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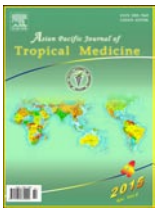

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Analysis of the *CHRNA7* gene mutation and polymorphism in Southern Han Chinese patients with nocturnal frontal epilepsy

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ABSTRACT

Objective: To detect the *CHRNA7* gene mutation and polymorphism in Southern Han Chinese patients with nocturnal frontal lobe epilepsy (NFLE). **Methods:** Blood samples were collected from 215 Southern Han Chinese patients with NFLE and 200 healthy Southern Han Chinese control subjects. Genomic DNA was extracted, and CHRNA7 whole genome exons were amplified by the polymerase chain reaction and subjected to Sanger sequencing. **Results:** No *CHRNA7* gene mutation was detected in all of the NFLE patients. However, five single nucleotide polymorphisms (SNPs) in sporadic cases were found, located in exons 5, 6, and 7 of the *CHRNA7* gene. Among them, c.690G>A and c.698A>G are known SNPs, while c.370G>A, c.654C>T, and c.497-498delTG were newly discovered SNPs. These SNPs were also found in some of the healthy controls. **Conclusions:** No *CHRNA7* gene mutation was identified in Southern Han Chinese patients with NFLE. The *CHRNA7* gene is probably not responsible for NFLE in this population.

1. Introduction

Nocturnal frontal epilepsy (NFLE) is idiopathic partial epilepsy, mainly characterized by clusters of frequent transient motion nocturnal seizures. NFLE occurs mostly at or shortly before awakening after sleep, and rarely during the daytime. Neuroimaging and interictal electroencephalography (EEG) examinations show no abnormalities in NFLE patients, so they are often misdiagnosed as having sleep disorders. In 1994, Scheffer *et al* first reported NFLE pedigrees; they showed that there was autosomal dominant inheritance in the pedigrees, which they named autosomal dominant nocturnal frontal epilepsy (ADNFLE)[1]. In the clinic, sporadic cases of ADNFLE are more common, and only 25% of NFLE patients have a family history. Notably, sporadic cases and cases with a family history of NFLE have similar clinical and EEG features[2].

Over the past decade, molecular genetics studies have demonstrated that neuronal nicotinic acetylcholine receptor (nAChR) is closely related to the pathogenesis of ADNFLE[2]. To date, the nAChR α 4, β 2, and α 2 subunits, encoded by the genes *CHRNA4*, *CHRNB2*, and *CHRNA2*, respectively, have been identified[3-5]. However, due to the high genetic heterogeneity of ADNFLE, mutations in the *CHRNA4*, *CHRNB4*, and *CHRNA2* genes are only found in approximately 10% of ADNFLE patients. Therefore, it is speculated that genes encoding other nAChR subunits may be responsible for NFLE.

In our previous studies, we could not detect mutations in CHRNA2, CHRNB2, or CHRNA4 in 215 Southern Han Chinese patients with sporadic NFLE[6,7]. In this study, we employed the

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same subjects to screen mutations in the *CHRNA7* gene, encoding the nAChR α7 subunit, in order to identify a new gene responsible for NFLE in the Chinese Han population.

2. Materials and methods

2.1. Subjects

A total of 215 sporadic cases of NFLE were collected from Guangdong, Hunan, Jiangxi, and Sichuan between 2006 and 2012. All of them were Han Chinese, the male:female ratio was 1.2:1, and the average age of onset was (10.71±0.62) years old. A detailed questionnaire regarding patient history was completed, and physical examination, long-range video EEG, brain magnetic resonance imaging, and other tests were performed.

All patients were diagnosed in strict accordance with the classification criteria for the diagnosis of epilepsy and epilepsy syndrome by the 2010 International League Against Epilepsy (ILAE)[8], At least 90% of the patients had clinical episodes during the night. The patients were diagnosed as having NFLE based on the clinical manifestations and laboratory examinations. The neurological examination was normal, and the family history was negative.

