A novel mutation of \textit{KCNQ3} gene in a Chinese family with benign familial neonatal convulsions

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\textbf{KEYWORDS}
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\textbf{Summary} Benign familial neonatal convulsions (BFNC, also named benign familial neonatal seizures, BFNS) is a rare autosomal dominant inherited epilepsy syndrome with clinical and genetic heterogeneity. Two voltage-gated potassium channel subunit genes, \textit{KCNQ2} and \textit{KCNQ3}, have been identified to cause BFNC1 and BFNC2, respectively. To date, only three mutations of \textit{KCNQ3}, all located within exon 5, have been reported. By limited linkage analysis and mutation analysis of \textit{KCNQ3} in a Chinese family with BFNC, we identified a novel missense mutation of \textit{KCNQ3}, c.988C>T located within exon 6. c.988C>T led to the substitution Cys for Arg in amino acid position 330 (p.R330C) in KCNQ3 potassium channel, which possibly impaired the neuronal M-current and altered neuronal excitability. Seizures of all BFNC patients started from day 2 to 3 after birth and remitted during 1 month, and no recurrence was found. One family member who displayed fever-associated seizures for two times at age 5 years and was diagnosed as febrile seizures, however, did not carry this mutation, which suggests that febrile seizures and BFNC have different pathogenesis. To our knowledge, this is the first report of \textit{KCNQ3} mutation in Chinese family with BFNC.

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Introduction

Benign familial neonatal convulsions (BFNC, also known as benign familial neonatal seizures, BFNS) is a rare autosomal dominant inherited epilepsy syndrome with clinical and genetic heterogeneity. Typical BFNC is characterized by unprovoked partial or generalized seizures which occur around the third day of life and remit spontaneously during 6 months. There are marked intrafamilial and interfamilial variations in the age of onset from the second day to the sixth month and the age of remission from 2 weeks to 24 months. The serum chemistry determinations, neuro-radiological examinations, interictal electroencephalogram (EEG) and psychomotor development are usually normal. Although BFNC is a benign epilepsy syndrome, there are 7% of patients with learning disorders, mild cognitive impairment or other neurological deficits, and 10—16% of patients with recurrence of seizures later in life which are mostly presented as generalized tonic or tonic–clonic seizures (Leppert et al., 1989; Ryan et al., 1991; Ronen et al., 1993; Burgess, 2005).

According to different loci, BFNC is classified as two forms, BFNC1 mapped to 20q13.3 and BFNC2 mapped to 8q24 (Leppert et al., 1989; Lewis et al., 1993). Two voltage-gated potassium channel genes, KCNQ2 on chromosome 20q13.3 and KCNQ3 on chromosome 8q24, have been identified as the genes responsible for BFNC1 and BFNC2, respectively (Singh et al., 1998; Charlier et al., 1998). Cytogenetic analysis raised the possibility of a new locus on chromosome 5 (Concolino et al., 2002). It cannot be excluded that additional unknown loci exist. Together with KCNQ1 mutations of which are associated with long QT syndrome-1 (LQT1), KCNQ4 mutations of which are responsible for autosomal dominant non-syndromic deafness, and KCNQ5 which has not yet been found to be associated with any disorder, KCNQ2 and KCNQ3 constitute a new subfamily of potassium channel genes. Every member of KCNQ channel subfamily consists of six transmembrane domains (S1—S6), the voltage sensor located in the S4 helix, an ion channel pore between S5 and S6, a short N-terminus and a long C-terminal domain. KCNQ2 and KCNQ3 constitute a heterotetrameric channel which produces the neuronal M-current and plays a crucial role in the regulation of neuronal excitability (Jentsch, 2000).

To date, 38 mutations of KCNQ2 have been found, whereas only three mutations of KCNQ3 have been identified (Singh et al., 1998, 2003; Charlier et al., 1998; Hirose et al., 2000; Coppola et al., 2003; Pereira et al., 2004; Borgatti et al., 2004; Richards et al., 2004; Claes et al., 2004; Tang et al., 2004). Here, we describe a novel mutation of KCNQ3, c.988C>T located within exon 6 in a Chinese family with BFNC. Typically in clinical features of this BFNC family, seizures of all patients started from day 2 to 3 after birth and remitted during 1 month, and no recurrence of seizures was found. One individual with febrile seizures but without BFNC did not carry the mutation.

Materials and methods

Family

The Chinese family had 17 members over four generations including seven with BFNC, one with FS. The pedigree is shown in Fig. 1. Clinical data collection, including detailed clinical history enquiry and neurological examination, were performed by at least two neurologists, respectively, for all members except I-1 who was died. The diagnosis of affected individuals who displayed febrile seizures from day 2 to 3 after birth then remission during 1 month without recurrence, were done according to the diagnostic scheme of ILAE (Engel, 2001). Correspondingly, individuals who had seizures associated with fever under 6 years old and had not other abnormality were diagnosed as febrile seizures based on the diagnostic scheme of ILAE (Engel, 2001). Serum biochemical determinations, interictal electroencephalogram (EEG), and computed tomography (CT) were performed on the proband (III-7), III-3, III-4 and III-5. Chromosome karyotypes were obtained in the proband and II-8.

