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Next-generation screening of a panel of genes associated with periodic fever syndromes in patients with Familial Mediterranean Fever and their clinical characteristics

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ABSTRACT

Familial Mediterranean Fever (FMF) is a hereditary fever syndrome that primarily affects Mediterranean populations. For the study, total number of 182 patients with FMF disease were enrolled and screening of a panel of genes , called “fever panel” which comprises 17 genes, was performed. The most common mutations in *MEFV* gene were homozygous M694V missense mutation (4.3%) and R202Q missense mutation (4.9%). The most common heterozygous mutations were R202Q (26.5%), M694V (25.9%) and E148Q (11.9%). Compound heterozygous and homozygous mutations were also detected. Also, different types of mutations were identified in *NOD2, CARD14, NLRP12, NLRP3, NLRP7, IL1RN, LPIN2, TNFRSF1A, MVK* and *PSTPIP1* genes. Two novel missense variations in the *MEFV* gene, Gln34Pro and Ile247Val, which have not been previously reported in the databases, were identified. Also, Thr91Ile missense variation in the *NOD2* gene, Gly461Cys missense variation in *NLRP3* and Tyr732Stop nonsense variation in *LPIN2* were firstly identified. The results of the current study suggest that in addition to the MEFV gene which has an important roles in FMF, molecular screening of other genes related to other autoinflammatory diseases might provide support in suspected cases and provide detailed information about the course of the disease.

1. Introduction

Familial Mediterranean Fever (FMF; MIM 249100) is an autosomal recessive, autoinflammatory disease which is frequently seen in populations of Mediterranean origin. FMF is characterized by recurrent fever, peritonitis, pleuritis, arthritis and erysipelas-like skin lesions [1,2]. Although FMF is an autosomal recessive disease, individuals with heterozygous mutations have also shown to exhibit the classic symptoms of the disease [3]. FMF primarily affects populations of Mediterranean countries such as Turkey, Syria, Armenia, Tunisia, Morocco, Israel, Iran, Greece and Italy. FMF is accompanied by a significant reduction in daily life quality due to the recurrent attacks of fever and subclinical inflammation in the attack-free periods. Untreated patients or patients who have not received adequate treatment are under risk of developing amyloidosis causing renal failure, which is a leading cause of morbidity and mortality [4].

Identification of mutation in *MEFV* gene is an important factor supporting the diagnosis in the patient whose clinical findings are compatible with FMF [5]. Another finding that supports the diagnosis is the increase in acute phase reactants, which are known to increase in inflammatory events during attack periods. C-reactive protein (CRP), erythrocyte sedimentation rate, fibrinogen and leukocyte count increase in attacks. In addition, serum amyloid-A (SAA), ceruloplasmin, haptoglobulin and some cytokines have been reported to increase during the attack period [6].

MEFV (MEFV innate immuity regulator, pyrin) gene, which is responsible from the disease, is located on chromosome 16p13.3 region and consists of ten exon regions [7]. The *MEFV* gene encodes “pyrin”, a 781 amino acid long protein involved in the regulation of inflammation and apoptotic processes [8,9]. Pyrin forms an element of the NLRP3 inflammatory inflammasome complex, which regulates the production of pro-inflammatory cytokine interleukin-1β (IL- 1β) [9]. FMF can therefore be classified as an inflamasomepathy [10]. To date, more than 100 mutations and more than 330 sequence variants have been identified in the *MEFV* gene. The majority of these variations are located in exon 2 and 10. Four missense mutations detected in exon 10 (M680I,

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Table 1
Genes analyzed with next-generation sequencing in the "fever panel".

No	Symbol	Full name	Associated disease	Location	Ensembl ID
1	MEFV	MEFV, innate immuity regulator, pyrin	Familial Mediterranean Fever	16p13.3	ENSG00000103313
2	LPIN2	Lipin 2	Majeed syndrome	18p11.31	ENSG00000101577
3	ELANE	Elastase, neutrophil expressed	Cyclic neutropenia	19p13.3	ENSG00000197561
4	MVK	Mevalonate kinase	Mevalonate kinase deficiency	12q24.11	ENSG00000110921
5	NLRP3	NLR family pyrin domain containing 3	Neonatal Onset Multisystem Inflammatory Disease Muckle-Wells syndrome Familial Cold Autoinflammatory Syndrome	1q44	ENSG00000162711
6	PSTPIP1	Proline-serine-threonine phosphatase interacting protein 1	Pyogenic Sterile Arthritis, Pyoderma Gangrenosum, And Acne, PAPA Syndrome	15q24.3	ENSG00000140368
7	TNFRSF1A	TNF receptor superfamily member 1A	TNF receptor-associated periodic fever syndrome (TRAPS)	12p13.31	ENSG00000067182
8	IL1RN	Interleukin 1 receptor antagonist	Osteomyelitis, Sterile Multifocal, With Periostitis And Pustulosis	2q14.1	ENSG00000136689
9	NLRP12	NLR family pyrin domain containing 12	Familial Cold Autoinflammatory Syndrome	19q13.42	ENSG00000142405
10	NOD2	Nucleotide binding oligomerization domain containing 2	Blau Syndrome, Yao Syndrome, Crodn Disease, Cancer	16q12.1	ENSG00000167207
11	CARD14	Caspase recruitment domain family member 14	Familial Pityriasis Rubra Pilaris Generalized Pustular Psoriasis Psoriatic Arthritis	17q25.3	ENSG00000141527
12	NLRP7	NLR family pyrin domain containing 7	Hydatidiform Mole	19q13.42	ENSG00000167634
13	TNFRSF11A	TNF receptor superfamily member 11a	Osteopetrosis Paget disease of bone	18q21.33	ENSG00000141655
14	IL10RA	Interleukin 10 receptor subunit alpha	Inflammatory bowel disease 28	11q23.3	ENSG00000110324
15	CECR1	Adenosine deaminase 2, ADA2	Adenosine Deaminase 2 Deficiency	22q11.1	ENSG00000093072
16	IL10RB	Interleukin 10 receptor subunit beta	Inflammatory bowel disease 25	21q22.11	ENSG00000243646
17	PSMB8	Proteasome subunit beta 8	Nakajo-Nishimura Syndrome	6p21.32	ENSG00000204264

M694V, M694I, and V726A) which account for approximately 75–85% of *MEFV* gene mutations in the Mediterranean region [11,12]. The number and types of mutations in the *MEFV* gene varies among populations. M694V mutation is most common among non-ascenazi Jews and Turks. The most common *MEFV* gene mutation in Armenians is M680I. In previous studies, it has been reported that V726A mutation is common in Askenazi and Iraqi Jews and M694I mutation is common in Arabs. M694V mutation has been reported to be the most common in many studies conducted in Turkey [12].

