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MEditerranean FeVer (*MEFV*) gene mutations in glomerulonephritides: a clinicopathological study

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Background/aim: The aim of this study is to determine the *ME*diterranean *FeV*er (*MEFV*) gene mutation carrier rate in patients with glomerulonephritis and to investigate the association between disease features and MEFV variants.

Materials and methods: Medical records regarding clinical, laboratory, histopathological, and prognostic features of 200 adult patients with biopsy-proven glomerulonephritis were evaluated retrospectively. Exons 2 and 10 of the *MEFV* gene of each patient were sequenced by next-generation sequencing. Variants were detected and compared with disease features.

Results: *MEFV* mutation carrier rate was 25%, similar among disease subgroups, and higher than the previously reported rates for normal populations. Demographic, clinical, and laboratory features at diagnosis did not differ in patients with and without mutations. Refractory disease rates were 73% and 40% in carriers and noncarriers of E148Q (P = 0.051). Percentage of global sclerotic glomeruli was higher in M694V carriers than noncarriers (medians 24% vs. 0%, P = 0.047). Tubulointerstitial fibrosis was also more severe in M694V carriers. The carrier rate of M694V was 14.3% in patients eventually needing chronic renal replacement therapy (RRT) (n = 21), whereas it was 2.8% in the group without RRT (OR = 5.8 [1.28–26.3], P = 0.040).

Conclusion: *MEFV* mutation carrier rate was higher than expected in our sample of Turkish patients with glomerulonephritis. The E148Q mutation may be associated with refractory disease. The M694V mutation was more frequent in patients who needed chronic RRT.

Key words: *MEFV* gene, mutation, glomerulonephritis

1. Introduction

The *ME*diterranean *FeV*er (*MEFV*) gene encodes an intracellular, cytoskeleton-associated protein, pyrin (also known as marenostrin) (1). Although the function of this protein is not fully understood, one hypothesis is that it regulates inflammation by inhibiting caspase-1-mediated activation of prointerleukin-1β (2). Thus, pyrin is classically proposed to prevent inappropriate secretion of interleukin-1β (IL-1β) and the resulting immune activation. Homozygous or compound heterozygous mutations of the *MEFV* gene result in defects in pyrin function and are responsible for the disease familial Mediterranean fever (FMF, OMIM 249100) (3). Patients with this autoinflammatory disease are also more prone to other rheumatic diseases like seronegative spondylarthropathies (4), vasculitides (5,6), Behçet's disease (7,8), glomerulonephritides (6), inflammatory bowel diseases (9), and multiple sclerosis (10), probably because they have defects in preventing inflammation. Evidence supports that, like patients with FMF, people carrying a heterozygous *MEFV* mutation have some defects in preventing inappropriate inflammation and have augmented inflammatory responses (11,12). Moreover, carrying a heterozygous *MEFV* mutation is known to modify the course of disease in patients with seronegative spondylarthropathies (13), rheumatoid arthritis (14–16), juvenile idiopathic arthritis (17), gouty arthritis (18), inflammatory bowel diseases (19–21), multiple sclerosis (22), systemic lupus erythematosus (23,24), Behçet's disease (25–27), and other vasculitides (5,28). In most of these studies, disease groups also had higher carrier rates of *MEFV* mutations than expected, which raises the question of whether these mutations might be genetic susceptibility factors for non-FMF rheumatic diseases, especially in regions endemic for FMF (13,14,17,20,25,29–31). Chromosomal region 16p13, which bears the *MEFV* gene,

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also bears other genes such as major histocompatibility complex class II transactivator (*MHC2TA*), which encodes proteins functioning in immune processes. Polymorphisms in the genes located in this chromosomal region are known to have predisposing/protective effects on immune diseases like multiple sclerosis and rheumatoid arthritis (32). Collectively, these findings made researchers think that there is a link between *MEFV* mutations and rheumatic diseases other than FMF.

Although it is known that patients with FMF have higher rates of coexistent glomerulonephritis (6), little is known about glomerulonephritides and non-FMF *MEFV* mutation carriers (33). The aim of this study is to determine *MEFV* mutation carrier rate in a sample of Turkish patients with glomerulonephritis and to investigate the association between disease features and *MEFV* variants.

2. Materials and methods

2.1. Patients

Patients older than 18 years of age with biopsy-proven glomerulonephritis, referring between August 2015 and June 2016 and followed up at the Department of Nephrology of the Ankara University Medical Faculty, a tertiary care center accepting patients from all over the country, were included in the study. Exclusion criteria were presence of coexistent FMF, amyloidosis, and allogeneic hematopoietic stem cell transplantation. Written informed consent was obtained from each patient. All procedures were in accordance with the ethical standards of the Institutional Review Board of Ankara University Medical Faculty (study approval number 03-90-16) and the 1964 Helsinki declaration.

