Arch Rheumatol 2023;38(2):238-248
doi: 10.46497/ArchRheumatol.2023.9468

ORIGINAL ARTICLE

Mevalonate kinase gene polymorphisms in ankylosing spondylitis patients: A cross-sectional study

Fatih Yıldız[1], Suzan Dinkçi[2], Eren Erken[3]

[1]Department of Internal Medicine, Division of Rheumatology, Kahramanmaraş Sütçü Imam University Faculty of Medicine, Kahramanmaraş, Türkiye
[2]Immunology and HLA Laboratory, Çukurova University Faculty of Medicine, Adana, Türkiye
[3]Department of Rheumatology and Immunology, Çukurova University Faculty of Medicine, Adana, Türkiye

ABSTRACT

Objectives: This study aimed to investigate the potential effect of the mevalonate kinase (MVK) gene polymorphisms on the pathogenesis and clinical findings in ankylosing spondylitis (AS) patients.

Patients and methods: This cross-sectional study was conducted with 103 participants (63 males, 40 females) between January 2013 and January 2014. Of these, 51 (32 males, 19 females; mean age: 37.3±10.2 years; range, 19 to 60 years) were adult AS patients who met the 1984 Modified New York Criteria, and 52 (31 males, 21 females; mean age: 33.8±12 years; range, 19 to 60 years) were healthy volunteers with similar demographics. MVK gene analysis was performed using polymerase chain reaction sequencing by isolating deoxyribonucleic acids from peripheral blood samples. We determined serum immunoglobulin (Ig)D levels using radial immunodiffusion. We performed physical examinations on the AS patients. The Bath Ankylosing Spondylitis Disease Activity Index and the Bath Ankylosing Spondylitis Functional Index forms were filled and erythrocyte sedimentation rate, C-reactive protein, and IgD levels were recorded.

Results: There was no statistically significant difference in the mean age between the groups (p=0.121). The frequency of symptomatic single nucleotide polymorphisms (SNPs), c.769-38 C>T heterozygous, c.769-7 T>G heterozygous, and c.769-38 C>T homozygous were similar between the groups (15/15; p=0.646). Nonsymptomatic SNPs were more common in the patient group, but the difference was not significant (83/58; p>0.05). The rate of having an MVK gene polymorphism was 36 (70.6%) in the AS compared to the 33 (63.4%) in the control group (p>0.05). There were no associations in clinical findings between the AS patients with or without MVK gene polymorphisms. New heterozygous SNPs, I56V A>G, E281D G>D, V80I G>A, and C173Y G>A, were present in four AS patients.

Conclusion: The frequency of MVK gene polymorphisms was higher in AS patients than in healthy controls. But there was no statistically significant difference. We determined no effect of the present polymorphisms on AS clinical and laboratory findings.

Keywords: Ankylosing spondylitis, autoinflammation, mevalonate kinase gene, periodic fever.polymorphism.

Ankylosing spondylitis (AS) is a chronic, progressive, inflammatory disease that often involves the axial skeleton but can also involve the hip, shoulder, and peripheral joints, such as the knee and ankle.[1] The prevalence of AS has been reported to be up to 1.4% depending on ethnicity, human leukocyte antigen (HLA)-B27 frequency, selection criteria for evaluation, and diagnostic criteria.[1,2] In an epidemiological study conducted by Onen et al.[2] in Türkiye, the prevalence of AS was 0.49%. Genetic factors play a fundamental role in the etiology of AS.[3] The major histocompatibility complex (MHC) locus, which includes the HLA-B27 and HLA-B genes, strongly relevant to genetic predisposition, contributes the most to the onset of the disease.

Received: December 30, 2021 ***Accepted:*** June 06, 2022 ***Published online:*** October 21, 2022

Correspondence: Fatih Yıldız, MD. Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi, Romatoloji Bilim Dalı, 46040 Onikişubat, Kahramanmaraş, Türkiye.
E-mail: drfatih75@gmail.com

Citation:
Yıldız F, Dinkçi S, Erken E. Mevalonate kinase gene polymorphisms in ankylosing spondylitis patients: A cross-sectional study. Arch Rheumatol 2023;38(2):238-248. doi: 10.46497/ArchRheumatol.2023.9468.

Various research groups have shown the role of the interleukin (IL)-1 gene complex in AS. Ankylosing spondylitis is commonly seen with familial Mediterranean fever (FMF), an autoinflammatory disease that causes IL-1 gene and receptor defects.[4] Ankylosing spondylitis can coexist with autoinflammatory diseases. Many studies have reported the association of AS and spondyloarthropathy (SpA) with FMF.[4-7] In a study, the frequency of juvenile SpA in pediatric FMF patients was 10.2%, and the M694V mutation was very common in both groups (FMF alone and FMF+juvenile SpA).[8]

