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Interleukin (IL)-1β, IL-1 Receptor Antagonist, IL-6, IL-8, IL-10, and Tumor Necrosis Factor α Gene Polymorphisms in Patients With Febrile Seizures

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Inflammation and genetics may play a role in the pathogenesis of febrile seizures (FSs). We aimed to test whether interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1 Ra), IL-6 promoter, IL-8, IL-10, or tumor necrosis factor (TNF) gene polymorphisms could be used as markers of susceptibility to FSs. An association study was performed among a cohort of 104 patients with FSs and 143 normal control subjects. There was no significant difference between patients and controls in the distribution of allele frequencies of the IL-1β promoter, IL-1β exon 5, IL-6 promoter, IL-8, IL-10, or TNF-α gene polymorphisms. In contrast, the IL-1 Ra-I homozygote was more frequent in patients with FSs than in healthy controls (93.2% vs. 83.92%, $\chi^2 = 4.51$, $P = 0.034$). In addition, individuals homozygous for the IL-1 Ra-I genotype were more than twice as likely to develop FSs than individuals heterozygous for the IL-1 Ra-I/II genotype (OR, 2.63, 95% CI: 1.08–6.39; $\chi^2 = 4.55$, $P = 0.033$). We conclude that the IL-1 Ra gene might be one of the useful markers for predicting susceptibility to FSs. J. Clin. Lab. Anal. 24:154–159, 2010.

Key words: interleukin; polymorphism; febrile seizure

INTRODUCTION

Febrile seizures (FSs), the most common form of childhood seizures, occur in 2–5% of children before the age of 5 (1). A FS is defined by the International League Against Epilepsy as a seizure in association with a febrile illness in the absence of a central nervous system infection or acute electrolyte imbalance in children older than 1 month of age without prior afebrile seizures (2). The pathogenesis of FSs remains unknown. There is compelling evidence that an inherited factor is critical in most cases. A family history of FSs is the most important risk factor, and the more relatives affected the greater the risk (3). In addition, the effects of endogenous pyrogens such as interleukin-1 (IL-1) on neuronal excitability are direct and related to the parallel rise of brain temperature during fever. Strategies of antipyretic management are applied in infants during fever crisis mainly to protect them from epileptic seizures. Therefore, the underlying genetic predisposition and the association of fever with the seizures suggest that some genes responsible for the regulation of the inflammatory process and fever play a role in the molecular pathogenesis of FSs.

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A burst of expression of proinflammatory cytokines (endogenous pyrogens) and their subsequent release are the common triggering events controlling fever and all the other phases of the innate acute-phase response. There is evidence that certain genes of the interleukin complex are associated with susceptibility to inflammation. Genetic variations have been shown to cause increased production of anti-inflammatory cytokines or decreased production of proinflammatory cytokines. Cytokine gene polymorphisms, which may be related to the amount of cytokine produced, have been found to be related to susceptibility to or disease activities of individual diseases. Cytokine polymorphisms that might be related to epilepsy include interleukin-1β (IL-1β) and IL-1 receptor antagonist (IL-1 RA). Peltola et al. (4) investigated cytokine gene polymorphisms in a group of patients with therapy-resistant localization-related epilepsy and found that those who were carriers of the promoter—511 T allele and non carriers of the IL-1 RA allele 2 had a propensity for increased inflammatory responses, which may lead to an increased susceptibility to develop therapy-resistant epilepsy. Kanemoto et al. reported a strong genotype association between a bi-allelic regulatory polymorphism in the gene promoter of IL-1β(511*2/2) and genetic susceptibility to temporal lobe epilepsy with hippocampal sclerosis (5). Furthermore, Virta et al. (6) found an increased frequency of IL-1β-511 T in children with FSs and concluded that this allele is associated with increased in vitro production of IL-1, which may intensify proinfalmmatory reactions during fever in such children.