2.2. Variables and definitions

Medical records regarding age, sex, definite pathological diagnosis, disease duration, presence of nephritic/nephrotic signs (edema, new-onset hypertension, or macroscopic hematuria) at diagnosis, laboratory findings (serum creatinine, albumin, C3 and C4 complement levels, creatinine clearance, urinary examination for microscopic hematuria, 24-h urine protein, antinuclear antibody [ANA], and antineutrophilic cytoplasmic antibody [ANCA] profiles) at diagnosis, kidney biopsy findings (presence of endocapillary, mesangial and extracapillary proliferation, crescent percentages, degree of tubulointerstitial inflammation, fibrosis and tubular atrophy, percentages of global and segmental sclerotic glomeruli, and positivity of antiimmunoglobulin and anticomplement stainings of the mesangium and glomerular basement membrane in direct immunofluorescence microscopy), previous serum creatinine if measured, immunosuppressive regimens if given, stabilization time for proteinuria, duration of immunosuppressive use, and serum creatinine and 24-h urine protein in remission were identified. For those with relapse, time from diagnosis to relapse, presence of second remission, and second relapse were identified. For those who needed eventual chronic renal replacement therapy (RRT), time from diagnosis to initiation of RRT was noted. Glomerular filtration rate (GFR) loss at diagnosis was defined as more than 25% nonchronic decrease in estimated GFR (eGFR) calculated by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (34). Stabilization time for proteinuria was defined as the time from diagnosis to achievement of the level of proteinuria with no further decrease in at least two consecutive measurements. Nephrotic proteinuria was defined as more than 3 g/day and hypoalbuminemia as serum albumin lower than 3.5 g/dL. Definitions of remission, relapse, and refractory disease are based on the Kidney Disease Improving Global Outcomes (KDIGO) 2012 clinical practice guidelines for glomerulonephritis (35).

2.3. Genetic analysis

About 5 mL of peripheral blood from each patient was drawn into tubes with ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from these samples by the spin column method and quality-checked. Exons 2 and 10 of the *MEFV* gene along with exon-intron junctions were amplified with polymerase chain reaction (PCR) (T100TM Thermal Cycler, Bio-Rad, USA) using RNA primers F: 5'-GACAGCTTCATCATTTTGCATCTGG-3', R: 5'-CTTTCTCTGCAGCCGATATAAAGTAGG-3' for exon 2 and F: 5'-TTCCAGAAGAACTACCCTGTCCCTG-3', R: 5'-TTTCCCATAGCAGCTAGCACCTAGTC-3' for exon 10 (Sentegen Biotechnology, Turkey). Mutations and polymorphisms of the two exons were detected using an automated genetic analyzer (3130 Genetic Analyzer, Applied Biosystems, USA) with next-generation sequencing.

2.4. Statistical analysis

All the statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp., USA). In the prestudy power analysis, 200 patients were found to be included in the study to detect a 1.5 times increased rate of *MEFV* mutations in the study group compared to the normal population with 80% power and alpha of 0.05. Data were described as numbers and percentages for categorical variables and as means ± standard deviations or medians with interquartile ranges for continuous variables. Analyses between 2 or more categorical variables were performed using chi-square or Fisher's exact tests. Odds ratios (ORs) with 95% confidence intervals were calculated where appropriate. After testing for normality, Mann–Whitney U or Kruskal–Wallis tests were used for comparison of variables with nonnormal distributions and t-tests or one-way ANOVA for normally distributed ones. Bonferroni corrections and adjusted significance levels for pairwise comparisons were

used for post hoc analyses. Stratifications and Mantel–Haenszel (M-H) estimates of common ORs were used in analyses including categorical confounders. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographic and clinical features

Demographic features and disease subgroups are given in Table 1. Ten patients (5%) had disease durations shorter than 6 months and minimum duration of disease was 4 months. As only biopsy-proven cases referring in a limited time period were included in the study, rates of specific diseases were not thought to reflect true incidences. Clinical and laboratory features of disease subgroups at diagnosis are given in Table 2.

3.2. Frequencies of *MEFV* variants

MEFV mutation carrier rates, mutant allele frequencies, and normal population data based on the results of the largest nationwide study (36) are given in Table 3. Allele frequency of R202Q, the only amino acid-changing polymorphism, was 74/400. There are no normal population data for the frequency of this polymorphism but it was comparable with the reported rates of healthy control groups of some small-scale regional studies (38,39). Carrier rates and allele frequencies did not differ between disease subgroups (Supplementary Table S1).

Table 1. Demographic features of patients and disease groups

Number of patients	200
Age (years)	46±16
Disease duration (months)	60.5 (IQR 99.5)
Sex n(%)	
Female	100(50)
Male	100(50)
Disease groups n(%)	
Membranous glomerulonephritis	46(23)
Focal segmental glomerulosclerosis	36(18)
Immunoglobulin A nephropathy	33(16.5)
Minimal change disease	20(10)
Membranoproliferative glomerulonephritis	17(8.5)
SLE glomerulonephritis	16(8)
Pauci-immune glomerulonephritis	12(6)
Other[a]	20(10)

SLE=systemic lupus erythematosus

Age is given as mean±standard deviation and disease duration as median with interquartile range (IQR).

[a]Chronic idiopathic glomerulonephritis (3%), mesangial proliferative glomerulonephritis (2%), IgM nephropathy (1.5%), anti-glomerular basement membrane disease (1.5%), C1q nephropathy (1%), cryoglobinemic glomerulonephritis (1%).

