

Analysis of TNFRSF1A Gene R92Q Mutation in Familial Mediterranean Fever

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ABSTRACT This study examines the frequency of TNFRSF1A gene R92Q mutation in patients with Familial Mediterranean Fever (FMF) and the role of this mutation in FMF. The study included 223 FMF patients with definite diagnosis, according to Tel-Hashomer criteria, carrying two mutations and 205 FMF patients as controls (symptomatically diagnosed) with definite diagnosis but without any of the MEFV gene mutations screened. The DNA samples of FMF patients and controls were genotyped with regard to TNFRSF1A gene R92Q mutation by PCR-RFLP method. Genotypes and allele frequencies of the TNFRSF1A gene R92Q mutation were similar in the two groups ($p=0.481$ and $p=0.48$, respectively). Because of the similarities between the symptoms of FMF and TNFRSF1A-associated periodic syndrome (TRAPS), the frequencies of the TNFRSF1A gene R92Q mutation was studied in patients with two MEFV gene mutations and also in patients without any of the twelve most common MEFV gene mutations. No significant difference was observed between the two groups. Despite sharing common symptoms, it seems that FMF is not confused with TRAPS as the TNFRSF1A gene R92Q mutation frequencies are similar between both groups.

INTRODUCTION

Hereditary Periodic Fever Syndromes (HPFS) are a group of diseases including Familial Mediterranean Fever (FMF), tumor necrosis factor receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndromes (CAPSs), pyogenic arthritis, pyoderma-gangrenosum and acne (PAPA) syndrome, pediatric granulomatous arthritis, hyperimmunoglobulinemia D with periodic fever syndrome (HIDS), chronic recurrent multifocal osteomyelitis (CRMO), deficiency of the IL-1 receptor antagonist (DIRA), and deficiency of the IL-10 receptor early-onset enterocolitis (IBD) (Henderson and Goldbach 2010). These monogenic diseases of the innate immune system are characterized with self-limited inflammatory attacks, and they are called autoinflammatory syndromes (Grateau and Duruoz 2010). Although auto-inflammatory diseases have some common clinical features such as fever attacks, serous mem-

brane inflammation, muscle-skeleton system involvement, various types of rashes and amyloidosis, they also show different characteristics such as age of onset, duration and type of treatment (Stjernberg-Salmela et al. 2004).

Familial Mediterranean Fever (FMF, OMIM #249100) is the most common inherited periodic fever syndrome, which is characterized by recurrent attacks of fever accompanied with peritonitis, pleuritis, arthritis, and a typical rash called erysipelas-like erythema. Severity of the disease results from the association of FMF with the formation of AA amyloidosis (Sohar et al. 1967). However, the introduction of colchicine treatment in the early 1970s changed the natural history of the disease (Zemer et al. 1974), completely abolishing the attacks in 60% of the patients, substantially decreasing them in 35%, and preventing the appearance of amyloidosis in almost all of them. Approximately 5% of the patients do not respond to colchicine despite the use of maximal doses (Pras and Kastner 1997).

Although there are cases, such as the three Japanese patients recently reported by Fukushima et al. (2014), of autosomal dominant inheritance, FMF generally has an autosomal recessive inheritance and affects populations of the Mediterranean basin such as Sephardic Jews, Armenians, Turks and Arabs. FMF has also been identified in European populations (Booth et al.

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1998). MEFV gene which causes Familial Mediterranean Fever is located on the short arm of chromosome 16. Up to date, 296 different sequence variants have been reported in the MEFV gene (Milhavet et al. 2008). A novel missense mutation (I247V) was recently reported from Hatay Province of Turkey (Gunesacar et al. 2014). Encoded by this gene, pyrin/marenostrin protein, which consists of 781 amino acids regulates the production of mature interleukin-1 α (IL-1 α) and is also involved in the pathogenesis of various autoinflammatory diseases (Yagel et al. 2010). It is believed that this protein plays an "auto regulator" role on leukocytes. Pyrin is expressed particularly in leukocytes, monocytes and to some extent in fibroblasts. Normally, an association is believed to exist between this protein and some cytokines that have important roles in inflammation and signal molecules responsible for apoptosis such as NF-KB. As in familial Mediterranean fever, if there is a mutation in MEFV gene, the formation of IL-1 β , which is an important factor in inflammation, will be stimulated and also apoptosis will be suppressed and thus, increased inflammatory response will arise as a result of minor stimulations (Kasapçopur and Arisoy 2006).

