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MVK, NLRP3, TNFRSF1A and MEFV gene mutation distributions in childhood autoinflammatory diseases: Experiences in North Anatolia

Engin ALTUNDAĞ[1,*], Ümmet ABUR [1], Ömer Salih AKAR [1], Alişan YILDIRAN [2] Mehmet Halil ÇELİKSOY [2], Alberta G. A. PAUL [3], Hatice MUTLU [4], Medine KARADAĞ ALPARSLAN [1] Gönül OĞUR [1]

[1]Department of Genetics , Faculty of Medicine , Ondokuz Mayıs University, Samsun, Türkiye
[2]Division of Pediatric Allergy and Immunology, Department of Pediatrics, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[3]Division of Asthma, Department of Allergy and Immunology, School of Medicine, University of Virginia, Charlottesville, VA, US
[4]Division of Pediatris Genetics, Department of Pediatrics, Faculty of Medicine, Ankara University, Ankara, Türkiye

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Abstract

Autoinflammatory diseases (AID) are characterised by recurrent fever and inflammation without an apparent infectious etiology and include Familial Mediteranean Fever (FMF), Tumour Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS), Hyper-IgD/ Mevalonate Kinase Deficiency Syndrome (HIDS/MKD), Cryopyrin-Associated Periodic Syndromes (CAPS) associated with MEFV, TNFRSF1A, MVK and NLRP3 gene variants. Totally 286 pediatric patients prediagnosed with AID were included in this study. Targeted sequence analysis of MEFV, TNFRSF1A, MVK and NLRP3 genes were performed with Sanger sequencing. Patients were grouped into two categories by the presence of MEFV variant: AIDgroup and MEFV-WTgroup.194 patients fell into AIDgroup and remaining 92 patients were in MEFV-WTgroup. Genetic variants were detected in 69% (135/194) of the patients in first group (AIDgroup). Of these patients, 62 (46%) had MEFV, 41 (30%) had MVK, 20 (15%) had NLRP3, 12 (9%) had TNFRSF1A variants. Pathogenic variants in these genes other than MEFV were detected in 6 (3%) of the 194 patients. Five of them had heterozygous variants in the MVK gene including V377I (four patients) and N205S variants (one patient). Also, in the TNFRSF1A gene N145S variant was detected in only one patient (0.5%). No pathogenic variant was detected in the NLRP3 gene. In second group (MEFV-WTgroup), 3% (3/92) of the patients had pathogenic variants including NLRP3 I313V variant (2 patients) and MVK V377I variant (1 patient). No pathogenic variant was detected in the TNFRSF1A gene. This is the first study to describe the distributions of variants in the MEFV, NLRP3, MVK and TNFRSF1A genes of the pediatric AID population in Central Black Sea region of Turkiye. Our results are consistent with the literature in terms of the variant distribution. However, pathogenic variant rates were lower than the literature data. The variant spectrum was also limited in this study possibly due to a smaller study size.

Keywords: autoinflammatory, MVK, MEFV, fever

1. Introduction

Autoinflammatory diseases are diseases that develop with abnormal activation of the innate immune system without an external stimulus and are characterized by inflammation-related clinical and acute phase elevation (1,2). The absence of autoantibodies or antigen-specific T cells during or between attacks distinguishes these syndromes from autoimmune diseases (3).

Autoinflammatory diseases can be inherited monogenically and polygenically (1,4). Familial Mediterranean Fever (FMF), Cryopyrin-Associated Periodic Syndrome (CAPS), TNF receptor-associated periodic syndrome (TRAPS), Mevalonate Kinase Deficiency/Hyperimmunoglobulin D Syndrome (MKD/HIDS) are common monogenic autoinflammatory diseases with periodic fevers. Variants in FMF related MEFV (exon 1-10), CAPS related NLRP3 (exon 1-10), TRAPS related TNFRSF1A (exon 1-10) and MKD/HIDS related MVK (exon 1-11) genes play a role in etiopathogenesis.

In this group of diseases, recurrent fever, inflammation attacks and a healthy period between attacks are observed. In systemic autoinflammatory diseases, periodic fever, elevated acute phase reactans, peritonitis, pleuritis, pericarditis, arthritis and skin rashes have been reported (1,4). In some patients, systemic amyloidosis accumulation is observed and patients are lost as a result of end-stage renal failure (4,5,6). Although it is frequently seen in the community, many patients are frequently misdiagnosed and treated due to the variability of clinical findings and overlapping clinical findings with different diseases. Therefore, molecular diagnosis in autoinflammatory diseases is very important for the patient to receive the correct diagnosis and treatment.

