Int J Clin Exp Med 2015;8(6):9063-9070
www.ijcem.com /ISSN:1940-5901/IJCEM0007271

Original Article

Mutational analysis of CHRNB2, CHRNA2 and CHRNA4 genes in Chinese population with autosomal dominant nocturnal frontal lobe epilepsy

Zhihong Chen[1*], Lingan Wang[2*], Chun Wang[1], Qian Chen[1], Qiongxiang Zhai[1*], Yuxiong Guo[1], Yuxin Zhang[1]

[1]Department of Pediatrics, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangdong Academy of Neuroscience ,Guangzhou 510080, China; [2]Southern Medical University, Guangzhou 510515, China. [*]Equal contributors.

Received February 23, 2015; Accepted June 10, 2015; Epub June 15, 2015; Published June 30, 2015

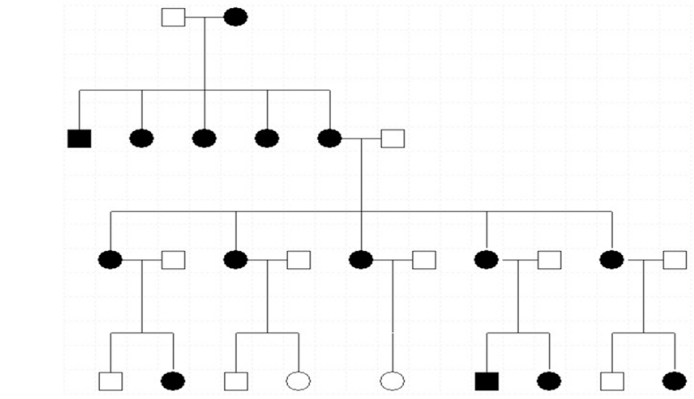
Abstract: Objective: The present study aims to investigate the gene mutations of CHRNB2, CHRNA2 and CHRNA4 in Chinese population with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). Methods: 257 ADNFLE patients (74 sporadic and 32 familial) were collected, including 42 pedigree patients and 215 sporadic cases. Exon mutational screening of CHRNB2, CHRNA2 and CHRNA4 was performed by direct PCR sequencing. Results: No published mutations of CHRNB2, CHRNA4 and CHRNA2 genes were detected in this study. Three kinds of c.SNP (c.66C> T, c.249C> T, c.375A> G) were detected on the 2nd and 5th exons of CHRNA2; six kinds of c.SNP (c.639T> C, c.678T> C, c.1209G> T, c.1227T> C, c.1659G> A, c.1629C> T) were detected on the 5th exon of CHRNA4. Three novel mutations were discovered, respectively locating on the exon 5 of CHRNA4 gene (c.570C> T), 5th and 6th exons of CHRNB2 gene (c.483C> T and c.1407C> G). The three mutations were absent in 200 healthy controls, indicating that the mutations were very rare. Conclusion: CHRNA4, CHRNB2 and CHRNA2 may be not the causative genes of Chinese ADNFLE population. Whether the three novel synonymous mutations were genetic factors of ADNFLE pathogenesis in Chinese Han population needs to be further studied.

Keywords: Nocturnal frontal lobe epilepsy, CHRNB2, CHRNA2 gene mutation

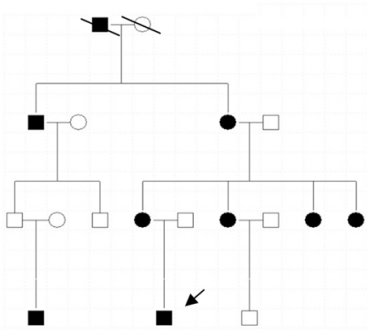
Introduction

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a kind of nocturnal frontal lobe epilepsy [1]. The clinical characteristics are nocturnal seizures, cluster seizures, and transient rolandic epilepsy, with repeated awakening and clustered rolandic seizures in nighttime sleep. The disease is usually onset in childhood, sustaining to adulthood. Neuroimaging structural inspection and interictal EEG are often normal [2-4].

