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CASE REPORT

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A case of recurrent intracranial hemorrhage in CADASIL caused by *NOTCH3* c.1759C>T heterozygous mutation

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Abstract

Background: Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a cerebrovascular disease that is closely related to the *NOTCH3* gene. Recurrent ischemic stroke, progressive cognitive dysfunction, and mental symptoms are the main clinical manifestations, whereas symptomatic intracranial hemorrhage is rare.

Methods: We detected a heterozygous mutation of c.1759C>T in exon 11 of the *NOTCH3* gene that caused recurrent intracranial hemorrhage in CADASIL.

Results: Second-generation sequencing of a sample of the patient's genome revealed a heterozygous mutation of c.1759C>T in exon 11 of *NOTCH3*, which resulted in amino acid changes (p.R587C). This variation may be rated as a CADASIL clinical variation.

Conclusion: The discovery of this mutation site provides an important theoretical basis for a gene-based diagnosis and treatment of recurrent intracranial hemorrhage.

KEYWORDS
CADASIL, gene mutation, intracranial hemorrhage, *NOTCH3*, stroke

1 | INTRODUCTION

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare familial hereditary cerebrovascular disease that first occurs in middle-aged individuals. Precursory migraine, recurrent ischemic stroke, transient ischemic attack, progressive cognitive dysfunction, and mental symptoms are the primary clinical manifestations of this disease. Few patients present with intracranial hemorrhage (ICH), epileptic attacks, and Parkinson's syndrome as the first clinical symptoms.[1] Imaging examination (head MRI) of this disease mainly reveal symmetrical large white matter hyperintensities involving the temporal pole and external capsule, micro-hemorrhage foci distributed along the brain stem and thalamus, lacunar cerebral infarction, and brain atrophy.[2] It is strongly associated with a mutation in the *NOTCH3* gene on chromosome 19.[3,4] NOTCH3 protein is expressed in mature vascular smooth muscle cells and plays a key role in maintaining the stability of vascular structure and function. Mutations in the *NOTCH3* gene result in the duplication or loss of cysteine residues in the epidermal growth factor duplication region, leading to changes in the structure and function of the encoding transmembrane protein. The mutant NOTCH3 protein aggregates are deposited on the surface of cerebral vascular smooth muscle cells, causing vascular muscle layer lesions, leading to the onset of stroke. The course of the disease,

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imaging examination, and *NOTCH3* gene detection are important bases for diagnosis of CADASIL. In this paper, we report a rare case of CADASIL with recurrent ICH caused by a *NOTCH3* c.1759C>T heterozygous mutation.

2 | MATERIALS AND METHODS

2.1 | Patient and family

We present the case of a patient with CADASIL, who was treated at the Department of Neurology, the First Affiliated Hospital of Dalian Medical University, China. Peripheral blood samples were collected for investigation. The study was approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University (Approval No: PJ-KS-KY-2022-52) and performed in accordance with the tenets of the Declaration of Helsinki. The patient provided written informed consent.

2.2 | Mutation analysis

Whole-exome sequencing (WES) was performed on DNA extracted from peripheral blood samples. PCR amplification and direct Sanger sequencing were used to verify the suspected gene mutation sites detected by WES. The amplified PCR products of *NOTCH3* were visualized on a 2% agarose gel. To identify harmful mutations, BLAST (https://blast.ncbi.nlm.nih.gov/) was used to align the sequence data with the *NOTCH3* reference DNA sequence.[5,6]

3 | CASE DESCRIPTION

3.1 | Disease history

A 59-year-old man complained of "sudden left limb disability for 8 h." Eight hours before admission, the patient showed inflexibility of the left limbs during a meal. The main symptoms were that he found it difficult to hold things using the left upper limb, he could not walk with the left lower limb, the left limbs were numb, and his speech was unclear. There was no dizziness, headache, nausea, swallowing dysfunction, falls, convulsions, incontinence, or loss of consciousness observed.

3.2 | Past history

In the preceding 8 years, he had four episodes of ICH, two of which were located in the thalamus. In the preceding 6 months, his blood pressure had increased, and the highest blood pressure recorded was 150/100 mmHg. The patient had not been regularly treated with oral antihypertensive drugs prior to admission. There was no history of diabetes, coronary heart disease, infectious disease, trauma, blood transfusion, smoking, or alcohol consumption.

