MEFV, TNFRSF1A and CARD15 mutation analysis in Behçet's disease

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ABSTRACT

Objectives. *Given the pathological similarities between Behçet's disease (BD), Familial Mediterranean fever (FMF), TNF receptor-associated periodic syndrome (TRAPS) and Crohn's disease (CD) we evaluated the frequency of mutations and polymorphisms in MEFV, TNFRSF1A and CARD15 in Israeli BD patients of either Jewish or Arab descent.*

Methods. *Fifty-four BD patients (11 Jews and 43 Arabs), evaluated with respect to the entire spectrum of BD disease manifestations, were granted a systemic severity score for BD. An association between BD manifestations and MEFV, TNFRSF1A and CARD15 variants was analysed.*

Results. *Twelve patients (20.7%) displayed a single MEFV mutation and four patients (7.4%) had two mutated FMF alleles. Two patients (3.8%) carried a CARD15 variation and none carried a TNFRSF1A polymorphism. The frequency and distribution of mutated alleles between patients and controls was comparable (p=0.27). No statistically significant differences between carriers and non-carriers with respect to disease manifestations and severity score were calculated. Arab patients were diagnosed earlier than Jewish patients (25.8±11.6 and 37.2±10.7, respectively, p=0.06).*

Conclusions. *The overall MEFV high carrier frequency in our cohort of BD patients seems to be attributed to their Mediterranean extraction rather than related to BD. The propensity of Arab patients (79.6%) in a cohort of BD patients from northern Israel is highlighted in face of their proportion (20%) in the general population lending further support to arguments that favour a genetic component for BD.*

Introduction

Behçet's disease (BD) is an inflammatory disorder traditionally described as a triad consisting of recurrent aphthous stomatitis, genital ulcerations, and ocular disease. BD is now recognised as a multi-system disorder, the clinical expression of which may be dominated by mucocutaneous, articular, neurologic, urogenital, vascular, intestinal, or pulmonary manifestations (1-3). Diagnosis of BD relies on the identification of clinical criteria outlined by the International Study Group for BD (ISBD) (3, 4). Its etiology seems to be multifactorial with environmental factors triggering the onset of the disease in individuals with a susceptible genetic background. Although BD commonly appears as a sporadic disease, its peculiar geographical distribution, along the old silk route through middle eastern countries, and the numerous reports of multiple affected relatives argue in favour of a genetic component (5, 6). A strong association with HLA B51/B5 in middle eastern and Mediterranean countries has been demonstrated for BD. Emerging evidence seems to suggest the presence of major still-unknown gene effects that either, cause, precipitate the onset and/or enhance the susceptibility of patients to BD (6, 7).

The prevalence of BD is higher in the middle eastern and Mediterranean populations. Familial Mediterranean fever (FMF) is another chronic inflammatory disease, highly prevalent in these populations. BD and FMF have common pathophysiological features, the clinical manifestations of both diseases can mimic each other and the coexistence of both diseases in the same patient has been reported (8-13). TNF receptor-associated periodic syndrome (TRAPS) and Crohn's disease are other inflammatory disorders, that share a number of features with both FMF and BD. FMF stems from mutations in the *FMF* gene (*MEFV*) whereas TRAPS and Crohn's disease are linked to mutations in the type 1 TNF receptor (*TNFRSF1A*) and *CARD15* genes, respectively (14-17).

BD and FMF both result from inappropriate activation of neutrophils. The pyrin domain of *MEFV* gene, the gene responsible for FMF, is a member of death-domain superfamily and has been proposed to regulate inflammatory signalling in myeloid cells (17). With this in mind several investigators have looked for an association between BD and *MEFV* mutations (8-13). Many studies have shown that *MEFV* mutations may be over-represented in BD patients. A genetic association between BD and mutations in the *TNFRSF1A* gene and *CARD15* was meagerly studied.

In this study we evaluated the influence of mutations in *MEFV*, *TNFRSF1A* and *CARD15* on the clinical expression and disease severity in Israeli BD patients of either Jewish or Arab descent.

Patients and methods

Patients and controls

Patients with BD were ascertained using the criteria proposed by the ISBD (4) that include oral aphthae (at least 3 episodes in one year) plus at least two of the followings: genital ulceration, erythema nodosum, necrotic folliculitis, uveitis and retinal vasculitis. Patients were recruited at the Department of Rheumatology, Bnei-Zion Medical Center Haifa and the Institute of Human Genetics, RAMBAM Health Care Campus during 2006-2008. The study was approved by the Institutional Review Board of the two medical centres. Data related to the entire spectrum of disease manifestations were collected from medical files and patient interviews. Patients were given educational information about the aims of the study, signed an informed consent form and a blood sample was drawn for molecular testing. The control group consisted of 317 healthy individuals who came to be screened for common genetic diseases as part of the screening policy in Israel. The controls were consecutively collected, at a ratio of six controls for each patient, matched for ethnic descent and collectively made up of 251 Arabs subdivided according to their religion (197 Muslim Arabs; 36 Druze Arabs and 18 Christian Arabs) and 66 Jews (24 Ashkenazi Jews and 42 non-Ashkenazi Jews) to match 54 patients (34 Muslim Arabs; 6 Druze Arabs, 3 Christian Arabs, 4 Ashkenazi Jews and 7 non-Ashkenazi Jews).

