys) variant were reported, additional related data were collected: presence of growth retardation, pancytopenia, hepatosplenomegaly, zincemia and calprotectinemia. Normal serum levels of zinc and calprotectin were defined as 11.5 to 21.4 µmol/L and 0.4 to 3.9 µg/ml, respectively (Holzinger et al. 2015; Mejbri et al. 2019).

Genetic results

Genetic data were reported according to the Human Genome Variant Society (http://varnomen.hgvs.org/) nomenclature based on the NM_003978.3 transcript. We retained 39 of the 63 variants listed in infevers as this paper focuses on rare variants (allele frequency <1% in controls or in GnomAD [https://gnomad.broadinstitute.org/ Accessed 04/25/2020]) in coding exons and intronic boundaries (±12 base pairs). Therefore, we did not include 16 common variants reported by Kumar et al. 2016 and Newman et al. 2004, and 8 variants outside these regions. All variants are accessible in the Infevers database (Milhavet et al. 2008). In total, 18/39 (46%) variants were already classified by ISSAID experts (Shinar et al. 2020; Van Gijn et al.

2018). We classified the remaining variants according to American College of Medical Genetics guidelines (Richards et al. 2015).

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at http://dx.doi.org/10.17632/m5wthxf8n8.2 and http://dx.doi.org/10.17632/cf9nzbvnfz.3 hosted at Mendeley Data (Boursier, 2020).

RESULTS

We identified 161 patients (114 probands and 47 relatives) with sequence variants in the *PSTPIP1* coding region (See datasets Table S1); 64 (40%) were female, 65 (40%) male and 32 (20%) of unknown gender.

Patient variants

Among the 39 different rare sequence variants identified in these patients, 7 (18%) were pathogenic or likely pathogenic, 23 (59%) were of uncertain clinical significance (VUS) and 9 (23%) were benign or likely benign. All pathogenic or likely pathogenic variants were located on exons 10, 11 or 14. The most frequent variants were p.(Glu250Lys), 26 families; p.(Ala230Thr), 19 families; and p.(Glu250Gln), 10 families.

Patient and relative phenotypes

The most frequent phenotypes identified were PAPA (n=75; 47%) and PAMI syndromes (n=27; 17%). We could retrieve the following information for PAPA and PAMI patients with (likely) pathogenic variants for whom clinical data were available (Figure 2). A biological inflammatory syndrome was evidenced in 95% (37/39) of informative patients with PAPA and in all (25/25) patients with PAMI. Fever was reported in all (5/5) PAMI, but in only 50% (7/14) of PAPA. Conversely, arthritis, acne, or features of PG were more prevalent in PAPA than in PAMI: 95%

(42/44) *vs* 56% (15/27), 73% (32/44) *vs* 19% (5/27), 58% (26/45) *vs* 41% (11/27), respectively. Age of onset of patients ranged from birth to 18 years old (median [Q1-Q3]: 3 [2-6] years and 2 [1-6] years for PAPA and PAMI patients respectively). CRP levels ranged from 5.2 to 251 mg/l (median [Q1-Q3]: 47 [28-79] mg/l and 114 [58-157] mg/l for PAPA and PAMI patients respectively). One PAPA patient with the p.(Ala230Thr) variant (P24) had also SH (Schellevis et al. 2011). SH was described in 2 members of one family with the p.(Tyr345Cys) variant and associated with PASH syndrome (Saito et al. 2018).

Other disorders (i.e., FMF, Behçet's syndrome, etc.) were not represented more than 6 times in the cohort (n=20, 12%). Among the remaining patients, twenty six (16%) had an undefined AID and four had a phenotype not associated with an AID (2 with common variable immunodeficiency and 2 with no AID) were described ; the phenotype was unknown for 6 patients; and 5 were described as asymptomatic.

Phenotype–genotype relation

Splitting the patients by pathogenicity score associated with their variants highlighted a clearly different distribution of phenotypes (Figure 2). Three phenotypes were associated with pathogenic or likely pathogenic variants: PAPA, PAMI and PASH syndromes. All variants except p.(Tyr345Cys) were located within the region that interacts with pyrin and PTP-PEST. Most variants were VUS, which likely stems from the fact that most often, segregation analysis was lacking in affected families. The same held true for patients with benign or likely benign variants.

