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Original article

Is there a relationship between gouty arthritis and Mediterranean fever gene mutations?



Ismail Sari^{a,b,*}, Ismail Simsek^b, Yusuf Tunca^c, Bunyamin Kisacik^{b,d}, Hakan Erdem^b, Salih Pay^b, Hasan Fatih Cay^b, Davut Gul^c, Ayhan Dinc^b

^a Department of Rheumatology, School of Medicine, Dokuz Eylul University, Izmir, Turkey

^b Department of Rheumatology, Gulhane Military Medical Academy, Ankara, Turkey

^c Department of Medical Genetics, Gulhane Military Medical Academy, Ankara, Turkey

^d Department of Rheumatology, School of Medicine, Gaziantep University, Gaziantep, Turkey

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ABSTRACT

Objective: Gouty arthritis and familial Mediterranean fever share some clinical and pathological features such as being classified as auto-inflammatory disease, association with inflammasome, short-lived intermittent arthritis, and good response to colchicine and anti-interleukin-1 treatments. As Mediterranean fever gene is the causative factor of familial Mediterranean fever, we aimed to investigate the prevalence of Mediterranean fever gene mutations and their effect on disease manifestations in Turkish gouty arthritis patients.

Methods: Ninety-seven patients diagnosed with primary gouty arthritis (93 M and 4 F, 54 [37–84] years) and 100 healthy controls (94 M and 6 F, 57 [37–86] years) were included in the study. All subjects were genotyped for the Mediterranean fever gene variations. Number of gout attacks, diuretic use, history of nephrolithiasis and presence of tophus were also recorded.

Results: The carriage rate of Mediterranean fever mutations for patients and controls was 22.7% ($n = 22$) and 24% ($n = 24$), respectively. The comparison of the patient and control groups yielded no significant difference in terms of the Mediterranean fever mutations' carriage rate ($p = 0.87$). The allelic frequencies of the Mediterranean fever mutations in patients were 11.9% ($n = 23$) and 14% ($n = 28$) in controls ($p = 0.55$). The presence of Mediterranean fever variants did not show any association with clinical features of gouty arthritis. The subgroup analysis of patients revealed that gouty arthritis patients with mutations had similar frequencies of tophus, history of nephrolithiasis and podagra compared to the ones without mutations ($p > 0.05$).

Conclusions: This study does not provide support for a major role of Mediterranean fever mutations in Turkish gouty arthritis patients.

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* Corresponding author.

E-mail: ismailsari35@gmail.com (I. Sari).

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Existe uma relação entre a artrite gotosa e as mutações genéticas da febre familiar do Mediterrâneo?

R E S U M O

Palavras-chave:

Artrite gotosa

Proteína MEFV

Febre familiar do Mediterrâneo

Objetivo: A artrite gotosa e a febre familiar do Mediterrâneo (FFM) compartilham algumas características clínicas e patológicas, como ser classificada como uma doença autoimune inflamatória, ter associação com o inflamassoma, manifestar artrite intermitente de curta duração e boa resposta a tratamentos com colchicina e anti-interleucina-1. Como o gene da febre familiar do Mediterrâneo (MEFV) é o fator causador da FFM, este estudo teve como objetivo investigar a prevalência de mutações do gene MEFV e seu efeito sobre as manifestações da doença em pacientes turcos com artrite gotosa.

Métodos: Foram incluídos no estudo 97 pacientes com diagnóstico de artrite gotosa primária (93 M e 4 F; 54 [37-84] anos) e 100 controles saudáveis (94 M e 6 F; 57 [37-86] anos). Todos os indivíduos foram submetidos à análise do genótipo à procura de variações no MEFV. Também foi registrado o número de crises de gota, o uso de diuréticos e a história de nefrolitíase e presença de tofos.

Resultados: A frequência de portadores de mutações no MEFV em pacientes e controles foi de 22,7% (n=22) e 24% (n=24), respectivamente. A comparação entre os pacientes e os controles não produziu diferença estatisticamente significativa em termos de frequência de portadores de mutações no MEFV (p=0,87). As frequências alélicas de mutações no MEFV nos pacientes foram de 11,9% (n=23) e 14% (n=28) nos controles (p=0,55). A presença de variantes do MEFV não mostrou qualquer associação com as características clínicas da artrite gotosa. A análise por subgrupos de pacientes revelou que aqueles com artrite gotosa com mutações tinham frequências semelhantes de tofo, história de nefrolitíase e podagra em comparação com os indivíduos sem mutações (p>0,05).

