Analysis of NLRP3, MVK and TNFRSF1A variants in adult Greek patients with autoinflammatory symptoms

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ABSTRACT

Objective. *Autoinflammatory diseases are characterised by abnormal hyperactivity of the innate immune system, causing systemic inflammation. The cryopyrin associated periodic syndrome (CAPS), the hyper IgD syndrome (HIDS) and the TNF receptor-associated periodic syndrome (TRAPS), are autoinflammatory conditions associated with mutations in the NLRP3, MVK and TNFRSF1A genes, respectively. We present the experience of our Department with these rare syndromes analysing genetic and clinical data of adult patients encountered between January 2011 and September 2017.*

Methods. *Eighty-eight adult patients with clinical suspicion of CAPS, HIDS and TRAPS were sequentially recruited and genetically tested for specific mutations in NLRP3, MVK and TNFRSF1A using Sanger sequencing. Clinical picture of mutation carriers was reviewed. Allele frequencies were compared to those described for the normal population by the 1000 Genomes project.*

Results. *Seventy-two of the 88 adult patients were found to be positive for mutations or polymorphisms. One patient carried two pathogenic MVK mutations (pV377I/c.1129G>A and c.850delG) and another one carried a pathogenic heterozygous pA439V/c.1316C>T NLRP3 mutation. Seventeen patients carried variants of uncertain significance. The pS434S/c.1302C>T NLRP3 mutation is slightly increased in our patients compared to the reference population and seems to correlate with severe symptom presentation.*

Conclusion. *In rare cases, periodic fever and inflammatory symptoms in adults can be attributed to mutations in NLRP3, MVK and TNFRSF1A. Clinical assessment and genetic analysis are critical for proper diagnosis and treatment of autoinflammatory diseases.*

Introduction

Recurrent episodes of fever where infection and neoplasias have been ruled out, can possibly result from autoinflammatory disorders, a heterogeneous group of disorders with both common and specific features. In these syndromes, episodes of fever and inflammation are usually due to hyperactive innate immunity, characterised by aberrant inflammasome activation and proinflammatory cytokine upregulation. Autoinflammatory syndromes often have genetic basis. Monogenic autoinflammatory conditions include the familial Mediterranean fever (FMF), the cryopyrin associated periodic syndrome (CAPS), the hyper IgD syndrome (HIDS) and the TNF receptor-associated periodic syndrome (TRAPS), associated with mutations in the MEFV, NLRP3, MVK and TNFRSF1A genes, respectively.

MEFV encodes a protein also known as pyrin, involved in the regulation of innate immunity and inflammation in response to interferon gamma (IFN-γ). Over 300 genetic variants of MEFV have been described, the majority benign or of unknown significance. Most pathogenic MEFV mutations causing FMF are located in exon 10 of the gene, which encodes the C-terminal B30.2 protein domain. MEFV mutations are more common in Mediterranean and Middle East populations.

The NLRP3 gene product, cryopyrin 2, is also implicated in inflammasome nucleation, cleavage and activation of caspase 1, and subsequently of proinflammatory cytokines interleukin (IL) 1b, IL-18, NFκB and other molecules. Most known pathogenic NLRP3 mutations causing CAPS are gain of function missense mutations in exon 3, resulting in aberrant activation of the molecule.

The MVK gene encodes the enzyme mevalonate kinase, which catalyses the phosphorylation of mevalonate, a

Table I. NLRP3, MVK and TNFRSF1A allele frequencies in healthy and diseased individuals.

