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REVIEW

Heterozygous Pathogenic and Likely Pathogenic Symptomatic *HTRA1* Variant Carriers in Cerebral Small Vessel Disease

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Abstract: High temperature requirement serine peptidase A1 (HTRA1) related cerebral small vessel disease (CSVD) includes both symptomatic heterozygous *HTRA1* variant carrier and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) patients. Presently, most reported symptomatic heterozygous *HTRA1* variant carrier cases are sporadic family reports with a lack of specific characteristics. Additionally, the molecular mechanism of heterozygous *HTRA1* gene variants is unclear. We conducted this review to collect symptomatic carriers of heterozygous *HTRA1* gene variants reported as of 2022, analyzed all pathogenicity according to American College of Medical Genetics and Genomics (ACMG) variant classification, and summarized the cases with pathogenic and likely pathogenic *HTRA1* variants gender characteristics, age of onset, geographical distribution, initial symptoms, clinical manifestations, imaging signs, *HTRA1* gene variant information and to speculate its underlying pathogenic mechanisms. In this review, we summarized the following characteristics of pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers: to date, the majority of reported symptomatic HTRA1 carriers are in European and Asian countries, particularly in China which was found to have the highest number of reported cases. The age of first onset is mostly concentrated in the fourth and fifth decades. The heterozygous *HTRA1* gene variants were mostly missense variants. The two variant sites, 166–182 aa and 274–302 aa, were the most concentrated. Clinicians need to pay attention to *de novo* data and functional data, which may affect the pathogenicity analysis. The decrease in HtrA1 protease activity is currently the most important explanation for the genetic pathogenesis.

Keywords: stroke, *HTRA1* gene, ACMG criteria, heterozygous variant, symptomatic carrier

Introduction

Cerebral small vessel disease (CSVD) is an age-related cerebrovascular disease. A variety of factors may affect the small arteries, arterioles, capillaries, and venules in the brain, leading to clinical, imaging, and pathological syndromes of CSVD.[1] The clinical symptoms associated with CSVD are complex and include cognitive impairment, stroke, spine disorders, gait disorders, alopecia, psychiatric disorders, migraine, epilepsy, and more.[2,3] The consequences of CSVD on the brain lesions were easily captured in magnetic resonance imaging (MRI). White matter hyperintensities (WMHs), lacunar infarctions (LIs), and cerebral microbleeds (CMBs) were the most common imaging manifestations.[2] With improvements in the understanding of genetic precision medicine, a number of disease-causing genes, including *NOTCH3* (OMIM 600276),[4] *HTRA1* (OMIM 602194),[5,6] *TREX1* (OMIM 606609),[7] *COL4A1* (OMIM 120130),[8] *COL4A2* (OMIM 120090),[9] *CTSA* (OMIM 613111),[10] and *GLA* (OMIM 300644),[11] have been discovered; among these, heterozygous *HTRA1* gene variant is related to autosomal dominant cerebral arteriopathy with subcortical infarcts and leukoencephalopathy type 2 (CADASIL2).[6] CADASIL caused by *NOTCH3* gene mutation, the most common

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dominantly inherited monogenic cerebrovascular disease, has typical abnormal temporal pole signals.[12] Additionally, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), also known as the Maeda syndrome, which pathogenic gene is the same as CADASIL2, and has more specific manifestations, such as alopecia and spine disorders.[13]

Presently, most reported symptomatic carriers of heterozygous *HTRA1* gene variants are sporadic family reports with a lack of specific characteristics. Moreover, the molecular mechanism of heterozygous *HTRA1* gene variants is unclear. Thus, in this review, we used PubMed, Scopus, Web of Science, and Google Scholar to collect symptomatic carriers of heterozygous *HTRA1* gene variants with complete index cases information reported as of 2022, analyzed all pathogenicity according to American College of Medical Genetics and Genomics (ACMG) variant classification,[14] excluded all variants of uncertain significance, and summarized the cases with pathogenic and likely pathogenic *HTRA1* variants gender characteristics, age of onset, geographical distribution, initial symptoms, clinical manifestations, imaging signs, *HTRA1* gene variant information and to speculate its underlying pathogenic mechanisms.