In this study, we aimed to determine the distribution of variants in MEFV, MVK, TNFRSF1A, NLRP3 genes in patients admitted to department of Medical Genetics with a pre-diagnosis of autoinflammatory disease and to define the prominent autoinflammatory diseases in North Anatolia.

2. Material and Methods

Between April 2014 and June 2015, 286 patients between the ages of 0-18 years who were consulted to Ondokuz Mayıs

*Correspondence: engin.altundag@omu.edu.tr

University Faculty of Medicine, Department of Medical Genetics from Pediatric Allergy and Immunology and Pediatric General outpatient clinics with a pre-diagnosis of autoinflammatory disease (AIH) were included in our study. Data collection was performed retrospectively. The 286 patients were divided into two subgroups. In the first study group, there were 194 pediatric patients with a prediagnosis of autoinflammatory disease (AID group). In the second group, there were 92 patients who had previously undergone MEFV gene analysis due to a prediagnosis of FMF and were found to be "variant negative" (MEFV-WT group) and then consulted to our department for investigation of other autoinflammatory diseases.

DNA was extracted from the peripheral blood samples of the patients with QIAamp DNA Kit (QiagenGmbH, Hilden, Germany). Targeted exons of MEFV, NLRP3, TNFRSF1A and MVK genes were studied by Sanger sequencing method. Exons 2, 3, 5 and 10 of the MEFV gene (NM_000243), exon 3 of the NLRP3 gene (NM_001243133), exons 2-7 of the TNFRSF1A gene (NM_001065) and exons 2-11 of the MVK gene (NM_000431) were amplified using a commertial trademark's primer kits (SeqFinder Sequencing System MEFV Kit, NLRP3 Kit, TNFRSF1A Kit, MVK Kit; GML A.G., Wallerau, Switzerland). PCR was performed in 35 cycles on Applied Biosystems GeneAmp PCR System 9700 (ThermoFisher Scientific Inc., Foster City, CA, USA) .

After the presence of PCR products was detected by gel electrophoresis, a "clean-up" step was performed by enzymatic reactions using the ExoSAP product of GML (GML A.G., Wallerau, Switzerland) to clean the PCR products from PCR artifacts. The PCR products were then prepared for sequence analysis using ABI PrismBigDyeTerminator v3.1 kit (ThermoFisher Scientific Inc. Foster City, CA, USA). ABI Genetic Analyzer 3500XL capillary electrophoresis sequencer (ThermoFisher Scientific Inc., Foster City, CA, USA) was used for this purpose. Sequence data were analyzed with the SeqScape® Software 3 (Applied Biosystems) analysis program using the Ensemble database. Variant frequencies were obtained through the gnomAD (http://gnomad.broadinstitute.org/). In addition, according to ACMG criteria, the pathogenicities of variants checked and added from Varsome (http://varsome.com), Franklin (http://franklin.genoox.com) and Qiagen Clinical Insight (http://variants.tr.qiagenbioinformatics.com) variant analyse tools.

2.1. Statistical Analysis

Mean, standard deviation, median, minimum, maximum value frequency, and percentage were used for descriptive statistics. SPSS 15.0 was used for statistical analysis.

3. Results

We screened the variants of MEFV, MVK, NLRP3 and TNFRSF1A genes of 286 patients. The mean age of the all the patients was 7.76± 4.6 years. In the AID group (194 patients), 111 were boys and 83 were girls. The mean age was 7.43±4.7 years. In the MEFV-WT group of 92 patients, there were 53 girls and 39 boys. The mean age of this group was 8.53±4.3 years.

When low-penetrance variants were included in the AIDgroup, a total of 135 (69%) variants were detected. Among the variants detected in 135 patients, 62 patients had variants in MEFV (46%), 41 patients had variants in MVK (30%), 20 patients (15%) had variants in NLRP3, and 12 patients (9%) had variants in TNFRSF1A gene (Fig. 1).