The diagnosis of FMF has long been made based only on clinical criteria. Cloning of the FMF gene (MEFV) and the identification of the mutations that causes the disease have increased hopes on quicker and more accurate diagnosis of FMF (The International FMF Consortium 1997). However, since molecular diagnosis does not have sufficient sensitivity, even after sequencing of all the genes, a definitive diagnosis of FMF can result in failure in some of the typical patients. Because of the changeable nature of the disease and because it is characterized by an age dependent penetrance, it can be extremely hard to make FMF diagnosis in the presence of typical findings, especially in the absence of family history and in the case of a late onset. Several sets of diagnostic criteria have been proposed, with their validity evaluated and compared with each other (Berkun and Eisenstein 2014). However, a definitive diagnosis enables the use of colchicine, which is an effective treatment in preventing amyloidosis and attacks, in suitable daily doses and for a life time (Zemer et al. 1986).

The tumor necrosis factor receptor - associated periodic syndrome (TRAPS, OMIM # 142680) was initially reported in Irish and Scot-

tish families (Williamson et al. 1982), but is now known to be more common than previously thought (Yagel et al. 2010). TRAPS, the most common autosomal dominant autoinflammatory disease (Rigante et al. 2014), is caused by mutations in the TNFRSF1A gene on chromosome 12 and encodes the 55-kD tumor necrosis factor (TNF)-alpha receptor 1 (TNFR1), which consists of 445 amino acids (McDermott et al. 1999).

TNF- α is the major inflammatory cytokine which induces the synthesis of other inflammatory cytokines such as interleukin-1 α (IL-1 α) and interleukin-6 (IL-6), activates leukocytes, increases the expression of adhesion molecules and regulates apoptosis while providing host defense against intracellular pathogens together with inflammation management (Karatay and Melikoglu 2007).

TRAPS is characterized by fever, abdominal pain, erythematous skin rash, muscle pain, conjunctivitis, irregular attacks of periorbital edema and amyloidosis. However, clinical findings differ (Hull et al. 2002). Similar to FMF, physical stress or trauma stimulates the attacks. Diagnosis is usually made by the analysis of mutations in the gene TNFRSF1 (Kasapçopur and Arisoy 2006). Because of the variability of the clinical findings, genetic confirmation is required for diagnosis. TRAPS patients may show different clinical manifestations as several mutations have been found in association with the disease (Cosan et al. 2013; Rigante et al. 2014).

Treatment of TRAPS with non-steroidal anti-inflammatory (NSAI) drugs, etanercept which is a TNF- α blocker, and anakinra which is an IL-1 α receptor antagonist, was found to be effective in most patients (Karatay and Melikoglu 2007; Yagel et al. 2010; Rigante et al. 2014).

Presently, 138 different sequence variants have been reported in the TNFRSF1A gene (Milhavet et al. 2008). However, the R92Q substitution is the most frequent and widespread mutation identified in patients suffering from TRAPS. Carriers of this genetic defect show milder and more heterogeneous symptoms (Kauffman et al. 2012).

Rationale and Aim of the Study

When the FMF patients who applied to this laboratory were examined, it was found that some patients who had definitive diagnosis according to Tel-Hashomer criteria and who carried

FMF clinical characteristics did not carry any of the screened MEFV gene mutations. Since the clinical characteristics of TRAPS and FMF are similar, the aim of this study was to analyze TNFRSF1A gene R92Q mutation in FMF patients.

