Pseudodominance of autoinflammatory disease in a single Turkish family explained by co-inheritance of haploinsufficiency of A20 and familial Mediterranean fever

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ABSTRACT

Objective. *We investigated a Turkish family with multiple patients presenting with familial Mediterranean fever (FMF) and Behçet's disease (BD)-like manifestations. The index case and the two daughters with Behçet-like disease, were previously found to have a TNFAIP3 frameshift mutation. The high number of affected cases in this expanded family could be consistent with a dominantly inherited inflammatory disease, although some individuals had clinical features more consistent with recessively inherited FMF. We sequenced DNA from members of this family to determine whether the TNFAIP3 frameshift mutation and/or MEFV variants could explain this autoinflammatory disease pedigree.*

Methods. *Patients were clinically diagnosed to have FMF or BD. Sanger sequence targeting TNFAIP3 exon 5 and MEFV exon 10 was carried out.*

Results. *The symptomatic mother of the index case and her affected maternal uncle had compound heterozygous FMF-associated MEFV mutations, p.Met680Ile and p.Arg761His. Two affected daughters of the maternal uncle also had compound heterozygous FMF-associated mutations, p.Met680Ile and p.Val726Ala. The index case and her two affected daughters had a TNFAIP3 frameshift mutation (c.799delG; p.Pro268Leufs*19), which is consistent with their HA20 diagnosis, and also carried a heterozygous MEFV p.Arg761His mutation.*

Conclusion. *Autoinflammatory disease manifestations in a Turkish family with multiple affected cases could be explained by co-inheritance of pathogenic MEFV variants and a heterozygous HA20-associated mutation. FMF-associated p.Arg761His allele carried with the loss of function TNFAIP3 mutation by all three HA20 patients may contribute to their autoinflammatory phenotype and could also be responsible for their favourable response to colchicine.*

Introduction

Familial Mediterranean fever (FMF) is an auto-inflammatory disorder that presents with recurrent fevers, painful serosal inflammation, and synovial inflammation in the joints. FMF is caused by bi-allelic mutations in exon 10 of the *MEFV* gene, although some patients may have only a single exon 10 mutation in this gene (1-3). Although these mutations are recessively inherited, they act as gain-of-function mutations on the protein function. The *MEFV* gene encodes pyrin, a key component of the pyrin inflammasome. The pyrin inflammasome plays a role in sensing bacterial induced Rho GTPase modifications. FMF-associated mutations affect pyrin inhibition and cause activation of the inflammasome, leading to caspase-1 mediated cleavage of pro-interleukin-1 beta (IL-1β) to its active form (4). Behçet's disease (BD) is an inflammatory disorder that mainly affects mucocutaneous tissues, eyes, joints, blood vessels, gastrointestinal and nervous systems. For the most part, BD cases present sporadically, and the disease is usually considered genetically complex or polygenic (5). However, early-onset familial BD-like disease has recently been reported to result from haploinsufficiency of the *TNFAIP3* gene encoding the ubiquitin-editing enzyme, A20. This enzyme plays a critical role in the regulation of many inflammatory signaling pathways including the canonical NF-κB and inflammasome pathways (6).

We studied a Turkish kindred with multiple FMF and BD-like cases. The index patient and her two daughters presented with BD-like manifestations and were previously found to have a heterozygous *TNFAIP3* frameshift mu-

tation (Family 6, *TNFAIP3*: c.799delG, p.Pro268Leufs*19) (6). The high frequency of affected individuals in this enlarged family (Fig. 1) could be consistent with a dominantly inherited inflammatory disease, although some individuals had clinical features consistent with FMF and others were more consistent with BD. We sequenced DNA from affected and unaffected members of this family to determine the roles of pathogenic *MEFV* mutations and the *TNFAIP3* frameshift mutation in the development of various autoinflammatory manifestations.

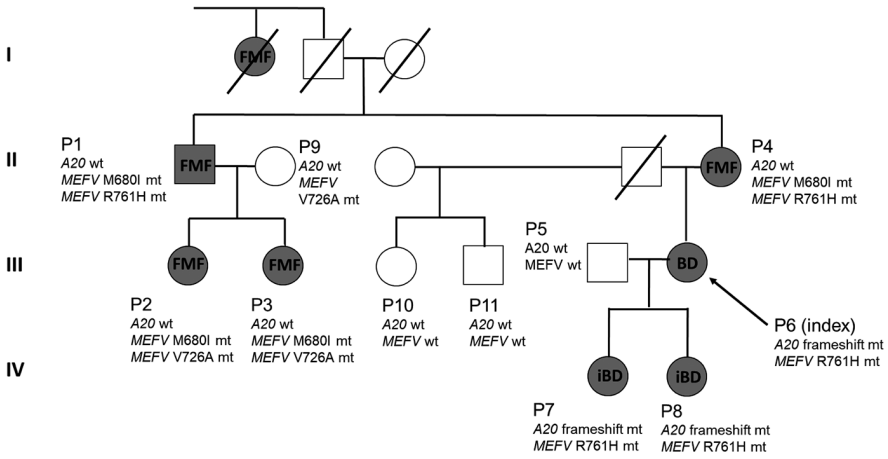

Fig. 1. Pedigree and variants.
FMF: Familial Mediterranean fever; BD: Behçet's disease; iBD: incomplete Behçet's disease.
P7 and P8 had oral ulcers, genital ulcer, and weakly positive Pathergy test.
A20: Tumour Necrosis Factor-Alpha-Induced Protein 3, wt: wild type, mt: mutation.

