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Research paper

Interleukin-1Ra rs2234663 and Interleukin-4 rs79071878 Polymorphisms in Familial Mediterranean Fever

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ABSTRACT

Objective: Familial Mediterranean Fever (FMF) is an autosomal recessively inherited auto inflammatory disorder. MEFV gene, causing FMF, encodes pyrin that is associated with the interleukin-1 (IL-1) related inflammation cascade. The aim of this study was to investigate the relationship of interleukin-1 receptor antagonist (*IL-1Ra*) and interleukin-4 (*IL-4*) polymorphisms with the risk of FMF in the Turkish population.
Methods: This study included 160 patients with FMF (74 men, 86 women) and 120 healthy controls (50 men, 70 women), respectively. Genotyping of *IL-1Ra* rs2234663 polymorphism was evaluated by gel electrophoresis after polymerase chain reaction (PCR). The *IL-4* rs79071878 polymorphism was determined by PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis. The results of analyses were evaluated for statistical significance.
Results: There was no significant difference in *IL-1Ra* genotype and allele distributions between FMF and the control groups ($p > 0.05$). However, a significant association was observed between FMF patients and control groups according to *IL-4* genotype distribution ($p = 0.016$), but no association was found in the allelic frequency of *IL-4* between FMF patients and the controls ($p > 0.05$, OR: 1.131, CI 95%: 0.71–1.81).
Conclusions: The *IL-4* rs79071878 polymorphism, was associated whereas the *IL-1Ra* rs2234663 polymorphism was not associated with FMF risk in the Turkish population. Larger studies with different ethnicities are needed to determine the impact of *IL-1Ra* and *IL-4* polymorphism on the risk of developing FMF.

1. Introduction

Familial Mediterranean Fever (FMF; OMIM 249100) is a recessively inherited auto-inflammatory disorder. FMF has been predominantly found in ethnic groups living around the Mediterranean basin (Jews, Arabs, Turks, and Armenians). FMF is clinically characterized by recurrent and self-limited attacks of fever, arthritis, erysipelas-like skin disease, abdominal pain, and inflammation of serous membranes (Yigit et al., 2014a). *MEFV*, the gene responsible for FMF, encodes the pyrin protein (Grattagliano et al., 2014). Pyrin takes a role in the events that are related to the innate immune system, which is responsible for primary defense against noxious agents and external pathogens (Portincasa et al., 2013). In a so-called inflammasome complex, pyrin and other proteins are likely to prompt the conversion of pro-interleukin (IL)-1β to interleukin-1β (IL-1β), and pyrin plays a fundamental role in the development of fever, inflammation and apoptosis (Portincasa et al., 2013). It is considered that variant forms of pyrin inappropriately trigger neutrophil activation, causing unprovoked and short-lived bursts of systemic inflammation that are clinically seen in FMF (Lachmann et al., 2006).

Interleukin-1 (IL-1) are molecules that have a regulatory role in initiating and modulating immunologic and inflammatory events (Cai et al., 2014). The interleukin-1 receptor antagonist (IL-1Ra) (also called IL-1RN) is included in the IL-1 family (Perrier et al., 2006). IL-1Ra is an important anti-inflammatory molecule that competes with interleukin-1 alpha (IL-1α) and interleukin-1 beta (IL-1β) thus inhibits the activities of these cytokines and modulates a number of IL1-related immune and inflammatory activities (Granowitz et al., 1991; Arend et al., 1998). *IL-1Ra* gene is a polymorphic that causes quantitative differences in IL-1Ra and IL-1β production (Kamenarska et al., 2014). In the second intron of the *IL-1Ra* gene, there is a variable tandem repeat polymorphism (VNTR) 86 base pairs in length (Jaiswal et al., 2012). The number of repetitions in this sequence ranges between 2 and 6. The most frequent is

Abbreviations: FMF, Familial Mediterranean Fever; IL-1, Interleukin-1; *IL-1Ra*, Interleukin-1 receptor antagonist; *IL-4*, Interleukin-4; VNTR, Variable T-Number Tandem Repeat; PCR, Polymerase chain reaction; PCR-RFLP, PCR-based restriction fragment length polymorphism.

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allele 1 (four repeats) followed by allele 2 (2 repeats) in general population. The other three alleles are rarely seen, i.e. less than 1% in most populations. The role of *IL-1Ra* VNTR polymorphism in the development of inflammatory diseases has been the subject of any research (Fischer et al., 1992).