MATERIAL AND METHODS

Study Population

The study included 223 unrelated FMF patients with a definitive diagnosis according to the Tel-Hashomer criteria. These patients have two of the twelve most commonly occurring MEFV mutations (E148Q, P369S, F479L, M680IG/C, M680IG/A, I692del, M694V, M694I, K695R, V726A, A744S, R761H). They applied to Samsun Ondokuz Mayıs University, Faculty of Medicine, Department of Medical Biology and a control group of 205 FMF patients who had a definitive diagnosis of FMF (symptomatically) but who did not carry any of the twelve MEFV gene mutations screened. All the individuals included in the study signed an informed and voluntary consent form. After signing the form, the patients were examined according to Tel-Hashomer criteria and the patients and the controls with definitive diagnosis were evaluated for TNFRSF1A R92Q mutation.

Genotyping

Genomic DNA was extracted from peripheral blood cells using Vivantis GF-1 Nucleic Acid Extraction Kit (Qiagen, Istanbul, Turkey) in accordance with the manufacturer's instructions.

R92Q mutation of the TNFRSF1A gene was tested by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique as described previously (Yagel et al. 2010). After digestion with BcnI (NciI), the three possible genotypes were defined by the three distinct banding patterns: RR (380 bp), RQ (153, 227 and 380 bp), and QQ (153 and 227 bp).

Statistical Analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium by using chi-square test. Allele frequency between groups and the difference in genotype distribution was analyzed for significance with χ^2 test.

RESULTS

DNA samples of the 223 FMF patients and the 205 controls were genotyped with regard to TNFRSF1A gene R92Q mutation. No difference was found between FMF patients with definitive diagnosis and two mutations and the control group, FMF patients with definitive diagnosis but no mutations, with respect to genotype and allele frequencies.

In this study, findings showed that 217 (97.3%) of 223 patients who had definitive diagnosis and carrying double mutations had RR genotype, while 6 (2.7%) of these patients had RQ genotype; 197 (96.1%) of 205 controls who had definitive diagnosis but did not carry any of the twelve MEFV gene mutations tested had RR genotype; while, 8 (3.9%) of these controls had RQ genotype (Table 1). Since QQ genotype was not seen in both of the groups, it was not included in the statistical analysis. When the genotypic frequencies of the TNFRSF1A gene R92Q mutation were compared, the results of the two groups were not significantly different ($\chi^2=0.50$; $p=0.481$). R allele had a frequency of 98.7% in FMF patients while it had a frequency of 98.0% in the control group. Q allele had a frequency of 1.3% in FMF patients while it had a frequency of 2.0% in the control group. However, the difference between the two values (1.3% and 2.0%) was not significant ($\chi^2=0.49$; $p=0.485$) (Table 2).

The basic clinical attributes of the FMF patients with definite diagnosis according to Tel-Hashomer criteria and carrying R92Q mutation as well as the patients in the control group were shown in Table 3. In all of these patients, fever and abdominal pain accompanying the fever were present. But none had amyloidosis, the most serious symptom of FMF.

Table 1: R92Q in patients with Familial Mediterranean Fever (FMF)

Group	No. of patients	No. of R92Q carriers
1. Patients with definite FMF with 2 mutations	223	6
2. Patients with definite FMF without mutation	205	8
Total	428	14 (3.3%)

Table 2: The comparison of R92Q polymorphism genotype and allele frequencies between FMF patients with definitive diagnosis and two mutations together with the control group, FMF patients with definitive diagnosis but no mutations

<i>TNFRSF1A</i> gene R92Q genotype	FMF patients with definitive diagnosis and two mutations (n=223)	FMF patients with definitive diagnosis but no mutations (n=205)	χ^2	P value
RR	217 (97.3%)	197 (96.1%)	0.50	0.481
RQ	6 (2.7%)	8 (3.9%)		
QQ	0	0		
R	440 (98.7%)	402 (98.0%)	0.49	0.485
Q	6 (1.3%)	8 (2.0%)		

Table 3: Clinical features of R92Q positive patients, with MEFV gene mutations and without MEFV gene mutations

<i>Clinical manifestation</i>	<i>R92Q positive patients with MEFV gene mutations (n=6)</i>	<i>R92Q positive patients without MEFV gene mutations (n=8)</i>
Fever	6	8
Peritonitis	6	8
Pleuritis	4	2
Amyloidosis	0	0
Colchicine response	5	7
Arthritis	4	7
Erysipelas-like erythema	2	2

DISCUSSION

Hereditary periodic fever syndromes constitute a rare disease group, and the most common member of this group is FMF. In Turkey, FMF is a very frequent disease (Tunca et al. 2005; Cosan et al. 2013).