Mevalonate kinase deficiency (MKD) is a monogenic autoinflammatory syndrome. It is also a multisystem inflammatory disease and a possible immunodeficiency disorder.[9] The mevalonate kinase (MVK) gene responsible for MKD (also known as hyper-immunoglobulin (Ig) D syndrome or HIDS) has been identified.[10,11] The disease was described by van der Meer et al.[12] in Dutch patients. MKD is a rare autosomal recessive disease belonging to the autoinflammatory syndrome group characterized by fever attacks.[13] The four most common mutations (V377I, I268T, H20P/N, and P167L) are responsible for 71.5% of all mutations. Typically, MKD patients have fever attacks lasting for three to seven days that start in the first year of life. Attacks may generally recur every four to six weeks. The most common symptoms accompanying fever are lymphadenopathy, abdominal pain, joint pain, diarrhea, vomiting, skin lesions, and aphthous ulcers.[13] Another important feature of the disease is systemic inflammation. Clinical symptoms also occur due to IL-1β irregularity.[14]

There is a strong relationship between the HLA gene and AS. Many other genes have been investigated until now, but the MVK gene has not been studied in AS patients. Potential association with the MEFV gene has been reported in AS patients, particularly in those who are HLA-B27 negative.[4,6,15] Except for the HLA gene and a few non-HLA genes that contribute to the pathogenesis of AS, it is still not fully elucidated. MVK gene polymorphisms in Behçet's disease has previously been investigated.[16,17] However, MVK gene polymorphisms in AS have remained unaddressed so far. Therefore, the present study investigated the possible contribution of MVK gene polymorphisms to pathogenesis and clinical findings in AS patients.

PATIENTS AND METHODS

This nonrandomized, cross-sectional, analytical study was conducted with 103 participants at the Çukurova University Faculty of Medicine, Department of Rheumatology between January 2013 and January 2014. Of these, 51 (32 males, 19 females; mean age: 37.3±10.2 years; range, 19 to 60 years) were consecutive AS patients who met the 1984 Modified New York Criteria for AS[18] and were followed up in our rheumatology outpatient clinic in 2013. The control group consisted of 52 (31 males, 21 females; mean age: 33.8±12 years; range 19 to 60 years) age-and sex-matched healthy volunteers.

Ankylosing spondylitis patients under 18 years of age, patients with a diagnosis or a family history of FMF and those with comorbid conditions (e.g., amyloidosis) or malignant disease were excluded from the study. Meanwhile, for the control group, any participants with inflammatory back pain, SpA findings, or a history of systemic autoimmune or autoinflammatory diseases were excluded.

The demographic characteristics and medical histories of the AS patients were queried and recorded. We used the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Bath Ankylosing Spondylitis Functional Index (BASFI) to assess disease activity and function in patients. For this purpose, we administered BASDAI and BASFI forms that were prepared and validated beforehand to the patients.[19,20] Afterward, we examined the AS patients, assessed their posture, and performed the measurements of Schober's test, fingertip-to-floor distance, occiput-wall distance, and chest expansion. We also checked the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and IgD levels in the AS patients. In addition, we recorded the MKD symptoms and signs, such as fever, lymphadenopathy, abdominal pain, splenomegaly, arthralgia/arthritis, cutaneous findings, serositis, orbital tendonitis, and secondary amyloidosis, in AS patients and the control group.

From each patient and control, we drew 3 mL of blood into an EDTA tube and 5 mL of blood into a biochemistry tube and determined IgD levels using the radial immunodiffusion method. We obtained deoxyribonucleic acids (DNAs) from peripheral blood samples in the immunology and HLA typing laboratory and performed MVK gene mutation analysis using the polymerase chain reaction (PCR) sequencing method.

Genetic analysis

We performed molecular genetic analysis on genomic DNA extracted from venous blood in EDTA stored at -20°C, using the RTA Genomic DNA isolation kit (RTA Laboratuvarları, Gebze, Kocaeli, Türkiye) according to the manufacturer's instructions. The genotyping of the MVK gene was achieved through the PCR sequencing method. We amplified the exonic regions and the exon-intron junction of this gene by PCR using the specific primers given in Figure 1.

The PCR proceeded with 35 cycles of 95°C for 45 sec, followed by 95°C for 10 min, 59°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 7 min. PCR amplification was performed on a Gradient Palm-Cycler (Corbett Life Science, Sydney, Australia). We treated the resulting PCR product with ExoSAP-IT (GML A.G., Wollerau, Switzerland) and then processed it with the BigDye Terminator version 3.1 Direct Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's instructions. We ran the sequencing reactions in an automated capillary DNA sequencer (ABI-3500 Genetic Analyzer 8-Capillary Array; Applied Biosystems, Warrington, UK). We used SeqScape Software version 2.7 and Sequencing Analysis Software version 5.4 for sequencing analysis (Thermo Fisher Scientific, Waltham, MA, USA). The IgD level was determined by radial immunodiffusion, and CRP was assayed in serum using the nephelometric method on Dade Behring BN II Nephelometer (Dade Behring Inc., Deerfield, IL, USA). For ESR, whole blood containing EDTA was analyzed using an autoanalyzer (flow kinetic analysis with capillary photometry).