Conclusões: As mutações no gene MEFV não exercem um papel relevante em pacientes turcos com artrite gotosa.

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Introduction

Gouty arthritis is one of the most frequently observed inflammatory arthritis in the world. Although its epidemiology shows significant ethnical variations, it is affecting at least 1–2% of men in the western world.¹ Gouty arthritis has some classical clinical findings such as acute painful attacks of arthritis in the joints (especially the first metatarsal joint of the foot), mono-articular involvement and intermittent pattern.² It is caused by the deposition of monosodium urate monohydrate (MSU) crystals in the joints. MSU crystals induce a variety of inflammatory cytokines particularly interleukin-1 (IL-1).³ In addition, recent reports revealed a major role for inflammasome activity in the development of gout attacks.³ On the other hand, familial Mediterranean fever (FMF) is the most commonly seen periodic fever syndrome.⁴ FMF is caused by the mutations (single substitutions) in the *Mediterranean Fever* (MEFV) gene at the short arm of the 16th chromosome.⁴ This gene encodes a protein called pyrin. Under normal circumstances, pyrin limits the activation of the NLRP3-inflammasome. It is presumed that the mutated pyrin protein in FMF is theoretically not able to suppress the inflammasome, and thus the inflammatory response develops.⁵ Both gouty arthritis and FMF share some clinical and pathogenic mechanisms such as short-lived and intermittent arthritis, association with inflammasome and response to colchicine and anti-IL-1 therapies.^{3,6} To the best of our knowledge, there

has been no previous study about the association between MEFV gene mutations and gouty arthritis. In this study, we aimed to investigate the prevalence of MEFV gene mutations and their effect on disease manifestations in Turkish gouty arthritis patients.

Methods

Sample size calculation

The sample size was calculated by using the results of previous studies that investigated the frequency of MEFV mutations in patients with inflammatory rheumatic diseases and healthy controls.^{7,8} According to the analysis, based on $\alpha=0.05$ and a power of 80%, at least 89 subjects were needed per group.

Patients and controls

Ninety-seven unrelated patients diagnosed with primary gouty arthritis were recruited from the outpatient clinic of Gulhane Military School of Medicine Department of Rheumatology (Ankara, Turkey). The clinical diagnosis of gout was established by the revised American College of Rheumatology classification criteria.⁹ Patients were also questioned for the presence of the Tel-Hashomer criteria for diagnosis of FMF.¹⁰ Sex, age, number of gout attacks, diuretic use, and history of nephrolithiasis and presence of tophus were also collected.

The control group included 100 unrelated healthy subjects without any history of chronic diseases and was recruited from the blood donors and relatives of hospital staff. The study was approved by the Ethics Committee and informed consent was obtained from all the participants.

MEFV gene mutation analysis

A total of 197 specimens collected were analyzed for the mutations of the MEFV gene. Genomic DNA from whole blood samples was isolated with QIAamp DNA blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Both exon 2 and exon 10 of the MEFV gene were amplified by polymerase chain reaction (PCR) using the following primers: 5'-GTGGGACAGCTTCATCATTTTG-3' and 5'-CCTTCTCTGCGTTTGCTC-3' (exon 2), 5'-TTACTGGGAGGTGGAGGTTG-3', and 5'-GAGG AGCTGTGTTCTCCC-TC-3' (exon 10). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen). Purified PCR amplicons were bidirectionally fluorescence sequenced using ABI BigDye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems) and run on a ABI 3100 automated sequencer (Applied Biosystems).