3.3. *MEFV* variants and clinical and laboratory findings at diagnosis

Rates of edema, hypertension, and hematuria as well as serum creatinine, eGFR, creatinine clearance, serum albumin, proteinuria, and rate of GFR loss at diagnosis were all similar between *MEFV* mutation carriers and noncarriers. Positivity and titers of ANA and ANCA, and serum levels of C3 and C4, were also similar between mutation carriers and noncarriers (data not shown). C3 but not C4 hypocomplementemia was more frequent in patients carrying R202Q polymorphism as compared to noncarriers (26% vs. 13%, OR = 2.25 [1.03–4.91], P = 0.037). This may be explained by the confounding effect of disease subgroups. Carrier rates of R202Q were higher in systemic lupus erythematosus (SLE) and membranoproliferative glomerulonephritis (MPGN) groups (56.3%, 35.3%, and 31.3% in SLE, MPGN, and all patients, respectively), which are characterized by higher rates of hypocomplementemia (68.8%, 47.1%, and 17% in SLE, MPGN, and all patients, respectively). After stratification according to disease subgroups, strata-specific ORs were homogeneous among groups (Tarone's statistic = 2.83, P = 0.83) and the M-H estimate of the common OR was 1.84 (0.7–4.83), P = 0.685.

3.4. *MEFV* variants and pathology findings

Mutation/polymorphism carriers and noncarriers had similar distributions of percentages of endocapillary, mesangial, and extracapillary (crescentic) proliferation. Positivity and severity of anticomplement and antiimmunoglobulin stainings of the mesangium and glomerular basement membrane were similar as well (data not shown). M694V mutation carriers had higher percentages of global glomerular sclerosis and more severe tubulointerstitial fibrosis compared to noncarriers (Table 4).

3.5. *MEFV* variants and prognostic parameters

The patient flowchart is given in the Figure. There was no difference between mutation/polymorphism carriers and noncarriers in terms of rates of remission, refractory disease, relapse, and need for eventual chronic RRT. Stabilization time for proteinuria, duration of immunosuppressive treatment, time from diagnosis to relapse, rates of second remission and second relapse, serum creatinine, eGFR, creatinine clearance, proteinuria in remission, and time from diagnosis to initiation of RRT were similar as well (data not shown). The relationship between E148Q mutation and prognostic parameters in 113 patients who completed first-line immunosuppressive treatment is shown in Table 5. Among 148 patients who achieved remission, refractory disease rates were 48% and 20% in carriers (n = 17) and noncarriers (n = 131) of E148Q mutation, respectively (OR = 3.6 [1.2–10.2], P = 0.027). Carrier rates of M694V mutation were 14.3% and

Table 2. Clinical and laboratory features at diagnosis

	MGN (n=46)	**FSGS** (n=36)	**IgAN** (n=33)	**MCD** (n=20)	**MPGN** (n=17)	**SLEN** (n=16)	**PIGN** (n=12)	**Other** (n=20)	**Total** (n=200)
Age (years)	47±13	40±16	35±15	32±19	29±18	34±19	43±18	37±21	39±17
Edema (%)	89	51	24	95	82	38	46	70	63
New-onset hypertension (%)	4	20	46	11	35	20	27	20	21
Macroscopic hematuria (%)	2	0	30	5	6	0	9	20	9
Microscopic hematuria (%)	17	37	46	26	82	88	82	50	45
Proteinuria (mg/day) (% nephrotic)	8907±4540 (91)	5442±3216 (80)	2902±2014 (47)	8967±4474 (100)	5777±4230 (75)	4376±3615 (50)	3167±2378 (40)	6029±3539 (81)	6049±4266 (74)
Serum albumin (g/dL) (% hypoalbuminemia)	2.4±0.8 (89)	3.2±0.8 (53)	3.5±0.6 (45)	1.9±0.7 (100)	2.9±1.1 (63)	3±1 (63)	2.8±0.6 (90)	2.5±0.7 (90)	2.8±0.9 (72)
Rate of GFR loss (%)	0	21	38	18	43	50	100	29	27

MGN=membranous glomerulonephritis; FSGS=focal segmental glomerulosclerosis; IgAN=immunoglobulin A nephropathy; MCD=minimal change disease; MPGN=membranoproliferative glomerulonephritis; SLEN=systemic lupus erythematosus glomerulonephritis; PIGN=pauci-immune glomerulonephritis; GFR=glomerular filtration rate.

Age, proteinuria, and serum albumin are given as means±standard deviations.

Proteinuria of 17 patients (8.5%) was not available. Status of GFR loss was not known for 35 patients (17.5%) due to lack of any previous serum creatinine measurements.

Table 3. Carrier rates and allele frequencies of MEFV gene mutations

	Observed	Reported[a]	P value
Carrier rate	50/200 (25%)	14.8%	N/C[e]
Mutant allele frequency	53[b]/400 (13.25%)	8.4%	N/C[e]
E148Q	22/400 (5.5%)	3.5%	0.029
V726A	9/400 (2.25%)	0.7%	<0.001
M694V	8/400 (2%)	2%	1.000
M680I	4/400 (1%)	0.2%	<0.001
A744S	3/400 (0.75%)	0.8%	0.91
K695R	2/400 (0.5%)	-	0.025
M694I	1/400 (0.25%)	-	0.114
R761H	-	0.4%	0.205
Other[c]	4/400 (1%)	0.8%	N/C[e]
Cumulative carrier rate[d]	48/200 (24%)	13.8%	<0.001
Cumulative mutant allele frequency[d]	51/400 (12.75%)	7.9%	<0.001

