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Genomic analysis of the interleukin-1β-511 and interleukin-6-174 gene polymorphisms in Turkish patients with epilepsy

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ABSTRACT. In this study, we examined the frequency of polymorphisms in the interleukin (IL) genes *IL-1β-511* and *IL-6-174* in patients with epilepsy as well as a control group in Kars, Turkey. A total of 100 patients diagnosed with epilepsy and 100 nonepileptic subjects as a control group were examined. Peripheral blood samples were acquired from patients and control subjects for DNA extraction. The target region was amplified using polymerase chain reaction and digested using the restriction enzymes *Sfa*NI and *Ava*I. Restriction products were extracted from agarose gel electrophoresis and polymorphisms were analyzed using gel images. For *IL-1β*, the most common genotype among the epilepsy group was the CT genotype with a 62% frequency; the T allele was the most common allele with a frequency of 34%. Among the control group, however, the CT genotype showed a frequency of 25% and the T allele had a 22% frequency. For *IL-6-174*, among the epilepsy group, the GG genotype prevalence was approximately 42%

and G allele prevalence was 46%. The GG genotype was approximately 50% and the G allele was 53% in the control group. Thus, changes in the allele frequency of the T allele of *IL-1β-174* may be associated with epilepsy. However, there was no significant difference for the G allele frequency of *IL-6-511*. A larger sample size should be examined to verify these relationships, which could help to improve the clinical diagnosis and treatment of epilepsy.

Key words: Epilepsy; Interleukin-1β gene; Interleukin-6 gene; Restriction fragment length polymorphism; Turkish population

INTRODUCTION

Epilepsy involves black-out events with somatic and psychic functions of neurons, and temporary and repetitive disorders in brain functions that occur together with paroxysmal motor, sensorial, and autonomic phenomena that are not provoked (Tekeli et al., 2012). Epileptic seizure refers to temporary symptoms caused by abnormal or over-synchronous neuronal activities. Although the same mechanism is not shared in all epileptic seizures, increased neural excitability and synchronism are common properties (Fisher et al., 2005). Although the prevalence of epilepsy is approximately 1% in the global population, active epilepsy prevalence has increased in many societies worldwide over the last 5 years (Fisher et al., 2005; Tekeli et al., 2012). Between 5 and 8 of 1000 people have epilepsy according to studies in the USA, China, Nigeria, Europe, and India (Bebek and Baykan, 2006; Wrona, 2006). Active epilepsy prevalence was found to have an average incidence of 5 in 1000 individuals (range: 2-10 in 1000), according to 8 studies conducted in Turkey between 1995 and 2010 (Banks, 2004; Bebek and Baykan, 2006). Etiological genetic factors play a role in idiopathic epilepsy, which is considered to account for 40% of epilepsy cases. Gene mutations may cause abnormal ionic channel disorders, resulting in abnormal network connections in patients with epilepsy (Banks, 2004; Ravizza and Vezzani, 2006). Various genetic studies have examined epileptogenesis and provided insight into the epilepsy pathophysiology. Correct diagnosis can be implemented by searching for candidate genes involved in epilepsy pathophysiology. Complex relationships between epilepsy and the immune system were recently identified (Browne and Holmes, 2004; Kalueff et al., 2004). Immune system reactions play important roles in epileptic epileptogenesis (Banks, 2004). Abnormalities were also observed in cytokine expression and in the immune cells of patients with epilepsy. Cytokines that act as immune modulators and inflammation regulators have been implicated in various psychiatric and neurological events (Li et al., 2011). Polymorphisms in cytokine genes may result in febrile seizures in patients with epilepsy (Virta et al., 2002; Kanemoto et al., 2003; Dubé et al., 2005). In this study, we examined polymorphisms in the cytokine interleukin (IL) genes *IL-1β* and *IL-6* in a Turkish population to determine the relationship between cytokine genes and epilepsy.

