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Association of Idiopathic Generalized Epilepsy With Polymorphisms in the Neuronal Nicotinic Acetylcholine Receptor Subunits

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Idiopathic generalized epilepsy (IGE) refers to a common group of epilepsies, and genetic factors play an important role in the pathogenesis of these disorders. Mutations in *CHRNA4* and *CHRNB2* are associated with some cases of familial epilepsies classified as autosomal-dominant nocturnal frontal lobe epilepsies. We aimed to evaluate whether polymorphisms of *CHRNA4* and *CHRNB2* are associated with IGE. A total of 75 children with IGE and 80 normal control subjects were included in the study. Each genetic polymorphism was typed by polymerase chain reaction (PCR)-based restriction analysis. The genotypes and allelic frequencies of each polymorphism were compared between the IGE patients and controls. The results showed that genotype and allelic frequency for *CHRNB2* did not differ significantly between the groups. However, the genotype proportion of the *CHRNA4* (Ser543-Ser) gene in both groups was significantly different ($P < 0.0001$). The T allele frequency was significantly higher ($P = 0.0126$) in patients with IGE compared to healthy controls. The odds ratio (OR) for developing IGE in individuals with the CHRNA4 (Ser543Ser)-T homozygote was 4.9 (95% confidence interval (CI), 1.71–14.04) compared to individuals with two copies of the *CHRNA4* (Ser543Ser)-C allele. This study demonstrates that the CHRNA4 gene may be one of the susceptibility factors for IGE. J. Clin. Lab. Anal. 21:67–70, 2007.

Key words: CHRNA4; CHRNB2; polymorphism; idiopathic generalized epilepsy

INTRODUCTION

Idiopathic generalized epilepsy (IGE) affects about 0.3% of the general population and accounts for 30% of all epilepsies (1). The term ''IGE'' refers to a group of epilepsies with no apparent neurological abnormalities or structural brain damage. Patients are prone to recurrent seizures involving both hemispheres of the brain, which usually present in childhood or adolescence. Seizure types include generalized tonic-clonic seizures, myoclonic seizures, and/or absences. Patients also exhibit generalized spike waves on electroencephalograms and may be photosensitive. IGE includes many common syndromes, such as juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), and epilepsy with generalized tonic-clonic seizures (GTCS) (2). There is considerable overlap between syndromes, and more than one IGE syndrome may be observed within families. Many IGEs cannot easily be placed into a particular category (3). IGEs are polygenic, as suggested by rapidly diminishing risks beyond first-degree relatives (4) and high concordance between monozygotic twins (5).

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In the past decade, studies of large families in which epilepsy has been inherited in an autosomal-dominant fashion have revealed several mutated genes, most of which encode ion channel subunits. One such epilepsy is autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE), which has been linked to mutations in human genes encoding subunits of the neuronal nicotinic acetylcholine receptor (nAChR) (6–11). The nAChR is a pentameric assembly of homologous subunits that mediates rapid synaptic transmission. The major isoform in the brain is composed of α-4 and β-2 subunits encoded by the CHRNA4 and CHRNB2 genes. Mutations in both of these subunits have been associated with the disorder ADNFLE, characterized by brief seizures during light sleep that originate in the frontal lobe. The functional defects in the mutated ligand-gated channels are predicted to increase the intrinsic excitability of neurons (12–15). Increased excitability could lead to increased neuronal firing and to episodes of synchronized firing by large numbers of neurons that constitute a seizure. These functional ion channel genes may be tested as candidate genes for many other epilepsies because of their relationship with the same process of electrophysiology.

In previous studies we used single nucleotide polymorphisms (SNPs) as a tool to search for the genetic markers of febrile seizure (FS) (16–19). SNPs are the most abundant type of DNA sequence variation in the human genome (20,21). It is a single base pair on the DNA that varies from person to person. The SNP markers may provide a new way to identify complex gene-associated diseases, such as IGE. We previously observed a strong association between CHRNA4 and FS (18), and found no association between CHRNB2 and FS (19). Based on these experiences, we tried to evaluate whether these polymorphisms are useful markers for predicting susceptibility to IGE. Two SNPs markers have been identified: SNP1044396 (Ser543Ser) in the CHRNA4 gene, and SNP2072659 in the CHRNB2 gene. These markers will be useful for detecting a disease-causing gene association (www.ncbi.nlm.nih.gov/SNP).

