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Identification and characterization of human neuronal voltage-gated calcium channel gamma 3 subunit gene

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Abstract By homologous expressed sequence tag (EST) searching, one EST (GenBank: W29095) was obtained, which shows 75% identity in 435 bp overlap with the coding sequence of mouse *Cacng2* gene. A 1 545 bp cDNA fragment was obtained from the nested polymerase chain reaction (PCR) and rapid applification of cDNA end (RACE) reaction in the human brain prefrontal cortex cDNA library and the human brain Ready cDNA with the primers designed on W29095. The fragment contained a 948-bp open reading frame (ORF) encoding 315 amino acids, and was named *CACNG3*. As it was identical to a BAC clone (GenBank: AC004125) from chromosome 16p12-p13.1, the *CACNG3* gene was mapped to human chromosome 16p12-p13.1, and the coding region was composed of 4 exons. Reverse transcription PCR (RT-PCR) analysis showed that the *CACNG3* gene expressed in human adult brain and fetal brain. Single strand comformation polymorphism (SSCP) analysis was performed in 3 pedigrees with autosomal recessive retinitis pigmentosa, 8 pedigrees with autosomal recessive retinitis pigmentosa accompanied by deafness and 2 pedigrees with epilepsy, but no mutation was detected.

Keywords: ***Cacng2*, *CACNG3*, gene cloning, SSCP, homologous searching.**

Highly selective and hydrophilic ion channels are composed of protein molecules mounted in the lipid layer of cell membrane, which maintain inorganic ions' concentration gradients between extracellular and intracellular matrixes, and play an important role in signal transduction. Various types

of cells have specific ion channels for specific cellular functions. Recent researches focus on the relationships between clinical neurology, human genetics and ion channel[1], from which the mutations of different calcium channel genes are verified to be responsible for seven kinds of hereditary diseases.

Letts and colleagues[2] recently identified in mouse a neuronal voltage-gated calcium channel gamma subunit named *Cacng2*, and found an early transposon mutation in *Cacng2* in stargazer mouse characterized by hereditary murine absent epilepsy and cerebellar disorder. In this study, we identified a putative human neuronal voltage-gated calcium channel gamma 3 subunit gene by homologous analysis and mapped it to chromosome 16p12-p13.1.

1 Materials and methods

(i) Analysis of EST database. Seqlab softwares of GCG (Genetics Computer Group, Madison, USA) were used to perform the data analysis. The coding sequence of mouse *Cacng2* gene was subjected to BLAST (Basic Local Alignment Search Tool) searching against the human dbEST of NCBI.

One EST (W29095) obtained from retina shows 75% identity in 435 bp overlap with the coding sequence of *Cancng2*. Further analysis was done to determine if the gene including W29095 has been cloned.

(ii) Expression of W29095. Amplification was performed in the human fetal liver, fetal kidney, fetal heart, placenta, brain prefrontal cortex, testis, hypophysis, pancreas and spleen cDNA libraries (Clontech Company), and also in the human placenta, fetal liver, fetal brain, adult liver, adult brain, adult kidney Ready cDNA (Clontech Company) using the primers Car/Caf (table 1) designed on W29095. The reaction components are as follows: 100 ng cDNA library or 200 ng Ready cDNA as template, 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs (Sangon Company), 10 μmol/L of each primer, 0.8 μ Taq polymerase (Sangon Company). The PCR condition was 30 cycles for 10 s at 95℃, 20 s at 58℃, 30 s at 72℃ on PE GeneAmp PCR system 9600.

Table 1 Sequence of PCR primers

Primer name	Primer sequence	position	Product length	Purpose
Caf	5′ tgtggaggacctgctgcctag3′	W29095	215 bp	expression of W29095
Car	5′ gacgttgtgtctgctgcggtg3′			
CaA	5′ gacgttgtgtctgctgcggtgg3′	W29095		nested PCR
CaB	5′ gtcctgttcgtagtcagcatcttc3′			
CaC	5′ aggcggtcaagttctcgctccac3′	W29095		3′RACE and 5′RACE
CaD	5′ cacaccatcccttccactgacatc3′			
CaF	5′ ccgtccagagtaccatgaag3′	*CACNG3*-5′UTR	1 003 bp	verification of *CACNG3* ORF
CaR	5′ caaggctgtgctgggcgtg3′	*CACNG3*-3′UTR		

(iii) cDNA cloning. The nested PCR was performed in the human brain prefrontal cortex cDNA library with the primers CaA/CaB or CaC/CaD (table 1) designed on W29095 and the primers λgt10—5/gt10—5 or λgt10—3/gt10—3 on the arm of vector. The 3′ and 5′ RACE reactions were performed in the human fetal brain and adult brain Ready cDNA with the primers CaA/CaB or CaC/CaD and AP1 or AP2 using touchdown PCR. The conditions of touchdown PCR were as follows: 5 cycles of 94℃ for 5 s, 72℃ for 3 min; 5 cycles of 94℃ for 5 s, 70℃ for 3 min; 25 cycles of 94℃ for 5 s, 68℃ for 3 min on PE GeneAmp PCR system 9600. The products were purified and sequenced with ABI Prism Dye Terminator Cycle Sequencing kit and ABI 377 DNA sequencer. The sequences and W29095 were assembled by assemble software of GCG.

