Case Report

A novel report of Cys1298Gly mutation in exon 24 of *NOTCH3* gene in a Chinese family with CADASIL

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Objectives: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common monogenic hereditary small cerebral vessel disease, which is caused by mutation of the neurogenic locus notch homolog protein 3 gene (*NOTCH3*). The exon 24 encodes EGF-like repeats, variants on this exon are rare. Here, we report a novel heterozygous variant c.3892 T >G (p. Cys1298Gly) on exon 24 of *NOTCH3* gene in a 57-year-old Chinese woman. *Materials and Methods:* We present a patient with clinical manifestations, laboratory examination and imaging reveal suspicion of CADASIL. The family and genetic test and pathological examination were performed. *Results:* Magnetic resonance imaging revealed diffuse leukoencephalopathy with hyperintense signals in the bilateral temporal poles, periventricular white matter, centrum semiovale, basal ganglia, frontal and parietal cortex and subcortical areas bilaterally. Molecular Genetic testing identified a heterozygous variant c.3892 T >G (p. Cys1298Gly) on exon 24 of *NOTCH3* gene. Her brother and his son were confirmed as subclinical carriers of the variant. The skin biopsy was negative, but the pathologic role of this mutation is predicted by using the DynaMut database and results showed the stability of the *NOTCH* gene is decreased. *Conclusions:* To the best of our knowledge, this is the second case of exon 24 mutations reported from China and the variant of c.3892 T >G (p. Cys1298Gly) on exon 24 of *NOTCH3* has not been reported so far. Our report broadens the mutation spectrum of the *NOTCH3* gene in CADASIL.

Keywords: CADASIL—NOTCH3 gene—Exon 24—Stroke—Dementia

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Introduction

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is an adult-onset autosomal dominant small cerebral vessel disease[1]. It is caused by a pathogenic mutation in the notch homolog protein 3 gene (*NOTCH3*) on Chromosome 19p13.2-p13.1[2]. The cardinal manifestations of CADASIL include migraine with aura (present in 20−40% of patients)[3], recurrent subcortical ischemic events, mood disturbances and cognitive impairment with an onset age of 40-50 years old[4]. Typical magnetic resonance imaging (MRI) abnormalities of CADASIL patients include diffusing leukoencephalopathy, multiple lacunar infarcts and cerebral micro-bleeds[5, 6].

Pathological evidence and *NOTCH3* mutation testing are critical for the diagnosis of CADASIL. The main pathogenic changes of CADASIL, presenting in leptomeningeal and penetrating small arteries of the grey and white matter, include vascular smooth muscle cells (VSMC) degeneration and granular osmiophilic material (GOM) deposits in the tunica media of vessels[7, 8]. It was reported that mutation in the extracellular domains ($NOTCH3^{ECD}$) leads to the accumulation of NOTCH3 in the brain vessels that may ultimately cause multifactorial toxicity[9]. Besides, GOM deposits are also found in dermal arterioles and other extra-cerebral vessels, making skin biopsy a diagnostic procedure[1]. The specificity of skin biopsy is high (approaching 100%), but the sensitivity is nearly 50%. With the development of biotechnology, genetic testing is becoming an increasingly accessible diagnostic tool. If conditions permit, the sensitivity of Sanger sequencing of EGFR encoding exons can approach 100%.

NOTCH3 gene contains 33 exons and encodes for a transmembrane receptor with an extracellular domain consisting of 34 epidermal growth factor-like repeats (EGFRs), which is expressed in vascular smooths muscle cells (VSMC) and pericytes[10]. There more than 200 *NOTCH3* variants were reported, and most of them were identified in a cluster in exon 3 and 4[2]. As we know, mutation on exon 24 of *NOTCH3* is rare. To date, there are no more than 7 families reported worldwide, most of them were reported from Italy[11-14].

Here, we identified a pathogenic mutation of c.3892 T >G (p. Cys1298Gly) in the *NOTCH3* gene and provided its clinical manifestations of CADASIL. we reviewed the clinical features and neuroimaging characteristics of this patient along with relevant literature.

