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Genetic Variations in Tumor Necrosis Factor Alpha, Interleukin-10 Genes, and Migraine Susceptibility

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Abstract

Objectives. Migraine is a very common headache disorder and pathogenesis of the disease is still largely unknown. Cytokine genes have been implicated in migraine susceptibility. The present study was designed to explore the associations of polymorphisms in the tumor necrosis factor alpha (TNF-α), interleukin-10 (IL-10) gene, and IL-10 haplotypes in Turkish migraine patients.

Methods. TNF-α –308G/A, IL-10 –1082G/A, –819C/T, and –592C/A polymorphisms in 203 migraine patients and 202 healthy subjects were analyzed by using amplification refractory mutation system-polymerase chain reaction.

Results. The –308G/A genotypic and –308A allelic frequency of TNF-α polymorphism was higher in migraine patients than healthy controls, and significant association was found between migraine and TNF-α –308G/A polymorphism (Bonferroni correction [Pc]: <0.0001, odds ratio: 2.16, 95% confidence interval: 1.44–3.28). No statistically significant association was found between IL-10 –1082G/A, –819C/T, and –592C/A polymorphisms and haplotypes containing these alleles and migraine.

Conclusions. This study reflect that TNF-α –308G/A polymorphism may be one of the many genetic factors for migraine susceptibility in the Turkish population.

Key Words. Migraine; TNF-α; IL-10; Genetic Susceptibility; Polymorphism

Introduction

Migraine is a common neurovascular disorder characterized by severe, often unilateral, pulsatile headache that can be accompanied by nausea, vomiting, photo- and/or phonophobia. Migraine has a complex etiology determined by genetic and environmental factors [1]. Although the pathophysiology of migraine remains unknown, it is thought that cytokines play an important role in the modulation of pain threshold and may be involved in the pathogenesis [2].

It has been postulated that Th1 lymphocytes release tumor necrosis factor alpha (TNF-α), interleukin (IL) 2, interferon gamma, and lymphotoxin, while Th2 lymphocytes release IL-4, IL-5 and IL-10, and an unbalance between Th1/Th2 cytokines may influence the spread of pain-producing processes in migraine and they could contribute to trigeminal nerve fiber sensitization [3]. Recently, several cytokines such as IL-1β, TNF-α, IL-10, IL-4, IL-13, and IL-2 have been suggested to be involved in the pathogenesis of migraine attacks [4].

TNF-α is a proinflammatory cytokine that plays a key role in the perpetuation of the inflammatory response, whereas IL-10 inhibits the production of the proinflammatory cytokines such as TNF-α [5–7]. TNF-α and IL-10 have opposing role in the inflammatory response. Furthermore, they also have a synergistic role in the control of the immune-inflammatory responses [8,9]. These cytokines stimulations exert strong control on the Th1/Th2 balance [10], and the perturbation of this balance may affect migraine pathogenesis [3].

Monozygotic twin studies suggest that 60% of TNF-α and 75% of IL-10 variation in the production capacity appears to be genetically determined [11]. TNF-α and IL-10 productions are tightly regulated both at the transcriptional

and posttranscriptional levels and several single nucleotide polymorphisms in these genes (TNF-α –308G/A, IL-10 –1082G/A, –819C/T, and –592C/A) have been found to be associated with altered levels of circulating IL-10 and TNF-α [12,13]. The –308A allele of TNF-α gene and –1082G allele of IL-10 gene have been shown to increase the expression of these genes [12,13]. IL-10 –1082G/A, –819C/T and –592C/A polymorphisms influence the amount of IL-10 secreted in cell cultures, the GCC haplotype has been associated with an increased production, while the ACC and the ATA haplotypes have been associated with intermediate and lower production, respectively [12]. According to this, GCC+/GCC+ genotype individuals have classified as high producers, while GCC+/GCC– and GCC-/GCC– genotype individuals have classified as intermediate and low producers, respectively. TNF-α –308A/G allele carrier individuals have classified as high producers, while –308G/G allele carrier individuals have classified as low producers [12,13].

