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ORIGINAL ARTICLE

Report of two pedigrees with heterozygous *HTRA1* variants-related cerebral small vessel disease and literature review

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Abstract

Background: Biallelic *HTRA1* pathogenic variants are associated with autosomal recessive cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). Recent studies have indicated that heterozygous *HTRA1* variants are related to autosomal dominant hereditary cerebral small vessel disease (CSVD). However, few studies have assessed heterozygous *HTRA1* carriers or the genotype–phenotype correlation.

Methods: The clinical data of two unrelated Chinese Han families with CSVD were collected. Panel sequencing was used to search for pathogenic genes, Sanger sequencing was used for verification, three-dimensional protein models were constructed, and pathogenicity was analyzed. Published *HTRA1*-related phenotypes included in PubMed up to September 2021 were extensively reviewed, and the patients' genetic and clinical characteristics were summarized.

Results: We report a novel heterozygous variant c.920T>C p.L307P in the *HTRA1*, whose main clinical and neuroimaging phenotypes are stroke and gait disturbance. We report another patient with the previously reported pathogenic variant *HTRA1* c.589C>T p.R197X characterized by early cognitive decline. A literature review indicated that compared with CARASIL, *HTRA1*-related autosomal dominant hereditary CSVD has a later onset age, milder clinical symptoms, fewer extraneurological symptoms, and slower progression, indicating a milder CARASIL phenotype. In addition, *HTRA1* heterozygous variants were related to a higher proportion of vascular risk factors ($p < .001$) and male sex ($p = .022$).

Conclusion: These findings broaden the known mutational spectrum and possible clinical phenotype of *HTRA1*. Considering the semidominant characteristics of *HTRA1*-related phenotypes, we recommend that all members of *HTRA1* variant families undergo genetic screening and clinical follow-up if carrying pathogenic variants.

KEYWORDS

Alzheimer's disease, CARASIL, cerebral small vessel disease, heterozygous variant, HTRA1

1 | INTRODUCTION

1.1 | Cerebral small vessel disease

Cerebral small vessel disease (CSVD) is a common and highly heterogeneous cerebrovascular disease. Depending on the location, CSVD can manifest clinically as acute small vessel stroke, latent cognitive disorder or dementia, emotional disturbance, motor and gait dysfunction, urinary incontinence, or be asymptomatic (Rutten-Jacobs & Rost, 2020). The diagnosis of CSVD is mainly based on clinical manifestations and brain imaging, including recent small subcortical infarcts, old lacunar infarcts, white matter hyperintensities, cerebral microbleeds, cerebral atrophy, and prominent perivascular spaces (Wardlaw et al., 2013). Stroke, the second-leading cause of death and acquired disability worldwide, is attributed to CSVD in up to 20–30% of ischemic strokes and the vast majority of hemorrhagic strokes (Litak et al., 2020). Monogenic disorders have been identified in a growing minority of patients, constituting up to 5% of all strokes (Marini et al., 2020). The most common single-gene CSVD is autosomal dominant cerebral artery disease with subcortical infarcts and leukoencephalopathy (CADASIL). To date, more than 300 variant sites have been reported worldwide. Autosomal recessive hereditary cerebral artery disease with subcortical infarcts and leukoencephalopathy (CARASIL) is a very rare but well-characterized autosomal recessive CSVD. The main neurological manifestations include rapid progressive cognitive decline, mood disorders and motor disabilities in association with extraneurological symptoms, such as spondylosis and alopecia. Cerebral magnetic resonance imaging (MRI) shows diffuse white matter hyperintensity (WMHs), multiple lacunar infarctions, cerebral atrophy, and microbleeds. Most of the reported cases have come from Japanese families, with some coming from Chinese, European, and American families. In 2009, Hara et al. identified that CARASIL was associated with high-temperature requirement A serine peptidase 1 (also called HtrA serine protease 1, *HTRA1*) by gene linkage analysis (Hara et al., 2009).

1.2 | HTRA1

The human *HTRA1* gene (OMIM: 602194) is located on chromosome 10q26.2, composed of nine exons, encoding a 50 kDa polypeptide of 480 amino acid residues, and is involved in various pathological processes, such as cancers, rheumatic diseases, osteoporosis, spinal disk degeneration, age-related macular degeneration, CARASIL, and Alzheimer's disease (AD). HTRA1 is a member of the mammalian HtrA serine protease family, and its members have dual chaperone and serine protease activities (Clausen et al., 2011; Li et al., 2020; Zurawa-Janicka et al., 2017). The HTRA1 protein consists of a signal peptide domain, an insulin-like growth factor binding protein (IGFBP) domain, a Kazal serine protease inhibitor domain, a trypsin-like serine protein domain, and a PDZ domain (Zurawa-Janicka et al., 2017). Among them, the L3 ring (amino acid positions 283 to 291) and LD ring (amino acid positions 301 to 314) in the trypsin-like serine protein domain play an important role in substrate activation and conformation selection (Nozaki et al., 2016; Zurawa-Janicka et al., 2017). Members of the HTRA1 family can inhibit signaling of the transforming growth factor-β (TGF-β) family (Oka et al., 2004). Studies have also found that the protease activity of CARASIL-related mutant HTRA1s is reduced and cannot inhibit TGF-β signaling. In addition, the concentration of TGF-β1 in the cerebral arterioles of CARASIL patients increased (Shiga et al., 2010). In CARASIL patients, the expression levels of ED-A fibronectin and versican induced by TGF-β signaling accumulate in the cerebral arteriole wall (Shiga et al., 2010). HTRA1 reduces the TGF-β1 signal triggered by pro-TGF-β1 in cells and reduces the number of mature TGF-β1(Shiga et al., 2011).

1.3 | CSVD and *HTRA1*

In the CARASIL families, some members with *HTRA1* heterozygous variants (p.P285L, p.G295R, p.E42fs, and p.A321T) also have white matter lesions (Bianchi

et al., 2014; Chen et al., 2013; Mendioroz et al., 2010). However, most parents of CARASIL patients are asymptomatic, indicating that *HTRA1* heterozygous carriers are not clinically affected in all cases. In 2015, Edgard Verdura et al. found that the heterozygous *HTRA1* variant caused autosomal dominant genetic CSVD (Verdura et al., 2015). Compared to CARASIL, the onset age of autosomal dominant genetic CSVD was late and lacked typical extraneurological features of CARASIL. In addition to family history, it was similar to sporadic cerebral small vessel disease. European cohorts have shown that approximately 5% of familial CSVDs with unknown etiology are associated with heterozygous *HTRA1* variants (Verdura et al., 2015). Heterozygous variant of *HTRA1* accounts for 2.08% of CSVD in Taiwan (Lee et al., 2018), and up to 1.3% of patients with lacunar stroke have potential pathogenic *HTRA1* heterozygous missense and nonsense variants (Tan et al., 2019). As of September 2019, Uemura M et al. had concluded that there were 22 variants in CARASIL and 28 variants in heterozygous *HTRA1*-related CSVD (Uemura et al., 2020).

1.4 | Unmet need and objective

A summary of previous clinical studies indicated a strong correlation between genotype of *HTRA1* variants and their clinical phenotype. Different variant types (missense mutation or frameshift mutation) and sites (different exons) can cause different effects on HtrA protease activity and signal regulation of the TGF-β family, resulting in different severities of clinical characteristics, which are highly related to the active structure of serine proteases (Truebestein et al., 2011). In this study, we report two interesting familial CSVDs with two different *HTRA1* heterozygous variants (one family with a novel variant and the other family with a reported variant plus early cognitive decline), reviewed, and compared the published cases of heterozygous *HTRA1*-related CSVD and CARASIL, summarized their epidemiological characteristics, explored the correlation between phenotype and genotype, and updated the literature on *HTRA1*-related CSVD.