In our previous study, we used single nucleotide polymorphisms (SNPs) as a tool to search for genetic makers of FSs (7–11). SNPs are the most abundant types of DNA sequence variation in the human genome (12,13). An SNP is a single base pair on the DNA that varies from person to person. SNP markers may provide a new way to identify complex gene-associated diseases such as idiopathic generalized epilepsies. We have found that polymorphisms of the γ-aminobutyric acid (GABA) type A receptor gene and the IL-1 RA gene might be associated with susceptibility to FSs (8,11). GABA terminals are abundant in areas containing thermosensitive neurons, and muscimol application causes reductions in both firing rate and thermo-sensitivity. IL-1β and other cytokines have been shown to influence signaling with many neurotransmitters, including norepinephrine, serotonin, GABA, acetylcholine, and adenosine (14,15). The balance between these cytokines influences the level of fever and could therefore play a role in the pathogenesis of FSs. In this study, we performed an association study of SNPs of IL-1β, IL-1 Ra, IL-6 promoter, IL-8, IL-10, and tumor necrosis factor (TNF) genes in patients with FSs and in healthy controls.

PATIENTS AND METHODS

A total of 100–104 Taiwanese children with FSs and 142–143 normal control subjects were included. This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. All parents provided informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity, and geographic location of origin. Subjects with FSs were recruited from the central and southern regions of Taiwan. Diagnosis of FSs followed the criteria established in the 1989 International Classification of Epileptic Syndromes. FSs were defined, as described previously, as seizures associated with a febrile illness. The age at first FS was between 4 months and 5 years of age (2.0 ± 1.3 years). The EEG was normal for all patients or showed mild nonspecific abnormalities. Patients with (a) afebrile seizures; (b) FSs beginning at the age of 6 years or later (FSs plus); (c) epileptiform EEG traits; or (d) evidence of intracranial infection were not included in the study.

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood by a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 μl containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM of each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA). For IL-Ra polymorphisms, PCR products were directly analyzed by electrophoresis. The IL-1β promoter, IL-1β exon 5, IL-6 promoter, IL-8, IL-10, and TNF-α polymorphisms were typed by the restriction fragment length polymorphism method (8,16–18). Details of each analysis are listed in Table 1. The PCR amplifications were performed using the GeneAmp PCR system 2400 programmable thermal cycler (Perkin-Elmer). For these five polymorphisms, PCR products were digested with appropriate restriction enzymes (Table 1) and the digested fragments were analyzed by electrophoresis on 3% agarose gels and then stained with ethidium bromide. Each allele was recognized according to its size.

Statistical Analysis

Allelic frequencies are expressed as a percentage of the total number of alleles. Differences in genotypic and allelic frequencies for the polymorphisms between the two groups were compared with the χ^2 test or Fisher's

TABLE 1. Association of Febrile Seizures (FSs) Patients With Interleukin (IL)-1β Promoter, IL-6 Promoter, IL-8, IL-10 and TNF-α Gene Genotypes