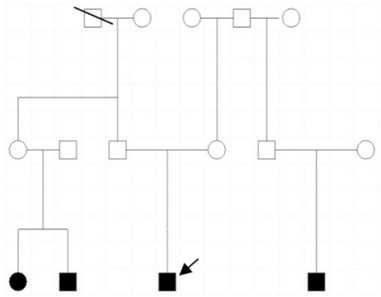
ADNFLE genetic penetrance is mostly 70-80% or lower in most knowns [5-7], which was also found in sporadic cases. Sporadic cases and pedigree patients have similar clinical and EEG features [5-7]. Molecular genetic studies indicated that neuronal nicotinic acetylcholine receptor (nAChR) was closely related to ADNFLE pathogenesis, and 11 pathogenic mutations of three nAChR subunits (CHRNA4, CHRNB4, CHRNA2) had been identified. Since Steinlein [8] found that the pathogenic gene of an Australia pedigree was located on 20q and first discovered mutations in CHRNA4 in 1995, it has been found five kinds of CHRNA4 gene mutations in nine pedigrees of different races and two sporadic cases, including three kinds of missense mutations: S252F (Australia [8, 9], Spain, Norway [10], Scotland [11]), S256L (Japan [12], South Korea [13], Poland [14], a sporadic case of Lebanon, [15]), T265I (Germany [16]), R308H (China, a sporadic case [17]); and one kind of insertion mutations: 263insL (Norway [18]). CHRNB2 is the second identified ADNFLE disease gene, and five kinds of missense mutations were found in eight pedigrees: V287L (Italy [19]), V287M (Scotland [20], Spain [21]), I312M (Switzerland [22], South Korea [23]), L301V (Turkey [24]), V308A (Scotland [24], England [24]). In recent years, I279N missense mutation of CHRNA2 gene located on the 6th exon was found again in an



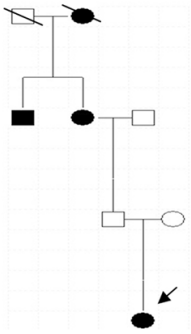
Pedigree A



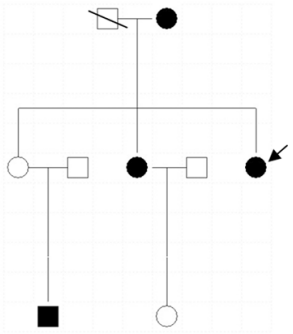
Pedigree B



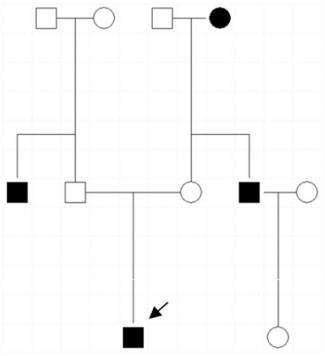
Pedigree C



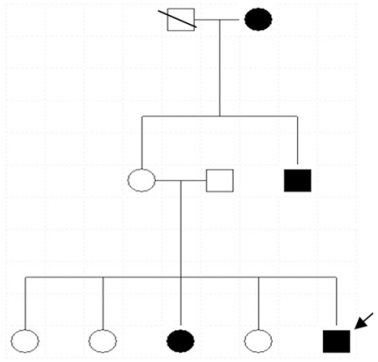
Pedigree D



Pedigree E



Pedigree F



Pedigree G

Italian family [25]. More and more studies showed that most of the above mutations mainly affected the hydrophobic transmembrane region of TM1-3 encoding nAChR, changing the function of ion channels to cause seizures [3, 4].

Despite sporadic cases and family cases were guessed to have the same genetic background, the majority of known nAChR gene mutations existed only in a minority of pedigree patients. However, so far there are no clear mutations of CHRNB2 and CHRNA2 found in sporadic cases, and there have been CHRNA4 gene mutations found in two sporadic cases. One case is the Lebanese sporadic patient carrying S256L [15], and the same mutation and disease were also found in his son, so sporadic cases can possibly evolve into family cases. In recent years, Yan Chen et al [17] firstly reported one sporadic case and his healthy mother carrying R308H mutation in CHRNA4 gene. However, ADNFLE pedigrees were rare in China and most were sporadic cases; the relevant domestic molecular biology research was still weak, lacking large sample ADNFLE gene screening studies. Whether genetic background of Chinese ADNFLE population is consistent with abroad is worthy of further discussion.

In this study, we collected a total of 257 cases of Han ADNFLE patients, including 215 sporadic cases and 42 pedigree patients from 11 unrelated familial, and mutation screening of CHRNA4,

