Common FMF alleles may predispose to development of Behcet's disease with increased risk for venous thrombosis

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Background: Behcet's disease (BD) is an inflammatory disorder of unknown cause, associated with vasculitis. Arterial or venous thrombosis occurs in about 25% of BD patients. Familial Mediterranean fever (FMF) is another inflammatory disorder, which stems from mutations in the FMF gene (MEFV) and shares a number of features with BD.
Objective: MEFV analysis in patients with BD suggests that mutated MEFV may act as a susceptibility gene in BD. We studied the rate and the clinical correlates of MEFV mutations in Israeli BD patients.
Methods: Included were 54 BD patients who satisfied the International Study Group criteria for BD. All BD patients were genotyped using polymerase chain reaction (PCR) and restriction enzyme analysis for the three most common MEFV mutations (M694V, V726A, and E148Q). The association between BD manifestations and MEFV alleles was analysed.
Results: Twenty-one BD patients were found to carry a single MEFV mutation and three additional patients were compound heterozygotes, a frequency significantly higher than that expected for ethnically matched healthy individuals. There were no statistically significant differences between carriers and non-carriers with respect to gender, frequency of HLA B5 antigen, cutaneous lesions, joint disease, and severity score. However, carriers did experience thrombosis more often [54% vs. 17%, $p<0.005$, odds ratio (OR)=6.9, 95% confidence interval (CI) 1.75–26.9] and uveitis less often (20% vs. 40%, $p<0.05$, OR=0.2, 95% CI 0.04–0.92).
Conclusions: MEFV appears to be a susceptibility and modifier gene in BD.

Behcet's disease (BD) is an inflammatory disease of unknown cause, characterized by a waxing and waning course in which oral and genital ulcers, skin lesions, uveitis, obstructive vasculopathy and other inflammatory manifestations may flare up and then ameliorate (1). The underlying pathogenesis is vasculitis (1, 2), and BD is one of the vasculitides found in patients with familial Mediterranean fever (FMF) with a frequency higher than that of the general population (3–5).

As part of the explanation for the association between BD and FMF, Touitou et al found that the A9 allele of MICA, an HLA region that has been associated with BD, is also a modifier gene in FMF (6). Indeed, genetically, most FMF-BD patients are heterozygous, carrying only one FMF mutation (7), suggesting that another modifier gene is the culprit that leads to the expression of the FMF arm of their disease.

However, the association between the two inflammatory diseases could possibly stem from FMF-related factors. For instance, the chronic inflammation characterizing FMF (8–10) could precipitate BD in individuals with an appropriate genetic background, who would otherwise remain clinically silent. Alternately, FMF-related genetic factors could underlie this association.

To determine whether a mutated pyrin, the FMF-related gene, has a modifying role in BD, we studied the frequency of FMF alleles among BD chromosomes, and determined the clinical sequela of such a genetic predisposition.

Methods

Patients

Fifty-four consecutive BD patients, who do not have FMF and who constitute a large majority of such patients currently under our care, were asked to donate blood for the study. In all patients the

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diagnosis of BD met the International Study Group criteria for diagnosis of BD (11). None of the BD patients fulfilled the criteria for the diagnosis of FMF (12) or had a family history of FMF affecting first-degree relatives. To determine the BD-related manifestations over the course of their disease, data were abstracted from the patient files and the patients were interviewed and examined. Ethnic origin, age, sex, disease duration, and a variety of manifestations, including oral and genital ulcers, uveitis or retinal vasculitis, cutaneous manifestations (pustular lesions and erythema nodosum), thrombotic events (venous or arterial), arthritis (episodic or chronic), gastrointestinal complaints (abdominal pain, diarrhoea, ulcerative disease), and any other organ involvement that could be related to BD, drug treatment directed specifically against disease manifestations, and estimates of disease activity and severity were all recorded, and constituted the basis for comparison between patient populations. The severity of the disease was estimated using a published key, in which manifestations are scored according to the potential damage and impairment they might inflict (13). The Human Experimentation Ethical Committee of our institution approved the study.

Detection of common MEFV mutations

Blood samples were drawn and subjected to DNA extraction, followed by mutation analysis, using polymerase chain reaction (PCR) amplification of the DNA segment of interest, and restriction enzyme digestion. The three most common mutations in our Jewish population were determined (M694V, V726A, and E148Q). These mutation analyses were performed as described previously (14).