MEFV gene mutations cause dysregulation of the inflammasome and increased IL-1 α response. This pathway is the major mechanism in FMF pathogenesis. In populations with high MEFV gene mutation carrier rates, it was shown that these mutations are associated with severe disease prognosis in the other inflammatory syndromes. The main cause of this effect is the dysregulation of IL-1 α activation (Cosan et al. 2013).

Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is an autosomal dominant inherited autoinflammatory disease. TRAPS occur as a result of the mutations in the TNFRSF1A gene, which encodes the TNF- α receptor (McDermott et al. 1999).

This study analyzed the frequency of TNFRSF1A R92Q mutation in patients who had definitive FMF diagnosis according to Tel-Hashomer criteria, but who did not carry any of the twelve MEFV gene mutations tested, since

the symptoms of FMF and TRAPS are similar and no significant difference was found between the groups.

First, FMF patients who came to the researchers clinic were evaluated by Tel-Hashomer criteria and the DNAs of the patients with definitive diagnosis were screened for the 12 most common MEFV gene mutations. The presence of patients who did not carry any of the scanned MEFV gene mutations and the similarity between the symptoms of FMF and TRAPS brought to mind that unexplained FMF cases may carry TNFRSF1A gene mutations. In fact, a recent case from Japan signifies this line of thought. Yasumura et al. (2014) identified a two-year old girl who had both TNFRSF1A gene T50M and MEFV gene E84K as well as P115R mutations.

FMF is an autosomal recessively inherited disease and being a double MEFV gene mutations carrier, causes most of the patients to experience clinical symptoms. However, some researchers have shown that in 25% of the patients, single MEFV gene mutation will probably be enough for the appearance of the disease (Yigit et al. 2008; Booty et al. 2009; Kone-Paut et al. 2009; Marek-Yagel et al. 2009). Nevertheless,

the presence of another mutant gene in patients who do not carry any mutation can account for the appearance of clinical symptoms. Eight (3.9%) of the 205 symptomatic FMF patients who did not carry mutation were found to be heterozygote for R92Q. Although the frequency is less than that for patients with mutation, yet the difference is not statistically significant ($p=0.481$).

A study by Yagel et al. (2010) analyzed the TNFRSF1A gene R92Q mutation frequency between 92 FMF patients and 250 controls and discovered that 3.2% of the FMF patients and 6% of the control group were found to be R92Q mutation carriers. In FMF patients who carried 20 single MEFV mutations, 2 patients were found heterozygous for R92Q while in FMF patients who carried 13 double MEFV mutations, 1 heterozygote patient was found (Yagel et al. 2010).

Another study by Booty et al. in 2009 found that 1 out of the 14 FMF patients was a R92Q mutation carrier (Booty et al. 2009). Another study published in the same year reported that 1 out of the 21 FMF patients who carried single mutation in MEFV gene had R92Q mutation (Kone-Paut et al. 2009).

The results of these three studies are similar to those of this study. None of the four studies found a statistically significant difference in the carrier frequencies between the patient and the control groups.

Renal amyloidosis of the AA type is the main complication of hereditary diseases such as FMF and more recently described disorders such as TRAPS and MWS (Muckle-Wells syndrome) (Grateau et al. 1999). These diseases are disorders of the inflammatory pathway characterized by recurrent attacks of fever with visceral, synovial, or cutaneous inflammation. Although FMF amyloidosis generally appears years after the beginning of inflammatory attacks, it may develop in some patients without or prior to other manifestations (Dode et al. 2002).

Two different studies suggested that existence of R92Q mutations can be a potential risk factor for development of amyloidosis (Dode et al. 2002; Aganna et al. 2004). However, according to these results amyloidosis was not observed in our R92Q mutation carriers ($n: 14$). Similarly, results of the Yagel et al.'s study showed no relation between R92Q mutation and amyloidosis. Although sharing common biochemical pathways, brings about the fact that the patient

and the control groups have a similar R92Q mutation frequency supports the notion that an interaction between TNFRSF1A and MEFV is minimal or nonexistent (Yagel et al. 2010).