MATERIALS AND METHODS

This study included Taiwanese children with IGE (group 1: $N = 75$ in CHRNA4, and $N = 77$ in CHRNB2) and normal control subjects (group 2: $N = 80$ in CHRNA4, and $N = 83$ in CHRNB2). The study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. The parents of the subjects signed informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity, and geographic location of origin. IGE subjects were recruited from the midland of Taiwan. Diagnosis of IGE followed the criteria established by the 1989 International Classification of Epileptic Syndromes. Probands with a clinical presentation consistent with IGE and a generalized spike wave and normal background rhythm on electroencephalography were identified by the Department of Neurology. The sample was unselected by subtype and consisted of patients with CAE, JAE, JME, GTCS, overlap syndromes, and unclassified cases. Our patients were 2–18 years old (year [mean ± SD]: 8.52 ± 4.23). The female-to-male ratio was 0.97.

Genotyping

All of the children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood with the use of 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 each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA). Four PCR primers were used to amplify the associated gene. The sequences of these primers were as follows (from 5′ to 3′ end): CHRNA4 (SNP1044396): upstream, CCTGGCCTCTCGCAACAC; downstream, TTGGT GCTGCGGGTCTTG; and CHRNB2 (SNP2072659): upstream, GGTGGAGGATGGACGAGTGA; downstream, GCAGCCAAAACAAAGCAGTTG. The PCR condition was as follows: 35 cycles at 94°C for 1 min, 59°C for 30 sec, and 72°C for 45 sec, then standing at 72°C for 30 min and holding at 4°C. The polymorphisms were analyzed by PCR amplification followed by restriction analysis: *Hha I* for CHRNA4 (SNP1044396) and *Dpn*II for CHRNB2 (SNP2072659). The PCR products were directly analyzed on 2% agarose gel by electrophoresis, and each allele was recognized according to its size. The heterozygotes of the PCR products were sequenced to avoid mistaking partial digestion for a heterozygote.

Statistical Analysis

Allelic frequencies were expressed as a percentage of the total number of alleles. Genotypes and allelic frequencies for CHRNA4 (SNP1044396) and CHRNB2 (SNP2072659) polymorphisms in both groups were compared. The SAS system with a χ^2 test was used for statistical analyses. A value of $P < 0.05$ was considered statistically significant. We compared the patients and controls using odds ratios (ORs) and their 95%

confidence intervals (CIs). Multiple comparisons among the TT, TC, and CC alleles were adjusted with the use of a Bonferroni test.

RESULTS

For each polymorphism, it was confirmed that the genotype proportions of the cases and controls fitted Hardy-Weinberg equilibrium, as estimated by the χ^2 test. The results showed that the genotype proportion and allele frequency for CHRNA4 (SNP1044396) were significantly different (Table 1). The most common genotype for CHRNA4 (SNP1044396) in groups 1 and 2 was the C/C homozygote. The proportions of C homozygote, C/T heterozygote, and T homozygote for CHRNA4 (SNP1044396) were respectively 58.7%, 12%, 29.3% in the IGE group, and 61.3%, 32.5%, and 6.2% in the control group. The proportion of patients with the CHRNA4 (SNP1044396) T homozygote for IGE was significantly greater than that of controls (29% vs. 6.2%, $P = <0.001$). The OR for developing IGE in individuals with the CHRNA4 (SNP1044396) T homozygote was 4.9 (95% CI, 1.71–14.04) compared to individuals with the CHRNA4 (SNP1044396) C homozygote. The allele C and T frequencies for CHRNA4 (SNP1044396) were respectively 64.7% and 35.3% in the IGE group, and 77.5% and 22.5% in the control group (Table 1). The CHRNA4 (SNP1044396) T allele frequency was significantly higher in IGE patients compared to controls ($P = 0.0131$). The OR for developing IGE in individuals with the T allele was 1.88 (95% CI, 1.02–3.46) compared to individuals with the C allele.

However, the genotype distributions or allelic frequency of the CHRNB2 gene (SNP2072659) in both groups were not significantly different (Table 2).