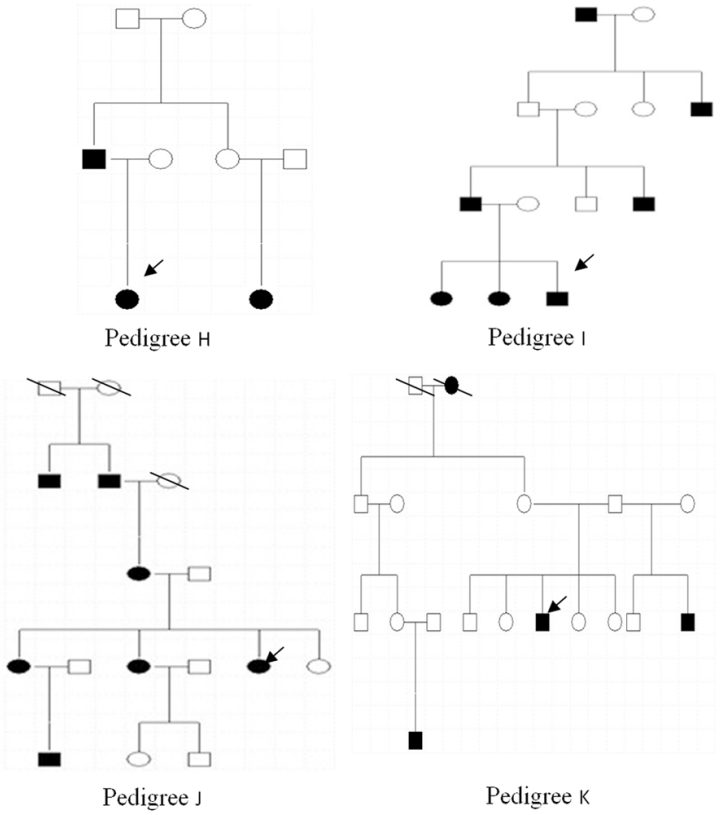


Figure 1. ADNFLE genetic pedigree chart. Arrow: Proband; Blank box: Male normal member; Black circle: Female patients; Blank circle: Female normal member. Slash indicated died.

CHRNB2 and CHRNA2 genes was conducted by direct PCR sequencing.

Subjects and methods

Subjects

257 ADNFLE patients were collected from January 2006-December 2011 in Guangdong Provincial People's Hospital, including 42 existing patients in 11 pedigrees and 215 sporadic cases. All patients were Han population. The control group included 200 cases of healthy Han individuals in the same region. Detailed clinical data of all patients were collected; the dynamic long-range EEG and head MRI were conducted. Diagnostic criteria were strictly in accordance with "epilepsy and epilepsy syndrome diagnostic criteria" proposed by the International League against Epilepsy in 1981 and 1989. The family history was dated back to more than three generations; each family included in the study had at least three patients, which with a single patient was considered as sporadic cases. All subjects or their legal guardians were required to sign an informed consent; the entire research was approved by the Ethics Committee of Guangdong Provincial People's Hospital.

In this study, 42 patients in 11 ADNFLE pedigrees were collected (**Figure 1**), including 11 cases of probands, male: female 1:1, with a average age of 5.5 ± 0.5 years old, patients per pedigree ≥ 3; Simple segregation analysis showed that the genetic pattern belonged to autosomal dominant inheritance. A total of 215 sporadic cases were selected, male:female 2:1, with an average age of 12.7 ± 0.8 years old. The clinical manifestations of all patients met the diagnostic criteria of nocturnal frontal lobe epilepsy, mainly performing as nocturnal sudden eyes, sat up, violent overactivity, incongruity movement of limb and trunk like dystonia and dyskinesia, abnormal posture, walk, fear and involuntary sound. Some patients may have secondary generalized seizures. Different seizure types existed in the pedigree patients, a few with automatism. EEG occurred in the second stage of non-REM phase, and abnormal EEG was mostly caused by unilateral or bilateral frontal epileptiform activity or increased limited slow wave. Antiepileptic drugs, such as carbamazepine, oxcarbazepine, valproate and topiramate, had good therapeutic effect on most patients.

Methods

DNA isolation: Peripheral blood of 257 patients and 200 controls were collected for genetic analysis. After EDTA anticoagulation, DNA was isolated using DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN, Shanghai, China).