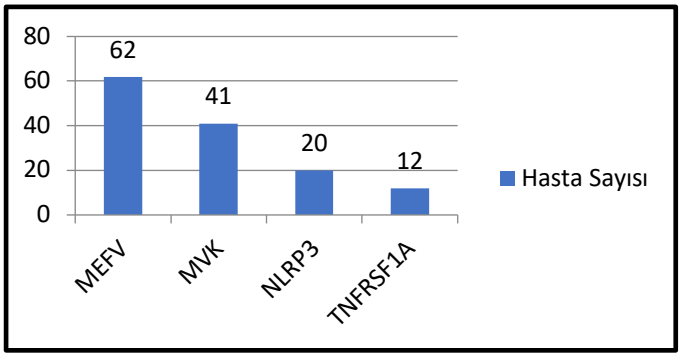

Fig. 1. Distribution of variants by genes in the AIDgroup

Table 1. Types of *MEFV* variants detected in the AIDgroup

Mutation	Number of Patients
M694V/-	16
E148Q/-	9
V726A/-	8
M680I/-	5
M694V/M694V	4
E148Q/M694V	2
A744S/-	2
K695R/-	2
R761H/-	2
P369S/-	1
L649V/-	1
T267I/-	1
Q778Sf*4/-	1
761_764 dup CCGC/-	1
M680I/V726A	1
M694V/A744S	1
M694V/R761H	1
E148Q/P369S	1
P369S/R408Q	1
P369S/R408Q/R501R Heterozygous	1
P369S/P369S/ R408Q/R408Q/R501R/R501R Homozygous	1
TOTAL	62

According to MEFV gene analysis, 32% (62/194) of the patients in the AIDgroup were variant. M694V homozygous in 4 patients, M694V heterozygous in 16 patients, E148Q heterozygous in 9 patients, V726A heterozygous in 8 patients, M680I heterozygous in 5 patients, A744S two, K695R two, R761H two, L649V one, P369S one, T267I one, Q778Sfs*4 one, c.761_764 dupCCGC heterozygous variants were found in one patient (Table 1). Compound heterozygous variants were found in 8 patients. Of these, 2 were E148Q/M694V compound heterozygous. The other 6 patients were M694V/R761H compound heterozygote, M694V/A744S compound heterozygote, M680I/V726A compound heterozygote, E148Q/P369S compound heterozygote, P369S/R408Q compound heterozygote, P369S/R408Q/R501R compound heterozygote, respectively. One patient was P369Shomozygous/ R408Qhomozygous homozygous. Pathogenic variants were detected in six of 194 patients (3%) in three genes other than the MEFV gene. N145S heterozygous variant in TNFRSF1A gene was detected in one patient (0.5%). Five patients had variants in the MVK gene. Four of these were V377I heterozygous and one was N205S heterozygous variants. No pathologic variant was detected in NLRP3 gene.

Table 2. Allele frequency and pathogenicity of all variants detected in our study

Gene	Variants		Allel Frequency (%)		Clinical significant ACMG variant classification
			This Study	gnomAD	
MEFV (NM_000243.3)	M694V	c.2080A>G	%6.18	%0.03	Pathogenic
	E148Q	c.442G>C	%3.09	%6.58	Benign
	V726A	c.2177T>C	%2.31	%0.2	Pathogenic
	M680I	c.2040C>G	%1.54	%0.01	Pathogenic
	P369S	c.1105C>T	%1.54	%1.47	Benign
	R408Q	c.1223G>A	%1.03	%1.34	Benign
	A744S	c.2230G>T	%0.77	%0.18	Pathogenic/ Likely Pathogenic
	R761H	c.2282G>A	%0.77	%0.02	Pathogenic
	K695R	c.2084A>G	%0.51	%0.58	Likely Pathogenic
	L649V	c.1945C>G	%0.25	N/A	Uncertain Significance
	T267I	c.800C>T	%0.25	%0.01	Likely Pathogenic
	Q778Sfs*4	c.2330dupG	%0.25	N/A	Likely Pathogenic
	N256Rfs*70	c.761_764dupCCGC	%0.25	%0.01	Likely Pathogenic
MVK (NM_000431.4)	V377I	c.1129G>A	%0.87	%0.16	Pathogenic
	N205S	c.614A>G	%0.17	N/A	Likely Pathogenic
	S52N	c.155G>A	%10.83	%10.33	Benign
NLRP3 (NM_001243133.2)	I313V	c.937A>G	%0.34	%0.02	Likely Pathogenic/ Uncetain Significance
	Q703K	c.2107C>A	%5.24	%3.84	Benign
	V198M	c.592G>A	%1.04	%0.83	Benign
TNFRSF1A (NM_001065.4)	N145S	c.434A>G	%0.17	%0.01	Likely Pathogenic /Uncertain Significance
	R121Q	c.362G>A	%1.92	%1.29	Benign

In the AIDgroup, besides pathogenic variants, so-called low-penetrance variants (Q703K, V198M, R121Q, S52N) which are the polymorphic variants that finding over %1 in healthy population, were also observed. 67 of 194 patients had a low-penetrance variant in one of the 3 genes. When these variants were included in the study, the number of variants in the 194 AID patient population increased to 73 and the variant rate was determined as 37% (73/194). This rate was 10% (20/194) for NLRP3 gene, 21% (41/194) for MVK gene and 6% (12/194) for TNFRSF1A. When only low-penetrance variant distribution was analyzed, the distribution for the entire AID population was 9% (17/194) for NLRP3 gene Q703K, 1.5% (3/194) for V198M; 19% (36/194) for MVK gene S52N; 5.6% (11/194) for TNFRSF1A gene R121Q.

