

I appreciate author's approach, to connecting novel manuscript with the topic. Interesting study to read.

I advise the author to make your writing compelling, concise, and clear to understand. Some of my recommendations are below:

The Frequency of Familial Mediterranean Fever Gene Mutations and ~~T~~he Correlations ~~B~~etween Phenotype and Genotype ~~I~~n Turkish Children.

- **Spell check** (E.g. attac - wrong; attacks -correct)
 - **Sentence Case Check** (E.g. department -wrong; Department)
 - **Use Full Form** (Familial Mediterranean Fever, Mediterranean fever, etc.) instead of Acronym (*FMF, MEFE, etc.*) – To make easy for readers
- Note:** do not change well known acronyms (E.g. DNA)

ABSTRACT

Aim: Familial Mediterranean Fever is an autosomal recessive disease characterized by recurrent **attacs** of serosal membranes. The aim of the current study was to determine the frequency of the Mediterranean fever (*MEFV*) gene pathogenic variants in 158 children (78 male, 80 female) diagnosed with Familial Mediterranean Fever (FMF) and to compare the phenotype-genotype correlation.

Methods: In our retrospective case-control study, 158 FMF patients (78 males, 80 females) who were diagnosed with **MEFV** gene mutation in Bursa Yüksek İhtisas Training and Research Hospital, ~~d~~Department of Pediatrics between January 2018 and June 2019 were included in the study. Mutation screening of the *MEFV* gene was performed for 12 mutations and the 8 most common mutations were taken into the study.

Results: Abdominal pain (77.8%), fever (74%), arthralgia (46.2%) were the most prevalent clinical features in our patients. The most frequent mutations were M694V, E148Q, V726A, M680I and P369S. In cases with M694 mutation, it is noted that the incidence of arthritis was 2.5 times, appendectomy frequency 3.1 times higher, and early diagnosis probability 3.2 times higher. The frequency of chest pain was 2.9 times higher in the M680I mutation, and the frequency of arthralgia was 2.2 times higher in the P369S mutation.

Conclusion: Patient's mutations in **FMF** patients are important for clinical expectations, and some mutations such as P369S are not as innocent as expected. However, reevaluation of phenotypes of mutations that are rare with more patients will be significant.

Key Words: Familial Mediterranean Fever, **MEFV** Gene Mutations, Phenotype-Genotype Correlations.

- **Try to convert Passive sentences into Active** – To make a clearer reading. Use strong active verbs and varied sentence structure.
- **Try to avoid needless words, phrases and/or repetitions**

INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disorder characterized by recurrent self-limited attacks of serosal membranes[1]. The disease primarily affects patients in the Mediterranean and Middle Eastern populations such as Arabs, Jews, Turks, Armenians, Cypriots, Italians, Spaniards with genetic prevalence of 1 to 6% [2]. The estimated prevalence of FMF is about 1/400-1/1000, and the carrier frequency is 20% in the Turkish population [3-4]. FMF is caused by mutations in the Mediterranean fever (MEFV) gene, which is located on the short(p) arm of chromosome 16 at position 13.3. The gene encodes a protein called pyrin/maronestrin that regulates neutrophil activity [5]. Phenotype–genotype correlations have not been completely determined in patients with FMF. Types of MEFV mutations and clinical picture of FMF change in different geographic regions and ethnic groups. Several researchers have reported an increased disease severity and the development of renal amyloidosis in patients having specific MEFV gene mutations such as homozygous M694V [6]. We conducted the current study to analyze the correlation of variable clinical presentations and MEFV genotypic distributions in Turkish children.

METHODS

Patients

In our retrospective case-control study, 158 FMF patients (78 males, 80 females) who were diagnosed with MEFV gene mutation in Bursa YuksekIhtisas Training and Research Hospital, department of Pediatrics between January 2018 and June 2019 were included in the study. Epidemiological features such as age, gender, ethnicity, FMF story in family, consanguinity between mother and father, age of diagnosis and clinical features as abdominal pain, chest pain, fever, arthritis, arthralgia, erysipelas like erythema, myalgia, oral aphthae, headache during attack, appendectomy story and response to colchicine are noted in the cases. The study has been approved by the ethics committee of Bursa YuksekIhtisas Training and Research Hospital and informed consent was obtained from all patients who participated in the study.