Table 1. The primers sequences of CHRNA2, CHRNA4, and CHRNB2 genes

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Temp (°C)
CHRNA2	GGACCCAGGACCCAGAAC	CAGCCTGGCTTGGAGATTC	57
	GGTTTCACCTGCAGAATCG	TAGGAGCAGGTGACATCCAG	57
	GGCCTTTTGCTCTCATTTCA	CAACACATCCACCTGCAGAC	57
	TGCTGTGCTCAGCTCTGATGA	AAGTCACTGCTGTGCGTGAG	57
	GTAAGGGTGGCAGGTTTCAG	TGCTGTTCCTTGGTCTTTCA	57
	TACTTGCTCAGAGCCCGTTC	GAGGCGAGGAAGCTGACAC	57
	CGGGGGAGACTTACACACTT	GAAGACGGTGAGTGACAGCA	57
	GAGCAGACTGTGGACCTGAA	CACATGCCCATCTGTCCTC	57
	ATCTTCGTCACCCTGTCCAT	TCCATCTAAGGACCATGACTGA	57
	CTTTGCTCCCCAAGGTCTCT	TTGAGGTCTGCATTCCCTTC	57
CHRNA4	GGTGCGTGCGCCATGGAGC	GCAGTCAGGAGCCTGCCTC	71.3
	ACCTGAGCCACTGGCCTGCC	CGACCTCAGTCACAGCGCAC	60
	CCCGTCCACCATATCTTGC	GGCAGTGCCCTCCCACTC	60
	CATCCAGGAGTGGCACGACTA	ACCAAGGCCCTGTAGAGGAC	60
	ACCCTTCGCTCTCTTCCTGC	CCACGATGACCCACTCGCC	60
	CGGCTCCTGGACCTACGAC	CAGGCAGGGGATGATGAGG	60
	GGCGAGTGGGTCATCGTGG	GATGACCAGTGAGGTGGACG	60
	CCTGCCCTCCGAGTGTGGC	GGGCATGGTGTGCGTGCGTG	60
	ACGCACGCACACCATGCCC	GCGGCAGGGTCCAGGCGAG	71.3
	CCTTCCTGCAAGTCACCCTCC	GTGCTTTGGTGCTGCGGGTC	56.9
	AAGGAGCCCTCTTCGGTGTC	CCCAAAGCGAAGCAGCCTGA	64.5
	GGCCGTGCTGGAGTGACG	GCCCCACAGAGTCCAGGG	60
CHRNB2	GAGGCAGCGAGCTATGCCCG	GCGGCGAGTCTTGGGCCGT	60
	GGATTGTCCCATTTGCCTG	ACCTTTCTGCCTGTTTGAG	56.9
	TTTCCTTAACCTTGAGCCCC	CTTATTTTCCCCAGAACCCC	60
	AGGGCTGACTGTGCCCATC	GTCCAGGCTGGCCACCTCA	60
	CACAGAGATCGACTTGGTGCT	TAGAAGACAAGGATGGCTAGCG	60
	TATGACTTCATCATTCGCCGC	ACGAGCGGCACGTCGAGG	60
	TCCAAGATCGTGCCTCCCAC	AGCAGCGCGGGCAGCTTC	60
	TCCAAGATCGTGCCTCCCAC	GGTCCCACCGTGCGGCACT	60
	CTCGTTTGTCTCCCATCCTG	TCCACCCAACACTACTGTGC	60

Primers: The primers were designed to amplify the exon regions of CHRNB2, CHRNA4 and CHRNA2 (**Table 1**). All primers were synthesized by Shanghai Sangon Biotechnology Company.

PCR reaction: The reaction system was totally 25 μL, including ddH_2O 18.7 μl, upstream and downstream respectively 0.5 μl, 1 × dNTP 2 μl, Taq enzyme 0.3 μl (50 U/μL), 10 × Buffer (containing 20 nmol $MgCl_2$) 2.5 μl and DNA 1 μl (30 ng/μL); PCR instrument was ABI9700 (Applied Biosystems, USA). The reaction program: predenaturation at 96°C for 5 min, denaturation at 96°C for 30 s, annealing at 56-71.3°C for 30 s (primer conditions were shown in **Table 1**), extension at 72°C for 40 s, totally 30 cycles, then extension at 72°C for 10 min, finally 4°C for preservation. After 2% agarose gel electrophoresis and EB staining, the PCR product bands were detected under UV light.

Sequencing analysis: The sequencing kit of Applied Biosystems (BigDye® Terminator v3.1) was used; 5 μl reaction system contained the purified PCR product 2 μl, unidirectional sequencing primer (1.6 pmol) 2 μl, BigDye Terminator reaction solution 1 μl. The reaction program: denaturation at 96°C for 10 s, annealing at 55°C for 5 s, extension at 60°C for 4 min, totally 25 cycles, 4°C for preservation. Product sequencing was performed by the 3730XL sequencer (ABI, USA); the results were interpreted by Chromas 1.62 package and compared with the standard sequences of corresponding genes in NCBI GenBank database (http://www.ncbi.nlm.nih.gov/).