DISCUSSION

Three main *PSTPIP1*-associated phenotypes emerged from this review: PAPA, PASH and PAMI syndromes.

PAPA syndrome

The variants originally identified, namely p.(Ala230Thr) and p.(Glu250Gln), are definitely classified as disease-causing because they segregate with PAPA syndrome in multiple affected family members and their effect was confirmed by functional studies (Shoham et al. 2003; Waite et al. 2009; Yu et al. 2007). The more recently found variants, p.(Asp246Asn) and p.(Glu257Gly), were classified as likely pathogenic because they are absent from large databases of controls, they are located in the same critical PSTPIP1 domain protein and they occurred *de novo* in patients with no family history (Fathalla BM 2014; Holzinger et al. 2015; Omenetti et al. 2016). In addition, p.(Glu257Gly) segregated with PAPA syndrome in 3 families (Holzinger et al. 2015; Omenetti et al. 2016). However, studies assessing the functional impact of these latter variants are still lacking.

Five patients with a diagnosis of PAPA syndrome carried likely benign variants (P103, P104 and P105) or VUS (P141 and P144). Whether these *PSTPIP1* variants truly cause the disease remains unknown, and evidence of recurrence in other families or functional studies are needed to resolve this issue.

A relative (P32) of a PAPA patient remained asymptomatic until age 16 (at the time of the case report) even though she carried the pathogenic variant p.(Ala230Thr) and experienced substantial trauma, a triggering factor of skin involvement (Demidowich et al. 2012). Indeed, because incomplete penetrance of p.(Ala230Thr) was identified in only this individual in our review, whether follow-up in adulthood would reveal tardive symptoms of PAPA syndrome is of interest.

PASH syndrome

SH was reported in 3 patients with likely pathogenic or pathogenic variants: a patient with PAPA syndrome (P24) who carried p.(Ala230Thr) and 2 relatives with PASH syndrome (P62, proband, and P63, mother) who carried the extremely rare p.(Tyr345Cys) variant (Saito et al. 2018; Schellevis et al. 2011). Tyrosine 345 (Y345) is critical for the dephosphorylation of PSTPIP1 by PTP-PESTs, which supports that p.(Tyr345Cys) is a likely pathogenic variant apparently associated with PASH syndrome (Côté et al. 2002; Saito et al. 2018). The youngest sister of this PASH family with the p.(Tyr345Cys) variant showed no symptoms, with no further indication of her age and follow-up. However, skin involvement in PAPA or PASH syndrome may start in adolescence or later in adulthood (Vinkel and Thomsen 2017).

An increased number (5 to 8) of CCTG repeats in the 5'UTR of *PSTPIP1* has been reported in patients with PASH syndrome (Braun-Falco et al. 2012; Duchatelet et al. 2015; Lin et al. 2011). However, we have no evidence that this variation has any functional effect. Furthermore, similar microsatellite expansion has been identified in unaffected people and in patients with pathogenic variants in *NCSTN* gene, which are also known to be associated with familial SH [MIM:142690] (André et al. 2010; Duchatelet et al. 2015). Thus, these microsatellite expansions may be involved in these inflammatory disorders and act as a modifier gene, although they are probably not causal because they are found in 10% to 78.8% of controls (André et al. 2010; Marzano et al. 2016).

PAMI syndrome

The sole genetic cause identified so far in this disorder is the substitution of a glutamic acid by a lysine at position p.Glu250 or p.Glu257 of PSTPIP1 (Holzinger et al. 2015). However, p.(Glu250Lys) was also detected in 6 patients with PAPA syndrome (P68, P69, P70, P72, P87

and P95) and p.(Glu257Lys) in 1 patient (P86). No clinical description was available for several of these patients. Thus, a phenotype overlap between PAPA and PAMI syndromes is likely because, for example, P72 had arthritis and PG, which are seen in both PAPA and PAMI syndromes. Indeed, absence of a clear phenotypical distinction between PAPA and PAMI syndromes could reflect variable expressivity. Patient P86 (Khatibi et al. 2016) was reported to have PAPA syndrome and an unusual cerebral arterial vasculopathy. However, the clinical signs of PAPA syndrome were not described in the paper. Because he carried the likely pathogenic variant p.(Glu257Lys), which was associated with PAMI syndrome in 2 other families, the dosage of zincemia and calprotectinemia would be of interest to better understand the genotype–phenotype relation in this patient (Holzinger et al. 2015). Indeed, data on zinc/calprotectin levels, pancytopenia, growth retardation and hepatosplenomegaly were mostly unavailable for these patients. Some of them such as P69 who had hyperzincemia, hypercalprotectinemia, anemia and splenomegaly may have a PAMI-like phenotype. This seems also relevant in PAPA patients with p.(Glu250Gln) and p.(Glu257Gln) variants because alterations at codons 250 and 257 are reported in both PAPA and PAMI and these data would be required to distinguish PAMI from PAPA.

