SHORT COMMUNICATIONS

Clinical and genetic analysis of two Chinese families with benign familial neonatal convulsions*

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Abstract Benign familial neonatal convulsions (BFNC) is a rare autosomal dominant inherited epilepsy syndrome. Two voltage-gated potassium channel genes, KCNQ2 and KCNQ3 have been identified as the genes responsible for BFNC. Here we report two Chinese families with clinical histories of typical BFNC. Using six microsatellite markers, two located at KCNQ2 locus and four at KCNQ3 locus, linkage analysis was performed in the two families, which excluded the linkage of BFNC to KCNQ3, but could not exclude the linkage to KCNQ2. Direct DNA sequencing of the KCNQ2 gene in the two families was performed, and two formerly unknown polymorphisms were identified, but no KCNQ2 mutation was found in the two families. Our study suggests the genetic heterogeneity in Chinese families with BFNC and proves the existence of a new gene locus for BFNC.

Keywords: BFNC, epilepsy, potassium channel, linkage analysis, mutation analysis.

Benign familial neonatal convulsions (BFNC) is a rare autosomal dominant inherited epilepsy syndrome characterized by unprovoked partial or generalized seizures. The seizures usually occur from the second day of birth to the sixth month and remit spontaneously after several weeks to months. Most individuals are seizure-free by the age of six months. The serum chemistry and neuroradiological examinations, interictal electroencephalogram (EEG), and psychomotor development are usually normal. However, 10% to 15% of patients have the risk of seizure recurrence later in life which are mostly described as generalized tonic or tonic-clonic seizures[1—4].

BFNC is a genetically heterogeneous disorder. In 1998, two voltage-gated potassium channel genes, KCNQ2 on chromosome 20q13.3 and KCNQ3 on chromosome 8q24 were identified as the genes responsible for BFNC[5,6]. Recent studies raise the possibility of other genes[7,8]. Both KCNQ2 and KCNQ3 genes were expressed in nearly every region of the central nervous system, their products assemble to form a functional hetero-tetrameric channel which plays a crucial role in the regulation of neuronal excitability[8]. So far, 31 mutations of KCNQ2 gene and 3 mutations of KCNQ3 gene have been reported[5,6,9—11], and we also recently reported a novel KCNQ2 mutation in a Chinese family with BFNC[12]. Here, we report the results of clinical and genetic analyses in the other two Chinese families with BFNC. Our study shows the genetic heterogeneity in Chinese family with BFNC, and proves the existence of a new gene locus for BFNC.

1 Materials and methods

1.1 Families

Family 1 had 7 affected individuals in three generations. Family 2 had 5 affected individuals in four generations. The pedigrees are shown in Figs. 1 and 2 respectively. The consent of involvement of the study was obtained from each subject. Except Ⅰ-1, Ⅲ-4 in family 1, and Ⅰ-1, Ⅰ-2, Ⅱ-3, Ⅱ-4, Ⅱ-5, Ⅱ-6, Ⅱ-7, Ⅲ-4, Ⅲ-5, Ⅲ-6, Ⅲ-7 in family 2, all the affected and unaffected individuals were examined by two experienced neurologists. The diagnosis of BFNC was based on the previously described clinical criteria[5,13]. Subjects were classified as the affected individuals if they had the following characteristics: the

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occurrence of brief seizures on or after the second day of life which disappeared spontaneously within a few weeks or months, positive family history with autosomal dominant inheritance, exclusion of other causes of neonatal seizures, normal serum chemistry determinations, interictal electroencephalography and neuroradiological examination, and normal subsequent neurodevelopment. Subjects without seizures or abnormal signs were classified as the unaffected individuals.

