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Clinical features and functional significance of the P369S/R408Q variant in pyrin, the familial Mediterranean fever protein

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Abstract

Objectives—Familial Mediterranean fever (FMF) is caused by mutations in *MEFV*, which encodes pyrin. The nature of substitutions P369S and R408Q in exon 3 remains unclear. Exon 3 encoding pyrin's B-box domain is necessary for interactions with PSTPIP1. We aimed to characterize the phenotype of patients with these substitutions and to determine their functional significance.

Methods—A database of genetic tests undertaken in our institution was interrogated. Symptoms and signs were classified according to Tel-Hashomer criteria. Co-immunoprecipitation techniques were employed to determine the variants' effects on pyrin/PSTPIP1 interactions.

Results—We identified 40 symptomatic and 4 asymptomatic family members with these substitutions. P369S and R408Q were found in *cis*, and co-segregated in all patients sequenced. Clinical details were available on 22 patients. Five patients had symptoms and signs fulfilling a clinical diagnosis of FMF. Fourteen received colchicine. In patients not reaching the criteria, trials of anti-TNF agents resulted in partial or no benefit; resolution of symptoms was noted in those receiving anakinra. The carrier frequency was higher in the patient cohort than in controls but was not statistically significant. Co-immunoprecipitation studies demonstrated that these pyrin variants did not affect binding to PSTPIP1.

Conclusions—P369S/R408Q substitutions are associated with a highly variable phenotype, and are infrequently associated with typical FMF symptoms, however a trial of colchicine is warranted in all. Functional and modeling studies suggest that these substitutions do not significantly affect pyrin's interaction with PSTPIP1. This study highlights the need for caution in interpreting genetic tests in patients with atypical symptoms.

Keywords

Familial Mediterranean Fever; Genetic Variants; Treatment; Inflammation; Biologic Therapy

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Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by acute attacks of fever, and serositis usually lasting 1–3 days. FMF is caused by mutations in *MEFV*, which encodes pyrin (marennosin).[1, 2]

To date over 40 *MEFV* variants have been reported in FMF patients.[3] However, the nature of substitutions P369S and R408Q, located in exon 3, remains unclear. Physicians encountering patients harboring these variants face significant challenges in determining if such substitutions are consistent with a diagnosis of FMF. There are limited data regarding the clinical features, response to colchicine, or incidence of amyloidosis in patients with these variants.

Pyrin contains a pyrin domain (PYD), B-box zinc-finger, coiled-coil and B30.2 domain. The PYD of pyrin engages in homotypic interactions with an adaptor protein called ASC (apoptosis associated speck-like protein with a CARD) influencing the activation of interleukin (IL)-1 β . Exon 3 of *MEFV* encodes the B-box domain (amino acid 370–412), which has been shown to interact with PYD and thereby blocks its interaction with ASC.[4] The B-box domain is both necessary and sufficient for pyrin's interactions with proline serine threonine phosphatase interacting protein (PSTPIP1), the gene mutated in patients with the syndrome of pyogenic arthritis, pyoderma gangrenosum and acne (PAPA).[5] No other FMF-associated mutations in the B-box domain have been reported.

Given the uncertainty surrounding the clinical significance of P369S and R408Q variants, we aimed to characterize the phenotypes of patients with these substitutions and to determine their functional significance.

PATIENTS AND METHODS

Patients

A database of all FMF genetic tests undertaken at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) was interrogated for individuals harboring the substitutions P369S and/or R408Q. Following retrospective chart review, clinical symptoms and signs were classified according to the Tel-Hashomer criteria as described in Table 1, a validated set of criteria with high specificity (99%) and sensitivity (98%) for the clinical diagnosis of FMF in a selected population.[6] We randomly selected mutation negative symptomatic patients for detailed chart review.

This research was conducted following approval by the Institutional Review Board and all patients or guardians provided written informed consent.