Several studies have examined cytokines levels in migraine patients [14,15], and the association of migraine with TNF-α polymorphism has been studied in different ethnic populations with inconsistent results [16–20]. Moreover, there was no data representing the association of IL-10 polymorphism/haplotypes with migraine susceptibility. IL-10 is a major endogenous downregulator of TNF-α production that, conversely, induces IL-10 synthesis and restricts its own production and effect on inflammation [8]. Thus, IL-10 –1082 G/A alleles or haplotypes containing these alleles may influence the Th1/Th2 balance, and hence may play a role in migraine susceptibility and increase the risk of developing disease. We, therefore, aimed to explore the associations of polymorphisms in the TNF-α, IL-10 gene, and IL-10 haplotypes in Turkish migraine patients

Materials and Methods

Subjects

The study group consisted of 203 patients with migraine (25 men and 178 women; mean age: 36.49 ± 9.55 standard deviation [SD] years), and 202 (28 men and 174 women; mean age: 38.18 ± 8.42 SD years) healthy hospital workers with no previous or current history of migraine, all of whom live in Tokat, Turkey. All migraine patients were registered at the outpatient clinic of the Neurology Department at Gaziosmanpasa Medical Faculty, and they all fulfilled the International Headache Society criteria for classification [21]. The control subjects matched for age and geographic area. The study protocol was approved by the ethics committee of Gaziosmanpasa Medical Faculty, and written informed consent was obtained from the study participants.

Methods

Genomic DNA was extracted from blood samples using an Invitrogen DNA isolation kit (Invitrogen Life Technologies, Carlsbad, CA, USA). The same methodology that has been described in previous reports [22–24] has been applied for the analysis of polymorphisms. Amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) was done in 25-μL volumes containing 25–50 ng of genomic DNA, 0.25 mM specific primers, and 0.1 mM internal control primers in the presence of 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 500 mM KCl, 200 mM Tris-HCl, 0.001% gelatin, and 0.5 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). ARMS-PCR for amplification of IL-10 (–1082G/A, –819C/T) alleles and TNF (–308G/A) allele was used under the following conditions: amplification consisted of a 1-minute denaturation step at 95°C; 10 cycles of 15 seconds at 95°C and 50 seconds at 65°C and 40 seconds at 72°C; and 20 cycles of 20 seconds at 95°C, 50 seconds at 59°C, and 30 seconds at 72°C, followed by cooling to 4°C [24,25]. IL-10 –592C/T polymorphism is in linkage disequilibrium with –819C/A polymorphism: allele C at –592 is always present when at position –819 is allele C and allele A is always present when at position –819 is allele T [25]. The amplified products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and the genotypes were determined under ultraviolet (UV) illumination.

Statistical Analysis

Statistical analysis was performed by using Epi Info Software Version 3.2.2 (CDC, Atlanta, GA, USA). The distribution of TNF-α, IL-10 genes polymorphisms between migraine patients and healthy controls were compared by using the χ^2 or Fisher's exact test. *P* values smaller than 0.05 were considered significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated whenever χ^2 or Fisher's exact test was significant. Goodness of fit χ^2 test was used to check Hardy–Weinberg equilibrium in the control population, Arlequin Software v. 2000 (University of Geneva, Geneva, Switzerland). Significant probability values obtained were corrected for multiple testing (Bonferroni correction; Pc). The correlation of mean age with groups was analyzed using the *t*-test for independent samples. The comparison of the categorical variables between the groups was made by the chi-square test (SPSS 15.0, SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the demographic characteristics of migraine patients and healthy controls. No significant difference in the mean ages and gender was observed between the two groups. For quality control, ~20% of the samples were randomly selected to be genotyped again by a different investigator, and the results were 100% concordant.

Allelic and genotypic distributions of the studied polymorphisms are shown in Tables 2 and 3. The –308G/A genotypic and –308A allelic frequency of TNF-α polymorphism was higher in migraine patients than healthy controls, and a significant association was found between migraine and

Table 1 The demographic characteristics of patients with migraine and healthy controls

Demographic Features	Migraine Patients N = 203	Healthy Controls N = 202
Age (years) mean (SD)	36.49 (9.55)	35.47 (8.18) (*t*:1.15, *P*:0.25)
Sex (female/male)	178/25	174/28 (χ2:0.21, *P*:0.64)
Migraine types		
MA	33	N/A
MO	170	

MA = migraine with aura; MO = migraine without aura; N/A = not assessed; SD = standard deviation.

TNF-α –308G/A polymorphism (Pc: <0.0001, OR: 2.16, 95% CI: 1.44–3.28). TNF-α 308A/A genotype was not found in either the patients or the control group (Table 2).

We found remarkably similar frequencies in migraine patients compared with controls for IL-10 –1082G/A genotypes/alleles, and no association was observed between migraine and this polymorphism. The frequency of IL-10–819 C/T genotype was higher and C/C genotype was lower in migraine patients, while the frequency of C/T genotype was lower and C/C genotype was higher in healthy subjects, but no significant associations were found between these genotypes/alleles and migraine. The patient and the control group showed nearly same frequency of IL-10 haplotypes/genotypes. No significant differences in genotypic and haplotypic distribution of IL-10 gene polymorphism were observed among migraine patients and control subjects (Table 3).