HTRA1 Gene

The *HTRA1* gene (OMIM 602194) is located on the autosome 10q26.13, and its most commonly used transcript code is NM_002775.5. The *HTRA1* gene includes nine exons and a 1443 bp coding domain sequence, and it encodes the HtrA1 protease comprising 480 amino acids (aa).[15] HtrA1 protease can be widely expressed in normal human tissues, including four domains such as the insulin-like growth factor binding protein (33–98 aa), Kazal-type serine protease inhibitor (99–157 aa), trypsin-like serine protease (204–364 aa), and PDZ domains (365–467 aa).[16] Its linker region (158–203 aa), Loop D (283–291 aa), and Loop 3 (301–314 aa) are the key structures for activation of enzyme activity.[17]

In CSVDs, homozygous and heterozygous variants in the *HTRA1* gene cause different disease phenotypes.[17,18] Homozygous or compound heterozygous *HTRA1* gene mutations are related to CARASIL[19] while heterozygous *HTRA1* gene variants are related to CADASIL2;[6] however, the pathogenic mechanisms of both diseases are unclear till date.[20]

Clinical Manifestations of Symptomatic *HTRA1* Variant Carriers

In 2015, Verdura[6] used next generation sequencing to screen all candidate pathogenic genes carried by probands with familial cerebrovascular disease of unknown etiology. After verification by Standard PCR amplification and Sanger sequencing, it was first proposed that heterozygous *HTRA1* gene variants were associated with autosomal dominant cerebrovascular disease. In 2016, the Online Mendelian Inheritance in Man (OMIM) database officially named the CSVD caused due to heterozygous *HTRA1* gene variants as CADASIL2 (https://www.omim.org/). However, the correlation between the genotype of the heterozygous HTRA1 variant and the clinical phenotype has not been confirmed,[21] and not all carriers of the heterozygous *HTRA1* gene variant will have the corresponding clinical manifestations. The penetrance appears to be low.[17] Therefore, the naming of CADASIL2 is still controversial. Some scholars[17] believe that it is more suitable to use "symptomatic HTRA1 variant carriers" to describe this type of disease.

This study reviews a total of 76 symptomatic *HTRA1* variant carriers' family probands with relatively complete data from PubMed, Scopus, Web of Science, and Google Scholar databases as of 2022. We analyzed all pathogenicity according to ACMG variant classification,[14] and included 55 pathogenic and likely pathogenic variants (Table 1). The gender, age of first onset, nationality, initial symptoms, clinical manifestations, imaging signs, accompanying diseases, *HTRA1* gene variant information were analyzed by 55 pathogenic and likely pathogenic symptomatic carriers to summarize the characteristics of this type of disease.

Among 55 probands, no consanguinity of the proband's parents were found, and males accounted for about 56.36%. These indicate that a male sex advantage may exist, and similar to CARASIL.[22] Most probands are from Asian and European countries, especially from China (50.91%), followed by Japan, France, and Italy. In recent years, related cases have also been reported in African and American countries (Figure 1).

The age of onset of pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers was later than that of CARASIL. The former onsets mostly during the fourth to fifth decade while the latter onsets mostly during the second to third decade.[13] The initial symptoms of stroke (45%) the most common, followed by the cognitive impairment (Figure 2). The clinical manifestations of symptomatic HTRA1 variant carriers were mainly progressive cognitive impairment.