Materials and methods

Patients' clinical findings were collected using a standard form and evaluated for disease classification according to established criteria (7, 8).

Sanger sequencing targeting *TNFAIP3* exon 5 and *MEFV* exon 10 was carried out in a standard manner.

Results

The pedigree of the family is given in Figure 1, and clinical manifestations of the affected family members are summarised in Table I.

The proband, P6, fulfilled clinical criteria for BD with mucocutaneous manifestations. Her skin findings also included vesicular eruptions, which are not typical for BD. She also had Hashimoto thyroiditis. She has been doing moderately well with colchicine treatment and has not required additional systemic immunosuppressive or biologic treatments.

Her two daughters (P7, P8) had oral and genital aphthous ulcers, and their pathergy test results were inconclusive (Table I). Hence, they did not fulfill the ISG criteria for BD. They also had serologic findings for Hashimoto thyroiditis with normal thyroid function. Both siblings were positive for the *TNFAIP3* mutation (c.799delG, p.Pro268Leufs*19) identified in their mother; and all three (P6, P7, and P8) also carried a single allele (heterozygous) p.Arg761His mutation in the *MEFV* gene (Fig. 1). The affected mother of the index case did not fulfill ISG BD criteria and the father was deceased at the time of investigation. His past history included chronic obstructive pulmonary disease, rare oral ulcers and some skin findings with pruritus on his legs. The father had two children from his previous marriage (P10 and P11), and both had no relevant findings and were negative for both A20 and *MEFV* variants.

Four family members (P1-4) including the mother of the index case had a clinical history of FMF, and their diagnoses were confirmed after re-examination of their clinical findings (Table I). They all were on colchicine treatment with good response. The mother of the index case (P4) also had Hashimoto thyroiditis. Results of the *MEFV* mutation screen were compatible with the clinical diagnosis of FMF with an autosomal recessive inheritance pattern, all carrying two exon 10 pathogenic variations. Patients P1 and P4 had compound heterozygous FMF-associated mutations, p.Met680Ile and p.Arg761His. Patients P2 and P3 also had compound heterozygous FMF-associated mutations, p.Met680Ile and p.Val726Ala. The wife of P1 (P9) was heterozygous for p.Val726Ala, and she had no findings of FMF but suffered from recurrent oral ulcers since age 10.

Discussion

HA20 and FMF are two distinct autoinflammatory diseases with some overlapping clinical features. Both diseases manifest in early childhood and present with recurrent fevers and gastrointestinal complaints.

FMF is a common disease in multiple Mediterranean populations including Turkish, Armenian, Arab and Jewish, while it is far less common in Asian and Caucasian populations. On the other hand, HA20 is caused by *de novo* or dominantly inherited mutations in patients of diverse ancestries. FMF is characterised by short-duration recurrent attacks of systemic inflammation that manifest with severe abdominal and/or chest pain and arthralgia/arthritis. A minority of patients may present with mucocutaneous lesions, and increased risk of BD associated with penetrant *MEFV* variations may result in appearance of overlapping manifestations of BD and FMF (9-11). Other factors may contribute to disease variability and expression (12). In contrast, recurrent oral and/or genital and/or gastrointestinal ulcers, and cutaneous ulcers are the hallmark features of HA20, and its BD-like manifestations are considered challenging in the differential diagnosis (13). Some patients with HA20 also present with autoimmune features including lupus like manifestations or Hashimoto thyroiditis (13, 14). All three HA20 patients in this family had Hashimoto thyroiditis, and one of the FMF patients (P4, mother of the index case) also had findings of Hashimoto thyroiditis.

Most FMF patients have a good response to treatment with colchicine and less than 10% are refractory and require IL-1 blocking agents, while cytokine

