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RESEARCH ARTICLE

Novel mutations in *HTRA1*-related cerebral small vessel disease and comparison with CADASIL

Chen Zhang[1], Honghua Zheng[2], Xin Li[2], Shaowu Li[3], Wei Li[4], Ziwei Wang[2], Songtao Niu[1], Xingao Wang[1] & Zaiqiang Zhang[1]

[1]Department of Neurology, China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing, China
[2]Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, School of Medicine, Xiamen University, Xiamen, Fujian, China
[3]Beijing Neurosurgical Institute, Beijing Tiantan Hospital, Capital Medical University, Beijing, 100070, China
[4]Monogenic Disease Research Center for Neurological Disorders, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Correspondence
Zaiqiang Zhang, Department of Neurology, China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, No.119 South 4th Ring West Road, Fengtai District, Beijing, 100070, China. Tel: 86-10-599798322; Fax: 86-10-599798322; E-mail: zzhang2016@126.com

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Abstract

Objective: There is evidence showing both heterozygous *HTRA1* and homozygous *HTRA1* mutations as causal for familial cerebral small vessel disease (CSVD). The clinical and neuroimaging signs of heterozygous *HTRA1*-related CSVD can mimic cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). We aimed to characterize the genotypic and phenotypic features of *HTRA1*-related CSVD, and we compared the features of heterozygous *HTRA1*-related CSVD and CADASIL. **Methods**: We carried out genetic sequencing in a series of unrelated patients with suspected familial CSVD from China. Clinical and imaging characteristics of heterozygous *HTRA1*-related CSVD and CADASIL were compared. **Results**: We identified nine heterozygous *HTRA1* mutations and one homozygous *HTRA1* mutation, seven of which are novel. Compared with CADASIL, patients with heterozygous *HTRA1*-related CSVD had a higher proportion of spine disorders and a lower proportion of white matter hyperintensities involving the anterior temporal lobe ($p < 0.001$). **Interpretation**: This study shows that most *HTRA1*-related CSVD patients in China carry heterozygous *HTRA1* mutations. The specific extra-neurological features and neuroimaging features reveal informative differences between heterozygous *HTRA1*-related CSVD and CADASIL. We expand the mutational spectrum of *HTRA1*.

Introduction

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) was previously considered a unique nonhypertensive recessive form of cerebral small vessel disorder hallmarked by stroke, cognitive defects, lumbago, and alopecia.[1] Neuroimaging features are similar to cerebral small vessel diseases (CSVD), such as the presence of cerebral white matter hyperintensities (WMH), lacunes, visible perivascular spaces (PVS), cerebral microbleeds (CMBs), and cerebral atrophy.[2,3] Characteristic pathological changes in the vasculature include thickening of small arteries, loss of smooth muscle cells (SMCs), and splitting of the elastic lamina.[4–7]

There is growing data showing heterozygous *HTRA1* mutation as a cause of familial CSVD with a dominant inheritance pattern.[8-10] The clinical characteristics are different from classic CARASIL, including, for example, later age of onset and the absence of typical extra-neurological features of CARASIL, such as alopecia.[10] The dominant inheritance pattern, as well as the clinical and neuroimaging characteristics, resemble autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). There have been only three high-quality cohorts from Europe (10 probands),[10] Japan (6 index cases and 2 affected siblings),[9] and Taiwan (7 index cases and 2 affected siblings)[8] have characterized the disease features and the influence of the mutations on HTRA1 protease activities. The inheritance pattern, clinical, and

genetic features of *HTRA1*-related CSVD in the Chinese population remain unclear.

In the present study, we aimed to determine the genotypic and phenotypic features of *HTRA1*-related CSVD in a series of suspected familial CSVD from China. We also compared heterozygous *HTRA1*-related CSVD and CADASIL to help better differentiate these two types of autosomal-dominant CSVD.

Methods

Study population

One hundred and eighty-one unrelated Chinese patients were recruited from the Neurology Department of Beijing Tiantan Hospital between 2014 and 2019. The inclusion criteria were as follows: (1) the presence of moderate to severe white matter hyperintensities; (2) the presence of more than one of the following features: stroke-like episodes, cognitive disorders, gait disturbance, psychiatric disorder; and (3) a family history of stroke, cognitive decline, or white matter hyperintensities. Leukodystrophy and acquired causes (infections, autoimmune syndromes, etc.) were excluded. This study was approved and performed following the guidelines of the Ethics Committee of Beijing Tiantan Hospital (KY 2018-002-02) and informed consent was obtained from all patients or their families.