2 | METHODS

2.1 | Ethical compliance

This research was approved by the ethics committees of Xiangya Hospital, Central South University (202103054).

2.2 | Participants

The patients were from two unrelated families carrying different heterozygous *HTRA1* variants in Xiangya Hospital of Central South University. The clinical data of patients were collected, and their families were investigated. Written consent was obtained from participants prior to commencing the study.

2.3 | Methods

The medical history was obtained in detail. MRI of the head and spinal cord of the proband and his cousins with similar symptoms were performed. With informed consent, 2 ml of peripheral venous blood of the proband and his family members were extracted and anticoagulated with ethylenediamine tetraacetic acid. Xiangyin Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China) was entrusted to conduct a cerebrovascular disease panel of Family 1 and a dementia panel of Family 2, respectively, by high-throughput sequencing captured in the target region. A detailed description of these genes can be found in Supplementary file. AfterQC was used to evaluate the sequencing quality of the original sequencing data. Low-quality and connector-contaminated reads were removed. The filtered data were sequenced with the human hg19 reference genome using burrows wheel aligner (BWA), and the capture effect was then evaluated. The single nuclear variant (SNV) and indel (insertion and deletion) in the genome were analyzed by Genome Analysis Toolkit (GATK, Broad Institute, Cambridge, Massachusetts, USA). The SNVs and indels obtained from the analysis were screened using the *1000 human genome dataset* and *genome aggregation database dataset 2.1.1*. The dbNSFP database was used to predict the pathogenicity of missense mutations and splicing mutations. The Human Mendel genetic database (OMIM), human gene mutation database (HGMD) and ClinVar database were used to screen reported mutations. All variant sites were classified using The American Academy of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). Finally, all possible pathogenic sites were verified by Sanger sequencing. An HTRA1 3D protein model (PDB ID: 3NZi, 3NZi. 1A) was constructed using SWISS-MODEL software (https://swissmodel.expasy.org/). The model images were drawn and annotated using RasMol software (http://rasmol.org/OpenRasMol.html).

After obtaining informed consent from the patient and her families, we took the abdominal skin of III_3 in Family 1 after local injection of anesthetic. We cut it to the depth of subcutaneous tissue, took a piece of approximately 1.5 cm

×1.0 cm, and fixed it in 2.5% pentaerythritol phosphate buffer solution. Fixed skin tissue specimens were used to observe the morphology of small blood vessels and screen for pathological changes.

2.4 | Systematic search

We searched for all published articles in PubMed up to September 12, 2021, using the key words *HTRA1* variant. All published articles and their reference lists were reviewed. Two researchers screened the literature independently according to the following criteria: (1) reported patients with homozygous or heterozygous variants; (2) members with heterozygous variants in CARASIL families were excluded because most of them were asymptomatic (Nozaki et al., 2016); (3) detailed clinical and imaging materials were available; and (4) asymptomatic subjects with only *HTRA1* variants were not included. For the selected subjects, we sorted and counted, collected clinical and imaging data, recorded their variant sites and frequency in the normal population database gnomAD, analyzed protein activity, and predicted their pathogenicity using PolyPhen2 and SIFT.

2.5 | Statistical analyses

Statistical analyses were performed by SPSS version 25.0 (SPSS; IBM, Chicago, IL, USA). Variables such as epidemiology, clinical features, and variant locations were analyzed by *t* test and chi-square test. Statistical significance was defined as $p \leq .05$. When processing data, we used age at the time of study minus the average course of disease to replace the unknown age of onset. If the clinical features, such as alopecia, were not described, we interpreted it as no such symptom.

3 | RESULTS

3.1 | Case description: Clinical, neuroimaging, and genetic features

3.1.1 | Family 1

The clinical characteristics of the reported heterozygous *HTRA1*-related CSVD patients are summarized in Table 1. The proband (III_{12}) of Family 1 (Figure 1a) was a 45-year-old male who was admitted to the hospital because of dysphonia for more than 20 days, accompanied

TABLE 1 Clinical characteristics of heterozygous *HTRA1*-related CSVD patients we found

Characteristics	III_2, family 1	III_3, family 1	III_{12}, family 1	II_4, family 2
Sex	F	F	M	F
Age at time of study (years)	56	53	45	50
Age at onset (years)	52	52	35	49
Initial symptom	Gait disturbance	Gait disturbance	Spondylosis, alopecia	Dementia
Alopecia, age at onset (years)	N	N	Y, 35	N
Spondylosis, age at onset (years)	Y	Y	Y, 35	N
Dementia, age at onset (years)	Y	Y	Y,	Y, 49
Stroke, age at onset (years)	Y, 54	Y, 53	Y, 45	N
Gait disturbance, age at onset (years)	Y, 53	Y, 52	N	N
Hypertension	N	N	N	N
Diabetes mellitus	N	Y	N	N
Hyperlipidemia	N	Y	Y	N
Babinski sign	+	+	+	−
Oligoclonal band	NA	NA	−	NA
Microbleeds	N	Y, multiple	Y, multiple	N
Lacunes	N, multiple	Y, multiple	N	N
WMH	Y, Fazekas grade 3	Y, Fazekas grade 3	Y, Fazekas grade 2	Y, Fazekas grade 1
HTRA1 variant	c.920T>C p.L307P	c.920T>C p.L307P	c.920T>C p.L307P	c.589C>T p.R197X

Abbreviations: F, female; M, male; N, no; NA, not available; WMHs, white matter hyperintensities; Y, yes; yr, year.