Characteristic	IL-1β promoter	IL-1β exon 5	IL-1Ra	IL-6 promoter	IL-8	IL-10	TNF-α
Type of polymorphism	Single base C/T	Single base	86-bp VNTR	Single base C/G	Single base A/G	Single base C/A	Single base A/G
Site of polymorphism	Position-511	Exon 5	Intron 2	Position-572	Position-2767	Position-627	Position-308
PCR primers							
Upstream	5′-TGGCATTGAT CTGGTTCATC-3′	5′-GTTGTCATCA GACTTTGACC-3′	5′-CTCAGCAAC ACTCCTAT-3′	5′-GCAAAGTCCTC ACTGGGAGG A-3′	5′-CTTTAGTG TTTTTATGTG CTCTCCA-3′	5′-CCTAGGTCACA GTGACGTGG-3′	5′-AGGCAATAGGTT TTGAGGGCCAT-3′
Downstream	5′-GTTTAGGAAT CTTCCCACTT-3′	5′-TTCAGTTCAT ATGGACCAGA-3′	5′-TCCTGGTCTG CAGGTAA-3′	5′-TCTGACTCCA TCGCAGCCC-3′	5′-GCAAATAT GCTTAGGCTT TAACC-3′	5′-GGTGAGCACTA CCTGACTAGC-3′	5′-ACACTCCCCATC CTCCCGGCT-3′
PCR conditions							
Denaturation	95°C, 30 sec	95°C, 30 sec	95°C, 30 sec	95°C, 30 s	95°C, 30 s	95°C, 30 s	94°C, 10 s
Annealing	55°C, 30 sec	55°C, 30 sec	58°C, 30 sec	60°C, 30 s	55°C, 30 s	55°C, 30 s	60°C, 10 s
Extension	72°C, 45 sec	72°C, 45 sec	72°C, 45 sec	72°C, 45 s	72°C, 45 s	72°C, 45 s	72°C, 10 s
No. of cycles	35	35	35	35	35	35	30
Digestion	Yes (AvaI)	Yes (TaqI)	No	Yes (*Bsr*BI)	*Apa*LI	Yes (RsaI)	Yes (NcoI)
Allele size (bp)	C: 190+114 T: 304	E1: 135+114 E2: 249	I: 410 II: 240 III: 500 IV: 325 V: 595	G: 210+95 C: 296	A:387+131 G:518	A: 236+176 C: 412	A: 97+20 G: 117

IL-1β, interleukin-1β; IL-1Ra, interleukin-1 receptor antagonist; C/T, cytosine to thymine substitution; VNTR, variable number of tandem repeats; C/A, cytosine to adenine substitution; PCR, polymerase chain reaction.

TABLE 2. Distribution of Interleukin Genotypes in Patients with Febrile Seizures (FSs) ($n = 104$) and Healthy Control Subjects ($n = 143$)

Gene polymorphism	Genotype	FSs No. (%)	Controls No. (%)	*P*-value[a]
IL-1Ra	I/I	96 (93.20)	120 (83.92)	0.028[a]
	I/II	7 (6.80)	23 (16.1)	
	II/II	0 (0)	0 (0)	
IL-10	AA	17 (16.3)	61 (42.70)	<0.0001[a]
	AC	87 (83.6)	53 (37.1)	
	CC	0 (0)	29 (20.3)	
IL-1β promoter	CC	30 (29.1)	48 (33.6)	0.74
	CT	47 (45.6)	63 (44.1)	
	TT	26 (25.3)	32 (22.4)	
IL-1β exon 5	E1E1	100 (96.2)	139 (97.2)	0.50
	E1E2	4 (3.85)	3 (2.1)	
	E2E2	0 (0)	1 (0.7)	
IL-6 promoter	CC	64 (62.7)	88 (62.0)	0.92
	CG	32 (31.4)	47 (33.1)	
	GG	6 (5.9)	7 (4.9)	
TNF-α promoter	AA	1 (1.0)	3 (2.1)	0.87
	AG	21 (20.2)	29 (20.4)	
	GG	82 (78.8)	110 (77.5)	
IL-8	AG	47 (47.0)	69 (48.6)	0.80
	AA	11 (11.0)	12 (8.5)	
	GG	42 (42.0)	61 (43.0)	

[a]*P*-value were calculated by χ^2 test.

exact test. The corrected P values (P_c) were obtained by multiplying the uncorrected P values (P_u) with the number of comparisons, according to Bonferroni's method. Cases were compared with controls using odds ratios and 95% confidence intervals. A P value of <0.05 was considered to represent statistical significance.

