Original Article

Two novel mutations in NOTCH3 gene causes cerebral autosomal dominant arteriopathy with subcritical infarct and leucoencephalopathy in two Chinese families

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Abstract: Objective: To investigate the genetic pathogenic causes of cerebral autosomal dominant arteriopathy with subcritical infarct and leucoencephalopathy (CADASIL) in two Chinese families, to provide the molecular basis for genetic counseling and antenatal diagnosis. Methods: The genetic mutation of gene NOTCH3 of propositus and family members was analyzed in these two CADASIL families by polymerase chain reaction and DNA sequencing technology directly. At the same time, the NOTCH3 gene mutation point of 100 healthy collators was detected, to explicit the pathogenic mutation by function prediction with Polyphen-2 and SIFT. Results: Both propositus of the two families and patients with symptom were all accorded with the clinical features of CADASIL. It was shown by DNA sequencing that the 19th exon [c. 3043 T > A (p.Cys1015Ser)] in gene NOTCH3 of propositus, 2 patients (II3, III7), and a presymptomatic patient (IV1) in Family I all had heterozygosity missense mutation; and the 3rd exon [c.316T > G, p. (Cys106Gly)] in gene NOTCH3 of the propositus, a patient (IV3) and two presymptomatic patients (IV5, 6) in Family II all had heterozygosity missense mutation; and no mutations were detected in the 100 healthy collators. It was indicated by analyzing the function prediction that the mutation of [c. 3043 T > A (p.Cys1015Ser)] and [c.316T > G, p. (Cys106Gly)] may both influence encoding protein in NOTCH3. By analysis of the conservatism of mutation point in each species, these two basic groups were highly conserved. Conclusion: The heterozygosity missense mutation of 19th exon [c. 3043 T > A (p.Cys1015Ser)] and the 3rd exon [c.316T > G, p. (Cys106Gly)] in NOTCH3 gene are the new pathogenic mutations of CADASIL, and enriches the mutation spectrum of NOTCH3 gene.

Keywords: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leuкоencephalopathy, NOTCH3 gene, mutation

Introduction

Cerebral autosomal dominant arteriopathy with subcritical infarct and leucoencephalopathy (CADASIL) is a rare single-gene hereditary disease with small brain artery caused by mutation of NOTCH3 located in 19p13 with adult onset, no hazards of obvious cerebrovascular stroke, recurring cerebral ischemic attack. Multifocal lacunar cerebral infarction and extensive white matter osteoporosis are the major performances in head MRI. And it may be accompanied by cognitive disorder, migraine with aura and mental disturbance [1]. A total of more than 20 mutations of NOTCH3 were gradually found such as R110C and R153C after CADASIL families were firstly reported in China 2000 [2]. The NOTCH3 gene mutation of two CADASIL families were investigated in this paper, to identify 2 new mutations of NOTCH3 gene causing CADASIL attack to Han Chinese in China, and to explicit its genetic etiology.

Family I is from Hefei, Anhui Province. The propositus is 55-year-old, male, transient ischemic attack (TIA) comes repeatedly since the age of 48. There were three times that new cerebral infarction attacked in April, July and August in 2013, and then he was sent to our hospital. Head MRI showed extensive cerebral white matter lesions, and he had cognitive impairment, recent memory decline, depression and...
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migraine without aura. There were 5 patients with clinical symptoms in four generations in Family I, I1 and II1 died recurrent stroke at the ages of 65 and 67; II3 and III7 were attacked by repeatedly TIA and recurrent stroke at the ages of 43 and 41 (Figures 1, 2). Family II is from Yingshang, Anhui Province. The propositus is 46-year-old, female, migraine repeated frequently since the age of 43, recent memory declined and depressed at the age of 44, and cognitive impairment arose at the age of 45, dizziness happened since last month. Head MRI showed multifocal cerebral infarction and extensive cerebral white matter lesions. There were 6 patients with clinical symptoms in 4 generations in Family II. I1, II3, II5, and III5 died recurrent stroke at the ages of 69, 67, 59, and 63; IV3 was attacked by the age of 49, repeatedly headache, cognitive impairment, and cerebral infarction attacked at the age of 41 (Figures 1, 3). The patients in the 2 families have no history of hypertension, diabetes, hyperlipemia, hyperhomocysteinemia, amyloidosis, and vasculitis, and no history of smoking and alcohol. Both families accord with characteristic of autosomal dominant inheritance, and both propositus and patients accord with CADASIL.