Limited linkage analysis

After informed consent was obtained from each subject, blood samples were collected from 13 family members including all patients. Genomic DNA was extracted from peripheral blood leukocytes by routine methods.

Four microsatellite markers, D20S171, D20S173, D8S284 and D8S514 were amplified by fluorescent multiplexed PCR. PCR products were separated by capillary electrophoresis using an ABI PRISM 3100 Automated Sequencer (PE Applied Biosystems, Foster City, CA, USA). Microsatellite marker-allele data were analyzed by GENESCAN (version 3.7) and...
GENOTYPER (version 2.1) (PE Applied Biosystems, Foster City, CA, USA). Linkage analysis was performed under the assumption of an autosomal dominant mode with penetrance at 0.90 and frequency of the disease at 0.0001 by using the MLINK program of the LINKAGE PACKAGE (version 5.1).

**Mutation analysis of KCNQ3 gene**

All of 15 exons and the adjacent introns of **KCNQ3** of the proband were amplified by PCR with 15 pair of primers designed in terms of genomic DNA sequence of human **KCNQ3** (NT_008046). Nucleotide numbers refer to the published cDNA sequence: NM_004519. PCR was performed in 10 μl volume with 0.05U Hotstart DNA Polymerase on an MJ Research PTC-200 thermocycler. After digested by exonuclease I and shrimp alkaline phosphatase, PCR products were directly sequenced with dye terminator sequencing method using the same amplification primers on an ABI 3100 DNA sequencer (PE Applied Biosystems).

When c.988C>T in exon 6 of **KCNQ3** was found, DNASTar software was used to analyze the changing of restriction endonuclease cutting sites and we found that the mutation abolished a Hae III site. Then Hae III was used to identify the cosegregation of genotype with phenotype. Exon 6 of **KCNQ3** of all the 13 members in the family and 100 unrelated normal controls was amplified by PCR, then subjected to thorough digesting by Hae III at 37°C for 12 hours. After digesting, products were electrophoresed on 6% polyacrylamide gels in 0.5% TBE, followed by silver staining.

**Results**

**Clinical features**

Pedigree of the family indicated autosomal dominant inheritance mode. BFNC was present in seven patients. The proband, 6 years old now, was born well after 40 weeks’ pregnancy and the Apgar score was 10 at 1 min. On the third day of life, he showed frequent afebrile seizures characterized by partial clonic seizures affected one- or two-sided limbs. Seizures lasted from 30 s to 1 min, and were observed from 1 to 10 times a day following by drowsiness for several hours. In the interictal period, he had no abnormality. Treated with Phenobarbital, his seizures were well controlled. He had no further seizures after day 21. His serum biochemical determinations including kalium, sodium, calcium, magnesium and glucose, interictal EEG and cranial CT were all normal. I-2, II-5, II-7, III-4, III-5 of the pedigree manifested partial clonic seizures at age 2–3 days. Pedigree member II-3 did not manifest obvious paroxysmal limb clonic but presented paroxysmal squeals at day 3 and remitted at 1 month. Pedigree member III-3 displayed generalized tonic–clonic seizures for two times while fever raised at age 5 years, and no recurrence was observed again. He was correspondingly diagnosed as FS after exclusion of other central nervous system disorders. Serum biochemical determinations, interictal EEG, and cranial CT of III-3, III-4 and III-5 were all normal. Chromosome karyotypes of the proband and II-8 were normal. Physical and intellectual examinations were normal in all of the patients. Whether treated or not, the seizures spontaneously remitted between 2 weeks and 1 month. No recurrence of seizures was found in later life of all patients.

**Limited linkage analysis**

Two-point LOD scores for the four markers were shown in Table 1. When the recombination fraction (θ) is 0, two-point LOD scores for D20S171, D20S173 which are located in the locus of **KCNQ2** were −4.52, −4.06, respectively. Therefore, the linkage to **KCNQ2** was excluded. At θ = 0, two-point LOD scores for D8S284 and D8S514 which are located in the locus of **KCNQ3** were 1.98 and 1.16 respectively. This result significantly suggested the linkage to **KCNQ3** gene locus.

**Mutation analysis of KCNQ3 gene**

By DNA direct sequencing, a missense mutation, c.988C>T was found in exon 6 of **KCNQ3** of the proband (Fig. 2). The mutation led to the substitution Cys for Arg in amino acid position 330 (p.R330C), a conserved residue within the linker of pore region and S6 of KCNQ3 potassium channel. We then ascertained its cosegregation with BFNC but not with FS by restriction endonuclease cutting analysis (Fig. 1). Normally, the amplified fragment of exon 6 is 341 bp including two Hae III restriction sites. This mutation, however, abolished the second Hae III restriction site. After digested by Hae III, PCR products of exon 6 were cut into three fragments as 115bp, 43bp, 183bp and 226bp in the mutation ones. The abnormal band, 226bp presented in all of the patients with BFNC but not in the pedigree member III-3 with FS, the unaffected members of the family and 100 unrelated normal controls.