MKD-EX2-F: 5'- TGCTCTTCTCATTGGCTTTCGCT - 3'
MKD-EX2-R: 5'- TGATCTGCTCCCAAGCTGTAC - 3'
MKD-EX3-F: 5'- TAGTGGGAGAGCATGCCAAAGG- 3'
MKD-EX3-R: 5'- CCTTGCACTCACCCAGAATGG- 3'
MKD-EX4-F: 5'- GCACCAAAGTCCCTCTCACACT- 3'
MKD-EX4-R: 5'- CCTGGCACATGATGAGGACACCCT- 3'
MKD-EX5-F: 5'- CCCCTGGTTCAGTGCTGCGA- 3'
MKD-EX5-R: 5'- AGCACTAGCTGTGTGGCGCA- 3'
MKD-EX6-F: 5'- CTCCTATGCCCCTTGGGCTT- 3'
MKD-EX6-R: 5'- GCCCCAAGATTTCTCCCCAAGCC - 3'
MKD-EX7-F: 5'- GGCAAAATGAACCCAACCAC- 3'
MKD-EX7-R: 5'- CACTGTGAGGGCCACATTAA- 3'
MKD-EX9-F: 5'-AGACACACTGACTTTGCAGCTGCCT - 3'
MKD-EX9-R: 5'- AGCATAGGCCAGAGGCAAAG- 3'
MKD-EX10-F: 5'- ACAACTGTCAGATGGACGAGTGA- 3'
MKD-EX10-R: 5'- TTCTCCAGGTGGACCCCAGA- 3'
MKD-EX11-F: 5'- TGTTGGCTCAGGTGGGTCACTGA- 3'
MKD-EX11-R: 5'- CTGCAGAGCTTGGCTGGCCGG- 3'

Figure 1. Mevalonate kinase primers.

Statistical analysis

Data were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were taken as numbers and percentages, and continuous variables as mean ± standard deviation and median (minimum-maximum). The chi-square test was used to compare categorical variables between groups. The Kolmogorov-Smirnov test was used to determine whether sample data were normally distributed. The independent samples t-test was used for continuous variable analysis between independent groups. The one-way analysis of variance was used to compare continuous variables between groups. A p value of <0.05 was considered statistically significant.

RESULTS

There was no statistically significant difference between the two groups in age (p=0.121). The demographic characteristics of the patient and control groups are summarized in Table 1.

All patients had bilateral Grade ≥2 or unilateral ≥3-4 sacroiliitis on pelvic X-ray or sacroiliac spot imaging that met the New York radiological criteria for AS.[18] In 21 patients who underwent the HLA-B27 test, nine (42.8%) were positive, and 12 (57.2%) were negative. There were 18 (35.3%)

Table 1. Demographic characteristics of AS patients

	All cases (n=51)					AS (Man) (n=32)					AS (Woman) (n=19)					
Parameters	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	*p*
Age (year)			37.3±10.3					-					-			
Symptom age (year)			27±7.7					24.2±7.5					31.9±5.4			<0.001
Age at diagnosis (year)			30.8±8.2					27.9±8.0					35.7±5.9			0.001
Delay in diagnosis (year)			3.8±2.9					3.8±3.0					3.7±2.8			0.902
Peripheral arthritis	18	35.3				10	31.3				8	42.1				0.435
FFD (cm)*				13.82	0-40				17.5	0-40				5	0-38	0.003
OWD (cm)*				2.24	0-16				3.33	0-16				1.0	0-10	0.029
Schober (5> cm)			2.9±1.2					2.7±1.2					3.4±1.1			0.072
CE (>5 cm)			4.1±1.2					3.9±1.0					4.4±1.3			0.142
BASDAI (<4)			3.7±1.9					3.4±2.0					4.1±1.6			0.215
BASDAI (<4)																
Active	19	37.3				10	31.3				9	47.4				0.252
Remission	32	62.7				22	68.8				10	52.6				
BASFI			3.2±1.8					2.8±1.9					3.8±1.4			0.063
ESR (N <25 mm/h)				19	2-97				16	2-97				28	2-90	0.306
CRP (N <0.8 mg/dL)				0.60	0.1-3.8				0.600	0.1-3.7				0.790	0.1-3.8	0.547
IgD (N 5-50 mg/L)				22.2	4-152				22.2	4-152				22.0	4-100	0.852
Smoking																
Yes	18	35.3				16					2					<0.001
No	33	64.7				16					17					

AS: Ankylosing spondylitis; SD: Standard deviation; FFD: Fingertip-to-floor distance; OWD: Occiput-wall distance; CE: Chest expansion; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ESR: Erythrocyte sedimentation rate; CRP: C-Reactive protein; N: Normal value; p values: Independent samples t-test, statistical significance (p<0.05).

patients with peripheral arthritis. Peripheral arthritis was more common in female patients than males, but the difference was not statistically significant (p=0.43). The mean Schober's test, fingertip-to-floor distance, occiput-wall distance, and chest expansion measurements of the patients are given in Table 1.

The mean BASDAI of AS patients was 3.67±1.864 (male/female [M/F]: 3.42/4.09). Thirty-two (62.7%) patients with a BASDAI score <4 were considered in remission. The difference between the number of male and female patients in remission was not significant (p=0.252).

Drug use was as follows: only nonsteroidal anti-inflammatory drugs, 10 (19.6%); sulfasalazine (SSZ), 12 (23.6%); methotrexate+SSZ, one (1.9%); etanercept, 14 (27.5%); etanercept+SSZ, one (1.9%); adalimumab, eight (15.7%); infliximab, five (9.8%). Twenty-eight (64.4%) of the patients used tumor necrosis factor alpha antagonists. One (2%) male patient had secondary amyloidosis.