Mutation Analysis

2-3 ml peripheral venous blood was collected to hemogram tubes for DNA isolation from all individuals evaluated by FMF pre-diagnosis. Isolation was performed in the MagPurix DNA isolation robot using the MagPurix Blood DNA Extraction kit. The obtained DNA samples were subjected to Polymerase Chain Reaction (PCR). For each case 2 µl DNA sample and 23 µl PCR mix were used. PCR protocol was performed in GenePro (Bioer) brand gradient system thermal cycler.

PCR samples of all cases were loaded to the NanoChip 400 using the FMF 12 Kit (Savyon Diagnostics). The results of the analysis with the NanoChip Version 7 analysis program were evaluated and the mutation status of the 12 regions (M694V, V726A, E148Q, M680IG> A, M680IG> C, M694I, R761H, F479L, I692del, K695R, A744S, P369S) on the MEFV (Mediterranean Fever) gene associated with FMF disease was checked. M694I, I692del, F479L mutations with allele counts less than 5 were removed from the study. M680IG> A and M680IG> C were considered as a single mutation.

Statistical Analysis:

Descriptive analyzes of numerical data were shown as mean and standard deviation, and descriptive analyzes of categorical data were shown as frequency and percentages. Chi-square test was used for independent group comparisons between categorical data and independent samples t test was used for comparison between numerical data. In situations where the chi-square assumption in categorical data comparisons cannot be met, Fisher's precision test was applied. The relationship between MEFV gene mutations and clinical parameters was evaluated by logistic regression analysis. All statistical analyzes were performed using SPSS v21 software and the Type-I error margin was accepted as 5% in the whole study.

RESULTS

Among the 158 children, 78 (49.3%) were males and 80 (50.7%) were females. The patient's mean age was 9.37 ± 4.41 (ranged between 1–17 years). The mean age at onset of FMF in Turkish children was 4.2 ± 2.7 years and the age at diagnosis was 6.9 ± 3.5 . A positive family history of FMF was noted in 53 (33.6%) of the patients, whereas the parents of 15 (9.7%) patients proved to be relatives. The most frequent clinical findings were abdominal pain (77.8%), fever (74%), arthralgia (46.2%), myalgia (21.5%), arthritis (13.2%), headache

(13.2%), erysipelas-like erythema (12.6%), oral aphtha (12.6%) and chest pain (8.8). Colchicine was administered orally to all patients and a favorable therapeutic effect was seen in 122 patients (77.2%). The number of patients with appendectomy was 29 (18.3%). Amyloidosis was found only in a patient with homozygous M694V mutation (0.63%). Homozygous mutations were detected in 49 (30.8%), heterozygous mutations in 57 (36.5%), compound heterozygous mutations in 52 (32.7%) patients. The most common homozygous genotypes were M694V/M694V, heterozygous genotypes M694V/- and compound heterozygous genotypes M680I/M694V which were found in 37 (23.3%), 25 (15.7%), and 13 (8.2%) of these patients, respectively (Table 1). The most common mutation was M694V with a frequency of 50.5%, followed by E148Q (14.5%), V726A (12.9%), M680I (8.6%), P369S (4.1%), K695R (3.9%), R761H (3.1%) and A744S (1.9%) (Table 2). When patients were assessed by Logistic Regression analysis to evaluate genotype and phenotype association, it was found that arthritis frequency in M694V mutation was 2.5 times higher than other mutations and appendectomy frequency was 3.1 times higher and it was noted that determination is 3.2 times earlier than other mutations ($p < 0.05$). Similarly, arthralgia was found to be 2.2 times higher in the P369S mutation and chest pain was 2.9 times higher in the M680I mutation ($p < 0.05$) (Table 3).

- **Remove adverbs** (E.g. mainly, interestingly, etc.)

DISCUSSION

In this study, we evaluated the clinical features and the genotypes of FMF in 158 Turkish children. The hospital where the study was conducted is located in a migration-receiving region in Bursa. When the origins of the patients studied were examined, it was seen that they were from 7 regions of Turkey. Therefore, it is thought that this study reflects the general of Turkey.