Results

CHRNB2 mutation screening

In the 257 cases of patients in this study, there were 42 pedigree patients and 215 sporadic patients; all had completed CHRNB2 exon sequence screening; no reported missense mutations of CHRNB2 (V287L, V287M, I312M, L301V, V308A) were detected. We had not found nucleotide mutations in the pedigree patients, while H161H (c.483C> T), a new CHRNB2 synonymous heterozygous mutation, was found in a sporadic case (**Figure 2**). H161H (c.483C> T) located on the 5th exon of CHRNB2 gene, and did not cause changes in the 161th

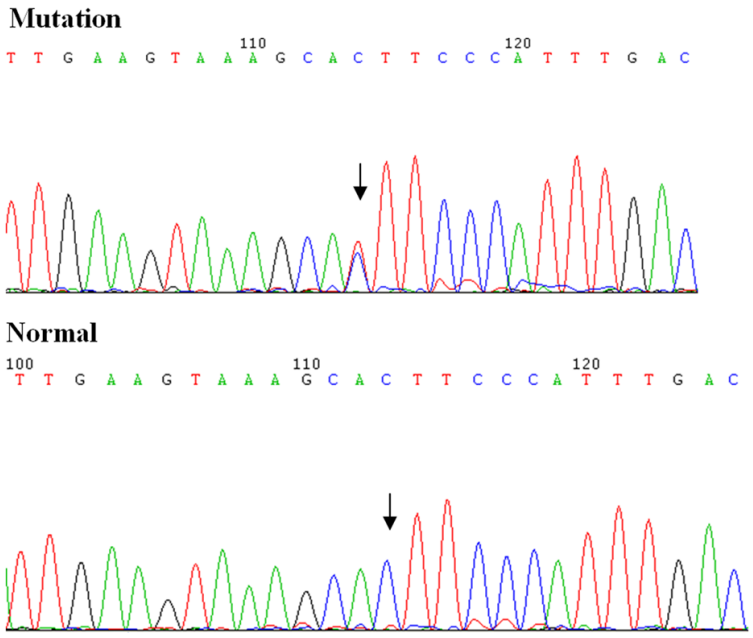


Figure 2. Sequence results of H161H (c.483C> T) mutation in CHRNB2 gene. Arrow: mutation locus.

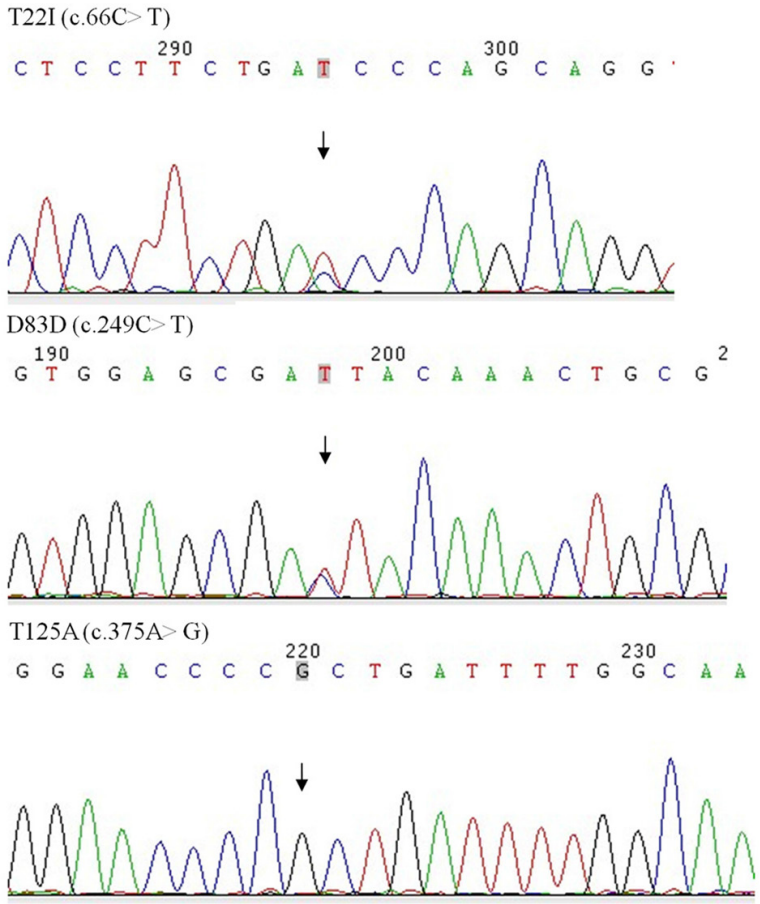


Figure 3. Sequence results of three mutations in CHRNA2 gene. Arrow: mutation locus.

amino acid (His) of the coding region.

