Editorial

How to classify PFAPA? No hard evidence for associated CAPS or CARD variants and not any for links with Behçet's syndrome

E. Ben-Chetrit[1], H. Yazici[2]

[1]*Rheumatology Unit, Hadassah Medical Centre and Faculty of Medicine, Hebrew University of Jerusalem, Israel;*
[2]*Academic Hospital (Rheumatology), Istanbul, Turkey.*

Eldad Ben-Chetrit, MD
Hasan Yazici, MD

Please address all correspondence to:
Eldad Ben-Chetrit,
Rheumatology Unit,
Hadassah-Hebrew University
Medical Centre, POB 12000,
91120 Jerusalem, Israel.
E-mail: eldad@hadassah.org.il

Hasan Yazici,
Academic Hospital Uskudar,
Istanbul 34668, Turkey.
E-mail: hasan@yazici.net

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Periodic fever, aphthous oral ulcers, pharyngitis and cervical lymphadenopathy (PFAPA) syndrome was first described in 1987 by Marshall *et al*. in a dozen American children (1). It is characterised by recurrent fever of early onset (under 5 years of age) and one or more of the following associated symptoms: oral aphthous ulcers, cervical lymphadenopathy or pharyngitis, in the absence of evidence of recurrent upper respiratory tract infections or cyclic neutropenia. Headache, gastrointestinal symptoms, rash and arthralgia, may also be rarely present (2, 3). The attacks of PFAPA last between 3–7 days with a frequency of about every 21 to 28 days. The extreme regularity of attacks is typical. Characteristically, children are completely well between attacks (4). PFAPA is by no means a disease limited to those of northern European descent and has been described among almost every population in the world (3, 5). Laboratory findings during flares show non-specific leukocytosis with neutrophilia, elevated erythrocyte sedimentation rate, C-reactive protein (CRP) and fibrinogen (6). Serum IgD can also be elevated in some patients (4).

PFAPA is diagnosed by excluding other probable causes of recurrent fever in children, such as infectious, autoimmune, and malignant diseases. The differential diagnosis also includes cyclic neutropenia and the hereditary periodic fever syndromes (HPFs) (5). The latter disorders are caused by genetic variants of the innate immune system and include familial Mediterranean fever (FMF), TNF receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndromes (CAPS) and mevalonate kinase deficiency (MKD), formerly called hyperimmunoglobulinaemia D syndrome (HIDS) (5, 7).

Steroid treatment is highly effective in controlling symptoms. One dose of glucocorticoids (2 mg/kg/d prednisone or 0.3 mg/kg of betamethasone) can lead to dramatic resolution of fever within 2 to 6 hours (2, 3). Cimetidine treatment is only effective in up to 30% of patients (8). Colchicine and Montelukast (Singulair) have also been tried with partial success (9). In patients with severe, recurrent attacks every 7–10 days for >3–4 months, tonsillectomy is an option following which 20–80% (depending on the study) have recovered completely (10, 11).

In general, the prognosis of PFAPA is good and most children will outgrow their symptoms within a decade. Although PFAPA is the most common periodic fever disease in children, some studies have also documented it in adults (12, 13).

The aetiology of PFAPA is unknown and its pathogenesis is still obscure. In a study by Kolly *et al*. it was shown that IL1b monocyte production was dysregulated in patients with PFAPA (14). It was suggested that one of the inflammasomes (NLRP3) may be involved in its pathogenesis.

Stojanov *et al*. showed that during PFAPA attacks, complement (C1QB, C2, SERPING1), IL-1–related (IL1B, IL-1RN, CASP1, IL18RAP), and IFN-induced (AIM2, IP-10/CXCL10) genes were significantly overexpressed, but T cell-associated transcripts (CD3, CD8B) were down-regulated (15). On the protein level, PFAPA flares were accompanied by significantly increased serum levels of chemokines for activated T lymphocytes (IP-10/CXCL10, MIG/CXCL9), G-CSF, and proinflammatory cytokines (IL-18, IL-6). PFAPA flares also manifested a relative lymphopenia. Based upon these observa-

tions, the authors suggested that an environmentally triggered activation of complement and IL-1β/-18 lead to PFAPA flares, with induction of Th1-chemokines and subsequent retention of activated T cells in peripheral tissues. A gene associated with PFAPA has not been found and, since the disease does not seem to be hereditary, its real place among the autoinflammatory diseases (AID) is still unclear. In a recent study Manthiram *et al*. suggested including PFAPA in the spectrum of Behçet's syndrome (BS) based upon some common clinical manifestations and few genetic similarities (16). The aim of this editorial is to critically review the different concepts proposed over the years to locate PFAPA among the autoinflammatory conditions including BS.

