Interleukin (IL)-1β, IL-1α, and IL-1 Receptor Antagonist Gene Polymorphisms in Patients with Temporal Lobe Epilepsy

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Proinflammatory cytokines, including interleukin (IL)-1β, are known to modulate effects of neurotoxic neurotransmitters discharged during excitation or inflammation in the central nervous system (CNS). They also regulate development of glial scars at sites of CNS injury. To elucidate a genetic predisposition of temporal lobe epilepsy with hippocampal sclerosis (TLE-HS$^+$), we studied polymorphisms in the IL-1β, IL-1α, and IL-1 receptor antagonist (IL-1RA) genes in 50 patients with TLE-HS$^+$ and in 112 controls. Fifty-three patients who had TLE without HS were also examined (TLE-HS$^-$) as disease controls. The distribution of the biallelic polymorphism in the promoter region at position −511 of the IL-1β gene (*IL-1B−511*) was significantly different both between TLE-HS$^+$ patients and controls and between TLE-HS$^+$ and TLE-HS$^-$ patients. The differences were due to overrepresentation of the homozygotes for *IL-1B−511*2*, which is suggested to be a high producer of IL-1β, in TLE-HS$^+$ patients compared with both controls and TLE-HS$^-$ patients. In contrast, there was no difference between TLE-HS$^-$ patients and controls. Our data suggest that, in the homozygotes for *IL-1B−511*2*, minor events in development such as febrile convulsions could set up a cascade leading to HS.

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After a longstanding controversy over the proposal of Falconer and Taylor,[1] medial temporal lobe epilepsy (MTLE) has been established as a definite clinical entity, and hippocampal sclerosis (HS) has been acknowledged as the most common histological finding of MTLE. The advent of magnetic resonance imaging (MRI) has made it possible to diagnose HS with only minimal false-positive results even before surgical intervention.[2]

Interleukin (IL)-1α and IL-1β are major proinflammatory cytokines that are synthesized during infection and inflammatory processes not only by macrophages but also by glial and neuronal cells.[3] IL-1 receptor antagonist (IL-1RA) competes for the same IL-1 receptor as that for IL-1α and IL-1β, thereby preventing activation of the target cells. Receptors for IL-1 have been located in the central nervous system, with the highest density reported in the molecular and granular layers of the dentate gyrus,[4] suggesting a physiological role for IL-1 in the hippocampus.

The genes for IL-1α, IL-1β, and IL-1RA are all located on the long arm of chromosome 2. Two biallelic base change polymorphisms in the IL-1β gene have been reported to influence the protein production.[5–7] One is in the promoter region at position −511 (*IL-1B−511*), and the other is in exon 5 at position +3953 (*IL-1B+3953*). A polymorphism in the IL-1α gene promoter region (position −889) (*IL-1A−889*) and a variable number tandem repeats (VNTR) polymorphism in the IL-1RA gene have been also associated with several inflammatory diseases.[8–10]

In the present study, we investigated whether these cytokine gene polymorphisms may be responsible in part for genetic susceptibility to TLE with HS.

Subjects and Methods

This study included all outpatients visiting the Kansai Regional Epilepsy Center from March to August, 1999. Each patient showed evidence of temporal electroencephalographic spikes or sharp waves and complex partial seizures. All subjects gave informed consent for the study. The patients were studied using MRI techniques described later, and these were designed to optimize the detection of HS. Patients with MRI evidence of unilateral HS were chosen as study subjects (TLE-HS$^+$ group). Patients with minor global or focal temporo-occipital brain atrophy were also included. Cases in which MRI suggested coexistent foreign tissue lesion and HS were excluded. Patients with essentially normal MRIs or foreign tissue lesions were selected as disease controls (TLE without HS; TLE-HS$^-$). The TLE-HS$^+$ group consisted of

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50 patients and the TLE-HS$^-$ group of 53 patients. One hundred twelve unrelated, healthy, randomly selected individuals from the same geographic area served as controls.

MRI was performed on a 1.5-T unit (Shimadzu Co, Kyoto, Japan). The protocol for imaging patients with TLE was as follows: (1) parallel to the lateral ventricle inferior horns (TLE axis) T1-weighted (500/15/4 [repetition time/echo time/excitations]) and T2-weighted (4000/22/2); and (2) transverse to the inferior horns (TLE coronal) T1-weighted and T2-weighted with the same width sections and intersection spacing. TLE coronal T2-weighted images were inversed black and white using software supplied by Shimadzu. Hippocampal atrophy was diagnosed when the summated hippocampus area on inverted TLE coronal T2-weighted images on the lesional side was smaller by 20% or more than the nonlesional side.

