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Study of candidate gene cHRNA4 for familial epilepsy syndrome

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Abstract. – OBJECTIVE: To screen a three-generation familial partial epilepsy with variable foci (FPEVF) family with epilepsy to identify the cHRNA4 gene (a candidate gene).

PATIENTS AND METHODS: A total of 18 members of the three-generation FPEVF family with partial epilepsy were selected, and 18 blood samples were collected for investigation. Among them, five members were affected by epilepsy, and another 13 members were not affected. A pedigree chart was mapped to comprehensively analyze the clinical characteristics of each member, including ictal semiology, electroencephalogram (EEG), past medical history, MRI features, neuropsychological MMSE (mini-mental state examination) scores, etc. PCR and Sanger sequencing method were used to screen the mutant gene cHRNA4.

RESULTS: cHRNA4 genes of all affected members were positively mutated, and that of the unaffected members were negative. The positive mutation was base A instead of base G.

CONCLUSIONS: cHRNA4 is the causative gene of FPEVF, and genes of the affected members are all heterozygotes mutations.

Key Words:
Familial partial epilepsy with variable foci (FPEVF), Candidate gene cHRNA4, Sanger sequencing method.

Introduction

Epilepsy (EP) is the third most common disease next to cerebrovascular disease and dementia in neurology. EP is divided into primary epilepsy and secondary epilepsy, of which primary epilepsy accounts for about 2/3[1]. In addition, the prevalence of EP is about 3.6-7.0% in all regions in our country. There are more than 9 million epileptics in China, among which 70-80% do not undergo formal treatment, and the mortality rate is as high as 79,000/100,000[2]. At present, the pathogenesis and the exact mechanism of EP are still not very clear. Thus, prevention and treatment results are less successful[3]. Although there are more studies supporting that genetic factors are important pathogenic factors of EP[4,5], there are still less large-scale studies on the genetic epidemiology of EP.

Currently, several familial partial epilepsy syndromes have been identified, such as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)[6], autosomal dominant partial epilepsy with auditory features (ADPEAF)[7], familial partial epilepsy with variable foci (FPEVF)[8], benign childhood occipital epilepsy (BOE)[9], benign childhood epilepsy with centrotemporal spikes (BECTS)[10] and so on. Since EP has a complex genetic background and very different clinical manifestations, it is very difficult to collect family data, especially in large families. Herein, we meticulously collected all clinical phenotypes of the affected and unaffected members in a FPEVF family from consulted literature and previous study results. We screened the mutant gene cHRNA4 by using PCR and Sanger sequencing method, which provides a scientific basis for the next genome-wide scan family-based linkage analysis, as well as the analysis of new pathogenesis of EP, and the early diagnosis, treatment, prognosis evaluation and new drug development of EP.

Patients and Methods

Patients

A three-generation FPEVF family with partial epilepsy was selected, and a pedigree chart was mapped. A total of 18 members were enrolled collected and investigated. Among them, there were 2 Generation I members, 8 Generation II members and 8 Generation III members. 5 of 18 members were affected by epilepsy. The member Generation II-1 was the propositus, and another 4 affected members were: Generation I-1, Generation II-5, Generation III-1 and Generation III-6 (Figure 1). Ethics Committee approval was given by our hospital. The informed consent of the respondent was obtained for EP diagnosis based

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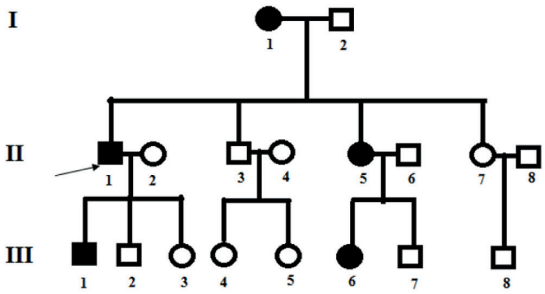

Figure 1. The pedigree chart of the three-generation FPEVF family with partial epilepsy (the member generation II-1 was the propositus and the black represented the affected member).

on classification criteria for epilepsy syndrome by the International League Against Epilepsy (ILAE, 2001).

Methods

Firstly, a question-based survey form was used to comprehensively analyze the social background and clinical manifestations of each affected member. The clinical manifestations included ictal semiology, past medical history, dynamic EEG, brain MRI features, neuropsychological data (by using MMSE scores, a simple method to check intelligence status) and other materials. Then, the cyrillic2.1 software was applied to draw the pedigree chart, and PCR and Sanger sequencing method was lastly used to screen the mutant gene cHRNA4.

