Behçet’s disease and hereditary periodic fever syndromes: Casual association or causal relationship?

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ABSTRACT

Objective. Mutations in the MEFV and the type 1 TNF receptor (TNFRSF 1A) genes have recently been linked to familial Mediterranean fever (FMF) and TNF receptor-associated periodic syndrome (TRAPS), respectively. A higher prevalence of Behçet’s disease (BD) among FMF patients has been described compared to the general population. The aim of this study was to evaluate whether FMF, TRAPS and BD could be genetically related.

Methods. We screened a cohort of 50 BD patients and 100 healthy subjects for the common MEFV and TNFRSF 1A mutations. An initial screening of exons 10 and 2 of the MEFV gene and exon 4 of the TNFRSF 1A was performed in all chromosomes.

Results. The heterozygous MEFV mutation (K695R) was found in one (2%) BD patient. Analysis for FMF mutations in the control group revealed that 5 (5%) individuals bore MEFV gene mutations (3 were heterozygous for the E148Q and 2 were heterozygous for the A744S). At codon 202, there were no differences in allele frequencies between BD and control population: 73%R 27%Q in the BD patients vs 75%R 25%Q in controls. Concerning mutations in the TNFRSF 1A gene, the R92Q mutation was present in heterozygous state in one (2%) BD patient and in 4 (4%) controls without differences between allele frequencies: 99%R 1%Q in BD patients vs 98%R 2%Q in controls, respectively. There was no association between the clinical manifestations of BD patients and the presence of a particular polymorphism or a mutation.

Conclusions. Neither FMF nor TRAPS are genetically associated with BD in our cohort of Spanish patients.

Introduction

The hereditary periodic fever syndromes (HPFS) are a subset of autoimmune inflammatory diseases that are characterised by episodes of fever with localised serosal, synovial and/or cutaneous inflammation (1). The most frequent of these syndromes is familial Mediterranean fever (FMF), which affects the population of the Mediterranean basin. It is characterised by recurrent episodes of fever, serosal inflammation resulting in sterile peritonitis, arthritis and pleurisy (2). FMF is recessively inherited and the responsible gene is MEFV (3, 4), which encodes pyrin/marenostrin, a protein of unknown function. TNF receptor-associated periodic syndrome (TRAPS) is a hereditary fever syndrome that resembles FMF (5). However, the mode of inheritance is dominant and the duration of the fever episodes is longer than in FMF. TRAPS results from mutations in the TNF receptor superfamily 1A (TNFRSF 1A) gene that has long been recognised to play an important role in inflammation and immunity (5).

Behçet’s disease (BD), a systemic vasculitis of unknown aetiology, can also cause periodic fever (6). BD has a world-wide distribution, although most cases are reported from Japan, the Middle East, and the Mediterranean basin. It is characterised by recurrent oral and genital ulcers and uveitis, but cutaneous, articular, neurologic, or vascular manifestations have also been observed (7). Unlike HPFS, BD is not a single-gene associated disease: it seems to be a multigenic disease with contributing environmental factors (8).

Lately, there has been suggested the association of BD and FMF. In 1997, Schwartz et al. found 39 patients with concurrent FMF and BD (9), 16 of them had the complete syndrome according to the International Study Group for Behçet’s Disease criteria (10). Birlik et al. (11) described a case involving coexistence of FMF and BD and suggested that both disorders may have a common etiopathogenic mechanism. Re-
cently, Ben-Chetrit et al. (12) evaluated the frequency of BD among FMF patients and the reverse association. Their screening of 353 FMF patients and 53 with BD revealed 2 individuals who had both diseases concomitantly. The statistical analysis supported the finding that the association between FMF and BD was higher than expected in both directions (FMF in BD and BD in FMF). Nevertheless, the small number of patients (only 2) with concomitant disease was of concern. Tougou et al (13) discovered a higher than expected frequency of MEFV mutations in BD compared with a controlled cohort of healthy individuals from the same ethnic origin. On the other hand, in the study of Ben-Chetrit et al. (12) there was no difference in any of the clinical manifestations of BD in patients heterozygous for MEFV mutations, as compared with those who had no mutations at all.