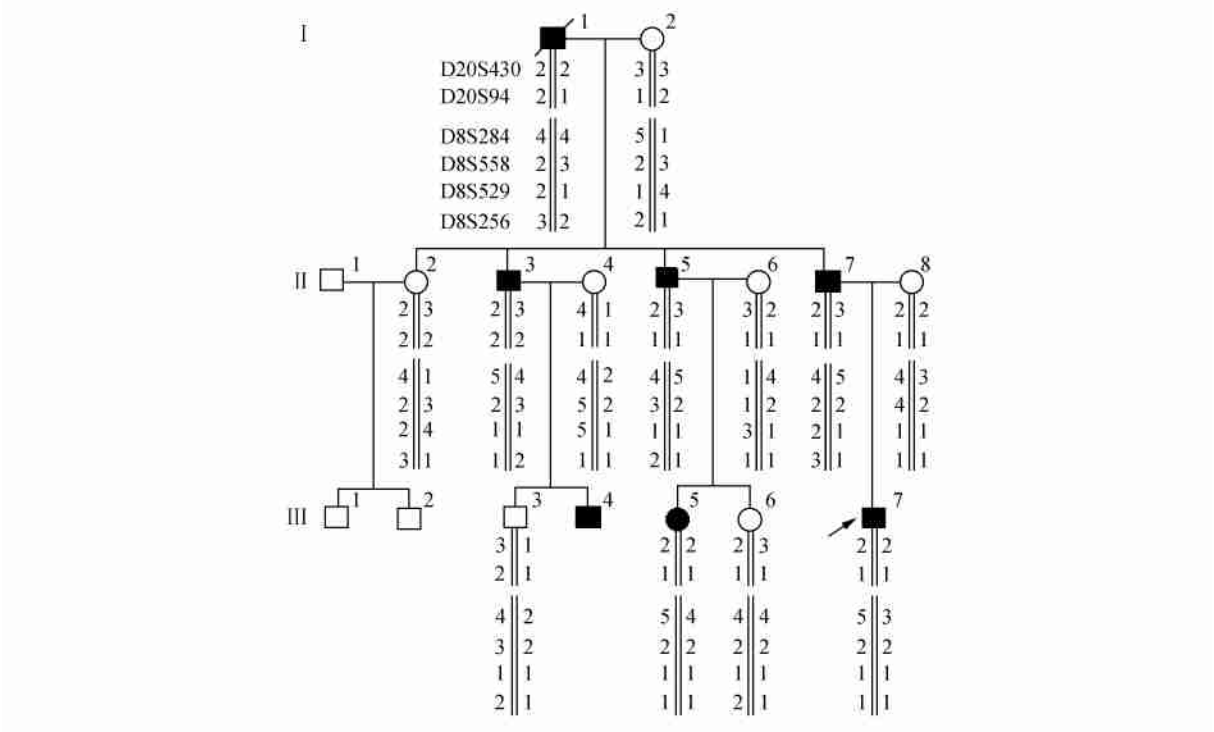

Fig. 1. Pedigree and haplotype of family 1.

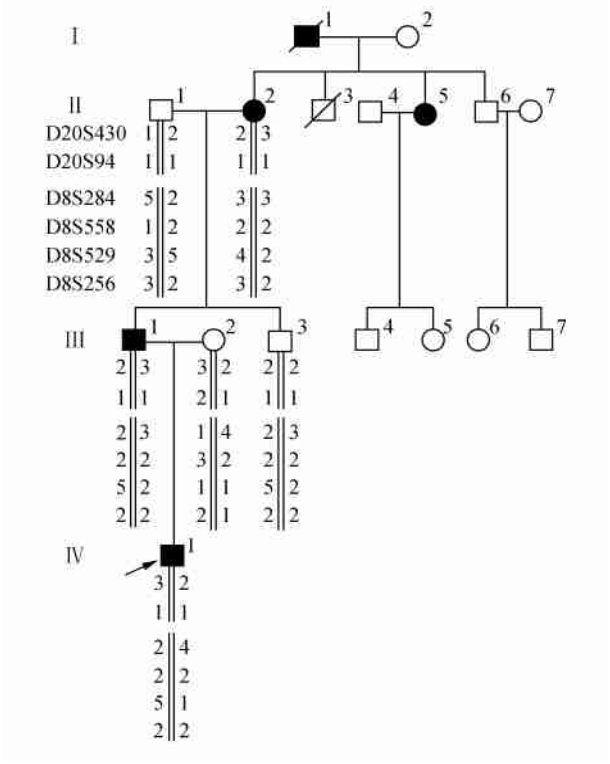

Fig. 2. Pedigree and haplotype of family 2.

1.2 Molecular genetic analysis

Blood samples were collected from 8 affected, 10 unaffected individuals of the two families with informed consent. Genomic DNA was extracted from peripheral blood leukocytes by routine methods.