As the control, 200 healthy Han Chinese were studied. All subjects or their legal guardians signed an informed consent form, and the protocol was approved by the Ethics Committee of Guangdong Provincial People's Hospital.

2.2. DNA Extraction

Blood samples (4 mL) were collected from 215 sporadic ADNFLE patients and 200 healthy controls and put into EDTA anticoagulant tubes. Genomic DNA was extracted using a DNA extraction kit (QIAampdna Kit, QIAGEN, Shanghai, China).

2.3. Polymerase Chain Reaction (PCR)

PCR was performed in a total volume of 25 μL, containing 13.9 μL of ddH_2O, 5 μL of betaine, 2.5 μL of 10× Buffer, 2 μL of 1×dNTP, 0.3 μL of Taq enzyme (50 U/μL), 0.3 μL of primers (50 M), and 1 μL of genomic DNA (15 ng/μL), on an ABI9700 instrument (Applied Biosystems, USA). The primers for all exons of CHRNA7 were designed using Primer 3.0 online software with the following sequences:

exon1 forward 5' gacagccgagacgtggag 3' and reverse 5' tgagtggttgcgagtcattg 3'

exon 2 forward 5' tgcttgtctgggctgcac 3', and reverse 5' tagcttggggccaactagag 3'

exon 3 forward 5' attggaagtgcttggtgcat 3', and reverse 5' tcttgtgcatgtgttgagca 3'

exon 4 forward 5' ttctctttggttttgcacttacc 3', and reverse 5' catatccagcatctctgtgaaa 3'

exon 5 forward 5' ggcccctctcaaggtcttt 3', and reverse 5' gaagcaaagagctggtccac 3'

exon 6 forward 5' tccatttgctctggactgtg 3', and reverse 5' caccggaaaggacagtgg 3'

exon 7 forward 5' aggactgtgcttgtgtgtgg 3', and reverse 5' ccaggaaccctgatggagt 3'

exon 8 forward 5' aatgcacacgcccatacata 3', and reverse 5' cagcaaatcatcaccttcca 3'.

The cycling program was as follows: initial denaturation at 95 ℃ for 10 min; 38 cycles of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 30 s, and extension at 72 ℃ for 40 s; and then a final extension at 72 ℃ for 10 min. PCR products were detected under UV light by 1.5% agarose gel electrophoresis with ethidium blue staining.

2.4. PCR product purification

The PCR product (2 μL) was mixed with 5 μL of purification mix containing 4.71 μL of ddH_2O, 0.25 μL of *Exo* Ⅰ, and 0.04 μL of FaSAP; and the mixture was incubated at 37 ℃ for 60 min and at 80 ℃ for 15 min.

2.5. Sequencing

Sequencing was performed in a 5-μL system containing 2 μL of purified PCR product, 2 μL of forward or reverse sequencing primer (1.6 M), and 1 μL of BigDye Terminator reaction solution (Applied Biosystems Company) on an ABI3730 sequencer. The cycling program was as follows: 96 ℃ for 1 min; 28 cycles of 96 ℃ for 20 s, 50 ℃ for 10 s, and 60 ℃ for 3 min; and then 15 ℃ for 10 min. The sequencing results were analyzed using DNA Sequencing Analysis 5.1.1 software, with the GenBank sequence for the *CHRNA7* gene as the standard.

3. Results

We screened all exon sequences of CHRNA7 in the 215 sporadic NFLE patients and failed to detect any gene mutations. Instead, we found five different SNPs in sporadic cases, which were located on exons 5, 6, and 7 of the *CHRNA7* gene (Table 1, Figure 1-5). Among them, c.690G>A and c.698A>G are known, while c.370G>A, c.654C>T, and c.497-498delTG were newly discovered SNPs. These SNPs were also found in some of the healthy controls.