MATERIAL AND METHODS

Study population

A total of 100 patients with epilepsy who were patients at the Kars State Hospital Neu-

rology Outpatient Clinic and 100 healthy volunteers were included in the study. All patients and controls visited the hospital between September 2012 and June 2013. All patients signed informed consent forms before beginning the study and the study was approved by the Ethics Committee of the hospital. Physical and neurological examinations and electroencephalography (Nihon Kohden; Tokyo, Japan) were performed for patients and controls. If necessary, neuroimaging was performed. The diagnosis of epilepsy followed the clinical guidelines proposed by the International League Against Epilepsy (ILAE). As defined by the ILAE (Engel, 2006), epilepsy is a condition characterized by 2 or more unprovoked seizures without an acute underlying cause. Age, gender, family history, and birth information of the patients and control group were recorded. In addition, head trauma, febrile seizures, and consanguinity, which may be a risk factor for epilepsy, were recorded. The mean (± SD) age of the patient group was 33 ± 13.65 years and that of the control group was 29 ± 7.56 years (Table 1). To isolate DNA, 8 mL blood was collected into tubes containing ethylenediaminetetraacetic acid from each patient and control subject.

Table 1. Demographic characteristics of patients and controls.

Epilepsy patient groups					Control groups				
Age (years)	Female (N)	%	Male (N)	%	Age	Female (N)	%	Male (N)	%
18-70	47	0.47	53	0.53	19-61	32	0.32	68	0.68
33± 13.65	47	0.47	53	0.53	29± 7.56	32	0.32	68	0.68

Genotyping

Each 8-mL blood sample was subjected to DNA using the salting-out method (Rapley and Walker, 2008). Isolated DNA samples were stored at -80°C after measuring the concentration using a Nanodrop spectrophotometer (ND1000; Thermo Scientific; Waltham, MA, USA) The primers used to identify *IL-1β-511* polymorphisms were F5'-TGGCATTGATCTGGTTCATC-3' and R5'-GTTTAGGAATCTTCCCACTT-3', and the *IL-6-174*-specifically designed primers were F5'-TGACTTCAGCTTTACTCTTTGT-3' and R5'-CTGATTGGAAACCTTATTAAG-3' (Kira et al., 2010; Tiwari et al., 2012). Two polymerase chain reaction (PCR) mixture of 25 µL were prepared for *IL-1β-511* and *IL-6-174* to amplify the genes from the DNA samples. Reaction mixtures contained 10X *Taq* polymerase buffer, 0.3 mM dNTP mixture, 10 pmol of each primer, 1-2 U *Taq* polymerase (Bioron; Ludwigshafen, Germany), and 50 ng genomic DNA. PCR conditions were as follows: initial denaturation for 3 min at 95°C, followed by 35 cycles at 94°C for 1 min, 53°C for 1 min, 74°C for 1 min, and final extension at 72°C for 5 min (Kira et al., 2010; Tiwari et al., 2012). PCR products were detected by agarose gel electrophoresis (90 V, 300 A for 1 h) on a 2.5% agarose gel containing ethidium bromide. The fluorescence intensity of each band was evaluated using an ultraviolet transilluminator (Kira et al., 2010) (Gel Logic Pro 2200; Ontario, Canada). PCR amplification bands were 300 and 240 bp in length for *IL-6-174* PCR. *IL-1β-511* amplification products were digested with 2 U *Ava*I and *IL-6-174* was digested with 2 U *Sfa*NI (New England Biolabs; Ipswich, MA, USA). *IL-1β-511* polymorphism showed 2 DNA fragments of 190 and 110 bp after restriction enzyme digestion. PCR products of *IL-1β-511* were fragmented by *Ava*I enzyme digestion. A band pattern of 300 bp was considered to be the TT genotype

(homozygote), while those showing band pattern of 300 + 190 + 110 bp were the CT genotype (heterozygote), and those with 190 + 110 bp bands were considered to be the CC genotype (wild-type). PCR products of *Il-6-174* were fragmented with *Sfa*NI enzyme digestion. A band pattern indicating a 70-bp band was considered to be GG (wild-type) genotype, while those showing a 30-bp band were considered to be GC genotype (heterozygote genotype), and those showing a 5-bp band were determined to be CC genotype (homozygote genotype) (Kira et al., 2010; Mansoori et al., 2010; Tiwari et al., 2012).

Statistical analysis

The Graphpad Prism 6.02 software was used for statistical analysis (San Diego, CA, USA). To evaluate the data, in addition to the descriptive statistical methods (mean, standard deviation), the Student *t*-test was used to compare patient and control groups, while the Fisher exact test was used to compare qualitative data. $P < 0.05$ was considered to be statistically significant.