The patient group's median ESR was 19 (2-97; M/F:16/28) mm/h, and the median CRP was 0.68 mg/dL (0.1-3.8; M/F: 0.6/0.79). The patients were divided into two as active and remission according to the BASDAI. The ESR, CRP, and IgD levels were compared for both groups. The ESR and CRP were significantly correlated with disease activity according to the BASDAI (p=0.002 and p=0.001, respectively), which was not the case with IgD levels (p=0.320; Table 1). There was a significant correlation between the ESR, CRP, and BASDAI in demonstrating disease activity. Male AS patients had more severe clinical symptoms than females.

Single nucleotide polymorphisms (SNPs) have been classified as symptomatic and nonsymptomatic according to the Infevers website (https://infevers.umai-montpellier.fr/web/).

The first group (symptomatic) SNPs, c.769-38 C>T heterozygous (intron-8), c.769-7 T>G heterozygous (intron-8), and c.769-38 C>T homozygous (intron-8) were classified into four groups as homozygous, compound heterozygous, heterozygous, and not present. There were 15 symptomatic SNPs in the patient and control groups each. The difference between the two groups was not statistically significant (p=0.646; Table 2).

An investigation of the second group (nonsymptomatic) SNPs revealed the presence of D170D C>T heterozygous (exon-5), c632-18 A>G heterozygous (intron-6), c.885+24 G>A heterozygous (intron-9), S52N G>A heterozygous (exon-3), c.371+8 C>T heterozygous (intron-4), c.78+61 A>G heterozygous (exon-5), c632-18 A>G homozygous (intron-69), and S135S G>A (exon-5). The difference between the patient and control groups regarding the distribution of nonsymptomatic SNPs was not statistically significant (p>0.05; Table 3).

Table 2. Mevalonate kinase SNPs

	All		AS		Control		
	n	%	n	%	n	%	*p*
Symptomatic SNP							
Homozygous	7	6.8	2	3.9	5	9.6	
Heterozygous	23	22.3	12	23.5	9	17.3	0.646
Compound heterozygous	1	1	1	2	1	1.9	
Not present	72	69.9	36	70.6	36	71.2	
All SNP							
Present	52	50.5	36	70.6	33	63.4	
Absent	51	49.5	15	29.4	19	36.6	>0.05
Total	103		51		52		

SNPs: Single nucleotide polymorphisms; AS: Ankylosing spondylitis; P value is statistical significance (p<0.05).

Table 3. Nonsymptomatic SNPs

	Yes				No			
	AS		Control		AS		Control	
SNP	n	%	n	%	n	%	n	%
c632-18 A>G Heterozygous	25	49.09	17	32.7	26	51	35	67.3
D170D C>T Heterozygous	19	37.3	13	25	32	62.7	39	75
c.885+24 G>A Heterozygous	11	21.6	10	19.2	40	78.4	42	80.8
S52N G>A Heterozygous	9	17.6	10	19.2	42	82.4	42	80.8
c.371+8 C>T Heterozygous	10	19.6	9	17.3	41	80.4	43	82.7
c.78+61 A>G Heterozygous	2	3.9	4	7.7	49	96.1	48	92.3
c632-18 A>G Homozygous	2	3.9	4	7.7	49	96.1	48	92.3
S135S G>A	5	9.8	4	7.7	46	90.2	48	92.3

SNPs: Single nucleotide polymorphisms; AS: Ankylosing spondylitis; Differences in all groups are not significant

A group of new SNPs was found in the patient group: I56V A>G heterozygous, E281D G>D heterozygous, V80I G>A heterozygous, and C173Y G>A heterozygous. None of the patients had clinical findings resembling MKD.

Nonsymptomatic SNPs were more common in the patient group than in the control group, but the difference was not statistically significant (p>0.05). Similarly, there was no statistically significant difference in the distribution of SNPs according to the type of AS involvement (p=0.646; Table 2).

An assessment of the total number of SNPs concerning smoking revealed that at least one SNP was present in 13 (72%) smokers and 23 (69%) nonsmokers; however, the difference was not statistically significant (p=0.558).

When AS patients were evaluated as having or not having MVK polymorphism, their clinical and laboratory characteristics were found similar, with no statistically significant difference (p>0.05; Table 4). Single nucleotide polymorphisms in the patient and control groups were also similar, with no statistically significant difference (p>0.05; Table 5).

An investigation of SNPs' copresence in the study population yielded the following results: c.769-38 C>T heterozygous and c.769-38 C>T homozygous were together with c.78+61 A>G heterozygous in 100% of the cases. The same was true for c.769-38 C>T heterozygous and c.632-18 A>G heterozygous. Similarly, c.769-38 C>T heterozygous and c.769-38 C>T homozygous were together with S135S G>A in %100 of the cases. The differences between the patient and control groups regarding these copresences were not statistically significant (p>0.05).