Febrile convulsion lasting more than 15 minutes was termed prolonged febrile convulsion. We defined simple febrile convulsion as a febrile convulsion with neither focality nor a duration of more than 15 minutes. Fundamental clinical data, including age, age at epilepsy onset, sex, and histories of febrile convulsions, are depicted in Table 1.

Genomic DNA was extracted from peripheral blood leukocytes, as described elsewhere.[11] Each polymerase chain reaction (PCR) reaction was carried out with 50 ng genomic DNA and 1.0 U AmpliTaq Gold polymerase (Perkin-Elmer Cetus, Branchburg, NJ). For optimal amplification, the Mg^{2+} concentration of the reaction buffer was adjusted to 1.5 mM.

A single base pair (bp) polymorphism at position −511 in the promoter region of the IL-1β gene was analyzed by the PCR-restriction fragment length polymorphism (RFLP) method.[10] A 304-bp PCR fragment of the IL-1β promoter region was amplified using the following primers: 5′-TGGCATTGATCTGGTTCATC-3′ and 5′-GTTTAGGA ATCTTCCCACTT-3′. PCR conditions were as follows: a denaturing step of 95°C for 10 minutes, then 36 cycles of 94°C for 45 seconds, 54°C for 50 seconds, 72°C for 1 minute, and final incubation at 72°C for 5 minutes. The products were digested with 5 U of *Ava*I (Takara, Shiga, Japan) at 37°C for 3 hours and were run on an ethidium bromide-stained 3% agarose gel. This gave products that either remained intact (allele 2; *IL-1B−511*2*) or were cut into two fragments of 190 and 114 bp (allele 1; *IL-1B−511*1*).

The polymorphic region containing the *Taq*I (New England Biolabs, Inc, Beverly, MA) restriction site at position +3953 within exon 5 of the IL-1β gene was amplified using the following primers: 5′-GTTGTCATCAGACTTTGAC C-3′ and 5′-TTCAGTTCATATGGACCAGA-3′.[10] The PCR conditions were the same as above. The products were digested with 5 U of *Taq*I at 65°C for 3 hours. *Taq*I digestions of the 249-bp fragments were cut into two fragments of 135 and 114 bp (allele 1; *IL-1B+3951*1*) or remained intact (allele 2; *IL-1B+3953*2*).

A C-T transition polymorphism at position −889 in the promoter region of the IL-1α gene was analyzed according to McDowell and colleagues.[8] Primers designed to create a recognition site for *Nco*I (Takara) in one allele but no site in the other were used in a PCR. Amplification conditions were as described earlier, but an annealing temperature of 46°C was used. The 99-bp PCR products were digested with *Nco*I, and run on a 11% polyacrylamide gel. Allele 1 (*IL-1A*1*) gave products of 83 and 16 bp, and allele 2 (*IL-1A*2*) gave a product of 99 bp.

The IL-1RA intron 2 contains a VNTR of an 86-bp length of DNA. Oligonucleotides 5′-CTCAGCAACACTCCTAT-3′ and 5′-TCCTGGTCTGCAGGTAA-3′ flanking this region were used as primers.[10] Amplification conditions were the same as above, but an annealing temperature of 60°C was used. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. Allele 1 (four repeats) was 410 bp, allele 2 (two repeats) was 240 bp, allele 3 (three repeats) was 325 bp, allele 4 (five repeats) was 500 bp, and allele 5 (six repeats) was 595 bp.

Data Analysis

The data were evaluated by χ^2 or Fisher's exact test, using the standard statistical software (Stat View 5.0, Abacus Concepts, Berkeley, CA). 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. Equality between groups was tested by the Mann-Whitney U test. The level of significance was $p < 0.05$.

Results

The clinical characteristics of TLE-HS$^+$ and TLE-HS$^-$ patients are summarized in Table 1. The age at onset of epilepsy was significantly earlier for TLE-HS$^+$ patients than for TLE-HS$^-$ patients (9.4 ± 5.9 years vs 18.7 ± 10.7 years, $p < 0.0001$). TLE-HS$^+$ was far more likely to be preceded by prolonged febrile convulsions than TLE-HS$^-$ (54.0% vs 5.7%, $p < 0.0001$). On the other hand, no difference was found between TLE-HS$^+$ and TLE-HS$^-$ patients with respect to the risk of antecedent simple febrile convulsions (see Table 1).

