Association of the Single Nucleotide Polymorphisms of the Genes Encoding IL-2 and IFN-γ With Febrile Seizure

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Abstract- Inflammatory elements and genetics have major roles in febrile seizures (FS) pathogenesis. Seventy patients were enrolled and compared with 139 controls. The allele and genotype frequency of the IL-2 gene at -330 and +166 positions and the IFN-γ at +874 position were determined. A significant positive association with GG genotype at position -330 in the patient group was found (*P*=0.003). Further, a positive association was detected in simple and complex FS groups at the same position (*P*=0.03, *P*=0.004). IL-2 GT haplotype was significantly more common in the patients compared to controls (*P*=0.0008). Higher frequency of GT haplotype was detected in simple FS patients in comparison to controls (*P*=0.0003). Contrary, IL-2 TG haplotype frequency was lower in complex FS group (*P*=0.005). Overrepresentation of certain alleles, genotypes and haplotypes in IL-2 gene in FS patients could predispose individuals to this disease.

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Keywords: Febrile seizure; Gene polymorphisms; Interleukin-2; IFN-γ; Etiology

Introduction

Under physiological conditions, very low levels of cytokines are produced constitutively expressed by and act on neurons of normal brain tissue, but their over expression in the brain is an important factor in the pathogenesis of neurotoxic and neurodegenerative disorders (1,2). There are several pro-inflammatory signals that are induced during epilepsy in rodent brain (3). We still do not know that these proinflammatory signals is a predisposing factor for seizure or are induced following epileptic activity.

Febrile seizures (FS) are the most common convulsive event in children with a prevalence of 2-5% that triggered with high fever. The etiology of FS is still unclear. The most important risk factor of FS is a positive family history suggesting that shared genes or environmental factors by family members play a causal role, as 17-30% of patients have a positive family history (4,5). This underlying genetic predisposition, along with the association of fever with the seizures, suggests the possibility of involvement of some gene or genes for the regulation of pro-inflammatory and anti-inflammatory cytokines (Interleukine (IL)-1, IL-2, IL-6 and tumor necrosis factor (TNF)) (2,6-8).

Several studies showed the interaction between the immune inflammatory system, cytokines and genetic factors which are involved in FS (8-12). In a study by Sinha *et al*., they did a serial analysis during the seizure-free period which revealed a decrease in cytokine levels: TNF-α (25% to 12.5%), IFN-γ (12.5% to 0%), IL-1 (25% to 0) and IL-2 (6.2% to 6.2%), IL-4 (18.8% to 0%)

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and IL-6 (18.8% to 6.2%) (13). In contrast, Choi *et al*., studied 41FS patients and found no significant differences in IFN-γ levels, among afebrile and febrile controls (14).

We have recently shown that there is a significant association between the presence of the +565A of IL-6, -590 C of IL-4, codon 10/CT (rs1982037) TGF-β, –238 G TNF-α, the IL-1Ra/C allele at position Mspa-I 11100, the IL-1Ra/T and FS (8,12,15-17). In the present study, two cytokine SNPs within the IL-2 gene at -330 and +166 positions, and one SNP within the IFN-γ at +874 position were investigated in Iranian children with FS.

Materials and Methods

Participants

Seventy children with confirmed diagnosis of FS who were referred to the Department of Pediatric Neurology and Pediatric Emergency Service of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran, were enrolled in this study. The results of the study on the patient group were compared to 139 control individuals from the same region (18).

Family histories were obtained through pediatricians' interviews with parents. Informed consent was obtained from the parents of each individual before blood sampling, and the study was approved by the Ethics Committee of Tehran University of Medical Sciences.

Diagnosis of FSs followed the criteria established in the 1989 International Classification of Epileptic Syndromes. The age at first FS was between 6 months and 6 years of age. The EEG was normal for all patients or showed mild nonspecific abnormalities. Patients with afebrile seizures, FS beginning at the age of 6 years or later, epileptiform EEG traits or evidence of intracranial infection were not included in this study. Cases and controls were matched for age and gender.

Genotyping

DNA was extracted from peripheral blood. Cytokine genotyping was performed, using polymerase chain reaction with sequence-specific primers (PCR-SSP assay kit, Heidelberg University). The method was previously explained in details (18). Briefly, the gene was amplified using a Tedane Flexigene thermal cycler (Roche). Thereafter, the availability of the PCR products was assessed using 2% agarose gel electrophoresis. The gel was placed on a UV transilluminator, and a digital image was taken for analysis and documentation. The frequencies of alleles, genotypes, and haplotypes of the IL-2 gene at -330 and +166 positions, and one SNP within the IFN-γ at +874 position were recorded.

Statistical analysis

We estimated allele frequencies by direct gene counting and compared them using the chi-square test. The odds ratio (OR) and Wald's 95% confidence interval (CI) were calculated for each allele, genotype, and haplotype. $P<0.05$ considered being statistically significant.

Results

IL-2 and IFN-γ allele polymorphisms

Table 1 presents the allele frequency (number and percentage), *P*, and OR with its 95% CI in FS patients and controls. We found no significant differences in frequency of G and T alleles at -330 and +166 positions, between patients and controls. There were no differences in genotype frequency between simple and complex FS patients.