Table 1

SNPs of the *CHRNA7* gene in sporadic ADNFLE patients

Base change	Exon	Amino acid change	acidrs number
c.370G>A	5	Ala→Thr	-
c.497_498delTG	6	Leu→Gln	-
c.654C>T	7	Pro	-
c.690G>A	7	Thr	rs201822909
c.698A>G	7	Tyr→Cys	rs142728508

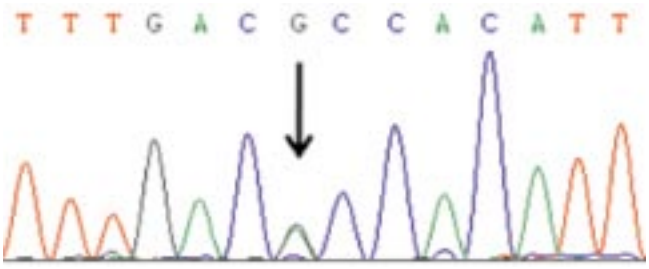

Figure 1. Sequence of c.370G>A in the *CHRNA7* gene of a sporadic ADNFLE patient.

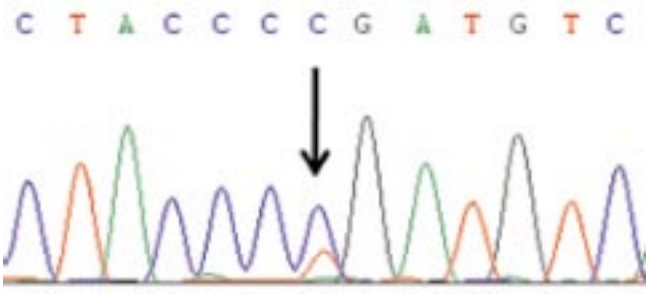

Figure 2. Sequence of c.497_498delTG in the *CHRNA7* gene of a sporadic ADNFLE patient.

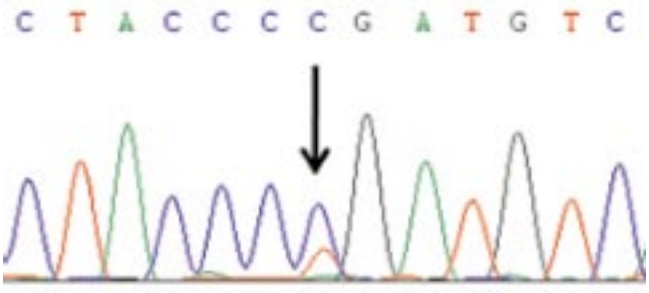

Figure 3. Sequence of c.654C>T in the *CHRNA7* gene of a sporadic ADNFLE patient.

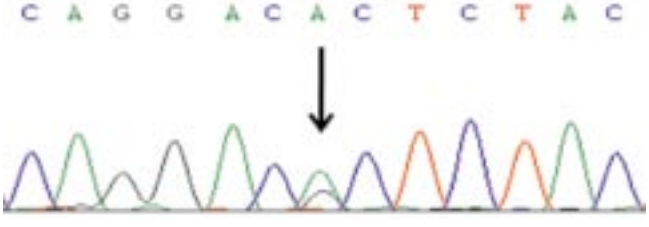

Figure 4. Sequence of c.690A>G in the *CHRNA7* gene of a sporadic ADNFLE patient.

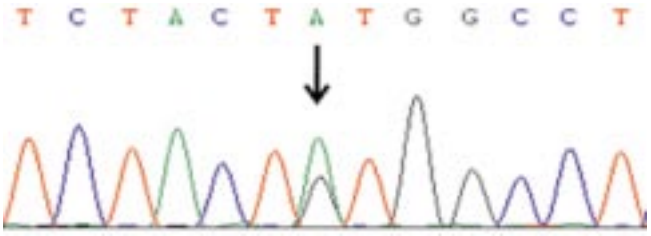

Figure 5. Sequence of c.698G>A in the *CHRNA7* gene of a sporadic ADNFLE patient.

