Periodic fevers in adult Greeks: clinical and molecular presentation

T.P. Karatsourakis, K.N. Oikonomaki, A.G. Tzioufas, H.M. Moutsopoulos

Department of Pathophysiology, School of Medicine, University of Athens, Athens, Greece.

Theofilos P. Karatsourakis
Katerina N. Oikonomaki
Athanasios G. Tzioufas, PhD
Haralampos M. Moutsopoulos, PhD

Please address correspondence to:
Theofilos P. Karatsourakis,
75 Mikras Asias Street,
Athens 11527, Greece.
E-mail: karatsourakis@yahoo.gr

Received on September 1, 2013; accepted in revised form on December 5, 2013.

Clin Exp Rheumatol 2014; 32 (Suppl. 84): S45-S48.

Key words: periodic fever, familial Mediterranean fever, hyper IgD syndrome, CAPS syndromes, Greeks

Funding: this work was supported by Founding Account for Research of The National and Kapodistrian University of Athens.

Competing interests: none declared.

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-

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 (Qiagen). 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: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, 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. TRAP***S**

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,

Table I. Clinical features of patients with presence of E148Q mutation.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
		Fulfilling Tel Hashomer Criteria			Not Fulfilling Tel Hashomer Criteria	
Sex	F	M	F	F	M	M
Age	21	18	39	48	58	22
Caucasian	Yes	Yes	Yes	Yes	Yes	Yes
E148Q						
Status	Heterozygous	Homozygous	Heterozygous	Heterozygous	Heterozygousous	Heterozygous
Body temperature >38^{O}C	Yes	Yes	Yes	No	Yes	No
Arthralgias	Yes	Yes	Yes	No	No	Yes
Arthritis	No	No	No	No	No	No
Pleuresy	No	No	Yes	No	Yes	No
Abdominal pain	Yes	Yes	Yes	Yes	No	Yes
Ascitis	Yes	No	Yes	No	No	No
Sensorineural hearing loss	Yes	No	No	No	No	No
Cervical lymphadenopathy/ Splenomegaly	No/Yes	No/Yes	Yes/No	No/No	No/No	No/No
Presence of leukocytosis	No	No	No	No	Yes	No
CRP levels >3Uln	Yes	Yes	Yes	No	Yes	No

Table II. Clinical features of patients with HIDS IVS8 c.769-38C>T mutayion.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Sex	M	M	F	F	M	F	F
Age	40	33	56	65		47	50
Caucasian	Yes	Yes	Yes	Yes	Yes	Yes	Yes
IVS8 c.769-38C>T Status	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Homozygous	Homozygous
Another mutation	pV377I/c.1129 G>A heterozygous, c.850delG heterozygous (new mutation)	FMF A744S heterozygous	IVS8 c.769-7T>G heterozygous				
Body temperature >38^{O}C	Yes	Yes	Yes	No	No	Yes	Yes
Arthralgias	Yes	No	Yes	No	Yes	Yes	Yes
Arthritis	Yes	No	Yes	No	No	No	No
Skin involvement	Yes	No	No	No	No	No	Yes (nodules)
Pleuresy	No	No	No	Yes	No	No	No
Pericarditis	No	Yes	Yes	No	No	No	No
Abdominal pain	Yes	No	Yes	Yes	No	No	No
Ascitis	Yes	No	No	No	No	No	No
Generalised lymphadenopathy	Yes	No	No	No	No	No	No
Presence of leukocytosis	Yes	No	No	No	No	No	No
CRP levels	Yes	No	Yes	No	No	No	No

corticosteroids, azathioprine and pericardiotomy. 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 pV377I/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, axilary and inguinal lymphadenopathy with periodic reoccurrence, frequent episodes of abdominal pain with ascites 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 (10), but the coexistence of sensorineural hearing loss and FMF may still be fortuitous or a coincidence.

We describe a patient with HIDS, positive for pV377I/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).

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 this diseases in the last years as well (14, 15).

References

1. BEN-CHETRIT E, TOUITOU I: Familial mediterranean Fever in the world. *Arthritis Rheum* 2009; 61: 1447-53.
2. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. The International FMF Consortium. *Cell* 1997; 90: 797-807.
3. FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
4. EISENSTEIN EM, Y BERKUN, BEN-CHETRIT E: Familial Mediterranean fever: a critical digest of the 2012-2013 literature. *Clin Exp Rheumatol* 2013; 31 (Suppl. 77): S103-S107.
5. GIAGLIS S, PAPADOPOULOS V, KAMBAS K *et al*.: MEFV alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever. *Clin Genet* 2007; 71: 458-67.
6. KONSTANTOPOULOS K, KANTA A, DELTAS C *et al*.: Familial Mediterranean fever associated pyrin mutations in Greece. *Ann Rheum Dis* 2003; 62: 479-81.
7. ZONIOS D, MAVRAGANI CP, GIAGLIS S, RITIS K.: A novel mutation of the familial Mediterranean fever gene in a Greek family related to a non-classical, variably expressed FMF phenotype. *Rheumatol Int* 2007; 28: 167-9.
8. LA REGINA M, BEN-CHETRIT E, GASPARYAN AY, LIVNEH A, OZDOGAN H, MANNA R: Current trends in colchicine treatment in familial Mediterranean fever. *Clin Exp Rheumatol* 2013; 31 (Suppl. 77): S41-S46.
9. BERKUN Y, EISENSTEIN E, BEN-CHETRIT E: FMF - clinical features, new treatments and the role of genetic modifiers: a critical digest of the 2010-2012 literature. *Clin Exp Rheumatol* 2012; 30 (Suppl. 72): S90-S95.
10. SINGH-GREWAL D, CHAITOW J, AKSENTIJEVICH I, CHRISTODOULOU J: Coexistent MEFV and CIAS1 mutations manifesting as familial Mediterranean fever plus deafness. *Ann Rheum Dis* 2007; 66: 1541.
11. D'OSUALDO A, PICCO P, CAROLI F *et al*.: MVK mutations and associated clinical features in Italian patients affected with autoinflammatory disorders and recurrent fever. *Eur J Hum Genet* 2005; 13: 314-20.
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.
13. ABREU TT: [Periodic fever: the first Portuguese case-report of hyper-IgD syndrome (HIDS)]. *Acta Med Port* 2004; 17: 391-4.
14. SORIANO A, MANNA R: Familial Mediterranean fever: new phenotypes. *Autoimmun Rev* 2012; 12: 31-7.
15. LA REGINA M, NUCERA G, DIACO M *et al*.: Familial Mediterranean fever is no longer a rare disease in Italy. *Eur J Hum Genet* 2003; 11: 50-6.