CHRNA2 mutation screening

Three kinds of c.SNP (c.66C> T, c.249C> T, c.375A> G) were detected on the 2nd and 5th exons of CHRNA2, shown in **Figure 3**.

CHRNA4 mutation screening

In all cases, no reported mutations of CHRNA4 (S248F, S284L, 263insL, T265I) were found; six kinds of c.SNP (c.639T> C, c.678T> C, c.1209G> T, c.1227T> C, c.1659G> A, c.1629C> T) were detected on the 5th exon of CHRNA4. In one patient in a new synonymous mutation of CHRNA4 (c.570C> T) was discovered, shown in **Figure 4**; this change was located on the 5th exon of CHRNA4, which did not cause a change in the encoded amino acids (D190D), and was negative in 200 healthy controls.

Discussions

ADNFLE is the firstly discovered familial, idiopathic and partial epilepsy with clear genetic mutations. In the last ten years, more than a hundred pedigree cases have been reported in order. The relationship between ADNFLE clinical phenotype and gene phenotype is not clear, and genetic heterogeneity of ADNFLE exists in patients of different races with the same clinical phenotype. Among or within families, the complexity and severity of clinical manifestations vary widely, and phenotypes vary widely in different individuals of the same family with the same mutation; while the mutant genes only exist in a small portion of the families.

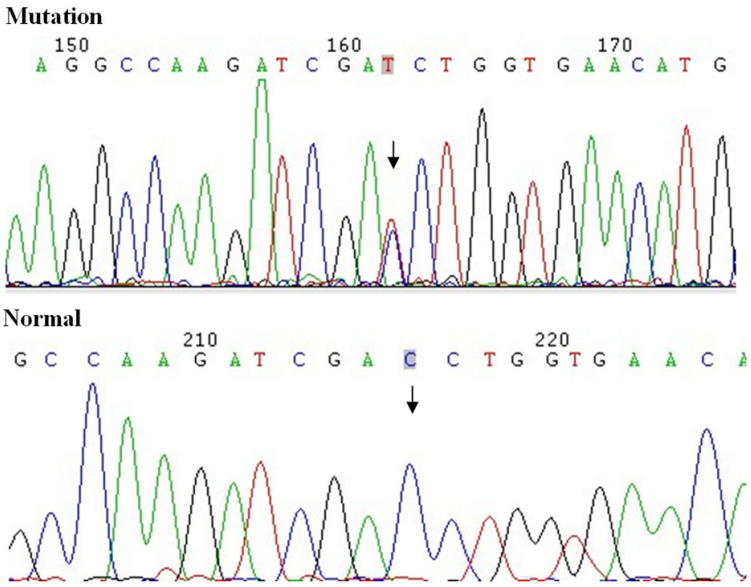


Figure 4. Sequence results of a novel mutations in CHRNA4 gene. Arrow: mutation locus.

Currently known CHRNA4, CHRNB2 and CHRNA2 mutations can only explain less than 10% of ADNFLE cases [2]; the majority of known nAChR gene mutations existed only in pedigree patients, and more than half of gene mutations can only be found in single-race pedigrees.

nAChR is a ligand-gated channel, which is a rose-like heterogeneous pentamer surrounded by five subunits; Each subunit consists of four hydrophobic transmembrane segments (TM1-TM4); binding sites of acetylcholine locate at the N-terminal of α subunit protruding outside of the plasma membrane; TM2 hydrophobic segments constitute the inner wall of ion channel to form the pore canal, involving in the ion channel composition and determining the ion-selective of the receptor and the energy level of allosteric transition (**Figure 4**). Presently known CHRNB2 mutations were found only in few pedigree cases, which were located on the 5t exon, mainly in the hydrophobic transmembrane region of TM2 or TM3 encoding the receptor. Electrophysiological studies have shown that the increased sensitivity of mutant receptor to acetylcholine, desensitization delay of ion channels and calcium dependent decrease of receptor may cause seizures [3, 4].

CHRNB2 mutations haven't reported yet in sporadic cases, which were found only in a small number of family cases. We screened the CHRNB2 exon sequences of all ADNLE patients in this study and failed to find the five known mutations of V287L, V287M, I312M, L301V and V308A. However, a new H161H synonymous mutation (c.483C> T) and SNP (c.1407C> G) were respectively found in two sporadic cases, which were not reported in domestic and abroad. The sporadic patients carrying H161H heterozygote mainly performed as frequent nocturnal motor seizures with automatism, and EEG prompted frontal discharge. The mutation was also found in their healthy mothers at the same time, but absent in the subsequent 200 cases of healthy controls, indicating that the mutation was very rare. H161H located on the 5th exon of CHRNB2, and it was in the N-terminal domain of the receptor rather than in the TM1-4. Although CAC and CAT both encoded histidine (His) and did not change the amino acid sequences of CHRNB2, whether they affect on the nAChR function and mRNA transcription remains to be further studied. The discovered c.1407C> G in the other sporadic case also was heterozygous mutation, locating on the 6th exon of CHRNB2 gene, without amino acid changes (Val); so we considered it as a single-nucleotide polymorphism change rather than potential ADNFLE pathogenic factor.