Clinical and Imaging data acquisition and analysis

Clinical and imaging data from all index patients and their available affected family members were collected and analyzed. Brain neuroimages were obtained using a 3.0T magnetic resonance imaging (MRI) scanner (Magnetom Trio Tim; Siemens) including the following scanning sequences: whole-brain axial T2-weighted images, fluid-attenuated inversion recovery images, sagittal T1-weighted images, and susceptibility-weighted images. All CSVD-related MRI analyses (WMH, PVS, lacunes, CMBs, and atrophy) were conducted in line with the Standards for Reporting Vascular Changes on Neuroimaging recommendations.[11] The total CSVD score was calculated as previously described (an ordinal score of total CSVD burden, range 0–4).[12] The experienced investigators were blinded to all clinical and genetic information.

Genetic sequencing, analysis, and interpretation

We performed whole-exome Illumina sequencing or gene-panel sequencing (genes included in the panel were *NOTCH3*, *HTRA1*, *COL4A1*, *TREX1*, *KRIT1*, *CCM2*, *PDCD10*, *GSN*, *APP*, *CST3*, *COL4A2*, *TGFBR1*, *TGFBR2*, *COL3A1*, *ABCC6*, *MTHFR*, *CBS*, *ITM2B*, and *PDE4D*) on samples obtained from the patients. Genomic DNA was isolated from peripheral leukocytes using a DNA Isolation Kit from Bioteke (catalog no. AU1802). The DNA libraries were sequenced on the HiSeq X10 platform (Illumina, San Diego, USA). The called variants were annotated with public databases (1000 genomes project, Genome Aggregation Database [gnomAD], Huaman Gene Mutation Database [HGMD], and an in-house database). Sanger sequencing was conducted in all probands and the available family members, including the affected members and the spouse, to confirm the detected variants and find out whether the variant cosegregated with the affected status. The functional impact of the candidate variants was predicted by Mutation Taster,[13] PolyPhen-2,[14] and SIFT.[15]

HTRA1 protease activity

Mutant constructs of human HTRA1 (p.A20V, p.V175M, p.F278L, p.P285L, p.Q318H, p.V339M, and p.G350E) were prepared from wild-type pcDNA3.1/myc-His.[9] The primers and their complementary primers are shown in Table S1. Transfection of HEK293 cells with mutant and wild-type HTRA1 was done as described previously.[8] Protease activity for wild-type and mutant HTRA1 were measured using the Pierce® Fluorescent Protease assay kit following previous reports.[1,8–10]

Statistical analysis

SPSS (version 22.0) and GraphPad Prism 7.0 were used for analyses.

Results

Mutations of the *HTRA1* gene

Among the 181 unrelated patients with suspected familial CSVD, a total of 10 *HTRA1* mutations were identified in 11 index patients (6.08%), including nine heterozygous mutations (eight missense mutations and one splicing mutation) and one homozygous missense mutation (Tables 1 and 2). Seven were novel *HTRA1* mutations, one of them was a homozygous missense mutation (c.59C>T), and the other six mutations were heterozygous (c.832T>C, c.834C>G, c.954G>C, c.973-2A>G, c.1015G>A, and c.1049G>A). Eight of the 10 detected *HTRA1* variants were not present in public data resources including 1000 Genomes,[16] HGMD,[17] or an in-house database comprising 200 healthy control individuals in

Table 1. Characteristics of patients with *HTRA1* mutations in this study.