Mutation detection

We screened 1075 samples for mutations in exons 2, 3 and 10 of *MEFV*. Five patients were screened for the presence of the P369S mutation using RFLP analysis, and their DNA samples were not available for further sequencing. All remaining samples were subjected to standard fluorescent sequencing on the coding regions and splice junctions of *MEFV*, as previously described.[7] Testing for mutations in exons 9 and 11 of *MVK*, the gene mutated in hyperimmunoglobulin-D with periodic syndrome (HIDS), and exons 2, 3, 4, and 5 of *TNFRSF1A*, gene mutated in TNF-receptor associated periodic syndrome (TRAPS), was performed in a subset of patients at the discretion of the treating physician.

Control DNA genotyping

The allele frequencies of P369S and R408Q substitutions were evaluated in Caucasian control DNA samples using mass spectrometry (Sequenom Inc., San Diego, CA).[8]

Transfection/Co-immunoprecipitation

Human embryonic kidney 293T cells were transfected with myc tagged pyrin (or mutant variants) and GST tagged PSTPIP1 (or mutant variants) using FuGENE 6 Transfection Reagent (Roche, Indianapolis, IN). Point mutations were made using the QuikChange Site-Directed Mutagenesis Kit (Stratagene Inc, La Jolla, CA). Immunoprecipitation was performed using Glutathione Sepharose-4B (GE, Piscataway, NJ) followed by western blotting.

In silico modeling of the pyrin B-box domain

A structure for the B-box domain of pyrin was modeled on the nuclear magnetic resonance structures of the TRIM29 B-box. Structural alignment was generated using the FUGUE algorithm with energy minimization performed using GROMOS in the program Swiss-Model.[9,10]

Results

Patient characteristics and response to therapy

We identified 40 patients carrying P369S or P369S+R408Q mutations (carrier frequency 0.037). P369S and R408Q appear to be in linkage disequilibrium as they co-segregated in 35 patients sequenced for exon 3 mutations. In cases where parental DNA was available, both alleles were transmitted from one parent. We identified four P369S(+)R408Q carriers as asymptomatic family members. For this study we excluded 2 patients bearing single copies of FMF mutations such as M694V in conjunction with P369S(+)R408Q. Two patients bore the R92Q variant in *TNFRSF1A*, none bore mutations in *MVK*. Sixteen patient samples were referred to our laboratory for genetic testing by outside physicians. Twenty-two patients were seen at NIAMS, thus only these patient records were available for this retrospective study.

None of the patients had typical episodes of peritonitis, pleuritis, pericarditis or monoarthritis as specified in the Tel-Hashomer criteria. We identified 5 patients who met criteria for the clinical diagnosis of FMF (Table 2). All had incomplete abdominal attacks or febrile episodes lasting from 1–3 days as the major criteria of the disease. We adopted a conservative approach in defining the abdominal attacks as incomplete, as none of the patients were seen during an attack. Three of the patients were noted to have cervical lymphadenopathy; all had normal serum immunoglobulin-D (IgD) levels and none had mutations in *MVK*. Two were of Ashkenazi Jewish descent and the remainder of Italian/mixed European ancestry. Of these 5 patients, four had received colchicine therapy resulting in partial benefit in three. Despite optimal dose adjustment and self-reported compliance, one patient denied benefit. The remaining patient had infrequent episodes, which responded to non-steroidal anti-inflammatory drugs (NSAIDs) and did not require colchicine prophylaxis. None of these patients required biologic therapies.

Seventeen patients with these variants did not reach a clinical diagnosis of FMF using the Tel-Hashomer criteria (Table 3). Median duration of symptoms was 5.25 days (range 2 hours to 3 months). Information regarding ethnicity was available in 15, of these six were of Ashkenazi Jewish descent, six of mixed Southern European descent, one of mixed Japanese/Scottish descent, one Iranian, and one of mixed European/Filipino descent. None of the pediatric patients reached recently proposed diagnostic criteria for FMF.[11] A clustering of symptoms was observed, enabling us to group patients into 4 distinct symptom sets; six patients had non-specific symptoms not amenable to further analysis.