Critical role of TNF in BD suggests that the interaction of both TNF and TNF receptor polymorphisms could contribute to the pathogenesis of BD (14). The prevalence of the TNFRSF 1A gene mutations in BD has been studied in only one series (15). This study found a high frequency of R92Q TNFRSF 1A mutation in European patients with BD.

The aim of the study is to evaluate whether the FMF, TRAPS and BD could be genetically related. To address this question, we screened a series of 50 BD Spanish patients for the common MEFV and TNFRSF 1A mutations.

Materials and methods

Patients

We studied 50 Spanish patients with BD (27 men and 23 women). No Jewish ancestry or other ancestry in populations of high FMF and TRAPS incidence was known for those patients. The mean (±SD) age at onset of the disease of the 50 BD patients was 27 ± 12 years (range, 10 to 58). The mean age at diagnosis was 32 ± 13 years (range, 16 to 62); and the mean age at study inclusion was 35 ± 14 years (range, 16 to 67). Follow-up ranged from 1 to 242 months, with a median of 36 months. All patients fulfilled three or more of the International Study Group criteria for the diagnosis of Behçet’s disease (10). Blood samples were collected a median of 15 months (range, 0 to 129) after diagnosis during an inactive phase of the disease. We also analysed a control group of 100 anonymous autochthonous mainland Spanish healthy unrelated subjects (54 women and 46 men; mean age, 41 ±18 years) without autoimmune disease. The study was approved by the Human Experimental Committee of the Hospital Clinic, Barcelona, Spain, and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Sampling

Samples for genotype studies were drawn in trisodium ethylene-diamine-tetraacetic acid tubes (Becton, Dickinson and Company), and 10 mL of whole blood was transferred into tubes containing lysis buffer (5 mol/L guanidine thiocyanate, 1.3% [w/v] Triton X-100, and 50 mM Tris-HCl, pH 6.4) and frozen at -70°C.

DNA extraction

Genomic DNA from whole blood samples was isolated using QIAmp DNA Blood Mini Kit (QIAGen, Germany) following the manufacturer’s instructions.

HPFS-associated genes mutational screening

A mutational screening of exons 10 and 2 of the MEFV gene and exon 4 of the TNFRSF 1A was performed in all patients and controls enrolled in this study. According our previous experience in the MEFV mutational analysis performed on the Spanish population (data not published), we have considered that exploring exons 2 and 10 accounts for more than 96% of the mutations detected. In particular, the mutations detected in exon 10 account for 75.7% of the mutated alleles and that mutations in exon 2 account for the 20.7% of mutated alleles. Exonic and intronic flanking regions were amplified by polymerase chain reaction (PCR) using specific intronic primers and amplification conditions previously published (4,5). The PCR products were purified using QIAquick PCR Purification Kit (QIAGen, Germany), according the manufacturer’s instructions, sequenced using an ABI BigDye Terminators v1.1 Cycle Sequencing Kit (Foster City, CA) and run on an ABI 3100 automatic sequencer.

Statistical analysis

Fisher’s exact test (2-sided) was used for comparison of proportions.

Results

All BD patients had oral ulcers. Genital ulcers were present in 66%, cutaneous involvement in 68%, ocular involvement in 55% (32% in form of posterior uveitis), and vascular involvement in 37%.

Only one (2%) BD patient was found to carry one single MEFV mutation, being heterozygous for the K695R mutation. Analysis of the genomic DNA for FMF mutations in the control group revealed that 5 (5%) individuals bore MEFV gene mutations. Three of them were heterozygous for the E148Q and the remaining two were heterozygous for the A744S mutation. At codon 202, there were no differences in allele frequencies between control and BD population: 75% R and 25% Q in the control group and 73% R and 27% Q in BD patients.