Because of the small sample size, statistical analyses with sufficient power could not be done between TNF-α and IL-10 polymorphisms and migraine subtypes and gender (migraine with aura vs migraine without aura; female vs male) in our study.

Table 3 Relationship between Il-10 polymorphisms, haplotypes, genotypes, and migraine

IL-10 Locus	Migraine Patients N = 203	Control Subjects N = 202	*P*
–1082 G/A			0.82
G/G	29 [14%]	25 [12%]	
G/A	81 [40%]	80 [40%]	
A/A	93 [46%]]	97 [48%]	
Allele frequency			0.268
G	139 [34%]	130 [32%]	
A	267 [66%]	274 [68%]	
–819C/T(592C/A)			0.0719
T/T (A/A)	20 [10%]	23 [11%]	
C/T (C/A)	124 [61%]	101 [50%]	
C/C (C/C)	59 [29%]	78 [39%]	
Allele frequency			0.121
T(A)	164 [40%]	147 [36%]	
C(C)	242 [60%]	257 [64%]	
Genotypes			0.053
GCC/GCC	29 [14%]	25 [12%]	
GCC/ACC	22 [11%]	32 [16%]	
GCC/ATA	59 [29%]	48 [24%]	
ACC/ACC	8 [4%]	21 [10%]	
ACC/ATA	65 [32%]	52 [26%]	
ATA/ATA	20 [10%]	24 [12%]	
Haplotypes			0.18
GCC	139 [34%]	130 [32%]	
ACC	103 [25%]	126 [31%]	
ATA	164 [41%]	148 [37%]	

IL-10 = interleukin-10.

Discussion

In our study, TNF-α –308G/A genotype and A allele frequency was over represented in migraine patients, showing significant difference with controls (Pc: <0.0001, OR: 2.16, 95% CI: 1.44–3.28). The association of the

Table 2 Relationship between TNF polymorphisms and migraine

TNF Locus, Polymorphism	Migraine Patients N = 203	Control Subjects N = 202	*P* (Pc)	OR (CI 95%)
TNF-α –308G/A				
A/A	0	0	<0.0001 (<0.0001)	2.53 (1.62–3.97)
G/A	78 [38%]	40 [20%]		
G/G	125 [62%]	162 [80%]		
Allele				
A	78 [19%]	40 [10%]	<0.0001 (<0.0001)	2.16 (1.44–3.28)
G	328 [81%]	364 [90%]		

Pc = Bonferroni correction; TNF = tumor necrosis factor; OR = odds ratio; CI = confidence interval.

Table 4 Characteristics of the individual studies included in the systematic review and overall analysis

Study (reference)	Population	Numbers Migraine Patients (M) Control Subjects (C)	Allele Frequency N (%) G	A	*P*
Mazaheri et al. [16] (2006)	Iran	221 (M)	265 (60)	177 (40)	<0.0001
		183 (C)	274 (75)	92 (25)	
Ghosh et al. [28] (2010)	India	216 (M)	391 (91)	41 (9)	0.092
		216 (C)	406 (94)	26 (6)	
Trabace et al. [29] (2002)	Italy	79 (M)	146 (93)	12 (7)	≥0.05
		101 (C)	189 (94)	13 (6)	
Rainereo et al. [18] (2004)	Italy	299 (M)	554 (93)	44 (7)	0.033
		306 (C)	502 (82)	110 (18)	
Lee et al. [30] (2007)	South Korea	439 (M)	816 (93)	62 (7)	0.40
		382 (C)	718 (94)	46 (6)	
Asuni et al. [19] (2009)	Italy (Sardinia)	299 (M)	570 (95)	28 (5)	0.67
		278 (C)	526 (95)	30 (5)	
Yilmaz et al. [27] (2010)	Turkey	67 (M)	97 (72)	37 (27)	<0.0001
		96 (C)	174 (91)	18 (9)	
Schürks et al. [17] (2009)	USA	4705 (M)	No data	No data	0.55
		21008 (C)	No data (83)	No data (17)	
Present study	Turkey	203 (M)	328 (81)	78 (19)	<0.0001
		202 (C)	364 (90)	40 (10)	
Total studies (one excluded)	Overall	1823 (M)	3167 (86.86)	479 (13.13)	*P* = 0.001
		1764 (C)	3153 (89.37)	375 (10.63)	OR = 1.27
					95% CI:1.10–1.47

CI = confidence interval; OR = odds ratio.