Interleukin-4 (IL-4) is the main cytokine secreted by T helper 2 lymphocytes (Th2), basophils and mast cells and is mapped within the cytokine gene cluster on chromosome 5q31.1 (Song et al., 2013). IL-4 plays a key regulatory role in humoral and adaptive immune responses and negatively regulates the production of pro-inflammatory cytokines (Cuneo and Autieri, 2009). IL-4 contains several polymorphisms. One of these is a VNTR polymorphism in its third intron which is associated with IL-4 production (rs79071878). This polymorphism contains three alleles: a P1 allele (2 repeats = 183 bp), a P2 allele (3 repeats = 253 bp) and a P3 allele (4 repeats) (Birbian et al., 2014; Kazemi, 2010). The allele with three repeats is the most common one, and the allele with two repeats is rare. It is reported that another rare allele of four repeats exists only in a few populations (Mout et al., 1991). It has been established that P1 allele causes an increase in IL-4 expression more than P2 allele (Yigit et al., 2014b). The purpose of this study was to evaluate the distribution of the *IL-1Ra* rs2234663 and *IL-4* rs79071878 polymorphisms in Turkish patients with FMF and to determine whether these polymorphisms are a risk factor for the development of FMF.

2. Materials and methods

2.1. Patients

160 patients with FMF (74 men and, 86 women) and 120 healthy controls (50 men and, 70 women) who presented to Samsun Training and Research Hospital and Giresun University, Faculty of Medicine, Department of Medical Genetic and Gaziosmanpasa University, Faculty of Medicine, Department of Internal Medicine were included in the study. All the participants were informed of the study protocol and their written informed consent was received. Subjects included in the study were of Turkish origin from the Central Black Sea region of Turkey. All patients were different from our previous study subjects (Yigit et al., 2014a) and had come from different regions. The diagnosis of FMF was carried out according to Tel Hashomer criteria. The protocol of the study was approved by the Clinical Studies Ethics Committee of Trabzon Kanuni Education and Research Hospital (2015/06-04), and the study was conducted in accordance with the Helsinki Declaration.

2.2. Genotyping

Genomic DNAs isolated from whole blood collected from FMF patients and the control groups by the standard procedures (Sigma-Aldrich, St. Louis, MI, USA) were stored at −20 °C. The rs2234663 polymorphism of *IL-1Ra* and the rs79071878 polymorphism of *IL-4* were analyzed according to the protocols described previously, respectively (Tarlow et al., 1993; Mout et al., 1991). The rs2234663 of *IL-1Ra* gene was analyzed by polymerase chain reaction (PCR). PCR reaction was performed in a 25 μL final volume containing 25 pM of each primer, 0.1 mM of dNTP, 0.5 μg of genomic DNA, 1.5 mM of MgCl, 2 and 2.5 μL of PCR buffer and 1.5 unit of Taq DNA polymerase according to the following protocols: initial denaturation at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 45 s, annealing at 51 °C for 30 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 5 min. Two oligonucleotide primers forward: 5′-CTC AGC AAC ACT CCT AT-3′ and reverse: 5′-TTC CAC CAC ATG GAA C-3′ based on flanking region of the *IL-1Ra* gene were used. PCR products were separated by electrophoresis on a 3% agarose gel and visualized by ethidium bromide staining. Five different alleles of *IL-1Ra* were described as follows: allele 1 four repeats (410 bp); allele 2, two repeats (240 bp); allele 3, five repeats (500 bp); allele 4, three repeats (325 bp) and allele 5, six repeats (595 bp).

For *IL-4*, PCR was performed in a 25 μl reaction mixture containing 50 ng DNA, 0.8 μM of each primer, 200 μM of each dNTP, 2.5 mM MgCl, 1.5 units Taq polymerase, 2.5 μl 10× KCl buffer. Amplification was performed using the forward 5′ AGG CTG AAA GGG GGA AAG C-3′ and reverse 5′-CTG TTC ACC TCA ACT GCT CC-3′ primers, with initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The PCR products were separated on a 3% agarose gel and visualized by ethidium bromide staining. The PCR products were of 183 bp for the P1 allele and 253 bp for the P2 allele.

2.3. Statistical analysis

All statistical analyses of data were performed using the computer software SPSS version 15.0 for Windows and OpenEpi Info software package program. Genotype distributions and allele frequencies were compared between FMF patient and controls by $\chi 2$ test and Fisher's exact test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. P values of 0.05 or less were considered statistically significant.