Family	Member	cDNA change	Protein change	Mutation type	Sex/age	Vascular risk factors	Main symptoms	MRI features
1	1	c.59C>T	p.A20V	Homozygous	M/53	Smoking and HTN	CH, recurrent IS, GD, and SD	WMH*, PVS, CMBs, lacunes, and atrophy
	2	c.59C>T	p.A20V	Heterozygous	M/29	Abs	Abs	PVS
	3	c.59C>T	p.A20V	Heterozygous	F/60	HTN	CH, CI, GD, and SD	WMH*, CMBs, and PVS
2	4	c.59C>T	p.A20V	Homozygous	F/64	Abs	IS and SD	WMH*, lacunes, PVS, and atrophy
	5	c.59C>T	p.A20V	Heterozygous	F/60	Abs	IS and SD	WMH*
3	6	c.59C>T	p.A20V	Heterozygous	F/31	Abs	SD	WMH*
	7	c.59C>T	p.A20V	Heterozygous	M/57	Abs	IS, CI, and SD	WMH and PVS
4	8	c.G523A	p.V175M	Heterozygous	M/44	HTN	IS, CI, GD, and SD	WMH*, PVS, CMBs, lacunes, and atrophy
5	9	c.832 T>C	p.F278L	Heterozygous	M/42	HTN	IS, CI, and SD	WMH, PVS, and lacunes
	10	c.832 T>C	p.F278L	Heterozygous	F/41	HTN	SD	WMH* and PVS
6	11	c.834C>G	p.F278L	Heterozygous	F/54	Abs	IS, CI, GD, and SD	WMH*, PVS, CMBs, lacunes, and atrophy
7	12	c.854C>T	p.P285L	Heterozygous	F/42	Abs	IS, CI, GD, and SD	WMH*, PVS, and lacunes
8	13	c.954G>C	p.Q318H	Heterozygous	F/66	Abs	IS, CI, and SD	WMH* and atrophy
	14	c.954G>C	p.Q318H	Heterozygous	M/46	Smoking and HTN	IS, CI, GD, and SD	WMH*, PVS, and lacunes
	15	c.954G>C	p.Q318H	Heterozygous	M/46	Smoking and HTN	IS, CI, GD, and SD	WMH*, PVS, CMBs, and lacunes
	16	c.954G>C	p.Q318H	Heterozygous	M/39	Smoking and HTN	IS and SD	WMH*, PVS, and lacunes
	17	c.954G>C	p.Q318H	Heterozygous	M/36	Smoking	SD	WMH* and PVS
9	18	c.1015G>A	p.V339M	Heterozygous	F/44	HTN	IS, CH, CI, migraine, GD, and SD	WMH*, PVS, CMBs, lacunes, and atrophy
10	19	c.973-2A>G	Splicing	Heterozygous	F/55	HTN	IS, CI, migraine, and GD	WMH*, PVS, CMBs, lacunes, and atrophy
	20	c.973-2A>G	Splicing	Heterozygous	M/69	DM	IS, CI, migraine, and GD	WMH*, PVS, lacunes, and atrophy
	21	c.973-2A>G	Splicing	Heterozygous	F/58	HTN	CI, migraine, and GD	WMH* and PVS
11	22	c.1049G>A	p.G350E	Heterozygous	M/39	Smoking	IS, CI, alopecia, and SD	WMH*, PVS, and lacunes

Abbreviations: Abs, absence; CH, cerebral hemorrhage; CI, cognitive impairment; CMBs, cerebral microbleeds; DM, Diabetes mellitus; GD, gait disturbance; HTN, hypertension; IS, ischemic stroke; MRI, magnetic resonance imaging; PVS, perivascular spaces; SD, spine disorders; WMH, white matter hyperintensities.

*White matter hyperintensities not involving the anterior temporal lobe.

China, with a low allele frequency in gnomAD[18] (Table 2); they were predicted as pathogenic by Mutation Taster,[13] PolyPhen-2,[14] and/or SIFT[15] (Table 2). The A protein structure of HTRA1 with the mutant positions is displayed in Figure 1A,B.

HTRA1 protease activity

To investigate the functional influence of these novel *HTRA1* missense mutations, the protease activity of wild-type and mutant HTRA1 proteins to hydrolyze fluorescein isothiocyanate-labeled casein was measured. All the HTRA1 mutants had dramatically decreased proteolytic activity than cells with a plasmid for wild-type HTRA1 (Fig. 1C; $p < 0.001$). To investigate whether these *HTRA1* mutations had a dominant negative effect on the HTRA1 enzymatic function, we transfected human embryonic kidney cells 293 with 1/2 dosage of wild-type and 1/2 dosage of each mutant HTRA1, then we measured the HTRA1 protease activities purified from the cells. The catalytic capacity of the HTRA1 mixture of wild-type and each mutant HTRA1 was lower compared with wild-type HTRA1(Fig. 1D; WT/V175M, WT/Q318H, WT/V339M, and WT/G350E, $p < 0.001$; WT/A20V, WT/F278L, and WT/P285L, $p < 0.01$).

Clinical and pathological characteristics

Twenty-two of the patients (11 index patients and 11 affected family members) carrying the *HTRA1* mutations

Table 2. *HTRA1* variants identified in this study.

cDNA change	Protein change	Exon	Protein domain	Mutation type	Mutation Taster	PolyPen-2	SIFT	1000 Genomes	HGMD	gnomAD
c.59C>T	p.A20V	1	Signal peptide	Homozygous	Polymorphism	Benign	Tolerated	5.07188×10^{-2}	Abs	1.29992×10^{-2}
c.59C>T	p.A20V	1	Signal peptide	Heterozygous	Polymorphism	Benign	Tolerated	5.07188×10^{-2}	Abs	1.29992×10^{-2}
c.G523A	p.V175M	2	—	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	4.06055×10^{-6}
c.832 T>C	p.F278L	4	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	4.06861×10^{-6}
c.834C>G	p.F278L	4	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	Abs
c.854C>T	p.P285L	4	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	4.06445×10^{-6}
c.954G>C	p.Q318H	4	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	4.07153×10^{-6}
c.973-2A>G	splicing	5	Serine protease	Heterozygous	Disease causing	NA	NA	Abs	Abs	4.06062×10^{-6}
c.1015G>A	p.V339M	6	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	Abs
c.1049G>A	p.G350E	6	Serine protease	Heterozygous	Disease causing	Probably damaging	Deleterious	Abs	Abs	Abs

Abbreviations: Abs, absence; NA, not available.