Group 1: Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome-like symptoms

Three patients had fever in association with aphthous ulcers, pharyngitis and cervical lymphadenopathy. Episodes lasted a mean of 4 days and occurred every 2–6 weeks, all patients had normal serum IgD levels. Cimetidine was not beneficial in 3 patients, but was of partial benefit in one. A single patient had resolution of symptoms following tonsillectomy. In one patient methotrexate, hydroxychloroquine, montelukast and etanercept had been employed without benefit, corticosteroid tapers resulted in partial benefit.

Group 2: Predominant serositis symptoms—One patient had recurrent episodes of pericarditis resulting in tamponade on one occasion. Treatment with colchicine, corticosteroids and infliximab were of partial benefit. Resolution of symptoms was noted with anakinra.

Group 3: Predominant joint symptoms—One patient had episodes of knee synovitis, another had recurrent febrile episodes with arthralgia.

Group 4: Uveal tract inflammation—One patient had a history of panuveitis in addition to short-lived fevers, abdominal pain and small joint arthritis. Treatments included colchicine, methotrexate, azathioprine and cyclosporine without benefit; adalimumab resulted in a flare of her eye symptoms. Another patient with non-granulomatous panuveitis associated with inflammatory bowel disease and fever had partial benefit from colchicine and etanercept. Significant benefit was noted with adalimumab.

Of the remaining 6 patients, three had prolonged episodes of fever with either vague abdominal discomfort or arthralgia. One had episodes of fever with profound fatigue onset in his 6th decade. Trials of high dose corticosteroids, methotrexate, and colchicine resulted in transient benefit. He had resolution of symptoms with anakinra.

Amyloidosis

One patient with a history of recurrent conjunctivitis developed localized amyloidosis affecting both eyelids but had no other clinical features of inflammation. None of the other patients had a diagnosis of amyloidosis or proteinuria detected on screening.

Mutation negative patients

We identified one out of 33 patients who met the Tel-Hashomer criteria, with an excess of patients with a range of atypical symptoms. Additional details are available in online supplementary material.

Use of biologic therapies

Biologic therapies were used in 5 patients, all of whom had atypical symptoms. Of the four patients treated with anti-TNF therapies, 3 had partial benefit. Resolution of symptoms was noted in two patients treated with anakinra.

Genetics

Complete *MEFV* sequencing failed to identify the second mutation in 22 patients. Among 40 carriers for P369S we identified 2 E148Q homozygotes and 11 E148Q carriers. In terms of age of onset, and symptoms experienced, we found no significant difference between E148Q carriers and those who bore P369S variants alone. A second possible disease-associated variant, I591T, was identified in one family of Scottish/Japanese ancestry. Additional details are available in online supplementary material.

In a panel of 310 healthy controls, 6 were carriers for both P369S and R408Q (carrier frequency of 1.9%), while one carried P369S alone. The P369S carrier frequency was higher in the cohort of symptomatic patients than in controls (3.7% vs. 1.9%) however this was not statistically significant.

Functional analysis of P369S, R408Q

We performed *in silico* modeling to estimate the impact of P369S and R408Q substitutions on the conformation of the B-box domain (Fig. 1). This model suggests that these variants do not result in significant alterations to the secondary structure elements potentially required to maintain the structural integrity of the B-box domain.

Co-immunoprecipitation studies confirm that pyrin is able to bind to PSTPIP1 and that pyrin interacts more avidly with a PSTPIP1 disease causing variant (A230T)[5] (Fig 2). A second disease causing variant (E250Q) was also increasingly bound by pyrin, but less so than A230T as quantitated by densitometry (Fig 2 D). We compared the effect of single pyrin variants P369S or R408Q and a construct bearing both variants on PSTPIP1 binding. Only minor variation compared to WT was observed, and these differences were not significant when compared to the effect of PSTPIP1 mutations.