Gene	Variant	Effect	Position	EUR	ALL PAT	CLIN PAT
NLRP3	pR170H (c.509G>A)	Not classified	chr1:247587260	NR	0.0078	**0.0217***
NLRP3	pT219T (c.657C>T)	Benign	chr1:247587408	0.0547	0.0938	0.0652
NLRP3	pA242A (c.726G>A)	Benign	chr1:247587477	0.5487	0.5859	0.6087
NLRP3	pR260R (c.780G>A)	Likely benign	chr1:247587531	0.0408	0.0703	**0.1304***
NLRP3	pD310D (c.930C>T)	Likely benign	chr1:247587681	0.0010	0.0078	
NLRP3	pL411L (c.1231C>T)	Benign	chr1:247587982	0.0099	0.0078	
NLRP3	pS434S (c.1302C>T)	Benign	chr1:247588053	0.1143	**0.1875***	0.2174
NLRP3	pA439V (c.1316C>T)	Pathogenic	chr1:247588067	0.0000	0.0078	**0.0217***
NLRP3	pQ703K (c.2107C>A)	Uncertain significance	chr1:247588858	0.0507	0.0938	**0.1304***
MVK	IVS2 (c.78+61A>G)	Likely benign	chr12:110012766	0.0795	0.0692	0.0952
MVK	IVS8 (c.769-38C>T)	Likely benign	chr12:110029008	0.1769	0.2154	0.1667
MVK	IVS8 c.769-7T>G	Likely benign	chr12:110029039	0.0109	0.0077	0.0238
MVK	pE284Kfs*17, (c.850delG)	Pathogenic	chr12:110029127	0.0000	0.0077	
MVK	IVS9 (c.885+24G>A)	Likely benign	chr12:110029186	0.1769	0.2000	0.1667
MVK	pV377I (c.1129 G>A)	Pathogenic	chr12:110034320	NR	**0.0154***	
MVK	1245-1246INSG (wt/c.*54_55insG)	Not classified	chr12:110034435-110034436	0.1769	**0.0846****	0.0714
TNFRSF1A	pR92Q, c.362G>A	Uncertain significance	chr12:6442643	0.0189	0.0316	0.0652

Effect described according to Infevers database. Chromosomal positions are based on GRCh37/hg19 Assembly. EUR: European population, 1000 Genomes Phase 3, ALL PAT: All Patients tested in our study, CLIN PAT: Patients with available clinical data. Asterisks indicate statistically significant differences (Fisher's exact test, *p<0.05, **p<0.01).

key step in isoprenoid and cholesterol biosynthesis. HIDS is usually caused by loss-of-function MVK mutations. MVK deficiency and inhibition of the mevalonate pathway has been proposed to impair Toll-like receptor (TLR)-induced phosphatidylinositol-3-OH kinase (PI3K) activation, due to defective protein geranylgeranylation, causing hyper-inflammatory conditions (1). Pathogenic HIDS mutations appear to be more prevalent in the Netherlands.

Finally, TNFRSF1A encodes a receptor of the tumor necrosis factor TNF superfamily. Most pathogenic mutations occur in exons 2, 3, and 4 and have been reported in families of various ethnic backgrounds. At a molecular level, various effects have been proposed for TNFRSF1A mutations, including aberrant folding and subcellular localisation or ligand binding, leading to defective autophagy and innate immunity regulation.

In this brief report we describe the experience of our Department with rare periodic fever syndromes encountered between January 2011 and September 2017. We do not present patients with FMF, as these have been previously reported (2).

Patients and methods

Eighty-eight adult patients with periodic fever and clinical suspicion of autoinflammatory diseases were sequentially recruited and tested for genetic mutations. In all cases, neoplasias and infections had been excluded. Patients presented primarily recurrent episodes of unknown aetiology fevers, arthralgias, serosal inflammation and/or cold-induced rash, and to a lesser degree myalgias, gastrointestinal and mild central nervous system symptoms, suggesting CAPS, HIDS or TRAPS as possible underlying conditions. Patients were either examined by a rheumatologist in our department or initially examined in other centres and referred to us for genetic testing. Whole peripheral blood samples were collected in EDTA containing tubes and DNA was extracted using QIAamp DNA blood mini kit (Qiagen). DNA was amplified using specific primers and subjected to Sanger sequencing (3). The most relevant regions of 3 genes were analysed, namely exons 2, 8, 9, 10, 11 of the MVK gene, exons 2, 3, 4 of TNFRSF1A and exon 3 of NLRP3, including intron/exon boundaries. Allele frequencies were compared with those reported for healthy European individuals sequenced in the context of 1000 Genomes project (4). Two-tailed Fisher's test was performed to compare variant allele frequencies. Available patient clinical data was analysed retrospectively.

Results

Seventy-two of the 88 adult patients were found to be positive for mutations of known or unknown significance or polymorphisms. Two patients carried pathogenic mutations. One patient carried two pathogenic mutations of the MVK gene, pV377I/c.1129 G>A and c.850delG. One patient carried a pathogenic heterozygous pA439V/c.1316 C>T mutation on the NLRP3 gene. Seventeen patients carried mutations of uncertain significance: twelve were heterozygous for the pQ703K/c.2107C>A mutation on the NLRP3 gene, while 5 were heterozygous for the pR92Q/c.362G>A mutation of the TNFRSF1A gene, which has been associated with a milder clinical phenotype of the syndrome (5). The pR170H/c.509G>A mutation of the NLRP3 gene that had not been registered in the Infevers database, was identified in 1 patient (3, 6). Clinical data were available for 24 of the 72 patients carrying any type of mutation or polymorphisms in the genes of interest, the rest being unavailable mainly because the patients were examined in other centres and referred to our Department for the genetic analysis. Available clinical files included 1 of the 2 patients with pathogenic mutations.