<table>
<thead>
<tr>
<th>Marker</th>
<th>LOD scores at different recombination fraction (θ)</th>
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<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>D8S284</td>
<td>1.98</td>
</tr>
<tr>
<td>D8S514</td>
<td>1.16</td>
</tr>
<tr>
<td>D20S171</td>
<td>−4.52</td>
</tr>
<tr>
<td>D20S173</td>
<td>−4.06</td>
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Figure 2  Double-stranded sequencing of exon 6 of KCNQ3 between nt979 and nt996 and deduced amino acid sequence changing. (A) The sequence of a normal control; (B) the sequence of the proband; (C) alignment of pore and S6 in KCNQ channel. (●) The reported mutations of KCNQ3; (✦) KCNQ3 p.R330C.

Discussion

At present, only 3 mutations of KCNQ3 have been identified for BFNC2, p.G310V in a Mexican American family, p.W309R in a Japanese family and p.D305G in an American family. All three mutations are located in exon 5 of KCNQ3 and encoded the pore region of KCNQ3 potassium channel (Charlier et al., 1998; Hirose et al., 2000; Singh et al., 2003). By linkage and mutation analysis in our work, a novel missense mutation c.988C>T in KCNQ3 was found in a Chinese family with typical BFNC. c.988C>T is situated in exon 6 of KCNQ3 and this nucleotide change caused the substitution Cys for Arg in 330 residue of KCNQ3 protein (p.R330C). p.R330C is located in the linker of pore region and S6 of KCNQ3 potassium channel. The mutation cosegregated with the phenotype of BFNC in the family and was absent in any one of 100 normal controls, which excludes the possibility of polymorphism. Our study shows that the pore of KCNQ3 potassium channel is not the only one mutation region. To our knowledge, this is the first report of KCNQ3 mutation in Chinese family with BFNC.

The Chinese family showed a typical BFNC features including autosomal dominant inheritance mode, febrile seizures starting around the age of 3 days and disappearing within 2 weeks to 1 month, normal physical and interictal examinations, no recurrence of seizures in later life in any patient. Compared with previously reported BFNC families with KCNQ3 mutations, the Chinese family had the same clinical features including the earlier age of onset and remission, no recurrence of seizures in juvenile and adult, no neurological defects and mental retardation (Charlier et al., 1998; Hirose et al., 2000; Singh et al., 2003). Therefore, our study confirms that the phenotype associated with KCNQ3 mutations is restricted to BFNC as described in our family, suggesting a strict genotype—phenotype correlation. The patients with BFNC mostly manifested tonic, clonic, tonic–clonic seizures and occasionally presented myoclonic, absence seizures (Ryan et al., 1991; Ronen et al., 1993; Steinlein, 2002; Schmitt et al., 2005). One patient (II-3) of the family had paroxysmal squeals started at day 3 and disappeared by 1 month. Because the age of onset and remission of paroxysmal squeals is compatible with typical BFNC, we think paroxysmal squeals may be a rare manifestation of seizures in BFNC patient. One family member with febrile seizures for two times at the age of 5 years old but not BFNC, did not carry c.988C>T mutation, which is consistent with previous reports and shows that FS and BFNC have different pathogenesis (Lerche et al., 1999; Hirose et al., 2000).

M-current formed by KCNQ2 and KCNQ3 potassium channel subunits has a key role in the stabilization of membrane potential and thus in neuronal excitability. Functional analysis of mutations in KCNQ2 or KCNQ3 so far suggests that a partial loss of activity in the M-current is sufficient to produce epilepsy, and that dominant negative mutations could result in a more severe phenotype (Burgess, 2005). The novel change of p.R330C is located within the linker of pore region and S6 of KCNQ3 potassium channel. R330 is a conserved amino acid among KCNQ subfamily. It can be speculated that KCNQ3 p.R330C possibly impairs the M-current by altering the structure near the pore region or S6 of KCNQ3 channel. Functional expression of KCNQ3 pore mutation p.G310V showed that p.G310V caused a 20% reduction in the maximum heteromeric channel current, while having no effect on the surface expression of KCNQ3 protein. Another KCNQ3 pore mutation, p.D305G reduced the maximal heteromeric current by 40% with no alterations in voltage dependence of activation or deactivation kinetics (Singh et al., 2003). Although the reduction of M-current caused by KCNQ2 or KCNQ3 mutations has been discovered, the pathogenesis of BFNC has not been well explained yet. In our study, c.988C>T as a novel mutation in KCNQ3 led to the substitution Cys for Arg in amino acid position 330 (p.R330C), a conserved residue within the linker of pore region and the sixth transmembrane domain of KCNQ3 potassium channel. This substitution might possibly alter the stabilization of membrane potential and neuronal excitability thereby to cause seizures by impairing the neuronal M-current. We are carrying out KCNQ3 function experiments to investigate how the novel mutation acts in pathogenesis of BFNC. Further studies about identifying new mutation and functional expression of these mutations are necessary for us to understand the pathogenesis of BFNC and exploit the new antiepileptic drugs.
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References