CHRNA2 is the newly discovered third pathogenic candidate nAChR subunit gene; heterozygous missense mutation of I192N was only found in an Italian family. I192N located on 6th exon of CHRNA2, affecting TM1 (a key function region of receptor); it has been confirmed to enhance the sensitivity of receptor to the acetylcholine like CHRNA4 and CHRNB2. TM1-4, encoding the receptor transmembrane regions, mainly located on exons 6 and 7 of CHRNA2 gene; in this study, we focused on the screening of the above sequences, however, whether in family or sporadic cases, mutations were not found, which was consistent with the reported multi-sample screenings of CHRNA2 mutations

in Germany and Italy populations in recent years [26, 27].

In summary, although clinical phenotype of patients in this study was similar with the majority of reported ADNFLE and the result was same as previous study. We failed to find the known mutation of CHRNB2 and CHRNA2, indicating that CHRNB2 and CHRNA2 were probably not a major causative gene in southern China ADNFLE patients, which further confirmed that ADNFLE had significant genetic heterogeneity. We have discovered synonymous mutation H161H (c.483C> T) in a sporadic case, but whether it will change the function of receptor and cause disease still need to be confirmed by further studies.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qiongxiang Zhai, Department of Pediatrics, Guangdong General Hospital, Guangdong Academy of Medical Sciences, 106 Zhongshan Second Road, Guangzhou 510080, China. Tel: +86-13719236388; Fax: +86-020-8328480; E-mail: zhaiqiongxiang@sina.com

References

[1] Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann F, Andermann E, Desbiens R, Cendes F, Manson JI. Autosomal dominant frontal epilepsy misdiagnosed as sleep disorder. Lancet 1994; 343: 515-7.

[2] Shi XB, Lang SY. Autosomal dominant nocturnal frontal lobe epilepsy. Zhonghua Shenjing Yi Xue Za Zhi 2006; 5: 1184-1188.

[3] Steinlein OK, Bertrand D. Neuronal nicotinic acetylcholine receptors: from the genetic analysis to neurological diseases. Biochem Pharmacol 2008; 76: 1175-83.

[4] Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. Prog Neurobiol 2009; 87: 41-57.

[5] Oldani A, Zucconi M, Asselta R, Modugno M, Bonati MT, Dalprà L, Malcovati M, Tenchini ML, Smirne S, Ferini-Strambi L. Autosomal dominant nocturnal frontal lobe epilepsy. A video-polysomnographic and genetic appraisal of 40 patients and delineation of the epileptic syndrome. Brain 1998; 121: 205-23.

[6] di Corcia G, Blasetti A, De Simone M, Verrotti A, Chiarelli F. Recent advances on autosomal dominant nocturnal frontal lobe epilepsy: "understanding the nicotinic aeetylcholine receptor (nAChR)". Eur J Paediatr Neurol 2005; 9: 59-66.

[7] Marini C, Guerrini R. The role of the nicotinic acetylcholine receptors in sleep-related epilepsy. Biochem Pharmacol 2007; 74: 1308-14.

[8] Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF. A missense mutation in the neuronal nicotinic acethylcholine receptor a4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 1995; 11: 201-3.

[9] Saenz A, Galan J, Caloustian C, Lorenzo F, Márquez C, Rodríguez N, Jiménez MD, Poza JJ, Cobo AM, Grid D, Prud'homme JF, López de Munain A. Autosomal dominant nocturnal frontal lobe epilepsy in a Spanish family with Ser-252Phe mutation in the CHRNA4 gene. Arch Neurol 1999; 56: 1004-9.

[10] Steinlein O, Stoodt J, Mulley J, Berkovic S, Scheffer IE, Brodtkorb E. Independent occurrence of the CHRNA4 Ser252Phe mutation in Norwegian family with nocturnal frontal lobe epilepsy. Epilepsia 2000; 41: 529-35.