PFAPA: is it a syndrome in the spectrum of CAPS?

Although PFAPA syndrome is a sporadic condition, some studies have reported familial susceptibility that emphasised a probable genetic cause (17, 18). In a genetic screening of PFAPA patients for carriage of typical mutations of the monogenic periodic fever syndromes, Hofer *et al*. found in 15 of 57 patients heterozygous variants of AID genes; NLRP3/CAPS in 12, MEFV in 4, TNFRSF1A and MVK 1in each. All variants were of uncertain clinical significance (mild, reduced penetrance or without functional effect) (19, 20). There were significantly higher frequencies of three NLRP3 variants (R488K, V198M, Q705K) in the PFAPA cohorts. The pathogenic role of the Q705K variant is a matter of debate because it is present in ~5% of the healthy population. Interestingly, carriers of the Q705K variant with a typical CAPS phenotype have been described, suggesting that additional genetic and/or environmental modifiers may be involved (21). Thus, the finding that in 20% of PFAPA patients there were NLRP3 variants led to the proposal that NLRP3 inflammasome is involved in PFAPA pathogenesis as the case with CAPS.

PFAPA: is it related to the CARD 8 variants?

In a study of 82 unrelated PFAPA patients, 12 were found to carry a heterozygous variant in the CARD8 gene. CARD8-FS variant was associated with more severe PFAPA (higher incidence of symptoms out of flares and aphthosis) (22). CARD8 is known to interact with NLRPs proteins that assemble into inflammasome complexes. CARD8-FS lost its ability to interact with NLRP3, indicating that, beyond the fact that CARD8-FS is probably expressed at very low levels, remaining protein products are unable to regulate inflammasome assembly. Moreover, it has been shown that CARD8-FS has no effect on caspase-1 cleavage compared with full-length CARD8, which could explain the excess inflammasome activation in CARD8-FS patients.

Altogether, these data suggest that PFAPA patients carrying a CARD8-FS allele have an overall decreased CARD8 activity and much more severe inflammatory phenotype.

In contrast to these, two observations regarding the possible genetic basis for PFAPA pathogenesis, a study of patients from 14 different families indicated that PFAPA was unlikely to be a monogenic condition (related to either NLRP3 or CARD8). Therefore, the authors suggested an underlying oligogenic or complex inheritance of variants as a genetic basis for the disease (18).

PFAPA: is it a part of the spectrum of BS?

As said, recently another effort to relocate PFAPA has been made (16). Mainly based on gene and proteomic analyses and some perceived clinical similarities, it has been suggested that PFAPA together with recurrent aphthous stomatitis and the fully developed BS form a construct which the authors decided to name the Behcet Spectrum of Disorders (BSD). It is suggested that these 3 conditions have similar disease mechanisms and depending on differing environmental and host factors (mainly in the form of different HLA associations), one may develop either recurrent oral ulceration (RAS), the least severe disease, PFAPA, a more severe disease, or the bone fide BS, proposed to be the most severe form in the BSD.

The authors studied 231 patients (from two European-American cohorts and one Turkish cohort) with PFAPA for common variants previously reported to be associated with RAS and BS (16). They also specifically studied the HLA region. The most significant association they found was with a variant upstream of IL12A (rs17753641). In functional studies related to this variant they report that monocytes from individuals who were heterozygous or homozygous for this risk allele produced significantly higher levels of IL-12p70 protein upon IFN-γ and LPS stimulation. Moreover, significant associations with variants near STAT4, IL10, and CCR1-CCR3 were found. In the HLA association studies they observed that significant genetic associations with the HLA alleles are modest among the PFAPA patients studied. This contrasts with the much stronger associations in BS and even weaker reported associations among RAS patients. The main conclusions were that the disease mechanisms of PFAPA involved abnormal antigen-presenting cell function and T cell activity and polarisation. These, in turn, and like in BS, implied the role of both innate and adaptive immune responses producing the aphthous ulcerations in the oral mucosa. The authors also suggested that differing kinds and degrees of the HLA associations probably determined the disease severity in the disease spectrum they propose

Issues for discussion

Related to CAPS and CARD 8 variants:

PFAPA is a non-hereditary periodic fever syndrome. The mere presence of NLRP3 variants of unknown significance in about 20% of PFAPA patients does not justify its inclusion among the CAPS family. Similarly, the proposal that PFAPA is associated with CARD 8 variants was not supported by additional studies (16).