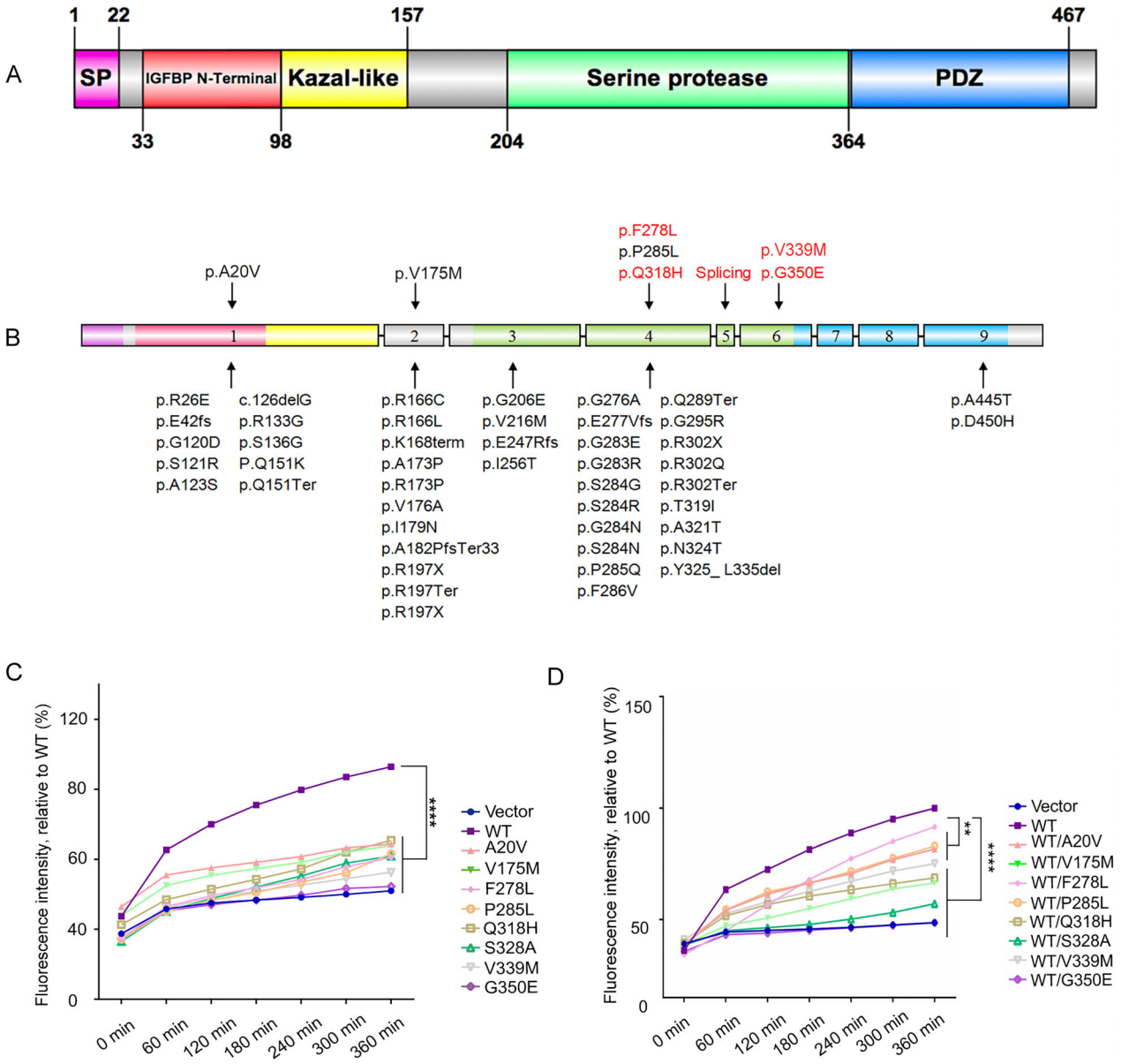

Figure 1. Heterozygous *HTRA1* gene mutations in this study. (A) Schematic for the known functional domains of the HTRA1 protein. Purple, signal peptide; red, insulin-like growth factor-binding protein domain; yellow, Kazal-type serine protease inhibitor domain; green, trypsin-like serine protease domain; blue, PDZ domain. (B) Heterozygous *HTRA1* mutational spectrum. The mutations found in this study are shown in the upper part, with novel mutations represented in red. The mutations reported in previous studies are shown in the lower part. (C, D) Protease activities of mutant HTRA1 proteins and HTRA1 protein complex. The active-site mutant S328A was used as a negative control.

with complete demographic, clinical, and MRI information were included; these data are presented in Table 1. The mean age (±SD) was 48.9 ± 11.3 years; 11 (50%) patients were male. Hypertension was the most frequent risk factor 11 (50.0%). Ischemic stroke and cognitive impairment were common neurological symptoms. Eighteen (95.5%) patients had spine involvement, and 1 (4.5%) had alopecia. Histopathologic analysis of the skin from the proband of family 7 showed an abnormal thickness of artery walls and aberrant loss of smooth muscle cells (Fig. 2).

