ABSTRACT

Objective. Hereditary periodic fever syndromes (HPFS) are rare diseases characterised by recurrent, self-limited episodes of fever and localised inflammation, which arise from monogenic defects. In the present study we describe the clinical features, laboratory parameters and genetic profile of adult patients.

Methods. Samples examined between May 2010 and December 2012 at the laboratory of genetic molecular diagnosis of the department of Pathophysiology of School of Medicine, National University of Athens.

Results. Of the MEFV gene variants the most frequent genotype was the E148Q heterozygosity, with patients presenting with the typical clinical picture, two patients were positive for the pR92Q/c.362G>A mutation in heterozygosity. The testing for the Hyper IgD Syndrome was positive for the pV377I/c.1129 G>A heterozygosity in a patient with the corresponding typical picture and the testing for the CAPS syndromes was positive for a new mutation, pR170H/c.509G>A in heterozygosity, in a case with less typical clinical features.

Conclusion. Availability of genetic testing in everyday clinical practice can provide valuable information regarding the clinical diversity, geographic distribution and genetic characteristics of these rare disease in all age groups.

Introduction

Hereditary periodic fever syndromes (HPFS) are rare diseases characterised by recurrent, self-limited episodes of fever and localised inflammation, which arise from monogenic defects and include familial Mediterranean fever (FMF), hyperimmunoglobulinemia D and periodic fever syndrome (HIDS), TNF receptor associated periodic syndrome (TRAPS) and the cryopyrin-associated periodic syndromes (CAPS). FMF, an autosomal recessive disease, the most prevalent among these diseases with an estimated prevalence of 1:400–1:1,000 in countries of the eastern Mediterranean basin (1), is caused by mutations in the MEFV gene (16p13) (2-4). HIDS is a rare disease, originally described in patients of Dutch ancestry with more than 90% of all cases of HIDS to have been reported from the Netherlands. It is caused by mutations in the gene MVK (12q24), which encodes mevalonate kinase. TRAPS is a disease caused by mutations in TNFRSF1A gene (12p13) which encodes the type I TNF Receptor (p55). Northern Europeans are mostly affected with a variable age of onset of symptoms. The cryopyrin-associated periodic syndromes are a group of diseases caused by mutations in NLRP3/CIAS1 gene which lead to NLRP3-dependent IL-1b excessive release. It affects all ethnic groups, with variable age of onset. Studies in the Greek population have described the spectrum of MEFV alterations in FMF patients (5, 6) and new mutations have been reported (7) while the other diseases are less frequent. This is the first brief report of the clinical manifestations and genetic testing results in the adult greek population of this group of diseases.

Patients and methods

Patients Between May 2010 and December 2012 a total number of 124 samples of peripheral blood of patients were examined for mutations of the genes of HPFS at the laboratory of genetic molecular diagnosis of the department of Pathophysiology of School of Medicine, National University of Athens. Of those patients, 54 (43.5%) were under 18 years. For the 70 samples of adult patients available data was collected retrospectively. Samples were sent from all the geo-
Periodic fever in adult Greeks / T.P. Karatsourakis et al.

graphic areas of Greece, based on the individual judgement of the physician for each patient, in a suggestive clinical context. In each case a workup was performed individually and patients who did not fulfill criteria for other diseases were examined for a set of genes of the periodic syndromes, depending on the most probable diagnosis and the previous testing.

The main demographic characteristics were recorded (age, sex, race), the clinical features of patients based on the presence or absence of unexplained fever (>38°C) with a periodic pattern and presence of all relevant clinical manifestations. The laboratory parameters were the results of the genotype analysis, leukocytosis and elevated levels of CRP (due to the different laboratory methods used, a significant rise in the serum levels was defined as a value greater than three times the upper limit of normal).

Mutation analysis

Whole blood was collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes and genomic DNA was isolated using the commercial kit QIAamp DNA blood mini kit (Qia-gen). For the mutational analysis of the MEFV gene, the FMF Strip Assay kit (ViennaLab) was applied for the detection of the 12 most common mutations: M680I(G/C) heterozygosity, 2 cases with homozygosity also recorded. E148Q heterozygosity, with a case of each of M694V, K695R, V726A, A744S and R761H. For the mutational analysis of the TNFRSF1A, NALP3 and MVK genes, selected coding sequences, which are considered as mutational hotspots, were analysed by polymerase chain reaction (PCR) and direct sequencing. The primers were designed to include exons and exon – intron boundaries in the respective amplimers. PCR products were purified using the Purelink PCR purification kit (Invitrogen). The purified PCR products were directly sequenced using the DTCS QUICK START kit (Beckman coulter) and the CEQ 8000 Genetic analysis system (Beckman coulter).

Results

124 samples were evaluated. Positive samples, for at least one mutation of known or unknown significance were as follows: 14 of 106 patients tested for the MEFV gene, 2 of 62 patients tested for TNFRSF1A gene, 20 of 59 patients tested for the MVK gene, and 2 of 46 patients tested for NLP3/CIAS1 gene mutations. 71 patients were positive for non-pathogenic mutations. Data for adults patients will follow.