In addition, several cases described in the literature demonstrate that these two variants should be interpreted in conjunction with clinical elements. For example, the mother (P71) of a “PAPA” patient carried p.(Glu250Lys) and was reported as asymptomatic, but she actually experienced chronic fatigue, arthralgia and myalgia (Lee et al. 2012). Although these symptoms are not specific, definitely claiming that these manifestations are not associated with p.(Glu250Lys) is difficult. In one PAMI family, there were some doubts about the status of a father (P99) who carried the variant p.(Glu250Lys). No clinical information other than acne during his teenage years and no follow-up were available.

CONCLUSIONS

This review describes the largest cohort of patients with *PSTPIP1* variants in that it integrated all known cases from the literature and our own clinical experience. It confirms that only 2 phenotypes (i.e., PAPA and PAMI syndromes) are clearly associated with *PSTPIP1*. As the clinical expressivity of these patients is very broad, ranging from no skin manifestations to debilitating skin features, it can be hypothesized that within the same family with the same pathogenic variant, incomplete penetrance may be more common than appreciated and may be under-reported in the literature. A recent taxonomy advised the use of the name of the mutated protein to describe AIDs (Ben-Chetrit et al. 2018). Like Holzinger *et al.*, the authors suggested a "roof" name, *PSTPIP1*-associated AIDs (PAIDs), with two subtypes: PAPA and PAMI (Ben-Chetrit et al. 2018; Holzinger et al. 2015). Such a general term can advantageously include any intermediate phenotype and reflect a continuum of *PSTPIP1*-associated disorders. Whether PASH syndrome could also belong to this PAIDs group must be confirmed because it has been strongly associated with *PSTPIP1* [variant p.(Tyr345Cys)] in only one family. Other PAIDs phenotypes may emerge in the future. For example, Janssen *et al.* reported 2 novel *PSTPIP1* variants in patients with common variable immunodeficiency but no classical PAPA syndrome characteristics underlining the close links between AIDs and immunodeficiency (Janssen et al. 2018).

We recorded 39 rare punctual variants in the coding sequence of *PSTPIP1* among 161 patients in 114 families. Of these sequence variants, only 3 are considered pathogenic [p.(Ala230Thr), p.(Glu250Gln) and p.(Glu250Lys)] and 4 are likely pathogenic [p.(Asp246Asn), p.(Glu257Gly), p.(Glu257Lys) and p.(Tyr345Cys)]. Among supportive data for pathogenicity are co-segregation of the variants with the disease in multiplex families and functional studies demonstrating a damaging effect on the gene regulation or gene product. The underlying molecular mechanism of

the variable expressivity of *PSTPIP1* variants is unclear. It is however shown that at a same position, 250 or 257, a glutamine increases PSTPIP1 tyrosine phosphorylation, whereas a lysine seems in addition to alter the electrostatic binding site of PSTPIP1, resulting in two phenotypes (i.e PAPA or PAMI syndromes) (Holzinger et al. 2015). However, to our knowledge, genotype-phenotype association data have not yet demonstrated their usefulness for treatment selection and understanding of the variable response to anti-TNF/IL1 biotherapies in PAIDs patients.

The limitation of our review is that we lacked some clinical and biological data and family history for many case reports. Furthermore, the apparent prevalence of sequence variants or phenotype identification may not reflect the true prevalence because of a reporting bias.

Of note, the phenotypes of patients with VUS were highly heterogeneous and included disorders (e.g., Behçet's disease or undefined AID) that have never been associated with *PSTPIP1* variants scored as pathogenic, which suggests that in these patients, the *PSTPIP1* variants were not causal, but does not dismiss their possible involvement as modifiers.