RESULTS

In this study, the characteristics of 100 patients who visited Kars State Hospital Neurology Polyclinics between September 2012 and June 2013 and had been diagnosed with epilepsy and 100 healthy individuals without epilepsy or any neurological disorders were analyzed. The *IL-1β-511* polymorphism distribution, determined using restriction enzyme digestion, revealed that the most common genotype was CT (62%) with the T allele being more frequent (56%) in patients with epilepsy. In the control group, the most common genotype was CC (55%) with a T allele frequency of 22.4%. For *IL-6-174*, the GG genotype and G allele accounted for 42 and 46%, respectively, of the patient group. In the control group, the GG genotype and G allele frequency was 50 and 53%, respectively.

Restriction fragment length polymorphism (RFLP) analysis results of the *IL-1β-511* gene with *Ava*I

The results of the genotype distribution of *IL-1β-511*, based on *Ava*I enzyme digestion, were evaluated with respect to allele frequency. Statistically significant differences in allele and genotype frequency were observed between the patient group (N = 100) and control group (N = 100). The T allele, which is thought to play a role in epileptogenesis, was observed at a frequency of 56% in the patient group and 22% in the control group (Table 2). The CT genotype was frequently observed in patients with a family history of epilepsy and the CC genotype was frequently observed in the control group. While the C allele was frequently observed in healthy individuals, the T allele was most frequently observed in individuals with a family history of epilepsy. The CT genotype was most frequently observed in both the patient group with febrile seizures and in healthy individuals; however, the patient group showed a higher TT genotype and lower CC genotype compared to controls. While the C allele was most frequently observed in the control group, the T allele was most frequently observed in patients with febrile seizure. The CC genotype was most frequently observed in the control group and in the patient group with head trauma. The C allele was more frequently observed than the T allele in the patient group with head trauma and in the control group. There was a statistically significant difference in allele frequency between the control group and patients who had a

difficult birth. While the C allele frequency was high in the control group, the T allele frequency was higher in the group who had experienced a difficult birth. Different aspects of the *IL-1β-511* region and genotypic and allellic analysis results are shown in Table 3.

Table 2. *IL-1β-511* genotype and allele frequencies in patients with epilepsy and controls.

Genotypes	Epilepsy patient genotype and allele frequencies						Control genotype and allele frequencies						
	CC	%	CT	%	TT	%	CC	%	CT	%	TT	%	P
Men	9	16.98	31	58.49	13	24.53	47	69.12	15	22.06	6	8.82	0.453
Women	4	8.51	31	65.96	12	25.53	18	56.26	10	31.26	4	12.5	0.447
Total	13	13.00	62	62.00	25	25.00	65	65.00	25	25.00	10	10.00	0.0001
Allele	0.66				0.34		0.78				0.22		

In epilepsy group, N = 53 men and N = 47 women. In control group, N = 68 men and N = 32 women.

Table 3. Clinical characteristic of *IL-1β-511* genotype and allele frequencies in patients with epilepsy and controls.

Genotypes	Epilepsy patient genotype and allele frequencies						Control genotype and allele frequencies						
	CC	%	CT	%	TT	%	CC	%	CT	%	TT	%	P
EPFH	2	8.00	17	68.00	6	24.00	65	65.00	25	25.00	10	10.00	0.0001
Allele	0.42				0.58		0.78				0.22		
EPFS	3	10.00	20	66.67	7	23.33	65	65.00	25	25.00	10	10.00	0.0001
Allele	0.43				0.57		0.78				0.22		
EPHT	8	50.00	5	31.25	3	18.75	65	65.00	25	25.00	10	10.00	0.0001
Allele	0.66				0.34		0.78				0.22		
EPDB	1	8.33	6	50.00	5	41.67	65	65.00	25	25.00	10	10.00	0.0001
Allele	0.33				0.67		0.78				0.22		

EPFH = epilepsy patients with family history; EPFS = epilepsy patients with febrile seizure; EPHT = epilepsy patients with head trauma; EPDB = epilepsy patients with difficult birth.