1. FMF

The most frequent genotype was the E148Q heterozygosity, with a case with homozygosity also recorded. Other genotypes included 2 cases of M680I(G/C) heterozygosity, 2 cases of R761H heterozygosity and one case of each of M694V, K695R, V726A, A744S heterozygosity. Two of the patients were compound heterozygotes for two different variants. There was not any patient with E148Q/V726A double mutation.

The clinical features of these patients are shown in Table I.

All patients presented with the typical clinical manifestations in mild intensity. The single unusual manifestation was sensorineural hearing loss in a 21 old female patient with periodic fever from early childhood, abdominal pain and ascites, heterozygous for E148Q mutation who gradually developed almost complete bilateral hearing loss, unexplained otherwise.

Patients were treated mainly with colchicine, with daily doses up to 1mg in most cases and 3 patients required addition of anakinra 100mg daily due to unsatisfactory control of their symptoms (8, 9). There was no case of amyloidosis.

2. TRAPS

Two patients were positive for the pR92Q/c.362G>A mutation of the TNFRSF1A gene in a heterozygous state. One of the two, who was the best described, was a 40-year-old male originating from South Africa from black father and Greek mother. He presented with recurrent episodes of fever with bilateral pleurisy and pericarditis which were treated with NSAIDS.
corticosteroids, azathioprine and pericardiectomy. There is no clear family history of periodic fever syndromes in either the paternal or maternal lineage. He is currently under anakinra with dosing being gradually reduced to twice weekly subcutaneous injections.

3. HIDS
The testing for the MVK gene was positive for pV377L/c.1129 G>A mutation in heterozygous state, in a 40 year old male with recurrent episodes of fever beginning from the age of 8 months, generalised macular rash of the trunk and limbs in the first year of his life, cervical, axillary and inguinal lymphadenopathy with periodic recurrence, frequent episodes of abdominal pain with ascesis and arthritis of the MCPs, PIPs, knees and ankles. In the first years, the periodic episodes of fever were up to 40°C, with duration of three to six days once every month approximately and maculopapular generalised rash, episodes of abdominal pain with diarrhea and arthritis of knees and ankles dominated the clinical picture. Later during the disease course, rash and arthritis became less frequent and generalised lymphadenopathy was present during the longer episodes of fever with the most intense abdominal pain. NSAIDS and corticosteroids were prescribed initially and later on colchicine was added with better results regarding the control of fever and the episodes of abdominal pain but with gastrointestinal intolerance in medium doses. In the last 6 years the patient is under anakinra with substantial improvement. To our knowledge, this is the first case of the Hyper IgD syndrome in the Greek population, diagnosed on the basis of the clinical picture and the genetic testing. The same patient also carries a new mutation c.850delG in heterozygous state. 27 of the examined patients carried non pathogenic variants and 19 mutations of unknown significance (Table II).

4. CAPS
After mutational analysis of the NLP3/CIAS1 gene, a new mutation, causing a substitution of guanine for adenine at position 609 leading to a substitution of arginine from a histidine at codon 170 (pR170H/c.509G>A) in heterozygous state was detected, in a 57-year old woman with recurrent episodes of daily fevers, polyarthritis, with episodes of pericarditis and abdominal pain, accompanied by raised CRP and ESR levels. She is treated with low doses corticosteroids and daily subcutaneous injections of anakinra with a better response. 44 of the examined patients carried non pathogenic variants.

Discussion
In our present study we describe patients from all geographic regions of Greece, tested or retested thoroughly. Of the FMF gene variants the most frequent genotype was the E148Q in heterozygosity. A case with sensorineural hearing loss was recorded, a feature more frequent for the infantile periodic syndromes but also reported in literature in other two cases with co-existent mutations in the CIAS1 and MEFV genes, but the coexistence of sensorineural hearing loss and FMF may still be fortuitous or a coincidence. We describe a patient with HIDS, positive for pV377L/c.1129 G>A mutation in heterozygous state with typical the clinical manifestations for HIDS as the first Greek patient carrier of this mutation to our knowledge. Although the majority of patients are of Dutch or northern European ancestry, there are several cases reported in other Mediterranean and southern European countries as well (11-13).

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<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Caucasian</th>
<th>IVS8.c.769-38C&gt;T Status</th>
<th>Another mutation</th>
<th>Body temperature &gt;38°C</th>
<th>Arthralgias</th>
<th>Arthritis</th>
<th>Skin involvement</th>
<th>Pleuressy</th>
<th>Pericarditis</th>
<th>Abdominal pain</th>
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Table II. Clinical features of patients with HIDS IVS8.c.769-38C>T mutation.
Two new mutations were found, one for the MVK gene and one for the NLP3/CIAS1 gene.

The present study reflects the importance of genetic testing in everyday medical practice as a source of collecting data on these diseases. Wider awareness of these syndromes and their wide clinical spectrum, in a national and international scale is essential to provide information for clinical and laboratory research in this relatively new field. There is an increase in diagnosing periodic fever syndromes, especially FMF, along with earlier diagnosis and better description of milder clinical phenotypes in other Mediterranean countries with similar prevalence of these diseases in the last years as well (14, 15).

References
12. TAs DA, DINKCI S, ERKEN E: Different clinical presentation of the hyperimmunoglobulin D syndrome (HIDS) (four cases from Turkey). Clin Rheumatol 2012; 31: 889-93.