The distribution of the *IL-1B−511* genotypes and

Table 1. Comparison of Clinical Data between MTLE and Non-MTLE Patients

	MTLE (n = 50)	Non-MTLE (n = 53)	p
Sex (F/M)	26/24	18/35	0.064
Age (yr)	34.0 ± 9.2	39.0 ± 11.5	0.014
Age at epilepsy onset (yr)	9.4 ± 5.9	18.7 ± 10.7	<0.0001
Simple febrile convulsion	10	9	0.80
Prolonged febrile convulsion	27	3	<0.0001

MTLE = medial temporal lobe epilepsy.

allele frequencies in TLE-HS$^+$ patients, TLE-HS$^-$ patients, and controls is shown in Table 2. The distribution of the *IL-1B−511* genotypes was significantly different between TLE-HS$^+$ patients and controls (χ^2 = 9.55, p_u = 0.0085, p_c = 0.017). The difference was due to the overrepresentation of the *IL-1B−511*2* homozygote in TLE-HS$^+$ patients (χ^2 = 9.49, p_u = 0.0021, p_c = 0.013). In contrast, there was no difference between TLE-HS$^-$ patients and controls. A significant difference of the *IL-1B−511* genotype was also found between TLE-HS$^+$ and TLE-HS$^-$ patients (χ^2 = 7.62, p_u = 0.022, p_c = 0.044). When compared with TLE-HS$^-$ patients, the frequency of the homozygote for *IL-1B−511*2* increased significantly in TLE-HS$^+$ patients (χ^2 = 7.59, p_u = 0.0059, p_c = 0.035). Although the increased frequency of *IL-1B−511*2* allele was observed in TLE-HS$^+$ patients compared with controls, it was not statistically significant (χ^2 = 3.80, p_u = 0.051, $p_c > 0.1$).

The frequencies of *IL-1B+3953* and *IL-1A−889* alleles in the TLE patients and controls are also summarized in Table 2. There were no differences of genotypes or allele frequencies in any of them. Concerning the frequencies of IL-1RA gene polymorphism, each TLE group and the control group showed a similar distribution in the frequency of *IL-1RA* alleles (Table 2).

Since the genes for IL-1RA, IL-1α, and IL-1β are all located in the same region of chromosome 2, we examined relationships among the alleles of these genes. The *IL-1B+3953*2* was in linkage disequilibrium with *IL-1A*2* (χ^2 = 32.6, $p < 0.0001$). We also detected the tendency of an association between *IL-1RA*2* and *IL-1B+3953*2*, but it was not significant after corrections for multiple comparisons.

Discussion

Predominance of histories of complicated febrile convulsions and relatively early onset of habitual seizures in the current TLE-HS$^+$ series have been suggested as the hallmarks of this type of TLE,[12–14] indicating that our series constitutes a representative group of MTLE.

To our knowledge, the present study is the first attempt to examine the polymorphisms of the IL-1α, IL-1β, and IL-1RA genes in TLE patients. Our aim was to study the genetic predisposition of the TLE-HS$^+$ patients to those cytokines that are supposed to modulate effects of neurotoxic neurotransmitters discharged during CNS excitation or inflammation. They are also reported to influence scar formation at sites of CNS injury,[15] which could initiate epileptic discharges through mechanisms involving reactive microglia. In this study, the homozygotes for allele 2 at position −511 of the IL-1β gene (*IL-1B−511*2*) increased significantly in TLE-HS$^+$ patients compared with both

Table 2. Associations of TLE Patients with Interleukin (IL)-1β, IL-1α, and IL-1RA Genotypes