RESULTS

The distribution of the IL-1β promoter, IL-1β exon 5, IL-Ra, IL-6 promoter, IL-8, IL-10, and TNF genotypes and allele frequencies in the patient group and control group are shown in Tables 2 and 3. Genotype proportions and allele frequencies for the IL-Ra in both groups were significantly different ($\chi^2 = 4.82$, $P = 0.028$; $\chi^2 = 4.51$, $P = 0.034$, respectively). The IL-1 Ra-I homozygote was more frequent in patients with FSs than in healthy controls (93.2% vs. 83.92%, $\chi^2 = 4.51$, $P = 0.034$). In addition, individuals homozygous for the IL-1 Ra-I genotype were more than twice as likely to develop FSs than individuals heterozygous for the IL-1 Ra-I/II genotype (OR, 2.63, 95% CI: 1.08–6.39; $\chi^2 = 4.55$, $P = 0.033$).

In contrast, there were no significant differences in genotypes of the IL-1β promoter, IL-1β exon 5, IL-6, IL-8, or TNF ($\chi^2 = 0.61$, $P = 0.74$; $\chi^2 = 1.38$, $P = 0.50$; $\chi^2 = 0.16$, $P = 0.92$; $\chi^2 = 0.45$, $P = 0.80$; $\chi^2 = 0.51$, $P = 0.78$, respectively) between patients and controls.

TABLE 3. Distribution of Interleukin Allele Frequencies in Patients With Febrile Seizures (FSs) ($n = 104$) and Healthy Control Subjects ($n = 143$)

Gene polymorphism	Allele	FSs No. (%)	Controls No. (%)	*P*-value[a]
IL-1Ra	I	199 (96.6)	263 (92.0)	0.0337[a]
	II	7 (3.4)	23 (8.0)	
IL-10	A	121 (58.2)	175 (61.2)	0.4995
	C	87 (41.8)	111 (38.8)	
IL-1β promoter	C	107 (51.9)	159 (55.6)	0.4225
	T	99 (48.1)	127 (44.4)	
IL-1β exon 5	E1	204 (98.1)	281 (98.3)	0.8859
	E2	4 (1.9)	5 (1.7)	
IL-6 promoter	C	160 (78.4)	223 (78.5)	0.9810
	G	44 (21.6)	61 (21.5)	
TNF-α promoter	A	23 (11.1)	35 (12.3)	0.6670
	G	185 (88.9)	249 (87.7)	
IL-8	A	69 (34.5)	93 (32.7)	0.6873
	G	131 (65.5)	191 (67.3)	

[a]*P*-values were calculated by χ^2 test.

The genotype proportions for the IL-10 in both groups were significantly different ($\chi^2 = 57.35$, $P < 0.0001$). However, there were no significant differences in allele frequencies of the IL-1β promoter, IL-1β exon 5, IL-6, IL-8, IL-10, or TNF ($\chi^2 = 0.64$, $P = 0.42$; $\chi^2 = 0.02$, $P = 0.89$; $\chi^2 = 0.0005$, $P = 0.98$; $\chi^2 = 0.16$, $P = 0.69$; $\chi^2 = 0.46$, $P = 0.50$; $\chi^2 = 0.19$, $P = 0.67$, respectively) between patients and controls.

DISCUSSION

In this study, we found that FSs are not associated with IL-1β promoter, IL-1β exon 5, IL-6 promoter, IL-8, IL-10, and TNF-α promoter gene polymorphisms. However, we found that the IL-1 Ra allele I is associated with a higher susceptibility to FSs. This finding is consistent with our previous report (19), and a recent study that demonstrated that the IL-1 Ra intron 2 variable tandem repeat polymorphisms are significantly associated with resistance to FSs (20). However, Haspolat et al. (21) found no significant effects of this polymorphism on FSs in Turkish children (73 with FSs and 152 healthy control subjects). Geographic and ethnic differences may partly account for the discrepancy between the results of Haspolat et al. from a Turkish population and our results from a Taiwanese population. Indeed, many SNPs have very different allele frequencies in different ethnic groups. Further replication in independent population studies is critical.