MEFV=MEditerranean FeVer; N/C=not comparable.
[a]Based on the results of the largest nation-wide population-based study by Soylemezoglu et al. (36).
[b]Three patients, with no Familial Mediterranean Fever related symptoms or signs, had bi-allelic (two compound heterozygous and one homozygous) MEFV gene mutations.
[c]Other mutations observed in our study: V704I (1/400), T177I (1/400), E251K (1/400), and c.761-764dupCCG (1/400); reported by Soylemezoglu et al: P369S (0.8%). All of the mutations detected in this study were previously reported to be either disease-causing or symptom-related (37).
[d]Cumulative carrier rates and mutant allele frequencies were calculated for the comparable 8 mutations (E148Q, V726A, M694V, M680I, A744S, K695R, M694I, and R761H).
[e]Bacause of methodological differences, these data were not comparable.

2.8% in patients who needed (n = 21) and did not need (n = 179) chronic RRT, respectively (OR = 5.8 [1.3–26.3], P = 0.04). Carrying an M694V mutation was not found to be associated with any other prognostic parameter.

4. Discussion

Although it is known that pyrin has a regulatory role on inflammatory processes via inflammasome- and/or cytoskeleton-associated intracellular mechanisms, its precise molecular interactions and functions still need to be clarified since controversies exist (1,2,40). Nevertheless, mutations and polymorphisms of its coding gene, *MEFV*, have been shown to modify the course of many non-FMF rheumatic diseases (13–28). In FMF, different mutations do not have the same impact on disease severity and prognosis, and even the same mutation may have different prognostic importance in different races and geographical regions (41), probably because each mutation alters pyrin function to a different degree and some environmental and genetic modifiers other than *MEFV* gene mutations exist (1). For the same reason, it is probably the case with *MEFV* gene mutation carriers with rheumatic diseases other than FMF. As an example, in Israeli patients with rheumatoid arthritis, carrying an *MEFV* gene mutation, particularly E148Q, is found to be associated with more severe disease (16), but in Japanese rheumatoid arthritis patients, E148Q or any other *MEFV* gene mutation had no association with severity or any other disease-related parameter (42) despite the frequency of E148Q mutation being much higher in Japanese rheumatoid arthritis patients. In Turkish patients with rheumatoid arthritis, on the other hand, carrying an *MEFV* gene mutation is associated not with higher disease activity scores but with more deformed joint count in a manner not primarily dependent on the presence of E148Q (15). Thus, *MEFV* mutations do not have fixed effects on the course of either FMF or non-FMF rheumatic diseases.

In the study by Kukuy et al. (33), they investigated the potential association of *MEFV* mutation carrier status and disease characteristics in 40 patients with immunoglobulin A nephropathy (IgAN) and 40 patients with other forms of primary glomerulonephritis from a multiethnic Jewish

Table 4. M694V mutation and pathology findings

	M694V mutation		P value
	present (n=8)	absent (n=182)	
Globally sclerotic glomeruli (%)	24(IQR 72)	0(IQR 20)	0.047
Tubulointerstitial inflammation			0.058
Absent (%)	12.5	49	
Mild-moderate (%)	75	37	
Severe (%)	12.5	14	
Tubulointerstitial fibrosis			0.046
Absent (%)	12.5	53	
Mild-moderate (%)	62.5	36	
Severe (%)	25	11	
Tubular atrophy			0.063
Absent (%)	37.5	56	
Mild-moderate (%)	25	36	
Severe (%)	37.5	8	

10 patients (5%) were excluded from the analysis due to lack of detailed pathology reports.
Percentages of globally sclerotic glomeruli are given as medians with interquartile ranges (IQRs).