Discussion

The ability to confirm a clinical suspicion of FMF using molecular diagnostics has provided physicians with greater certainty in both counseling and appropriately treating FMF patients. However, greater availability of genetic testing has resulted in sequencing of patients with atypical symptoms who have a lower pretest probability of disease. Significant challenges arise when variants of uncertain significance such as P369S are identified in these patients. To date, limited data were available to guide clinicians regarding the frequency of these variants in the wider population and their significance. P369S and R408Q have been reported in few FMF or FMF-like cases in the literature.[7,12–14] This study identified 44 individuals bearing these variant, of these 40 were symptomatic. Using the Tel-Hashomer criteria for the clinical diagnosis of FMF, just 5 individuals had symptoms consistent with a clinical diagnosis of FMF.

We noted a wide range of clinical symptoms among those who did not reach the Tel-Hashomer criteria. Colchicine therapy, a mainstay in the treatment of FMF, was beneficial in 3 patients with typical FMF symptoms. The Tel-Hashomer criteria include a favorable response to colchicine as one of the minor criteria to determine the diagnosis of FMF potentially biasing interpretation of response to colchicine in our examination of treatment response. However, after excluding this minor criterion, we determined that all 5 reached a diagnosis of FMF based on other clinical features. Therefore, it remains notable that 3 of the patients with typical FMF had a favorable response to colchicine. Colchicine was used in 10 patients with atypical disease. Five had no benefit, and of the 5 patients who had partial benefit, 3 required biologic therapies in addition. The need for biologic therapies to control disease symptoms among those with atypical disease is notable. None of the patients with typical FMF required biologic therapies, while 5 of 17 with atypical disease were commenced on either anti-TNF agents or IL-1 receptor antagonists. While both patients treated with anakinra demonstrated dramatic benefit, the use of anti-TNF agents resulted in, at best, a partial benefit. Case reports describe the use of anti-TNF agents in FMF with variable response.[15,16]

Of particular concern in FMF patients is the potential to develop AA amyloidosis. In our cohort, we identified one patient (172) as having a localized amyloidosis affecting her eyelid following episodes of conjunctivitis. She had no clinical features of FMF and no evidence of

systemic amyloidosis. Amyloid deposition in the eyelid is considered pathognomic of primary systemic amyloidosis, while conjunctival amyloidosis is usually a benign localized disease. However, on review of the literature, we found 3 case reports of patients presenting with nodular amyloid eyelid tumours without any evidence for systemic amyloidosis.[17–19] None of the other patients in this study had evidence of amyloidosis. However, given the cross sectional nature of this study, and low numbers of patients, it is not possible to definitively state that these variants are not associated with a risk for amyloidosis.

Our understanding of the genetics underlying FMF continues to evolve. While FMF is classically considered a recessive disorder, evidence is emerging that individuals bearing single mutations can present with typical symptoms and signs of disease.[20] In light of these reports we excluded those patients who carried one of the common *FMF* mutations and P369S. P369S and R408Q were recently found to be in strong linkage disequilibrium in the Japanese population ($D' = 1$).[14] Furthermore the frequency of R408Q mirrors that of P369S in Caucasian, Sub-Saharan African and Japanese populations.[21] In keeping with these observations we found that in those patients in whom we had parental DNA, P369S and R408Q co-segregated in *cis*.

The role that these variants may play in mediating human disease remains unclear. Using the conventional definition of what constitutes a polymorphism, i.e. an allele frequency exceeding 1% in healthy controls, P369S and R408Q are polymorphisms. We observed an allele frequency of 1.33% in our controls; other studies confirm a 1.3% frequency in Caucasians.[14] The allele frequency exceeds 5% in Asian/Japanese control populations.[21]

As part of our screen for coding region variants in *MEFV* we identified 10 individuals bearing E148Q. Controversy surrounds the clinical significance of the E148Q variant. Its allelic frequency varies widely, from 0.5%-5% in Caucasians to 23% in Japanese.[7,14,22] Observations that E148Q homozygotes are asymptomatic,[7,23] has lead some to conclude that E148Q is not disease associated.[24,25] In contrast, studies of Greek and Turkish patients support the hypothesis that E148Q is associated with an inflammatory phenotype.[26,27] In our study none of those bearing both P369S and E148Q had typical FMF symptoms. The R92Q variant in *TNFRSF1A*, is another controversial entity, with an allele frequency of 3.3% in Caucasians.[21] It has been reported in patients with a broad range of symptoms. None of the patients with atypical symptoms bore this variant, while two patients with typical FMF symptoms were carriers.

