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A rare mutation in exon 7 of the *NOTCH3* gene in a Chinese CADASIL family: Case report with a literature review

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Abstract

More than 300 mutations have been reported since *NOTCH3* was identified as the causal gene of CADASIL. However, mutation sites on exon 7 have rarely been reported in patients with CADASIL. We reported a 44-year-old female from a Chinese family with a clear family history presented with progressive dizziness and gait disturbance for more than 2 months and aggravated for 20 days. Whole-exome sequencing (WES) was used to identify a rare heterozygous missense variant (NM_000435.3: c.1136G>C) of *NOTCH3* in this patient. PolyPhen-2 and VarSite predicted that this mutation site was probably pathogenic with the highest score of 1.00 and highly conserved among species. Our case report first identified a rare C379S mutation in exon 7 of *NOTCH3* in a Chinese CADASIL family, expanding the ethnic spectrum of this condition. Moreover, in view of our report and literature, if patients have a clear family history and manifest uncommon clinical manifestations of CADASIL such as dizziness and atypical headache, clinicians could consider screening *NOTCH3*.

Keywords: CADASIL, *NOTCH3,* exon7, mutation, C379S

INTRODUCTION

CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, OMIM 125310) is a rare autosomal dominant monogenetic cerebral small vascular disorder characterized by recurrent stroke, progressive dementia, migraines, or psychiatric disorder in adults.[1] To-date more than 300 mutations have been reported since *NOTCH3* (Notch receptor 3) was identified as the causal gene of CADASIL in 1996.[2] *NOTCH3* gene consists of 33 exons, which encodes a single-pass transmembrane receptor with an extracellular domain (ECD) comprised of 34 epidermal growth factor-like repeats (EGFr), a transmembrane domain, and a Notch intracellular domain (NICD). The majority of CADASIL-associated mutations are located in exons 2-24 encoding the EGFr, of which exons 3 and 4 are the most common clustering mutation regions.[3,4] However, any type of mutation in exon 7 has never been reported in the Chinese population. Herein, we report the first Chinese CADASIL family with a rare heterozygous C379S mutation and a literature review on clinical features of mutations on exon 7 of the *NOTCH3* gene.

CASE REPORT

The proband (Figure 1A, family member III-9) was a 44-year-old, right-handed female labourer from Zhejiang province. She presented with progressive dizziness and gait instability for more than 2 months, aggravated for 20 days. The patient had a history of hypertension for nearly 8 years. Currently she was taking amlodipine besylate tablets 5 mg per day combined with losartan 50 mg per day for antihypertensive treatment and her blood pressure was controlled at a normal level. The patient denied any history of migraine, diabetes, or coronary heart disease. The neurological examination showed a positive Hoffmann's sign on the left. Fluid attenuated inversion recovery (FLAIR) imaging of Brain MRI (3.0T) indicated multiple lacunar infarcts and diffuse hyperintensity lesions in centrum semiovale, periventricular, and external