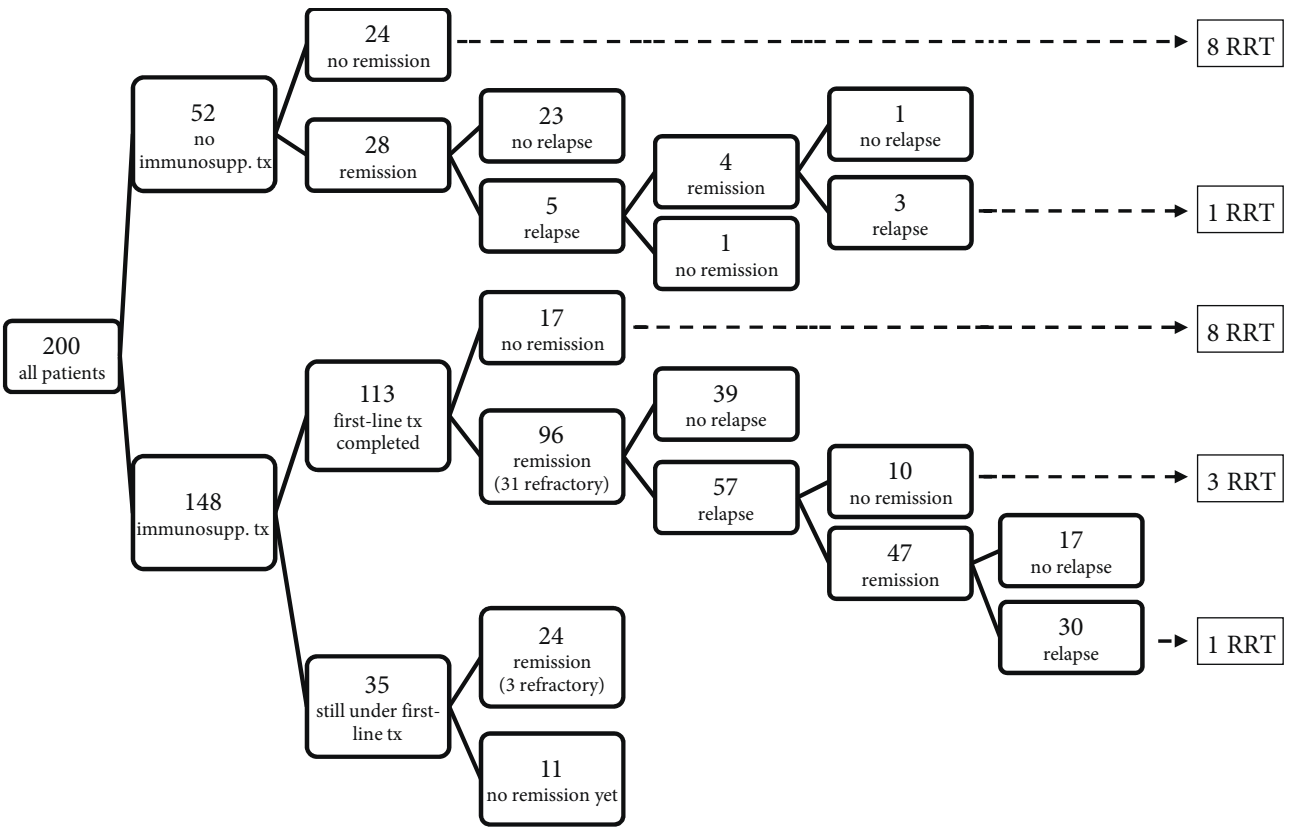

Figure. Patient flowchart. immunosupp. = immunosuppressive; tx = treatment; RRT = renal replacement therapy.

Table 5. Prognostic parameters in carriers and non-carriers of E148Q mutation in 113 patients who completed first-line immunosuppressive treatment

	E148Q mutation			
	present (n=11)	absent (n=102)	Odds ratio	P value
Remission (%)	82	85	0.7(0.1-3.9)	0.670
Stabilization time (month)	12(IQR 27)	14(IQR 17)	N/A	0.523
Remission proteinuria <1 g/day (%)	64	65	0.9(0.1-5.0)	0.995
Refractory disease (%)	73	40	4.1(1.0-16.5)	0.051
Relapse (%)	89	56	6.2(0.7-51.7)	0.078
Time from diagnosis to relapse (month)	65(IQR 68)	38(IQR 73)	N/A	0.795
Duration of steroid use[a] (month)	16(IQR 39)	20(IQR 20)	N/A	0.420
Second immunosuppressive use[a] (%)	91	61	6.4(0.8-52.3)	0.054
Duration of second immunosuppressive[a] (month)	19(IQR 17)	17(IQR 19)	N/A	0.619
Need for chronic RRT (%)	9	11	0.8(0.1-7.0)	1.000

RRT=renal replacement therapy; N/A=not applicable.
Continuous variables are given as medians with interquartile ranges (IQRs). Odds ratios are given with 95% confidence intervals.
[a]for the first-line treatment.

population. They did not find any difference in the rates of the three most commonly seen *MEFV* gene mutations (E148Q, M694V, V726A) between patients and a normal population. In the IgAN subgroup, they assessed clinical disease severity based on serum creatinine and proteinuria, clinical course (stable or deteriorating), and kidney biopsy pathology findings (focal, mesangial, or diffuse proliferative). They were not able to show any association of *MEFV* mutation carrier status and disease characteristics. In contrast, we investigated all of the possible exon 2 and exon 10 mutations and polymorphisms with sequence analysis. The most commonly seen three mutations in Turkish and Jewish populations are E148Q, M694V, and V726A. We identified 14 allelic *MEFV* gene mutations in addition to 39 with E148Q, M694V, and V726A (Table 3). Clinical, laboratory, and pathology findings in our study were also more detailed and quantity-based, allowing further statistical analyses. Very recently, a study from eastern Turkey reported a high *MEFV* mutation carrier rate (35.9%) in patients with primary glomerulonephritis but did not exclude cases with coexistent FMF, and the rate of coexistent FMF in the study group was quite high (18.7%) (43). Detailed prognostic and histopathological evaluations were also lacking. To our knowledge, there exists no other study in the literature investigating the potential effect of *MEFV* mutations on clinical, laboratory, histopathological, and prognostic features of patients with glomerulonephritis. In this study, we report higher refractory disease rates in E148Q mutation carriers and more severe tubulointerstitial fibrosis, more global glomerulosclerosis, and higher RRT rates in M694V mutation carriers compared to noncarriers in a sample of Turkish patients with various types of glomerulonephritides. Although refractory disease rates were higher in E148Q carriers, eventual remission and RRT rates were similar, probably because of more aggressive use of immunosuppressive agents (Table 5). Interestingly, laboratory and histopathological findings at diagnosis as well as proteinuria and creatinine clearances in remission were also similar in E148Q carriers compared to noncarriers. Thus, with no effect on eventual outcomes and predictors of outcomes at diagnosis, E148Q mutation may modify the response to immunosuppressive treatment as carriers have higher refractory disease rates. The higher carrier rate of M694V mutation among patients who needed RRT may be related with more severe histopathological chronicity findings in M694V carriers at diagnosis (Table 4). Since the indications and timing of kidney biopsy were not standard, it is difficult to interpret why M694V carriers had more severe global glomerulosclerosis and tubulointerstitial fibrosis at diagnosis. It was also not possible to make further statistical analyses regarding remission, relapse, and refractory disease rates in patients who were given immunosuppressive treatment as only a total of 8 patients carried the M694V mutation.