By evaluating clinical picture in combination with genetic results, we observed the following. The heterozygous patient for the pathogenic pA439V/c.1316C>T NLRP3 mutation presented with fever, arthralgia, rash and Raynaud's phenom-

enon. Treatment with an interleukin 1 receptor antagonist relieved rash but not the other symptoms, so colchicine was added. Six out of 7 heterozygous patients for the mutation of unknown significance pQ703K/c.2107C>A in NLRP3 exhibited bouts of recurrent episodes of pericarditis. Of these 7 carriers, three were responsive to anti-interleukin 1 treatment, three were responsive to colchicine and one was not responsive to either treatment. Interestingly, pericarditis and in some cases arthralgias or aseptic meningitis were also present in 8 out of 9 patients carrying the pS434S/c.1302C>T mutation of the NLRP3 gene, which was previously considered benign. All these patients were also carriers of mutations pA242A/c.726G>A and pR260R/c.780G>A of the same gene, in a homozygous or heterozygous state. Although the two latter alleles display similar frequencies between the healthy and the diseased individuals compared in our study, pS434S/c.1302C>T seems to be slightly increased in our patients compared to the reference European population (4) (Table I). Finally, the heterozygous patient for the NLRP3 mutation pR170H/c.509G>A also presented bouts of pericarditis and arthralgia, although it needs to be noted that the patient was also heterozygous for pS434S/c.1302C>T of the same gene. This patient responded well to anti-interleukin 1 treatment.

Allele frequency comparisons (Table I) revealed statistically significant differences for the pS434S/c.1302C>T variant of the NLRP3 gene and the pV377I/c.1129G>A and 1245-1246INSG/c.*54_55insG variants of the MVK gene between the European population and the total group of our patients. Moreover, when comparing the group of patients with available clinical data and the European population from the 1000 Genome project, we identified significantly increased frequency of the pR170H/c.509G>A, pR260R/c.780G>A, pA439V/c.1316C>T, and pQ703K/c.2107C>A variants of the NLRP3 gene.

Discussion

Autoinflammatory conditions are heterogeneous and accurate diagnosis is often challenging. Genetic testing complements clinical evaluation towards understanding the underlying pathology and guide proper treatment. In some cases, the correlation between genotype and phenotype is quite straightforward, while in others the link seems to be more complex. Given that these conditions are rare, population data from different ethnic groups can be very helpful in increasing statistical power.

We analysed Greek patients with clinical symptoms suggesting CAPS, HIDS and TRAPS as possible underlying conditions for mutations in the genes associated with these diseases. Detection of pathogenic mutations in our patient group supports that periodic fever syndromes, like HIDS and CAPS can occur in Greek adults and should be considered by clinicians as a possible diagnosis.

Comparison with other studies reveals a similar clinical presentation in our group of patients with that reported for other ethnic groups (8-12). Some apparent differences include lower frequency of lymphadenopathy and gastrointestinal symptoms in our group of patients with HIDS mutations as well as lower prevalence of hearing defects, eye and cold-related symptoms in our group of patients with CAPS mutations compared with patients in other studies. Our patients also presented increased incidence of serositis compared with other reports.

Our results identify R92Q/c.362G>A as the most common TNFRSF1A mutation and A439V/c.1316C>T as the most common pathogenic NLRP3 mutation, which is in agreement with other adult autoinflammatory disease patient studies (11, 13). The other pathogenic mutation identified in our patients, MVK V377I/c.1129G>A, has also been reported as the most common pathogenic MVK mutation in HIDS patients in other studies (8, 9, 13).

In addition to the pathogenic mutations, we have identified a number of MVK, NLRP3, and TNFRSF1 variants considered benign or of unknown significance. Given our targeted analysis, we cannot exclude the presence of other pathogenic mutations, that could account for the presented symptoms. However, our data suggest that variants considered benign could be associated with clinical features, especially when occurring in a combinatorial fashion, which requires medical intervention (7). One such example could be the pS434S/c.1302C>T NLRP3 variant, which is slightly increased in our patient group and appears to associate with specific symptoms and disease severity. This is a synonymous mutation, therefore if proved to be pathogenic, it would be interesting to further examine the mechanisms that would not depend on protein sequence alterations.

Finally, given the rarity of these autoinflammatory syndromes, genetic and clinical data of different ethnic groups could be useful in better understanding their pathologic implications.

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