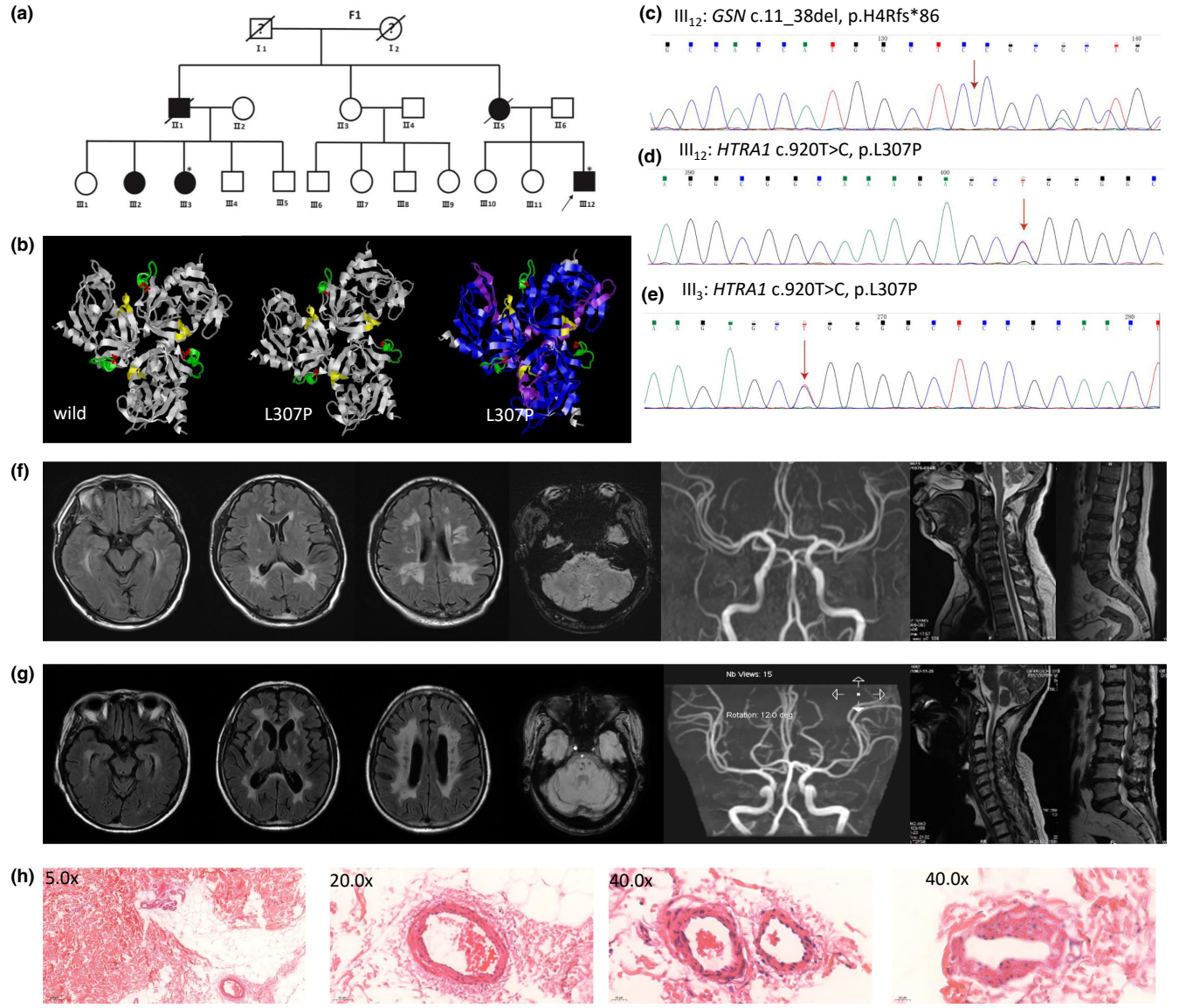

FIGURE 1 Characteristics of neuroimage, genetic and microangiopathology in family 1. (a) Pedigrees of family 1. F1, family 1; square, male; circle, female; diagonal black line, deceased individual; question mark, unknown status; full black-filled symbol, clinically or MRI-proven affected individual; empty symbol, clinically healthy relative; asterisk, variant carrier individuals. (b) SWISS-MODEL prediction of advanced structural changes (PDB ID: 3NZi). Red, mutant site; yellow, LD ring; green, L3 ring; blue, trypsin region; purple, linker region. (c) Sequence chromatograms of heterozygous variant *GSN* c.11_38del p.H4Rfs*86 in proband of family 1. (d) Sequence chromatograms of heterozygous variant *HTRA1* c.920T>C p.L307P in proband of family 1. (e) Sequence chromatograms of heterozygous variant *HTRA1* c.920T>C p.L307P in variant carriers of III3 in family 1. (f) Neuroimages of III12. The cerebral MRI scan showed bilateral diffuse white matter abnormalities involving deep frontal parietal lobes and subcortical and periventricular areas (Fazekas grade 2), with multiple microhemorrhage lesions in the right frontal lobe, left parietal lobe, and pons. There was no obvious abnormality in brain magnetic resonance angiography (MRA). Spinal MRI showed multilevel degenerative disc disease (C3/4, C4/5, C6/7, C7/T1, L2/3, L3/4, L4/5, and L5/S1). (g) neuroimages of III3. The cerebral MRI scan showed the following: 1. High signal intensities in the corpus callosum, deep white matter, and periventricular area (Fazekas grade 3); 2. Brain atrophy, bilateral ventricular dilatation, and hydronephrosis accompanied by Para-interstitial brain edema; 3. Multiple lacunar foci in both frontal lobes and basal ganglia; 4. Multiple intracranial microbleeds; 5. No obvious abnormality on brain MRA. Her spine MRI showed multilevel degenerative disc disease (C2/3, C3/4, C4/5, C5/6, C6/7, T12/L1, L2/3, L3/4, L4/5, and L5/S1). (h) HE staining of a skin biopsy of III3. The vascular wall of the dermal fat layer was thickened without normal structure; instead, it was surrounded by eosinophilic transparent denatured clumps, and there were nucleus-like substances surrounded by empty halos scattered in the center of the clumps

by drooping of the angle of the mouth and dizziness. His past medical history included blurred vision, chronic bronchitis, gallstones, and low back pain for more than 10 years. In recent years, he suffered from gradual hair loss and progressive memory deterioration. No history of hypertension or diabetes mellitus was reported. He

drunk liquor 0.25–0.5 kg every week. Physical examination showed that the bilateral palmomandibular reflex and Babinski sign were positive. Laboratory tests indicated that his glucose (6.5 mmoL/L, normal range is 3.90–6.10 mmoL/L) and triglyceride (1.86 mmoL/L, normal range is less than 1.70 mmoL/L) levels were slightly high. Serum total cholesterol (4.48 mmoL/L, normal range is less than 5.18 mmoL/L), high-density lipoprotein (HDL) (1.18 mmoL/L, normal range is 1.04–1.55 mmoL/L) and low-density lipoprotein (LDL) (2.66 mmoL/L, normal range is 1.55–3.79 mmoL/L) were normal. Blood count and blood chemistry were normal. Both immune and cerebrospinal fluid (CSF) analyses were unremarkable. The cerebral MRI scan (Figure 1f) showed bilateral diffuse white matter abnormalities involving deep frontal parietal lobes and subcortical and periventricular areas (Fazekas grade 2), with multiple microhemorrhage lesions in the right frontal lobe, left parietal lobe, and pons. There was no obvious abnormality in brain magnetic resonance angiography (MRA). Spinal MRI showed multilevel degenerative disc disease (C3/4, C4/5, C6/7, C7/T1, L2/3, L3/4, L4/5, and L5/S1). Magnetic resonance spectroscopy (MRS) showed that the NAA/Cr was 1.64 and Cho/Cr was 1.06 in the left frontal lobe, and the spectral morphology was roughly normal, consistent with demyelinating changes.

The mother of the proband (II_5) gradually became confused and had gait disturbances in her fifties. Her symptoms deteriorated stepwisely. She became paralyzed and had dementia in the last years of her life. She died at the age of 59 years.

The uncle of the proband (II_1) had gait disturbances and low back pain in his thirties and died at the age of 63 years.

His 56-year-old cousin (III_2) had gait disturbances and mood changes at the age of 52 years. Her cerebral MRI showed diffuse leukoencephalopathy.

His 53-year-old cousin (III_3) suffered from numbness, pain, and weakness in the four limbs in her early fifties, accompanied by recent memory loss and mood change, without dysphagia or alopecia. The above symptoms gradually worsened. In addition, she had a history of lower back pain since her forties and diabetes for 2 years. Physical examination showed that she had a wide-based gait, and her Romberg sign and bilateral Babinski sign were both positive. Laboratory tests indicated that her triglyceride was 2.83 mmol/L (normal range is less than 1.70 mmoL/L), serum total cholesterol was 5.07 mmoL/L (normal range is less than 5.18 mmoL/L), HDL was 1.17 mmoL/L (normal range is 1.04–1.55 mmoL/L), LDL was 3.16 mmoL/L (normal range is 1.55–3.79 mmoL/L), glucose was 8.83 mmoL/L (normal range is 3.90–6.10 mmoL/L), and HbA1c was 9.4% (normal range is 4.20–6.20%). Other biochemical and immunological indicators were normal. Her thyroid color Doppler ultrasound showed multiple nodules. The cerebral MRI scan (Figure 1g) showed the following: 1. High signal intensities in the corpus callosum, deep white matter, and periventricular area (Fazekas grade 3); 2. Brain atrophy, bilateral ventricular dilatation, hydrocephalus, and possibly accompanied by parainterstitial brain edema; 3. Multiple lacunar foci in both frontal lobes and basal ganglia; 4. Multiple intracranial microbleeds; 5. No obvious abnormality on brain MRA. Her spine MRI showed multilevel degenerative disc disease (C2/3, C3/4, C4/5, C5/6, C6/7, T12/L1, L2/3, L3/4, L4/5, and L5/S1). Electromyography showed neurogenic damage to the right lower limb (L4-L5 level), and there was no obvious abnormality in the sympathetic reflex of limb skin or anal sphincter (no obvious abnormality in the sympathetic function of the Onuf nucleus in the lumbosacral segment). HE staining of an abdominal skin biopsy (Figure 1h) showed that the vascular wall of the dermal fat layer was thickened without normal structure; instead, it was surrounded by eosinophilic transparent denatured clumps, and there were nucleus-like substances surrounded by empty halos scattered in the center of the clumps.