Table 1 Worldwide Distribution of P and LP Symptomatic *HTRA1* Variant Carriers

Cases	Gender/ Onset Age	Nationality	Clinical Manifestations	Imaging	Accompanying Diseases	Variants (NM_002775.5)	Exon	HtrA1 Protease Activity	ACMG Criteria
Muthusamy[27]	F/54	America	Stroke, cognitive impairment, migraine, urinary incontinence	WMHs	Hypertension	c.184_185delTG p.C62Arffs*106	Exon 1	NA	LP (PVS1 +PM2_Supporting)
Lee[30]	F/52	Taiwan, China	Stroke, cognitive impairment	WMHs, LIs, ICH	Hypertension	c.359G>A, p. G120D	Exon 1	↓ [30]	LP (PS3 +PM2_Supporting +PP3)
Thaler[31]	F/25	Germany	Stroke, gait disorders, migraine, spine disorders	WMHs, CMBs	Hypertension, ex-nicotine abuse	c.451C>T, p. Q151X	Exon 1	NA	LP (PVS1 +PM2_Supporting)
Chen[36]	F/31	China	Alopecia, spine disorders	WMHs, LIs	Hypertension	c.472+1G>A	Intron1	NA	LP (PVS1 +PM2_Supporting)
Bougea[53]	M/29	Hellenic Republic	Cognitive impairment, alopecia, migraine	WMHs	No	c.496C>T, p. R166C	Exon 2	↓ [41]	LP (PS3 +PM2_Supporting +PM5)
Liu[18]	F/35	China	Stroke, cognitive impairment, gait disorders, psychiatric disorders, alopecia, spine disorders, urinary incontinence, diplopia	WMHs, LIs, CMBs	No	c.496C>T, P. R166C	Exon 2	↓ [41]	LP (PS3 +PM2_Supporting +PM5)
Favaretto[24]	M/41	Italy	Stroke, cognitive impairment, psychiatric disorders	WMHs, LIs, CMBs	Vitiligo, autoimmune hyperthyroidism	c.496C>T, p. R166C	Exon 2	↓ [41]	LP (PS3 +PM2_Supporting +PM5)
Verdura[6]	M/66	France	Stroke, cognitive impairment, spine disorders	WMHs, LIs	Dyslipidemia	c.497G>T, p. R166L	Exon 2	↓ # [6,41]	LP (PS3 +PM2_Supporting +PM5)
Cao[54]	M/57	China	Stroke, cognitive impairment	WMHs, LIs, CMBs	No	c.497G>T, p. R166L	Exon 2	↓ # [6,41]	LP (PS3 +PM2_Supporting +PM5)
Verdura[6]	F/65	France	Cognitive impairment, gait disorders	WMHs, LIs	Hypertension	c.517G>C, p. A173P	Exon 2	↓ # [6,41]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Donato[55]	M/59	Italy	Stroke, cognitive impairment, gait disorders	WMHs, LIs	Hypertension, dyslipidemia	c.523G>A, p. V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Liu[18]	M/49	China	Stroke, cognitive impairment, psychiatric disorders, alopecia, spine disorders	WMHs, LIs, CMBs, dilated perivascular spaces	Hypertension, diabetes	c.523G>A, p. V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)

(*Continued*)

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Table 1 (Continued).