3.3 | Physical examination of the nervous system

The patient had clear consciousness, dysarthria, normal intelligence, and left central facial paralysis. The muscle strength of the left upper limb was scored as 3, and that of the lower limb was scored as 4. The muscle tension of the left limbs were increased, the left side showed hypoesthesia, and both sides showed positive Babinski signs. No other nervous system abnormalities were observed.

3.4 | Additional examinations

Head CT scan: Acute cerebral hemorrhage of the right thalamus (Figure 1A).

Head MRI/SWI: (1) acute cerebral hemorrhage of the right thalamus (Figure 1B); (2) old cerebral hemorrhage lesions in the left thalamus and the posterior limb of the internal capsule (Figure 1B); (3) multiple intracranial hemorrhages and microhemorrhages (Figure 1C); and (4) severe demyelination of white matter (Figure 1D).

MMSE score: 27.

3.5 | Genetic analysis

Genomic DNA was extracted from the patient's peripheral blood sample. One heterozygous variation, c.1759C>T, was identified in exon 11 of *NOTCH3* (Figure 1D). And no other genetic mutations in neurodegenerative disease-related genes were found. The quality control data of the WES are listed in Table 1. To further verify the gene test results, PCR amplification and direct Sanger sequencing were used to detect variants in the mutational hotspot sequence exons. The sequences of the primers used were as follows: F-5′-ACCACTGTGCCCCACTAGAT-3′ and R-5′-GTCCGAGGCCTCAC TTGT-3′. No pathogenic gene mutations were found in other hotspots of this gene. Unfortunately, due to privacy, other family members of the patient refused the genetic test.

4 | DISCUSSION

The human NOTCH3 protein is specifically expressed in mature vascular smooth muscle cells and plays a key role in maintaining the stability of vascular structure and function.[7] The c.1759C>T mutation causes the arginine in its encoded receptor to be replaced by cysteine (p.R587C), forming an odd cysteine, resulting in the damage of the NOTCH3 dimer or the formation of inappropriate disulfide bonds, thus causing an imbalance in the NOTCH3 signal pathway,[8,9] resulting in NOTCH3-mediated vascular dysfunction. CADASIL

