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Association of Idiopathic Generalized Epilepsy with a Polymorphism in the Neuronal Nicotinic Acetylcholine Receptor Subunit α4

Abstract

Studies provided evidences that the protein coded by the α4 subunit gene of the neuronal nicotinic acetylcholine receptor (CHRNA4) is one of the most abundant subunits of the neuronal nicotinic acetylcholine receptors in mammalian brain, and mutations of CHRNA4 seem to cause neuronal excitation. Idiopathic generalized epilepsy (IGE) comprises a common group of epilepsies and genetic factors play an important role in pathogenesis. The present study assessed the distribution of genotypes of CHRNA4 in patients with IGEs. A total of 110 children with IGEs and 80 normal control subjects were included in the study. Polymerase chain reaction was used to identify the C/T polymorphism of the CHRNA4 gene. Genotypes and allelic frequencies for the CHRNA4 gene polymorphisms in both groups were compared. The genotype proportion of the CHRNA4 (Ser543Ser) gene in both groups was significantly different (P=0.0001). T allele frequency was significantly higher (P= 0.0041), in patients with IGEs compared to healthy controls. The odds ratio for developing IGEs in individuals with the CHRNA4 (Ser543Ser)-T homozygote was 5.22 (95% confidence interval, 1.90-14.36) compared to individuals with two copies of the CHRNA4 (Ser543Ser)-C allele. This study has demonstrated an association between the CHRNA4 gene and IGEs. Individuals with the T allele

had a higher incidence of IGEs. The CHRNA4 gene might be one of the susceptibility factors for IGEs.

Introduction

Idiopathic generalized epilepsy (IGE) is a common disorder. It affect about 0.3% of the general population and account for 30% of all epilepsies (Sander et al., 2003). IGE comprises 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 electroencephalogram 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) (Engel, 2001). There is considerable overlap between syndromes and more than one IGE syndrome may be observed within families, and many IGEs cannot easily be placed into a particular category (Janz et al., 1992). A strong family history of IGEs exists in siblings and parents, suggesting a genetic predisposition. In twin studies, there is a higher concordance for monozygotic pairs than for dizygotic pairs, where concordant monozygotic pairs usually share the same syndrome (Berkovic et al., 1994; and 1998).

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. Three types of Mendelian epilepsies have been linked to mutations in human genes encoding subunits of the neuronal nicotinic acetylcholine receptor (α4, CHRNA4, and β2, CHRNB2, subunits) (Steinlein et al., 1997; Hirose et al., 1999; Ito et al., 2000; De Fusco,. et al., 2000; Phillips et al., 2001), the voltage-gated potassium (KCNQ2, KCNQ3) (Biervert et al., 1998; Singh

et al., 1998; Biervert et al., 1999; Charlier et al., 1998) and the voltage-gated sodium (SCN1A, SCN2A, SCN1B) channels (Escayg et al., 2000; Sugawara et al., 2001; Wallace et al., 1998). The CHRNA4 has been identified as the first gene underlying an idiopathic partial epilepsy syndrome in human, autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE). CHRNA4 is assembled with at least one type of acetylcholine receptor (nAChR) α subunits into a hetero-pentamer that functions as a nAChR. The nAChRs are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. The nAChR is considered to function as an excitatory element in central nervous system and mutations of CHRNA4 seem to cause neuronal excitation (Weiland et al., 1996; Kuryatov et al., 1997; Unwin, N., 1995; Matsushima et al., 2002). The discovery of CHRNA4 as the first gene responsible for a rare form of idiopathic epilepsy suggests it might also be involved in epilepsies with a more complex pathogenesis.

The etiology of IGEs remains obscure. The genetic susceptibility to IGEs seems to involve multiple genes in most instances. IGEs have been studied intensively with regard to genetic variation associated with illness. To date, about thirty different association studies have been published, examining the relationship between common forms of IGE and variation in a number of plausible candidate genes. However, the majority of these studies report non association.