(iv) Verification of the open reading frame (ORF). Amplification was performed in the human brain prefrontal cortex cDNA library with primers CaF and CaR (table 1) designed on the assembled cDNA sequence including a complete open reading frame.

RT-PCR was carried out in the total RNA of human placenta, fetal nerve, fetal spinal cord, fetal liver, fetal heart and fetal brain using the primers CaF and CaR, with β-actin as the internal control.

(v) Structural analysis of nucleotide sequence and amino acids. Seqlab softwares of GCG, such

as Blast, Fasta and Bestfit, were used to perform the homologous analysis; PeptideSort, PepPlot, Motif and Isoelectric were used to perform protein analysis.

(vi) Mutation detection. We designed six pairs of primers on the basis of the genomic sequence of the gene and performed SSCP analysis of 3 pedigrees with autosomal recessive retinitis pigmentosa, 8 pedigrees with autosomal recessive retinitis pigmentosa accompanied by deafness and 2 pedigrees with epilepsy.

2 Results

(i) Expression of W29095. By PCR amplification, a 215-bp specific fragment was obtained from the human brain prefrontal cortex cDNA, hypophysis cDNA, fetal brain Ready cDNA and adult brain Ready cDNA. In GenBank database the cDNA source of W29095 is retina. So the EST W29095 and its relevant gene should be expressed in human brain and retina.

(ii) cDNA cloning and verification of ORF. A 620-bp specific product was amplified from the human brain prefrontal cortex cDNA by the nested PCR. A 600-bp specific product was amplified from the human brain prefrontal cortex cDNA by the 3′ RACE reaction. The two sequences and W29095 were assembled into a 1 545-bp cDNA sequence, in which a 948-bp ORF encoding a predicted 315-aa polypeptide was assigned, with a start codon at nt 390—392 and a stop codon at nt 1335—1337.

A 1 013-bp fragment amplified from the human brain prefrontal cortex cDNA library using primers CaF and CaR was consistent with the assembled sequence. The 1 545-bp cDNA sequence was submitted to GenBank and the accession number is AF134640. It was named *CACNG3* (neuronal voltage gated calcium channel gamma 3 subunit) by the HUGO Nomenclature Committee.

RT-PCR analysis showed a 1-kb specific product which was only amplified from the total RNA of human fetal brain (fig. 1).

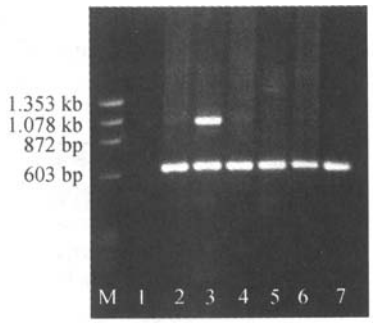

Fig. 1. RT-PCR analysis of *CACNG3* (the 600-bp fragment is the internal control). M, Marker; 1, negative control; 2, fetal spinal cord; 3, fetal brain; 4, fetal nerve; 5, placenta; 6, fetal liver, 7, fetal heart.

(iii) Genomic structure and analysis of nucleotide and amino acids. By searching Genemble database, the *CACNG3* gene was found to be identical to a BAC clone (GenBank: AC004125), which was mapped to 16p12-p13.1. So the *CACNG3* gene was mapped to the same region. The genomic sequence of the *CACNG3* gene was about 100 kb and the coding region was composed of four exons: nt 390—600 is exon 1, nt 601—684 is exon 2, nt 685—826 is exon 3, and nt 827—1 337 is exon 4.

The putative complete coding sequence of the *CACNG3* gene shows 72.1% identity in 751 bp overlap with that of the *Cacng2* gene and the predicted polypeptide shows 74.3% identity in 323 aa with the Cacng2 protein. The predicted polypeptide is a basic polypeptide and the theoretical isoelectric point is 10.25. The polypeptide has no specific motif. PepPlot analysis revealed that the polypeptide is composed of four transmembrane regions, N-terminal being hydrophobic and C-terminal being hydrophilic, which is similar to the polypeptides of the mouse *Cacng2* gene and the human voltage-dependent calcium channel gamma-1 subunit (CACNLG) (fig. 2).