Concerning mutations in the TNFRSF 1A gene, the R92Q mutation located in exon 4, was present in heterozygous state in one (2%) of the BD patient and in 4 (4%) of the controls (p not significant) without differences between allele frequencies: 99% R and 1% Q vs 98% R and 2% Q in BD patients and controls, respectively. There was no apparent association between the clinical manifestations and the presence of a polymorphism or a mutation.

Discussion

Our study demonstrates that neither FMF nor TRAPS are genetically associated with BD in our cohort of patients. In addition, R202Q polymorphism is not associated with FMF in Spanish population and does not confer a
significant risk at suffering FMF, as previously reported by our group (16). Although it is true that the frequency of MEFV mutations are somewhat increased among BD, more recent epidemiological data were not supportive of the proposed association between BD and FMF. In the one hand, a clinical relationship between FMF and BD has been described in Israel, with a slightly higher prevalence of BD among FMF patients compared to the general population (9). Furthermore, some MEFV mutations were shown to be more frequent in BD patients from Turkey than in controls, suggesting that they act as additional susceptibility factors in BD (17). These authors suggested that MEFV mutations are associated with vascular involvement in these BD patients. Livneh et al. (18) evaluated the effect of BD on the expression of MEFV phenotype in carriers of a single mutated MEFV allele. The authors suggested that the MEFV phenotype was associated with the simultaneous presence of BD.

In the other hand, Ben Chetrit et al. (12) screened 353 charts of patients with FMF to detect individuals with concomitant BD. In addition, they studied 53 patients with BD, looking for FMF and for their MEFV mutations. None of 353 patients with FMF was found to have concomitant BD. Sixteen patients with BD bore MEFV mutations, 2 of whom were symptomatic homozygotes and had concomitant FMF. The authors did not find differences between both BD groups (with or without MEFV mutations) in their clinical manifestations and disease course.

In our cohort of BD patients, both FMF and TRAPS are not genetically associated with BD. These results are in accordance with the study of Touitou (13). These authors stratified their cohort of BD patients into two groups: those commonly affected by FMF (Arabs, Turks, and non- Ashkenazi Jews) and those not commonly affected by FMF (Italians and French). The MEFV gene mutations were more frequent in the group of BD patients commonly affected by FMF. The fact that MEFV mutations are found in BD patients from populations with a high carrier rate is not surprising. It is still possible that this finding is related to the ethnic origin of the groups rather than to their FMF disease. Our group compared the Spanish MEFV mutation spectrum with those of 10 other populations and found that the Spanish are closest to the French and Italian MEFV spectra; this genetic distance is half to that to next closest population, the Turks. Then, a Western Mediterranean MEFV mutation spectrum can be defined, which is at least quantitatively distinct from that of so-called ancestral populations (16). The prevalence of the TNFRSF1A gene mutations in BD has been studied in only one series (15). It found a high frequency of R92Q mutation in European BD patients, most of them from Paris and its area, and it was associated with an increased risk of extracranial venous thrombosis. It is possible that the results of polymorphisms studies differ according to the ethnic origin of population studied. Furthermore, it is likely that the phenotypic manifestations of R92Q in BD depend on other linked or unlinked modifying genes and/or on modifying environmental factors.

The different ethnic origins may explain differences in terms of genetic susceptibility to autoimmune diseases. Our study demonstrates that neither FMF nor TRAPS are genetically associated with BD in our cohort of Spanish patients. The low carrier rate of MEFV and TNFRSF1A mutations in our population and the low prevalence of both BD and HPFS (19) in our country may explain this lack of correlation. Further studies on MEFV and TNFRSF1A mutations in other ethnic groups will be required to determine the influence of these mutations in BD pathogenesis.

References

17. ATAGUN DZ, EROGLU T, DIREKSENDIL H: MEFV mutations are increased in Behçet’s disease (BD) and are associated with vascular involvement. Clin Exp Rheumatol 2003; 21(suppl 30): S35-37.