When the patients in the AIDgroup were evaluated in terms of disease-associated variants in four genes including the MEFV gene, disease-associated variants in one of the four genes were found in 52 patients (27%). Of the 52 patients, 46 (88%) were variants in MEFV, five (10%) in MVK, and one (2%) in TNFRSF1A gene.

Our second patient group (FMF-WT) consisted of 92 pediatric patients. NLRP3, TNFRSF1A and MVK gene variant analyses were performed in these patients. In 92 patients, the rate of disease-associated variants in the three genes was 3% (3/92). Two of these were I313V heterozygous variants in the NLRP3 gene for CAPS diseases. One was a V377I heterozygous variant in the MVK gene. No pathogenic variant was detected in TNFRSF1A gene analysis.

When low-penetrance variants were taken into account, the total number of variants in 92 FMF-WT patients was 45, with a variant rate of 49% (45/92). The variant rate for NLRP3 was 20% (18/92) [Q703K heterozygous variant in 13 patients (14%), V198M heterozygous variant in 3 patients (3%)]. The variant rate for MVK gene was 29% (27/92) [S52N in 26 patients].

In addition, on the outside of the rates calculated based on the number of patients in the previous paragraphs, the allele frequencies and pathogenicity of all variants detected in our study are shown in table 2

4. Discussion

Autoinflammatory Diseases (AID) are a group of diseases characterized by involvement of serosal membranes with recurrent episodes of fever, inflammation attacks and a healthy period between attacks, elevated acute phase reactants and accompanied by periodic fever, peritonitis, pleuritis, pericarditis, arthritis and skin rashes. Genetically, it covers a wide range of diseases from monogenic inheritance to multifactorial diseases. Diagnosis of this group of diseases is difficult due to overlapping clinical findings.

In our study, 194 of 286 patients with a clinical diagnosis of autoinflammatory disease underwent MEFV, NLRP3, TNFRSF1A and MVK gene analysis and disease-associated variants were detected in approximately one quarter. The majority of these were associated with the MEFV gene. The rate of disease-associated pathogenic variants detected for the other three genes was 3% in both the autoinflammatory group in which four genes were analyzed, in the MEFV-WT group and in all 286 patients analyzed. In our study, disease-associated MEFV gene variants were predominant in autoinflammatory diseases.

While the total variant rates of our study and the study by Federici et al. (7), which is one of the large studies conducted in the field of autoinflammatory diseases in the world, were almost similar, the pathogenic variant rate was lower in our study. The distribution of pathogenic variants in our study was very narrow compared to the study of Federici et al. In the same study, a special group was formed for these diseases. The admission requirements for this group were the presence of two variants (except R202Q) in the MEFV gene, at least one of which involved exon 10 for FMF, the presence of one of the variants except V198M and Q703K in the NLRP3 gene for CAPS, the presence of variants in both alleles of the MVK gene (except S52N, P165L and H20Q) for HIDS, and the presence of a variant except R121Q (the old name of this variant [R92Q] is used in this article) and P46L in the TNFRSF1A gene for TRAPS. The proportion of patients in this special group was 18%: 5% (67/1215) for CAPS, 6% (74/1215) for HIDS and 7% (86/1215) for TRAPS. Our study included a small number of patients for this type of scoring, but when an appropriate group was created with similar criteria, 0.7% (2/286) of patients for CAPS and 0.3% (1/286) for TRAPS fell into this group. There were no eligible patients for HIDS. In our study, the total variant rate eligible for the group was 1%. In the same study, the rate of patients with MEFV gene variant was 41% (7). The rate of patients in the special group was 24%. In our study, the rate of MEFV gene variant in the AIDgroup was 32% and the rate of patients fitting into this group was 5%.