3 Discussion

The *CACNG3* gene showed a high identity to the mouse *Cacng2* gene in both nucleotide sequence and putative polypeptide. The predicted polypeptide has four hydrophobic transmembrane domains which resemble the mouse *Cacng2* and the human *CACNLG*. So we speculated that the *CACNG3* gene is a member of the calcium channel gemma subunit family. The mouse *Cacng2* gene is only expressed in the cerebrum of adult mouse, not in heart, spleen, lung, liver, kidney and skeletal muscle[2]. Our studies showed that the *CACNG3* gene is expressed in human brain and retina, not in other tissues. We conclude that the *CACNG3* gene is a kind of human neuronal voltage-gated calcium channel.

The ion channels mounted in the cell membrane play a critical role in maintaining ion

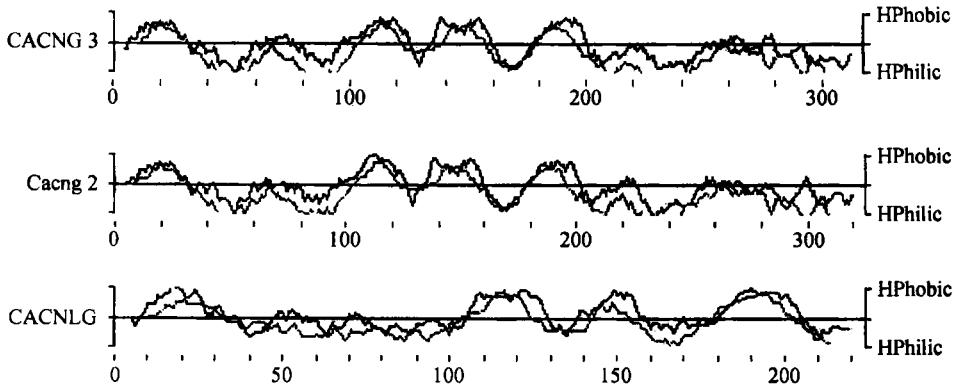

Fig. 2. PepPlot analysis of CACNG3 polypeptide. The abscissa is numbers of amino acid and the ordinate is hydrophilism (HPhilic)/hydrophobicity (HPhobic). The CACNG3 polypeptide has four transmembrane regions. N-terminal is hydrophobic and C-terminal is hydrophilic, similar to the polypeptides of the mouse Cacng2 and the human CACNLG.

concentration gradients and signal transduction. The calcium channels regulate the flux amount of calcium ion and participate in a series of Ca^{2+}-dependant physiological actions such as muscular contraction, releasing of hormone and neurotransmitter, and signal transduction. The mutations of many ion channel genes lead to neuromuscular disorder. So far, seven kinds of human hereditary diseases are found associated with the mutations of calcium channel genes. The mutations of P/Q type voltage-gated calcium channel α1A subunit result in familial hemiplegic migraine, episodic ataxia type 2[3] and spinocerebellar ataxia type 6[4], respectively. The hypokalemic periodic paralysis[5–7] is attributed to the mutations of C type voltage-gated calcium channel α1S subunit. Mutations in the human ryanodine receptor gene are associated with the central core disease[8] and malignant hyperthermia[9]. There exist three mechanisms that can be used to explain the dysfunction of calcium channels[10]. i) The mutation increases the calcium channel activity. ii) The normal functional calcium channel is not adequate to maintain physiological functions. The mutants interfere with the activity of normal protein. Many channel auxiliary subunits make important contributions to channel functions, although they are incapable of forming pores themselves. For example, Lethergic mice have a frameshift mutation in the calcium channel β4 subunit[11]. An early transposon insertion in the intron 2 of the mouse calcium channel γ subunit *Cacng2* is responsible for the stargazer seizure disorder in mice. It is possible that the mutation of the *CACNG3* gene may lead to calcium channel dysfunction. Expression of the *CACNG3* gene only in human brain and retina revealed that the mutation of the *CACNG3* gene was associated with nervous hereditary disease or retinal disease.

In 1997, four pedigrees with autosomal dominant infantile convulsive disorder accompanied by paroxysmal choreoathetosis were localizd to 16p12-q12[12]. Lee[13] also mapped a Chinese inherited infantile convulsive disorder with paroxysmal choreoathetosis to the same region in 1998. Finckn[14] identified a locus for autosomal recessive retinitis pigmentosa at 16p12.1-p12.3. Therefore, the *CACNG3* gene is a candidate gene for these disorders. We then performed the SSCP analysis at three pedigrees with autosomal recessive inherited retinitis pigmentosa, eight pedigrees with autosomal recessive inherited retinitis pigmentosa accompanied by deafness and two pedigrees with epilepsy, but no mutation was found. Previous studies have indicated that retinitis pigmentosa and epilepsy have a higher genetic heretogeneity, therefore, mutation analysis of more families is necessary.

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