Genotypes	TLE-HS$^+$ (total = 50) n (%)	TLE-HS$^-$ (total = 53) n (%)	Controls (total = 112) n (%)	*p*	
				TLE-HS$^+$ vs Controls	TLE-HS$^-$ vs Controls
IL-1B−511[a,b]					
1/1	9 (18.0)	13 (24.5)	31 (27.7)	p_u = 0.0085[f]	NS
1/2	19 (38.0)	30 (56.6)	58 (51.8)	p_c = 0.017	
2/2	22 (44.0)[d,e]	10 (18.9)	23 (20.5)		
IL-1B+3953[a]					
1/1	45 (90.0)	49 (92.5)	105 (93.8)	NS	NS
1/2	5 (10.0)	3 (5.7)	7 (6.3)		
2/2	0 (0.0)	1 (1.9)	0 (0.0)		
IL-1A−889[a]					
1/1	38 (76.0)	44 (83.0)	87 (77.7)	NS	NS
1/2	10 (20.0)	8 (15.1)	25 (22.3)		
2/2	2 (4.0)	1 (1.9)	0 (0.0)		
IL-1RA[c]					
1/1	46 (92.0)	52 (98.1)	102 (91.9)	NS	NS
1/2	3 (6.0)	1 (1.9)	6 (5.4)		
1/3	1 (2.0)	0 (0.0)	1 (0.9)		
1/4	0 (0.0)	0 (0.0)	2 (1.8)		

[a] *p* value was calculated by χ^2 test with a 2 × 3 contingency table (genotype) and a 2 × 2 table (allele).
[b] The distribution was significantly different between TLE-HS$^+$ and TLE-HS$^-$: χ^2 = 7.62; p_u = 0.022, p_c = 0.044 (2 × 3 table).
[c] *p* value was calculated by χ^2 test with a 2 × 4 contingency table (genotype) and a 2 × 2 table (allele).
[d] Significantly increased compared with controls: χ^2 = 9.49; p_u = 0.0021, p_c = 0.013 (2 × 2 table).
[e] Significantly increased compared with TLE-HS$^-$: χ^2 = 7.59; p_u = 0.0059, p_c = 0.035 (2 × 2 table).
[f] The distribution was significantly different between TLE-HS$^+$ and controls: χ^2 = 9.55; p_u = 0.0085, p_c = 0.017 (2 × 3 table).

TLE = temporal lobe epilepsy; TLE-HS$^+$ = temporal lobe epilepsy with hippocampal sclerosis; TLE-HS$^-$ = temporal lobe epilepsy without hippocampal sclerosis; NS = not significant; p_u = uncorrected *p* value; p_c = corrected *p* value.

TLE-HS$^-$ patients and controls. Although there were some discrepancies concerning the functional significance of the genetic marker,[5–7] *IL-1B−511∗2* carriers have been regarded to be higher producers of IL-1β than *IL-1B−511∗1* carriers.[16]

Most authors agree that histories of prolonged febrile convulsion during early childhood or infancy are highly associated with the later development of MTLE.[12–14] On the other hand, most studies have failed to confirm any association between simple, self-limited febrile convulsions and TLE.[17,18] The explanation for this discrepancy would be that patients destined to develop TLE with HS later in life have some genetic predisposition for otherwise harmless, self-limited excitations of the CNS, such as febrile convulsions.

Maher and McLachlan[17] suggested that fever and convulsions interact to cause mesial temporal sclerosis through excitatory amino acid neurotransmitters and their receptors. Vezzani and co-workers[19] reported that injection of IL-1β intrahippocampally before focal application of kainic acid doubled duration of the seizures induced by kainate in animal models. This effect was blocked by co-injection of a selective antagonist of NMDA (*N*-methyl-D-aspartate) receptor, suggesting that application of IL-1β prolonged kainate-induced hippocampal seizures by enhancing glutamatergic neurotransmission. However, they have dealt with seizures in adult animals rather than in developing animals, which may be a different situation from febrile convulsions early in life.

Our data do not directly support the argument suggested by Maher and McLachlan[17] that prolonged febrile seizures are a cause of HS, because the competing hypothesis that HS is developmental could also be interpreted in our molecular genetic findings. Indeed, the supplemented analysis of the current series revealed that TLE-HS$^+$ patients without prolonged febrile seizures were equally as often *IL-1B−511∗2* carriers as those with prolonged febrile seizures (data not shown). This indicated that other less dramatic events in early childhood could also lead to HS in *IL-1B−511∗2* carriers.

Recently, Fernández and associates[20] reported a striking familial accumulation of an asymmetry of hippocampal volume in unaffected relatives of patients with MTLE, suggesting the role of a preexisting, genetically determined anomaly in the genesis of MTLE.

In conclusion, we have shown that the functional polymorphism in the IL-1β gene was associated with TLE-HS$^+$ patients but not with TLE-HS$^-$ patients. This suggests that a subtle anomaly or minor events in development or early infancy could set up a cascade leading to HS in patients with such a genetic predisposition.

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