Imaging characteristics

MRI data are presented in Table 1. Neuroimaging evaluation revealed that all the patients had WMH. Only two

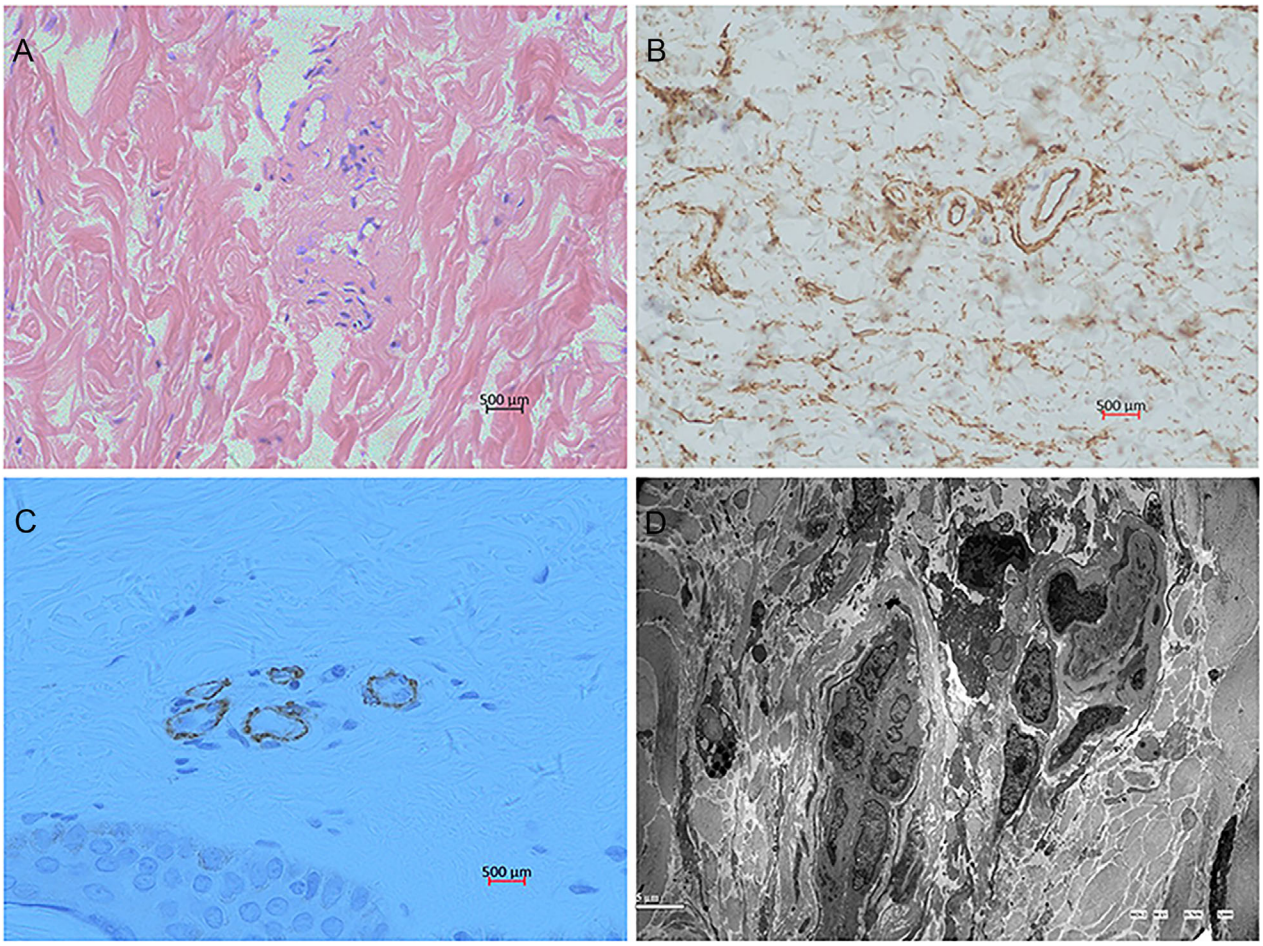

Figure 2. Histopathologic findings in this study. (A–D) Skin artery biopsy of the proband from family 7 with heterozygous P285L mutation. (A) Hematoxylin–eosin staining shows obviously fibrotic and thickened arteries. (B) CD34 immunohistochemical staining shows thickening of the vascular walls. (C) α-actin staining shows the wall of arterial wall irregularly decreased and the degeneration of smooth muscle cells. (D) Electron micrographs shows marked luminal narrowing of an artery.