3. Results

In the present study, a total of 280 subjects, including 160 FMF patients and 120 adult healthy controls were genotyped for the *IL-1Ra* and *IL-4* polymorphisms. Baseline clinical and demographic features of the patient and control groups are shown in Table 1. The mean age ± standard deviation (SD) was 19.69 ± 12.69 in patients and 19.53 ± 12.60 in the control group. There were 86 (53.9%) women and 74 (46.1%) men in the patient group and 70 (58.3%) women and 50 (41.7%) men in the control group. Five alleles were observed in patients and control subjects. The overall distribution of *IL-1Ra* genotypes did not differ significantly between FMF cases and controls. The most frequent genotype observed was 1.1 (50.0%) followed by 1.2 (37.22%) in the patient group. Two alleles, 1 and 2, were the most frequent ones. In study, we detected the following *IL-1Ra* alleles in the patient group: *IL-1Ra* 1 (70.83%), *IL-1Ra* 2 (26.94%), *IL-1Ra* 3 (0.55%), *IL-1Ra* 4 (1.66%), and *IL-1Ra* 5 (0%). No significant difference was observed in the *IL-1Ra* allele frequencies between patients and the control group

Table 1
Demographic and clinical characteristics of the study and control groups.

Characteristic	Control group, n (%)	Study group, n(%)
Gender, male/female	50/70 (41.7/58.3)	74/86 (46.1/53.9)
Age, mean ± SD, year	19.53 ± 12.60	19.69 ± 12.69
Age of first symptoms, mean ± SD, year		10.47 ± 6.86
Age of onset, mean ± SD, year		16.27 ± 9.40
The frequency of attacks, mean ± SD, day		24.88 ± 14.53
Usage of colchicine		
No/Yes		54/106 (33.8/66.2)
Family history		
No		83 (51.9)
Yes		
1. degree		53 (33.1)
2. degree		19 (11.7)
3. degree		5 (3.2)
Fewer		
No/Yes		27/133(16.9/83.1)
Abdominal pain		
No/Yes		14/146 (9.1/90.9)
Thoracic Pain		
No/Yes		114/46 (71.4/28.6)
Joint involvement		
No/Yes		52/108 (32.5/67.5)
Appendicitis		
No/Yes		142/18 (89/11)
Erythema		
No/Yes		133/27 (83.1/28.6)

SD: Standard deviation.

Table 2
Genotype and allele frequencies of IL-1Ra rs2234663 gene polymorphism in FMF patient and control groups.

Genotypes											p
	1.1	1.2	1.3	1.4	1.5	2.2	2.3	2.4	4.4	5.5	
IL-1Ra											>0.05
Patients (n:180)	90 (50)	67 (37.22)	2 (1.11)	6 (3.33)	0	15 (8.33)	0	0	0	0	
Controls (n:120)	58 (48.33)	39 (32.50)	1 (0.83)	6 (5)	1 (0.83)	10 (8.33)	1 (0.83)	2 (1.66)	1 (0.83)	1 (0.83)	

Alleles						p
	1	2	3	4	5	
Patients	255 (70.83)	97 (26.94)	2 (0.55)	6 (1.66)	0	>0.05
Controls	163 (67.91)	62 (25.80)	2 (0.83)	10 (4.16)	3 (1.25)	

($p > 0.05$). The distribution of *IL-1Ra* genotypes and allele frequencies in the study and control groups were shown in Table 2. The three genotypes of *IL-4* were classified as follows: P1P1 (183 bp), P2P2 (253 bp) and P1P2 (both 183 and 253 bp fragments). The frequencies of P1P1, P2P2, and P1P2 genotypes of rs79071878 polymorphism in patients were 6.60%, 76.11%, and 17.22% and in the controls were 0.83%, 73.33%, and 25.83%, respectively. The distribution of genotype of *IL-4* rs79071878 polymorphism was statistically different between FMF patients and control group ($p = 0.016$). The frequency of P1P1 genotypes was also significantly higher in FMF patients than healthy controls. P1 and P2 allele frequencies were respectively 15.27% and 84.72% in the patient group and 13.75% and 86.25% in the control group. There was no significant difference in the allele frequencies of *IL-4* between the study and control groups ($p > 0.05$, OR: 1.131, CI 95%: 0.71–1.81). The distribution of genotype and allele frequencies of *IL-4* gene rs79071878 polymorphism are shown in Table 3.