Strikingly, there was a frameshift variant in the proband's *GSN* gene: c.11_38del, p.H4Rfs*86 (the deletion of nucleotides 11 to 38 in the coding region led to the mutation of amino acid No. 4 from histidine to arginine and a new reading frame, which ended at codon 86 downstream) (Figure 1c). The frequency in the gnomAD database was 0.00009. The clinical significance of this locus is unknown. There was a heterozygous missense variant in the *HTRA1* gene: c.920T>C, p.L307P (nucleotide 920 in the coding region was changed from thymine to cytosine, resulting in amino acid 307 being changed from leucine to proline) (Figure 1d,e). This was a novel variant without frequency reports in the gnomAD database. The variant was predicted by SIFT software to affect protein function and predicted by Polyphen-2 software as probably damaging. The heterozygous variant of *HTRA1* was carried by III_2 and III_3, and it was possibly pathogenic according to ACMG guidelines. SWISS-MODEL prediction of advanced structural changes with *HTRA1* c.920T>C was shown in Figure 1b.

3.1.2 | Family 2

The proband of Family 2 (Figure 2a) was a 50-year-old female. She complained of memory loss at the age of 49 accompanied by anxiety and depression. She had a history of allergic rhinitis. Her Mini Mental State Examination (MMSE) score was 22/30 (Primary education level), Montreal Cognitive Assessment (MoCA) score was

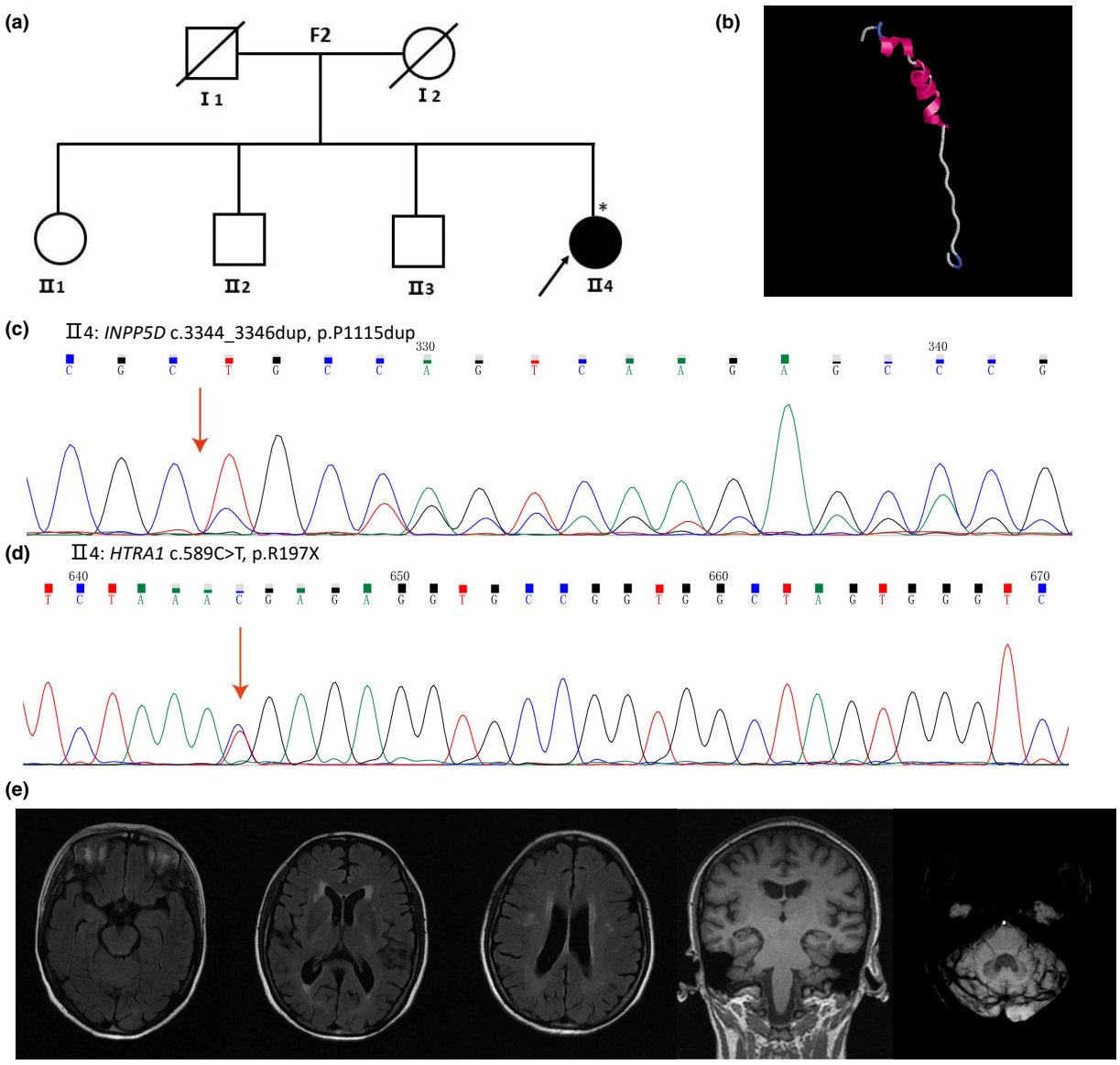

FIGURE 2 Characteristics of neuroimage and variant in family 2. (a) Pedigrees of family 2. F2, family 2; square, male; circle, female; diagonal black line, deceased individual; full black-filled symbol, clinically or MRI-proven affected individual; empty symbol, clinically healthy relative; asterisk, variant carrier individuals. (b) SWISS-MODEL prediction of advanced structural changes (PDB ID: 3NZi. 1A). (c) Sequence chromatograms of heterozygous variant *INPP5D* c.3344_3346dup p.P1115dup in variant carriers. (d) Sequence chromatograms of heterozygous variant *HTRA1* c.589C>T p.R197X in variant carriers. (e) Neuroimages of the proband in family 2. Her cerebral MRI scan showed high signal intensities in periventricular areas and supratentorial deep white matter (Fazekas grade 1) without microbleeds or lacunar infarction. Medial temporal lobe atrophy (MTA) scale 1

12/30, Clinical Dementia Rating (CDR) score was 0.5, Neuropsychiatric Inventory (NPI) score was 24, Activity of Daily Living Scale (ADL) score was 24, Hamilton Anxiety Scale (HAMA) score was 15, and Hamilton depression scale (HAMD) score was 14. Her neuropsychological evaluation suggested mild dementia. Blood lipids: triglyceride was 0.92 mmol/L (normal range is less than 1.70 mmoL/L), serum total cholesterol was 6.07 mmoL/L (normal range is less than 5.18 mmoL/L), HDL was 1.84 mmoL/L (normal range is 1.04–1.55 mmoL/L), and LDL was 3.5 mmoL/L (normal range is 3.5 mmoL/L). Her cerebral MRI scan (Figure 2e) showed high signal intensities in periventricular areas and supratentorial deep white matter (Fazekas grade 1) without microbleeds or lacunar infarction, and coronal T1 thin-layer imaging showed that her choroid fissure was widened (medial temporal lobe atrophy [MTA] scale 1). The concentration of CSF amyloid beta (Aβ) 1–42 was 548.78 pg/ml (<550 pg/ml suggests amyloidosis), Aβ1-42 / Aβ1-40 was 0.10 (≤0.1 represents positive), phosphorylated Tau protein 181 (p-Tau 181) was 65.63 pg/ml (>61 pg/ml suggests neurofibrillary tangles), and total Tau protein was 243.39 pg/ml (<290 pg/

ml is normal). A genetic screen indicated that she carried two variants. One variant was in the *INPP5D* gene: c.3344_3346dup, p.P1115dup (nucleotide 3344–3346 in the coding region was repeated, resulting in repetition of the 1115th amino acid), which is a whole-code mutation (Figure 2c). The frequency in the gnomAD database was 0.00003. The clinical significance of this locus is unknown. Studies have shown that *INPP5D* is a susceptibility gene for AD (Lambert et al., 2013). In addition, there was a variant in the *HTRA1* gene: c.589C>T, p.R197X (nucleotide 589 in the coding region was changed from cytosine to thymine, leading to amino acid 197 being changed from arginine to termination), which is a nonsense variant (Figure 2d). The frequency in the gnomAD database was 0.000008. The locus was listed as a pathogenic locus by Clinvar. Her ApoE classification was ε3ε3. SWISS-MODEL prediction of advanced structural changes with *HTRA1* c.589C>T was shown in Figure 2b.