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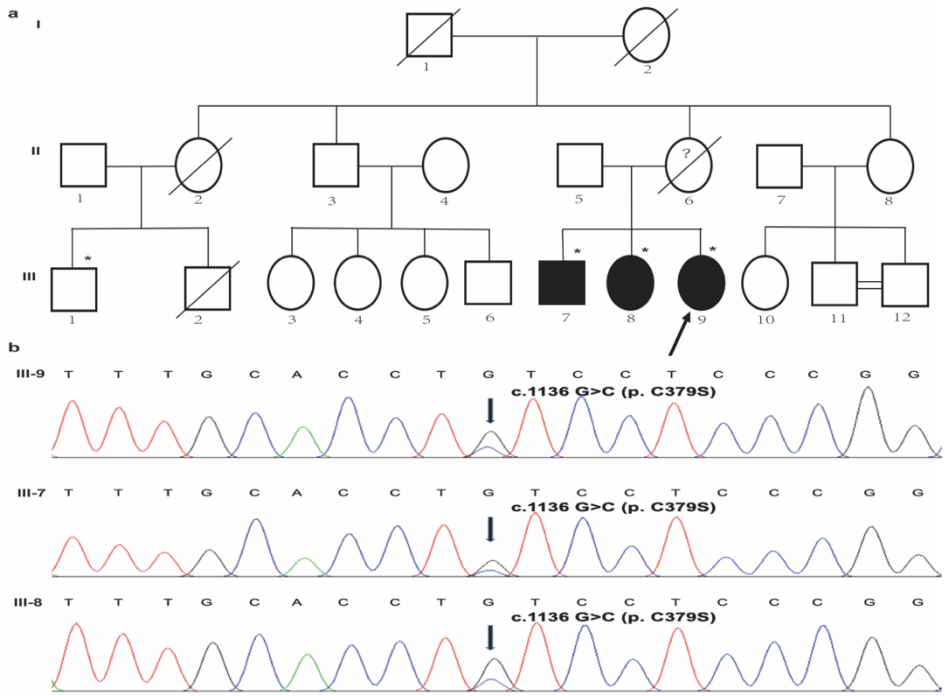

Figure 1. A rare NOTCH3 mutation found in a Chinese CADASIL family.
The genealogy of this Chinese CADASIL family was shown in (A). The square and circle represent males and females, respectively. The arrow points to the proband. The black-filled symbol indicates an affected subject. The white-filled symbol indicates a normal subject. The diagonal line through the symbol represents a deceased individual. Gene-tested individuals are marked with an asterisk. A question mark indicates an uncertain disease status. Gene sequence analysis of III-7, III-8, III-9 and an arrow indicating the mutation site (B).

capsular (Figure 2A, A1-A4). Susceptibility-weighted imaging (SWI) showed multiple microbleeds in bilateral thalami and basal ganglia (Figure 2A, A5). Transcranial Doppler (TCD) ultrasound foaming test displayed positive results supporting a right-to-left shunt of the heart, but the patient refused further transesophageal echocardiography and right heart angiography. Magnetic resonance angiography (MRA), Doppler ultrasound of cervical vessels, and 24-hour Holter electrocardiogram were normal. Laboratory tests showed an abnormal increase in total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). No obvious abnormalities were found in the blood routine examination, routine urine test, routine stool test, thyroid function glycosylated haemoglobin and cardiolipin antibodies.

The family pedigree is shown in Figure 1A. Her mother died of cerebral vascular disease at the age of 54 years (Figure 1A, family member II-6). Her elder brother began to have slurred speech when he was 40 years (Figure 1A, family member III-7). T2-weighted and SWI MRIs showed multiple leukoaraiosis in centrum semiovale, periventricular, external capsular and thalamic (Figure 2B, B1-B5), and microbleeds in bilateral basal ganglia and thalamic (Figure 2B, B6), respectively. Her elder sister (Figure 1A, family member III-8) had similar symptoms of gait instability (No MRIs were obtained).

The patient's family history and clinical presentation suggested the diagnosis of CADASIL, and genetic testing was conducted. Genetic analysis showed a heterozygous nucleotide mutation c.1136G>C in exon 7 of the *NOTCH3* gene of the proband, leading to the amino acid cysteine being replaced by serine at loci 379 (Figure 1B). This mutation was also verified in her family members III-7 and III-8 (Figure 1B). This mutation site was not detected in proband's cousin (III-1) with normal phenotype. The detected mutation frequency was absent in ExAC, 1000G, GnomAD, and Esp6500 databases. Mutation Taster (www. mutationtaster.org) predicted that this mutation was disease-causing, and the Polyphen-2 database showed a damaging score of 1.00 (Figure 3A). In addition, VarSite, an online tool for predicting disease variants and