The patient group's median IgD was 22.2 (5-50; M/F: 22.2/22) mg/L, and the difference between sex was not statistically significant (p=0.852). The IgD levels were classified into three groups: Group 1, <50 mg/L; Group 2, 50-100 mg/L; Group 3, >100 mg/L. There were 43 (84.3%) patients in Group 1, six (11.8%) patients in Group 2, and two patients in Group 3. The mg/L to IU/mL conversion factor for the IgD level (25 mg/L=17.7 U/mL) was 0.70.[21] In our study, 15.3% of AS patients had high serum IgD levels, which were not correlated with disease activity. One patient with IgD >100 IU/mL had no autoinflammatory clinical presentation or MVK gene mutation.

Three (5.9%) subjects in the whole patient group had clinical symptoms resembling an autoinflammatory disease. There were no subjects with similar clinical presentations in the control group. The clinical and genotypic characteristics of the three cases mentioned are presented in Table 6.

Table 4. Clinical and laboratory features of AS patients according to MVK SNPs

	Mutation present (n=36)		Mutation absent (n=15)		
Parameters	n	Mean	n	Mean	*p*
Sex					NS
Male	23		9		
Female	13		6		
Age (year)		37.81		36.13	0.602
Symptom age (year)		26.86		27.53	0.781
Age at diagnosis (year)		30.83		30.73	0.969
Delay in diagnosis (year)		4.03		3.27	0.409
Schober test (cm)		2.925		3.100	0.640
FFD (cm)		15.39		10,07	0.183
OWD (cm)		3.69		1.60	0.137
CE (cm)		3.90		4.43	0.137
ESR (mm/h)		24.92		26.80	0.795
CRP (mg/dL)		0.891		1.122	0.410
IgD (mg/dL) (n=5-50)		28.801		40.281	0.227
BASFI		3.236		3.067	0.766
BASDAI		3.75		3.46	0.616

AS: Ankylosing spondylitis; MVK: Mevalonate kinase; SNPs: Single nucleotide polymorphisms; FFD: Fingertip-to-floor distance; OWD: Occiput-wall distance; CE: Chest expansion; ESR: Erythrocyte sedimentation rate; CRP: C-Reactive protein; Ig: Immunoglobulin; BASFI: Bath Ankylosing Spondylitis Functional Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index.

Table 5. Mevalonate kinase SNPs

SNP	Features	Polymorphisms (n,%)
c.769-38 C>T (rs35191208)	Location: Intron 8 Disease-related symptoms: Symptomatic Associated phenotype: Recurrent fever Common index variant in the general population Folding in connection with A C885 +24 G>A	51 AS group: 17 (36.5%); 2 homozygous and 15 heterozygous 52 Control group: 15 (28.8%); 5 homozygous and 10 heterozygous
c885+24G>A (rs35191208)	Location: Intron 9 Disease-related symptoms: not symptomatic Associated phenotype: Unknown	51 AS group: 11 (21.6%) heterozygous 52 Control group: 10 (%19.2) heterozygous
c.769-7T >G (rs104895331)	Location: Intron 8 Disease-related symptoms: Symptomatic Associated phenotype: Unknown There is always folding associated with c769-38C> T and c885 +24 G> A	51 AS group: 2 (4%) heterozygous 52 Control group: 1 (%1.9) heterozygous

SNPs: Single nucleotide polymorphisms; AS: Ankylosing spondylitis.

DISCUSSION

In this study, the detected MVK gene polymorphisms were recorded as symptomatic, non-symptomatic, or new SNPs. In addition, we measured the serum IgD levels. We also investigated correlations with AS clinical findings and laboratory parameters and differences between the patient and control groups in terms of SNPs. AS can coexist with FMF, which is the prototype of autoinflammatory diseases. More MEFV genes have been reported in AS patients who are HLA-B27 negative.[15] There are more than 100 genes that cause AS development, but still, the most important association has been reported

Table 6. Clinical summary of autoinflammatory resembling clinical symptoms of there patients

Case 1	A 51-year-old male AS patient with axial involvement who had cervical LAP and joint pain but no abdominal pain or other clinical findings. He was using adalimumab for AS. He had ESR: 36 mm/h and normal levels of CRP and IgD. MVK gene SNPs were determined as c.769-38 C>T heterozygous, c.632-18 A>G heterozygous (NS), and c.885+24 G>A heterozygous (NS). Infevers website specifies c.769-38 C>T SNP as symptomatic. However, it has been reported to be a common variant index in the general population, seen together with c.885+24 G>A.
Case 2	A 35-year-old male patient who was using NSAID for AS. He had abdominal pain but no fever attacks. He had normal levels of ESR and CRP, IgA: 273 (N) and IgD: 65 mg/L. E148Q heterozygous was present in the MEFV, and c.769-38 C>T homozygous in the MVK gene. NS SNPs were D170D C>T heterozygous, c.371+8 C>T heterozygous, c.371+8 C>T heterozygous, and c.632-18 A>G homozygous. E148Q heterozygous positivity is a common condition in some populations. The patient did not have a typical autoinflammatory clinical picture.
Case 3	A 40-year-old male patient who was using adalimumab for AS. He had ESR: 66 mm/h, CRP: 1.7 mg/dL and IgD: 82 mg/L. He had complaints of fever, abdominal pain, joint swelling and pain. The MVK gene SNP was c.769-38 C>T heterozygous. NS SNPs were D170D C>T heterozygous, c.632-18 A>G heterozygous, and S52N G>A heterozygous. The symptoms of fever and abdominal pain had not begun in childhood and did not manifest in the form of attacks. The case was not considered a typical MKD.