Case report

A 57-year-old right-handed woman has been admitted to our hospital and complained of an 18-year history of migraines with aura and recurrent ischemic strokes. At the age of 39 years, she suffered her first migraine attack of the right temporal. With a series of medical examinations, she was diagnosed with multiple sclerosis (MS) and was treated with a pulse therapy of methylprednisolone sodium succinate. Besides, MRI scanning revealed multiple ischemic lesions near the lateral ventricles. Since then, recurrent migraine attacks bothered her. She developed left limb weakness at the age of 53 years old and was treated as MS and outmoded cerebral infarction. Recently, she presented with progressive cognitive decline including impaired memory, calculation, and comprehension. Her past medical history was significant for dyslipidemia. She had hypertension for more than ten years and her blood pressure was not well controlled. She did not smoke and never drank alcohol. Both of her parents died of cerebrovascular accidents. Her son died of leukemia.

The patient's physical and neurological examination was unremarkable. The neuropsychological evaluation showed mild cognitive decline including impairments of memory and apraxia. Her performance on the mini-mental status examination (MMSE) showed a slight cognitive impairment (a score of 25 out of 30). Neck vessel Doppler sonography revealed calcification of the right common carotid artery wall and left carotid bulb plaque. Echocardiography evaluation showed enlargement of the left atrium. Brain MRI and fluid attenuation inversion recovery (FLAIR) imaging revealed diffused white matter hyperintense mostly involving the bilateral temporal poles, thalamus, bilateral basal ganglia region, bilateral paraventricular white matter areas, bilateral semicircular centers, bilateral frontal and parietal cortex and subcortical areas (Fig. 1A-D). Particularly, the diffusion-weighted imaging (DWI) and apparent diffusion coefficient (ADC) showed massive acute cerebral infarction in the right paraventricular white matter area and the right temporal parietal lobe (Fig. 1E-H). Brain magnetic resonance angiography (MRA) revealed macrovascular sclerosis with severe stenosis of the horizontal segments of the bilateral middle cerebral arteries (Fig. 1I). Susceptibility-weighted imaging (SWI) showed minimal bleeding in the left thalamus (Fig. 1J). High-resolution magnetic resonance vessel wall imaging (HR-VWI) presented severe stenosis (79%-99%) of the right middle cerebral artery and right anterior cerebral, moderate stenosis (30%-69%) of the left middle cerebral artery, accompanied by intravascular thrombosis, inflammation and neovascularization of the vascular wall (Fig. 1K-L). Based on the image findings, the patient was considered to have vasculitis and underwent routine laboratory examinations including an autoimmune antibody test and we also perform a lumbar puncture However, no significant results were found. Based on clinical and neuro-imaging findings, she was clinically suspected of CADASIL.

After consent, the skin biopsy was used to find the GOM which was the characteristic of CADASIL, but the result was negative (Fig. 2). The Whole exome sequencing was done on the patient and variants analysis revealed a heterozygous mutation c.3892 T >G on exon 24 of *NOTCH3* responsible for the replacement of a cysteine at