Further functional and familial studies are needed to understand how variants affect the PSTPIP1–pyrin axis and possibly identify new syndromes within the continuum of PAIDs in the future, with variable clinical expression depending on the variants.

CONFLICTS OF INTEREST

The authors state no conflict of interest.

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AUTHORS CONTRIBUTION

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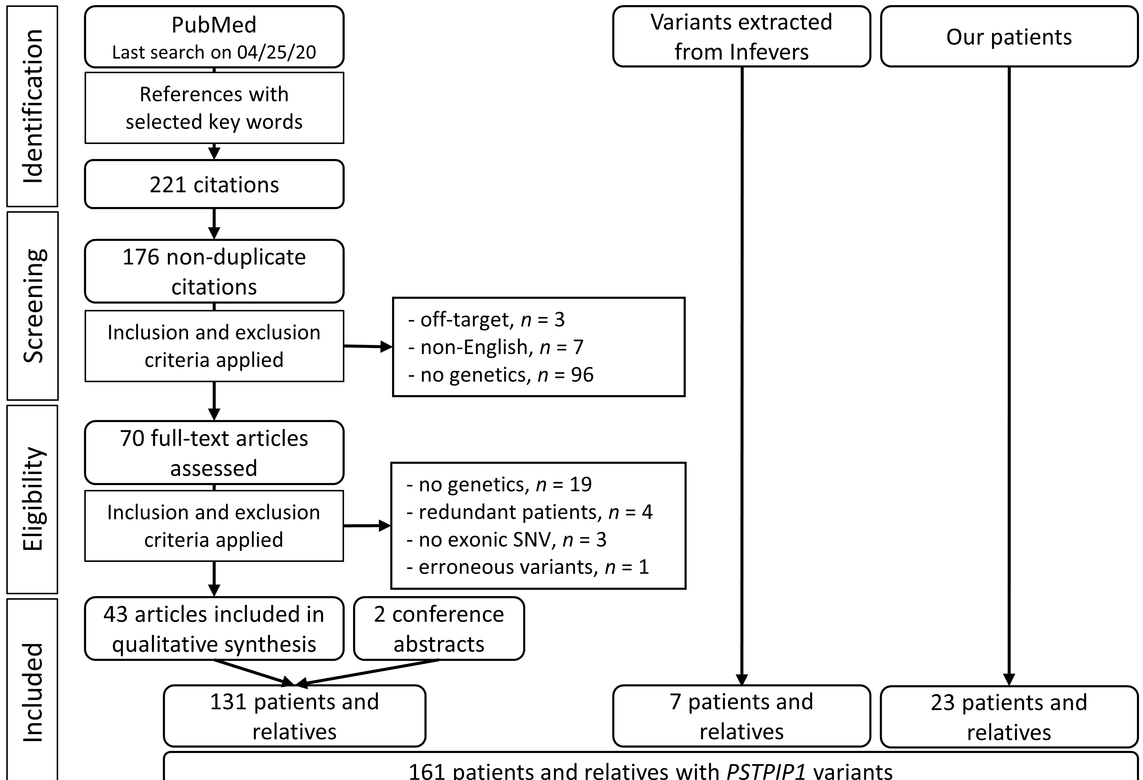
FIGURE LEGENDS

Figure 1. **Flow diagram for the identification of patients with *PSTPIP1* variants.** We used the PRISMA recommendations. Sources were references from PubMed and Infevers databases, and patients tested in our genetic lab. Number of single nucleotide variants (SNVs): 39 (datasets are hosted at http://dx.doi.org/10.17632/cf9nzbvnfz.3 on Mendelay data). Infevers is the reference database for variants in autoinflammatory disease-associated genes available at https://infevers.umai-montpellier.fr/web/.

Figure 2. Phenotype-genotype associations. Clinical picture of the 2 main phenotypes among genetically confirmed patients (i.e. with pathogenic or likely pathogenic variants): (a) PAPA syndrome and (b) PAMI syndrome. Twelve of the patients diagnosed as PAPA were not considered for this figure because 5 patients had a variant classified as VUS or probably benign, and 7 patients had a pathogenic variant generally associated with PAMI syndrome. Inflammation was considered when presence of an inflammatory syndrome was mentioned in the clinical description of the patient or when a C-reactive protein ≥ 5mg/l was reported. Cytopenia included anemia and/or neutropenia. Data on growth retardation, cytopenia, hepatosplenomegaly (includes splenomegaly only), hyperzincemia and hypercalprotectinemia were collected only when PAMI syndrome was reported. **Distribution of *PSTPIP1*-associated phenotypes.** The proportion of patients in each phenotypic group greatly varied according to the variant pathogenicity class: pathogenic or likely pathogenic (c), variant of uncertain significance (VUS) (d) or benign or likely benign variants (e).