CONCLUSION

Despite their symptomatic similarities, the fact that the TNFRSF1A gene R92Q mutation was seen in both groups with an approximate frequency shows that FMF is not confused with TRAPS.

RECOMMENDATIONS

The researchers hope that future studies will address to these limitations. They also hope that by constructing a new set of diagnostic criteria, cases of possible misdiagnosis can be reduced or totally eliminated.

LIMITATIONS OF THE STUDY

There are mainly three limitations in this study. First, researchers have only analyzed the most common TNFRSF1A gene mutation in FMF patients. Because of financial constraints we were not able to assay other mutations, which might have an effect on the appearance of the FMF symptoms in our control group. Second, they did not assay other genes causing hereditary periodic fever syndromes. Finally, the size of their cohort was not large enough to yield any number of QQ genotype.

REFERENCES

- Aganna E, Hawkins PN, Ozen S, Pettersson T, Bybee A, Mc-Kee SA et al. 2004. Allelic variants in genes associated with hereditary periodic fever syndromes as susceptibility factors for reactive systemic AA amyloidosis. *Genes Immun*, 5: 289-293.
- Berkun Y, Eisenstein EM 2014. Diagnostic criteria of Familial Mediterranean Fever. *Autoimmunity Reviews*, 13: 388-390.
- Booth DR, Gillmore JD, Booth SE, Pepys MB, Hawkins PN 1998. Pyrin/Marenostrin mutations in Familial Mediterranean Fever. *Q J Med*, 91: 603-606.
- Booty MG, Chae J, Masters SL, Remmers EF, Barham B, Le JM et al. 2009. Familial Mediterranean Fever with a single MEFV mutation: Where is the second hit? *Arthritis Rheum*, 60: 1851-1861.
- Cosan F, Ermence Z, Erbag G, Azakli H, Yilmazer B, Yazici A et al. 2013. The association of TNFRSF1A gene and MEFV gene mutations with adult onset-Still's disease. *Rheumatol Int*, 33: 1675-1680.