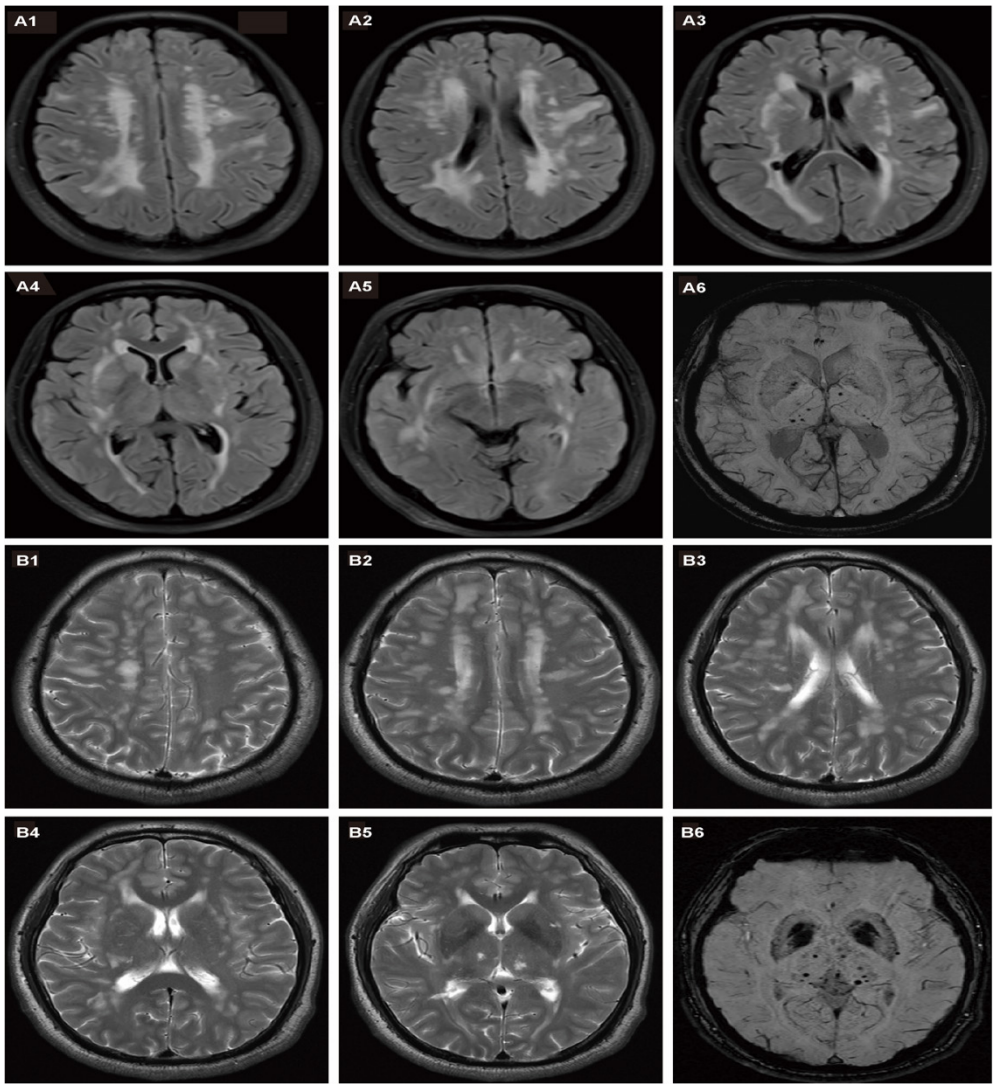

Figure 2. Brain MRI images of the proband (III-9) and her elder brother (III-7).
FLAIR and SWI MRIs of the proband (III-9) revealing multiple lacunar infarcts and diffuse hyperintensity lesions in centrum semiovale, periventricular, and external capsular (A1-A5), and multiple microbleeds in bilateral basal ganglia and thalamic (A6). T2-weighted and SWI MRIs of III-7 indicating multiple leukoaraiosis in centrum semiovale, periventricular, external capsular and thalamic (B1-B5), and microbleeds in bilateral basal ganglia and thalamic (B6).

protein structure, demonstrated that the alignment sequences were highly conserved among species (Figure 3A). Unfortunately, except for the proband's cousin (Figure 1A, family member III-1), the other members of this family refused to provide blood samples for genetic analysis.

