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LACK OF ASSOCIATION BETWEEN CHRNA4 C.651 C>T GENE POLYMORPHISM AND GENETIC GENERALIZED EPILEPSY

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ABSTRACT

Background: The neuronal nicotinic acetylcholine receptor (nAChR) channels become more sensitive to acetylcholine due to mutations of $CHRNA_4$ gene, thereby channels open more easily resulting in ion flow across the cell membrane that leads to changes in signaling between neurons. The variants of the $CHRNA_4$ gene have been associated with several psychiatric and brain disorders including autosomal dominant nocturnal frontal lobe epilepsy.
Aim: The present study was aimed to evaluate the possible association of $CHRNA_4$ c.651 C>T gene polymorphism with genetic generalized epilepsy (GGE).
Methods: The genotyping of CHRNA4 gene polymorphism was performed using PCR-RFLP assay.
Results: In majority patients, GGE onset was <18 years (185 patients 61.6%) and remaining 115 patients (38.3%) had the onset above 18 years. The absence seizure and epilepsy type were predominantly seen in patients with <18 years age group (36.4% and 28.1% respectively) compared to >18 years group (2.6% and 6.08 respectively). However, the myoclonus seizures were more predominantly seen in patients with >18 years age group (80%) compared to < 18 years group (7.2%). Significant variation was observed with the frequency of tonic-clonic type seizures in <18 year age group as compared to >18 years group. The molecular analysis revealed no significant difference in the genotypic distribution as well as allelic frequency between patients and controls ($p>0.05$). The genotypes, CT and TT were not observed in controls [(TT vs. CC, $\chi^2=0.3$; $p=0.30$; T *vs* C, $\chi^2 = 17.23$; $p < 0.05$)].
Conclusion: No significant association was found between CHRNA4 c.651 C>T gene polymorphism with IGE in the South Indian population.

KEYWORDS*:* Genetic generalized epilepsy, CHRNA4 gene polymorphism, Neuronal nicotinic acetylcholine receptor, Absence seizures, Myoclonus

INTRODUCTION

Genetic generalized epilepsies (GGEs) (Erstwhile known as idiopathic generalized epilepsy-IGE) constitute roughly 1/3rd of all epilepsies affecting about 0.3% of the general population.[1,2] The acetylcholine receptor, neuronal nicotonic, alpha-4 subunit (CHRNA4) gene encodes the α-4 subunit of the neuronal nicotinic acetylcholine receptor (nAChR), that belongs to ligand-gated ion channels. The nAChR channels become more sensitive to acetylcholine due to mutations of CHRNA4 gene, thereby channels open more easily resulting in ion flow across the cell membrane that leads to changes in signaling between neurons. The variants of the CHRNA4 gene have been associated with several psychiatric and brain disorders including autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [3-5], attention deficit /hyperactivity disorders [6,7], Alzheimer's disease [8,9] and febrile seizures [10] etc. It has been suggested that the CHRNA4 gene polymorphisms confer susceptibility to the GGE syndromes. Therefore, the present study was carried out with an aim to evaluate the possible association of CHRNA4 C>T gene polymorphism with GGE in the South Indian population, India.

MATERIALS AND METHODS

Three hundred patients male: female (195:105) with GGE evaluated in the Neurology clinic of Nizam's Institute of Medical Sciences, Hyderabad were enrolled for study, with their written informed consent. The clinical diagnosis was made according to the admitted criteria by the International League Against Epilepsy by an expert epileptologist from Nizam's Institute of Medical Sciences, Hyderabad, on the basis of age of onset, predominant type of seizures being generalised seizures in addition to myoclonic seizures, absence seizures and on EEG findings. In addition patients had normal intelligence and the MRI Brain was normal. The seizures usually were infrequent. Classical

EEG findings were normal background with generalized spike and wave discharges typical of individual syndrome type and having photosensitivity and activation during hyperventilation. For childhood absence/juvenile absence epilepsy, patients had typical absences along with classical generalized spike and wave or polyspike and wave discharges of 3 Hz (2.5 to/4.5 Hz). For juvenile myoclonic epilepsy, the patients had myoclonic jerks with poly spike and wave discharges of [4.5 Hz frequency. For GGE, the discharges were 3–4.5 Hz frequency. Patients with major renal, hepatic, cardiac disease, hypoxic-ischemic encephalopathy, trauma, cerebrovascular disease/infection, congenital CNS abnormalities, metabolic disorders, CNS malignant tumors and other degenerative disorders were excluded from the study.