3.2 | Variant characteristics of the *HTRA1* gene

Considering that the clinical phenotype of different variants is different and that the genotype of *HTRA1* variant has a strong correlation with its clinical phenotype, it is important to summarize the epidemiological characteristics and clinical manifestations of *HTRA1* variants to better understand CARASIL and symptom carriers and guide clinical practice. The *HTRA1* variants identified are summarized in Table 2. There were 19 papers including 83 patients were consistent with the symptomatic *HTRA1* heterozygous variant in the literature. There were 87 patients in total including the four patients we found. Among the 87 patients, 32 (36.78%) were from China; 14 (16.09%) were from Japan; 14 (16.09%) were from France; 12 (13.79%) were from Italy; 12 (13.79%) were from the UK; and 1 (1.15%) was from Germany, Greece, and Egypt. There were 46 heterozygous variants, including 39 missense variants (including the novel variant we found), 5 nonsense variants, 1 frameshift mutation, and 1 splice site abnormality. In the protein structure, 27 variants were in the trypsin region (9 in the LD ring and 4 in the L3 ring), 7 variants were in the Kazal region, 9 variants were in the linker region, and only 3 variants were in the PDZ region.

We also found 29 CARASIL patients reported by scholars from different countries, including 10 from Japan; 6 from China; 3 from India; 3 from Iran; 2 from Portugal; and 1 from America, Italy, Pakistan, Poland, and Turkey. There were 21 pathogenic variants in CARASIL, including 10 missense variants, 4 nonsense variants, 4 frameshift variants, 2 compound heterozygous variants, and 1 splice site abnormality. In the protein structure, 14 variants were in the trypsin domain (2 in the LD loop and 1 in the L3 loop), 3 variants were in the linker region, 1 variant was in the PDZ domain, and 1 variant was in the IGFBP domain. Two compound heterozygous variants (p.D320N/p.G341J and p.A321T/p.E42fs) were not analyzed because of their distribution characteristics.

The distribution of variants only in symptomatic carriers or in CARASIL is summarized in Table 3 and Figure 3. There were no significant differences in the structural distribution of CARASIL and heterozygous *HTRA1*-related dominant CSVD. Most of the variants are in the trypsin and linker regions. Compared with CARASIL, symptom carriers were more likely to be distributed in the Kazal region.

3.3 | Clinical features

Clinical and imaging findings from the included patients are summarized in Table 4. Compared with typical CARASIL, patients with heterozygous variants were more likely to be associated with male sex ($p = .022$) and other vascular risk factors ($p < .001$). The median onset age of heterozygous *HTRA1*-related CSVD was much later than that of CARASIL (51.90 vs. 19.13 years old, $p < .001$). In terms of family history, there were no significant differences between symptomatic carriers and CARASIL patients (59.8% vs. 58.6%, $p = .913$), but CARASIL patients usually had a history of consanguineous marriage (69%). In terms of extraneurological signs, the proportion of spinal degeneration in heterozygous *HTRA1*-related CSVD patients was lower than that in CARASIL (14.9% vs. 79.3% for alopecia; 19.5% vs. 93.1% for spondylosis). In terms of neurological symptoms, the proportion of psychiatric disorders and gait disturbance in heterozygous *HTRA1*-related CSVD patients was much lower than that in CARASIL patients (23.0% vs. 62.1% for psychiatric disorders; 41.4% vs. 82.8% for gait disturbance). There were no obvious differences in the proportion of cognitive impairment, stroke, or headache. Other clinical findings, including leukemia, have been reported in two symptomatic carriers. Here, we report an interesting case (Family 2) of a heterozygous variant carrier of the *HTRA1* gene in combination with early cognitive decline. On cerebral MRIs, the proportion of confluent WMHs was significantly higher in CARASIL patients than in *HTRA1*-related CSVD patients (96.6% vs. 72.4%, $p = .006$). The frequencies of microbleeds and lacunes were similar between the two groups.

4 | DISCUSSION

We identified the novel heterozygous missense variant p.L307P of the *HTRA1* gene in a family clinically suspected

TABLE 2 Summary of variants identified in patients with *HTRA1*-related CSVD and CARASIL

No.	cDNA	Amino acids	Domain	Trimerization	Protease activity	Allele frequency of gnomAD	PolyPhen2	SIFT	Patients	Families	Reference
Symptom carriers											
1	359G>A	G120D	Kazal	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Lee et al. (2018)
2	361A>C	S121R	Kazal	Missense	NA	NA	Probably damaging	Deleterious	1	1	Verdura et al. (2015)
3	367G>T	A123S	Kazal	Missense	NA	NA	Probably damaging	Neutral	1	1	Verdura et al. (2015)
4	397C>G	R133G	Kazal	Missense	NA	NA	Probably damaging	Deleterious	1	1	Verdura et al. (2015)
5	NA	S136G	Kazal	Missense	NA	NA	Benign	Neutral	1	1	Di Donato et al. (2017)
6	451C>A	Q151K	Kazal	Missense	NA	0.0001572	Probably damaging	Deleterious	2	2	Di Donato et al. (2017) Pati et al. (2018)
7	451C>T	Q151X	Kazal	Nonsense	NFM	NA	/	/	1	1	Thaler et al. (2018)
8	497G>T	R166L	Linker	Missense	Decreased	NA	Probably damaging	Deleterious	3	1	Verdura et al. (2015)
9	517G>C	A173P	Linker	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Verdura et al. (2015)
10	521A>C	D174A	Linker	Missense	NA	0.000003976	Probably damaging	Deleterious	1	1	Tan et al. (2019)
11	523G>A	V175M	Linker	Missense	NA	0.000003976	Probably damaging	Deleterious	3	2	Di Donato et al. (2017); Zhang et al. (2021)
12	527T>C	V176A	Linker	Missense	NA	NA	Probably damaging	Deleterious	6	1	Zhang et al. (2018)
13	536T>A	I179N	Linker	Missense	Decreased	NA	Probably damaging	Deleterious	2	1	Lee et al. (2018)
14	543delT	A182fs	Linker	Frameshift	Decreased	NA	/	/	1	1	Lee et al. (2018)

(Continues)

TABLE 2 (Continued)