Genetic testing

PCR and RFLPs for the predominant mutations in *MEFV*, *TNFRSF1A* and *CARD15* were performed.

MEFV mutations: Predominant mutations in *MEFV* gene namely, M694V, V726A, E148Q, M694I and M680I were determined as described by Gershoni-Baruch *et al*. 1999 (18). Predominant mutations in *MEFV* were studied based on the ethnic descent of individuals tested as published earlier by Gershoni-Baruch *et al*. 2001 (19).

CARD15 mutations: Three predominant mutations in *CARD15*/*NOD2*, known as the Leu1007fsinsC, R702W and G908R were tested. PCR for LfinsC1007P was performed using forward 5'-GGCTAACTCCTGCAGTCTCT-3' and reverse 5'-GGAGAGCTAAAACAGGCCTG-3' primers yielding a product of 205 bp. Restriction by NLAIV separated the mutant and the wild-type alleles. PCR for R702W was performed using forward 5'-TTCAGATCACAGCAGCCTTC-3' and reverse 5'- GGTGCAGCTGGCGGGATGG-3' primers yielding a product of 170 bp. Restriction by MspI separated the mutant and the wild-type alleles. PCR for G908R was performed with mismatched forward 5'-TTTGGCCTTTTCAGATTCC*TGG-3' and reverse 5'-CTCTTCACCTGATCTCC-3' primers yielding a product of 149 bp. Restriction by NciI separated the mutant and the wild-type alleles.

TNFRSF1A mutations: Two predominant mutations in TNFRSFA1, known as P46L and R92Q were tested. PCR was performed using forward 5'-TGTGTTCTCACCCGCAGCCTAAC-3' and reverse 5'- CTGAGGCCAAGCCCTCTC-3' primers yielding a product of 574 bp. Restriction by MspI separated the mutant and the wild-type alleles of both the P46L and the R92Q mutation.

Data management and statistical analysis

The prevalence of mutations between ethnically matched BD patients was compared using the simple Chi-square test. Severity score was calculated according to Krause *et al*. (20). Mild, moderate and severe symptoms were awarded one, two, or three points, respectively. Pearson correlation coefficient (r) and its significance (p) were calculated between age at diagnosis and severity score for each patient. Chi-square test or Fisher's exact test was performed, when appropriate, to analyse a statistically significant relationship between demographic, genetic and categorical variables (*e.g*. origin, gender and various clinical manifestations such as genital ulcers, ocular disease, arthritis, etc.). T-test analysis of variance was done, using the Duncan multiple comparison option, to test statistically significant differences in mean continuous variables (*e.g*. age at disease onset, disease duration, or severity score) in carriers and non-carriers. P-values <0.05 were considered statistically significant.

Results

Table I describes the demographic and clinical manifestations of 54 patients with BD who fulfilled the ISBD criteria for BD. Two patients suffered from both BD and FMF (BD-FMF). The cohort consisted of 27 (50%) males and 27 (50%) females. Most patients (n=43; 79.6%) were of Arab descent (31 sporadic and 12 familial cases) and 11 patients (20.4%) were of Jewish descent (all sporadic cases). Mean age at diagnosis was 27.9±12.1 years. Other than ulcers (oral and genital), the most common clinical manifestations, in descending order were arthralgia 38 (70.4%); skin lesions (erythema nodosum, folliculitis, papulopustular lesions or other) 32 (59.3%); arthritis 27 (50.0); and ocular disease (anterior uveitis or pan uveitis) 22 (40.8%). Vein thrombosis was reported by 11 (20.4%).

Predominant mutations in *MEFV* were identified in 16 patients (29.6%; Table II) of whom two carried the M694V mutation, two carried the V726A mutation, six were E148Q heterozygotes and two had the V726A/E148Q complex allele (double heterozygotes). Four patients (7.4%) had two mutated FMF alleles. In the control group 72 *MEFV* carriers (22.7%) were identified.

Table I. Demographic and clinical characteristics of the 54 patients with BD.

Demographic	Male/female ratio	27/27 (1.0)
Ethnic descent	Jews (%)	11 (20.4)
	Ashkenazi	4 (7.4)
	Non-Ashkenazi	7 (13.0)
	Arabs (%)	43 (79.6)
	Muslims	34 (63.0)
	Druze	6 (11.1)
	Christians	3 (5.5)
Clinical manifestation according to ISBD	Age at disease onset (yrs)	
	Range	1-50
	Median	28.0
	Mean±SD	27.9 ± 12.18
	Years since initial diagnosis	
	Range	1-35
	Median	10.0
	Mean±SD	11.18 ± 8.0
	Oral aphtosis (%)	54 (100.0)
	Genital ulcer (%)	44 (81.5)
	Skin lesion (%)	32 (59.3)
	Arthralgia (%)	38 (70.4)
	Recurrent headaches (%)	7 (13.0)
	Epididymitis (%)	3 (5.6)
	Bowel angina (%)	12 (22.2)
	Pleuritic pain (%)	None
	Superficial vein thrombosis (%)	2 (3.7)
	Arthritis (%)	27 (50.0)
	DVT (%)	6 (11.1)
	Anterior Uveitis (%)	17 (31.5)
	Gastrointestinal bleeding (%)	None
	Panuveitis (%)	5 (9.3)
	Arterial thrombosis (%)	None
	Major vein thrombosis (%)	3 (5.6)
	Neuro Behçet (%)	None
	Bowel perforation (%)	None