RFLP analysis results of the *IL-6-174* gene with *Sfa*NI

Genotype distributions and allele frequencies were examined after RFLP analysis in the *IL-6-174* polymorphic region using *Sfa*NI enzyme. No statistically significant differences were obtained between the epilepsy group (N = 100) and the control group (N = 100). No statistically significant difference was observed in the frequency of the 3 genotypes (GG/GC/CC) when epilepsy patients and the healthy control group were compared. The G allele, which is thought to play a role in epileptogenesis, was observed in 46% of patients and in 53% of controls. There was no statistically significant difference between genotype frequencies in male and female subjects in the patient group (Table 4). The GG genotype was most frequently observed in control subjects and in patients with a family history of epilepsy. The G allele was most frequently observed in healthy subjects and in individuals with a family history of epilepsy. While the GC genotype frequency was decreased in patients, the CC genotype was increased. Allele frequency of G allele was determined to be higher in subjects with febrile seizure than the control group. The GC genotype was not observed in the group with head trauma. GG was the most frequently observed genotype for both the head trauma and control groups. The G allele frequency was most common in patients with head trauma and in the control group. The GC genotype was not observed in patients with a difficult birth. While the GG and CC genotype distribution were similar in both groups, the GG genotype distribution was higher in the patient group with difficult birth. The G allele frequency in the patient group with difficult birth was higher than that in the control group (Table 5).

Table 4. *IL-6-174* genotype and allele frequencies in patients with epilepsy and controls.

Genotypes	Epilepsy patient genotype and allele frequencies						Control genotype and allele frequencies						
	GG	%	GC	%	CC	%	GG	%	GC	%	CC	%	P
Men	19	35.85	5	9.43	29	54.72	31	45.59	3	4.41	34	50.00	0.405
Women	23	48.90	3	6.40	21	44.70	19	59.37	2	6.25	11	34.38	0.340
Total	42	42.00	8	8.00	50	50.00	50	50.00	5	5.00	45	45.00	0.438
Allele	0.46				0.54		0.53				0.47		

In epilepsy group, N = 53 men and N = 47 women. In control group, N = 68 men and 32 women.

Table 5. Clinical characteristics of *IL-6-174* genotype and allele frequencies in epilepsy patients and controls.

Genotypes	Epilepsy patient genotype and allele frequencies						Control genotype and allele frequencies						
	GG	%	GC	%	CC	%	GG	%	GC	%	CC	%	P
EPFH	28	56.00	2	4.00	20	40.00	50	50.00	5	5.00	45	45.00	0.172
Allele	0.58				0.42		0.53				0.47		
EPFS	13	54.17	1	4.17	10	41.66	50	50.00	5	5.00	45	45.00	0.002
Allele	0.56				0.44		0.53				0.47		
EPHT	16	59.26	0	0	11	40.74	50	50.00	5	5.00	45	45.00	0.0001
Allele	0.59				0.41		0.53				0.47		
EPDB	11	78.57	0	0	3	21.43	50	50.00	5	5.00	45	45	0.0001
Allele	0.78				0.22		0.53				0.47		

EPFH = epilepsy patients with family history; EPFS = epilepsy patients with febrile seizure; EPHT = epilepsy patients with head trauma; EPDB = epilepsy patients with difficult birth.

DISCUSSION

Genomic studies have been increasingly used to examine epilepsy patients, revealing a complex relationship between neural system and the immune system (Cavalleri et al., 2005; Ravizza et al., 2008; Bauer et al., 2009; Mansoori et al., 2010). Polymorphic changes in cytokine genes could contribute to the development of epileptic seizures, which involves interactions between various factors. These changes may vary in different geographic regions and populations. As this condition shows relatively high heritability, genetic changes are likely involved (Jin et al., 2003; Ozkara et al., 2006; Alapirtti et al., 2009). Understanding the etiology of epilepsy would improve the ability to clinically treat epileptic events from a genomic perspective and improve treatment methods. In this study, the genetic aspects of epilepsy, in particular, its relationship with cytokine genetic polymorphisms, were examined by genomic methods. Stratified by gender, 47% of subjects in the patient group were women and 53% were men; in the control group, 32% were women and 68% were men. Previous studies report conflicting results regarding whether epilepsy is more common in men or women (Meisel et al., 2005; Brietzke et al., 2009). In this study, there was no statistically significant difference in the ratio of women to men in the patient group, but gender may nonetheless play a role in epileptogenesis. The cytokine genes (*IL-1β* and *IL-6*) examined in this study are pro-inflammatory cytokines involved in epilepsy and febrile seizures (Peltola et al., 2002; Haspolat et al., 2005). In this study, we found that the T allele affects epileptogenesis, while the G allele does not. In our study, 24% of the patient group had a family history of epilepsy, 23.3% had febrile seizure, 18.7% had head trauma, and 12% had a difficult birth. The T allele was observed in 58% of patients with a family history, 57% of patients with febrile seizure, 41.6% of patients with head trauma, and 67% of patients who had a difficult birth (Serdaroglu et al., 2009; Balcerzyk