Statistical analysis

Results were expressed as mean \pm standard deviation (SD) and proportions as percentages. A chi-square test or Fisher's exact test was used, when appropriate, to assess the difference in the prevalence of MEFV variants between gouty arthritis patients and healthy controls. Spearman's rho test was used to describe correlations. All *p* values were 2-tailed, and confidence intervals (CIs) were set at 95%. *p*-values less than 0.05 were considered significant. The statistical analysis was carried out by using Statistical Package for the Social Science (SPSS), version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

There were 97 gouty arthritis patients (93 male [M] and 4 female [F], 54 [37-84] years) and 100 healthy controls (94 M and 6 F, 57 [37-86] years). The sex and age distributions were not different between the groups (*p* values are 0.75 and 0.09, respectively). The median number of gout attacks of the patients were 2 (1-40); 10.8% of the patients had tophus, 21.5% had renal stone history, 23.7% of the patients were receiving diuretics and 18.3% had a history of alcohol intake.

There were 22 patients and 24 healthy controls that were carrying at least one mutated MEFV allele. Exon 2 mutations observed in this study were E148Q, R202Q, E230K, T267I and T177I. The detected exon 10 mutations were M694V, M680I, V726A, R761H, A744S and K695R. Distribution of the mutations in the patients and healthy controls are summarized in Table 1. The carriage rate of MEFV mutations for patients and controls were 22.7% and 24%, respectively. The comparison of the patient and control groups yielded no significant difference in terms of the MEFV mutations carriage rate (*p*=0.87; 95% CI=0.57-1.57). The sub-group analysis revealed that 31 (15.7%) subjects were carrying exon 2 and 16 (8.1%) were

Table 1 – Distribution of the MEFV gene mutations in the gouty arthritis and healthy control groups.

	Gouty arthritis patients, n=97	Healthy controls, n=100
M694V/M694V	1	
M694V/WT	4	2
M694V/R202Q		1
E148Q/E148Q		2
E148Q/WT	9	14
V726A/WT	1	
R761H/WT	1	1
M680I/WT	1	
A744S/WT	1	1
K695R/WT	1	1
R202Q/WT	2	
R202Q/E230K		1
T267I/WT	1	
T177I/WT		1

WT, wild type.

carrying at least one exon 10 mutations. The gouty arthritis patients and controls did not show any significance regarding the carriage rate of exon 2 (12 [12.4%] vs. 19 [19%]; *p*=0.24; 95% CI=0.33-1.27, respectively) and exon 10 (10 [10.3%] vs. 6 [6%]; *p*=0.31; 95% CI=0.65-4.55, respectively) mutations (Table 2).

The allelic frequencies of the MEFV mutations in patients were 11.9% (*n*=23) and 14% (*n*=28) in controls. The allele frequencies did not show any significance when the patients and controls were compared (*p*=0.55; 95% CI=0.51-1.42). Total exon 2 and exon 10 mutations were observed in 34 (8.6%) and 17 (4.3%) subjects respectively. The gouty arthritis patients and controls did not show any significance regarding the distribution of exon 2 (12 [6.2%] vs. 22 [11%]; *p*=0.11; 95% CI=0.28-1.1, respectively) and exon 10 (11 [5.7%] vs. 6 [3%]; *p*=0.22; 95% CI=0.71-5.01, respectively) mutations (Table 2).

When we analyzed E148Q separately, neither carriage rate nor allelic frequencies were different between gouty arthritis patients and healthy controls (*p*=0.2; 95% CI=0.23-1.28; *p*=0.11; 95% CI=0.21-1.12, respectively). Similarly, M694V mutations were not differed significantly between patients and controls (carriage rate *p*=0.49; 95% CI=0.41-7.56 and allele frequency *p*=0.33; 95% CI=0.52-8.5).

The subgroup analysis of gouty arthritis patients regarding their MEFV gene mutation status revealed that gouty arthritis patients with mutations had similar frequencies of tophus (11.3% vs. 9.1%, respectively, *p*=1), history of nephrolithiasis (22.5% vs. 18.2%, respectively, *p*=0.77) and podagra (42.3% vs. 45.5, respectively, *p*=0.81) compared to the ones without mutations. In addition the number of gout attacks were also similar between the patients with and without mutations (2 [1-8] vs. 2 [1-40], respectively, *p*=0.38).

The presence of MEFV mutations and the presence of tophus, history of nephrolithiasis, diuretic usage and number of attacks did not show any correlation (*p*=0.61; 0.73; 0.81 and 0.38, respectively).

Discussion

In this study we showed that both the carriage rate and allelic frequencies of MEFV gene mutations were not different

Table 2 – Allele frequencies and carriage rates of MEFV variants in patients with gouty arthritis as compared with healthy controls.