Two-point linkage analysis was performed with the MLINK program of the LINKAGE package (version 5.1) using microsatellite markers D8S284, D8S558 linked to KCNQ3 locus and D20S430, D20S94 linked to KCNQ2 locus in 18 members of the two families.

The entire coding region of KCNQ2 of the two probands were amplified by PCR using 20 pairs of primers designed according to genomic DNA sequence of human KCNQ2 gene (GenBank accession number: NT_011333). PCR was performed in a 40 μL volume on an MJ Research PTC-200 and carried out for 34 cycles at 94 ℃ for 40 s, 56—69 ℃ for 45—55 s and 72 ℃ for 1 min after an initial denaturation of 5 min at 94 ℃, and a final extension at 72 ℃ for 10 min. After being purified, PCR products were directly sequenced by a dye terminator sequencing method using the amplification primers on an ABI

377 DNA sequencer (PE Biosystems).

2 Results

2.1 Clinical features

The pedigree of family 1 indicated a profile of autosomal dominant inheritance. The proband (Ⅲ-7) is 4 years old now. He was born well at term and his Apgar score was 10 at 2 min. On the third day of life, he developed frequent afebrile seizures affecting one or two-sided limbs. Seizures lasted from 30 s to 2 min and were observed from one to ten times a day. He often had drowsiness for 2 to 3 hours after seizures. He was normal in the interictal period. At the age of 9 days, he was treated with phenobarbital and the seizures were well controlled. The medication was stopped at the age of 21 days when he had no seizures any more. His serum biochemical determinations (electrolytes, glucose and amino acids), chromosome karyotype, interictal EEG at day 12 and computed tomographic scan at day 9 were all normal. The members of Ⅰ-1, Ⅱ-3, Ⅱ-5, Ⅱ-7, Ⅲ-4, Ⅲ-5 in this family manifested partial or generalized clonic seizures on the third day of life, none of them were administrated with antiepileptic drugs and their seizures disappeared by 20 days to one months of life. The physical and intellectual examinations of all patients were normal. Although subject Ⅲ-3 had no BFNC, he had two generalized tonic-clonic seizures with febrile when he was 7 years old.

The pedigree of family 2 also indicated an autosomal dominant inheritance. The proband (Ⅳ-1), aged 6 months, was a healthy, term neonate with an uncomplicated gestation. On the second postnatal day, he had frequent clonic seizures which affected two up-limps or four limbs without febrile. Seizures usually associated with eye deviation, apnea and lip cyanopathy, lasted 1—2 minutes and occured 10 to 20 times a day. He was normal in the interictal period. Phenobarbital was given to him on the 3rd day and the seizures were well controlled at that time. The medication was stopped at the age of 17 days. Because seizures reoccurred at the age of 18 days, he was treated with valproate instead of phenobarbital. Valproate was discontinued on the age of 45 days. After having one seizure on the 46th day, he had no further seizure up to now. His serum biochemical determinations, chromosome karyotype, interictal EEG at day 8 and MRI scan at day 3 were all normal. Ⅰ-1, Ⅱ-2, Ⅱ-5, Ⅲ-1, Ⅳ-1 of this pedigree manifested partial or generalized clonic seizures on postnatal 3 to 10 days. The seizures disappeared by 18 days to one month in the four affected individuals except Ⅱ-2, who had generalized febrile seizures at 6 years of age. The physical and intellectual examinations of all patients were normal.

2.2 Molecular findings

The haplotype of two families is shown in Figs. 1 and 2, and lod scores from two-point linkage analysis are presented in Tables 1 and 2. For the two families, the data excluded the linkage of this disease to KCNQ3 locus, but did not exclude linkage to KCNQ2 locus. Then KCNQ2 gene mutation analysis was performed for the two probands. Two formerly unknown variants, 543G→A and 912C→T were found. Because they did not cause amino acid changing, they are two new polymorphisms. No KCNQ2 mutation was found in the two families.