Although FMF disease is seen **mainly** in the Mediterranean region, it has become a problem of the whole world due to travels and migrations. In recent studies, it is reported that the highest prevalence is among the Turks with 1 per 400 and 1 per 1000 [3-4]. One of the important reasons for this is the consanguineous marriage, which is still a serious problem for Turkey even though it has decreased over the years. Rates of consanguineous marriages vary according to regions in Turkey (The East of Turkey: 34.4%, The West of Turkey: 11-13%)[7-

8]. In the present study, consanguinity rate was 9.7% (Bursa is in West of Turkey). Although some studies have reported a male predominance, the frequency of FMF was similar in both genders [9]. In our study, the frequency of FMF was found to be the same in both genders (female to male ratio was 1.02). In studies conducted in countries where FMF is frequent and in our country, abdominal pain frequency is expressed as 91-96%, fever frequency is 92-100%, chest pain frequency is 31-84%, arthritis frequency is 33-70% and erysipelas like erythema frequency is 3-40% [10]. In our study, abdominal pain (77.8%), fever (74%) and joint findings (59.4%) were most common findings. Joint findings are present in the most common form of asymmetric, non-destructive arthritis in about 75% and the initial symptom in about 10% of the FMF patients. The incidence of arthritis in Turks, Arabs, and Armenians is significantly lower than that reported in Jews [6,11]. In our patients, it was found that the rate of patients with arthralgia clinic was 46.2% and the incidence of arthritis was 13.2%. Only in a patient with genotype M694V Homozygous (0.63%), amyloidosis was detected.

Mutation analysis in FMF disease, which depends on criteria such as typical clinical findings for diagnosis, response to family history and colchicine, is of great importance in terms of confirmation of diagnosis. In the current study, 12 MEFV mutations were screened among 158 Turkish children. The most common mutation was M694V (allele frequency was 50.5%). The most frequent mutation reported in Turkey in multicenter studies is reported as M694V and the allele frequency is reported at various rates of 14.6- 51.4% [8,12,13]. Although the ranking according to ethnicity is different in studies conducted both in Turkey and in countries where FMF is frequent, the 5 most common mutations are reported as M694V, E148Q, V726A, M680I, M694I [4,6,14]. In our study, the frequency of M694I was found to be low, and instead of it the P369S mutation was detected among the first 5 mutations (allele frequency was 4.1%). In 2017, Cekin and et al. conducted a study with 514 patients in Istanbul, indicating that the P369S allele frequency was 3.3%, the fifth most frequent mutation [15]. When genotypes of our patients were evaluated, M694V/M694V (23.3%), M694V/- (15.7%) and M694V/M680I (8.2%) were the most common genotypes. E148Q was the second most common mutation in our study (14.5%). The second most common mutation also in the majority of studies conducted in Turkey was E148Q [15,16,17]. In the multicenter study conducted by the FMF Working Group with 2838 patients in 2005, the second most frequent mutation was found M680I (14.4%) [8].

When our patients were evaluated by multiple regression analysis in terms of phenotype / genotype, in patients with M694V mutation, arthritis (OR: 2.58, CI: 1.74-7.85), appendectomy (OR: 3.15, CI: 0.89 -5.12) was found to be more frequent than other mutations, and the diagnosis was earlier (OR:3.22, CI:1.27-7.82) ($p<0.05$). In 158 children with FMF, only 1 patient developed amyloidosis, in which M694V homozygous mutation was detected. Currently, the most numerous phenotype-genotype data available relate to M694V. The penetrance of M694V is very high. This genotype has been demonstrated to be correlated with a severe disease course Jews, Arabs, Armenians and Turks (early disease onset, frequency of attacks, arthritis, high frequency of amyloidosis, high dose colchicine requirement) [18,19].The results of our study also support this. Another mutation with severe clinical signs is reported as M680I [20,21]. M680I was the third most common mutation in our study with a rate of 8.6%. Chest pain frequency was 2.9 times higher in M680I mutations than in other mutations (OR:2.91, CI:1.54-5.66) ($p<0.05$).In previous studies, P369S has been described mild phenotype or incomplete penetrance in patients with FMF [6]. In our study, when joint findings were evaluated, arthritis was common in the M694V mutation and arthralgia was more frequent in the P369S mutation (OR: 2.21, CI: 1.29-5.17).Cekin and et al. also found that the incidence of abdominal pain and chest pain in patients with P369S mutation was higher than expected [15].In both studies, although the P369S allele frequency was low (3.3% and 4.1%), the phenotype was not considered to be very slight in the P369S mutation. E148Q and V726A are the least penetrant FMF mutations and is recognized to have a mild effect on FMF patients in all reports. Especially in homozygous mutations of E148Q, 55% of the patients are asymptomatic, and amyloidosis is not reported in these patients [22,23,24].In our study, when allele frequencies and clinical findings were evaluated by logistic regression analysis, no statistically significant relationship was found between E148Q and A726A mutations and clinical findings ($p<0.05$).