Three hundred healthy individuals matched for sex and age formed the control group. The subjects were included in the study with their written informed consent. The study was approved by the ethical committee of the study hospital. None of the controls had any history of cerebrovascular disease, CNS disorder or any other medical disorders. Demographic features including age, gender, duration of the disease, frequency of seizures and family history were collected by using a structured questionnaire.

The genomic DNA was extracted from blood collected in EDTA tubes, using standard phenol-chloroform method. The genotyping of CHRNA4 gene polymorphism was performed using PCR-RFLP assay. PCR was done using the forward (5'-CGG CTC CTG GAC CTA CGA C-3') and reverse (5'-GAT GAC CAG TGA GGT GGA CG-3') primers. The amplified PCR products of 373 bp were digested with *Cfo* restriction enzyme (Fermentas Fast digest) by incubating at 37^0 C for 8 min followed by separation of polymorphic fragments on 2.5% agarose gel. The rare (homozygous) 'T' allele lacks the *Cfo* restriction site, it was detected as a single fragment of 373 bp, whereas it was present in the (homozygous normal) 'C' allele and was detected as two fragments of 232 and 141 bps. The heterozygous 'CT' was detected as 3 fragments of 373, 232 and 141 bps.

Statistical analysis

Hardy-Weinberg Equilibrium was tested for the CHRNA4 gene polymorphism. The association between genotypes and GGE was examined by Odds ratio with 95% CI and Chi-square analysis, using open-epi 6 software (Open-epi version 2.3.1 from the department of epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA). Allelic frequencies were calculated according to the number of different alleles observed and total number of alleles examined.

RESULTS

The mean age of GGE patients was 24.7±15.4 years where as in controls it was 22.9±16.5. The ratio between male and female was 195:105 and the family history of GGE was observed in 23% of patients. The mean age (in years) of the patients with IGE for age group<18 years and healthy controls was 8.6±2.46 and 10.7±3.85; and for patients in the age group >18 years and controls was 39.8±11.4 and 38.0±10.6 respectively (Table1).

Out of 300 GGE patients, in majority the age of onset was <18 years (185 patients 61.7%) and remaining 115 patients (38.3%) had the onset above the age of 18 years. The mean time since diagnosis was 14.8 years. The type of seizures as well as epilepsy types in different age groups have been given in table 2.

The absence seizure and absence epilepsy type were predominantly seen in patients with <18 years age group (36.4% and 28.1% respectively) compared to >18 years group (2.6% and 6.08 respectively). Conversely, the myoclonus seizures were more predominantly seen in the patients with >18 years age group (80%) compared to < 18 years group (7.1%). Similarly significant variation was observed in the frequency of tonic-clonic type seizures in <18 year age group compared to >18 years age group (Table 2).

The genotypic distribution of CHRNA4 c.651 C>T polymorphism and allelic frequency of C and T in patients and controls have been given in table 3.

We did not find significant difference in the genotypic distribution between patients and controls ($p>0.05$). Similarly, no significant difference was noticed in the allelic frequencies between patients and controls ($p>0.05$). The genotypes, CT and TT were not observed in controls.

DISCUSSION

Epilepsy is a disorder of CNS, characterized by recurrent spontaneous seizures affecting about 0.3% of the general population. Patients with GGE have no underlying structural or brain lesions and are neurologically normal.[11] Acetylcholine, an important neurotransmitter plays an important role in the neurotransmission of peripheral as well as CNS. Acetylcholine is released not only into synaptic clefts but also at non-synaptic sites. Activation of pre-synaptic nicotinic receptors causes release of more neurotransmitter whereas post synaptic nicotinic acetylcholine receptors are associated with fast excitatory transmission. Non-synaptic nicotinic acetylcholine receptors bring about diffuse volume transmission that influences neuronal excitability and control over different neurotransmitter systems.[12] The α-4 subunit assembles with the β-2 subunit and constitutes the major high-affinity nicotinic receptor in the brain. Such receptors are widely expressed both in thalamus and cortex. [13]

Several studies have been carried out to evaluate the association between nAChR genes and nicotine dependence. Animal studies with knock-in mice have demonstrated that alteration in the AChR genes can dramatically change

nicotine sensitivity.[14] Scott et al., found that SNP representing CHRNB3, the β3 nicotine receptor subunit gene, has the strongest association with nicotine dependence.[15] This additive effect of nicotine can be mediated via α4β2 units of nAChR by releasing more mesolimbic dopamine leading to nicotine dependence.[16] In addition, nAChRs can mediate the neuronal mechanisms associated with development and maintenance of drug addiction by modulating the release of many other neurotransmitters.