TNF-α A allele with high level of TNF gene expression and elevated serum levels have been reported previously [26]. Furthermore, TNF-α has been shown to induce headache, and TNF-α antibody can reduce pain in clinical trials [15]. Based on these reports, our results implicate that migraine might be associated with inflammation and TNF-α A allele may be one of the many genetic factors for migraine susceptibility. A number of studies have been published regarding the association of TNF-α –308G/A polymorphism in migraine. Similar to our findings, these studies also found significant association between migraine and this polymorphism. Mazaheri et al. and Yılmaz et al. found that TNF-α –308G/A genotype frequency in migraine patients without aura was significantly higher than controls [16,27]. Ghosh et al. reported a significant association between migraine patients with aura and controls for 308G/A genotype [28]. Because of the small size of the migraine patients with aura, we could not be able to compare the subtypes of the disease and the control group for TNF-α –308G/A polymorphism; however, our result agrees with the above-mentioned results. In contrast to these studies, Rainero reported that TNF-α G allele was associated with migraine [18]. Further studies also failed to show an association between migraine and TNF-α –308G/A polymorphism [29,30]. The sample sizes and methodology of these studies may account for contradictory results or may emphasize ethnic difference. Therefore, in order to overcome the limitations of individual studies and to reduce the random likelihood that random errors may be responsible for false-positive or false-negative association, overall analysis has been performed. Seven relevant studies that investigated the relationship between TNF-α –308G/A polymorphism and migraine were identified (Table 4); one study was excluded due to the lack of allele frequency data. Overall analysis of the TNF-α –308G/A polymorphism in 1,323 patients and 1,764 control subjects revealed significant association between migraine and TNF-α –308 A allele ($P = 0.001$, OR = 1.27, 95%CI = 1.10–1.47). Overall analysis has concluded that TNF-α –308 A allele confers a 1.27-fold risk for developing migraine. Not forgetting the complex etiology of migraine, it is suggested that multiple susceptibility genes with small or modest effect contribute to the development of migraine. As a number of studies have observed significant associations between TNF-α –308G/A polymorphism and various diseases, we have thought that TNF-α –308G/A polymorphism is not disease specific and seems to be common in immune-mediated diseases.

In addition, the overproduction of TNF-α in migraine may arise as a result of other TNF polymorphisms such as –1031T/C or posttranscriptional mechanisms. The TNF-α –1031 C allele has been associated with a high level of TNF-α gene expression. The individual with a TNF-α 1031 CC genotype is capable of producing more TNF-α [31] and also may explain the high TNF-α serum level in migraine patients. Furthermore, TNF-α production may also result from complex *cis* and *trans* interactions among other cytokines. As the cytokines in the circulation interact

with each other, it results in the association between TNF-α –308G/A polymorphism and migraine. Alternatively, this association may be due to linkage disequilibrium between TNF-α –308G/A and other susceptibility genes in this region such as HLA, which might have an indirect effect on differential TNF-α production. It is still unknown whether the lifestyle characteristics of individuals influence the migraine outcome; the unconsidered factors mixed together may cover the role of TNF-α –308G/A polymorphism on migraine.

Several reports implicated that IL-10 may be involved in the pathogenesis of migraine attacks [3,4,32,33]. To date, no association studies have been performed evaluating polymorphisms in the IL-10 gene for association with migraine. To our knowledge, this is the first report on whether polymorphisms and haplotypes of IL-10 gene are associated with the risk of migraine. IL-10 is a major endogenous downregulator of TNF-α production, and it has several effects on inflammation [34,35]. IL-10 –1082G/A, –819C/T, –592C/A polymorphisms influence the amount of IL-10 secreted in cell cultures [12]. The –1082 G allele and haplotypes containing this allele have been associated with high IL-10 production, whereas the A allele and the ATA haplotype have been associated with low IL-10 production [35]. We found no significant association between IL-10 1082 G/A genotypes, haplotypes containing this alleles and migraine. Our results suggest that IL-10 –1082G/A, –819C/T, –592C/A polymorphisms and haplotypes may not be related to migraine susceptibility.

Conclusions

The present study reflects that TNF-α –308G/A polymorphism may be one of the many genetic factors for migraine susceptibility in Turkish population. IL-10 polymorphism does not have an impact on the risk of developing migraine. As several genetic factors are involved in migraine, present study is the first that report the association of IL-10 genotypes/haplotypes with migraine, and our results need to be verified with further studies having larger sample size.

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