Table 1. Pairwise lod scores of family 1

Locus	Lod scores in different recombination fraction (θ)								Z_{max}	θ
	0.0	0.01	0.05	0.1	0.15	0.2	0.25	0.3		
D20S430	−4.26	−2.59	−1.28	−0.78	−0.52	−0.37	−0.26	−0.19	−0.19	0.3
D20S94	−0.05	−0.04	−0.02	0.00	0.01	0.02	0.02	0.01	0.02	0.2
D8S284	−4.06	−1.15	−0.50	−0.26	−0.15	−0.08	−0.04	−0.02	−0.02	0.3
D8S558	−3.35	−3.32	−2.55	−1.85	−1.40	−1.07	−0.81	−0.60	−0.60	0.3
D8S529	−0.48	−0.44	−0.32	−0.22	−0.14	−0.09	−0.05	−0.03	−0.03	0.3
D8S256	−4.17	−2.83	−1.50	−0.96	−0.67	−0.48	−0.35	−0.24	−0.24	0.3

Table 2. Pairwise lod scores of family 2

Locus	Lod scores in different recombination fraction (θ)								Z_{max}	θ
	0.0	0.01	0.05	0.1	0.15	0.2	0.25	0.3		
D20S430	−0.74	−0.67	−0.47	−0.32	−0.22	−0.15	−0.1	−0.06	−0.06	0.3
D20S94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
D8S284	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
D8S558	−4.56	−1.70	−1.0	−0.70	−0.52	−0.40	−0.30	−0.22	−0.22	0.3
D8S529	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	0
D8S256	−4.96	−2.37	−1.47	−1.02	−0.75	−0.55	−0.40	−0.28	−0.28	0.3

3 Discussion

Since the original description of Rett and Teubel in 1964, more than 50 families with BFNC have been reported. Here, we describe two Chinese families which fulfil the clinical diagnostic criteria for BFNC: partial or generalized seizures began on day 2 to 10, remitted on day 18 to 46; the serum chemistry and neuroradiological examinations, interictal EEG, and psychomotor development were normal; the patients have good response to antiepileptic drug treatment; positive family history with autosomal dominant inheritance. This clinical profile indicates that the two families are typical BFNC[14, 15]. Of the 67% seizures in the two families began on day 3 and 75% of the patients had the remitted seizures in the first 6 weeks, that is higher than previously reported that 42% of patients suffered from seizures on day 3 and 60%—68% remitted in the first 6 weeks[4]. The risk of subsequent epilepsy was 10%—15% in previous reports[2, 14]. In our reported families, the risk is 8.3%. These differences can be caused by the fact that the two families are relatively small, and the number of the studied families is not enough for the difference of race and terrain. The seizure phenotype in BFNC is various which can be generalized or partial tonic, clonic, myoclonic, or tonic-clonic, the most is clonic seizure, and the least is myoclonic[2, 4, 14]. In our two families, all of the patients manifested clonic seizures which confirmed that clonic seizure is the most phenotype.

Up to date, the majority of reported BFNC families are linked to KCNQ2 locus, only four families were found to be linked to KCNQ3 locus[9, 10, 14]. A recent report described a family in which pericentric inversion of chromosome 5 cosegregated with BFNC and raised the possibility of a new locus on chromosome 5[7]. But for some other families, the underlying genetic defect seems to be located elsewhere[9]. In our study, the chromosome karyotypes of the two probands are normal, so pericentric inversion of chromosome 5 is not the genetic defect of the two families. We have previously found a KCNQ2 gene mutation in a Chinese family with BFNC[12]. To investigate whether this disorder is genetically heterogenous in Chinese family with BFNC, we performed linkage analysis and KCNQ2 gene mutation analysis in the two Chinese families. Linkage analysis excluded linkage of BFNC to KCNQ3, but did not exclude linkage to KCNQ2. So, we further carried out KCNQ2 gene mutations analysis including the whole coding region in the two probands, but we did not find KCNQ2 gene mutation except two unreported polymorphisms. This shows neither KCNQ2 nor KCNQ3 is responsible for the BFNC in the two families. Together with previous studies, our data confirm the genetic heterogeneity in Chinese families with BFNC, and suggest that there is a new gene involved in BFNC besides KCNQ2 and KCNQ3. We are performing genomewide scan to localize new BFNC locus in the two Chinese families now, and through mutation analyses of candidate genes, it will help us to identify new BFNC gene(s).

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