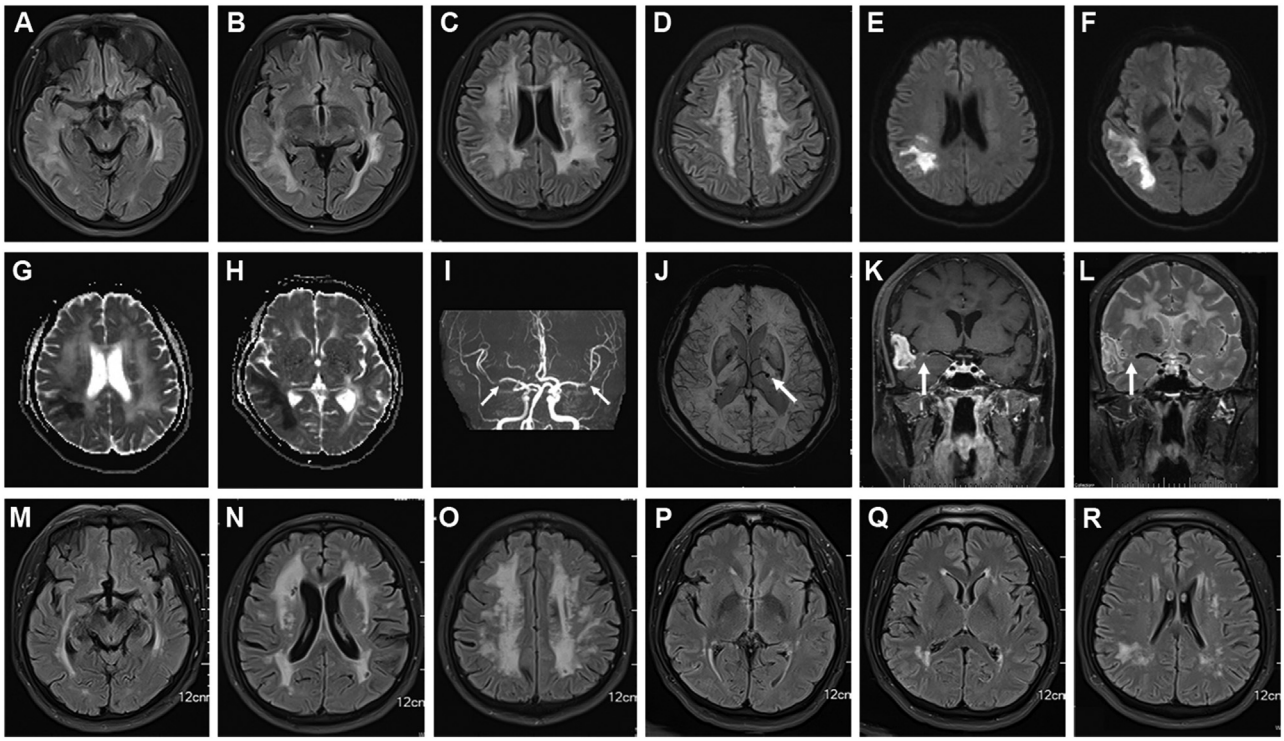

Fig. 1. *Brain MRI of the proband. (A-D) FLAIR images showed diffused white matter hyperintensities mostly involving the bilateral temporal poles, thalamus, bilateral basal ganglia region, bilateral paraventricular white matter areas, bilateral semicircular centers, bilateral frontal and parietal cortex and subcortical areas. (E-H) The DWI and ADC showed massive acute cerebral infarction in the right paraventricular white matter area and the right temporoparietal lobe. (I) MRA revealed macrovascular sclerosis with severe stenosis of the horizontal segments of the bilateral middle cerebral arteries. (J) Susceptibility-weighted imaging (SWI) showed minimal bleeding in the left thalamus. (K-L) High-resolution magnetic resonance vessel wall imaging (HR-VWI) presented severe stenosis (79%-99%) of the right middle cerebral artery and right anterior cerebral, moderate stenosis (30%-69%) of the left middle cerebral artery, accompanied by intravascular thrombosis, inflammation and neovascularization of the vascular wall. (M-O) Brain MRI of FLAIR(II-4: M-O; III-2: P-R)revealed diffused white matter hyperintensities mostly involving the bilateral temporal lobes, bilateral paraventricular white matter regions, bilateral semicircular centers, bilateral frontoparietal cortex, and subcortical regions.*