The 100 collators are healthy medical men in MEC of our hospital, they do not have the his-
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Figure 3. Brain MRI images of patient III9 in Family II. A: Bilateral lateral ventricle narrator osteoporosis; B: Leuko-araiosis bilateral ectocyst, bilateral basal ganglia region multifocal lacunar cerebral infarction; C: The anteromedial temporal leukoaraiosis.

Figure 4. Sequence diagram of NOTCH3. A: c.3043T > A hybrid missense mutation of propositus in Family I (arrow); B: Healthy collator, no mutations found; C: c.316T > G hybrid missense mutation of propositus in Family II (arrow); D: Healthy collator, no mutations found.

tory of nervous system diseases, vasculitis diseases, and mental diseases, no cerebrovascular disease with high risk, and family history. Every person been tested in this research had signed informed consent, and this research is authorized by Ethics Committee of Anhui Provincial hospital.

Methods

DNA extraction and NOTCH3 gene sequencing

Draw venous blood 5 mL (anti-freezing by sodium citrate), extract genome DNA by QIAamp DNA Blood Mini Kit (QIAamp, Germany). The primer, designed by Primer Premier5, covered the code area and splice site of NOTCH3 gene, and was composed by Sangon Biotech (Shanghai). Reaction condition of PCR includes: 95°C Initial denaturate for 5 min; 94°C denaturate for 30 s; 65°C anneal for 1 min; 72°C extend for 1 min, 35 cycles in total, and 72°C extend for 7 min. After purified, the target DNA amplified by PCR would be sequenced by Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, America).

Function prediction of gene mutation

The biological function of amino acid changing the protein structure and function of NOTCH3 were evaluated by software PolyPhen-2 and SIFT.

Conservation analysis of mutation site

NOTCH3 protein of human being were compared to isogeny AA seq of many species
in Uniprot (http://www.uniprot.org/uniprot/Q9UM47). The amino acid sequence of functional domain was analyzed in where mutation located by PolyPhen-2, especially the coincidence degree of species of which mutation site was representative.

Results

Detection result of mutation in NOTCH3 gene

After DNA sequencing, hybrid missense mutation of 19th exon [c. 3043 T > A (p.Cys1015Ser)] in NOTCH3 gene was detected in propositus and 2 patients (II3 and III7) in Family I, the presymptomatic patient (IV1) also carries the mutation; hybrid missense mutation of 3rd exon [c.316T > G, p. (Cys106Gly)] in NOTCH3 gene was detected in propositus, IV3, and 2 presymptomatic patients in Family II; and no mutations mentioned above detected in the 100 healthy collators (Figure 4).

Result of function prediction of gene mutation

The two assay value of PolyPhen-2 and SIFT were 1 and 0, it meant that the 2 locus mutations mentioned above might lead to the structural failure of protein in NOTCH3, and was likely to be the pathogenic locus mutations (Figure 5).

Result of conservation analysis of mutation site

This locus of human race in all the transcript of NOTCH3 was conserved sequence. Compared with other species, the basic group of the two locus mutations are highly conserved (Figure 6).

Discussions

The primary pathogenesis of CADASIL is the degenerative change of vascular smooth muscle cells (VSMC) related to the mutation of NOTCH3 gene [3]. Human NOTCH3 gene mainly expresses on VSMC, it is made up by 33 exons, and encodes a transmembrane protein made up by 2321 amino acid, which has the function of receptor and signal transduction. There are 34 repetitive sequence of epidermal-growth factor like (EGF-like) on the receptors of the lateral structure domain of NOTCH3, there are 6 cysteine residues combined each other with 3 disulfide bond in each domain of EGF-like. The 34 repetitive sequence of EGF-like out of the cell combine with the ligand, the 6 repetitive sequence of ankyrin mediate signal transduction inside the cell, and play an important role in cell differentiation. The pathogenic mutation of NOTCH3 lead to oddizing the number of cysteine residues in EGF-like sequence, ruined the normative pairing of disulfide bond, changed the protein conformation and the interaction between receptor and ligand, and made the maturity and differentiation of VSMC abnormal and pathogenic. At present there are over 200 CADASIL pathogenic mutation of NOTCH3 having been reported, most of them are Caucasians [4]. Most of the mutations of NOTCH3 related to CADASIL are in the repetitive sequence of EGF-like, and most of them are hybrid missense mutation, which are mainly centralize in 2-24th exon, related to cysteine, some homozgyous mutations and mutations out of the repetitive sequence of EGF-like were reported in recent years [5, 6]. The hot spots of NOTCH3 gene mutations are on the 3rd and 4th exon in the CADASIL families in Chinese Mainland [2].