The variants of the CHRNA2, CHRNA4 and CHRNB2 genes have been found to cause autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) by affecting the kinetics of ion pore. ADNFLEs are a group of idiopathic partial epilepsies characterized by brief violent motor seizures mostly occurring during non–rapid eye movement sleep.[17] Usually these affect childhood or adolescence age and have autosomal dominant type of inheritance. The mutations in CHRNA4 might result in either gain-of-function (increased affinity to acetylcholine) or loss-of-function (acceleration of desensitization and decreased calcium permeability). It has been reported that CHRNA4 gene mutation is associated with Autosomal Dominant Nocturnal frontal Lobe Epilepsy (ADNFLE) and it was the first observation that the genetic modification of ligand-gated channels can cause seizures [18] and which was confirmed by following studies.[19,20,21]

Apart from the mutations of CHRNA4 associated with ADNFLE, some of the polymorphisms of CHRNA4 also confer susceptibility to GGE subtypes especially Juvenile Myoclonic Epilepsy (JME).[22] Genes encoding the nAChR subunits have emerged as the candidates for idiopathic epilepsies. Significant evidence was found for linkage of JME to polymorphic loci encompassing the region on chromosome 15q14, in which the CHRNA7 is localized.[23,24] However, subsequently two independent studies have failed to confirm these findings.[25,26]

In the present study, we did not find a significant difference in the genotypic distribution as well as allelic frequency between patients and controls for CHRNA4 c.651 C>T gene polymorphism indicating that the polymorphism is not associated with GGE in the study population. Steinlein has studied the polymorphisms of CHRNA4 c.555C>T and c.594C>T, located in exon5, and two others, 1674+11C>T and 1674+14A>G, within intron5, associated with epilepsy and reported that only the c.594C>T variant was associated with IGE.[22] However, Chioza et al., (in a case-control, family-based study) and Agata et al., have not implicated the CHRNA4 c.594C>T polymorphism in susceptibility to epilepsy and no significant differences were found in T-allele distribution between the patients and the control group [27,28]. Though the CHRNA4 gene has been identified to be a candidate gene for IGE, Haider et al., reported that the missense mutation (Ser 248 Phe) in CHRNA4 gene was not found in Kuwaiti GGE children and also reported that the incidence of Fok I polymorphism in CHRNA4 gene was different from that reported in Caucasian population.[29] In 2007, Lee et al. evaluated the association of GGE with CHRNA4 and CHRNB2 polymorphisms and concluded that the CHRNA4 (Ser 543 Ser) variant may be one of the susceptibility factors for IGE.[30] In another study, Agata et al., concluded that 1674^{+11} C>T polymorphism may be a susceptibility factor for GGE, particularly the subtype JME [28] and also stated that the c.555 A>T polymorphism was not associated with GGE subtype, JME. In the present study, no significant association was found between CHRNA4 c.651 C>T gene polymorphism and genetic generalized epilepsy in the South Indian population, India. Apparently, more studies are needed to evaluate an association between CHRNA4 gene polymorphism and GGE in other ethnic groups.

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Table 1: Demographic profile of IGE patients and controls

Demographic features	**Patients (n=300)** (Mean±SD)	**Controls (n=300)** (Mean±SD)
Age in years (4-69)	24.7±15.4	22.9±16.5
<18 years (n=185)	8.6±2.46	10.7±3.85
>18 years (n=115)	39.8±11.4	38.0±10.6
Gender (male: female)	195 : 105	203 : 107
Family history (%)	23 %	2 %

Table 2: Distribution of seizure and epilepsy types in different age groups IGE patients

<18 years age group IGE patients (n=185)		**>18 years age group IGE patients (n=115)**	
Seizure type	**Epilepsy type**	**Seizure type**	**Epilepsy type**
Absence 71 (36.4%)	Absence 52 (28.1%)	Absence 3 (2.6%)	Absence 7 (6.08%)
Myoclonus 14 (7.17%)	Myoclonic 71 (38.4%)	Myoclonus 92 (80%)	Myoclonic 36 (31.3%)
Tonic clonic 181 (92.8%)	TC seizures only 62 (33.5%)	Tonic clonic 112 (97.3%)	TC seizures only 72 (62.6%)

Table 3: Distribution of CHRNA4 genotypes and allelic frequencies of the study population

Genotypes	**CC**	**CT**	**TT**	**Total**	**C**	**T**	**Total**
Patients (%)	284 (94.7%)	15 (5.0%)	1 (0.3%)	300	583 (0.9)	17 (0.1)	600
Controls	300	0	0	300	600	0	600

For TT vs CC, χ^2 =1.053; p=0.3; OR-unidentified
TT vs CC+CT, χ^2 =1; p=0.3; OR-unidentified
T vs C, χ^2 = 17.23, p<0.05; OR-unidentified