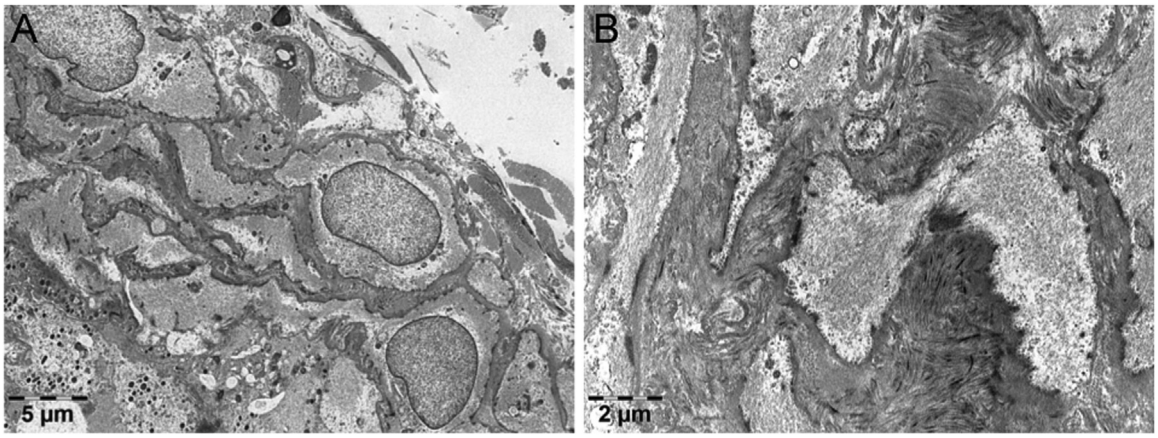

Fig. 2. *Skin biopsy of proband. (A-B) the absence of characteristic granular osmiophilic material(GOM) in the media of small arteries by electronic microscopy. A, Scale bar=5μm, B, scale bar=2μm.*

position 1298 with a glycine (p. Cys1298Gly). Sanger sequencing was then used to confirm the variant (Fig. 3B). One of her brothers (II-4) has a hypertension history and was diagnosed with coronary heart disease at the age of 40 years. Her nephew (III-2), a 36-year-old young male, also has a ten-years history of hypertension. Both of them were confirmed as sub-clinical carriers of the same mutation, yet without typical clinical features (Fig. 3B). Interestingly, their brain MRI and FLAIR imaging revealed diffused white matter hyperintensities mostly involving the bilateral temporal lobes, bilateral paraventricular white matter regions, bilateral semicircular centers,

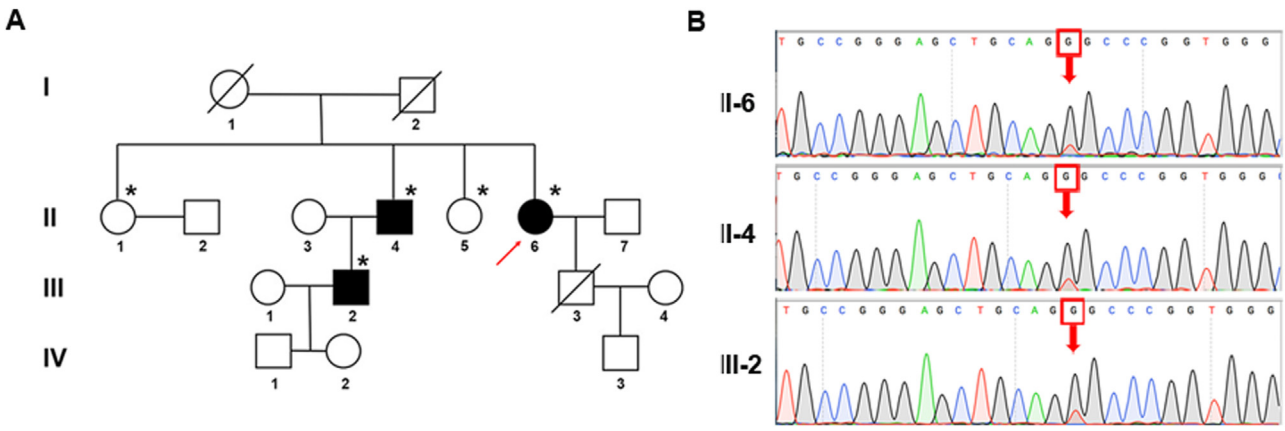

Fig. 3. *Family pedigree and genetic analysis of* NOTCH3 *gene. (A) Family pedigree. Squares represent men and circles represent women. The arrow indicates the proband. Filled symbols indicate affected members. Diagonal lines through symbols represent deceased members. Gene-tested subjects are marked with an asterisk. (B) Sanger sequencing read of* NOTCH3 *gene. The NOTCH3 genetic analysis identified the variant of c.3892T>G (p. Cys1298Gly) mutation in exon 24 was detected in the proband(II-6) and two other family members (II-4, III-2).*

bilateral frontoparietal cortex, and subcortical regions. **(II-4:** Fig. 1**M-P; III-2:** Fig. 1**Q-R)**. The variant was cosegregated with this disease in the family.