No.	cDNA	Amino acids	Domain	Trimerization	Protease activity	Allele frequency of gnomAD	PolyPhen2	SIFT	Patients	Families	Reference
15	589C>T	R197X	Linker	Nonsense	NFM	0.000007952	/	/	2	2	Zhang et al. (2018)
16	614C>G	S205C	Trypsin	Missense	decreased	NA	Probably damaging	Deleterious	2	1	Zhuo et al. (2020)
17	NA	G206E	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	1	1	Di Donato et al. (2017)
18	632A>C	E211A	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	1	1	Tan et al. (2019)
19	646G>A	V216M	Trypsin	Missense	NA	0.000007952	Probably damaging	Neutral	2	1	Kono et al. (2018)
20	679C>T	R227W	Trypsin	Missense	NA	0.00007777	Probably damaging	Deleterious	4	4	Tan et al. (2019)
21	767T>C	I256T	Trypsin	Missense	Decreased	0.00001193	Probably damaging	Deleterious	1	1	Lee et al. (2018)
22	824C>T	P275L	Trypsin	Missense	NA	0.00001195	Probably damaging	Deleterious	3	1	Zhang et al. (2021)
23	827G>C	G276A	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Lee et al. (2018)
24	834C>A	F278L	Trypsin	Missense	NA	0.000003984	Probably damaging	Deleterious	1	1	Tan et al. (2019)
25	848G>A	G283E	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Nozaki et al. (2016)
26	847G>A	G283R	Trypsin	Missense	NA	0.000003981	Probably damaging	Deleterious	1	1	Oluwole et al. (2020)
27	852C>A	S284R	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Verdura et al. (2015)
28	850A>G	S284G	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	3	1	Verdura et al. (2015)
29	851G>A	S284N	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	1	1	Kono et al. (2018)
30	854C>A	P285Q	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Verdura et al. (2015)

TABLE 2 (Continued)

No.	cDNA	Amino acids	Domain	Trimerization	Protease activity	Allele frequency of gnomAD	PolyPhen2	SIFT	Patients	Families	Reference
31	856T>G	F286V	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	2	1	Verdura et al. (2015)
32	865C>T	Q289X	Trypsin	Nonsense	Decreased	NA	/	/	2	1	Lee et al. (2018)
33	905G>A	R302Q	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	8	4	Ito et al. (2018); Nozaki et al. (2016); Wu et al. (2018)
34	920T>C	L307P	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	3	1	–
35	940A>G	M314V	Trypsin	Missense	NA	0.000007965	Probably damaging	Deleterious	1	1	Tan et al. (2019)
36	956C>T	T319I	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Nozaki et al. (2016)
37	958G>A	D320N	Trypsin	Missense	NA	0.00001596	Probably damaging	Deleterious	1	1	Tan et al. (2019)
38	971A>C	N324T	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	2	2	Lee et al. (2018); Liu et al. (2020)
39	973-1G>A	Y325_L335del	Trypsin	Splice site	Splice site	NA	/	/	1	1	Verdura et al. (2015)
40	1103A>G	H368R	PDZ	Missense	Decreased	NA	Benign	Deleterious	1	1	Tan et al. (2019)
41	1348G>C	D450H	PDZ	Missense	Decreased	NA	Probably damaging	Deleterious	2	2	Tan et al. (2019); Verdura et al. (2015)
CARASIL											
1	126delG/961G>A	Q42fs/A321T		Compounds heterozygous	Decreased		/	/	1	1	Bianchi et al. (2014)
2	161_162insAG	G56fs	IGFBP	Frameshift	NFM	NA	/	/	1	1	Cai et al. (2015)
3	502A>T	K168X	Linker	Nonsense	Decreased	NA	/	/	1	1	Preethish-Kumar et al. (2017)
4	517G>A	A173T	Linker	Missense	NA	0.000003976	Probably damaging	Deleterious	1	1	Khaleeli et al. (2015)

(Continues)

TABLE 2 (Continued)

No.	cDNA	Amino acids	Domain	Trimerization	Protease activity	Allele frequency of gnomAD	PolyPhen2	SIFT	Patients	Families	Reference
5	616G>A	G206R	Trypsin	Missense	NA	0.00003184	Probably damaging	Deleterious	1	1	Ibrahimi et al. (2017)
6	739delG	E247Rfs	Trypsin	Frameshift	Decreased	NA	/	/	1	1	Preethish-Kumar et al. (2017)
7	754G>A	A252T	Trypsin	Missense	Decreased	0.000003977	Probably damaging	Deleterious	2	1	Hara et al. (2009)
8	805insG	S270Lfs*69	Trypsin	Frameshift	Decreased	NA	/	/	3	1	Ziaei et al. (2019)
9	821G>A	R274Q	Trypsin	Missense	NA	0.000007086	Probably damaging	Deleterious	3	2	Nishimoto et al. (2011)
10	830_831delAG	E277Vfs	Trypsin	Frameshift	NA	NA	/	/	1	1	Preethish-Kumar et al. (2017)
11	847G>T	G283X	Trypsin	Missense	Decreased	NA	Probably damaging	Deleterious	1	1	Yu et al. (2020)
12	889G>A	V297M	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	5	2	Hara et al. (2009)
13	958G>A/1021G>A	D320N/ G341R	Trypsin	Compounds heterozygous	NA	0.00001596	Probably damaging	Deleterious	1	1	Xie & Zhang (2018)
14	983C>A	S328X	Trypsin	Nonsense	NFM	NA	/	/	1	1	Gündüz et al. (2019)
15	1005+1G>T	NA	Trypsin	Splice site	Splice site	NA	/	/	1	1	Roeben et al. (2016)
16	1091T>C	L364P	Trypsin	Missense	NA	NA	Probably damaging	Deleterious	2	1	Wang et al. (2012)
Both											
1	496C>T	R166C	Linker	Missense	Decreased	NA	Probably damaging	Deleterious	7	4	Bougea et al. (2017); Favaretto et al. (2019); Liu et al. (2020); Menezes Cordeiro et al. (2015)

TABLE 2 (Continued)

No.	cDNA	Amino acids	Domain	Trimerization	Protease activity	Allele frequency of gnomAD	PolyPhen2	SIFT	Patients	Families	Reference
2	854C>T	P285L	Trypsin	Missense	Decreased	0.00000398	Probably damaging	Deleterious	3	3	Chen et al. (2013); Nozaki et al. (2016)
3	883G>A	G295R	Trypsin	Missense	Decreased	0.00001194	Probably damaging	Deleterious	5	2	Di Donato et al. (2017); Mendioroz et al. (2010)
4	904C>T	R302X	Trypsin	Nonsense	Decreased	NA	/	/	5	4	Hara et al. (2009); Ohta et al. (2020); Tan et al. (2019); Tateoka et al. (2016)
5	1108C>T	R370X	PDZ	Nonsense	NFM	0.00001591	/	/	3	3	Bayrakli et al. (2014); Hara et al. (2009); Mishra et al. (2019)

Notes: Symptomatic carriers, heterozygous *HTRA1*-related CSVD; CARASIL, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; NFM, nonsense/frameshift mutation; NA, not available; /, not applicable; ExAC, Exome Aggregation Consortium; IGFBP, insulin-like growth factor binding domain; LD, loop D; L3, loop 3.

TABLE 3 Summary of *HTRA1* variants identified in only one group

Items	Symptomatic carriers	CARASIL	*p* value
Total variants	46	19	
Missense variants	39 (84.8)	10 (52.6)	.006
Kazal-like (99–157)	6 (13.0)	0 (0.0)	.098
Linker region (158–203)	7 (15.2)	2 (10.5)	.618
LD (283–291)/L3 (301–314)	11 (23.9)	2 (10.5)	.220
Trypsin (204–364) except L3/LD	13 (28.3)	6 (31.6)	.789
PDZ (365–467)	2 (4.3)	0 (0.0)	.356
Nonsense/frameshift variants	6 (13.0)	8 (42.1)	.010
IGFBP	0 (0.0)	1 (5.3)	.117
Kazal-like (99–157)	1 (2.2)	0 (0.0)	.517
Linker region (158–203)	2 (4.3)	1 (5.3)	.873
LD (283–291)/L3 (301–314)	2 (4.3)	1 (5.3)	.873
Trypsin (204–364) except L3/LD	0 (0.0)	5 (26.3)	<.001
PDZ (365–467)	1 (2.2)	1 (5.3)	.512
Variants in the splice site	1 (2.2)	1 (5.3)	.512

Notes: Two compounds heterozygous *HTRA1* variants of CARASIL are not involved. Symptomatic carriers, heterozygous *HTRA1*-related CSVD CARASIL, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; IGFBP, insulin-like growth factor binding domain; LD, loop D; L3, loop 3.