Statistical evaluation

Statistical analysis was performed using the χ^2 analysis for discrete variables and Student t-test methods for continuous variables. A multiple logistic regression was applied to test the differences between carriers and non-carriers of the MEFV mutated alleles, adjusting for confounders. A p-value of less than 0.05 was considered statistically significant. All tests were two-tailed.

Results

Of the 54 BD patients, 24 carried one or two mutations in their FMF gene. The distribution of the mutated alleles in relation to the ethnic origin of the patient is displayed in Table 1. Most of the patients were non-Ashkenazi Jews, originating from North Africa (27 patients) and other Sephardic communities (23 patients). Compared to the expected distribution of FMF-mutation carriers among non-FMF individuals in these ethnic groups (15–17), BD patients have a higher frequency of the FMF-associated alleles ($p < 0.005$, Table 1).

The MEFV alleles in the carriers included M694V in 14 patients, E148Q in six patients, and V726A in one. In addition, three patients were compound heterozygous to the M694V/E148Q mutations. They all had recurrent oral ulcers. One patient, a 26-year-old man, had a positive pathergy test, episodes of erythema nodosum, and episodes of non-febrile wrist arthralgia. Another patient, a 40-year-old woman, had vaginal aphthae, folliculitis and afebrile arthralgia, involving several large and small joints at a time, each lasting several weeks. The third patient, a 43-year-old female, experienced recurrent events of vaginal aphthae, erythema nodosum, and wrist pain. None of the double mutation carriers conformed to the criteria for the diagnosis of FMF (12).

Of the 27 FMF alleles, 17 carried the M694V mutation. The ethnic distribution of M694V carriers included nine patients of North African descent, one Iraqi, and seven with other non-Ashkenazi ethnicities.

Table 2 shows clinical, demographic, and genetic characteristics of BD patients of our cohorts, stratified by their MEFV carrier status. Of these, thrombotic manifestations, neuro-BD, and uveitis stood out as the most distinctive determinants separating the two subgroups. Compared to non-carriers, carrying an MEFV mutation predisposed BD

Table 1. Rate of MEFV-mutated alleles in the BD ethnic subgroups.

Jewish origin	Number of patients	Number of carriers	Actual frequency	Expected frequency*	Expected number of carriers
Ashkenazi	4	0	0	(1:7) 0.142	0.57
North African	27	14	0.52	(1:5) 0.2	5.4
Iraqi	8	4	0.5	(1:3.5) 0.29	2.32
Other non-Ashkenazi	10	4	0.4	(1:6) 0.17	1.7
Yemenite	5	2	0.4	(1:7) 0.142	0.71
Combined	54	24	0.44†	0.2	11

*Expected frequencies of MEFV mutated alleles were derived from references 10, 16, and 39. †The actual frequency is significantly higher than the expected frequency of mutations ($p < 0.005$).

Table 2. Features of BD patients by their MEFV carrier status.

Characteristics	Carriers (24)	Non-carriers (30)	Total (54)	p-value*
Mean age (years)	45±14	42±13	43±14	
Male gender	50	33	41	
Disease duration (years)	16±7	17±9	16±9	
Sephardic ethnicity	83	100	93	
HLAB5	63	50	56	
Uveitis	21	40	31	<0.05
Cutaneous	54	47	50	
Genital ulcers	27	29	28	
Arthritis	79	67	72	
Gastrointestinal	17	23	20	
Thrombosis	54	27	33	<0.025
Neuro-BD	13	37	26	<0.025
Severity (points)	4.9±1.7	4.7±1.5	4.8±1.6	

Figures denote the percentage of patients with the specific character among carriers or non-carriers of MEFV mutations, unless otherwise specified. *Only significant differences are marked by the p-value.

patients to develop thrombosis ($p<0.025$), while it protected them from uveitis ($p<0.05$) and neuro-BD ($p<0.025$), with odds ratios (ORs) of 6.9, 0.2, and 0.27, respectively (Table 3). As expected, most of the thrombotic manifestations (17 of 21) were limited to the venous tree. The number of patients with arterial involvement was too small to draw distinctions between the two groups (carriers and non-carriers).

Discussion

We present 54 patients with BD. Twenty-four of them had one or two MEFV mutations. This frequency is higher than expected for a population of the same ethnic background, and suggests that MEFV may be perceived as a susceptibility gene for BD (Table 1).

Most MEFV mutated alleles carried the M694V mutation (63%). Compared to the expected frequencies in populations of similar ethnic composition, our finding implies that BD is located halfway from the frequency of M694V in the general population (40% of all alleles), towards the frequency of the M694V in FMF (75% of all alleles) (14–19). Although changes in the rate of M694V forming this shift are statistically insignificant, probably because of the small number of patients studied, such a trend favours a role for M694V in predisposing individuals to develop BD. Of note, and in support of this analysis, is our previous finding that most FMF-BD patients are carriers of the M694V mutation (7).