Jesus et al. (8) evaluated 102 patients for autoinflammatory diseases in 2012. Based on clinical findings, 28 patients were considered as CAPS, 31 as TRAPS, 17 as FMF, 17 as Mevalonate Kinase Deficiency (MKD) and 9 as Blau syndrome. Gene analysis of diseases related to clinical findings (NLRP3, TNFRSF1A, MEFV, MVK and NOD2, respectively) was performed and genetic diagnosis was confirmed in 21% (6/28) of patients with clinical diagnosis of CAPS, 23% (7/31) of TRAPS, 18% (3/17) of FMF, 18% (3/17) of MKD and 89% (8/9) of Blau syndrome. Thus, 27% of all patients were genetically confirmed. They did not exclude low-penetrance variants in the study. They accepted homozygous or compound heterozygous variants as genetic diagnosis for FMF and MKD. According to these study criteria, in our study, 13% of patients were genetically confirmed for CAPS, 4% for TRAPS and 8% for FMF. The proportion of genetically confirmed patients in our study was very low compared to the study of Jesus et al.

In another study, Federici et al. (9) performed MEFV gene

analysis (exons 2, 3, 5 and 10) in 1118 patients with a clinical diagnosis of FMF and found variants in 43%. TNFRSF1A gene analysis (exons 2, 3 and 4) was performed in 286 patients with a clinical diagnosis of FMF and variants were found in 6.6%. Homozygous or compound heterozygous variants were found in 8% for NLRP3 (exon 3) and 2% for MVK (exons 2-9). As a result, they obtained genetic results associated with CAPS, TRAPS and HIDS in 7% of the patients. In our study, this rate was 17% (50/286) according to the same criteria.

Genetic studies on CAPS, TRAPS and HIDS are very limited in Turkey. Berdeli et al. (10) performed NLRP3 gene analysis (all exons for NLRP3) in 194 patients with autoinflammatory disease findings who had negative MEFV gene analysis in 2013. They found disease-associated variants in 25 patients (13%). 19 patients had Q703K, two patients had S726G, one patient had V198M, 3 patients had A154G, S726G and K610fsX613 frameshift variants. In our study, variants were detected in 38 of 286 patients (13%). Of these patients, 30 had Q703K, 6 had V198M, and 2 had I313V variants.

92 patients out of 286 patients were in the group without MEFV gene variant. Our NLRP3 variant rate in this group was 20%. The majority of the variants detected in both Berdeli's study and our study were low-penetrance variants.

In the study of Akdeniz S.(11), TRNFRSF1A gene analysis (all exons of TNFRSF1A gene) of 30 patients with autoinflammatory findings revealed no clinically relevant variant in any patient. In our study, we found a TRAPS-associated variant with a rate of 4% and only one patient had a disease-associated variant. However, none of the patients in the second group of the study, MEFV-WT (n:92), had TNFRSF1A variant.

Studies conducted in our population show similarities with the variant rate found in our study. When the literature data related to different ethnic groups are analyzed, our variant rate was found to be lower compared to the literature data. The reason for this may be population-based, but factors such as different patient selection criteria and non-standardization of the gene regions studied may also have led to this situation.

The strength of our study is that we did not restrict the selection of autoinflammatory patients and included patients in the broadest spectrum. We thought that this would give us the most realistic gene and variant distribution of autoinflammatory patients we will encounter. The weakness of our study is that it does not cover all of the auto inflammatory disease-related genes reported to date and does not cover all exons and introns of the genes studied.

In conclusion, MEFV gene variants and FMF disease were predominantly found among autoinflammatory diseases in North Anatolia. It is understood that the other three autoinflammatory diseases are rarely seen in our region. In our study, two thirds of the patients with autoinflammatory diseases were undiagnosed. Autoinflammatory diseases can be overlooked and difficult to diagnose, but they are urgent due to the risk of multiorgan involvement and amyloidosis. Clinical diagnostic criteria, known gene variant analyses (especially outside the FMF group) and the diagnostic efficiency of direct variant analysis techniques are not fully adequate. As a result of our study, our recommendation is to perform MEFV gene analysis as a first-line genetic test in patients with autoinflammatory findings in our region, and if no disease-associated variant is detected, next-generation whole exome or whole genome sequencing analyses should be recommended to patients. We hope that these difficulties will be overcome with the new molecules, molecular pathways and genes to be identified in the future and with the advanced technology next generation whole gene/exome/genomic sequencing and analysis systems.

Our study reflects the first results related to MEFV, NLRP3, MVK and TNFRSF1A gene variant distribution in the pediatric AID population of the Central Black Sea region. More similar studies are needed to clarify the variant distribution related to AID.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.A, G.O., A.Y. Design: E.A, G.O., U.A Data Collection or Processing: E.A, M.H.C. Analysis or Interpretation: E.A, U.A., O.S.A., M.K.A. Literature Search: E.A., A.G.A.P. Writing: E.A, G.O., U.A., O.S.A.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 10/09/2015 and the number of ethical committee decisions is 2015/335.

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