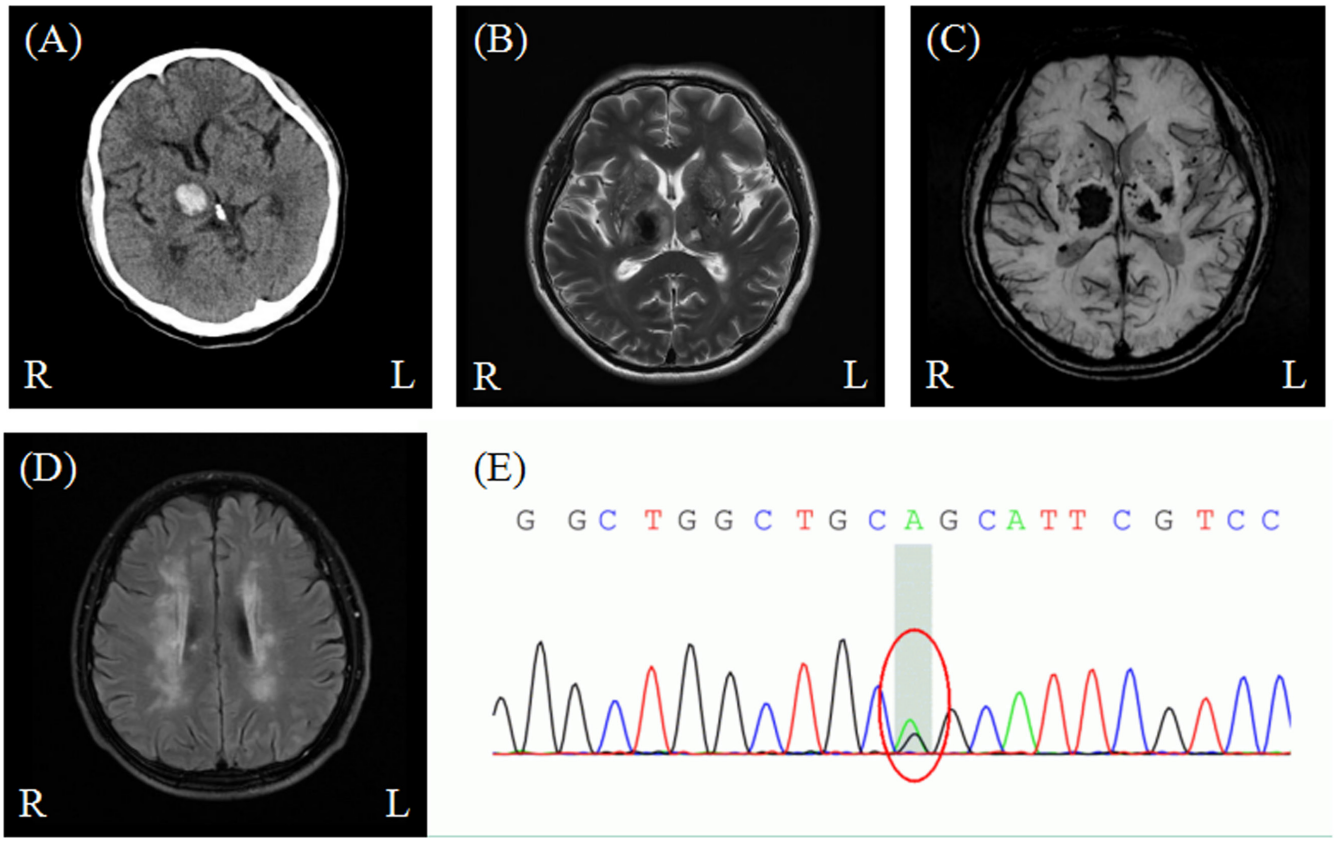

FIGURE 1 Brain image exam and genotype results. (A) CT scan reveals acute hemorrhage of the right thalamus; (B) T2-weighted MR imaging showed low signal in the right thalamus and high signals in the left thalamus and the posterior limb of the internal capsule, suggesting that the right thalamus was an acute cerebral hemorrhage, and the left thalamus and the posterior limb of the internal capsule were old cerebral hemorrhage lesions; (C) SWI revealing multiple intracranial hemorrhages and microbleeds; (D) Severe diffuse leukoencephalopathy in deep white matter; (E) Sequence the heterozygous mutation c.1759C>T in exon 11 of the *NOTCH3* gene.

caused by mutations at this site is often characterized by recurrent subcortical ischemic stroke and progressive dementia, and cases with recurrent symptomatic ICH as the first symptom have not been reported. In this report, the patient carried the *NOTCH3* gene c.1759C>T heterozygous mutation, with an 8-year history recurrent ICH, of which two occurred in the thalamus. Bleeding may be caused by the use of anticoagulants or antiplatelet drugs. In addition, the patient denied the existence of hypertension when having ICH in the past, and no clear risk factors for ICH were found. Therefore, recurrent ICH is associated with this *NOTCH3* mutation.

We reviewed previous reports on ICH as a clinical manifestation of CADASIL and found that most patients had hypertension.[10] Choi et al. also confirmed that ICH in patients was significantly associated with hypertension. The most commonly affected area in the nine reported ICH patients was the basal ganglia, followed by the thalamus, which was not different from the common bleeding area in hypertensive intracerebral hemorrhage.[11] In addition, 31%–69% of patients with CADASIL have cerebral microbleeds (CMB), which is believed to be related to an increased risk of ICH.[12] Lee et al. further studied the relationship between ICH, hypertension, and CMB in CADASL in 2017, and the results showed that hypertension and CMB were independent related factors of ICH in patients with CADASIL, and having ≥9 CMB was more likely to lead to ICH.[13] In addition, Lee et al. pointed out that the thalamus is the most common area to develop CMB, followed by the cerebral lobe and basal ganglia, which is inconsistent with the susceptible regions for ICH. The authors of that paper put forward the "two site hypothesis" for this phenomenon. They believed that the site of ICH is the vascular smooth muscle, while the site of CMB is the blood–brain barrier. Of course, this statement requires a lot of research for further confirmation.[13] According to previous reports, in East Asia, the prevalence of ICH in CADASIL patients is relatively high,[14] and the incidence rate of ICH in China and South Korea can reach 12.3%–25%.[15] Therefore, it is necessary to strictly control blood pressure in patients with CADASIL and hypertension, especially in Asians.