Dismissing any of these variants on the basis of population allele frequency risks ignoring the role they may play in modulating the inflammatory response to environmental factors.[28] Individuals bearing single copies of *MEFV* variants may mount a more robust inflammatory response to seemingly innocuous environmental pathogens resulting in a range of inflammatory symptoms atypical for FMF. In addition individuals bearing these variants may develop clinical FMF only in the presence of one or more modifying alleles in related genes. It is possible that, as yet unidentified modifier genes could contribute to a pro-inflammatory milieu potentiating the effect of the P369S variant in susceptible individuals.

The B-box domain via its interactions with the PYD domain of pyrin appears to act as an intra-molecular inhibitor preventing interactions with the adaptor protein ASC and influencing the activation of IL-1 β . [4] Thus it could be postulated that mutations in this region alter the auto-inhibitory role of this domain. Our structural modeling highlights that these mutations are unlikely to affect the global structure of the protein or affect its ability to modulate IL-1 β production. Mutations in PSTPIP1 that cause PAPA syndrome are known to influence binding to pyrin, and thus mutations in the B-box domain of pyrin may also affect

this interaction. Our co-precipitation studies were unable to demonstrate that P369S/R408Q variants affected pyrin's binding to PSTPIP1. However we cannot discount the possibility that alterations in binding that are too subtle to detect using *in vitro* techniques may have more pronounced effects *in vivo*. Indeed identifying the functional consequences of susceptibility alleles in many diseases remains a significant challenge.[29]

We conducted this study in light of the recently advocated model that 'variants of uncertain significance' such as P369S may be considered to be "rare variants" capable of synergistic effects in pathogenesis of systemic inflammation.[30] As each individual is predicted to bear 10 rare variants throughout our genome, it is likely that these patients may have additional rare pro-inflammatory variants in other known or unknown genes. In ideal circumstances, the clinical significance of such variants would be clarified by undertaking a large prospective cohort study. However, for now we must rely upon observational studies to provide clues as to their significance. Although hereditary recurrent fevers are considered monogenic diseases, recent reports have described patients who are double heterozygotes for 'rare variants' in two recurrent fever genes.[31–33] In some cases, patients had symptoms suggestive of two autoinflammatory diseases, or more severe disease.

Our genetic and functional data suggest that the P369S/R408Q variants are unlikely to represent a classical FMF disease-associated mutation. The majority of patients had an atypical clinical presentation and a relative lack of response to colchicine therapy similar to patients who are mutation negative for FMF and other known periodic fever diseases. The inability to identify a unifying diagnosis for a wide range of clinical features is difficult for both physicians and patients. In this setting, it may be prudent to consider symptomatic individuals as having a 'mixed autoinflammatory syndrome' and to focus on treating the most troublesome clinical features. Our data should not dissuade the physician from an empiric trial of colchicine. In this regard a therapeutic response would appear to trump molecular diagnostics. In those particularly disabled by attacks, IL-1 blockade with anakinra appeared to be effective.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. The International FMF Consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell*. 1997; 90(4):797–807. [PubMed: 9288758]
2. The French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nat Genet*. 1997; 17(1):25–31. [PubMed: 9288094]
3. Infevers: an online database for autoinflammatory mutations. Copyright. Available from: <http://fmf.igh.cnrs.fr/infevers/>
4. Yu J, Fernandes-Alnemri T, Datta P, et al. Pyrin activates the ASC pyroptosome in response to engagement by autoinflammatory PSTPIP1 mutants. *Mol Cell*. 2007; 28(2):214–27. [PubMed: 17964261]