New techniques of molecular analysis could help the dissection of genes for epilepsies with complex inheritance. Single nucleotide polymorphisms (SNPs) are the most abundant types of DNA sequence variation in the human genome (Collins et al., 1997; Kwok et al., 1999). The SNP marker has gained more and more popularity for its quick, accurate, and inexpensive properties for the genetic analyses of different diseases. For this reason, the SNP marker provides a new way for the

identification of complex gene-associated diseases such as IGEs. In our previous studies, we have investigated an association between CHRNA4 (SNP1044396) and febrile seizures in Taiwanese children (Chou et al., 2003). The gene for monogenic epilepsies may contribute to the lowered seizure threshold in idiopathic epilepsies. The CHRNA4 gene may have a role in the development of IGEs. Base on these results, we try to investigate the distribution of the polymorphisms for CHRNA4 in IGE probands, and evaluated whether these CHRNA4 polymorphisms are useful markers for predicting susceptibility to IGEs. Two SNPs markers have been identified in SNP 1044394 (Cys226Cys), at nucleotide position 678, and SNP1044396 (Ser543Ser), at nucleotide position 1629, respectively, allowing researchers to detect disease-causing gene association (Internet at http:// www.ncbi.nlm.nih.gov/SNP). In our previous study (Chou et al., 2003), we have found that neither the patients nor control population carried the SNP1044394 polymorphism in the CHRNA4 gene, so the SNP1044396 (Ser543Ser) was evaluated in this study.

Subjects and Methods

The study included Taiwanese children with IGEs (group 1; n=110) and normal control subjects (group 2; n=80). This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan. All parents signed informed consent before blood tests were performed. Cases were matched with controls according to age, sex, ethnicity and geographic location of origin. IGEs subjects were recruited from the midland of Taiwan. Diagnosis of IGEs followed the criteria established in the 1989 International Classification of Epileptic Syndromes. Probands with a clinical presentation consistent with IGE, and generalized spike wave and normal background rhythm on electroencephalography were identified via the department of pediatric neurology. The sample was unselected by subtype and consisted of patients with CAE, JAE, JME, GTCS, overlap syndromes and unclassified cases.

Genotyping

All children underwent peripheral blood sampling for genotype analyses. Genomic DNA was isolated from peripheral blood using 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 ul containing 10mM Tris-hydrochloride, pH 8.3; 50mM potassium chloride; 2.0mM magenesium chloride; 0.2mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, Calif., USA). Two 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, TTGGTGCTGCGGGTCTTG. The PCR condition was as follows: 35 cycles at 94ºC for one minute, 59º C for 30 sec**,** and 72ºC for 45 sec, then standing at 72°C for 30 minutes and holding at 4°C. The polymorphisms were analyzed by PCR amplification followed by restriction analysis: Hha I for CHRNA4 (SNP1044396**).** 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, for avoiding partial digestion mistaken 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) polymorphisms in both groups were compared. The SAS system with χ^2 test was used for statistical analyses. A value of $P<0.05$ was considered statistically significant. Cases were compared with controls using odds ratios and their 95% confidence intervals. The multiple comparisons among TT, TC and CC alleles were adjusting by Bonferroni test.

RESULTS

For each polymorphism of the CHRNA4 (SNP1044396), 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 genotype proportion and allele frequency for CHRNA4 (SNP1044396) were significantly different (Table). The most common genotype for CHRNA4 (SNP1044396) in group 1 was C/C homozygote, and in group 2 was also C/C homozygote. Proportions of C homozygote, C/T heterozygote and T homozygote for CHRNA4 (SNP1044396) were as follows: in the IGEs group, 56.4%, 13.6%, 30.0%, respectively; and in the control group, 61.3%, 32.5%, and 6.2%, respectively. The allele C and T frequencies for CHRNA4 (SNP1044396) in the IGEs group were 63.2% and 36.8%, respectively, and in the control group were 77.5% and 22.5%, respectively (Table). The proportion of individuals with the CHRNA4 (SNP1044396) T homozygote for IGEs was significantly greater than for controls (30.0% versus 6.2%, p=0.0001) and the CHRNA4 (SNP1044396) T allele frequency was significantly higher (p= 0.0041), in patients with IGEs compared to controls. The odds ratio for developing IGEs in individuals with the CHRNA4 (SNP1044396) T homozygote was 5.22 (95% CI, 1.90-14.36) compared to individuals with the CHRNA4 (SNP1044396) C homozygote.