Furthermore, significant advances have been made towards understanding and diagnosing the molecular basis of FMF through rapidly developing molecular genetic methods. In particular, much broader analyzes are performed instead of mutational analysis of a single gene or certain regions of *MEFV* gene. More recently, in order to detect FMF disease, a next generation sequencing (NGS) screening panel called "fever panel" was created and the variations detected by screening all regions of the 17 genes associated with autoinflammatory disease. In particular, in this comprehensive study, we analyzed sequence variations of *CARD14, LPIN2, NLRP3, NLRP7, NOD2, NLRP12, TNFRSF11A, PSTPIP1, MEFV, TNFRSF1A, MVK, IL10RA, CECR1, IL10RB, IL1RN, PSMB8,* and *ELANE* genes. The main objective of our study is to reveal variations associated with the FMF disease and impact of these variations in the clinical course of FMF.

2. Materials and methods

2.1. Study population

The present study included 182 independent individuals with diagnosis of FMF who referred to Tekirdag Namik Kemal University Health Application and Research Center between Between 1 January 2017 and 1 January 2019. Local ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Tekirdag Namik Kemal University in accordance with the Helsinki Declaration (Protocol No: 2019.12.01.12). Data obtained from Medical Genetics and Rheumatology clinics were used for genotype-phenotype comparisons.

2.2. DNA isolation

For the next generation sequencing screening, peripheral blood samples of patients were obtained using standard Vacutainer tubes and stored at −20 °C. DNA isolations were performed with QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the recommendations of the manufacturer. Measurements were performed in NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to determine the quantity and quality of the DNAs obtained. DNA samples were preserved at −20 °C for further experiments.

2.3. Sanger sequencing

Some of the patients included in the study were screened by Sanger sequencing method. Only the 2nd and 10th exons of the *MEFV* gene were screened. Briefly, primary amplification of the the regions were achived using gene specific primers and subsequently enzymatic purification of amplified PCR products were performed using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Following cleanup, sequence PCR was performed by using single primer for the gene of interest and samples were run in 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.4. Next-generation sequencing

For the analysis of "fever panel" in which sequence variations of *CARD14, LPIN2, NLRP3, NLRP7, NOD2, NLRP12, TNFRSF11A, PSTPIP1, MEFV, TNFRSF1A, MVK, IL10RA, CECR1, IL10RB, IL1RN, PSMB8,* and *ELANE* genes were screened (Table 1) by using next generation sequencing method and whole gene sequencing was performed. Briefly, a library was created with the Sophia Genetics FAID Panel Kit (Sophia Genetics, USA) for mutational screening of 17 genes in the "fever panel". Genomic DNA library preparation was achived by Custom Bundle Solution by Sophia Genetics and then capture and sequencing were performed. These operations were carried out in accordance with the recommendations of the manufacturer. Finally, the NextSeq 500 was operated with the Denature and Dilute Libraries Guide (Illumina, San Diego, California, USA). The results were analyzed by Sophia DDM analysis program and mutation types and heterozygosity/homozygosity

Table 2
Clinical and demographic chracteristics of patients.

		Wild type (n = 66)		Mutation (n = 116)		Total (n = 182)	
Age (Mean ± SD)		36.97 ± 12.15		33.88 ± 11.41		35.00 ± 11.74	
		Frequency	Percentage (%)	Frequency	Percentage (%)	Frequency	Percentage (%)
Gender	Male	27	40.9	57	49.1	84	46.2
	Female	39	59.1	59	50.9	98	53.8
Indications	Abdominal pain	10	15.2	12	10.3	22	12.1
	Joint pain	9	13.6	13	11.2	22	12.1
	Arthritis	2	3.0	2	1.7	4	2.2
	Heredofamilyal Amyloidosis	18	27.3	48	41.4	66	36.3
	Amyloidosis	5	7.6	9	7.8	14	7.7
	Inflammatory Spondylopathy	3	4.5	9	7.8	12	6.6
	Other	19	28.8	23	19.8	42	23.1

Table 3
Distribution of mutations detected in *MEFV* gene in patients with FMF.