Questionnaire of the Ictal Semiology of EP

The questionnaire included the type and clinical manifestations of seizures, with or without spasm, convulsions or consciousness, the loss of consciousness, specific predisposing factors, the duration of the factor and the epilepsy, memory loss during or after epileptic seizures, falling down and the injury due to it, or falling of things in the hands, obvious emotional changes (such as fear, anger or joy), illusions, auditory hallucinations, olfactory hallucinations, visual hallucination, etc.

Also, it included type and clinical manifestations of seizures with or without hydrostomia, strange body feelings (such as palpitation, chest pain, hunger, etc.), stiff arms during epileptic seizures, stiff legs, respiratory arrest, eyelid twitch, smack, lips-licking, chewing, swallowing, laughing, walking, stepping, speaking or other accompanying actions, with or without odaxesmus, gatism, fatigue and headache, with or without impact on language, vision and movement; the frequency of epileptic seizures, with or without different symptoms that were aggravated or relieved, with or without other complications; with or without antiepileptic drugs treatment (if any, the specific type, dosage and duration of antiepileptic drugs were described) or surgical treatment of epilepsy.

Gene Sequencing Method

3 ml of peripheral venous blood was drawn and then genomic DNA was extracted by using Promega DNA purification kit (Sigma-Aldrich Company, St. Louis, MO, USA). The primer design was completed by Sangon Biotech Co., Ltd. (Shanghai, China) with the use of the Primer3 online software according to the candidate genomic sequence in NCBI. The exons included 5'UTR, 3'UTR and adjacent partial intron. Moreover, the length of the primer was 20bp, and the length of the amplified fragment was 428-2,372bp. PCR amplification of the genomic DNA included template thermal denaturation, melting, annealing, complementary pairing, and primer extension. 35 cycles were repeated and then dideoxy chain termination DNA sequencing method was used. The 3'-position of 2', 3'-dideoxynucleoside triphosphates (ddNTP) lacked one hydroxyl group. It could be incorporated into a growing DNA strand via 5' triphosphate group under the reaction of DNA polymerase. When the terminal base was ddMP, the chain extension reaction was terminated. The chain extension was completed with an unexpected and specific chain termination, and thus a series of nucleotide strands were produced. The length of the above nucleotide strand depended on the distance from the end of the starting DNA synthesis primer to the position at which the premature chain termination occurred. As a result, 4 kinds of oligonucleotide were produced. These oligonucleotides were located in each A, C, G, or T position of the template strand, respectively. Next, denaturing polyacrylamide gel electrophoresis was used to separate and identify, and the instrument automatically detected DNA specific sequences. The sequencing results were analyzed by using Chromas 2.22 software and the positive loci were screened.

Statistical Analysis

SPSS20.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for data recording and statistical analysis. Measurement data, which were in line with a normal distribution, were expressed as mean ± standard deviation (SD) and compared by using *t*-test;

enumeration data were indicated as percentage and compared by using χ^2-test. The difference was statistically significant if $p<0.05$.

Results

Clinical Phenotype Analysis of FPEVF

Generation I-1 was a female, 70-years-old. The age of the first onset was 55-years-old. Symptoms: grand mal systemic spastic tonic EP without obvious causative factors, with the loss of consciousness; duration was 3-5 min; a total of 3 seizures, with the same type. Past medical history: no traumatic brain injury, tumor and surgery history. Sodium valproate and phenytoin were taken for treatment. Brain MRI features: circumscribed atrophy of the temporal lobe was found. EEG: there were visible temporal spikes in the period of onset. MMSE scores: a mild to moderate cognitive impairment was indicated.

Generation II-1 was a male, 42 years old. The age of the first onset was 42 years old, namely 5 years after the craniocerebral trauma surgery. He was diagnosed as complex epileptic seizures, with dizziness, transient loss of consciousness, mild auditory hallucinations and fear. Brain MRI features: the formation of occipital scars was found. EEG: there were visible focal occipital spikes, with persistent discharge in period of onset and without recurrence after removing the lesion by minimally invasive positioning and surgery. MMSE scores: normal.

Generation II-5 was a female, 38 years old. The age of the first onset was 36 years old. Symptoms: tonic-clonic of unilateral limb during sleep at night, with normal consciousness, without urinary incontinence and other accompanying symptoms; the duration was several tens of seconds; a total of 1 seizure. Past medical history: no treatment. Brain MRI features and EEG: no abnormalities. MMSE scores: normal.