DISCUSSION

In this report, a rare heterozygous missense mutation c.1136G>C (p. C379S) in exon 7 of the *NOTCH3* gene was first identified in three siblings from a family of Chinese descendants. Free online prediction software PolyPhen-2 and VarSite suggested that the p. C379S may damage the function and structure of the NOTCH3 protein (prediction score=1.00) and this locus is highly conserved among species, respectively. Thus, our study suggested the *NOTCH3* c.1136G>C mutation site may be a disease-causing mutation of CADASIL.

The proband and her elderly sister suffered progressive gait disturbance, and her elderly brother presented with recurrent slurred speech. MRI scans of the proband and her elderly brother

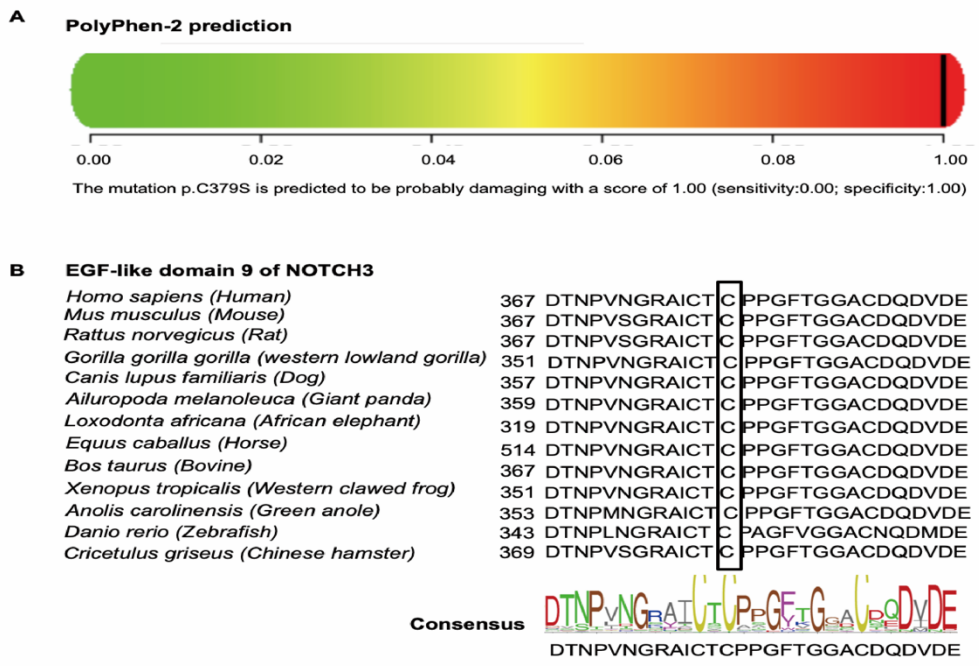

Figure 3. Pathogenicity and conservation prediction of this rare mutation.
Polyphen-2 prediction indicating the mutation p.C379S is probably damaging with the highest score of 1.00 (A). The cysteine at position 379 is highly conservative among species and the consensus sequences are analyzed with the online prediction tool VarSite (B).

were highly consistent with typical neuroimaging features of CADASIL, including bilateral subcortical infarcts and extensive hyperintensity lesions in centrum semiovale, periventricular and external capsular, and microbleeds in bilateral basal ganglia and thalamic. The subjects in our study reported no history of migraine with or without aura, which is in accordance with a low incidence of migraine in patients with CADASIL from the Asian population.[4,5] Combined with the typical imaging results, clinical presentation, and positive family history, the proband met the clinical diagnostic criteria for CADASIL. The diagnosis of CADASIL was genetically confirmed by the detection of a rare heterozygous mutation in exon 7 of the *NOTCH3* gene, which caused a substitution of cysteine with serine (p. C379S) in the encoded receptor.