Whether ICH in patients with CADASIL is related to specific gene mutation sites or to related disease processes caused by hypertension and CMB remains unknown. When combined, these factors are more likely to cause ICH. Therefore, it is necessary to further study and determine the pathogenesis of ICH in patients with CADASIL to conduct appropriate management to prevent the occurrence of stroke events.

TABLE 1 Quality control data of WES.

Total	
Raw_data (Mb)	12,137.47
Clean_data (Mb)	11,671.85
Aligned (%)	99.92
Initial bases on target	38,841,470
Base covered on target	38,814,566
Coverage of target region	99.90%
Total effective yield (Mb)	9796.78
Effective sequence on target (Mb)	5290.08
Fraction of effective bases on target	54.00%
Average sequencing depth on target	136.2
Fraction of target covered with at least 4×	99.90%
Fraction of target covered with at least 10×	99.90%
Fraction of target covered with at least 20×	99.70%
Duplication rate (%)	15.6

Abbreviation: WES, whole-exome sequencing.

5 | CONCLUSION

The heterozygous mutation c.1759C>T in exon 11 of *NOTCH3* may be associated with ICH in CADASIL. The discovery of this mutation site provides an important theoretical basis for gene-based diagnosis and treatment of recurrent intracranial hemorrhage.

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CONFLICT OF INTEREST

The present study does not have any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Chabriat H, Vahedi K, Iba-Zizen MT, et al. Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Lancet*. 1995;346:934-939.
2. Kalaria RN, Low WC, Oakley AE, et al. CADASIL and genetics of cerebral ischaemia. *J Neural Transm Suppl*. 2002;63:75-90.
3. Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser M. Cadasil. *Lancet Neurol*. 2009;8:643-653.
4. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996;383:707-710.
5. Dai Y, Liang S, Dong X, et al. Whole exome sequencing identified a novel DAG1 mutation in a patient with rare, mild and late age of onset muscular dystrophy-dystroglycanopathy. *J Cell Mol Med*. 2019;23(2):811-818.
6. Zheng Y, Xu J, Liang S, Lin D, Banerjee S. Whole exome sequencing identified a novel heterozygous mutation in HMBS gene in a Chinese patient with acute intermittent porphyria with rare type of mild anemia. *Front Genet*. 2018;9:129.
7. Wang T, Baron M, Trump D. An overview of Notch3 function in vascular smooth muscle cells. *Prog Biophys Mol Biol*. 2008;96:499-509.
8. Joutel A, Monet M, Domenga V, Riant F, Tournier-Lasserve E. Pathogenic mutations associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy differently affect Jagged1 binding and Notch3 activity via the RBP/JK signaling pathway. *Am J Hum Genet*. 2004;74:338-347.
9. Wang W, Prince CZ, Mou Y, Pollman MJ. Notch3 signaling in vascular smooth muscle cells induces c-FLIP expression via ERK/MAPK activation. Resistance to Fas ligand-induced apoptosis. *J Biol Chem*. 2002;277:21723-21729.
10. Lee YC, Liu CS, Chang MH, et al. Population-specific spectrum of NOTCH3 mutations, MRI features and founder effect of CADASIL in Chinese. *J Neurol*. 2009;256:249-255.
11. Choi JC, Song SK, Lee JS, Kang SY, Kang JH. Diversity of stroke presentation in CADASIL: study from patients harboring the predominant NOTCH3 mutation R544C. *J Stroke Cerebrovasc Dis*. 2013;22:126-131.
12. Choi JC, Kang SK, Kang JH, Park JK. Intracerebral hemorrhages in CADASIL. *Neurology*. 2006;67:2042-2044.
13. Lee JS, Ko K, Oh JH, et al. Cerebral microbleeds, hypertension, and intracerebral hemorrhage in cerebral autosomal-dominant Arteriopathy with subcortical infarcts and leukoencephalopathy. *Front Neurol*. 2017;8:203.
14. Liao YC, Hsiao CT, Fuh JL, et al. Characterization of CADASIL among the Han Chinese in Taiwan: distinct genotypic and phenotypic profiles. *PLoS One*. 2015;10:e0136501.
15. Tan QC, Zhang JT, Cui RT, Xu QG, Huang XS, Yu SY. Characteristics of CADASIL in Chinese mainland patients. *Neurol India*. 2014;62:257-261.

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