	Gouty arthritis patients, n = 97	Healthy controls, n = 100	p-value (95% CI)
<i>Carriage rate, %</i>			
Total exon 2 variants	12 (12.4)	19 (19)	0.24 (0.33–1.27)
Total exon 10 variants	10 (10.3)	6 (6)	0.31 (0.65–4.55)
Total MEFV variants	22 (22.7)	24 (24)	0.87 (0.57–1.57)
<i>Allele frequency, %</i>			
Total exon 2 variants	12 (6.2)	22 (11)	0.11 (0.28–1.1)
Total exon 10 variants	11 (5.7)	6 (3)	0.22 (0.71–5.01)
Total MEFV variants	23 (11.9)	28 (14)	0.55 (0.51–1.42)

between the gouty arthritis patients and healthy controls. Furthermore the presence of MEFV variants did not show any association with clinical features of gouty arthritis. In addition, the severity of the gout did not show any difference between the patients with and without MEFV mutations.

Gouty arthritis is one of the commonest forms of inflammatory arthropathy in the elderly population. MSU crystals trigger the inflammatory cascade, which ultimately result in pain and inflammation. In recent years several reports showed an association between NLRP3 inflammasome and gouty arthritis.³ It is hypothesized that MSU crystals increase NLRP3-induced IL-1 β and dysregulated production of the cytokine plays an important role in the clinical features of gout.³ Recently gout is classified as a form of auto-inflammatory diseases.¹¹

FMF is a hereditary auto-inflammatory disorder characterized by acute attacks of fever and serosal inflammation. It is prevalent among certain ethnic groups such as Jews, Armenians, Turks, and Arabs.⁴ FMF is caused by mutations in the MEFV gene, which encodes the pyrin protein. Mutant pyrin is associated with uncontrolled inflammatory cascade, probably by dysregulated inflammasome function and excessive IL-1 β production.⁵

In recent years there is a considerable interest regarding MEFV gene mutations and its association with different inflammatory diseases. In this respect MEFV mutations found to be increased in ankylosing spondylitis,¹² juvenile idiopathic arthritis,¹³ inflammatory bowel disease,⁸ palindromic rheumatism,¹⁴ Behcet's disease,⁷ polyarteritis nodosa¹⁵ and Schoenlein-Henoch purpura.¹⁶ Furthermore it was shown that these mutations were associated with severe disease prognosis in other inflammatory syndromes such as rheumatoid arthritis.¹⁷

Because of the similarities between gout and FMF (auto-inflammatory disease, short-lived intermittent arthritis, and good response to colchicine and anti-IL-1 treatments) we have undertaken this study to find out whether there is a relation with MEFV mutations, causative gene of FMF, in gouty patients. We revealed that carriage rate and allelic frequencies were not different between the patient and control groups. In literature, a case of a Japanese patient with atypical symptoms of gouty arthritis was reported to carry heterozygous E148Q mutation.¹⁸ To the best of our knowledge there is no other report regarding the association of MEFV and gout. It is known that certain MEFV mutations have severe clinical outcome such as the association of amyloidosis with M694V genotype in FMF.¹⁹ On the other hand, some reports emphasized the

over-representation of E148Q in several inflammatory disorders.¹⁴ In our sub-group analysis neither M694V alone nor total exon 10 mutations were different in the study group. Similarly E148Q alone and total exon 2 mutations were also not different between the patient and control groups. We also did not show any association with MEFV mutations and disease phenotype such as nephrolithiasis and tophus. In addition our subgroup analysis revealed that the number of attacks, presence of tophus, history of nephrolithiasis and podagra were not different between the patients with and without mutations. Based on the latter finding, someone may suggest that the presence of mutations in the gouty arthritis patients may not have an effect on disease severity.