9.1% of patients had WMH involving the anterior temporal lobe, and 19/22 (86.4%) patients showed PVS, followed by lacunes 14/22 (63.6%), CMBs 7/19 (36.8%), and brain atrophy 8/22 (36.4%). It was noteworthy that patient III-4 with heterozygous *HTRA1* mutation (c.954G > C) from family 8 had an arachnoid cyst in the right temporal and frontal lobe, which is a phenotype that has not been previously reported for heterozygous HTRA1-related CSVD (Fig. 3). Lumbar and cervical MRI revealed variable levels of retrogression of vertebrae and intervertebral discs, prolapse of intervertebral discs, and canal stenosis (Fig. 3).

Genotypic and phenotypic features

We found that individuals carrying both homozygous and heterozygous *HTRA1* mutations could manifest CSVD-related symptoms, while the healthy family members had no *HTRA1* mutations. Although patients from three different families (Family 1 to Family 3) carried the same heterozygous *HTRA1* mutation (c.59C>T, p.A20V), the clinical manifestations were different, some presented with cerebral hemorrhage, whereas others manifested as ischemic stroke or cognitive decline. A similar phenotypic heterogeneity phenomenon was observed among the hemorrhagic and ischemic individuals harboring the homozygous mutation (c.59C>T). Note that there was no obvious difference in the severity of phenotypes between the heterozygous carriers and homozygous carriers.

Comparison between heterozygous *HTRA1*-related CSVD and CADASIL

Among the 181 patients of suspected familial CSVD, there were 34 patients with (1) complete clinical and MRI data who were also (2) diagnosed with CADASIL and (3) had