Conclusion

In conclusion, phenotype-genotype association in FMF patients is important in terms of clinical expectations. In our study, compatible with the literature, M694V mutation was the most common mutation and it was found to be progressed with severe clinical findings. **Interestingly**, the result was that the P369S mutation did not progress with very slight clinical findings as expected. However, studies with more patients are needed to evaluate the phenotype-genotype relationship.

- Write references and manuscript in proper format mentioned.

REFERENCES

1. Sohar E, J.Gafni, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. *Am J Med.* 1967; 43(2): 227-253. doi:10.1016/0002-9343(67)90167-2
2. Sarı I, Birlik M, Kasifoğlu T. Familial Mediterranean Fever: An updated review. *Eur J Rheumatol.* 2014;1(1): 21–33. doi:10.5152/eurjrheum.2014.006
3. The international FMF consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause Familial Mediterranean Fever. *Cell.* 1997; 90 (4): 797–807. doi:10.1016/S0092-8674(00)80539-5
4. Yilmaz E, Ozen S, Balci B, Duzova A, Topaloğlu R, Besbas N, et al. Mutation frequency of familial Mediterranean fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet.* 2001; 9(7): 553–555. doi:10.1038/sj.ejhg.5200674
5. French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nature Genetics.* 1997; 17(1): 25-31. doi:10.1038/ng0997-25
6. Touitou I. The spectrum of Familial Mediterranean Fever mutations. *Eur J Hum Genet.* 2001; 9(7) :473–483. doi:10.1038/sj.ejhg.5200658
7. Akbayram S, Sari N, Akgün C, Dogan M, Tuncer O, Caksen H, et al. The frequency of consanguineous marriage in eastern Turkey. *Genet Couns.* 2009; 20(3): 207–214.
8. Tunca M, Akar S, Onen F, Ozdoğan H, Kasapcopur O, Yalcinkaya F, et al. Turkish FMF Study Group. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine (Baltimore).* 2005; 84(1): 1–11. doi:10.1097/01.md.0000152370.84628.0c
9. Onen F. Familial Mediterranean fever. *Rheumatol Int.* 2006; 26(6): 489–496. doi:10.1007/s00296-005-0074-3

10. Ece A, Cakmak E, Uluca U, Kelekçi S, Yolbaş I, Guneş A, et al. The MEFV mutations and their clinical correlations in children with familial Mediterranean fever in southeast Turkey. *Rheumatol Int.* 2014; 34(2): 207–212. doi:10.1007/s00296-013-2858-1
11. Mansour A. Familial Mediterranean fever, review of the literature. *Clin Rheumatol.* 2017; 36(8): 1707-1713. . doi: 10.1007/s10067-017-3715-5
12. Coskun S, Kurtgoz S, Keskin E, Sonmez F, Bozkurt G. Frequency of mutations in Mediterranean fever gene, with gender and genotype–phenotype correlations in a Turkish population. *J. Genet.* 2015; 94(4): 629–635.
13. Oztuzcu S, Ulasli M, Ergun S, Iğci YZ, Bayraktar R, Nacarkahya G, et al. Screening of common and novel familial Mediterranean fever mutations in south-east part of Turkey. *Mol Biol Rep.* 2014; 41(4): 2601–2607. doi:10.1007/s11033-014-3118-5
14. Yigit S, Bagci H, Ozkaya O, Ozdamar K, Cengiz K, Akpolat T. MEFV mutations in patients with familial Mediterranean fever in the Black Sea region of Turkey: Samsun experience (corrected). *J Rheumatol.* 2008; 35(2):106–113.
15. Cekin N, Akyurek ME, Pinarbasi E, Ozen F. MEFV Mutations and Their Relation to Major Clinical Symptoms of Familial Mediterranean Fever. *Gene.* 2017; 30: 626:9-13. doi:10.1016/j.gene.2017.05.013
16. Battal F, Silan F, Topaloğlu N, Aylanç H, Yıldırım Ş, Köksal BF, et al. The MEFV gene pathogenic variants and Phenotype-Genotype correlation in children with Familial Mediterranean Fever in Canakkale population. *BJMG.* 2016; 19(2): 23-28. doi: 10.1515/bjmg-2016-0032
17. Ozdemir O, Sezgin I, Kurtulgan HK, Candan F, Köksal B, Sumer H, et al. Prevalence of known mutations in the MEFV gene in a population screening with high rate of carriers. *Mol Biol Rep.* 2011; 38(5): 3195–3200. doi:10.1007/s11033-010-9991-7
18. Coskun S, Ustyol L, Bayram Y, Selçuk BM, Gulsen S, Cim A, et al. The spectrum of MEFV gene mutations and genotypes in Van province, the eastern region of Turkey, and

report of a novel mutation (R361T). *Gene*. 2015; 562(1): 128–131. doi: 10.1016/j.gene.2015.02.059