Generation III-1 was a male, 19 years old. The age of the first onset was 15 years old. Symptoms: petit mal absence; a total of 3 seizures, with the same type. Past medical history: sodium valproate was taken. Brain MRI features and EEG: no abnormalities. MMSE scores: normal.

Generation III-6 was a female, 16 years old. The age of the first onset was 15 years old. Symptoms: mixed epileptic seizures, with transient loss of consciousness and memory impairment after seizures; the duration was 1min; a total of 1 seizure. Past medical history: sodium valproate and phenytoin were taken for treatment. Brain MRI features: abnormal frontal lobe lesions were found. EEG: there were visible epileptic waves in frontal lobe and central zone in the period of onset, with normal intermediate stage. MMSE scores: normal.

Gene Sequencing of cHRNA4

cHRNA4 genes of all affected members were positive mutation, and that of the unaffected members were negative. The positive mutation was base A instead of base G (Figure 2).

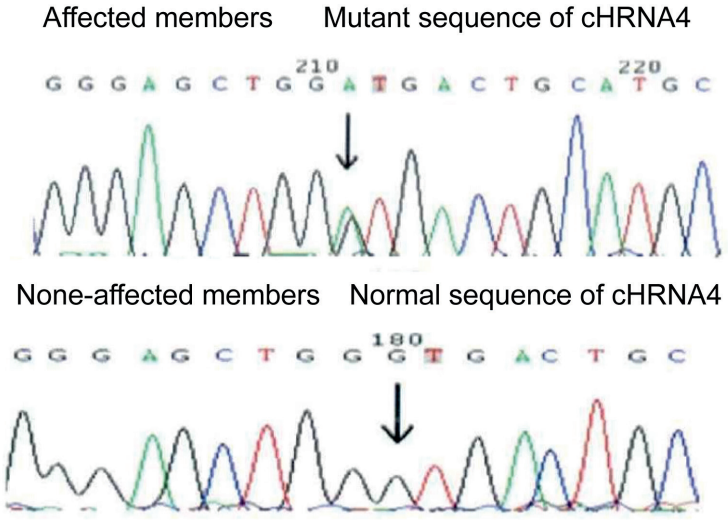

Figure 2. Diagram of the cHRNA4 sequence in gene sequencing (Gene G of the cHRNA4 of the affected members was replaced by Gene A).

Discussion

The main task of the genetic epidemiology is to analyze whether the disease is of familial aggregation, causes of the disease (such as the common living environment, genetic susceptibility or cultural education, etc.), transmission ways of the disease in the family (such as monogenic inheritance or polygenic inheritance), whether there is the effect of key-genes, interactions of genetic and environmental factors, etc., and to put forward prevention and intervention measures. The EP, Parkinson's disease and other neurological diseases are of polygenic inheritance. Moreover, there are a variety of theories on the pathogenesis of EP, including cell theory, neurotransmitter theory, ion channel theory, immune theory and theory of heredity[11,12]. All above theories consider that EP is characterized by familial aggregation, genetic factor is the important pathogenesis of EP, and EP is the result of interactions of internal factors (i.e., genetic factors) and external factors (i.e., various environmental factors leading to brain damages)[13,14].

Pathogenic genes of the idiopathic epilepsy are mainly distributed in ion channels (such as voltage-dependent or ligand-dependent ion channel genes); therefore, EP is also an ion channel disease. The following genes are proved by studies: Ca^{2+} channel-encoding genes[15], CACNAlA, CACNB4, etc., Na+ channel-encoding genes[16], SCNlA, SCN2A, etc., Cl- channel-encoding gene[17], CLCN2, and K+ channel-encoding genes[18], KCNQ2, KCNQ3, etc. At the same time, ligand-dependent ion channels include r-aminobutyric acid (GABA) receptor channel genes[19], GABRAl, GABRG2, etc., and acetylcholine receptor genes[20], CHRNA4, CHRNB2, etc. These mutant genes can cause epileptic seizures by affecting the excitability of neurons or lowering the seizure threshold.

Conclusions

In our study, clinical manifestations and gene sequencing performed on the candidate gene cHRNA4 of members in the three-generation FPEVF family with partial epilepsy confirmed that cHRNA4 was the pathogenic gene of FPEVF, in which not only the base A was mutated by the base G, but also genes of the affected members were mutant heterozygotes. It is noteworthy that the relationship between genetic manifestations and ethnic distributions is very close, and the known report on the relationship between various pathogenic genes of EP and Asians, especially the Chinese, is few. In this study, more members of the large family were affected with simplex phenotype, which is the ideal genetic resource. It is expected to find the pathogenic gene so as to provide reference for the study of genetic causes of the Chinese EP.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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