Mutation Type		Nucleotide change	Codon change	Amino acid change	rs ID	Patients [a] (n)	Frequency (%)
Homozygous	Missense	c.2040 G > A	AT**G** > AT**A**	p.Met680Ile	rs28940580	2	1.1
	Missense	c.2080 A > G	**A**TG > **G**TG	p.Met694Val	rs61752717	8	4.3
	Missense	c.2082 G > A	ATG > ATA	p.Met694Ile	rs28940578	1	0.5
	Missense	c.605 G > A	C**G**G > C**A**G	p.Arg202Gln	rs224222	9	4.9
Heterozygous	Missense	c.1105C > T	**C**CC > **T**CC	p.Pro369Ser	rs11466023	3	1.6
	Missense	c.149C > T	C**C**G > C**T**G	p.Pro50Leu	rs144716190	1	0.5
	Missense	c.2040 G > A	AT**G** > AT**A**	p.Met680Ile	rs28940580	14	7.6
	Missense	c.2080 A > G	**A**TG > **G**TG	p.Met694Val	rs61752717	48	25.9
	Missense	c.2084 A > G	AAG > AGG	p.Lys695Arg	rs104895094	3	1.6
	Missense	c.2282 G > A	AT**G** > AT**A**	p.Arg761His	rs104895097	5	2.7
	Missense	c.2177 T > C	G**T**T > G**C**T	p.Val726Ala	rs28940579	9	4.9
	Missense	c.442G > C	**G**AG > **C**AG	p.Glu148Gln	rs3743930	22	11.9
	Missense	c.605 G > A	C**G**G > C**A**G	p.Arg202Gln	rs224222	49	26.5
	Missense	c.101 A > C	C**A**G > C**C**G	p.Gln34Pro	Novel[c]	1	0.5
	Missense	c.1223 G > A	C**G**G > C**A**G	p.Arg408Gln	rs11466024	2	1.1
	Missense	c.739 A > G	**A**TT > **G**TT	p.Ile247Val	Novel[d]	1	0.5
	Missense	c.1503C > T	CG**C** > CG**T**	p.Arg501Arg	rs76464258	1	0.5
	Missense	c.1437C > G	TT**C** > TT**G**	p.Phe479Leu	rs104895083	1	0.5
	Missense	c.501G > C	GA**G** > GA**C**	p.Glu167Asp	rs104895079	1	0.5
	Missense	C.443A > T	GAG > G**T**G	p.Glu148Val	rs104895076	1	0.5
	Missense	c.311C > G	T**C**C > T**G**C	p.Ser104Cys	rs151306047	1	0.5
	Missense	c.586G > T	**G**GG > **T**GG	p.Gly196Trp	rs104895179	1	0.5
	Missense	c.329 T > C	C**T**G > C**C**G	p.Leu110Pro	rs11466018	1	0.5
Mutation type		Nucleotide change		Amino acid change		Patients [b](n)	Frequency (%)
Compound heterozygotes		c.2040G > C; c.2080A > G; 605G > A		p.Met680Ile; p.Met694Val; p.Arg202Gln		4	5.9
		c.2080A > G; c.442G > C		p.Met694Val; p.Glu148Gln		6	8.8
		c.2080A > G; c.442G > C; 605G > A		p.Met694Val; p.Glu148Gln; p.Arg202Gln		5	7.4
		c.2080A > G; 605G > A		p.Met694Val; p.Arg202Gln		13	19.1
		c.1105C > T; c.1223G > A; c.1503C > T		p.Pro369Ser; p.Arg408Gln; p.Arg501Arg		1	1.5
		c.1105C > T; c.1223G > A		p.Pro369Ser; p.Arg408Gln		1	1.5
		c.2177 T > C; c.2080A > G		p.Val726Ala; p.Met694Val		11	16.2
		c.2177 T > C; c.2040G > C		p.Val726Ala; p.Met680Ile		3	4.4
		c.2080A > G; c.2082G > A		p.Met694Val; p.Arg761His		3	4.4
		c.2040G > C; c.442G > C		p.Met680Ile; p.Glu148Gln		4	5.9
		c.1437C > G, c.2177 T > C, c.501G > C		p.Phe479Leu; p.Val726Ala; p.Glu167Asp		1	1.5
		c.1105C > T; c.1223G > A		p.Pro369Ser; p.Arg408Gln		1	1.5
		c.2040G > C, c.2080A > G		p.Met680Ile; p.Met694Val		1	1.5
		c.2080A > G, c.442G > C; c.329 T > C		p.Met694Val; p.Glu148Gln; p.Leu110Pro		1	1.5
		c.2084A > G, c.2080A > G, c.605G > A		p.Lys695Arg; p.Met694Val; p.Arg202Gln		2	2.9
		C.586G > T, c.605G > A		p.Gly196Trp; p.Arg202Gln		1	1.5
		c.2040G > C, c.443A > T		p.Met680Ile; p.Glu148Val		1	1.5
		c.442G > C, 605G > A		p.Glu148Gln; p.Arg202Gln		1	1.5
Compound homozygotes		c.2080A > G; 605G > A		p.Met694Val; p.Arg202Gln		5	7.4
Complex genotype (Homozygotes/heterozygotes)		c.2080A > G; 605G > A		p.Met694Val; p.Arg202Gln		3	4.4

The bold letters indicate nucleotide change.

[a] Since more than one mutation is detected in a patient, the number of patients is different. Only those with a homozygous or heterozygous mutation are included.

[b] Only samples with compound heterozygotes, compound homozygotes, and complex genotypes were included.

[c] A novel variation in the *MEFV* gene was found in the region between rs767848974 (c.99 G > A; GT**G** > GT**A**; p.Val33Val) and rs1310258078 (c.102 G > A; CA**G** > CA**A**; p.Gln34Pro).

[d] c.739A > G; **A**TT > **G**TT, p.Ile247Val heterozygous missense variation, which is found in the same location with rs1472692347 (c.739 A > C; **A**TT > **C**TT; p.Ile247Leu), was detected in the *MEFV* gene.

Table 4
Distribution of mutations detected in fever panel genes in patients with FMF.

Gene	Nucleotide change	Codon change	Amino acid change	Zygosity	Mutation type	rs ID	Patientes (n)	Frequency[a] (%)
NOD2	c.271 A > G	ACC > GCC	p.Thr91Ala	Heterozygous	Missense	Novel[b]	1	0.9
NOD2	c.2722 G > C	GGC > CGC	p.Gly908Arg	Heterozygous	Missense	rs2066845	4	3.4
NOD2	c.2555 A > G	AAC > AGC	p.Asn852Ser	Heterozygous	Missense	rs104895467	1	0.9
NOD2	c.315 G > T	GCG > GCT	p.Ala105Ala	Heterozygous	Synonymous	rs104895419	1	0.9
NOD2	c.3017 dupC/	CTT > CCTT	p.Leu1007Profs	Heterozygous	Frameshift	rs2066847	2	1.7
CARD14	c.2648 G > A	CGC > CAC	p.Arg883His	Heterozygous	Missense	rs2289541	1	0.9
CARD14	c.956G > A	CGA > CAA	p.Arg319Gln	Heterozygous	Missense	rs138991161	1	0.9
CARD14	c.2044C > T	CGG > TGG	p.Arg682Trp	Heterozygous	Missense	rs117918077	1	0.9
CARD14	c.1264 G > A	GAG > AAG	p.Glu422Lys	Heterozygous	Missense	rs61751629	1	0.9
CARD14	c.526 G > C	GAC > CAC	p.Asp176His	Heterozygous	Missense	rs144475004	1	0.9
CARD14	c.1583 T > C	CTA > CCA	p.Leu528Pro	Heterozygous	Missense	Novel[c]	1	0.9
NLRP12	c.779C > T	ACG > ATG	p.Thr260Met	Heterozygous	Missense	rs150280940	1	0.9
NLRP12	c.2788 G > A	GCC > ACC	p.Ala930Thr	Heterozygous	Missense	rs146368839	1	0.9
NLRP12	c.1054C > T	CGT > TGT	p.Arg352Cys	Heterozygous	Missense	rs199881207	1	0.9
NLRP3	c.2113C > A	CAG > AAG	p.Gln705Lys	Heterozygous	Missense	rs35829419	4	3.4
NLRP3	c.598 G > A	GTG > ATG	p.Val200Met	Heterozygous	Missense	rs121908147	2	1.7
NLRP3	c.1381 G > T	GGC > TGC	p.Gly461Cys	Heterozygous	Missense	Novel[d]	1	0.9
NLRP7	c.1614 T > A	TTT > TTA	p.Phe538Leu	Heterozygous	Missense	rs200193926	1	0.9
IL1RN	c.78 G > A	ACG > ACA	pThr26Thr	Heterozygous	Synonymous	rs2232353	1	0.9
IL1RN	c.316 G > A	GCC > ACC	p.Ala106Thr	Heterozygous	Missense	rs45507693	1	0.9
LPIN2	c.1786 G > A	GGT > AGT	p.Gly596Ser	Heterozygous	Missense	rs769806854	2	1.7
LPIN2	c.1510C > T	CTT > TTT	p.Leu504Phe	Heterozygous	Missense	rs104895500	2	1.7
LPIN2	c.2196C > G	TAC > TAG	p.Tyr732Stop	Heterozygous	Nonsense	Novel[e]	1	0.9
LPIN2	c.1876C > T	CCC > TCC	p.Pro626Ser	Heterozygous	Missense	rs150806357	1	0.9
TNFRSF1A	c.362 G > A	CGG > CAG	p.Arg121Gln	Heterozygous	Missense	rs4149584	2	1.7
TNFRSF1A	c.878_895del TCACCTCCAGCTCCACCT	TCACCTCCAGCTCCACCT		Heterozygous	Frameshift	rs775216961	1	0.9
TNFRSF1A	c.123 T > G	GAT > GAG	p.Asp41Glu	Heterozygous	Missense	rs104895271	1	0.9
MVK	c.118C > T	CGG > TGG	p.Arg40Trp	Heterozygous	Missense	rs1055952433	1	0.9
PSTPIP1	c.203C > T	ACG > ATG	p.Thr68Met	Heterozygous	Missense	rs201872851	2	1.7
PSTPIP1	c.262G > A	N/A	Non Coding Transcript Variant	Heterozygous		rs534702768	1	0.9
IL10RA	c.1235G > A	C**G**G > C**A**G	p.Arg412Gln	Heterozygous	Missense	rs117423374	1	0.9
IL10RA	c.1072G > A	**G**AC > **A**AC	p.Asp358Asn	Heterozygous	Missense	rs78753252	1	0.9
ELANE	c.225_239delAAACGTGG	AAACGTGG (Deletion)		Heterozygous	Frameshift	Novel[f]	1	0.9