Discussion

In the present study, we found that children with the CHRNA4 (SNP1044396)- T allele had a higher incidence of IGEs. This evidence indicates that the CHRNA4 (SNP1044396)- T allele is a candidate genetic marker for IGEs. This finding is consistent with a recent study demonstrating an allelic association between CHRNA4 polymorphisms and idiopathic generalized epilepsy (Steinlein et al., 1997). The previous study included a sample of IGEs probands, comprising three common subtypes, CAE, JAE, and JME. In contrast, three of the CHRNA4 polymorphisms were investigated in 182 Caucasian patients with IGEs, compared with 178 controls. None of the three polymorphisms exhibited any association with IGEs (Chioza et al., 2000). Indeed, many single nucleotide polymorphisms have very different allele frequencies in different ethnic groups. Therefore, any association obtained by case-control studies, should be regarded as provisional, and that replication in independent population studies is critical. Geographical and ethnic differences may partly account for the discrepancy between those results. Furthermore, given that the SNP involved in the association of our study does not change an amino acid, the disease associated allele must be in linkage disequilibrium with the DNA change, as yet unidentified.

The neuronal nicotinic acetylcholine receptors are pentameric ion channels, comprised of various hetero- or homologous combinations of eight α-subunits and three β-subunits (α2-α9; β2-β4) (Sargent et al., 1993). Different subunits can have different or overlapping expression patterns in brain. Although 11 distinct subunits have been identified in different species, most of which are expressed in human brain, and 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 (Weiland et al., 1996; Kuryatov et al., 1997). Electrophysiological characteristics of nAChR bearing S284L are similar to those of S280F (Unwin, 1995; Matsushima et al., 2002). In contrast, AChR bearing L301-302 exhibited normal receptor function but a higher affinity for AChR than the wild type receptor. In addition, reduced Ca^{2+} permeability of the mutant nAChR was noted (Steinlein, 1998). Since nAChR functions as a pentamer, nAChR harboring any number of dysfunctional subunit can be deficient.

In conclusion, the present study suggests that CHRNA4 gene or a closely linked gene might be one of the susceptibility factors for IGEs. The discovery of CHRNA4 as the first gene responsible for a rare form of idiopathic epilepsy suggests that it might also be involved in epilepsies with a more complex pathogenesis. The gene for monogenic epilepsies may contribute to the lowered seizure threshold in IGEs. Further studies could be focuses on the analysis of CHRNA4 RNA and protein in children with IGEs. The study may provide the basis for further survey of CHRNA4 polymorphisms.

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Table. Genotypes and allele frequencies for CHRNA4 (SNP1044396) polymorphisms in idiopathic generalized epilepsy (IGE) patients and normal controls

Genotype	IGE patients No.(%) n = 110	Normal controls No.(%) n = 80	Odds ratio (95% CI)	P
Genotype				
C/C	62(56.4)	49(61.3)	1.00	0.0001*
T/C	15(13.6)	26(32.5)	0.46(0.13-0.95)	
T/T	33(30.0)	5(6.2)	5.22(1.90-14.36)	
Allelic frequency				
Allele C	139(63.2)	124(77.5)	1.00	0.0041*
Allele T	81(36.8)	36(22.5)	2.01(1.27-3.18)	

* p-value were calculated by chi-square test

Abbreviation: CI = Confidence interval