5. Shoham NG, Centola M, Mansfield E, et al. Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(23):13501–6. [PubMed: 14595024]
6. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum*. 1997; 40(10):1879–85. [PubMed: 9336425]
7. Aksentijevich I, Torosyan Y, Samuels J, et al. Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population. *Am J Hum Genet*. 1999; 64(4):949–62. [PubMed: 10090880]
8. Mitchell MK, Gregersen PK, Johnson S, et al. The New York Cancer Project: rationale, organization, design, and baseline characteristics. *J Urban Health*. 2004; 81(2):301–10. [PubMed: 15136663]
9. Shi J, Blundell TL, Mizuguchi K. FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties. *Journal of molecular biology*. 2001; 310(1):243–57. [PubMed: 11419950]
10. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*. 1997; 18(15):2714–23. [PubMed: 9504803]
11. Yalcinkaya F, Ozen S, Ozcakar ZB, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. *Rheumatology (Oxford)*. 2009; 48(4):395–8. [PubMed: 19193696]
12. Cazeneuve C, Sarkisian T, Pecheux C, et al. MEFV-Gene analysis in armenian patients with Familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-genetic and therapeutic implications. *Am J Hum Genet*. 1999; 65(1):88–97. [PubMed: 10364520]
13. Touitou I. The spectrum of Familial Mediterranean Fever (FMF) mutations. *Eur J Hum Genet*. 2001; 9(7):473–83. [PubMed: 11464238]
14. Sugiura T, Kawaguchi Y, Fujikawa S, et al. Familial Mediterranean fever in three Japanese patients, and a comparison of the frequency of MEFV gene mutations in Japanese and Mediterranean populations. *Modern rheumatology / the Japan Rheumatism Association*. 2008; 18(1):57–9. [PubMed: 18097735]
15. Daysal S, Akcil G, Goker B, et al. Infliximab therapy in a patient with familial Mediterranean fever and chronic hip arthritis. *Arthritis Rheum*. 2005; 53(1):146–7. [PubMed: 15696552]
16. Seyahi, E.; Yazici, H.; Ozdogan, H. Alternative Treatments in Colchicine-Resistant Patients with Familial Mediterranean Fever (FMF). *FMF and Beyond -The Fourth International Congress on Systemic Autoinflammatory Diseases*; Bethesda, MD. 2005.
17. Fett DR, Putterman AM. Primary localized amyloidosis presenting as an eyelid margin tumor. *Arch Ophthalmol*. 1986; 104(4):584–5. [PubMed: 3954666]
18. Pelton RW, Desmond BP, Mamalis N, et al. Nodular cutaneous amyloid tumors of the eyelids in the absence of systemic amyloidosis. *Ophthalmic Surg Lasers*. 2001; 32(5):422–4. [PubMed: 11563787]
19. Mahajan D, Yadava U, Khurana N, et al. Primary localised nodular amyloidosis of the eyelid and conjunctiva: a case report. *Pathology*. 2006; 38(2):189–90. [PubMed: 16581667]
20. Booth DR, Gillmore JD, Lachmann HJ, et al. The genetic basis of autosomal dominant familial Mediterranean fever. *QJM*. 2000; 93(4):217–21. [PubMed: 10787449]
21. Database of Single Nucleotide Polymorphisms (dbSNP). National Center for Biotechnology Information, National Library of Medicine; Bethesda (MD): Accessed at <http://www.ncbi.nlm.nih.gov/SNP/>
22. Booth DR, Lachmann HJ, Gillmore JD, et al. Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148. *QJM*. 2001; 94(10):527–31. [PubMed: 11588211]
23. Ben-Chetrit E, Lerer I, Malamud E, et al. The E148Q mutation in the MEFV gene: is it a disease-causing mutation or a sequence variant? *Hum Mutat*. 2000; 15(4):385–6. [PubMed: 10737995]