The bold letters indicate nucleotide change.

[a] Frequencies were calculated for 116 patients with mutations.

[b] A novel variation in the *NOD2* gene was found in the region between rs771336423 (c.270C > T; GA**C** > GA**T**; p.Asp90Asp) and rs1356111500 (c.272C > T; A**C**C > A**T**C; p.Thr91Ile).

[c] A novel variation in the *CARD14* gene was found in the region between rs1424016462 (c.1582; **C**TA > **A**TA; p.Leu528Ile) and rs764792856 (c.1588A > G; **A**CG > **G**CG; p.Thr530Ala).

[d] In the *NLRP3* gene, G > T novel variation was detected in the region at the same position (G > A, G > C) as rs939724059.

[e] In the *LPIN2* gene, C > G novel nonsense variation close to the region rs761674505 (c.2193G > C; CT**G** > CT**C**; p.Leu731Leu) was detected.

[f] A novel deletion in the *ELANE* gene was found in the region between rs1486125123 (indel CGCGCG) and rs1241337454 (intronic variation).

status were reported.

2.5. Statistical analysis

Descriptive statistical analyzes were used to determine the frequencies and distributions of the data. Percentages and ratios were used for the evaluation of categorical data, and mean and standard deviations were used for the evaluation of continuous data. Chi-Square test was used for the analysis of categorical variables. Pearson correlation was used to determine the relationship between the variables. For all results, $p < .05$ was considered statistically significant.

3. Results

Of the 182 patients, 84 (46.2%) of them were males and 98 (53.8%) were females. In 66 (36.3%) patients, no mutation was detected, whereas in 116 (63.7%) of the patients, different types of mutations were detected (Table 2). The mean age of patients without genetic variation and patients having genetic variation were 36.97 and 33.88, respectively. Abdominal pain and joint pain were the most common complaints in patients referred to our clinic. Demographic and clinical chracteristics of patients were summarized in the Table 2.

3.1. Distribution of mutations in MEFV gene

Variations in the *MEFV* gene that are frequently mutated and associated with the clinical course of the disease are shown in Table 3. The most common homozygous mutations were M694V missense mutation (4.3%) and R202Q missense mutation (4.9%). Other common variations were M680I (1.1%) and M694I (0.5%) missense mutations. The R202Q (26.5%) missense mutation is the most common heterozygote mutation, which is followed by M694V (25.9%) and E148Q (11.9%) heterozygous mutations. Among the heterozygous variations, a novel variation of Q34P [c.101A > C (CAG > CCG)] was detected in a 27-year-old female patient. In addition, in a 44-year-old male patient, an E148Q heterozygote mutation and a novel variation of I247V [c.739 A > G (ATT > GTT)] in the *MEFV* gene and a P626S heterozygous variation in the *LPIN2* gene were detected (Table 3). M694V and R202Q (19.1%) were the most common mutations among compound heterozygotes mutations detected in *MEFV* gene. Following this, M694V, E148Q, R202Q compound heterozygotes variation was found to be second most common compound heterozygote variation. In this study, compound homozygotes M694V; R202Q mutations were seen together in 5 patients. In addition, 3 patients had a complex genotype (M694V, homozygous; R202Q, heterozygous). Also, in a 31-year-old female patient, M694V; R202Q compound heterozygous variations and

Table 5
Frequency of patients with variation in both the *MEFV* gene and the genes in the fever panel.