Moreover, no diversity was found between patient group and controls for IFN-γ/A and T alleles at position +874. Table 1

IL-2 and IFN-γ genotype polymorphisms

The significant differences in genotype frequency between the groups are shown in Table 2. A positive association with GG genotype at position -330 in the patient group was observed (OR, 4.17; 95%CI, 1.53-11.61; $P=0.003$). Further, a positive association was detected in patients with simple and complex FS at the same position (OR, 3.51; 95% CI, 0.98-12.35; $P=0.03$ and OR, 4.85; 95% CI, 1.49-15.87; $P=0.004$), thus revealing that patients were more susceptible to FS. There were no differences in genotype frequency between simple and complex FS patients.

No significant association was found between IFN-γ genotypes and FS. Table 1.

IL-2 haplotype polymorphisms

The frequency of haplotypes of IL-2 (-330, +166) in patients with FS and controls are shown in Table 2. IL-2 GT haplotype was significantly more common in the patient group compared to controls (5.8% in patients vs. 0.3% in controls, $P=0.0008$). Additionally, higher frequency of GT haplotype was detected in simple FS patients in comparison to controls (9% in patients vs. 0.3% in controls, $P=0.0003$). In contrast, IL-2 TG haplotype frequency was lower in patients with complex

FS (21.4% in patients vs. 40.6% in controls, *P*=0.005).

Table 1. Alleles and genotype frequencies in patients with FS and controls

Gene polymorphism	Alleles/Genotypes	Patients (n=70) N%	Controls (n=139) N%	OR (95%CI)	*P*
		FS and control			
IL-2 (-330)	G	68(49.3)	110(39.6)	1.48(0.96-2.29)	0.07
	T	70(50.7)	168(60.4)	0.67(0.44-1.04)	0.07
	GG	14(20.3)	8(5.8)	4.17(1.53-1.61)	0.00
	GT	40(58)	94(67.6)	0.66(0.35-1.25)	0.22
	TT	15(21.7)	37(26.6)	0.77(0.36-1.6)	0.55
IL-2 (+166)	G	101(73.7)	219(78.8)	0.76(0.46-1.25)	0.3
	T	36(26.3)	59(21.2)	1.32(0.8-2.19)	0.3
	GG	36(52.9)	82(59)	0.78(0.42-1.46)	0.5
	GT	28(41.2)	55(39.6)	1.07(0.57-2.01)	0.94
	TT	4(5.9)	2(1.4)	4.28(0.65-4.64)	0.09
IFN-γ (+874)	A	82(51.25)	140(50.7)	1.02(0.68-1.54)	0.99
	T	78(48.75)	136(49.3)	0.98(0.65-1.47)	0.99
	AA	22(27.5)	43(31.2)	0.84(0.44-1.61)	0.67
	AT	38(47.5)	54(39.1)	1.41(0.78-2.55)	0.28
	TT	20(25)	41(29.7)	0.82(0.42-1.59)	0.63
		Simple FS and controls			
IL-2 (-330)	G	31(45.6)	110(39.6)	1.28(0.72-2.26)	0.44
	T	37(54.4)	168(60.4)	0.78(0.44-1.38)	0.44
	GG	6(17.6)	8(5.8)	3.51(0.98-2.35)	0.03
	GT	19(55.9)	94(67.6)	0.61(0.26-1.39)	0.27
	TT	9(26.5)	37(26.6)	0.99(0.39-2.49)	0.84
IL-2 (+166)	G	51(76.1)	219(78.8)	0.86(0.44-1.7)	0.75
	T	16(23.9)	59(21.2)	1.16(0.59-2.28)	0.75
	GG	18(54.5)	82(59)	0.66(0.3-1.45)	0.34
	GT	14(42.4)	55(39.6)	1.13(0.49-2.59)	0.91
	TT	1(3)	2(1.4)	2.14(0-31.52)	0.47
IFN-γ (+874)	A	46(54.8)	140(50.7)	1.18(0.7-1.98)	0.6
	T	38(45.2)	136(49.3)	0.85(0.51-1.43)	0.6
	AA	14(33.3)	43(31.2)	1.1(0.5-2.44)	0.94
	AT	18(42.9)	54(39.1)	1(0.48-2.09)	0.86
	TT	10(23.8)	41(29.7)	0.74(0.31-1.75)	0.58
		Complex FS and controls			
IL-2 (-330)	G	37(52.9)	110(39.6)	1.71(0.98-3)	0.06
	T	33(47.1)	168(60.4)	0.58(0.33-1.02)	0.06
	GG	8(22.9)	8(5.8)	4.85(1.49-5.87)	0.004
	GT	21(60)	94(67.6)	0.72(0.31-1.65)	0.51
	TT	6(17.1)	37(26.6)	0.57(0.19-1.59)	0.34
IL-2 (+166)	G	50(71.4)	219(78.8)	0.67(0.36-1.27)	0.25
	T	20(28.6)	59(21.2)	1.48(0.79-2.79)	0.25
	GG	18(51.4)	82(59)	0.74(0.33-1.65)	0.53
	GT	14(40)	55(39.6)	1.02(0.45-2.31)	0.88
	TT	3(8.6)	2(1.4)	6.42(0.83-7.78)	0.05
IFN-γ (+874)	A	36(47.4)	140(50.7)	0.87(0.51-1.5)	0.69
	T	40(52.6)	136(49.3)	1.14(0.67-1.96)	0.69
	AA	8(21.1)	43(31.2)	0.59(0.23-1.48)	0.31
	AT	20(52.6)	54(39.1)	1.73(0.79-3.79)	0.19
	TT	10(26.3)	41(29.7)	0.84(0.35-2.02)	0.83