So far, 5 other missense mutations and 2 synonymous mutations were reported within the exon 7 of the *NOTCH3* gene in CADASIL patients.[3,6-11] We summarized the clinical characteristics of our case and the reported cases with mutations in exon 7 of the *NOTCH3* (Table1). Of note, the identified mutation p. Cys388Tyr reported in 2006 was a 38-year-old Japanese woman with a nonspecific headache, typical imaging presentation, and granular electron-dense osmiophilic material (GOM) in the skin.[6] In 2011, a study reported a novel 15-base pair duplication in exon 7 of the *NOTCH3* gene in a male African American CADASIL patient with typical CADASIL presentation of dementia and recurrent strokes.[12] To our knowledge, only two published studies identified this rare mutation, one study reported p.C379S in one out of 125 unrelated German patients with biopsy-proven CADASIL, and another study reported p.C379S in one out of 411 patients from 125 families with CADASIL.[8,13] Unfortunately, the former two studies were both from European descendants and provided no detailed clinical manifestations of this rare mutation. Hence, our study was the first to report this rare mutation in a Chinese CADASIL family with detailed clinical data.

There are several weaknesses in the present study. 1. Because the remained family members refused to provide blood samples, genetic analysis was not performed in all family subjects. 2. The clinical data were incomplete due to the inability to obtain the imaging data of the proband's sister. 3. No skin biopsy was performed in patients to further support the diagnosis of CADASIL. 4. Cellular or animal studies were not conducted to verify the function of this rare mutation.

Table1: Summary of the clinical features of CADASIL cases with mutation sites in exon 7 of NOTCH3 gene

References	Race	Gender/ age	Onset	Clinical presentation	Family history	Neuroimaging feature	GOM deposition	Mutation site	Mutation sites function
Ishida *et al*. 2006[6]	Japanese	F/38	38	Headache	Yes	T2-weighted and FLAIR MRI: diffuse hyperintensities in white matter and basal ganglia, characteristically in anterior temporal pole and external capsule	Positive	p.Cys388Tyr	Not provided
Pradotto *et al*. 2008[7]	Italian	F/60	50	Progressive subcortical dementia	No	Axial proton-density (PD)-weighted MRI: confluent extensive white matter abnormalities in external capsule and temporal lobe	Negative	p.Cys366Trp	Not provided
Lee *et al*. 2011[12]	African American	M/78	60	Dementia, recurrent strokes	Yes	White matter hyperintensities in anterior temporal and external capsule	Positive	c.1057_1071dup	Predicted insertion of 5 aa at position 353 to 357, including a cysteine residue
Alexander *et al*. 2013[10]	German	F/68	66	Progressive personality and behavioural change	No	FLAIR: confluent white matter hyperintensity in cerebral hemisphere	NA	p. Cys366Arg	Not provided
Current case	Chinese	F/44	44	Progressive dizziness and unstable walking	Yes	FLAIR: multiple lacunar infarcts and diffuse hyperintensity lesions in centrum semiovale, periventricular, and external capsular	NA	p.Cys379Ser	Not provided

GOM, granular osmiophilic electron-dense material; FLAIR, fluid attenuated inversion recovery; aa, amino acid.

In conclusion, this study is the first to report a rare heterozygous mutation on exon 7 of the *NOTCH3* gene in a Chinese CADASIL pedigree with detailed clinical presentation and MRI imaging data. We suggest that if patients have a clear family history and manifest with uncommon clinical manifestations of CADASIL such as dizziness and atypical headache, clinicians could consider screening *NOTCH3* to avoid missing the diagnosis.

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DISCLOSURE

Ethic approval: This study has been approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University. Informed consent was obtained from the patient, her two siblings and one cousin for the publication.

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Conflict of interest: None

Availability of data: The imaging and sequencing data are available from the corresponding author upon request.

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