Age	Gender	Indications	MEFV gene mutation		Mutations in fever panel genes		
			Amino acid change	Zygosity	Gene name	Amino acid change	Zygosity
25	M	HFA	M680I	Homozygous	NOD2	T91A	Heterozygous
					CARD14	R883H	Heterozygous
45	F	HFA	E148Q	Heterozygous	NLRP12	T260M	Heterozygous
30	F	IS	E148Q	Heterozygous	NLRP3	Q705K	Heterozygous
					NLRP3	Q200M	Heterozygous
31	F	HFA	R202Q	Heterozygous	NLRP7	F538L	Heterozygous
14	F	Joint pain	E148Q	Heterozygous	NOD2	L1007Profs	Heterozygous
33	M	Abdominal pain	P369S	Heterozygous	NRLP12	A930T	Heterozygous
			R408Q	Heterozygous	NRLP3	G461C	Heterozygous
			R501R	Heterozygous			
41	F	NV	R202Q	Heterozygous	LPIN2	G596S	Heterozygous
34	M	HFA	M680I	Heterozygous	LPIN2	L504F	Heterozygous
			M694V	Heterozygous			
			R202Q	Heterozygous			
63	M	CA	R202Q	Heterozygous	TNFRSF1A	R121Q	Heterozygous
56	M	Joint pain	M680I	Homozygous	TNFRSF1A	R121Q	Heterozygous
					MVK	R40W	Heterozygous
					PSTPIP1	T68M	Heterozygous
25	M	HFA	R202Q	Homozygous	TNFRSF1A	G461C	Heterozygous
47	F	HFA	K695R	Heterozygous	NLRP3	Q705K	Heterozygous
44	M	HFA	E148Q	Heterozygous	LPIN2	P626S	Heterozygous
24	M	HFA	E148Q	Heterozygous	NLRP3	Q705K	Heterozygous
51	F	HFA	M694V	Heterozygous	LPIN2	L504F	Heterozygous
			M694I	Heterozygous			
46	M	HFA	M694V	Heterozygous	NOD2	G908R	Heterozygous
			E148Q	Heterozygous			
53	M	HFA	M694V	Heterozygous	CARD14	D178H	Heterozygous
			E148Q	Heterozygous			
48	F	HFA	S104C	Heterozygous	NOD2	G908R	Heterozygous
24	F	HFA	M694V	Heterozygous	NOD2	G908R	Heterozygous
			V726A	Heterozygous			
38	M	HFA	M680I	Heterozygous	CARD14	R319Q	Heterozygous
			M694V	Heterozygous			
			R202Q	Heterozygous			
47	M	HFA	R202Q	Heterozygous	PSTPIP1	T68M	Heterozygous
40	F	IS	R202Q	Homozygous	NLRP3	Q705K	Heterozygous
44	M	IS	M694V	Heterozygous	NOD2	R439C	Heterozygous
21	M	Amyloidosis	M694V	Heterozygous	NOD2	N852S	Heterozygous
			V726A	Heterozygous	TNFRSF11A	K54R	Heterozygous
18	M	HFA	M680I	Heterozygous	LPIN2	P626S	Heterozygous
			E148Q	Heterozygous			
51	F	Abdominal pain	M694V	Heterozygous	IL1RN	A106T	Heterozygous
			R202Q	Heterozygous			
18	F	IS	M694V	Homozygous	NLRP3	V198M	Heterozygous
			R202Q	Homozygous			
36	M	HFA	E148Q	Heterozygous	NOD2	R702T	Heterozygous
					NOD2	G908R	Heterozygous

M: Male; F: Female; IS: Inflammatory Spondylopathy; HFA: Heredofamilyal amyloidosis; NV: Necrotizing Vasculopathy; CA: Crystal Arthropathy.

NM_000243.2(MEFV):c.1261-28A > G (rs104895140) heterozygous variation in the intronic region of *MEFV* gene was identified. This variation has already been identified by other researchers and has been clinically associated with FMF in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/97439/).

3.2. Distribution of mutations in the "fever panel"

In the present study, 17 different genes associated with periodic fever syndromes were screened in the fever panel by NGS method. Table 4 shows the distribution of mutations detected in the genes in the fever panel in patients with FMF.

Particularly, in a 25-year-old male patient, Met680Ile homozygous mutation in *MEFV* gene, Arg883His heterozygous variation in *CARD14* gene as well as a novel variation of T91A [c.271 A > G (ACC > GCC)] heterozigot missense variation in *NOD2* gene was detected. Also, in a 33-year-old male patient, P369S, R408Q, R501R compound heterozygote in *MEFV* gene and A930Y heterozygous variations in *NLRP12* gene as well as a novel variation of G461C [c.1381 G > T (GGC > TGC)] heterozigot missense variation in *NLRP3* gene was detected. In addition, in a 41-year-old male patient, V726A, M680I compound heterozygote in the *MEFV* gene as well as a novel variation of Y732*Stop [c.2196C > G (TAC > TAG)] nonsense variation in LPIN2 gene was identified.

Some of the other genes in the fever panel associated with mutations in the *MEFV* gene were also found to have different variations in patients with FMF. These variations were summarized in Table 5 in detail. Interestingly, most patients with mutations in the *MEFV* gene and other genes have heredofamilial amyloidosis.

4. Discussion

Although the clinical symptoms and course of the disease is still the cornerstone for the diagnosis of FMF, genetic confirmation of this condition helps to make differential diagnosis, especially in suspicious cases. Significant advances have been made by the help of rapidly developing molecular genetic techniques to understand the molecular basis of FMF disease and make differential diagnosis. The number and

Table 6
Syndromes associated with detected variations and their clinical significance.