et al., 2012). In our study, when *IL-1β-511* polymorphisms were examined in febrile seizure in epilepsy patients, the T allele frequency was 57% higher in patients than in controls. Kira et al. (2010) conducted a study in Japan and showed similar results. In addition, Serdaroglu et al. (2009) conducted a study in children and found that the T allele was higher in the patient group than in the control group (Kira et al., 2010; Yu et al., 2012).

Our study involved subjects older than 18 years, and thus our results may not be applicable to children. Kanemoto et al. (2003) conducted a study in Japan and found that the T allele frequency was higher in subjects without febrile seizure, supporting our results (Kanemoto et al., 2003). Nur et al. (2012) reported that the GG genotype was most frequently observed in Turkish patients with a family history of epilepsy (Dubé et al., 2005; Nur et al., 2012). The results of Kanemoto et al. (2003) agree with our results in many aspects. In contrast, no association between epilepsy and *IL-1β* polymorphisms was noted by Tiwari et al. (2012) in India, Chou et al. (2010) in Taiwan, Haspolat et al. (2005) in Turkey, Matsuo et al. (2006) in Japan, and Capurso et al. (2010) in the USA. In addition, Tilgen et al. (2002) and Tsai et al. (2002) found that the C allele was most frequently observed in febrile seizure patients in German and Taiwanese populations, respectively. These results are not consistent with ours; this can be explained based on genetic variations among populations. In studies examining different populations, the T allele frequency was found to be 46% in Japanese, 34% in European, and 60% in African populations (Shibata et al., 2002; Pola et al., 2002; Kauffman et al., 2008). The T allele frequency was found to be 42% in Turkish children with epilepsy (Haspolat et al., 2005). In this study, the T allele frequency was 28% in the control group. Chuo et al. (2010) in Taiwan, Mansoori et al. (2010) in India, and Capurso et al. (2010) in Italy found that the G allele does not affect epilepsy in febrile seizure patients. These findings agreed with the results of our study. Other studies found that the G allele affected the progress of febrile seizures (Shibata et al., 2002; Pola et al., 2012). These results do not agree with the results of our study. We found that the GG genotype was observed at a higher frequency in patients with a family history of seizures, indicating that it could be a risk factor for epilepsy. Reasons for these differences may include geography, ethnicity, and genomic heterogeneity (Tilgen et al., 2002; Chou et al., 2010; Wu et al., 2012). In this study, the GG genotype was the most frequently observed genotype in patients with head trauma and difficult birth, and the G allele frequency was the most common allele. However, the CC genotype was also the most frequently observed in the healthy group. Thus, the GG genotype may not be a risk factor in situations of head trauma or difficult birth. When the allele frequency of the *IL-1β-511* polymorphic region was compared between patient and control groups, the T allele frequency was higher in patients with a family history, particularly in first-degree relatives. These results were not obtained for the group with head trauma, where the C allele was observed most frequently. This may be because head trauma causes epilepsy because of environmental reasons rather than genetic reasons, and thus the polymorphism present has no effect. T allele frequency was determined to be higher in epilepsy patients with a difficult birth compared to healthy subjects.

We examined polymorphic regions in *IL-6-174* and found that the most common genotypes were GG and CC in all subjects, although the G allele frequency was lower in the patient group than in the healthy group. Thus, *IL-6-174* polymorphisms are not involved in epileptogenesis. When febrile seizure patients were compared with the healthy control group, no difference was detected in G allele frequency. Thus, epileptogenesis is not an indicator of genetic information in patients with febrile seizure.

In conclusion, we examined 2 separate polymorphic regions and compared gene polymorphisms in healthy and patient groups. For *IL-1β-511*, we found that the T allele affects epileptogenesis and seizures, and may be a risk factor affecting febrile seizures. For *IL-6-174* gene polymorphisms, in individuals with a family history, the G allele (GG genotype) was frequently observed in both groups. However, the high ratio of GG genotypes in the control group may be related to epileptogenesis, although further studies are required to confirm this association.

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