The action of IL-1 is complex and is regulated in part by its naturally occurring inhibitor, IL-1Ra. The IL-1 Ra is structurally related to IL-1α and IL-1β and competes with these molecules for occupation of IL-1β cell surface receptors. The presence of the IL-1 Ra allele

II has been shown to be associated with enhanced IL-1β production in vitro. Helminen and Vesikari (22) have shown increased IL-1 production of peripheral blood mononuclear cells from FS patients after stimulation with lipopolysaccharide. Tutuncuoglu et al. (23) reported increased plasma IL-1β and CSF TNF-α levels during the acute phase of FSs. In contrast, Lahat et al. (24) reported no difference in serum or CSF IL-1β levels between FSs and control children. These findings suggest that the balance between IL-1β and IL-1 Ra during seizures plays a significant role in altering neuronal network excitability, thus affecting the maintenance and spread of seizures.

The balance between proinflammatory and anti-inflammatory cytokines may be more critical than the concentration of a single cytokine in the regulation of inflammation. Cytokines are modified, modulated, or substituted by other cytokines. Proinflammatory cytokines such as IL-1β, TNF, and IL-6 participate in inducing acute-phase reactions including fever. Anti-inflammatory cytokines such as IL-1RA have a negative feedback effect during febrile response. Cells from children prone to seizures may produce higher levels of proinflammatory cytokines that may induce seizures or, alternatively, higher levels of anti-inflammatory cytokines as a defense mechanism against seizure. This may occur by direct action on ionic currents or indirectly by enhancing extracellular glutamate concentrations or reducing $GABA_A$ receptor function (25–27).

In conclusion, our study suggests that IL-Ra is positively associated with FSs, findings that support the hypothesis that the cytokine network is associated with that seizure disorder in children. Whether elevated levels of those cytokines play a role in the pathogenesis of FSs or reflect seizure activity must be investigated further.