The finding of increased carrier rate of *MEFV* mutations in glomerulonephritis (one out of four patients) raises the question of whether these mutations may be genetic susceptibility factors for glomerulonephritis. Heterozygous carriers of *MEFV* mutations are known

to have subclinical inflammation and augmented inflammatory responses (11,12) and hey may potentially have a predisposition, as in the case of other non-FMF rheumatic diseases (13,14,17,20,25,29–31). One point to be clarified is whether there is a true association or a haplotypic relationship between *MEFV* mutations and glomerulonephritis or other rheumatic diseases as the chromosomal region 16p13 bears *MHC2TA* and probably other genes regulating inflammatory processes (32). Further genetic studies are needed to conclude properly on this issue. Genome-wide association studies in patients with IgA nephropathy and lupus nephritis did not reveal any association with the *MEFV* gene or chromosomal region 16p13 before (44,45). Notably, these were not whole-exome sequencing studies and the *MEFV* gene was not identified as a target gene in these studies. Interestingly, we did not find any difference in *MEFV* mutation carrier rates of disease subgroups although not all types of glomerulonephritides are IL-1β-mediated (46,47). IL-1β, produced by phagocytes, is one of the major cytokines of the innate immune system, but it is still involved in adaptive immune processes like stimulation of T helper 17 (Th17) cells (48), which may have a pathogenetic role in some types of primary glomerulonephritis (47). The adaptive immune system uses effector mechanisms of the innate immune system as well (48). SLE, which is characterized primarily by overactivation of the adaptive immune system, is a good example to understand this interrelation. It is thought that FMF or the presence of *MEFV* gene mutations confer some protection from SLE and the two diseases rarely overlap (49), but in the case of their presence, *MEFV* mutations are still associated with higher disease activity indices and early onset disease (23), and exon 10 mutations with more severe disease and nephritis in patients with SLE (24). Thus, *MEFV* variants may potentially modify the course of glomerulonephritides not primarily mediated by IL-1β and the innate immune system like membranous nephropathy, immunoglobulin A nephropathy, and lupus nephritis.

In geographical regions and races with a high frequency of FMF and hence heterozygous *MEFV* mutations, it is worth further studying the possible predisposing and modifying effects of these mutations on specific types of glomerulonephritides and other non-FMF rheumatic diseases in large prospective cohorts for both a better understanding of the pathogenesis of autoimmune diseases and potential therapeutic implications. Numbers of patients in disease subgroups in this study were mostly not enough for subgroup analyses for specific types of glomerulonephritides. The number of patients with specific *MEFV* gene mutations was also low for further analysis. It was not possible for us to look for mutations in the whole *MEFV* gene, and normal population data for rare exon 2 and 10 mutations were also lacking (Table 3). Since geographical differences in *MEFV* mutation rates and profiles are of major concern in Turkey (36), studies with a multigeographical design are needed for more conclusive interpretations.

In conclusion, the *MEFV* mutation carrier rate was higher than expected in our sample of Turkish patients with glomerulonephritis. The E148Q mutation may be associated with refractory disease. The M694V mutation was more frequent in patients who needed chronic RRT.

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References

1. Manukyan G, Aminov R. Update on pyrin functions and mechanisms of familial mediterranean fever. Front Microbiol 2016; 7: 456.
2 Simon A, van der Meer JWM, Drenth JPM. Familial autoinflammatory syndromes. In: Firestein G, Budd R, Gabriel SE, McInnes IB, O'Dell J, editors. Kelley's Textbook of Rheumatology. 9th ed. Philadelphia, PA, USA: Elsevier Saunders; 2012. pp. 1597-1615.
3. [No authors listed.] Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. The International FMF Consortium. Cell 1997; 90: 797-807.
4. Kasifoglu T, Calisir C, Cansu DU, Korkmaz C. The frequency of sacroiliitis in familial Mediterranean fever and the role of HLA-B27 and MEFV mutations in the development of sacroiliitis. Clin Rheumatol 2009; 28: 41-46.
5. Aksu K, Keser G. Coexistence of vasculitides with familial Mediterranean fever. Rheumatol Int 2011; 31: 1263-1274.
6. Tunca M, Akar S, Onen F, Ozdogan H, Kasapcopur O, Yalcinkaya F, Tutar E, Ozen S, Topaloglu R, Yilmaz E et al. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. Medicine (Baltimore) 2005; 84: 1-11.
7. Ben-Chetrit E, Yazici H. Thoughts on the proposed links between Behcet's disease and familial Mediterranean fever. Clin Exp Rheumatol 2002; 20: S1-2.
8. Schwartz T, Langevitz P, Zemer D, Gazit E, Pras M, Livneh A. Behcet's disease in familial Mediterranean fever: characterization of the association between the two diseases. Semin Arthritis Rheum 2000; 29: 286-295.

