

## Mediterranean Fever Gene Mutations in Greek Patients with Behcet's Disease

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### ABSTRACT

**Objective:** It is known that clinical similarities between Behcet's disease and Familial Mediterranean Fever have led to the hypothesis of a common pathogenesis. Familial Mediterranean Fever is caused by MEFV gene mutations coding for pyrin. Therefore, we examined whether these pyrin mutations are also associated with Behcet's disease.

**Methods:** Molecular testing for pyrin mutations was performed in 96 unrelated Greek patients with an established diagnosis of Behcet's disease. The results were compared with an analysis for pyrin mutations in 140 unrelated healthy Greek controls.

**Results:** We found no pyrin mutations among the Behcet cases tested; this result is comparable with the control group.

**Conclusions:** Pyrin gene mutations in Greek patients with Behcet's disease are not more common than those in the general population. This finding is not in agreement with the findings in other populations. It is suggested that screening for pyrin mutations not be included in the evaluation of Greeks suspected to have Behcet's disease.

**Keywords:** Behcet's disease, familial mediterranean fever, Greece, pyrin

## Mutaciones en el Gen de la Fiebre Mediterránea en Pacientes Griegos con la Enfermedad de Behçet

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### RESUMEN

**Objetivo:** Se sabe que las similitudes clínicas entre la enfermedad de Behçet y la fiebre mediterránea familiar han llevado a la hipótesis de una patogénesis común. La fiebre mediterránea familiar es causada por mutaciones en el gen MEFV que codifica la pirina. Por lo tanto, examinamos si estas mutaciones de la pirina se hallan también asociadas con la enfermedad de Behçet.

**Métodos:** La prueba molecular para la detección de las mutaciones de la pirina se realizó en 96 pacientes griegos no relacionados, y diagnosticados con la enfermedad de Behçet. Los resultados se compararon con un análisis de las mutaciones de la pirina en 140 controles formados por individuos griegos saludables.

**Resultados:** No se encontraron mutaciones de pirina entre los casos de Behçet sometidos a prueba. Este resultado es comparable con el grupo control.

**Conclusiones:** Las mutaciones del gen de la pirina en los pacientes griegos con la enfermedad de Behçet no son más comunes que las de la población general. Este hallazgo no concuerda con los hallazgos en otras poblaciones. Se sugiere que el tamizaje para la detección de las mutaciones de pirina no se incluya en la evaluación de pacientes griegos sospechosos de padecer la enfermedad de Behçet.

**Palabras claves:** Enfermedad de Behçet, fiebre mediterránea familiar, Grecia, pirina

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## INTRODUCTION

Behcet's disease (BD), is a chronic inflammation characterized by recurrent oral and genital ulcers, ocular and skin lesions. Involvement of brain, blood vessels and the gastrointestinal tract is less frequent but may be life-threatening. Aetiology and pathogenesis is unclear but it probably occurs as a result of environmental triggering in genetically susceptible subjects (1). Histocompatibility genes play an important role; infectious agents may also be involved (2, 3). High titers of serum *Saccharomyces cerevisiae* antibodies were reported in BD but their role remains unclear (4).

Behcet's disease prevails along the old silk trading routes in Central Asia and the Middle East; however, many cases have been reported all over the world (5, 6). As no specific markers or laboratory findings exist, diagnosis relies on clinical criteria accepted by the International Study Group for BD (7). As disease manifestations vary in different parts of the world, it is obvious that diagnosis may pose difficulties and it may not be universally accepted in all cases. It is also evident that until the established diagnostic criteria are fulfilled, diagnosis may be delayed, even for years (4). This may be the case in the early stages when not all diagnostic criteria are met. Consequently, any marker facilitating diagnosis before a full spectrum of manifestations may be of practical value (4).

Familial Mediterranean Fever (FMF) is an "auto-inflammatory" disease, characterized by recurrent attacks of fever associated with aseptic serositis (peritonitis, arthritis, pleuritis), skin lesions and eventually complicated by amyloidosis (8). It is transmitted as an autosomal recessive trait and it is caused by point mutations of MEFV gene coding for pyrin protein. Pyrin normally plays an elusive, albeit crucial role in inflammation. In areas with a high prevalence of FMF, many heterozygotes are found which implies a heterozygote selection advantage (9). The commonest FMF associated MEFV gene mutations are roughly similarly distributed among populations affected, suggesting that these mutations date back to times before 2500 BC when all affected population groups lived together across the major Middle East area (10, 11).

As FMF and BD share common epidemiological and clinical features, clinical presentation may give rise to incorrect diagnosis in either direction in populations where they prevail (12). Co-existence of the two entities due to common pathogenesis or common genetic background is proposed by several authors.

We tested the prevalence of MEFV gene mutations among Greek patients with BD. Information of this type to the best of our knowledge is lacking for most European populations including Greeks.