4. Discussion

Auto-inflammatory diseases are clinically heterogeneous and have a chronic nature. Auto-inflammation occurs as a result of natural immune system dysfunction and influences multiple organs, including joints, skin, and nervous system. FMF, considered a prototype of auto-inflammatory diseases, is common in the Turkish population (Yigit et al., 2014a). Pyrin, expressed predominantly in innate immune cells such as neutrophils, monocytes, and dendritic cells, is a factor that controls inflammation under normal conditions. Pyrin is involved in the activation of caspase-1 and the processing and release of active IL-1β. It was reported that pyrin-deficient mice have increased caspase-1 activation, increased IL-1β maturation, and defective macrophage apoptosis (de Jesus et al., 2015). It is considered that impaired cytokine regulation underlies the pathogenesis of FMF. Therefore, studies were conducted to examine some cytokines and their receptors in patients with remission and attacks of FMF disease (Yigit et al., 2014a; Haznedaroglu et al., 2005). Recent studies indicate that about 30% of FMF patients have subclinical inflammation during attack-free periods (Orbach and Ben-Chetrit, 2001; Ben-Zvi and Livneh, 2011). In a study performed in Turkey, serum levels of interleukin-17 (IL-17) and interleukin-18 (IL-18), two pro-inflammatory cytokines, were found to be significantly higher in FMF patients during and without acute attack compared to control group (Haznedaroglu et al., 2005). In another study, mRNA levels of four pro-inflammatory cytokines [tumor necrosis factor alpha (TNF-α), IL-1β, interleukin-6 (IL-6) and interleukin-8 (IL-8)] were compared in FMF patients in clinical remission and in healthy controls. It was reported that these cytokines were more elevated in attack-free FMF patients than in controls (Notarnicola et al., 2002).

IL-1β, the strongest endogenous pyrogen, is a potent recruiter and activator of neutrophils and macrophages (de Jesus et al., 2015). IL-1Ra, a natural anti-inflammatory molecule, competes for binding and downregulates the IL-1α and IL-1β signaling. In a study, total IL-1, IL-1Ra, and the IL-1Ra/IL-1 ratio were measured in intestinal mucosa cells obtained from control group, patients with Crohn's disease, and ulcerative colitis patients. IL-1Ra/ IL-1 ratio was found to decrease significantly in the intestinal mucosa in both patients with Crohn's disease and ulcerative colitis when compared with control subjects (Casini-Raggi et al., 1995).

The purpose of this study was to evaluate the distribution of the IL-1Ra rs2234663 and IL-4 rs79071878 polymorphisms in Turkish patients with FMF and to determine whether these polymorphisms are a risk factor for the development of FMF. To our knowledge, this is the first study evaluating the prevalence of the *IL-1Ra* rs2234663 among FMF patients in Turkey. 86 bp VNTR polymorphism in the second intron of the *IL-1Ra* (rs2234663) contains three potential protein binding sites which affect the control of cell proliferation activity, resulting in potential regulation of *IL-1Ra* production (Zhang et al., 2012). This polymorphic site has five alleles described as 1–5, the *IL-1Ra* 1 and 2 alleles have been associated with variable transcription rates and circulating levels of both IL-1Ra and IL-1β (Patwari et al., 2008). When the complexity of cytokine interactions, the diversity of the environment and the pathology of the populations studied are considered, it may be expected that *IL-1Ra* 2 has been associated with increased (Danis et al., 1995), decreased (Tountas et al., 1999) or similar (Mwantembe et al., 2001) IL-1Ra protein levels, compared to other alleles.

The *IL-1Ra* polymorphism has been correlated with increased inflammatory responses and is found to be associated with several pathologic disorders including with inflammatory bowel disease (Bioque et al., 1995; Andus et al., 1997; Tountas et al., 1999), lichen sclerosus (Clay et al., 1994), periodontitis (Tai et al., 2002), psoriasis (Tarlow et al., 1997), alopecia areata (Cork et al., 1995), ulcerative colitis (Mansfield et al., 1994), systemic lupus erythematosus (Blakemore et al., 1994), juvenile idiopathic arthritis (Vencovský et al., 2001), Graves' disease (Blakemore et al., 1995), rheumatoid arthritis (Carreira et al., 2005), Sjögren's syndrome (Perrier et al., 1998), Henoch–Schönlein nephritis (Liu et al., 1997) as well as diabetic complications (Blakemore et al., 1996). According to our results, no significant differences were observed in the *IL-1Ra* genotype distribution and allele frequencies between FMF patients and control.