AS: Ankylosing spondylitis; LAP: Lymphadenopathy; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Ig: Immunoglobulin; MVK: Mevalonate kinase; SNPs: Single nucleotide polymorphisms; NS: Non-symptomatic; NSAID: Non-steroidal anti-inflammatory drugs; MKD: Mevalonate kinase deficiency.

to be with HLA-B27. There are several non-MHC genes, such as endoplasmic reticulum aminopeptidases (ERAP) 1 and 2, or pathways that are directly related to the pathogenesis of AS. However, the full pathogenesis of AS is still not explained. The frequency of HLA genes varies in different geographies and populations. AS is known to be strongly associated with MHC region variants and HLA alleles. The HLA-B27 and other MHC genes contribute to less than a third of the genetic risk of AS. The non-MHC variants have been investigated as contributing to disease susceptibility. The non-MHC genes are only held responsible for 7.2% of AS pathogenesis.[22] The MVK gene has not been studied in AS patients; therefore, we studied the MVK gene in AS patients.

The symptomatic SNPs in our study were similar regarding the AS and control groups. There was no difference between patients with and without SNPs in terms of clinical findings. The symptomatic SNPs did not differ significantly by peripheral arthritis. The nonsymptomatic SNPs were also similar in both groups. The clinical and laboratory features of AS patients did not differ significantly when their MVK genes were assessed for having SNPs or not. For the relevant SNPs with linkage disequilibrium, they are provided on the Infevers website.[15] The copresent SNPs observed in the present study were as on the Infevers website.

Mevalonate kinase gene polymorphisms in Behçet's disease have previously been investigated. Koné-Paut et al.[16] carried out a genetic analysis for the MVK on samples from 97 Behçet's disease patients and 51 healthy controls. MVK gene mutations (genotype V377I/V377I and V377I/S135L) were present in two patients. These patients had typical features of Behçet's disease and MKD. The investigators reported no remarkable increase in the MVK mutation rate in the Behçet's disease group compared to the healthy controls.

Tas et al.[17] also investigated MVK gene mutations in Behçet's disease by studying 50 patients and 51 healthy controls. The first group SNP c.769-38 C>T was detected in 21 patients and 15 controls; however, the difference between the patient and control groups was not significant. All subjects with the c.769-38 C>T mutation additionally had a c885+24 G>A polymorphism. The third symptomatic SNP reported was c.769-7 T>G. There was no significant difference between the patient and control group in terms of MVK mutations. The c.769-38 C>T SNP was more common in patients with neurological involvement. Tas et al.[23] investigated only three MKD-related mutations in their study. In our study, all MKD-related SNPs were investigated. We identified four new undeclared SNPs. The number and frequency of c.769-38 C>T, c885+24 G>A, and c.769-7 T>G are similar in

Behçet patients and AS patients. The detailed results are displayed in Table 5. The detected SNP was not associated with clinical findings or laboratory parameters.

Although the age of onset of HIDS is early childhood, some studies have reported late-onset cases with MVK polymorphisms and clinical presentations resembling HIDS.[24] Tas et al.[23] reported several adult cases with MVK polymorphisms or HIDS/MKD clinical findings. No AS/SpA clinical presentations were reported in any of the cases. In the present study, we detected the same SNPs, but they were nonsymptomatic. Although Tas et al.[23] considered these symptomatic SNPs as HIDS, they may be a sequence variant common in the population.

Various diseases and conditions affect serum IgD levels. Like IgM, IgD levels also increase in the early stages of infections. There is no uniform pattern of IgD production in primary immunodeficiencies, as some individuals have increased IgD levels while others have decreased levels. For instance, IgD level increases in cases of Nezelof syndrome or cellular immunodeficiency. In autoimmune and allergic diseases, serum IgD level increases along with other immunoglobulin levels. The increase is two-fold in smokers compared to nonsmokers. The increase in serum IgD levels does not necessarily imply the production of IgD antibodies.[21] In the present study, the level of IgD was significantly high in six (11.8%) patients and high in two (3.9%) patients according to reference intervals. However, the difference between AS patients with or without MVK gene polymorphisms was not statistically significant. A previous study recommended measuring the patients' IgD levels twice with an interval of one month and considering the result significant for MVK if the mean value was >100 IU/mL.[25] For the diagnosis of MKD, high serum IgD concentrations are characteristic but not a condition (www.hids.net). In the present study, we checked the IgD level once. The case with an IgD level of 141.2 mg/L was nonsymptomatic and had no MVK gene polymorphisms. In another study, Drenth et al.[26] reported a persistent elevation of IgD (>100 U/mL) in all 50 HIDS cases included.