Table II. The predominant mutations in the *MEFV* gene in 54 patients with BD.

Mutation	n. of patients (%)	n. of alleles (%)
Total	16 (30)	20 (18.5)
M694V/0	2 (3.7)	2 (1.85)
V726A/0	2 (3.7)	2 (1.85)
E148Q/0	6 (11)	6 (5.5)
V726A- E148Q/0	2 (3.7)	2 (1.85)
M694V/ M694V	1 (1.85)	2 (1.85)
E148Q/ E148Q	2 (3.7)	4 (3.7)
V726A/E148Q- M694V	1 (1.85)	2 (1.85)

The prevalence of *MEFV* mutations in controls was not statistically different than in BD patients (p=0.27).

Mutations in *CARD15* were detected in two patients (3.8%) (one patient carried the G908R mutation and the other had the R702W mutation). No patient carried a *TNFRSF1A* polymorphism. No statistically significant differences between carriers and non-carriers with respect to gender, age of disease onset, disease manifestations and severity score were calculated. However, Arab patients were diagnosed earlier than Jewish patients (25.8±11.6 and 37.2±10.7, respectively, p=0.06). Arthritis was more frequently diagnosed in Jewish than in Arab BD patients (8 of 11, 72.7% and 19 of 43, 44.2%, respectively, p=0.088 0.175), however with no statistical significance. Deep vein thrombosis was more frequent in Jewish than in Arab BD patients (3 of 11, 27.3% and 3 of 43, 7.0%, respectively, p=0.09).

Discussion

Although BD commonly appears as a sporadic disease, accumulating evidence argues in favour of a strong genetic component (5, 6). BD has been shown to be marginally associated with mutations in *MEFV*. In this study, 20 (18.5%) mutated FMF alleles were indentified in our cohort of 54 BD patients, a fraction trendially higher (p=0.068) than that observed in a control group which was meticulously matched for ethnicity. FMF is a genetic autosomal recessive auto-inflammatory disease that affects primarily people of Mediterranean origin with a high carrier frequency that varies from 1:3.5 to 1:4.7 in the different ethnic groups of the Israeli Jewish and Arab population (19). Thus, the overall carrier frequency, in our cohort of patients diagnosed with BD, may be attributed to their Mediterranean extraction rather than related to BD (8-12). The observation, that the high frequency of *MEFV* mutations is population related rather than disease associated, is further strengthened by the finding that no statistically significant differences between carriers and non-carriers with respect to disease manifestations and severity score were found. Our results sustain the observation made by Ben Chetrit *et al*. (21), that maintains that BD and FMF are two separate entities that have a mild trend toward a higher than expected association with no mutual effect of FMF on BD or *vice versa*. Conversely, Rabinovitch *et al*. (2007) (12). have shown that 50% of their BD patients carried an *MEFV* mutation significantly higher than in the general Israeli population. This difference, again, seems to be population related in view of the fact that all their patients were of Jewish descent (mainly non-Ashkenazi) while our BD patients were mainly of Arab descent.

Disease severity score was similar in both our Arab and Jewish groups of patients. Jewish patients, although rare (n=11; non-Ashkenazi Jews n=8; Ashkenazi Jews n=3) had higher rates of articular and thrombotic events compared to Arab BD patients. Rabinovich *et al*. (12) have previously commented on the relative expression of BD among the different ethnic groups in Israel and have shown that among patients

with BD, those originating from North African countries manifested a more severe disease.

Otherwise, mutations causing other related diseases, such as TNF receptor-associated periodic syndrome and Crohn's disease, were not over-represented in our cohort of BD patients.

Our results have not awarded either of *MEFV TNFRSF1A* or *CARD15* a susceptibility potential to BD. However, since many previous studies did detect an association between *MEFV* mutations and BD we cannot disclaim that *MEFV* may play a role in BD. As the study population is small the results could gain further strength if enlarged. The propensity of Arabs (79.6%), in our cohort of BD patients from northern Israel, should be highlighted, in face of their proportion (20%) in the general population. The familial pattern of BD in our Arab patients is attributable to the extremely high rate of consanguinity in this population, lending further support to arguments that favour a genetic component for BD, which in inbred populations does not reclaim the contribution of modifiers. Ultimately, the presence of major still-unknown gene effects that either, cause, precipitate the onset and/or enhance the susceptibility of patients to BD remain to be identified.

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