TABLE 1. Genotypes and allele frequencies for CHRNA4 (SNP1044396) polymorphisms in IGE patients and normal controls

	IGE patients number (%) (n = 75)	Normal controls number (%) (n = 80)	*P*-value*	Odds ratio (95% CI)[a]
Genotype				
C/C	44 (58.7)	49 (61.3)	<0.001*	1.00
T/C	9 (12.0)	26 (32.5)		0.39 (0.14–1.10)
T/T	22 (29.3)	5 (6.2)		4.9 (1.36–17.66)
Allelic frequency				
Allele C	97 (64.7)	124 (77.5)	0.0131	1.00
Allele T	53 (35.3)	36 (22.5)		1.88 (1.02–3.46)

*P-values were calculated by χ^2 test.
[a]Bonferroni adjustment has been made.
CI, confidence interval; IGE, idiopathic generalized epilepsy.

DISCUSSION

In the present study we found that children with the CHRNA4 (SNP1044396)-T allele had a higher incidence of IGE. This evidence indicates that the CHRNA4 (SNP1044396)-T allele is a candidate genetic marker for IGE. This finding is consistent with a recent study that demonstrated an allelic association between CHRNA4 polymorphisms and IGE (22). That study included a sample of IGE probands consisting of three common subtypes: CAE, JAE, and JME. However, given that the SNP involved in the association does not change an amino acid, the disease-associated allele must be in linkage disequilibrium with the DNA change, as yet unidentified in linkage disequilibrium with the DNA change.

Therefore, the existence of a second epilepsy-related gene in the vicinity of CHRNA4 cannot be excluded at this time. In a previous study the missense mutation (c.839C > T; S280F) in CHRNA4 was found in an Australian family with ADNFLE (6). This was followed by a report of an insertional mutation of CHRNA4 (c.873-874insGCT; L301-302) in a Norwegian family (7) and another point mutation of CHRNA (c.851C > T; S284L) in a Japanese family with ADNFLE (8,9). All of these mutations were heterozygous.

However, IGE is not associated with CHRNB2 (SNP2072659) gene polymorphisms. Even though this study cannot completely exclude the involvement of CHRNB2 in the pathogenesis of IGE, polymorphisms in this gene involved in familial epilepsies may not contribute to the pathogenesis of IGE.

The nAChRs are pentameric ion channels that consist of various hetero- or homologous combinations of eight α-subunits and three β-subunits (α2-α9; β2-β4) (23). Different subunits can have different or overlapping expression patterns in the brain. Although 11 distinct subunits have been identified in different species, most

TABLE 2. Genotypes and allele frequencies for CHRNB2 (SNP2072659) polymorphisms in IGE patients and normal controls

	IGE patients number (%) (n = 77)	Normal controls number (%) (n = 83)	*P*-value*
Genotype			
CC	8 (10.4)	4 (4.82%)	0.258
CG	21 (27.3)	30 (36.14%)	
GG	48 (62.3)	49 (59.04%)	
Allelic frequency			
Allele C	37 (24.0)	38 (22.89%)	0.580
Allele G	117 (75.8)	128 (77.11%)	

*P-values were calculated by χ^2 test.
IGE, idiopathic generalized epilepsy.

of which are expressed in human brain, not much is known about their specific function. Electrophysiological characteristics of AChR bearing either S280F or 301-302insL in CHRNA4 have been examined in *Xenopus oocytes*. S280F leads to faster desensitization upon activation by Ach, and the recovery is much slower than in the wild-type receptor (24,25). The electrophysiological characteristics of nAChR bearing S284L are similar to those of S280F (26,27). In contrast, AChR bearing L301-302 exhibits normal receptor function but a higher affinity for AChR than the wild-type receptor. In addition, a reduced Ca^{2+} permeability of the mutant nAChR has been noted (28). Since nAChR functions as a pentamer, any nAChR that harbors a dysfunctional subunit may be deficient.

In conclusion, the present study suggests that the CHRNA4 gene (or a closely linked gene) may be one of the susceptibility factors for IGE. The discovery of CHRNA4 as the first gene responsible for a rare form of idiopathic epilepsy suggests that it may also be involved in epilepsies with a more complex pathogenesis. The gene for monogenic epilepsies may contribute to the lower seizure threshold in IGE. Further studies could focus on analyzing CHRNA4 RNA and protein in children with IGE. This study may provide the basis for further surveys of CHRNA4 and CHRNB2 polymorphisms.

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