There were three cases with clinical pictures resembling autoinflammatory diseases. In the first case, Infevers website specifies c.769-38 C>T SNP as symptomatic. However, it has been reported to be a common variant index in the general population, seen together with c.885+24 G>A. In the second case, the patient did not have a typical autoinflammatory clinical picture. Finally, the third case was not considered a typical MKD.

A total of 264 MVK sequence variants/SNPs have been identified on the Infevers website. Three hundred MKD/HIDS patients have been reported in the literature.[23] The present study revealed four new MVK gene SNPs that had previously not been reported: I56V A>G heterozygous, E281D G>D heterozygous, V80I G>A heterozygous, and C173Y G>A heterozygous. The relevant patients had no clinical symptoms such as periodic fever. The new polymorphisms indicated were found neither on the Infevers website nor in the literature review.

Autoinflammatory diseases are a heterogeneous group of monogenic inherited diseases with overlapping clinical findings. Advanced genetic tests are widely used in the diagnosis of autoinflammatory diseases. In a new study, 15 variant genes were evaluated by next-generation sequencing in 196 pediatric and adult patients with one or more suspected autoinflammatory clinics without typical FMF patients or with atypical FMF.[27] Out of 59 patients with suspected MKD at the initial evaluation, five were positive for a MVK mutation, and two patients, one of whom was an adult, were diagnosed with MKD at the final evaluation. The MVK gene mutations, c.G1129A (p.V377I) and c.G431T (p.G144V), were reported symptomatically in a two-year-old male patient with a prediagnosis of CAPS/MKD, whereas in a 25-year-old male patient with a prediagnosis of DADA2 (deficiency of adenosine deaminase 2), c.G1129A (p.V377I) and possible symptomatic c.G52A (p.G18R) heterozygous mutations were reported. MKD patients were heterozygous for V377I, one of the four commonly reported MVK mutations.

In the absence of clinical findings, the MVK gene SNPs were considered sequence variants. In some populations, hereditary autoinflammatory diseases may progress with mild clinical presentations. Such variations in

disease progression depend on the response to genetic and environmental factors.[28]

Genetic testing alone can lead to false results in regions and populations with a large number of variants. In our study, sequence variants were common in both the patient and control groups. They were not considered significant in the absence of clinical findings. If some SNPs are reported as symptomatic, they may not show symptoms due to the effect of other genes or the environment. Detailed clinical and genetic evaluation for each disease and patient is important in the diagnosis and treatment of autoinflammatory diseases.[27]

The limitations of the study are the relatively small sample size of the patient and control groups and the single-center design. In addition, the IgD levels were not checked twice. Measuring the serum IgD levels in the control group would enable comparison with the patient group. Furthermore, the percentage of HLA-B27 positive AS patients was low. Studying MEFV mutations in AS patients could be compared to other studies.

In conclusion, all MVK gene polymorphisms were higher in AS patients compared to healthy controls in this study. There is no correlation between these SNPs and the AS clinic presentation. The number of symptomatic SNPs was the same, and nonsymptomatic SNPs were not different in the patient and control groups. The four most common MVK gene mutations of the AS patients and the control groups were not detected in our study. We found four new nonsymptomatic SNPs that had previously not been reported. Further studies in large-scale patient groups and different populations are required to analyze the reasons for the presence of more MVK gene polymorphisms in AS and investigate the effect of this phenomenon on clinical findings.

Acknowledgments: We would like to thank Dr. Ertan Kara from the Department of Public Health, Cukurova University, who contributed to the statistical analysis.

Ethics Committee Approval: The study protocol was approved by the Cukurova University Clinical Research Ethics Committee approval (No 3, dated 02.01.2013). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Collection data: F.Y.; Made genetic analysis: S.D.; Contributed to the writing of the article: F.Y., E.E.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: This study was supported by Cukurova University Scientific Research Projects (BAP) fund as a project no TF2013LTP3 (2013).

REFERENCES

1. Pedersen SJ, Maksymowych WP. The pathogenesis of ankylosing spondylitis: An update. Curr Rheumatol Rep 2019;21:58.
2. Onen F, Akar S, Birlik M, Sari I, Khan MA, Gurler O, et al. Prevalence of ankylosing spondylitis and related spondyloarthritides in an urban area of Izmir, Turkey. J Rheumatol 2008;35:305-9.
3. Thomas GP, Brown MA. Genetics and genomics of ankylosing spondylitis. Immunol Rev 2010;233:162-80.
4. Cosan F, Ustek D, Oku B, Duymaz-Tozkir J, Cakiris A, Abaci N, et al. Association of familial Mediterranean fever-related MEFV variations with ankylosing spondylitis. Arthritis Rheum 2010;62:3232-6.
5. Akkoc N, Gul A. Familial Mediterranean fever and seronegative arthritis. Curr Rheumatol Rep 2011;13:388-94.
6. Merashli M, Noureldine MHA, Tfayli Y, Jawad A, Uthman I. Ankylosing spondylitis among familial mediterranean fever patients. Endocr Metab Immune Disord Drug Targets 2018;18:148-54.
7. Yazici A, Ozdemir Isik O, Temiz Karadag D, Cefle A. Are there any clinical differences between ankylosing spondylitis patients and familial Mediterranean fever patients with ankylosing spondylitis? Int J Clin Pract 2021;75:e13645.
8. Ozer E, Seker D, Taner E, Adrovic A, Sahin S, Barut K, et al. The frequency of juvenile spondyloarthropathies in childhood familial Mediterranean fever. Clin Exp Rheumatol 2018;36(6 Suppl 115):141-5.
9. Bader-Meunier B, Florkin B, Sibilia J, Acquaviva C, Hachulla E, Grateau G, et al. Mevalonate kinase deficiency: A survey of 50 patients. Pediatrics 2011;128:e152-9.
10. Vuch J, Marcuzzi A, Bianco AM, Tommasini A, Zanin V, Crovella S. Evolutionary hypothesis of the mevalonate kinase deficiency. Med Hypotheses 2013;80:67-9.