Abbreviations: AID, autoinflammatory disease; CD, Crohn disease; CRMO, Chronic recurrent multifocal osteomyelitis; CVID, common variable immunodeficiency; FMF, familial Mediterranean fever; PAC, pyoderma gangrenosum with acne and ulcerative colitis; PAMI,

PSTPIP1-associated myeloid-related proteinemia inflammatory syndrome; PAPA, pyogenic sterile arthritis, pyoderma gangrenosum, and acne; PG, pyoderma gangrenosum; PASH, pyoderma gangrenosum, acne and suppurative hidradenitis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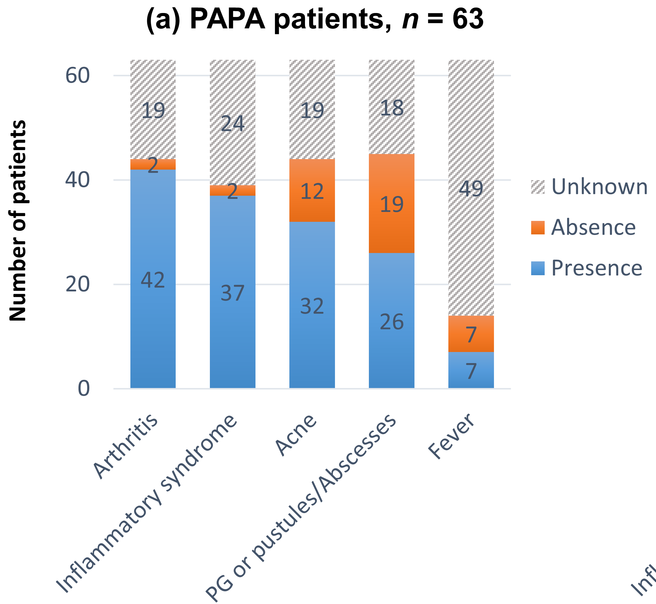
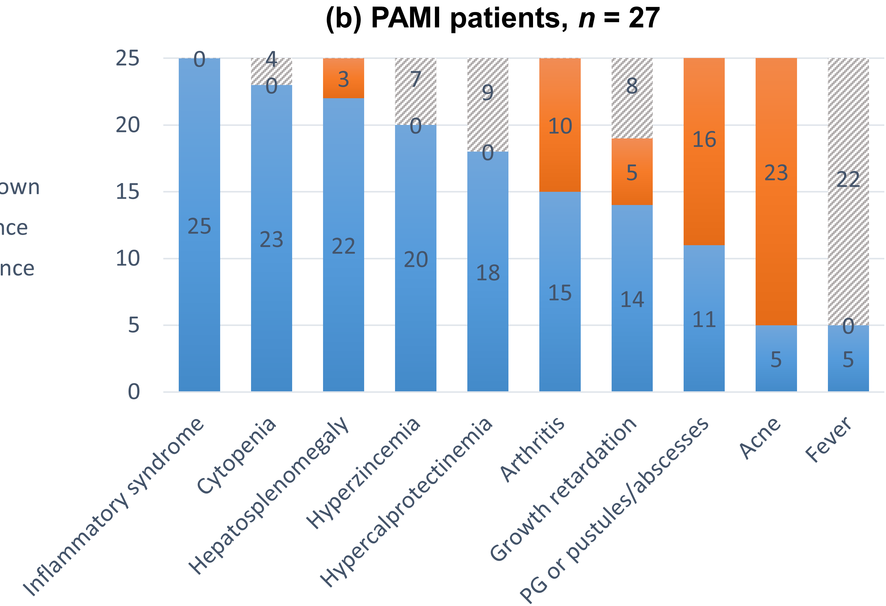
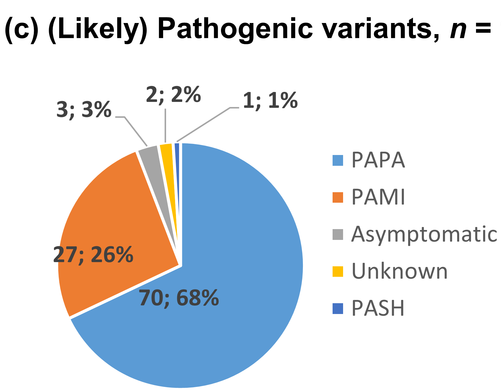
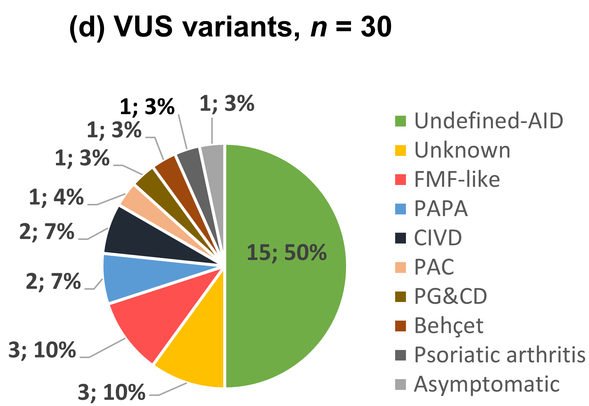
Screening
Eligibility
Included
PubMed
Last search on 04/25/20
References with selected key words
221 citations
176 non-duplicate citations
Inclusion and exclusion criteria applied
- off-target, n = 3
- non-English, n = 7
- no genetics, n = 96
70 full-text articles assessed
Inclusion and exclusion criteria applied
- no genetics, n = 19
- redundant patients, n = 4
- no exonic SNV, n = 3
- erroneous variants, n = 1
43 articles included in qualitative synthesis
2 conference abstracts
131 patients and relatives
Variants extracted from Infevers
Our patients
7 patients and relatives
23 patients and relatives
161 patients and relatives with PSTPIP1 variants