## SUBJECTS AND METHODS

We studied a total of 96 BD patients of Greek origin (60 males, 36 females) aged between 17 and 70 years (mean age 38.5). All cases fulfilled the standard International Study Group criteria for BD: recurrent oral ulceration plus two of the following (a) recurrent genital ulceration, (b) ocular lesions, (c) skin

lesions and (d) pathergy test (7). The spectrum of manifestations in the cases tested was as follows: oral ulcers in 100%, genital ulcers in 65.4%, skin lesions (including erythema nodosum and pseudofolliculitis) in 58%. No patient reported paroxysmal symptoms of any kind reminiscent of FMF or clinical signs that point to bowel involvement; no patient reported a family history of FMF; pathergy test was positive in 38% and the HLA B51 antigen was positive in 77% of the cases. For comparison, a group of 140 healthy Greek individuals tested for FMF-associated pyrin mutations (as already published) was used as controls (12). Informed consent and approval by the Local Ethics Committee were given.

Peripheral blood DNA was tested for MEFV gene mutations in BD cases by the standard FMF ViennaLab strip assay (ViennaLab, Vienna, Austria). This assay is based on the reverse-hybridization principle, and includes three successive steps, namely: (i) DNA is isolated from anticoagulated peripheral blood; (ii) MEFV gene sequences are simultaneously *in vitro* amplified and biotin-labelled in a single («multiplex») amplification reaction; (iii) amplification products are selectively hybridized to a test strip containing oligonucleotide probes (wild type and mutant-specific). The bound biotinylated sequences are detected using streptavidin alkaline phosphatase and colour substrates.

The following MEFV gene mutations were tested: E148Q, P369S, F479L, M680I [G/C], M680I [G/A], I692del, M694V, M694I, K695R, V726A, A744S, R761H. These twelve mutations cover the vast majority of FMF-associated mutations of the MEFV gene.

Testing of the controls for pyrin mutations was performed after NIRCA (non-isotopic RNase cleavage assay) and sequencing of *MEFV* exons 2 and 10 (12). The validity of both methods in detecting the MEFV mutations is fairly equivalent. Statistical analysis was performed with the online statistical tool freely available at <http://statpages.org>.

## RESULTS

No case carrying pyrin mutation of those tested was detected in the 96 BD cases whereas two were found to be carriers of *MEFV* mutations within the 140 healthy controls (12). Accordingly, in regard to the frequency of these mutations, it is obvious that no statistically significant difference exists between the BD patients and the population of the country (Yates' corrected Chi-square  $p = 0.650$ ).

## DISCUSSION

According to many authors, the clinical differences between BD and FMF outnumber their similarities (13). However, several common features in the epidemiological patterns, together with common clinical symptoms, may confuse clinicians. Furthermore, colchicine, the drug of choice for FMF, may prove to be of some benefit for many manifestations of BD thus leading to a further diagnostic confusion (14). The beneficial effect of other immunomodulators like thalidomide in both BD and FMF may also add to the confusion (15).

In view of all these facts, some authors concluded that the concomitant occurrence of FMF and BD is higher than expected in the populations studied (16). The authors suggest that in FMF-BD co-sufferers, the severity of FMF is moderate and the spectrum of BD manifestations is limited. A study from Israel revealed that 44.4% of BD cases carried one or two MEFV gene mutations (17). Interestingly, these BD sufferers were found to be at higher risk of venous thrombosis, a known complication of BD. In another study, pyrin mutations were detected in 16 out of 53 BD cases of Jewish and Arab origin (18).

In people of Turkish origin, some 26% of BD cases were found to be heterozygotes for pyrin mutation, as opposed to 9.1% of controls (19). In another report from Turkey, 36% of BD cases carried pyrin gene mutations compared to 11% in healthy controls (20). The authors concluded that pyrin gene mutation is involved in the pathogenesis of BD in Turkey.

High frequency pyrin mutations compared to controls were also reported in BD cases of mixed French, Arab and Turkish origin (21). Some authors reported a high frequency of pyrin mutation heterozygosity (> 40%) among BD cases in Palestinians, including pyrin mutations not as yet described in FMF patients. The authors claim that these mutations may be a factor of additional susceptibility to BD (22).

The criticism about all such studies focusses on the appropriate controls. The fact that pyrin mutations are frequently encountered among BD cases from populations where the incidence of FMF is high is not surprising. In such populations, high frequency of pyrin mutations could be expected in practically every disease one could study (13). Therefore, for a clearer understanding of the putative relation between FMF and BD, one should consider BD cases from areas with a rarity of FMF. To this effect, a study in a Spanish population reporting no correlation between pyrin mutations and BD is of importance (23).

Two recent genome-wide association studies (GWAS) point to the association of two genomic areas with BD, namely MHC class I region and IL23R-IL12RB2 region (24, 25). In these two GWAS, pyrin gene (MEFV) area was not covered, although it is well-known that this area affects cytokine profile. In this context, it becomes evident that the list of the candidate genomic areas involved in BD has not been exhausted (24, 25). Familial Mediterranean Fever is uncommon in Greeks although in recent times the disease is considered to be commoner than believed in the past; however, most authors conclude that it remains considerably less frequent than in countries such as Israel, Turkey or Armenia (26). The epidemiology of Behçet's disease in Greece is limited but local surveys estimate a frequency for BD of 400/100 000 (27). Therefore, BD appears to be less common than considered to be in the East. Due to the rarity of both entities in this country, should a link in molecular pathogenesis exist, their co-occurrence could be detected more easily.

## CONCLUSIONS

This work provides an interesting perspective on lack of connection between the two disease entities BD and FMF. A practical point from this study may be that in doubtful BD cases, pyrin mutation testing should not be part of the initial evaluation of the patient.

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