Cases	Gender/ Onset Age	Nationality	Clinical Manifestations	Imaging	Accompanying Diseases	Variants (NM_002775.5)	Exon	HtrA1 Protease Activity	ACMG Criteria
Muthusamy[27]	F/46	America	Stroke, cognitive impairment, gait disorders	WMHs, LIs, CMBs	Hypertension	c.523G>A, p. V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Shang[56]	F/40	Eritrea	Stroke, cognitive impairment	WMHs, CMBs	Hypertension	p.V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Zhang[26]	F/39	China	Stroke	WMHs, LIs, CMBs	Hypertension	c.523G>A, p. V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Zhang[37]	M/44	China	Stroke, cognitive impairment, gait disorders, spine disorders	WMHs, LIs, CMBs	Hypertension	c.523G>A, p. V175M	Exon 2	↓ # [37]	LP (PS3 +PM2_Supporting +PM5_Supporting)
Lee[30]	M/48	Taiwan, China	Stroke, spine disorders	WMHs, LIs	Hypertension, dyslipidemia, coronary artery disease	c.536T>A, p. I179N	Exon 2	↓ [30]	LP (PS3 +PM2_Supporting +PP3)
Lee[30]	M/62	Taiwan, China	Stroke, cognitive impairment, spine disorders, psychiatric disorders	WMHs, LIs, CMBs, ICH	Hypertension, smoking	c.543delT, p. A182Pfs*33	Exon 2	↓ * [30]	P (PVS1+PS3 +PM2_Supporting)
Chen[36]	M/40	China	Stroke, cognitive impairment, spine disorders, psychiatric disorders, alopecia	WMHs, LIs, CMBs	No	c.543delT, p. A182Pfs*33	Exon 2	↓ * [30]	P (PVS1+PS3 +PM2_Supporting)
Zhang[32]	M/77	China	Stroke, cognitive impairment	WMHs, LIs, CMBs	Hypertension	c.589C>T, p. R197X	Exon 3	NA	P (PVS1+PM1 +PM2_Supporting)
Zhou[33]	F/49	China	Cognitive impairment	WMHs	No	c.589C>T, p. R197X	Exon 3	NA	P (PVS1+PM1 +PM2_Supporting)
Zhuo[42]	F/46	China	Stroke, cognitive impairment	WMHs, LIs,	No	c.614C>G, p. S205C	Exon 3	↓ [42]	LP (PS3 +PM2_Supporting +PP3)
Lee[30]	M/54	Taiwan, China	Cognitive impairment, psychiatric disorders, spine disorders	WMHs, LIs	Hypertension	c.767T>C, p. I256T	Exon 3	↓ # [30]	LP (PS3 +PM2_Supporting +PP3)
Lee[30]	F/49	Taiwan, China	Stroke, cognitive impairment, spine disorders, psychiatric disorders	WMHs, LIs, CMBs, ICH	Dyslipidemia	c.827G>C, p. G276A	Exon 4	↓ [30]	LP (PS3 +PM2_Supporting +PP3)

Zhang[37]	M/42	China	Stroke, cognitive impairment, spine disorders	WMHs, LIs	Hypertension	c.832T>C, p. F278L	Exon 4	↓ # 37	LP (PS3 +PM2_Supporting +PP3)
Zhang[37]	F/54	China	Stroke, cognitive impairment, gait disorders, spine disorders	WMHs, LIs, CMBs	No	c.834C>G, p. F278L	Exon 4	↓ # 37	LP (PS3 +PM2_Supporting +PP3)
Nozaki[20]	M/49	Japan	Cognitive impairment, gait disorders, spine disorders	WMHs	No	c.848G>A, p. G283E	Exon 4	↓ # 20,41	LP (PS3 +PM2_Supporting +PP3)
Verdura[6]	F/49	France	Migraine	WMHs, LIs,	Hypertension	c.852C>A, p. S284R	Exon 4	↓ # 6,41	LP (PS3 +PM2_Supporting +PM5)
Verdura[6]	M/50	France	Stroke, gait disorders	WMHs, LIs	No	c.854C>A, p. P285Q	Exon 4	↓ 6,41	LP (PS3 +PM2_Supporting +PM5)
Nozaki[20]	M/20	Japan	Stroke, cognitive impairment, gait disorders, alopecia, spine disorders	WMHs, CMBs	Hypertension	c.854C>T, p. P285L	Exon 4	↓ # 20,37	LP (PS3 +PM2_Supporting +PM5)
Nozaki[20]	M/51	Japan	Stroke, cognitive impairment, gait disorders, spine disorders	NA	No	c.854C>T, p. P285L	Exon 4	↓ # 20,37	LP (PS3 +PM2_Supporting +PM5)
Chen[36]	F/32	China	Stroke, cognitive impairment, gait disorders, alopecia, psychiatric disorders	WMHs, LIs, CMBs	Hypertension	c.854C>T, p. P285L	Exon 4	↓ # 20,37	LP (PS3 +PM2_Supporting +PM5)
Zhang[37]	F/42	China	Stroke, cognitive impairment, gait disorders, spine disorders	WMHs, LIs	No	c.854C>T, p. P285L	Exon 4	↓ # 20,37	LP (PS3 +PM2_Supporting +PM5)
Verdura[6]	M/49	France	Stroke, cognitive impairment, gait disorders	WMHs, LIs	No	c.856T>G, p. F286V	Exon 4	↓ 6,41	LP (PS3 +PM2_Supporting +PP3)
Lee[30]	F/55	Taiwan, China	Stroke, cognitive impairment, spine disorders, alopecia, psychiatric disorders	WMHs, LIs, CMBs	No	c.865C>T, p. Q289X	Exon 4	↓ * 30	P (PVS1+PS3 +PM2_Supporting)
Donato[55]	M/65	Italy	Cognitive impairment, gait disorders	WMHs	No	c.883G>A, p. G295R	Exon 4	↓ # 41	LP (PS3 +PM2_Supporting +PP3)
Ragno[23]	M/44	Italy	Cognitive impairment, migraine	WMHs, cerebral atrophy, CMBs	Hypertension	c.889G>A, p. V297M	Exon 4	↓ 20	LP (PS3 +PM2_Supporting +PP3)