Gene	Nucleotide change	Amino acid change	rs ID	ClinVar	Phenotype MIM number	Inheritance	Clinical Significance	Ref
MEFV	c.2040 G > A	p.Met680Ile	rs28940580	Familial Mediterranean Fever	249100	AR	Pathogenic	[17]
MEFV	c.2080 A > G	p.Met694Val	rs61752717	Familial Mediterranean Fever	249100	AR	Pathogenic /Likely pathogenic	[2]
MEFV	c.2082 G > A	p.Met694Ile	rs28940578	Familial Mediterranean Fever	249100	AR	Uncertain significance	
MEFV	c.605 G > A	p.Arg202Gln	rs224222	Familial Mediterranean Fever	249100	AR	Likely benign/ benign	Illumina
MEFV	c.1105C > T	p.Pro369Ser	rs11466023	Familial Mediterranean Fever	249100	AR	Pathogenic	[18,19]
MEFV	c.149C > T	p.Pro50Leu	rs144716190	Not Reported				
MEFV	c.2084 A > G	p.Lys695Arg	rs104895094	Familial Mediterranean Fever	249100	AR	Pathogenic /Likely pathogenic	[20]
MEFV	c.2282 G > A	p.Arg761His	rs104895097	Familial Mediterranean Fever	249100	AR	Likely pathogenic	[21]
MEFV	c.2177 T > C	p.Val726Ala	rs28940579	Familial Mediterranean Fever	249100	AR	Pathogenic	[22]
MEFV	c.442G > C	p.Glu148Gln	rs3743930	Familial Mediterranean Fever	249100	AR	Conflicting interpretations of pathogenicity	[17,18]
NOD2	c.3017 dupC/	p.Leu1007Profs	rs2066847	Inflammatory bowel disease 1 (Crohn Disease), Blau syndrome	266600, 186580	Mu, AD	Risk factor, Likely benign	[23,24]
NOD2	c.2722 G > C	p.Gly908Arg	rs2066845	Inflammatory bowel disease 1 (Crohn Disease), Blau syndrome	266600, 186580	Mu, AD	Risk factor, Likely benign	[24,25]
NOD2	c.2146C > T	p.Arg716Cys	rs776025574	Not Reported				
NOD2	c.1515delG	pSer506Profs	rs767278572	Inflammatory bowel disease 1 (Crohn Disease), Blau syndrome	266600, 186580	Mu, AD	Uncertain significance	[26]
CARD14	c.2648 G > A	pArg883His	rs2289541	Pityriasis rubra pilaris	173200	AD	Benign	[27]
CARD14	c.892C > T	p.Arg298*Stop	rs772958714	Not Reported				
CARD14	c.1356 + 5G > A	Intronic	rs376524884	Not Reported				
CARD14	c.2172C > A	pTyr724*Stop	rs141122143	Not Reported				
NLRP12	c.779C > T	p.Thr260Mellt	rs150280940	Familial Cold Autoinflammatory Syndrome	120100	AD	Likely benign	Illumina
NLRP12	c.2788 G > A	p.Ala930Thr	rs146368839	Not Reported				
NLRP12	c.1922 T > C	p.Ile641Thr	rs1405519522	Not Reported				
NLRP3	c.2113C > A	p.Gln705Lys	rs35829419	Familial Cold Autoinflammatory Syndrome	120100	AD	Benign	[28]
NLRP3	c.2861C > T	p.Thr954Met	rs139814109	Familial Cold Autoinflammatory Syndrome	120100	AD	Likely benign	Illumina
NLRP3	c.410G > A	p.Arg137His	rs138946894	Not specified			Uncertain significance	GeneDx
NLRP3	c.937A > G	p.Ile313Val	rs180177501	Muckle-Wells syndrome	191900	AD	Uncertain significance	Invitae
NLRP3	c.598 G > A	p.Val200Met	rs121908147	Familial Cold Autoinflammatory Syndrome	120100	AD	Pathogenic/Likely benign	[29,30]
NLRP7	c.1137G > C	p.Lys379Asn	rs10418277	Hydatidiform mole	231090	AR	Not provided	CHRU Montpellier
NLRP7	c.574A > C	p.Met192Leu	rs104895529	Hydatidiform mole	231090	AR	Not provided	CHRU Montpellier
NLRP7	c.930 G > T	p.Gln310His	rs145973556	Not Reported				
NLRP7	c.1614 T > A	p.Phe538Leu	rs200193926	Not Reported				
IL1RN	c.78 G > A	pThr26Thr	rs2232353	Interleukin 1 Receptor Antagonist Deficiency	612852	AR	Uncertain significance	Illumina
LPIN2	c.1786 G > A	p.Gly596Ser	rs769806854	Not Reported				
LPIN2	c.1685 G > A	p.Arg562Gln	rs1476056180	Not Reported				
LPIN2	c.2621 G > T	p.Cys874Phe	rs201160155	Majeed syndrome	609628	Unknown	Uncertain significance	[26]
LPIN2	c.1510C > T	p.Leu504Phe	rs104895500	Majeed syndrome	609628	Unknown	Likely benign	GeneDx
LPIN2	c.1876C > T	p.Pro626Ser	rs150806357	Majeed syndrome	609628	Unknown	Likely benign	GeneDx
TNFRSF1A	c.362 G > A	p.Arg121Gln	rs4149584	TNF receptor-associated periodic fever syndrome (TRAPS)	142680	AD	Pathogenic	[31]
TNFRSF1A	c.123 T > G	p.Asp41Glu	rs104895271	TNF receptor-associated periodic fever syndrome (TRAPS)	142680	AD	Pathogenic	[32]
MVK	c.118C > T	p.Arg40Trp	rs1055952433	Not Reported				
PSTPIP1	c.203C > T	p.Thr68Met	rs201872851	Behcet's syndrome, Pyogenic arthritis, pyoderma gangrenosum and acne	109650 604416	[a]AD	Likely benign	Illumina
PSTPIP1	c.1115C > T	p. Ala372Val	rs200188483	Not specified			Uncertain significance	ARUP Laboratories
PSMB	c.208 A > T	p.Thr70Ser	rs17220206	Nakajo syndrome	256040	AR	Benign	[26]
IL10RA	c.716 T > C	p.Phe239Ser	rs541386535	Not Reported				

AD: Autosomal dominant; AR: Autosomal recessive; Mu: Multifactorial.
[a] Behcet's disease; familial cases reported, but probably not Mendelian.

structure of mutations in the *MEFV* gene, which plays a role in the etiopathogenesis of FMF cases varies between populations. The allele heterogeneity of the *MEFV* gene is particularly high in Turkish populations. In addition, the prevalence of this disease in Turkey was reported as 1/1000 and carrier rate was 1/5 and this percentage is quite high [13]. M694V, E148Q, M680I, V726A mutations are frequently identified variations in *MEFV* gene [5,14]. In line with these, in our study, the most common homozygous mutations were M694V (4.76%) and R202Q (4.88%) missense mutations. In addition, the most common heterozygous mutations include R202Q (42.35%), M694V (16.47%) and E148Q (12.94%) missense mutations. M680I and V726A mutations were less common than these.

In FMF, the distribution of mutations in *MEFV* gene has been shown in several studies in different populations. However, other gene mutations associated with FMF have been identified with the adaptation of next-generation sequencing methods to the routine clinic laboratories and increased genomic regions that need to be screened. In our study, mutation screening of genes that play important roles in the pathology of fever-related autoinflammatory diseases in patients with FMF was performed using NGS method and interesting results were obtained. Since this screening method is new and not widely used in both Turkish populations and Mediterranean countries, the data we obtained here is unique and provides a novel perpective for this disease. Routine use of molecular genetic tests provides important contributions to the differential diagnosis of autoinflammatory diseases as well as for the treatment planning. Previous studies have suggested that screening of *MEFV* gene mutations is sufficient for the genetic testing of FMF. Notably, in this study, we found that mutations in the *MEFV* gene show different frequencies and for the first time, along with *MEFV* gene mutations, we identified novel variations in genes associated with periodic fever syndromes that have not been previously reported (Table 6).

Berkun et al. have identified frequencies of G908R (8.7%) and R702W (1%) mutations in the *NOD2* gene (formerly known as *CARD15*) in FMF patients [15]. They suggested that *NOD2* variations do not show high sensitivity for FMF development, but the presence of related mutations in these patients may be associated with a certain phenotypic effect and course of the disease [15]. In this study, we demonstrated a new heterozygous variation in the *NOD2* gene together with *MEFV* M680I mutation in 1 patient with heredofamilial amiliodosis. In addition, in another patient with joint pain, the L1007Profs frame shift variation in the *NOD2* gene was detected together with the *MEFV* E148Q heterozygote mutation. When these results are evaluated together, mutations in the *MEFV* gene together with the mutations in the *NOD2* gene might be associated with different phenotypic effects.