Table I. Patients' characteristics.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Gender	M	F	F	F	M	F	F	F	F	F	M
Current age	59	37	33	63	44	41	22	18	54	69	67
Age of onset	19	15	15	20	-	29	13	7	10	-	-
Symptoms (FMF- & BD-related)											
Fever (Y/N)	Y	Y	Y	Y	N	Y	N	N	N	N	N
Duration of fever	1-3d	1-3d	1-3d	1-3d		1d					
Rash (Y/N)	N	N	N	N	N	Acne-like	N	N	N	N	N
Ocular lesion (Y/N)	N	N	N	N	N	N	N	N	N	N	N
Arthritis/arthralgia?	N	N	N	Arthritis	N	Arthralgia	N	N	N	N	N
FMF-related symptoms											
Peritonitis (Y/N)	Y	Y	Y	Y	N	N	N	N	N	N	N
Pleuritis (Y/N)	N	N	N	N	N	N	N	N	N	N	N
Pericarditis/Carditis?											
BD-related symptom											
Oral ulcer (Y/N)	Y	N	N	N	N	Y	Y	Y	Y	N	Rare
Genital ulcers (Y/N)	N	N	N	N	N	Y	Y	Y	N	N	N
Pathergy (Y/N/not done/inconclusive)	ND	ND	ND	ND	ND	Y	ic	ic	ND	ND	ND
Ocular lesion											
HLA B-51 (Y/N/not done)	ND	ND	ND	ND	ND	N	ND	ND	ND	ND	ND
Clinical diagnosis	FMF	FMF	FMF	FMF Hashimoto thyroiditis	Healthy	BD Hashimoto thyroiditis	iBD Hashimoto thyroiditis	iBD Hashimoto thyroiditis	ROU	Healthy	Healthy
Treatment, effectiveness, clinical course	Colchicine Effective	Colchicine Effective	Colchicine Effective	Colchicine Effective	-	Colchicine Effective	Colchicine Effective	Colchicine Effective	-	-	-

FMF: familial Mediterranean fever. BD: Behçet's disease. iBD: incomplete Behçet's disease; ROU: recurrent oral aphthous ulcer.

inhibitors have been successfully used to effectively suppress systemic inflammation in HA20 patients. In this family, similar to FMF patients, the HA20 patients had a moderate to favourable disease course and responded well to colchicine treatment, which is compatible with the recent review that concluded it may be effective for one fourth of HA20 patients (15).

The autoinflammatory disease in this pedigree with apparently dominant inheritance is explained by the segregation of three FMF-associated bi-allelic *MEFV* mutations in four affected individuals and one loss-of-function *TNFAIP3* mutation in three affected individuals. Pseudodominance is a type of inheritance in which the inheritance of a recessive trait imitates a dominant pattern of inheritance.

MEFV mutations are common in Turkey. Kirino *et al*. reported their prevalence in healthy individuals as follows: M680I 2%, M694V 4%, V726A 4%, and R761H 0.6% (9). Therefore, in Turkey a FMF patient has a considerably high risk of marrying an *MEFV* variant carrier without clinical manifestations of FMF, explaining the apparent dominant inheritance of FMF in P2 and P3 of this pedigree (Fig. 1).

Patients P6, P7, and P8 were recently shown to carry a loss-of-function mutation in *TNFAIP3* and therefore were diagnosed with HA20 (7). Although the mother of P6 (P4) has autoinflammatory symptoms, her symptoms are clinically consistent with FMF and not with HA20. We showed that the HA20 associated mutation in P6 was not inherited from her symptomatic mother (P4) who instead carried two FMF-associated *MEFV* mutations. The father of P6 was not available to determine his affection status and whether he transmitted the *TNFAIP3* mutation or if it was a *de novo* event in P6. He had two other children from his previous marriage, and those children were negative for the A20 and *MEFV* variants. Some autoinflammatory symptoms in the father of P6, such as rare oral ulcers and skin lesions described by his relatives, leave the possibility of his carrying the variant, which subsequently became more penetrant in his daughter and granddaughters with the co-inheritance of the *MEFV* variant.

Although HA20 displays dominant inheritance, as more patients and particularly more families with haploinsufficiency of A20 have been identified and evaluated, it has become clear that the clinical phenotype of HA20 is remarkably varied, even among family members sharing the same A20 mutation. In fact, HA20 patients have had initial diagnoses as varied as PFAPA, RA, RF-negative polyarticular JIA, systemic JIA, Hashimoto thyroiditis, Kawasaki disease, lupus, and FMF, as well as Behçet's (13, 14) and five mutation positive patients in one report had only recurrent aphthous stomatitis (14). This broad phenotypic variation may be explained at least in part by contributions of additional genetic or environmental factors. All three HA20 patients in this family co-carry the *MEFV* p.Arg761His mutation and if the HA20 mutation was inherited from the seemingly unaffected father of the proband, his mild phenotype could be explained by absence of the FMF mutation. Unfortunately, we cannot test this hypothesis because the father of

P6 had already died. Additional studies are required to determine whether the FMF-associated p.Arg761His allele carried with the loss of function *TNFAIP3* mutation by all three HA20 patients contributes to their autoinflammatory phenotype and could also be responsible for their favourable response to colchicine.

Common *TNFAIP3* polymorphisms (SNPs) are known susceptibility loci or risk factors for several autoimmune diseases (16). Therefore, the presence of additional autoimmune conditions may be a clue for suspecting A20 variants in patients with BD-like manifestations.

Our study demonstrates that HA20 should be considered even in patients from countries such as Turkey with a high prevalence of typical polygenic BD, especially when symptoms are very early onset or when multiple family members are affected. Molecular diagnostic investigations are essential for confirming diagnosis, genetic counseling in families, and for choosing an appropriate treatment.

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