To our best knowledge, the variant of p. Cys1298Gly was reported for the first time and its pathological function is not clear. We reviewed the literatures and summarized CADASIL patients with a mutation on exon 24 of *NOTCH3* (Table 1). Notably, mutations on exon 24 are less common and clinical features are atypical[11, 13, 15-17]. The DynaMut2[18] database was then used to predict the possible impacts on protein dynamics and structural stability of NOTCH3. The results showed a decreased stability of protein structure and support the pathogenic role of this variant (Fig. 4). The variant was curated as Likely pathogenic and CADASIL was thus diagnosed. The patient was treated with aspirin, cholesterol-lowering agents, glucocorticoid, and cilnidipine.

Discussions

CADASIL is a hereditary monogenic neurological disease caused by a pathogenic mutation in *NOTCH3*. According to the previous reports, exons 2-24 of *NOTCH3* encoding the EGF-like domains harbor the majority of the mutations[19, 20]. Typical mutations are mainly localized on exons 3, 4, 5, and 8, while less frequent mutations are localized on exons 2, 6, 11 and 18, resulting in a gain or loss of a cysteine residue within one of the 34 EFG-like repeats of the NOTCH3 receptor. Despite the exon 24 encodes EGF-like repeats, variants on this exon are rare[11, 13-17]. The present study reported a Chinese family with a pathogenic mutation of c.3892 T >G (p. Cys1298Gly) located on exon 24 of *NOTCH3*. To the best of our knowledge, this variant has not been reported so far.

High genotypic variability could be responsible for the high phenotypical variability of the disease It was believed that specific mutation sites are critical for clinical features[21, 22]. Individuals with a mutation in EGF-like domain 1-6 have an earlier stroke onset, higher brain lesion load and lower survival rates than those with a mutation in EGF-like repeats 7-34 that had a much milder clinical feature[23]. Notably, in previous reports, patients with exon 24 mutations presented diverse initial symptoms including parkinsonism[11], depression[16], chronic renal failure[15], and transient global amnesia[13]. In our case, the proband complained of an 18-year history of migraines with aura, recurrent ischemic strokes and cognitive impairment. Although the other two carriers lack typical clinical symptoms, both of them have a history of hypertension. Hypertension and myocardial infarction are not infrequent in CADASIL patients and are possibly caused by the presence of microvascular alteration[11, 24, 25]. Other factors, such as traditional vascular risk factors, environmental differences, diets, exercises, and medical therapy, also play an important role in the disease's progress and development[26, 27]. These findings supported that CADASIL patients have a high vascular risk profile. In addition, hypertension can also generate small vessel disease. Therefore, after the diagnosis of CADASIL, we should actively prevent and control blood pressure, as well as treatment of other risk factors and underlying diseases, thus slowing the progression of CADASIL disease. Long-term follow-up and progressive clinical examination of this CADASIL family are also needed. Accumulation of more cases is helpful to elucidate the genotype-phenotype correlations of exon 24 mutations on *NOTCH3*.

Another point of interest concerns the MRI findings of the patients. MRI images of the proband showed typical CADASIL features including symmetrical and extensive white matter hyperintense, multiple lacunar infarcts and cerebral micro-bleeds. Interestingly, her MRI images showed severe stenosis and vasculitis of the middle cerebral arteries, arteriosclerosis of the great vessels, as well as

Table. 1. *Clinical Overview of CADASIL patients with a Mutation in Exon 24 of NOTCH3.*