In a multi-center study, Karacan et al., showed that in addition to *MEFV* gene mutations, *CARD14* gene mutations were present in FMF patients [16]. In patients with *MEFV* M694V mutation, P510fs (frame shift mutation as a result of duplication) and A364V variation were detected in *CARD14* gene. Also, in addition to M694V and V726A mutations, which are common in a *MEFV* gene, R151Q mutation was detected in *CARD14* gene [16]. In our study, in addition to M680I (*MEFV*) and T91A (*NOD2*) mutations, R883H mutation was detected in *CARD14* gene. In addition to *MEFV* gene mutations, variations in *NLRP* (*NLRP3, NLRP7, NLRP12*) genes was also identified. Karacan et al., also identified M694V (*MEFV*) and G52S (*NLRP12*) mutations in a patient with vasculitis [16]. Our study is somewhat more comprehensive in this respect as *NLRP* gene mutations were detected in addition to *MEFV* gene in FMF patients (Tablo 5). Interestingly, the majority of patients (4 out of 6 patients) who have mutations in the *NLRP* genes along with the *MEVF* gene have heredofamilial amiliodosis. These results suggest that *NLRP* genes might be associated with amiliodosis.

5. Conclusions

In conclusion, the findings of this study suggest that mutations in genes associated with other autoinflammatory diseases together with the *MEFV* gene mutations contribute to the molecular pathophysiology of FMF disease. Partiuclarly, we identified two novel missense variations in the *MEFV* gene, Gln34Pro and Ile247Val for a first time in FMF disease. Also, novel variations of Thr91Ile missense variation in the *NOD2* gene, Gly461Cys missense variation in *NLRP3* and Tyr732Stop nonsense variation in *LPIN2* were firstly identified. Current study will provide a new perspective for the differential diagnosis of FMF.

Along with the *MEFV* gene mutations, variations detected in other genes associated with other autoimflammatory diseases should be taken in consideration by the clinicians in dealing with FMF patients. Although Sanger sequencing is a gold standard technique, the value of NGS, which enables screening of large number of genes in a single run, should not be neglected. In Sanger sequencing, limited number of genomic regions are currently screened for the FMF disease and patients that have no detected mutations in certain exons of *MEFV* gene are false-negatively considered as non-FMF. Therefore, we concluded that NGS analysis will be useful in the differential diagnosing FMF cases that are missed during rutin screening of *MEFV* gene variations.Also, in most genetic centers, only analysis of exon 2 and 10 of *MEFV* gene are analyzed. However, NGS analysis allows screening of all exons of *MEFV* gene in addition to other genes involved in autoimflammatory diseases.

Moreover, our study has some limitations. On the major limitation is that these results should be supported by multi-center studies with larger samples and standardization of the study population should be enhanced. In addition, lack of pathogenicity information of newly identified variants in the databases is insufficient to interpret the results. Further studies are needed to better understand clinical importance of these variations. Also, another important limitation of our study is that variations detected by NGS were not verified with Sanger sequencing method and familial segregation was not established.

Author statement

All authors confirmed that they have contributed to the intellectual content of this paper and have met the requirements of authorship including conception and design, analysis and interpretation of data, drafting and revising of the manuscript.

Declaration of Competing Interest

The author declares no conflicting interests in this work.

References

[1] E. Ben-Chetrit, M. Levy, Familial mediterranean fever, Lancet 351 (1998) 659–664.
[2] E. Ben-Chetrit, I. Touitou, Familial mediterranean fever in the world, Arthritis Rheum. 61 (2009) 1447–1453.
[3] Hafize Emine Sönmez, Ezgi Deniz Batu, Seza Özen, Familial Mediterranean fever: current perspectives, J. Inflamm. Res. 9 (2016) 13–20, https://doi.org/10.2147/JIR.S91352.
[4] Seza Ozen, Yelda Bilginer, A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin, Nat. Rev. Rheumatol. 10 (2014) 135–147, https://doi.org/10.1038/nrrheum.2013.174.
[5] E. Yilmaz, S. Ozen, B. Balcı, A. Duzova, R. Topaloglu, N. Besbas, U. Saatci, A. Bakkaloglu, M. Ozguc, Mutation frequency of familial Mediterranean fever and evidence for a high carrier rate in the Turkish population, Eur. J. Hum. Genet. 9 (2001) 553.
[6] H. Peru, A.M. Elmacı, A. Yorulmaz, B. Altun, F. Kara, The Evaluation of Patients with Familial Mediterranean Fever in Konya Region: A Retrospective Clinic and Genetic Study Genel Tip Dergisi, 18, (2008).
[7] E. Pras, I. Aksentijevich, L. Gruberg, J.E. Balow Jr., L. Prosen, M. Dean, A.D. Steinberg, M. Pras, D.L. Kastner, Mapping of a gene causing familial Mediterranean fever to the short arm of chromosome 16, N. Engl. J. Med. 326 (1992) 1509–1513.
[8] G. Manukyan, R. Aminov, Update on pyrin functions and mechanisms of familial Mediterranean fever, Front. Microbiol. 7 (2016) 456.
[9] O. Schnappauf, J.J. Chae, D.L. Kastner, I. Aksentijevich, The Pyrin Inflammasome in health and disease, Front. Immunol. 10 (2019) 1745.
[10] K. Migita, T. Asano, S. Sato, T. Koga, Y. Fujita, A. Kawakami, Familial Mediterranean fever: overview of pathogenesis, clinical features and management, Immunol. Med. 41 (2018) 55–61.
[11] E. Coşkunpınar, A. Özvarnalı, K. Çefle, A. Palanduz, A. Gül, D. Öztürk, Ş. Öztürk,