4. Discussion

ADNFLE is the first identified gene whose mutations are implicated in familial idiopathic partial epilepsy. A lot of studies have suggested that ADNFLE is highly correlated with the *nAChR* gene mutation, but there are racial and geographical differences due to significant genetic heterogeneity of the *nAChR* genes[2]. Because sporadic cases and familial cases of NFLE have a similar incidence, clinical presentation, EEG features, and treatment outcomes, sporadic cases are likely to have the same genetic causes as familial cases. However, the vast majority of *nAChR* gene mutations have been reported only in a small number of familial cases but rarely found in sporadic cases[9]. The molecular genetics of ADNFLE has mainly focused on the genes *CHRNA4*, *CHRNB2*, and *CHRNA2*, while genes encoding other *nAChR* subunits remain uncharacterized.

nAChR is an important synaptic signaling ligand-gated ion channel protein, consisting of more than 10 subunits to form a receptor superfamily. α 4 β 2 and α 7 are the main nAChR receptor subtypes distributed in the central nervous system[10]. Among the nAChR receptor subtypes, α 7nAChR is a unique subtype that consists of five identical subunits, has a very high permeability for calcium ions, and regulates calcium activation and the release of the neurotransmitter acetylcholine[11]. The *CHRNA7* gene, which encodes the α 7 subunit, is located on chromosome 15q14. In the nervous system, α 7nAChR is mainly distributed in the hippocampus, the medial and lateral geniculate nucleus, and the thalamic reticular nucleus, which are implicated in the pathophysiology of epilepsy[12]. In addition, α 7nAChR is expressed in peripheral $CD4^+$ T lymphocytes, bronchial epithelial cells, and macrophages. α 7nAChR regulates neuronal excitability to maintain normal behavioral responses, and participates in the release of neuropeptides and the inhibition of inflammation. The *CHRNA7* gene has been implicated in a variety of neurological disorders such as epilepsy, Alzheimer's disease, and schizophrenia[13]. Moreover, Helbig *et al* have proposed that CHRNA7 may contribute to the pathogenesis of idiopathic generalized epilepsy[14].

In previous studies, we examined the mutations of the genes *CHRNA4*, *CHRNB2*, and *CHRNA2* in 215 patients with sporadic NFLE, and we found no known mutations in these genes[6,7].

Therefore, in this study, we focused on the *CHRNA7* gene as a candidate gene. After sequencing all exons of the *CHRNA7* gene in sporadic NFLE patients, we found no mutations but five SNPs in exons 5, 6, and 7. Two of them (c.690G>A and c.698A>G) are known SNPs, while the other three (c.370G>A, c.654C>T, and c.497-498delTG) are newly discovered SNPs. It has been reported that the "repeat α7nAChR" gene *CHRFAM7A* exists in peripheral lymphocytes[15]. However, Gault *et al* have found that in CHRFAM7A, a polymorphism (c.497-498delTG) could cause a reading frame shift, resulting in a stop codon in exon 6 and dysfunction of the truncated α7 subunit[16]. The CHRFAM7A polymorphism c.497-498delTG is likely to be a risk factor for idiopathic generalized epilepsy[17]. In this study, we found a deletion variant (c.497_498delTG) in the *CHRNA7* gene in both patients and controls, indicating that it is a SNP but not a mutation. Further studies are necessary to understand whether the amino acid deletion variant has any impact on the transcription and/or function of the nAChR receptor, thus contributing to the pathogenesis of ADNFLE.

In summary, in the present study, we found no mutations but five SNPs in the *CHRNA7* gene in the Southern Han Chinese population. We speculate that the *CHRNA7* gene is probably not the major gene responsible for NFLE in this population. Notably, we identifed three new SNPs (c.370G>A, c.654C>T, and c.497_498delTG), providing candidate sites for subsequent gene mapping and gene function studies on epilepsy.

Conflict of interest statement

We declare that we have no conflict of interest.

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