This study has some limitations such as small sample size of total 197 cases and cross-sectional collection of data. In conclusion, despite these limitations, the results presented in this study do not provide support for a major role of MEFV mutations in Turkish gouty arthritis patients. Further replication studies in different populations with larger number of patients are needed to confirm our results.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- Birlik M, Gurler O, Akar S, Sari I, Onen F, Akkoc N. The prevalence of gout in an urban area of Izmir, Turkey: a population-based epidemiological study. *Int J Clin Pract*. 2014;68:775–82.
- Perez-Ruiz F, Castillo E, Chinchilla SP, Herrero-Beites AM. Clinical manifestations and diagnosis of gout. *Rheum Dis Clin North Am*. 2014;40:193–206.
- Pope RM, Tschopp J. The role of interleukin-1 and the inflammasome in gout: implications for therapy. *Arthritis Rheum*. 2007;56:3183–8.
- Sari I, Birlik M, Kasifoglu T. Familial Mediterranean fever: an updated review. *Eur J Rheumatol*. 2014;1:21–33.
- Samuels J, Ozen S. Familial Mediterranean fever and the other autoinflammatory syndromes: evaluation of the patient with recurrent fever. *Curr Opin Rheumatol*. 2006;18:108–17.
- Ozen S, Bilginer Y, Aktay Ayaz N, Calguneri M. Anti-interleukin 1 treatment for patients with familial Mediterranean fever resistant to colchicine. *J Rheumatol*. 2011;38:516–8.
- Atagunduz P, Ergun T, Direskeneli H. MEFV mutations are increased in Behcet's disease (BD) and are associated with

- vascular involvement. *Clin Exp Rheumatol*. 2003;21 4 Suppl. 30:S35-7.
8. Giaglis S, Mimidis K, Papadopoulos V, Thomopoulos K, Sidiropoulos P, Rafail S, et al. Increased frequency of mutations in the gene responsible for familial Mediterranean fever (MEFV) in a cohort of patients with ulcerative colitis: evidence for a potential disease-modifying effect? *Dig Dis Sci*. 2006;51:687-92.
 9. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum*. 1977;20:895-900.
 10. Pras M. Familial Mediterranean fever: from the clinical syndrome to the cloning of the pyrin gene. *Scand J Rheumatol*. 1998;27:92-7.
 11. Punzi L, Scanu A, Ramonda R, Oliviero F. Gout as autoinflammatory disease: new mechanisms for more appropriated treatment targets. *Autoimmun Rev*. 2012;12:66-71.
 12. Akkoc N, Sari I, Akar S, Binicier O, Thomas MG, Weale ME, et al. Increased prevalence of M694V in patients with ankylosing spondylitis: additional evidence for a link with familial mediterranean fever. *Arthritis Rheum*. 2010;62:3059-63.
 13. Ayaz NA, Ozen S, Bilginer Y, Erguven M, Taskiran E, Yilmaz E, et al. MEFV mutations in systemic onset juvenile idiopathic arthritis. *Rheumatology (Oxford)*. 2009;48:23-5.
 14. Canete JD, Arostegui JI, Queiro R, Gratacos J, Hernandez MV, Larrosa M, et al. An unexpectedly high frequency of MEFV mutations in patients with anti-citrullinated protein antibody-negative palindromic rheumatism. *Arthritis Rheum*. 2007;56:2784-8.
 15. Ozen S, Ben-Chetrit E, Bakkaloglu A, Gur H, Tinaztepe K, Calguneri M, et al. Polyarteritis nodosa in patients with Familial Mediterranean Fever (FMF): a concomitant disease or a feature of FMF? *Semin Arthritis Rheum*. 2001;30: 281-7.
 16. Ozdogan H, Arisoy N, Kasapcapur O, Sever L, Caliskan S, Tuzuner N, et al. Vasculitis in familial Mediterranean fever. *J Rheumatol*. 1997;24:323-7.
 17. Koca SS, Etem EO, Isik B, Yuce H, Ozgen M, Dag MS, et al. Prevalence and significance of MEFV gene mutations in a cohort of patients with rheumatoid arthritis. *Joint Bone Spine*. 2010;77:32-5.
 18. Nonaka F, Migita K, Haramura T, Sumiyoshi R, Kawakami A, Eguchi K. Colchicine-responsive protracted gouty arthritis with systemic inflammatory reactions. *Mod Rheumatol*. 2014;24:540-3.
 19. Kasifoglu T, Bilge SY, Sari I, Solmaz D, Senel S, Emmungil H, et al. Amyloidosis and its related factors in Turkish patients with familial Mediterranean fever: a multicentre study. *Rheumatology (Oxford)*. 2014;53:741-5.