24. Tchernitchko DO, Gerard-Blanluet M, Legendre M, et al. Intrafamilial segregation analysis of the p E148Q MEFV allele in familial Mediterranean fever. *Ann Rheum Dis.* 2006; 65(9):1154–7. [PubMed: 16439437]
25. Tchernitchko D, Legendre M, Cazeneuve C, et al. The E148Q MEFV allele is not implicated in the development of familial Mediterranean fever. *Hum Mutat.* 2003; 22(4):339–40. [PubMed: 12955725]
26. Konstantopoulos K, Kanta A, Lilakos K, et al. Familial Mediterranean fever and E148Q pyrin gene mutation in Greece. *International journal of hematology.* 2005; 81(1):26–8. [PubMed: 15717684]
27. Topaloglu R, Ozaltin F, Yilmaz E, et al. E148Q is a disease-causing MEFV mutation: a phenotypic evaluation in patients with familial Mediterranean fever. *Ann Rheum Dis.* 2005; 64(5):750–2. [PubMed: 15458961]
28. Schaner P, Richards N, Wadhwa A, et al. Episodic evolution of pyrin in primates: human mutations recapitulate ancestral amino acid states. *Nat Genet.* 2001; 27(3):318–21. [PubMed: 11242116]
29. Yamamoto K, Yamada R. Lessons from a genomewide association study of rheumatoid arthritis. *N Engl J Med.* 2007; 357(12):1250–1. [PubMed: 17804837]
30. Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet.* 2008; 40(6):695–701. [PubMed: 18509313]
31. Singh-Grewal D, Chaitow J, Aksentijevich I, et al. Coexistent MEFV and CIAS1 mutations manifesting as familial Mediterranean fever plus deafness. *Ann Rheum Dis.* 2007; 66(11):1541. [PubMed: 17934081]
32. Touitou I, Perez C, Dumont B, et al. Refractory auto-inflammatory syndrome associated with digenic transmission of low-penetrance tumour necrosis factor receptor-associated periodic syndrome and cryopyrin-associated periodic syndrome mutations. *Ann Rheum Dis.* 2006; 65(11):1530–1. [PubMed: 17038455]
33. Stojanov S, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Current opinion in rheumatology.* 2005; 17(5):586–99. [PubMed: 16093838]

Figure 1. Modeling of B-Box domain. Substitutions at P369S, and R408Q highlighted, neither are predicted to have a significant effect on protein conformation.

Figure 2.

P369S, R408Q and P369S/R408Q variants do not affect pyrin's binding to wildtype or mutant PSTPIP1. GST tagged PSTPIP1, wild type and variants known to affect binding to pyrin, were co-transfected with myc-tagged pyrin, wild types and variants of interest. Lanes 6 and 7 demonstrate that the A230T and to a lesser extent E250Q GST tagged PSTPIP1 mutants tightly bind to myc-tagged pyrin and act as a positive control. We also note a change in size of the immunoprecipitated pyrin band when co-expressed with the A230T PSTPIP1 mutation, a finding that was repeated in multiple experiments. This band of increased size is not observed in the protein lysate (Fig. 2C). None of the pyrin variants in exon 3 or the highly penetrant mutation in exon 10 (M694V) (lanes 3–5) bound PSTPIP1 with greater avidity than wild type (lane 2).

Table 1

Tel-Hashomer criteria for the clinical diagnosis of familial Mediterranean fever

<u>Major Criteria</u>	
1-4	Typical Attacks
1.	Generalized peritonitis
2.	Unilateral pleuritis or pericarditis
3.	Monoarthritis of hip, knee or ankle
4.	Fever alone
5	Incomplete abdominal attack
<u>Minor Criteria</u>	
1-2	Incomplete attacks involving 1 or more of the following
1.	Pleuritis or pericarditis
2.	Monoarthritis of hip, knee or ankle.
3	Exertional leg pain.
4	Favorable response to colchicine.
A diagnosis of FMF is reached if the patient has 1 or more major criteria, or 2 or more minor criteria.	
A typical FMF attack is defined as 3 or more episodes lasting 12 hours to 3 days, each of the same type, with fever of 38°C or more.	
An attack may be considered "incomplete" if it differs from the definition of a typical attack in only 1 or 2 of the following features: temperature less than 38°C, attack duration of 6 hours to 1 week, no signs of peritonitis during an abdominal attack, or signs are localized, atypical distribution of arthritis.	
Attacks not fulfilling these specific criteria are not counted towards a diagnosis of FMF.	