Related to BS

1. Apparently, one important reason why Manthiram *et al*. (16) carried out this study was the prior evidence that BS and PFAPA were related. In their discussion they say: "Many adult patients with Behçet's disease have re-

ported symptoms earlier in childhood that fulfil the diagnostic criteria for PFAPA" (23, 24). When we checked the actual reference 24 (ref. 20 in the original text), we found out that these adult patients had recalled having PFAPA at a mean age of around 3 years. Furthermore, in this study 80 adult Behçet's patients were interviewed separately by a rheumatologist, a paediatrician and an internist. The rheumatologist found that 30% of the 80 patients had had PFAPA as a child, the paediatrician 10% and the internist 7.5%. These findings hardly represent hard data. The other study (23) (ref 3 in the original text), on the other hand, is about a long-term follow-up of 60 paediatric patients with PFAPA for up to 21 years and only 1/60 developed BS.

2. The authors underline that the oral mucosa is the main target organ in their study. On the other hand, around 40% of the patients with criteria fulfilling PFAPA did not have oral ulcers (25). It follows that, like in any meaningful genetic association study, we need to know more about the phenotypes of these reported patients.
3. As a corollary to the previous point, all these patients, by definition had recurrent bouts of fever. It would have been very informative if the authors compared the genetic association they observed with the other monogenic recurrent fever syndromes in the spectrum of autoinflammatory diseases (5). Here we should remind ourselves that neither RAS nor BS are classified among the recurrent fever syndromes and there is no reason they should be.
4. The authors justifiably do functional studies to better define the significance of the rs17753641 variation they observe. They analyse the statistical significance of their findings by null hypothesis testing (NHT). When they search for increased IL12 production by measuring the number of CD4 + cells carrying subunits of IL12, their significant results during the PFAPA flares are based on 7 patients with a flare, 12 without a flare and 8 healthy controls. They do get a statistically significant *p*, which, however, is very fickle due to the small numbers at hand (26). We do not find a limitation alert for this finding or for that matter any other attempt at acknowledging limitations in the manuscript.

There are two important generalisations we can make about genotype-phenotype studies related to the issue under discussion:

a. Similar genotype-phenotype associations, by themselves, are by no means sufficient to cluster diseases. In fact, they might have exactly the opposite consequences. A notable example is that TLR4 and NOD 2 variants were found to be protective in BD and risky for Crohn's disease (27).
b. Totally different genetic background can produce very similar phenotypes. A good example is how A20 haplotype insufficiency is associated with a clinical picture closely resembling BS (28). From the clinical point of view, IBD and HA20 haploinsufficiency resemble BD much more than PFAPA, and MKD resembles PFAPA much more than BS and yet no one suggested lumping them together.

Finally, the same group of investigators which proposed including PFAPA in BSD, found a nonsense mutation in FUT2 which exists in BD and in Crohn's disease (29). The FUT2 (rs601338) variant among Caucasians impairs the secretion of ABO antigens at mucosal surfaces. This and other non-secretor alleles modulate risk not only for BD but also for Crohn's disease and some intestinal infections. So here again an example of a common functional variant (FUT2) in two diseases, Crohn's and BD, and yet these authors did not propose including them in the same spectrum (BSD).

We still do not know the cause(s) and the disease mechanism(s) of BS. Some consider it might even not be a single condition and thus prefer to call this condition a syndrome rather than a disease (30, 31). Still others propose re-lumping BS with the seronegative spondyloarthropathy group of diseases mainly based on some perceived clinical similarities and the involvement of the same canonical immune inflammatory pathways and the close association of MHC-I group of antigens irrespective of the specific allele associated (32). This is the so-called MHC-I-opathy concept. There are reasons why this concept is not sound either (33).

In brief, we need perhaps more wisdom than knowledge and more splitting than lumping to solve the riddles of both PFAPA and BS (34) .

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