[11] McLellan A, Phillips HA, Rittey C, Kirkpatrick M, Mulley JC, Goudie D, Stephenson JB, Tolmie J, Scheffer IE, Berkovic SF, Zuberi SM. Phenotypic comparison of two Scottish families with mutations in different genes causing autosomal dominant nocturnal frontal lobe epilepsy. Epilepsia 2003; 44: 613-7.

[12] Matsushima N, Hirose S, Iwata H. Mutation (Ser284Leu) of neuronal nicotinic acetylcholine receptor a4 subunit associated with frontal lobe epilepsy causes faster desensitization of the rat receptor expressed in oocytes. Epilepsy Res 2002; 48: 181-6.

[13] Cho YW, Motamedi GK, Laufenberg I, Sohn SI, Lim JG, Lee H, Yi SD, Lee JH, Kim DK, Reba R, Gaillard WD, Theodore WH, Lesser RP, Steinlein OK. A Korean kindred with autosomal dominant nocturnal frontal lobe epilepsy and mental retardation. Arch Neurol 2003; 60: 1625-32.

[14] Rozycka A, Skorupska E, Kostyrko A, Trzeciak H. Evidence for S248L mutation of the CHRNA4 in a white family with autosomal dominant nocturnal frontal lobe epilepsy. Epilepsia 2003; 44: 1113-7.

[15] Phillips HA, Marini C, Scheffer IE, Sutherland GR, Mulley JC, Berkovic SF. A de novomutation in sporadic nocturnal frontal lobe epilepsy. Ann Neurol 2000; 48: 264-7.

[16] Leniger T, Kananura C, Hufnagel A, Bertrand S, Bertrand D, Steinlein OK. A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy. Epilepsia 2003; 44: 981-5.

[17] Chen Y, Wu L, Fang Y, He Z, Peng B, Shen Y, Xu Q. A novel mutation of the nicotinic acetylcho-

line receptor gene CHRNA4 in sporadic nocturnal frontal lobe epilepsy. Epilepsy Res 2009; 83: 152-6.

[18] Steinlein O, Magnusson A, Stoodt J, Bertrand S, Weiland S, Berkovic SF, Nakken KO, Propping P, Bertrand D. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. Hum Mol Genet 1997; 6: 943-7.

[19] De Fusco MD, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, Ballabio A, Wanke E, Casari G. The nicotinic receptor beta2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet 2000; 26: 275-6.

[20] Phillips HA, Favre I, Kirkpatrick M, Zuberi SM, Goudie D, Heron SE, Scheffer IE, Sutherland GR, Berkovic SF, Bertrand D, Mulley JC. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. Am J Hum Genet 2001; 68: 225-31.

[21] Diaz-Otero F, Morales J, del Mar Quesada M. A Spanish family with autosomal dominant nocturnal frontal lobe epilepsy and a mutation in the CHRNB2 gene. Epilepsia 2001; 42: 21.

[22] Bertrand D, Elmslie F, Hughes E, Trounce J, Sander T, Bertrand S, Steinlein OK. The CHRNB2 mutation I312M is associated with epilepsy and distinct memory deficits. Neurobiol Dis 2005; 20: 799-804

[23] Cho YW, Yi SD, Lim JG, Kim DK, Motamedi GK. Autosomal dominant nocturnal frontal lobe epilepsy and mild memory impairment associated with CHRNB2 mutation I312M in the neuronal nicotinic acetylcholine receptor. Epilepsy Behav 2008; 13: 361-365.

[24] Hoda JC, Gu W, Friedli M, Phillips HA, Bertrand S, Antonarakis SE, Goudie D, Roberts R, Scheffer IE, Marini C, Patel J, Berkovic SF, Mulley JC, Steinlein OK, Bertrand D. Human noctumal frontal lobe epilepsy: pharmocogenomic profiles of pathogenic nicotinic acetylcholine receptor beta-subunit mutations outside the ion channel pore. Molphannacol 2008; 74: 379-391.

[25] Aridon P, Marini C, Di Resta C, Brilli E, De Fusco M, Politi F, Parrini E, Manfredi I, Pisano T, Pruna D, Curia G, Cianchetti C, Pasqualetti M, Becchetti A, Guerrini R, Casari G. Increased sensitivity of the neuronal nicotinic receptor a2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. Am J Hum Genet 2006; 79: 342-350.

[26] Gu W, Bertrand D, Steinlein OK. A major role of the nicotinic acetylcholine receptor gene CHRNA2 in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is unlikely. Neurosci Lett 2007; 422: 74-6.

[27] Combi R, Ferini-Strambi L, Tenchini ML.CHRNA2 mutations are rare in the NFLE population: evaluation of a large cohort of Italian patients. Sleep Med 2009; 10: 139-42.