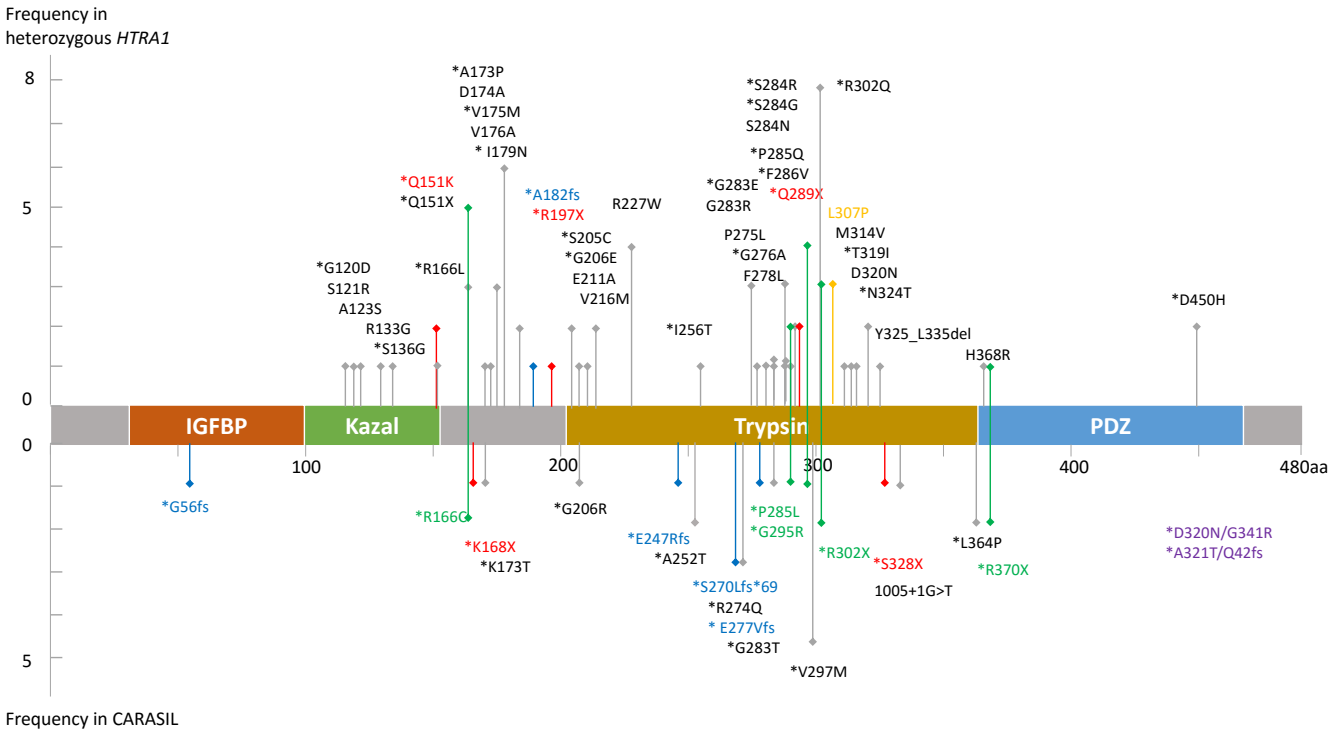

FIGURE 3 Distribution and frequency of HTRA1 variants in autosomal dominant CSVD and CARASIL patients. The gray bar stands for missense variants; red bar, nonsense variant; blue bar, frameshift variant; green bar, variants both in heterozygous and homozygous; Orange bar, the novel variant of family 1 found in this study. The asterisk indicates that the variant was pathogenic predicted by ACMG. Compounds heterozygous HTRA1 variants in CARASIL patients are not located in the diagram and are listed separately with violet font. HTRA1: High-temperature requirement serine peptidase A1; aa: amino acid

TABLE 4 Clinical characteristics of patients with CSVD related to heterozygous *HTRA1* variants and typical CARASIL

Characteristics	Heterozygous *HTRA1* ($n = 87$)	CARASIL ($n = 29$)	*p* value
Age of diagnosis, years, median (mean ± SD)	57.87 ± 10.67	34.90 ± 8.47	<.001
Age of onset, years, median (mean ± SD)	51.90 ± 12.50	19.13 ± 7.51	<.001
Gender, Male, n (%)	57 (66.5)	12 (41.4)	.022
Vascular risk factors, n (%)	42 (54.5)	2 (6.9)	<.001
Family history, n (%)	52 (59.8)	17 (58.6)	.913
Consanguineous parents, n (%)	0 (0)	20 (69.0)	<.001
Extra-neurological symptoms/signs			
Alopecia, n (%)	13 (14.9)	23 (79.3)	<.001
Spondylosis, n (%)	17 (19.5)	27 (93.1)	<.001
Neurological symptoms/signs			
Cognitive impairment, n (%)	51 (58.6)	21 (72.4)	.185
Psychiatric Disorders, n (%)	20 (23.0)	18 (62.1)	<.001
Stroke, n (%)	48 (55.2)	13 (44.8)	.334
Gait disturbance, n (%)	36 (41.4)	24 (82.8)	<.001
Headache, n (%)	10 (11.5)	2 (6.9)	.481
MRI findings			
Confluent WMHs, n (%)	63 (72.4)	28 (96.6)	.006
Microbleeds, n (%)	19 (21.8)	11 (37.9)	.087
Lacunes, n (%)	52 (59.8)	15 (51.7)	.447

Abbreviations: CARASIL, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; WMHs, white matter hyperintensities.

of showing CSVD. According to ACMG guidelines, the novel variant found in Family 1 was judged as likely pathogenic (PM1 + PM2 + PP2 + PP3) (Richards et al., 2015). The other variant we found in the proband was *GSN* c.11_38del, p.H4Rfs*86, with a frequency of 0.00009 in the gnomAD database. *GSN* is related to familial amyloidosis of the Finnish type, characterized by corneal lattice dystrophy, neurodegeneration, and cutis laxa, which is inconsistent with the phenotype of the proband (Solomon et al., 2012). It is not located in the key functional areas (Zorgati et al., 2019). Before genetic testing, the proband was diagnosed with demyelinating disease of the central nervous system (CNS) and received immunotherapy, but his symptoms continued to progress slowly. During the literature review, we did notice that some cases were misdiagnosed as multiple sclerosis (MS) because of early onset age and severe white matter lesions (Cai et al., 2015; Thaler et al., 2018; Yu et al., 2020). Two of his female cousins (III_2 and III_3 in Family 1) both had lumbago, unstable walking, mood change, and memory decline. Their cerebral and spinal MRI also showed similar abnormities as those of the proband (neuroimages of III_2 are not shown). As expected, they carried the same heterozygous missense variant of the *HTRA1* gene as the proband. The difference was that III_3 also had diabetes and hyperlipidemia. In addition to age and sex, this could be one reason why her cognitive impairment and neuroimaging findings were more severe than the proband. The etiology of CSVD is complex and includes many mechanisms depending on the type of CSVD, including arteriosclerosis-related, amyloid-related, inflammatory mediated, genetic-related, venous collagenosis, and other reasons (Litak et al., 2020). Clinically, the most common is arteriosclerosis-related CSVD due to hypertension, diabetes, and hyperlipidemia. Family history data interpretation suggests a hereditary predisposition for hypertension and diabetes. Therefore, we should not ignore the involvement of monogenetic factors in patients with CSVD who have a family history of hypertension or diabetes, especially for those with early onset age, obvious neuroimaging abnormalities, and other characteristic clinical manifestations outside the nervous system. Like III_3 in Family 1, who had diabetes, hyperlipidemia, and multilevel degenerative disc disease for many years, which are common among middle-aged and elderly groups, her large blood vessels are normal, suggesting us to further search for other possible etiologies responsible for her symptoms and intracranial lesions. Therefore, based on clinical manifestations, neuroimaging, and

genetic findings, we found one new unreported variant site in the *HTRA1* gene (c.920T>C, p.L307P), leading to autosomal dominant CSVD.