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REFERENCES

1. Baumann RJ, Duffner PK. Treatment of children with simple febrile seizures: the AAP practice parameter. American Academy of Pediatrics. Pediatr Neurol 2000;23:11–17.
2. Commission on Epidemiology and Prognosis, International League Against Epilepsy. Guidelines for epidemiologic studies on epilepsy. Epilepsia 1993;34:592–596.
3. Addy DP. Nosology of febrile convulsions. Arch Dis Child 1986;61:318–320.
4. Peltola J, Keränen T, Rainesalo S, Hurme M. Polymorphism of the interleukin-1 gene complex in localization-related epilepsy. Ann Neurol 2001;275–276.
5. Kanemoto K, Kawasaki J, Yuasa S, et al. Increased frequency of interleukin-1beta-511 T allele in patients with temporal lobe epilepsy, hippocampal sclerosis, and prolonged febrile convulsion. Epilepsia 2003;44:796–799.
6. Virta M, Hurme M, Helminen M. Increased frequency of interleukin-1beta (-511) allele 2 in febrile seizures. Pediatr Neurol 2002;26:192–195.
7. Chou IC, Tsai FJ, Huang CC, Lin CC, Tsai CH. The voltage-gated potassium channel KCNQ2 in Taiwanese children with febrile convulsions. Neuroreport 2002;13:1971–1973.
8. Chou IC, Peng CT, Huang CC, Tsai JJ, Tsai FJ, Tsai CH. Association analysis of gamma 2 subunit of gamma-aminobutyric acid type A receptor polymorphisms with febrile seizures. Pediatr Res 2003;54:26–29.
9. Chou IC, Lee CC, Huang CC, et al. Association of the neuronal nicotinic acetylcholine receptor subunit alpha4 polymorphisms with febrile convulsions. Epilepsia 2003;44:1089–1093.
10. Peng CT, Chou IC, Li CI, Hsu YA, Tsai CH, Tsai FJ. Association of the nicotinic receptor beta 2 subunit and febrile seizures. Pediatr Neurol 2004;30:186–189.
11. Tsai FJ, Hsieh YY, Chang CC, Lin CC, Tsai CH. Polymorphisms for interleukin 1 beta exon 5 and interleukin 1 receptor antagonist in Taiwanese children with febrile convulsions. Arch Pediatr Adolesc Med 2002;156:545–548.
12. Collins FS, Guyer MS, Charkravarti A. Variations on a theme: Cataloging human DNA sequence variation. Science 1997; 278:1580–1581.
13. Kwok PY, Gu Z. Single nucleotide polymorphism libraries: why and how are we building them? Mol Med Today 1999;5: 538–543.
14. Rothwell NJ, Hopkins SJ. Cytokines and the nervous system II: actions and mechanisms of action. Trends Neurosci 1995;18:130–136.
15. Luk WP, Zhang Y, White TD, et al. Adenosine: a mediator of interleukin-1beta-induced hippocampal synaptic inhibition. J Neurosci 1999;19:4238–4244.
16. Chou HT, Tsai CH, Chen WC, Tsai FJ. Lack of association of genetic polymorphisms in the interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 genes with risk of rheumatic heart disease in Taiwan Chinese. Int Heart J 2005;46:397–406.
17. Huang CM, Huo AP, Tsai CH, Chen CL, Tsai FJ. Lack of association of interleukin-6 and interleukin-8 gene polymorphisms in Chinese patients with systemic lupus erythematosus. J Clin Lab Anal 2006;20:255–259.
18. Chen HY, Chen WC, Hsu CM, Tsai FJ, Tsai CH. Tumor necrosis factor alpha, CYP 17, urokinase, and interleukin 10 gene polymorphisms in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. Eur J Obstet Gynecol Reprod Biol 2005;122:73–78.
19. Tsai FJ, Hsieh YY, Chang CC, Lin CC, Tsai CH. Polymorphisms for interleukin 1 beta exon 5 and interleukin 1 receptor antagonist in Taiwanese children with febrile convulsions. Arch Pediatr Adolesc Med 2002;156:545–548.
20. Serdaroğlu G, Alpman A, Tosun A, et al. Febrile seizures: interleukin 1beta and interleukin-1 receptor antagonist polymorphisms. Pediatr Neurol 2009;40:113–116.
21. Haspolat S, Baysal Y, Duman O, Coşkun M, Tosun O, Yeğin O. Interleukin-1alpha, interleukin-1beta, and interleukin-1Ra polymorphisms in febrile seizures. J Child Neurol 2005;20: 565–568.

22. Helminen M, Vesikari T. Increased interleukin-1 (IL-1) production from LPS-stimulated peripheral blood monocytes in children with febrile convulsions. Acta Paediatr Scand 1990;79:810–816.
23. Tutuncuoglu S, Kutukculer N, Kepe L, Coker C, Berdeli A, Tekgul H. Proinflammatory cytokines, prostaglandins and zinc in febrile convulsions. Pediatr Int 2001;43:235–239.
24. Lahat E, Livne M, Barr J, Katz Y. Interleukin-1beta levels in serum and cerebrospinal fluid of children with febrile seizures. Pediatr Neurol 1997;17:34–36.
25. Zeise ML, Espinoza J, Morales P, Nalli A. Interleukin-1beta does not increase synaptic inhibition in hippocampal CA3 pyramidal and dentate gyrus granule cells of the rat in vitro. Brain Res 1997;768:341–344.
26. Kamikawa H, Hori T, Nakane H, Aou S, Tashiro N. IL-1beta increases norepinephrine level in rat frontal cortex: involvement of prostanoids, NO, and glutamate. Am J Physiol 1998;275:R803–R810.
27. Wang S, Cheng Q, Malik S, Yang J. Interleukin-1beta inhibits gamma-aminobutyric acid type A (GABA(A)) receptor current in cultured hippocampal neurons. J Pharmacol Exp Ther 2000;292:497–504.