IL-4, the prototypic immunoregulatory cytokine, is a potent anti-inflammatory cytokine. IL-4 plays a significant role in the regulation of

Table 3
Genotype and allele frequencies of IL-4 rs79071878 gene polymorphism in FMF patient and control groups

Gene	FMF patients n = 180	Controls n = 120	p	OR (CI 95%)
IL-4 (70 bp VNTR)				
Genotypes				
P1/P1	12 (6.6 %)	1 (0.83 %)		
P1/P2	31 (17.22 %)	31 (25.83 %)	**0.016**	
P2/P2	137 (76.11 %)	88 (73.33 %)		
Alleles				
P1	55 (15.27 %)	33 (13.75 %)	>0.05	1.131 (0.71-1.81)
P2	305 (84.72 %)	207 (86.25 %)		

The results that are statistically significant are typed in bold.

antibody production, hematopoiesis and inflammation, and in the development of effector T-cell responses (Brown and Hural, 1997), and is thus an important factor in both humoral and cell-mediated immunity. Polymorphisms in the *IL-4* gene may cause alterations in its levels and therefore a disturbance in immune functioning, which is an indicator of its role in some autoimmune diseases. *IL-4* rs79071878 polymorphism is likely to alter messenger ribonucleic acid splicing, which results in different splice variants (Inanir et al., 2013). It was stated that while the pro-inflammatory cytokines up-regulated monocyte MEFV message levels, anti-inflammatory cytokines down-regulated monocytic MEFV expression (Centola et al., 2005). Also, the IL-4 gene that is an anti-inflammatory cytokine, P1P1 genotype exacerbates the pathogenesis of FMF (Aypar et al., 2003). *IL-4* gene polymorphisms have not only been associated with many infectious diseases but also reported to be associated with non-infectious diseases such as preeclampsia (Salimi et al., 2014), lupus (Wu et al., 2003), oral cancer (Tsai et al., 2005), knee osteoarthritis (Yigit et al., 2014b), cervical cancer (Shekari et al., 2012), rheumatoid arthritis (Cantagrel et al., 1999), fibromyalgia syndrome (Yigit et al., 2013), ischemic stroke (Tong et al., 2013), asthma (Birbian et al., 2014), childhood immune thrombocytopenic purpura (Makhlouf and Elhamid, 2014), diabetic peripheral neuropathy (Basol et al., 2013), recurrent aphthous stomatitis (Kalkan et al., 2013a), multiple sclerosis (Karakus et al., 2013) and deep venous thrombosis in Behçet's disease (Inanir et al., 2013). There are also studies suggesting that *IL-4* VNTR polymorphism is not associated with ankylosing spondylitis (Yigit et al., 2015), alopecia areata (Kalkan et al., 2013b), recurrent pregnancy loss (Saijo et al., 2004), idiopathic thrombocytopenic purpura (Chen et al., 2007), and steroid sensitive nephrotic syndrome (Liu et al., 2005). This may be related to the fact that the allelic distribution of cytokins have shown difference in ethnic and geographic populations.

It was showed the possible association between P1 allele and higher expression of the *IL-4* (Fang et al., 2011). Other studies showed that the P1 allele of the IL-4 gene was found with a significantly higher frequency in rheumatoid arthritis (Cantagrel et al., 1999), alopecia areata (Kalkan et al., 2013b), fibromyalgia (Shekari et al., 2012) patients compared with controls. In addition, there are some studies reporting that IL-4 P2 allele has protective role for destructive rheumatoid arthritis (Buchs et al., 2000), cancer (Duan et al., 2014) and apthous stomatitis (Kalkan et al., 2013a). However, it was reported that P2 allele is associated with preeclampsia susceptibility (Salimi et al., 2014).

On one hand, there are studies indicating that P2P2 genotype increases the risk of coronary artery disease (Basol et al., 2014), end-stage renal disease (Mittal and Manchanda, 2007). On the other hand, some studies associate P1P1 genotype with progressive IgA nephropathy (Masutani et al., 2003) and fibromyalgia (Yigit et al., 2013). In this study, we found that the genotype distribution of *IL-4* VNTR polymorphism had significantly increased the risk of FMF. The P1P1 genotype was significantly associated with FMF ($p = 0.016$). In a similar study, it was also found that P1P1 genotype was associated with FMF (Yigit et al., 2014a).

5. Conclusion

Our findings suggest that there is an association of the *IL-4* gene rs79071878 polymorphism with the susceptibility for development of FMF. However, the *IL-1Ra* gene rs2234663 polymorphism is not associated with FMF within the same population. Larger studies with different ethnicities are needed to find out the impact of IL-1Ra and *IL-4* polymorphism on FMF.

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