9. Fidder HH, Chowers Y, Lidar M, Sternberg M, Langevitz P, Livneh A. Crohn disease in patients with familial Mediterranean fever. Medicine (Baltimore) 2002; 81: 411-416.

10. Yahalom G, Kivity S, Lidar M, Vaknin-Dembinsky A, Karussis D, Flechter S, Ben-Chetrit E, Livneh A. Familial Mediterranean fever (FMF) and multiple sclerosis: an association study in one of the world's largest FMF cohorts. Eur J Neurol 2011; 18: 1146-1150.

11. Kalyoncu M, Acar BC, Cakar N, Bakkaloglu A, Ozturk S, Dereli E, Tunca M, Kasapcopur O, Yalcinkaya F, Ozen S. Are carriers for MEFV mutations "healthy"? Clin Exp Rheumatol 2006; 24: S120-122.

12. Lachmann HJ, Sengul B, Yavuzsen TU, Booth DR, Booth SE, Bybee A, Gallimore JR, Soytürk M, Akar S, Tunca M et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford) 2006; 45: 746-750.

13. Durmus D, Alayli G, Cengiz K, Yigit S, Canturk F, Bagci H. Clinical significance of MEFV mutations in ankylosing spondylitis. Joint Bone Spine 2009; 76: 260-264.

14. Inanir A, Yigit S, Karakus N, Tekin S, Rustemoglu A. Association of MEFV gene mutations with rheumatoid factor levels in patients with rheumatoid arthritis. J Investig Med 2013; 61: 593-596.

15. Koca SS, Etem EO, Isik B, Yuce H, Ozgen M, Dag MS, Isik A. Prevalence and significance of MEFV gene mutations in a cohort of patients with rheumatoid arthritis. Joint Bone Spine 2010; 77: 32-35.

16. Rabinovich E, Livneh A, Langevitz P, Brezniak N, Shinar E, Pras M, Shinar Y. Severe disease in patients with rheumatoid arthritis carrying a mutation in the Mediterranean fever gene. Ann Rheum Dis 2005; 64: 1009-1014.

17. Ayaz NA, Ozen S, Bilginer Y, Erguven M, Taskiran E, Yilmaz E, Besbas N, Topaloglu R, Bakkaloglu A. MEFV mutations in systemic onset juvenile idiopathic arthritis. Rheumatology (Oxford) 2009; 48: 23-25.

18. Balkarli A, Tepeli E, Balkarli H, Kaya A, Cobankara V. A variant allele of the Mediterranean-fever gene increases the severity of gout. Int J Rheum Dis 2018; 21: 338-346.

19. Fidder H, Chowers Y, Ackerman Z, Pollak RD, Crusius JB, Livneh A, Bar-Meir S, Avidan B, Shinar Y. The familial Mediterranean fever (MEVF) gene as a modifier of Crohn's disease. Am J Gastroenterol 2005; 100: 338-343.

20. Giaglis S, Mimidis K, Papadopoulos V, Thomopoulos K, Sidiropoulos P, Rafail S, Nikolopoulou V, Fragouli E, Kartalis G, Tzioufas A 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: 687-692.

21. Karban A, Dagan E, Eliakim R, Herman A, Nesher S, Weiss B, Berkowitz D, Shamir R, Gershoni-Baruch R. Prevalence and significance of mutations in the familial Mediterranean fever gene in patients with Crohn's disease. Genes Immun 2005; 6: 134-139.

22. Shinar Y, Livneh A, Villa Y, Pinhasov A, Zeitoun I, Kogan A, Achiron A. Common mutations in the familial Mediterranean fever gene associate with rapid progression to disability in non-Ashkenazi Jewish multiple sclerosis patients. Genes Immun 2003; 4: 197-203.

23. Shinar Y, Kosach E, Langevitz P, Zandman-Goddard G, Pauzner R, Rabinovich E, Livneh A, Lidar M. Familial Mediterranean FeVer gene (MEFV) mutations as a modifier of systemic lupus erythematosus. Lupus 2012; 21: 993-998.

24. Deniz R, Ozen G, Yilmaz-Oner S, Alibaz-Oner F, Erzik C, Aydin SZ, Inanc N, Eren F, Bayalan F, Direskeneli H et al. Familial Mediterranean fever gene (MEFV) mutations and disease severity in systemic lupus erythematosus (SLE): implications for the role of the E148Q MEFV allele in inflammation. Lupus 2015; 24: 705-711.

25. 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: S35-37.

26. Rabinovich E, Shinar Y, Leiba M, Ehrenfeld M, Langevitz P, Livneh A. Common FMF alleles may predispose to development of Behcet's disease with increased risk for venous thrombosis. Scand J Rheumatol 2007; 36: 48-52.

27. Tasliyurt T, Yigit S, Rustemoglu A, Gul U, Ates O. Common MEFV gene mutations in Turkish patients with Behcet's disease. Gene 2013; 530: 100-103.