The mutation [c. 3043 T > A (p.Cys1015Ser)] in Family I is located in the 19th exon of NOTCH3 gene, and the mutation [c.316T > G (p. Cys106Gly)] in Family II is in the 3rd exon, hot spot of NOTCH3 mutations, both of them are missense mutation inside of the repetitive sequence of EGF-like. The cause of 1015th codon transformation from cysteine to serine is the happening of T > A shift in the 3034th nucleotide of NOTCH3 gene in Family I. And the T > A shift in 316th nucleotide of NOTCH3 lead to the transformation of the 106th amino acid residue from cysteine to glycine. The analysis by PolyPhen-2 and SIFT shows that these mutation may cause the protein structure destruction of NOTCH3. The 100 healthy collators without genetic connection were directly sequenced, and no mutations mentioned above were found, therefore the possibility of single nucleotide polymorphism was excluded. By the conservation analysis of multi-source sequences to these two mutations, the sequences were in the conserved region, and were deduced to be pathopoiesis mutations, and were inherited pathogenic factor of the two families. After searching [Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and NOTCH3 gene Mutation Database (http://www.
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Figure 5. Pathogenicity of c.3043 T > A and c.316T > G mutations (A: c. 3043 T > A; B: c.316T > G, prediction result by Bioinformatics software, PolyPhen-2, showed high possibility that it maybe the pathoadaptive mutation.
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LOVD.nl/NOTCH3] at the same time, the mutation of [c. 3043 T > A (p.Cys1015Ser)] and [c.316T > G (p.Cys106Gly)] were not found being reported, these were new pathopoiesis mutations, and enriched the NOTCH3Gene mutation spectrum.

At present, some researches find that there are less congruent relationship between genotype and phenotype of CADASIL patients [7], complex clinical features, much more difference in different races and regions. Team of Wang Jun compared the clinical features of CADASIL families in China and other countries, found that cerebral ischemic stroke and TIA were common and the first symptom in China, a few patients were attacked by cerebral hemorrhage, and the morbidity of migraine were lower than the other countries obviously; Head MRI showed lacunar infarction and (or) leukoaraiosis dispersivity. Features of CADASIL were reported to be the T2 signal of the temporal pole white matter shown by Head MRI abroad [8]. There were also the positive reported, with low sensibility in our country, the sensibility was lower than 50% in reports of mainland and Taiwan in China [2, 9]. The first symptom of 6 patients with clinical symptoms in the two families is transient cerebral ischemic attack, three of them had cognitive decline, and two had depression, Head MRI of all the six patients showed extensive cerebral white matter damage and multifocal lacunar cerebral infarction, accord with CADASIL in China. The further study to the mutations of NOTCH3 in recent years shows that the clinical features can be different by the same mutation, in the same country. For example, in Italy, with the same mutation of R1006C located to 19th exon, 5 of patients showed late-onset vascular Parkinson's, and the other three patients didn't showed that [10]; different patients have different characteristics even in one family [11].

Because of there being no effective cure to CADASIL, early diagnosis and genetic counseling become more and more important. With the development of molecular genetics diagnosis technology, genetic diagnosis becomes easy to go, and have the clinical practical value to make a definite diagnosis to CADASIL. Therefore, clinical suspected patients of CADASIL and family members should take a NOTCH3 mutation detection to clarify the diagnosis, and provide gist to antenatal diagnosis and genetic counseling, to avoid giving birth to new babies with birth defects.

Disclosure of conflict of interest

None.

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