11. Ozen S, Hoffman HM, Frenkel J, Kastner D. Familial Mediterranean Fever (FMF) and beyond: A new horizon. Fourth International Congress on the Systemic Autoinflammatory Diseases held in Bethesda, USA, 6-10 November 2005. Ann Rheum Dis 2006;65:961-4.
12. van der Meer JW, Vossen JM, Radl J, van Nieuwkoop JA, Meyer CJ, Lobatto S, et al. Hyperimmunoglobulinaemia D and periodic fever: A new syndrome. Lancet 1984;1:1087-90.
13. van der Hilst JCH, Bodar EJ, Barron KS, Frenkel J, Drenth JPH, van der Meer JWM, et al. Long-term follow-up, clinical features, and quality of life in a series of 103 patients with hyperimmunoglobulinemia D syndrome. Medicine (Baltimore) 2008;87:301-10.
14. Simon A, Drenth JP, Matern D, Goetzman ES, Hager EJ, Gibson KM. Long chain fatty acid (Lcfa) abnormalities in hyper Igd syndrome (Hids) and Familial Mediterranean Fever (Fmf): New insight into heritable periodic fevers. Mol Genet Metab 2013;108:166-71.
15. Li Z, Akar S, Yarkan H, Lee SK, Çetin P, Can G, et al. Genome-wide association study in Turkish and Iranian populations identify rare familial Mediterranean fever gene (MEFV) polymorphisms associated with ankylosing spondylitis. PLoS Genet 2019;15:e1008038.
16. Koné-Paut I, Sanchez E, Le Quellec A, Manna R, Touitou I. Autoinflammatory gene mutations in Behçet's disease. Ann Rheum Dis 2007;66:832-4.
17. Arslan Taş D, Erken E, Yildiz F, Dinkçi S, Sakalli H. Mevalonate kinase gene mutations and their clinical correlations in Behçet's disease. Int J Rheum Dis 2014;17:435-43.
18. Raychaudhuri SP, Deodhar A. The classification and diagnostic criteria of ankylosing spondylitis. J Autoimmun 2014;48-49:128-33.
19. Yanik B, Gürsel YK, Kutlay S, Ay S, Elhan AH. Adaptation of the Bath Ankylosing Spondylitis Functional Index to the Turkish population, its reliability and validity: Functional assessment in AS. Clin Rheumatol 2005;24:41-7.
20. Karatepe AG, Akkoc Y, Akar S, Kirazli Y, Akkoc N. The Turkish versions of the Bath Ankylosing Spondylitis and Dougados Functional Indices: Reliability and validity. Rheumatol Int 2005;25:612-8.
21. Vladutiu AO. Immunoglobulin D: Properties, measurement, and clinical relevance. Clin Diagn Lab Immunol 2000;7:131-40.
22. Wu X, Wang G, Zhang L, Xu H. Genetics of ankylosing spondylitis-focusing on the ethnic difference between East Asia and Europe. Front Genet 2021;12:671682.
23. Tas DA, Dınkcı S, Erken E. Different clinical presentation of the hyperimmunoglobulin D syndrome (HIDS) (four cases from Turkey). Clin Rheumatol 2012;31:889-93.
24. Combemale P, Denis-Thely L, Crozes P, Estival JL, Dupin M, Chouvet B. Auto-inflammatory disease with hyperimmunoglobulinemia D of late onset. Ann Dermatol Venereol 2006;133:369-74.
25. Rodrigues F, Philit JB, Giurgea I, Anglicheau D, Roux JJ, Hoyeau N, et al. AA amyloidosis revealing mevalonate kinase deficiency: A report of 20 cases including two new French cases and a comprehensive review of literature. Semin Arthritis Rheum 2020;50:1370-3.
26. Drenth JP, Haagsma CJ, van der Meer JW. Hyperimmunoglobulinemia D and periodic fever syndrome. The clinical spectrum in a series of 50 patients. International Hyper-IgD Study Group. Medicine (Baltimore) 1994;73:133-44.
27. Karacan İ, Balamir A, Uğurlu S, Aydın AK, Everest E, Zor S, et al. Diagnostic utility of a targeted next-generation sequencing gene panel in the clinical suspicion of systemic autoinflammatory diseases: A multi-center study. Rheumatol Int 2019;39:911-9.
28. Ryan JG, Goldbach-Mansky R. The spectrum of autoinflammatory diseases: Recent bench to bedside observations. Curr Opin Rheumatol 2008;20:66-75.