Table 3. Calculated risk of mutation carriers to develop the indicated manifestation (by a multiple regression model).

Manifestation at risk	Odds ratio	95% CI	p-value
Thrombosis	6.9	1.75–26.9	0.006
Uveitis	0.2	0.04–0.92	0.05
Neuro-BD	0.27	0.06–1.2	0.08

It also seems that carrying a pyrin mutation not only predisposes to BD but also modifies the expression of BD to a certain extent. Our results show that patients with pyrin mutations, while sharing major clinical features with their counterparts, had significantly more venous occlusive episodes (Tables 2 and 3 with an OR of 6.9). The exact pathogenesis of thrombosis in BD is poorly understood, but genetic defects and acquired changes affecting thrombosis, fibrinolysis, and endothelial function have been proposed as part of, or in addition to, vasculitis (20–24). Because vasculitis underlies BD and thrombosis is its direct or indirect consequence, our findings suggest that mutated pyrin may indeed be directly involved in the essence of the pathology that develops into BD.

Our findings are in close concert with those of Ben-Chetrit et al (4), Touitou et al (25), Atagunduz et al (26), and Imirzalioglu et al (27), who found an increased frequency of MEFV mutations in cohorts of patients with BD, with a rate reaching 26% and 36% in the later studies. Of note, Atagunduz et al (26) found that BD patients carrying MEFV mutations tend to experience vascular manifestations in a significantly higher frequency compared to non-carriers, an observation very similar to our findings. However, not all observers found an association between MEFV mutations and clinical manifestations (4). The reason for this discrepancy is unclear and could be methodological, possibly due to different patient selection. However, the overwhelming evidence is in favour of the MEFV as a susceptibility gene, possibly implicated in the pathogenesis of BD.

Pyrin seems to be a modifier of several other inflammatory or autoimmune diseases. In rheumatoid arthritis (RA), multiple sclerosis (MS), and

Crohn's disease, it was found to cause a more severe disease (28–31). A severity score established to measure suffering and disability of RA patients was higher in carriers than in non-carriers of MEFV mutations (28). MS patients having a pyrin mutation reached a landmark 6 at the expanded disability status scale (EDSS) 17 years earlier than non-carriers (29). In Crohn's disease, carriers of the MEFV mutations were observed as suffering more often from stricturing diseases (30). In a cohort of 80 patients with a variety of glomerulonephritides, pyrin was found to be mutated in higher proportion than expected (Livneh et al, unpublished observation). Similarly, high rates of MEFV mutations were found in Henoch–Schonlein purpura and in patients with rheumatic heart disease (32, 33). Our paper joins all previous papers on MEFV in BD that added this disease to the list of diseases affected by a mutation in MEFV. Contrary to most of these disease entities, in BD a much stronger association with mutated MEFV was found, as it seems that pyrin affects both the precipitation and the phenotype of the disease.

Could ethnicity rather than MEFV mutation status cause clinical manifestations to diverge in MEFV mutation carriers? Indeed, Krause et al found significant differences in the clinical picture of BD among different Jewish ethnic groups (34). For instance, North Africans had a higher frequency of thrombosis, uveitis, and arthritis, and a higher severity score compared to other ethnic groups (Turkish or Iraqi Jewish patients). However, in our study, carriers and non-carriers shared almost the same ethnic composition (inferred from Table 1), and therefore ethnicity might be excluded as the cause of clinical differences in our patients.

Our understanding of the MEFV–BD association is limited because the role of pyrin is yet to be more clearly defined. However, recent data suggest that pyrin is strongly linked to metabolic pathways that regulate interleukin (IL)-1 expression (35–37). This function of pyrin is interrupted in cases with mutated pyrin proteins. As a result, higher than needed IL-1 levels are generated and induce inflammatory activity. Indeed, IL-1 has been noted in several studies to play a role in BD (38–40). Our finding therefore joins previous line of evidence to shed a light on a possible IL-1-mediated pathogenic mechanism, which has not been fully appreciated. However, we can offer no explanation for the protective effect of the carrier status against the occurrence of uveitis and neuro-BD.

In conclusion, the spectrum of the association between FMF and BD that was noted previously has now been expanded to include the findings of this work also, namely that MEFV carriers are prone to develop BD with a higher likelihood, and that their disease course may include large-vessel thrombosis.

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