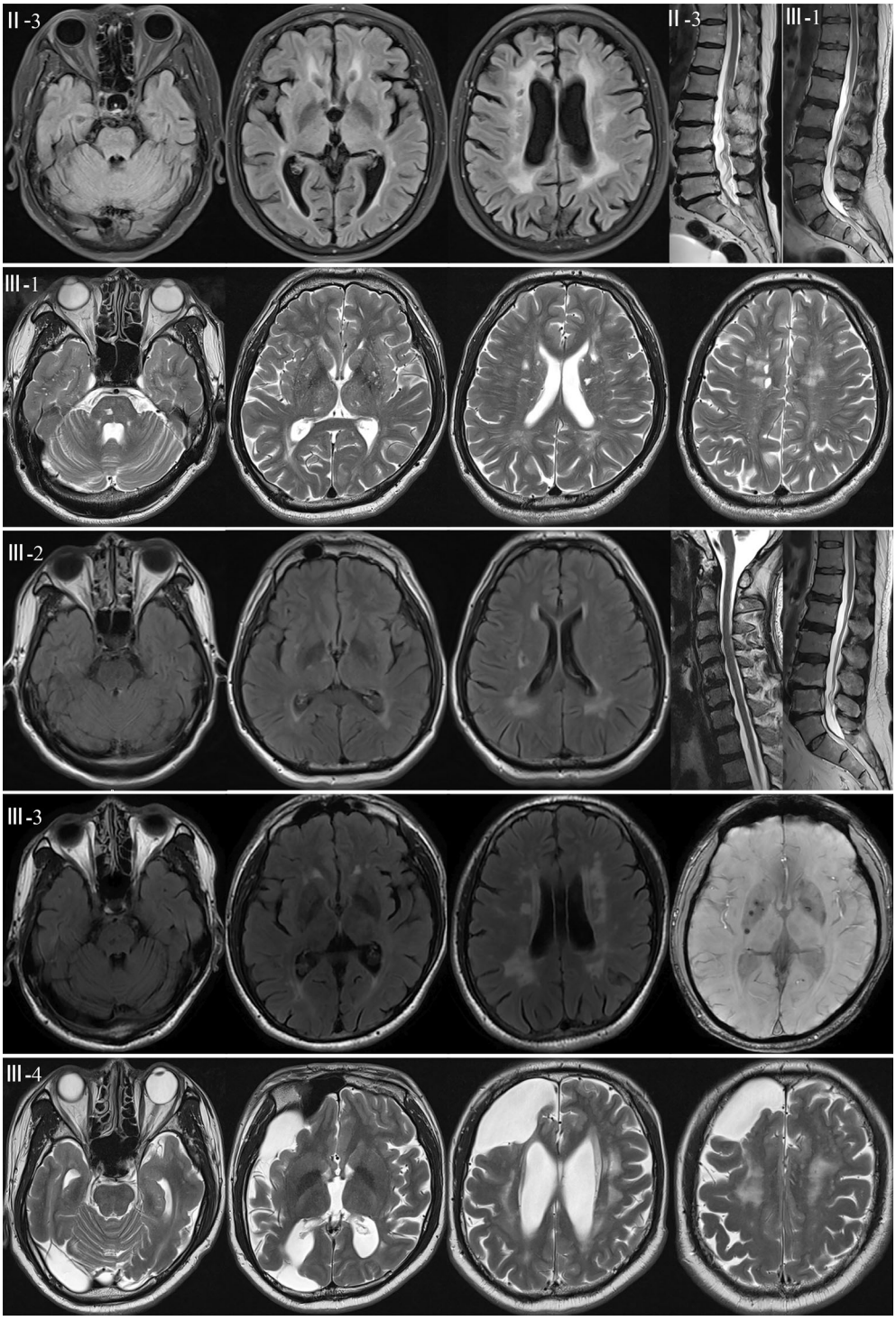

Figure 3. MRI findings in patients with heterozygous *HTRA1* mutation. MRI from family 8 with heterozygous *HTRA1* mutation. Fluid-attenuated inversion recovery images of the brain show symmetrical hyperintensities in the periventricular area, whereas anterior temporal poles were not obviously involved (II-3, III-2, and III-3); lacunar infarcts in the deep brain white matter and basal ganglia (II-3, III-2, and III-3); T2-weighted images of the brain revealing visible perivascular spaces in the basal ganglia and deep brain white matter (III-1). Susceptibility-weighted images demonstrate microbleeds in the basal ganglia (III-3); the arachnoid cyst is noted in the right temporal and frontal lobe of III-4; T2-weighted cervical and lumbar MRI revealed multilevel degenerative disease, including disc herniation, canal stenosis, and degeneration of vertebral bodies (II-3, III-1, and III-2).

confirmed *NOTCH3* pathogenic mutations (Table S2). These patients were examined with clinical history and neuroimaging to enable comparative analysis with heterozygous *HTRA1*-related CSVD individuals. Based on the demographic and clinical characteristics between heterozygous *HTRA1*-related CSVD and CADASIL, we detected no differences in age, gender, vascular risk factors, neurological symptoms, or alopecia. However, patients with heterozygous *HTRA1*-related CSVD had a higher proportion of spine disorders compared with CADASIL ($p = 0.001$). For the CSVD neuroimaging features, patients with heterozygous *HTRA1*-related CSVD showed a lower proportion of WMH involving the anterior temporal lobe compared with CADASIL (both $p < 0.001$) (Table 3); there were no other CSVD neuroimaging feature differences between the two groups.

Table 3. Comparison of the heterozygous *HTRA1*-related CSVD and CADASIL.

Characteristics	Heterozygous *HTRA1*-related CSVD ($n = 20$)	CADASIL ($n = 34$)	p value
Age, years, mean ± SD	47.9 ± 11.3	47.2 ± 9.3	0.807
Male, *n* (%)	10 (50)	16 (47.1)	0.835
Smoking, *n* (%)	5 (25.0)	7 (20.6)	0.970
Medical history, *n* (%)			
Hypertension	10 (50.0)	11 (32.4)	0.199
Diabetes mellitus	2 (10)	2 (5.9)	0.583
Clinical features, *n* (%)			
Migraine	4 (20.0)	7 (20.6)	1.000
Ischemic stroke	14 (70.0)	28 (82.4)	0.474
Cerebral hemorrhage	2 (10.0)	4 (11.8)	0.841
Gait disturbance	11 (55.0)	12 (35.3)	0.157
Cognitive impairment	14 (70.0)	26 (76.5)	0.600
Alopecia	1 (5.0)	0 (0.0)	0.370
Spine disorders	19 (95.0)	16 (47.1)	<0.001
CSVD neuroimaging features, *n* (%)			
WMH involves the anterior temporal lobe	2 (10.0)	30 (88.2)	<0.001
Presence of lacunes	12 (60.0)	25 (73.5)	0.301
Presence of visible PVS	17 (85.0)	31 (91.2)	0.492
Presence of cerebral microbleeds*	6 (35.3)	19 (63.3)	0.064
Presence of cerebral atrophy	6 (30.0)	17 (50.0)	0.151
Total CSVD score, median (IQR)	2 (1–4)	3 (2–4)	0.075

Abbreviations: CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CSVD, cerebral small vessel disease; IQR, interquartile range; PVS, perivascular spaces; SD, standard deviation; WMH, white matter hyperintensities.
*Three heterozygous *HTRA1*-related CSVD and four CADASIL patients missing the susceptibility-weighted images data.