Age/Sex	Initial age	Country	*NOTCH3* mutation	Clinical presentation	Brain MRI image High-intensity lesion	Microbleeds	Refs.
64/M	60	Italy	p.Cys1315Tyr	Parkinsonism Mirgraine Cognitive impairment	Periventricular Deep white matter External capsules	Not performed	[11]
61/M	39	Italy	p.Cys1298Phe	Speech disturbance Ischemic stroke Psychomotor slowness Memory defificits	Temporal lobes Right parietal lobe	Interpeduncular cistern subarachnoid hemorrhage	[12]
73/F	52	Italy	p.Cys1298Phe	Transient global amnesia Cognitive impairment Psychiatric disorders Delirium.	Diffuse white matter Brain stem Temporal poles Left external capsule	Right basal ganglia regions	[13]
71/F	30	Italy	p.Cys1315Tyr	Anxious-depressive symptoms Parkinsonism Behavioral disturbances Cognitive impairment Loss of autonomy in daily living activities	Mdian pons Right cerebral peduncle Basal ganglia	Not clear	[14]
62/M	35	Japan	p.Gly1347Arg	Chronic renal failure	Bilateral temporal poles External capsule	Bilateral basal ganglia and subcortical areas	[15]
50/F	30	Japan	p.Cys1293Trp	Depression	Bilateral external capsule Diffuse white matter Brain stem Bilateral anterior temporal poles	Bilateral basal ganglia and thalamus	[16
58/F	51	China	p.Gly1347Arg	Ischemic stroke Cognitive impairment	Periventricular Temporal poles External capsule	Not clear	[17]
57/F	39	China	p.Cys1298Gly	Migraines Ischemic stroke Cognitive impairment	Periventricular and deep white matter	Left thalamus	Present case

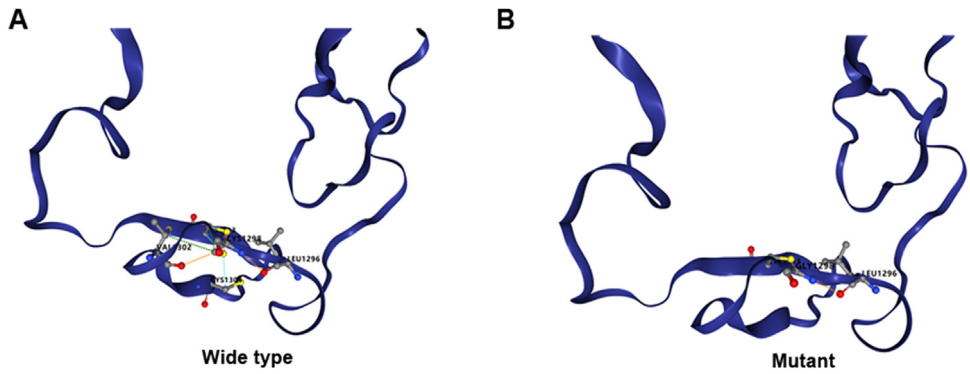

Fig. 4. *Analysis of NOTCH3 protein structures. (A)The protein structures of wide type NOTCH3 and mutant NOTCH3 (B) were predicted with the DynaMut2 database. ΔG (Gibbs free energy variation): -0.86 kcal/mol (Destabilizing). pLDDT score: 53.70 (low)*

massive acute cerebral infarction in the right lateral paraventricular white matter area and the right temporal parietal lobe. Those findings were uncommon in CADASIL since it was defined as a small cerebral vessel disease. A study reviewed 49 CADASIL patients, 23 of them had cerebral infarctions and seven of them had infarctions associated with cerebral large artery disease, which illustrated that infarction in association with the intracranial arterial disease may be a manifestation of CADASIL[28]. In our cases, considering the patient has a history of dyslipidemia and hypertension, the coexistence of CADASIL and other cardio cerebral vessel disorders should be taken into account. The other two subclinical carriers' MRI images were typical, indicating brain MRI abnormality is a potential surrogate marker for early diagnosis and providing a time window for potential primary stroke prevention.