- Dode C, Hazenberg BP, Pecheux C, Cattani D, Moulin B, Barthelemy A et al. 2002. Mutational spectrum in the MEFV and TNFRSF1A genes in patients suffering from AA amyloidosis and recurrent inflammatory attacks. *Nephrol Dial Transplant*, 17: 1212-1217.
- Fukushima Y, Obara K, Hirata H, Sugiyama K, Fukuda T, Takabe K 2013. Three Japanese patients (mother and two children) with familial Mediterranean fever associated with compound heterozygosity for L110P/E148Q/M694I and an autosomal true dominant inheritance pattern. *Asian Pac J Allergy Immunol*, 31: 325-329.
- Grateau G, Drenth JPH, Delpech M 1999. Hereditary fevers. *Curr Opin Rheumatol*, 11: 75-78.
- Grateau G, Duruoz MT 2010. Autoinflammatory conditions: When to suspect? How to treat? *Best Pract Res Clin Rheumatol*, 24: 401-411.
- Gunesacar R, Celik MM, Arica V, Elmacioglu S, Ozturk OH 2014. Frequency of MEFV gene mutations in Hatay province, Mediterranean region of Turkey and report of a novel missense mutation (I247V). *Gene*, S0378-1119(14)00683-0. doi: 10.1016/j.gene. 2014.06.019.
- Henderson C, Goldbach-Mansky R 2010. Monogenic auto-inflammatory diseases: New insights into clinical aspects and pathogenesis. *Curr Opin Rheumatol*, 22: 567-557.
- Hull KM, Drewe E, Aksentijevich I, Singh HK, Wong K, McDermott EM et al. 2002. The TNF receptor-associated periodic syndrome (TRAPS): Emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)*, 81: 349-368.
- Karatay S, Melikoglu MA 2007. TRAPS: Otozomal Dominant Bir Otoinflamatuvar Sendrom. *The Eurasian Journal of Medicine* (December), 39.
- Kasapçopur Ö, Arisoy N 2006. Ailesel Akdeniz Ateşivedigerotoenflamatuar hastalıklar. *Türk Pediatri Arsivi*, 41: 9-17.
- Kauffman MA, Gonzalez-Moro'n D, Garcea O, Villa AM 2012. TNFSFR1A R92Q mutation, autoinflammatory symptoms and multiple sclerosis in a cohort from Argentina. *Mol Biol Rep*, 39: 117-121.
- Kone-Paut I, Hentgen V, Guillaume-Czitrom S, Compeyrot-Lacassagne S, Tran TA, Touitou I 2009. The clinical spectrum of 94 patients carrying a single mutated MEFV allele. *Rheumatology (Oxford)*, 48: 840-842.
- Marek-Yagel D, Berkun Y, Padeh S, Abu A, Reznik-Wolf H, Livneh A et al. 2009. Clinical disease among patients heterozygous for familial Mediterranean fever. *Arthritis Rheum*, 60: 1862-6.
- McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M et al. 1999. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1 define a family of dominantly inherited autoinflammatory syndromes. *Cell*, 97: 133-144.
- Milhavet F, Cuisset L, Hoffman HM, Slim R, El-Shanti H, Aksentijevich I et al. 2008. The infEVERS auto-inflammatory mutation online registry: Update with new genes and functions. *Hum Mutat*, 29(6): 803-808.
- Pras M, Kastner DL 1997. Familial Mediterranean Fever. In: JH Klippel, PA Dieppe (Eds.): *Rheumatology*. 2nd Edition. London: Mosby, pp. 23: 1-4.
- Rigante D, Lopalco G, Vitale A, Lucherini OM, De Clemente C et al. 2014. Key facts and hot spots on tumor necrosis factor receptor-associated periodic syndrome. *Clin Rheumatol*, 33: 1197-1207.
- Sohar E, Gafni J, Pras M, Heler H 1967. Familial Mediterranean Fever: A survey of 470 cases and review of the literature. *Am J Med*, 43: 227-253.
- Stjernberg-Salmela S, Pettersson T, Karenko L, Blazevic V, Nevela H, Pitkanen S et al. 2004. A novel tumour necrosis factor receptor mutation in a Finnish family with periodic fever syndrome. *Scandinavian Journal of Rheumatology*, 33: 140.
- The International FMF Consortium 1997. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell*, 90: 797-807.
- Tunca M, Akar S, Onen F, Ozdogan H, Kasapçopur O, Yalcinkaya F et al. 2005. Familial Mediterranean fever (FMF) in Turkey: Results of a nationwide multicenter study. *Medicine (Baltimore)*, 84(1): 1-11.
- Williamson LM, Hull D, Mehta R, Reeves WG, Robinson BH, Toghiani PJ 1982. Familial Hibernian fever. *Q J Med*, 51: RA179-RA187.
- Yagel DM, Berkun Y, Padeh S, Lidar M, Shiinar Y, Bar-Joseph I et al. 2010. Role of the R92Q TNFRSF1A mutation in patients with Familial Mediterranean Fever. *American College of Rheumatology*, 62(9): 1294-1298.
- Yasumura J, Wago M, Okada S, Nishikomori R, Takei S, Kobayashi M 2014. A 2-year-old Japanese girl with TNF receptor-associated periodic syndrome: A case report of the youngest diagnosed proband in Japan. *Mod Rheumatol*, 20: 1-4.
- Yigit S, Bagci H, Ozkaya O, Ozdamar K, Cengiz K, Akpolat T 2008. MEFV mutations in patients with familial Mediterranean fever in the Black Sea region of Turkey: Samsun experience [corrected]. *J Rheumatol*, 35(1): 106-113.
- Zemer D, Revach M, Pras M, Modan B, Schor S, Sohar E et al. 1974. A controlled trial of colchicine in preventing attacks of familial Mediterranean fever. *N Engl J Med* 291: 932-934.
- Zemer D, Pras M, Sohar E, Modan M, Cabili S, Gafni J 1986. Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever. *N Engl J Med*, 314: 1001-1005.