Discussion

Our study shows that most *HTRA1*-related CSVD patients in China carry heterozygous *HTRA1* mutations. We broaden the genotypic spectrum of *HTRA1*-related CSVDs. We found that ischemic stroke and cognitive impairment are frequent neurological presentations of *HTRA1*-related CSVD, which is similar to CADASIL. However, spine disorders are the most common extra-neurological manifestation in *HTRA1*-related CSVD, with alopecia seen less frequently. Spine disorders, when combined with neuroimaging features, may indicate differential trends between the two similar familial CSVDs.

To date, only four cohorts have reported the heterozygous *HTRA1*-related CSVD. Verdura et al.[10] first reported 11 probands harboring deleterious heterozygous *HTRA1* mutations from French familial CSVD in 2015 and found all of them resulted in impaired protease activity. In 2016, Nozaki et al.[9] identified four heterozygous *HTRA1* mutations upon screening 113 unrelated Japanese CSVD cases; they investigated potential molecular mechanisms and found that heterozygous *HTRA1* exerted a dominant negative effect. In 2017, Donato et al.[2] identified five heterozygous *HTRA1* mutations from five Italian families. In 2018, Lee et al.[8] identified seven heterozygous *HTRA1* mutations among 337 unrelated Taiwanese CSVD patients and found that these mutations led adding to impaired HTRA1 protease activity. These findings support that heterozygous *HTRA1* mutations can cause familial CSVD. Our results further expand the *HTRA1* genetic spectrum and validate a causative role for heterozygous *HTRA1* mutation in familial CSVD.

Regarding the heterozygous *HTRA1* clinical phenotypes in our study, the main clinical features include ischemic stroke, cognitive decline, and spine disorders, finding in line with the previous cohort study.[8] It has been reported that the frequency of extra-neurological manifestations in dominant CSVD is lower in patients with heterozygous missense mutations than in CARASIL.[19] In our study, patients with heterozygous *HTRA1* mutations had a high frequency of spine disorders and a low frequency of alopecia, which is congruent with previous studies.[8,20] Intracranial arachnoid cyst is commonly considered a congenital lesion; it occurs most commonly in the temporal fossa and is relatively more prevalent in male patients.[21] The pathogenic mechanisms for cyst remain unclear,[21] and whether this manifestation is associated with *HTRA1* needs to be further elucidated.

Multiple features detected in patients with heterozygous *HTRA1* resemble CADASIL, including, for example, the dominant inheritance pattern, main neurological symptoms including stroke and cognitive decline, and typical CSVD-related neuroimaging markers including white

matter hyperintensities, lacunes, and cerebral microbleeds.[8] Although migraine has been considered a hallmark of CADASIL, patients with heterozygous *HTRA1*-related CSVD can also present with this symptom,[22,23] which can make clinical diagnosis challenging. It is true that genetic screening and skin biopsy can support a definite diagnosis and can distinguish the two diseases, but these methods are high cost[24] and invasive.[25] In our study, we found patients with heterozygous *HTRA1*-related CSVD had a higher proportion of skeletal disorders and less involvement of the anterior temporal lobe compared with CADASIL, the combined features indicate informative differences between these two similar forms of inherited CSVD. Nevertheless, only genetic screening can definitely discriminate the inherited CSVD.

HTRA1 functions in protein quality control.[26] Most of the variants identified in our study affect residues located within HTRA1's serine protease domain,[26-28] It is noteworthy that we found three families carrying homozygous or heterozygous p.A20V. Although this variant was predicted to be benign using in silico tools, all the affected family members (family 1, family 2, and family 3) presented with neurological symptoms that carried this mutation, although the clinical manifestations are varied. The p.A20V (c.59C>T) mutation affects residues within HTRA1's signal peptide domain,[26-28] and the function of HTRA1's N-terminal domain is not completely understood;[26] it has been observed that the autolysis of the N-domain in vivo does not result in decreased protease activity.[27] Future studies to elucidate the pathogenic impact(s) of this mutation are warranted.

In conclusion, our genetic, clinical, and neuroimaging study of a large cohort of CSVD cases in China broadens the genetic spectrum of *HTRA1* and sheds light on the substantial heterozygous genetic etiology of this neurological disease. Patients with heterozygous *HTRA1*-related CSVD had a higher proportion of spine disorders and a lower proportion of WMH involving the anterior temporal lobe compared with CADASIL.

Author Contributions

ZQZ and CZ formulated the study concept; ZQZ acquired funding for the study. CZ, SWL, STN, WL, XGW, and ZQZ collected the data. CZ, ZQZ, SWL, HHZ, and XL analyzed the data. CZ wrote the manuscript. ZQZ revised the manuscript.

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Conflict of Interest

The authors have no potential conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Primers for site-directed mutagenesis of *HTRA1*.

Table S2. *NOTCH3* variants from 34 patients with cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