28. Ozcakar ZB, Yalcinkaya F, Cakar N, Acar B, Kasapcopur O, Uguten D, Soy D, Kara N, Uncu N, Arisoy N et al. MEFV mutations modify the clinical presentation of Henoch-Schonlein purpura. J Rheumatol 2008; 35: 2427-2429.

29. Cosan F, Ustek D, Oku B, Duymaz-Tozkir J, Cakiris A, Abaci N, Ocal L, Aral O, Gül A. Association of familial Mediterranean fever-related MEFV variations with ankylosing spondylitis. Arthritis Rheum 2010; 62: 3232-3236.

30. Cinar M, Dinc A, Simsek I, Erdem H, Koc B, Pay S, Tunca Y, Kilic S, Gul D. The rate and significance of Mediterranean fever gene mutations in patients with ankylosing spondylitis: a three-month, longitudinal clinical study. Rheumatol Int 2008; 29: 37-42.

31. Unal A, Dursun A, Emre U, Tascilar NF, Ankarali H. Evaluation of common mutations in the Mediterranean fever gene in multiple sclerosis patients: is it a susceptibility gene? J Neurol Sci 2010; 294: 38-42.

32. Martinez A, Perdigones N, Cenit MC, Espino L, Varade J, Lamas JR, Santiago JL, Fernandez-Arguero R, de la Calle H, Arroyo R et al. Chromosomal region 16p13: further evidence of increased predisposition to immune diseases. Ann Rheum Dis 2010; 69: 309-311.

33. Kukuy OL, Kopolovic J, Blau A, Ben-David A, Lotan D, Shaked M, Shinar Y, Dinour D, Langevitz P, Livneh A. Mutations in the familial Mediterranean fever gene of patients with IgA nephropathy and other forms of glomerulonephritis. Clin Genet 2008; 73: 146-151.

34. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604-612.

35. KDIGO. Clinical Practice Guideline for Glomerulonephritis (GN). Cranford, NJ, USA: KDIGO; 2012.

36. Soylemezoglu O, Kandur Y, Gonen S, Duzova A, Ozcakar ZB, Fidan K, Saatci U, Bakkaloglu A, Ozguc M. Familial Mediterranean fever gene mutation frequencies in a sample Turkish population. Clin Exp Rheumatol 2016; 34: 97-100.

37. Touitou I. The Registry of Hereditary Auto-inflammatory Disorders Mutations. Montpellier, France: University of Montpellier; 2018.

38. Öztürk A, Özçakar B, Ekim M, Akar N. Is *MEFV* gene Arg202Gln (605 G>A) a disease-causing mutation? Turk J Med Sci 2008; 38: 205-208.

39. Yigit S, Karakus N, Tasliyurt T, Kaya SU, Bozkurt N, Kisacik B. Significance of MEFV gene R202Q polymorphism in Turkish familial Mediterranean fever patients. Gene 2012; 506: 43-45.

40. Sonmez HE, Batu ED, Ozen S. Familial Mediterranean fever: current perspectives. J Inflamm Res 2016; 9: 13-20.

41. Touitou I. The spectrum of familial Mediterranean fever (FMF) mutations. Eur J Hum Genet 2001; 9: 473-483.

42. Migita K, Nakamura T, Maeda Y, Miyashita T, Koga T, Tanaka M, Nakamura A, Komori H, Ishibashi T, Oruguchi H et al. MEFV mutations in Japanese rheumatoid arthritis patients. Clin Exp Rheumatol 2008; 26: 1091-1094.

43. Huzmeli C, Candan F, Bagci G, Alaygut D, Yilmaz A, Gedikli A, Bagci B, Timucin M, Sezgin I, Kayatas M. Frequency of familial Mediterranean fever (MEFV) gene mutations in patients with biopsy-proven primary glomerulonephritis. Clin Rheumatol 2017; 36: 2589-2594.

44. Saka S, Hirawa N, Oka A, Yatsu K, Hirukawa T, Yamamoto R, Matsusaka T, Imai E, Narita I, Endoh M et al. Genome-wide association study of IgA nephropathy using 23 465 microsatellite markers in a Japanese population. J Hum Genet 2015; 60: 573-580.

45. AlFadhli S, Ghanem AA, Nizam R. Genome-wide differential expression reveals candidate genes involved in the pathogenesis of lupus and lupus nephritis. Int J Rheum Dis 2016; 19: 55-64.

46. Hutton HL, Ooi JD, Holdsworth SR, Kitching AR. The NLRP3 inflammasome in kidney disease and autoimmunity. Nephrology (Carlton) 2016; 21: 736-744.

47. Wang L, Li Q, Wang L, Li C, Yang H, Wang X, Tao H. The role of Th17/IL-17 in the pathogenesis of primary nephrotic syndrome in children. Kidney Blood Press Res 2013; 37: 332-345.

48. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 4th ed. Philadelphia, PA, USA: Elsevier Saunders; 2012.

49. Lidar M, Zandman-Goddard G, Shinar Y, Zaks N, Livneh A, Langevitz P. Systemic lupus erythematosus and familial Mediterranean fever: a possible negative association between the two disease entities--report of four cases and review of the literature. Lupus 2008; 17: 663-669.