

Original Article

Functional implication of KCNJ10 gene polymorphism in childhood epilepsy

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Abstract: As a commonly occurred brain dysfunction, epilepsy has a sudden onset and is believed to be related with abnormal potassium level and firing pattern of brain neurons. The genetic factor has been implicated under the pathogenesis of epilepsy especially in children patients. KCNJ10 gene locates on chromosome 1q22-q23 and encodes for one important rectifying potassium channel. This study thus aimed to investigate the correlation between KCNJ10 gene polymorphism and childhood epilepsy. A total of 90 core families consisting of Chinese epilepsy children (Han ethnic group) were analyzed with their parents for single nucleotide polymorphism (SNP) at loci rs1186679 using PCR-restriction fragment length polymorphism (RFLP). The genotype frequency distribution at loci sr1186679 follows Hardy-Weinberg equilibrium. Haplotype relative risk (HRR) analysis revealed correlation between sr1186679 loci and childhood epilepsy ($P < 0.005$). Transmission disequilibrium test (TDT) showed significant difference in the genotype frequency distribution of between the transmissions from heterozygous parents to affected children and that in the transmission of unrelated alleles. There is a potential relationship between sr1186679 loci of KCNJ10 gene and childhood epilepsy in Chinese Han people.

Keywords: Genetics of epilepsy, gene polymorphism, restriction fragment length polymorphism, KCNJ10 gene

Introduction

Caused by abnormal synchronized hyperpolarization firing of brain neurons, epilepsy is manifested with sudden onset and temporary brain dysfunction. More than half epilepsy patients had their first onset during childhood, suggesting the involvement of genetic factors in the pathogenesis. Traditional genetic research using whole genome screening has revealed some evidences of genetic predisposing factors of epilepsy [1, 2]. With advancement of genomic and proteomic techniques, epilepsy-related genes such as $\gamma 2$ subunit of GABA_A receptor, KCNQ2/3 potassium channel, SCN1A/B genes have been identified. Recent studies have established the participation of calcium ions in certain phosphorylation/de-phosphorylation mechanisms in the systemic epilepsy onset with fever and seizure [3]. As these processes

involve calcium-dependent sodium influx and potassium efflux [4, 5], the role of related potassium channel is worth further studies.

KCNJ10 gene locates chromosome 1q22-q23 and encodes for one inwardly rectifying potassium channels, which can be activated by endogenous adenosine tri-phosphate (ATP) and helps to maintain the cytosolic potassium level of glial cells in the brain [6, 7]. This study therefore investigated the relationship between KCNJ10 gene polymorphism and childhood epilepsy.

Materials and methods

Research objects

We recruited 90 core families, which consisted of Chinese childhood epilepsy patients and