Figure 1


(a) PAPA patients, n = 63
Number of patients
60
40
20
0
19
2
42
24
2
37
19
12
32
18
19
26
49
7
7
Arthritis
Inflammatory syndrome
Acne
PG or pustules/Abscesses
Fever
Unknown
Absence
Presence


(b) PAMI patients, n = 27
25
20
15
10
5
0
0
25
4
0
23
3
22
7
0
20
9
0
18
10
15
8
5
14
16
11
23
5
22
0
5
Inflammatory syndrome
Cytopenia
Hepatosplenomegaly
Hyperzincemia
Hypercalprotectinemia
Arthritis
Growth retardation
PG or pustules/abscesses
Acne
Fever


(c) (Likely) Pathogenic variants, n = 103
3; 3%
2; 2%
1; 1%
27; 26%
70; 68%
PAPA
PAMI
Asymptomatic
Unknown
PASH


(d) VUS variants, n = 30
1; 3%
1; 3%
1; 3%
1; 3%
1; 4%
2; 7%
2; 7%
3; 10%
3; 10%
15; 50%
Undefined-AID
Unknown
FMF-like
PAPA
CIVD
PAC
PG&CD
Behçet
Psoriatic arthritis
Asymptomatic

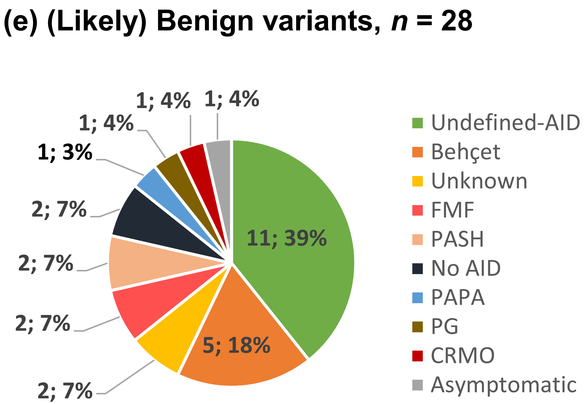

(e) (Likely) Benign variants, n = 28
1; 4%
1; 4%
1; 4%
1; 3%
2; 7%
2; 7%
2; 7%
2; 7%
11; 39%
5; 18%
Undefined-AID
Behçet
Unknown
FMF
PASH
No AID
PAPA
PG
CRMO
Asymptomatic

Figure 2