Modified from Livneh et al.[6]

Table 2

Clinical features and genotypes of patients bearing the P369S and R408Q variants in *MEFV* who reached the Tel-Hashomer Criteria.

Patient ID	Clinical features	Treatment	Additional features	<i>TNFRSF1A</i>	<i>MVK</i>
941	Fever 40°C, abdominal pain, 3–4 days duration.	Colchicine-partial benefit	Persistent cervical lymphadenopathy.	R92Q	Neg
1455	Fever 38°C, localized abdominal pain, 2–3 days duration	Colchicine-no benefit	None	Neg	Neg
1780	Fever 39.7°C, localized abdominal pain, 1–2 days duration	NSAIDs-no benefit	Mouth ulcers, pharyngitis, enlarged tonsils, headache and cervical lymph-adenopathy with some episodes	Neg	Neg
1817	Localized peritonitis-tunica vaginalis, 2 days duration	Colchicine-partial benefit	None	R92Q	Neg
1905	Fever 40.5°C, diffuse abdominal pain, arthralgia, myalgia, 3 days duration.	Colchicine-partial benefit	Cervical lymphadenopathy	Neg	Neg

Patient ID codes correspond to anonymized database reference numbers.

Table 3

Clinical features of symptomatic individuals bearing P369S and R408Q variants in *MEFV* not reaching the Tel-Hashomer criteria.

Patient	Age at onset	Fever	Biologic	Clinical features	Additional <i>MEFV</i> variants	<i>TNFRSF1A</i>	<i>MVK</i>
"PFAPA-LIKE" SYMPTOMS							
980	4 y	Y	N	Aphthous ulcers, pharyngitis, cervical lymphadenopathy.	E148Q +E148Q	Neg	Neg
1110	2 y	Y	N	Aphthous ulcers, pharyngitis, cervical lymphadenopathy.	E148Q(+) I591T	Neg	Neg
1874	1 mo	Y	Y	Aphthous ulcers, pharyngitis, cervical lymphadenopathy.		Neg	Neg
PREDOMINANTLY SEROSITIS SYMPTOMS							
1300	6 mo	Y	N	Pleuritis, lower limb arthralgia	E148Q	Neg	Neg
1688	14 y	N	Y	Pericarditis- tamponade, pleuritis, erysipeloid erythema		Neg	Neg
1818	12 y	Y	N	Single episode pleuritis			
1880	14 y	N	N	Pleuritis, arthralgia, nausea, vomiting.			
JOINT SYMPTOMS							
83	Adulthood	N	N	Recurrent knee synovitis		Neg	Neg
945	5 y	Y	N	Arthralgia-upper limb & jaw, abdominal pain, rash with fever			
UVEITIS							
122	50 y	Y	Y	Panuveitis, inflammatory bowel disease, lethargy	E148Q	Neg	Neg
1408	20 y	N	Y	Uveitis, dacrylitis, abdominal pain.	E148Q		
OTHER							
172	30 y	N	N	Unilateral eyelid amyloidosis following conjunctivitis.		Neg	Neg
1569	59 y	Y	Y	Profound fatigue, arthralgia		Neg	Neg
1793	11 y	Y	N	Arthralgia, myalgia, abdominal pain, oral ulcers.	E148Q	Neg	Neg
1827	35 y	Y	N	Diarrhea, cervical lymphadenopathy.	E148Q	Neg	Neg
1879	18 mo	N	N	Large joint arthralgia and myalgia			
1883	Infancy	N	N	Abdominal discomfort, arthralgia.	E148Q		