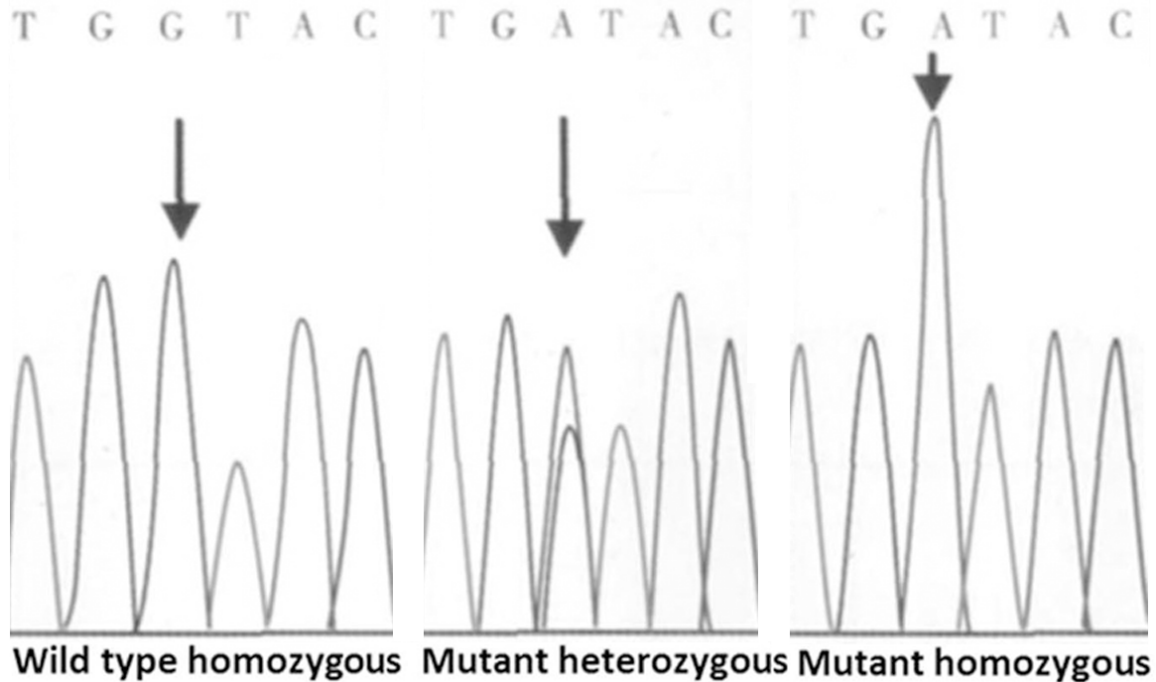


Figure 1. Sequencing results of restrictive digestion.

Table 1. Genotype frequency distribution at loci rs1186679

	The genotypic frequency			Chi-square value	P value
	AA	AG	GG		
Transmitted	94	76	9	1.234	0.038
Non-transmitted	7	38	46		

Table 2. DTT analysis at loci rs1186679

Allele	Transmitted frequency	Chi-square value	P value
G	15	1.203	0.026

their biological parents (all in Han ethnic group). Peripheral venous blood (5 mL) were drawn and stored at -20°C for extracting genomic DNA. This study has been pre-approved by the ethical committee of our hospital. Written consents have been obtained from all children's guardians.

Genomic DNA extraction

2 mL venous blood was extracted for genomic DNA using a whole-blood DNA extraction kit (Tiangen Biochem, China) following manual

instruction. Extraction products were measured using ultraviolet spectrometer for concentration and purity, and were stored at -20°C for further use.

PCR amplification of target gene fragments

Specific designed primers (Forward: 5'-TAG GAA AGG GCT CAG CGT AG-3'; Reverse: 5'-CCT AAG ACG GGG AAA GAA GC-3') were used to amplify target loci of KCNJ10 gene using PCR buffered mix (Invitrogen, US). The amplification parameters were: pre-denature for 5 min at 94°C; 94°C denature for 1 min, 60°C annealing for 1min and 72°C elongation for 3 min, repeated for 30 cycles; ended with 72°C elongation for 3 min. PCR products were separated by 1% agarose gel electrophoresis.

Restriction enzyme digestion

PCR products were processed by restriction enzymes according to the cutting pattern and were further separated by agarose gel electrophoresis. For those 300 bp length fragments, three genotypes have been identified: (1) uncut homozygous (A/A); (2) heterozygous with cutting (A/G) and homozygous (G/G).

Statistical analysis

Using chi-square method, we firstly tested if the distribution of genotype frequency follows Hardy-Weinberg equilibrium. Then haplotype relative risk (HRR) and transmission disequilibrium test (TDT) were performed. In HRR analysis, non-transmitted alleles in patient's parents were established as a reference for the calculation of disease allele transmission. TDT was based on the linkage analysis in a disequilibrium scenario to observe the probability of transmission of predisposing genes. SPSS 13.0 software package was used to perform all analysis.

Results

PCR amplification

Extracted DNA had a satisfactory concentration, with 260/280 ratio at 1.7~1.9, suggesting a high purity. Target gene KCNJ10 fragments were amplified by PCR and sequenced at loci sr1186679 as shown in **Figure 1**.

Hardy-Weinberg equilibrium

We analyzed the genotypes of both parents and affected children using Hardy-Weinberg equilibrium test. The obtained *P* value is larger than 0.05, suggesting that the frequency distribution of genotype basically follow Hardy-Weinberg equilibrium

Allele frequency analysis

We further employed HRR method to compare the allele frequency distribution between patients and their families. Results showed significant relationship between loci rs1186679 and childhood epilepsy due to significant difference of allele frequency ($P < 0.05$), as shown in **Table 1**.

Transmission disequilibrium test

Within all core families that we studied, TDT method was applied to analyze the transmission pattern for those at least one of parents had heterozygous genotype. Results showed significant difference between frequencies of two alleles that were transmitted from parents to their children (**Table 2**). Our study thus suggested the correlation between rs1186679 and childhood epilepsy.