Ş. Palanduz, Molecular diagnosis experience in familial Mediterranean fever: the Most frequent mutations in the MEFV gene, Haseki Tip Bulteni 56 (2018) 42.
[12] E. Gumus, The frequency of MEFV gene mutations and genotypes in Sanliurfa province, south-eastern region of Turkey, after the Syrian civil war by using next generation sequencing and report of a novel exon 4 mutation (I423T), J. Clin. Med. 7 (2018) 105.
[13] M. Tunca, S. Akar, F. Onen, H. Ozdogan, O. Kasapcopur, F. Yalcinkaya, E. Tutar, S. Ozen, R. Topaloglu, E. Yilmaz, Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study, Medicine 84 (2005) 1–11.
[14] A. Olgun, S. Akman, I. Kurt, A. Tuzun, T. Kutluay, MEFV mutations in familial Mediterranean fever: association of M694V homozygosity with arthritis, Rheumatol. Int. 25 (2005) 255–259.
[15] Y. Berkun, A. Karban, S. Padeh, E. Pras, Y. Shinar, M. Lidar, A. Livneh, Y. Bujanover, NOD2/CARD15 gene Mutations in Patients with Familial Mediterranean Fever, Seminars in Arthritis and Rheumatism, Elsevier, 2012, pp. 84–88.
[16] İ. Karacan, A. Balamir, S. Uğurlu, A.K. Aydın, E. Everest, S. Zor, M.Ö. Önen, S. Daşdemir, O. Özkaya, B. Sözeri, Diagnostic utility of a targeted next-generation sequencing gene panel in the clinical suspicion of systemic autoinflammatory diseases: a multi-center study, Rheumatol. Int. 39 (2019) 911–919.
[17] A. Bernot, C. da Silva, J.L. Petit, C. Cruaud, C. Caloustian, V. Castet, M. Ahmed-Arab, C. Dross, M. Dupont, D. Cattan, N. Smaoui, C. Dode, C. Pecheux, B. Nedelec, J. Medaxian, M. Rozenbaum, I. Rosner, M. Delpech, G. Grateau, J. Demaille, J. Weissenbach, I. Touitou, Non-founder mutations in the MEFV gene establish this gene as the cause of familial Mediterranean fever (FMF), Hum. Mol. Genet. 7 (1998) 1317–1325.
[18] I. Aksentijevich, Y. Torosyan, J. Samuels, M. Centola, E. Pras, J.J. Chae, C. Oddoux, G. Wood, M.P. Azzaro, G. Palumbo, R. Giustolisi, M. Pras, H. Ostrer, D.L. Kastner, Mutation and haplotype studies of familial Mediterranean fever reveal new ancestral relationships and evidence for a high carrier frequency with reduced penetrance in the Ashkenazi Jewish population, Am. J. Hum. Genet. 64 (1999) 949–962.
[19] J.G. Ryan, S.L. Masters, M.G. Booty, N. Habal, J.D. Alexander, B.K. Barham, E.F. Remmers, K.S. Barron, D.L. Kastner, I. Aksentijevich, Clinical features and functional significance of the P369S/R408Q variant in pyrin, the familial Mediterranean fever protein, Ann. Rheum. Dis. 69 (2010) 1383–1388.
[20] S. Oztuzcu, M. Ulasli, S. Ergun, Y.Z. Igci, M. Igci, R. Bayraktar, G. Nacarkahya, A. Tamer, M.O. Cevik, E.A. Cakmak, A. Arslan, Screening of common and novel familial mediterranean fever mutations in south-east part of Turkey, Mol. Biol. Rep. 41 (2014) 2601–2607.
[21] M. Bonyadi, M. Esmaeili, H. Jalali, M.H. Somi, A. Ghaffari, M. Rafeey, K. Sakha, N. Lotfalizadeh, A. Pourhassan, M. Khoshbaten, M.R. Ardalan, N. Laghaeian, MEFV mutations in Iranian Azeri Turkish patients with familial Mediterranean fever, Clin. Genet. 76 (2009) 477–480.
[22] F. Yalcinkaya, N. Akar, M. Misirlioglu, Familial Mediterranean fever–amyloidosis and the Val726Ala mutation, N. Engl. J. Med. 338 (1998) 993–994.
[23] R. Parkhouse, T.P. Monie, Dysfunctional Crohn's disease-associated NOD2 polymorphisms cannot be reliably predicted on the basis of RIPK2 binding or membrane association, Front. Immunol. 6 (2015) 521.
[24] I. Peter, A.A. Mitchell, L. Ozelius, M. Erazo, J. Hu, D. Doheny, M.T. Abreu, D.H. Present, T. Ullman, K. Benkov, B.I. Korelitz, L. Mayer, R.J. Desnick, Evaluation of 22 genetic variants with Crohn's disease risk in the Ashkenazi Jewish population: a case-control study, BMC Med. Gen. 12 (2011) 63.
[25] S. Yazdanyar, M. Weischer, B.G. Nordestgaard, Genotyping for NOD2 genetic variants and crohn disease: a metaanalysis, Clin. Chem. 55 (2009) 1950–1957.
[26] K. Nykamp, M. Anderson, M. Powers, J. Garcia, B. Herrera, Y.Y. Ho, Y. Kobayashi, N. Patil, J. Thusberg, M. Westbrook, S. Topper, Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria, Genet. Med. 19 (2017) 1105–1117.
[27] T. Takeichi, K. Sugiura, T. Nomura, T. Sakamoto, Y. Ogawa, N. Oiso, Y. Futei, A. Fujisaki, A. Koizumi, Y. Aoyama, K. Nakajima, Y. Hatano, K. Hayashi, A. Ishida-Yamamoto, S. Fujiwara, S. Sano, K. Iwatsuki, A. Kawada, Y. Suga, H. Shimizu, J.A. McGrath, M. Akiyama, Pityriasis Rubra Pilaris type V as an autoinflammatory disease by CARD14 mutations, JAMA Dermatol. 153 (2017) 66–70.
[28] T.V. Ting, L.V. Scalzi, P.J. Hashkes, Nonclassic neurologic features in cryopyrin-associated periodic syndromes, Pediatr. Neurol. 36 (2007) 338–341.
[29] E. Aganna, F. Martinon, P.N. Hawkins, J.B. Ross, D.C. Swan, D.R. Booth, H.J. Lachmann, A. Bybee, R. Gaudet, P. Woo, C. Feighery, F.E. Cotter, M. Thome, G.A. Hitman, J. Tschopp, M.F. McDermott, Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis, Arthritis Rheum. 46 (2002) 2445–2452.
[30] H.M. Hoffman, J.L. Mueller, D.H. Broide, A.A. Wanderer, R.D. Kolodner, Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and muckle-Wells syndrome, Nat. Genet. 29 (2001) 301–305.
[31] H. Lee, J.L. Deignan, N. Dorrani, S.P. Strom, S. Kantarci, F. Quintero-Rivera, K. Das, T. Toy, B. Harry, M. Yourshaw, M. Fox, B.L. Fogel, J.A. Martinez-Agosto, D.A. Wong, V.Y. Chang, P.B. Shieh, C.G. Palmer, K.M. Dipple, W.W. Grody, E. Vilain, S.F. Nelson, Clinical exome sequencing for genetic identification of rare Mendelian disorders, Jama 312 (2014) 1880–1887.
[32] J. Havla, P. Lohse, L.A. Gerdes, R. Hohlfeld, T. Kumpfel, Symptoms related to tumor necrosis factor receptor 1-associated periodic syndrome, multiple sclerosis, and severe rheumatoid arthritis in patients carrying the TNF receptor superfamily 1A D12E/p.Asp41Glu mutation, J. Rheumatol. 40 (2013) 261–264.