Table 2. IL-2 haplotype polymorphism in patients with FS and controls

Gene polymorphism	Alleles/Genotypes	Patients (n=70) N%	Controls (n=139) N%	OR (95%CI)	*P*
		FS and controls			
IL-2 (-330, +166)	GG	59(43.1)	107(38.8)	1.19(0.77-1.85)	0.5
	TG	42(30.7)	112(40.6)	0.65(0.41-1.02)	0.06
	TT	28(20.4)	56(20.3)	1.01(0.59-1.73)	0.9
	GT	8(5.8)	1(0.3)	17.05(2.14-67.34)	0.0008
		Simple FS and controls			
IL-2 (-330, +166)	GG	24(35.8)	107(38.8)	0.88(0.49-1.59)	0.8
	TG	27(40.3)	112(40.6)	0.99(0.55-1.76)	0.9
	TT	10(14.9)	56(20.3)	0.69(0.31-1.5)	0.4
	GT	6(9)	1(0.3)	27.05(3.15-07.14)	0.0003
		Complex FS and controls			
IL-2 (-330, +166)	GG	35(50)	107(38.8)	1.58(0.9-2.77)	0.1
	TG	15(21.4)	112(40.6)	0.4(0.2-0.77)	0.005
	TT	18(25.7)	56(20.3)	1.36(0.7-2.61)	0.4
	GT	2(2.9)	1(0.3)	8.09(0.57-228.76)	0.1

Discussion

Seizures result from a dynamic interplay between epileptogenic, endogenous and precipitating factors. Under normal circumstances like high fever, the normal brain is capable of developing seizures (2). There is a time dependent, cell, and region specific change pattern during seizures for many cytokines (19-21).

Several studies highlighted the role of cytokine activation in FS associated with specific interleukin alleles, and the importance of various polymorphic alleles in affecting the ability of cytokine genes to encode cytokines. Virta *et al*., detected a significant allelic association between the IL-1B _511 allele 2 and FS which increased the risk of FS (22). Also, Kira *et al*., found that sporadic simple FS patients exhibited significantly higher frequencies of IL1B−31C/−511T alleles and homozygotes than controls, while no differences were observed in patients with all or familial simple FS versus controls. There were no significant differences in the frequencies of −31C/T and −511C/T in the IL-1B promoter gene between complex FS patients and controls (23). Tsai *et al*., reported a higher frequency of IL-1 receptor antagonist allele 1 and no differences in the IL-1B _511 polymorphism in patients with FS (24). In contrast, Tilgen *et al*., did not observe an association between the IL-1B polymorphism and increased risk of FS (25). In a study on cytokines levels in patients with epilepsy and new onset seizure, Sinha *et al*., found a decrease in cytokine levels during the

seizure-free period : TNF-alpha (25% to 12.5%), IFN-gamma (12.5% to 0%), IL-1 (25% to 0) and IL-2 (6.2% to 6.2%), IL-4 (18.8% to 0%) and IL-6 (18.8% to 6.2%) (13).

To the best of our knowledge, the present study demonstrated for the first time the association between certain allele, genotype, and haplotype frequencies in IL-2 and IFN-γ genes with FS in Iranian population. There were no significant differences in frequency of G and T alleles of IL-2 at -330 and +166 positions as well as A and T of IFN-γ alleles at position +874, between patients and controls. A significant positive association was observed a positive association with GG genotype at position -330 in the patient group. Additionally, a positive association was detected in patients with simple and complex FS at the same position, thus revealing that patients were more susceptible to FS. Further, IL-2 GT haplotype was significantly more common in the patient group compared to controls. Additionally, higher frequency of GT haplotype was detected in simple FS patients in comparison to controls. In contrast, IL-2 TG haplotype frequency was lower in patients with complex FS. No significant association between IFN-γ and FS was found which is inconsistent with results of Choi *et al*., (14) study.

Genetic factors play a pivotal role in the etiology of epilepsies (26). Genetic studies of multifactorial diseases, such as FS and epilepsy, are difficult to approach because of the uncertainty of a polygenic trait (27). Also, geographic and ethnic variations and molecular heterogeneity may account for different results. Only with the advent of more consisted methodologies, it will be possible to define a fine line between advantageous and adverse effects of cytokines in seizures. A limitation of current study was the relatively small sample size. Further studies on ethnicities from different parts of the world, will provide additional understanding of the possible role of cytokine gene polymorphisms in the susceptibility to FS and progression to epilepsy.

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