Discussion

Epilepsy had an averaged incidence at about 7.0% in china [5], making it one of most commonly occurred neurological diseases and causing heavy burdens for patient family and the whole social health care system. It is believed that epilepsy, especially those with childhood onset, has certain genetic basis under multiple genes, as it show certain family concentrated pattern from epidemiology study. Besides the genetic factor, other influences from environment or postnatal diseases may also cause epilepsy. Overall speaking, epilepsy has a very complex pathogenesis mechanism and no single gene has been confirmed to play a critical role in epilepsy. Current genetic studies mainly compared the clinical patients and family history to determine the epidemiology. Recent reports performed correlation analysis between chromosome 1 and childhood epilepsy risk gene factors using the third generation of single nucleotide polymorphism (SNP) approach [1, 5]. With advancement of proteomic and genomic methods, data analysis related with epilepsy predisposing factors has been extensively explored. With the help of bioinformatics, epilepsy gene microarray data have been processes to obtain some genes/loci that were crucial, such as those encoding voltage gated calcium/sodium/potassium channel and ligand-receptor signal pathways.

Locates within 1q22-23, KCNJ10 gene encodes for one transmembrane inwardly rectifying potassium channel that can be activated by adenosine tri-phosphate (ATP) which is widely distributed and evolutionally conserved among all mammalian cells, thereby playing a certain role in buffering intracellular potassium level in brain glial cells. This study employed both TDT and HRR linkage analysis to test one certain SNP loci (rs1186670) of KCNJ10 gene and found a close correlation of these loci with childhood epilepsy.

Studies have found prominent expression of various potassium channels in GABA-ergic interneurons within cerebral cortex and hippocampus [6-10]. As those neurons have certain roles in inhibiting activity of pyramidal neurons [6-8], it is highly likely that potassium channels participate in the epilepsy onset under certain conditions. Recent studies have found the possible correlation between potassium channel

and selective serotonin reuptake inhibitor (SSRI) targets. It is well known that serotonin (5-HT) mainly modulates the potassium channel open in an attempt to regulate the excitability of neurons, thereby playing a certain role in epilepsy onset [9, 10]. Animals studies showed that potassium channel gene knockout mice, when treated with glutamate receptor agonist (such as digenic acid) and GABA receptor antagonist (pentylenetetrazole, PTZ) to induce epilepsy onset, had more severe disease, higher mortality rates and maximal onset strength compared to controlled animals [11-13], suggesting the involvement of potassium channel in epilepsy onset. Further electrophysiological experiments found that those potassium channel gene knockout mice had significantly higher frequency and amplitude of bilateral spike firing compared to those in wild type mice. Moreover, c-fos, a biochemical marker for neuronal activity, showed elevated expression in those knockout mice. All these results support the role of potassium channel in suppressing neuronal excitability and thus may work as a target for epilepsy onset [14, 15].

It is once believed that one inducible endogenous mechanism existed in the brain that can protect neurons from damages from ischemia. This mechanism may consist of potassium channels as supported by previous studies in cerebral ischemia models [16-18]. Based on the interaction between cerebral ischemia and epilepsy, it is believed that potassium channel plays a certain role in epilepsy onset, but with unclear mechanisms so far. Recent studies showed some inhibitory function of potassium channels in hippocampal GABAergic neurons [19-21]. Based on numerous *in vivo* and *in vitro* studies, potassium channel may indirectly induce the abnormal firing of neurons via its modulation on neuronal excitability, thereby playing a role in epilepsy onset.

Although great achievements have been obtained regarding pathogenesis of epilepsy, the detailed mechanism underlying this neural disorder is still unclear. Past studies and our result both point to the potential involvement of potassium channel. With further in-depth work, especially those for pathological mechanisms, related genes and drug target screening, clinical treatment of epilepsy will be benefited from basic researches. So far little has been known about the direct relationship between epilepsy

and potassium ions. Our future work therefore will be concentrated in the dynamic change of cytosolic potassium level during the pathogenesis process of epilepsy, thereby identifying direct evidence and potential drug targets for clinical treatment.

On the other hand, the correlation between Cyp450 gene polymorphism and refractory epilepsy has been established. For example, one study analyzed the SNP pattern of Cyp3A4 gene in Chinese Han children with refractory epilepsy using PCR-RFLP technique. They found that 7% of mutant heterozygous in certain loci on affected children, but not in any of epilepsy children who had satisfactory response to medications. Further statistical analysis found significantly higher genotype/mutant allele frequency at Cyp3A4-18A polymorphism loci, compared to those patients who are responded to drugs. Their results suggested certain correlation between Cyp3A4-18A gene polymorphism and drug resistance of epilepsy, indicating the potential significance of genetic diagnosis in optimizing treatment plan.

In summary, this study found possible relationship between sr1186679 loci of KCNJ10 gene and childhood epilepsy in Chinese Han people, thus further suggesting the role of potassium channel in the pathogenesis of epilepsy. Our results suggest one possible genetic diagnosis method for early identification of childhood onset epilepsy and are worth for further exploration regarding the gene polymorphism, channel function and epilepsy pathology.

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Disclosure of conflict of interest

None.

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