19. Shohat M, Magal N, Shohat T, Chen X, Dagan T, Mimouni A, et al. Phenotype–genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. *Eur J Hum Genet*. 1999; 7(3): 287–292. doi:10.1038/sj.ejhg.5200303

20. Albayrak F, Selcuk NY, Odabas AR, Cetinkaya R, Pirim I. Genotype-phenotype correlation in patients with familial Mediterranean fever in East Anatolia. *Genet Test Mol Biomarkers*. 2010; 14(3): 325-328. doi:10.1089/gtmb.2009.0189.

21. Ben-Chetrit E, Touitou I. Familial Mediterranean Fever in the World. *Arthritis Rheum*. 2009; 61(10): 1447–1453. doi: 10.1002/art.24458

22. Tamir N, Langevitz P, Zemer D, Pras E, Shinar Y, Padeh S et al. Late-onset Familial Mediterranean fever (FMF): a subset with distinct clinical, demographic, and molecular genetic characteristics. *Am J Med Genet*. 1999; 87(1): 30-35.

23. Ben-Chetrit E, Lerer I, Malamud E, Domingo C, Abeliovich D. The E148Q mutation in the MEFV gene: Is it a disease-causing mutation or a sequence variant? *Hum Mutat*. 2000; 15(4): 285-286. doi:10.1002/(SICI)1098-1004(200004)15:4<3C385::AID-HUMU22%3E3.0.CO;2-A

24. Touitou I, Ben-Chetrit E, Notarnicola C, Domingo C, Dawalle M, Dross C, et al. Familial Mediterranean Fever (FMF) clinical and genetic features in Druzes and Iraqi-Jews: a preliminary study. *J Rheumatol*. 1998; 25(5): 916-919.

- Calculations in table (1, 2) are not appropriate -- Advice to thorough recheck.
- Table check -- decimal (.) replaced by comma (,)

Table 1. Genotype Distribution of Patients.

Mutations	Genotypes	n	%
Homozygous (n=49, 31%)	M694V/M694V	37	23,3
	E148Q/E148Q	7	4,4
	V726A/V726A	2	1,3
	M680I/M680I	2	1,3
	R761H/R761H	1	0,6
	<i>Subtotal</i>	49	30,8
Heterozygous (n=57, 36%)	M694V/-	25	15,7
	E148Q/-	11	6,9
	V726A/-	7	4,4
	P369S/-	6	3,8
	K695R/-	4	2,5
	A744S/-	3	1,9
	M680I/-	1	0,6
	<i>Subtotal</i>	57	36,5
Compound Heterozygous (n=52, 32.9%)	M680I/M694V	13	8,2
	M694V/V726A	10	6,3
	E148Q/M694V	5	3,1
	M680I/V726A	4	2,5
	E148Q/P369S	3	1,9
	E148Q/V726A	3	1,9
	K695R/M680I	3	1,9
	R761H/V726A	3	1,9
	A744S/R761H	1	0,6
	A744S/V726A	1	0,6
	E148Q/R761H	1	0,6
	K695R/M694V	1	0,6
	K695R/P369S	1	0,6
	K695R/V726A	1	0,6
	M680I/R761H	1	0,6
	M694V/P369S	1	0,6
<i>Subtotal</i>	52	32,7	34.4
TOTAL	158	100,0	99.5

Table 2. Allele Frequencies of the MEFV Gene Mutations.

Allele	Number of Alleles	Frequency (%)
M694V	129	50.5
E148Q	37	14.5
V726A	33	12.9
M680I	22	8.6
P369S	11	4.1
K695R	10	3.9
R761H	8	3.1
A744S	5	1.9
	255	100

Table 3. Logistic Regression Models Between MEFV Gene Mutations and Clinical Parameters

Symptom	MEFV Gene Mutations	OR	%95 Confidence Interval	p
Arthritis	M694V	2,582	1,745 - 7,852	0,040
Appendectomy	M694V	3.15	0.892 - 5.127	0.028
Early Diagnosis	M694V	3.22	1.275 - 7.825	0,029
Arthralgia	P369S	2,214	1,296 – 5.177	0,044
Chest Pain	M680I	2,918	1,545 - 5,660	0,045

Note : Comments with red ink are only for reference, author should thoroughly do corrections.

Hope the author will not take my comments otherwise.

All the very best.

Thanks!