It would be difficult to distinguish CADASIL from multiple sclerosis (MS)in some cases. Because, abnormal high signals in white matter can be observed, as well as clinical manifestations can be progressive and relapsing in both diseases[29]. In our case, the proband was diagnosed and treated as an MS patient for many years. Previous literature has reported several cases of CADASIL that have been followed up and misdiagnosed as MS[30-32]. Typical radiographic CADASIL changes include a predominance of T2 hyperintense in the bilateral temporal lobes and external capsules. In contrast, confluent periventricular hyperintense or other atypical features can be observed in MS. Besides, cerebrospinal fluid (CSF) examination of the oligo-clonal band (OCB), *NOTCH3* gene sequencing and skin biopsy are critical to distinguish these two diseases. The coexistence of CADASIL and MS is exceedingly rare. Although an individual presenting with cognitive decline was diagnosed with MS and CADASIL[33], the coexistence of these two diseases is still controversial.

Another point of confusion in this disease is, imaging showed suspicion of cerebral arteritis in the patient. We performed lumbar puncture and autoimmune antibody tests but the results were negative. We tried methylprednisolone pulse therapy according to the patient's permission and then changed to an oral dose with decreasing discontinuation. After treatment, the patient showed partial improvement in cognitive function, but for personal financial reasons, the patient was not reviewed for MRA. Therefore, we were not able to know the recovery of subsequent vasculitis. Autoimmune cerebral vasculitis is mainly diagnosed by pathology, and we did not perform further tests to determine whether the patient had CADASIL in combination with this disease.

CADASIL is an incurable and life-threatening disease. Despite the improved diagnosis, the pathogenesis of CADASIL is unclear. No treatment for controlling the progress of the disease has been reported except for some symptomatic treatments. Efforts have been made on stroke prevention, including anti-platelets, anti-hypertension, and cholesterol-lowering agents[34]. Based on that, our proband was treated with aspirin, statin and cilnidipine. Moreover, in vitro and in vivo studies of immunotherapy, growth factors administration, and antisense oligonucleotides are currently under investigation[35]. Accumulation of pathological variants, clinical data as well as further in vivo studies may provide a better insight into CADASIL and help in the optimization of therapy.

NOTCH3 encodes a transmembrane receptor belonging to the Notch family which is involved in stem cell differentiation and arterial vessel remodeling[36]. It is not yet fully understood how *NOTCH3* mutation results in the pathological changes of CADASIL. It has been reported that mutations of *NOTCH3* change the three-dimensional structure of the protein, and lead to multimerization of NOTCH3[37]. Deposition and aggregation of abnormal protein around the vascular smooth muscle of the brain are possible mechanisms[1]. Skin biopsies were performed on our patient, however, no deposition of GOM material was observed under electron microscopy and the skin biopsies were negative. However, genetic sequencing confirmed the disease. This result also proves that the sensitivity of skin biopsy is not as high as that of genetic diagnosis. The poor sensitivity of biopsy may also be related to the sampling site. Therefore, If the clinical symptoms and imaging

signs reveal high suspicion of CADASIL, genetic diagnosis is recommended if conditions permit.

Considering the variant is novel and the function of the mutation is not clear, we predicted the possible impacts on protein dynamics and structural stability of NOTCH3 with the DynaMut2 database. The results showed a decreased stability of protein structure and may support the pathogenic role of this variant.

Conclusion

To sum up, the 24 exon mutation is rather rare, and this case represents a novel report of a heterozygous *NOTCH3* c.3892 T >G (p. Cys1298Gly) mutation and clinical features in CADASIL from China. This is the first time to clarify the clinical features of this novel variant. The obvious clinical symptom of our patients is classic, while other patients with mutations on exon 24 are not. This pathologic mutation also widened the genetic spectrum of CADASIL.

Ethics approval and consent to participate

We have obtained the patient's permission and informed consent for the publishing of their information and images. This research is approved by the Institutional Review Board of People's Hospital of Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, China

Consent for publication

Written informed consent was obtained from the patient for publication of this Case report and any accompanying images.

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None

Authors' contributions

JHH and JQ analysis and interpretation of data and wrote the manuscript. ZHC contributed to genetic analysis.BT and YL gave the clinical information containing medical history, neurological findings, hematological examination and treatment. XZL and QG revised the manuscript critically. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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