The chief complaint of proband in Family 2 was cognitive disorder and the level of her CSF biomarkers (Aβ1-42, Aβ1-42/Aβ1-40, and p-Tau) were abnormal. The mild atrophy of the medial temporal lobe on coronal T1 thin-layer MRI imaging, which was not matched with her age, suggested the loss of cells. According to ATN framework and her clinical characteristics, she might develop into AD in the future (Jack et al., 2018) and we will continue to follow up this patient. Interestingly, we found two mutant genes (*HTRA1* c.589C>T, p.R197X and *INPP5D* c.3344_3346dup, p. P1115dup) that may both be pathogenic. The same missense variant of the *HTRA1* gene was reported as pathogenic variant from a family in 2018 (Zhang et al., 2018). AD is the most common type of dementia, characterized by neurofibrillary tangles and Aβ deposits. Apoε4 is the strongest risk factor for late-onset AD (LOAD). Biochemical research has revealed that Tau proteins and amyloid precursor protein (APP) are both HTRA1 substrates (Grau et al., 2005; Tennstaedt et al., 2012). HTRA1 is a candidate Apoε4 modulating enzyme in vitro (Chu et al., 2016). Furthermore, research has indicated that, when HTRA1 expression is elevated, the accumulation of neurofibrillary tangles and neuritic plaques in neuronal cells and patient brains decreases, respectively (Poepsel et al., 2015; Tennstaedt et al., 2012), indicating that HTRA1 also degrades aggregated and fibrillar tau protein. Xiao X et al. revealed that *HTRA1* was nominally associated with AD according to gene-based aggregation testing (Xiao et al., 2021). AD has significantly higher prevalence of vascular pathology than α-synucleinopathy, FTLD-Tau and -TDP, prion disease and unremarkable brain, which was more prevalent at younger ages, and the presence of CSVD would increase the risk of AD (Toledo et al., 2013). More summaries of clinical characteristics and fundamental research are needed to clarify this area. In addition, studies have shown that the other variant, *INPP5D*, is associated with LOAD, and the expression of INPP5D increases as AD progresses, predominantly in plaque-associated microglia (Tsai et al., 2021). The premature onset age of the proband may be the result of the joint action of these factors. Further follow-up and more case studies are needed to illustrate whether this cognitive disorder is amyloid-related, genetic-related, or due to mixed etiologies.

We found 46 heterozygous *HTRA1* variants related to CSVD and 21 pathogenic variants of CARASIL in the literature. There was no significant difference in the structural distribution of CARASIL and heterozygous *HTRA1*-related CSVD. Most of the variants are in trypsin (especially LD/L3) and the linker region, and few are distributed in the IGFBD and Kazal regions, which is consistent with the fact that neither the IGFBD nor Kazal-type motif influences the activity of the protease in vitro (Zurawa-Janicka et al., 2017). Studies have also shown that, compared with non-LD/L3 domain mutations, LD/L3 domain mutations have more harmful effects on trimerization, resulting in lower protease activity (Uemura et al., 2019). In heterozygous *HTRA1*-related CSVD and CARASIL, very few variants were located in the PDZ domain, which was consistent with the fact that the PDZ domain was not necessary to activate HTRA1 (Truebestein et al., 2011). This showed that the distribution of variant sites was consistent with the significance of gene structure.

At present, it is generally believed that decreasing residual HTRA1 activity might increase the risk of CSVD based on the molecular mechanism of symptomatic carriers. However, the exact pathogenic mechanism of heterozygous *HTRA1*-related CSVD is not clear. Variant of the *HTRA1* gene may affect angiogenesis by affecting TGF-β signal transduction. The four parents of CARASIL patients suffered from moderate leukoencephalopathy and were considered heterozygote carriers (p.P285 L, p.G295R, p.E42fs, and p.A321T) (Bianchi et al., 2014; Chen et al., 2013; Mendioroz et al., 2010). However, most parents of CARASIL patients were asymptomatic. These data indicate that carriers of *HTRA1* heterozygotes are clinically affected in some cases but not in others. Recently, studies have shown that *HTRA1* with a heterozygous variant has a significant negative effect on the protease activity of wild-type *HTRA1* because it cannot form a stable trimer (Nozaki et al., 2016). Uemura et al. found that *HTRA1* variants identified in symptomatic carriers have the capability of interfering with the trimer-dependent activation cascade of HTRA1 (Uemura et al., 2019). Fasano A., et al. discovered that each heterozygous *HTRA1* missense variant exhibits a different and distinct HTRA1 expression mode and that the CSVD phenotype may also result from 50% HTRA1 expression (Fasano et al., 2020). More research is needed to clarify the exact role of HTRA1.

We concluded that the onset age of symptomatic *HTRA1* heterozygous variant-related CSVD was much later than that of CARASIL, the clinical symptoms were milder, the frequency of extraneurological symptoms such as alopecia and spinal diseases was lower, and the WMHs were lower on MRI. Heterozygous variants in *HTRA1* patients have more cerebrovascular risk factors, which might be related to age. The pathogenesis of CARASIL is related to homozygous or compound heterozygous variants, and the double allele variant results in the loss of HTRA1 activity. Meanwhile, the heterozygous

variants of *HTRA1* only results in partial loss of HTRA1 activity. At present, there is no consistent conclusion to determine whether the *HTRA1* variant is pathogenic, and there are no functional studies or common separation criteria to evaluate *HTRA1* in vitro or in vivo in the literature. Therefore, we did not judge the pathogenicity of every gene, which may have led to differences in clinical features. In addition, regarding the slight phenotypic expression of heterozygous *HTRA1* variants, it is uncertain whether the carriers of these variants form a unique clinical entity (Bougea, 2018). CADASIL type 2 (OMIM 616779) has been proposed as the name of the carrier of symptomatic *HTRA1* variant (Uemura et al., 2020). As CSVD does not always occur in variant carriers, these variants may only be a risk factor for CSVD. Before elucidating the pathogenesis of this variant, it may be reasonable to use the term "heterozygote *HTRA1*-related CSVD spectrum" instead of a new disease.

5 | CONCLUSION

Taken together, we report a new heterozygous *HTRA1* variant p.L307P, whose main clinical phenotype is CSVD and spondylosis. We reported another patient with early cognitive decline who carried two variants (*HTRA1* and *INPP5D* genes). After a literature review, we conclude that heterozygous *HTRA1*-related CSVD is a mild phenotype of CARASIL. In addition, phenotypic severity is influenced by the variant domain and other vascular risk factors. To pursue effective preventive and therapeutic measures for this uncurable disease, it is necessary to further study the pathogenic mechanism of heterozygote *HTRA1* variants to determine whether other elements, in addition optimal control of modifiable vascular risk factors, might delay the disease progression.

AUTHOR CONTRIBUTIONS

Hui Zhou collected data, searched the database, wrote the initial draft, and did the final editing; Liangjuan Fang designed the study, searched the database, analyzed the data, and revised the manuscript; Ziyu Ouyang followed up with the two families, collected data, searched the database, and analyzed the data; Bin Jiao designed the study, analyzed the data, and revised the manuscript; Qihui Wu and Lu Shen revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Xiangya Hospital, Central South University (202103054). Written informed consent was obtained from all participants or their legal guardians.

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