(*Continued*)

Table 1 (Continued).

Cases	Gender/ Onset Age	Nationality	Clinical Manifestations	Imaging	Accompanying Diseases	Variants (NM_002775.5)	Exon	HtrA1 Protease Activity	ACMG Criteria
Muthusamy[27]	M/41	America	Stroke, cognitive impairment, seizures, TGA	WMHs, LIs, CMBs	Hypertension, dyslipidemia	c.889G>A, p. V297M	Exon 4	↓ [20]	LP (PS3 +PM2_Supporting +PP3)
Chen[36]	M/51	China	Stroke, cognitive impairment, gait disorders, psychiatric disorders	WMHs, LIs, CMBs	Hypertension	c.889G>A, p. V297M	Exon 4	↓ [20]	LP (PS3 +PM2_Supporting +PP3)
Ohta[34]	M/49	Japan	Stroke, gait disorders	WMHs, LIs	No	c.904C>T, p. R302X	Exon 4	↓ * [41]	P (PVS1+PS3 +PM2_Supporting)
Tateoka[35]	M/59	Japan	Stroke, cognitive impairment	WMHs, LIs, CMBs	No	c.904C>T, p. R302X	Exon 4	↓ * [41]	P (PVS1+PS3 +PM2_Supporting)
Wu[57]	M/35	China	Stroke, cognitive impairment	WMHs, LIs	No	c.905G>A, p. R302Q	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Ito[51]	M/30	Japan	Stroke, cognitive impairment, spine disorders	WMHs, LIs, CMBs	No	p.R302Q	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Nozaki[20]	M/63	Japan	Stroke, cognitive impairment, gait disorders, spine disorders	NA	Hypertension	c.905G>A, p. R302Q	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Nozaki[20]	M/40	Japan	Cognitive impairment, gait disorders, spine disorders, alopecia	WMHs, CMBs	No	c.905G>A, p. R302Q	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Mahale[58]	F/37	India	Migraine, seizures, cognitive impairment	WMHs, CMBs	No	C.905G>A, p. R302Q	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Zhang[37]	F/66	China	Stroke, cognitive impairment, spine disorders	WMHs	No	c.954G>C p. Q318H	Exon 4	↓ # [37]	LP (PS3 +PM2_Supporting +PP3)
Nozaki[20]	M/53	Japan	Stroke, cognitive impairment, gait disorders, spine disorders	NA	Hypertension, dyslipidemia	c.956C>T, p. T319I	Exon 4	↓ # [20]	LP (PS3 +PM2_Supporting +PP3)
Muthusamy[27]	F/64	America	Stroke, cognitive impairment, migraine	WMHs, CMBs	Hypertension, diabetes	c.958G>A, p. D320N	Exon 4	NA	LP (PS3 +PM2_Supporting +PP3)

Lee[30]	M/60	Taiwan, China	Stroke, cognitive impairment, spine disorders	WMHs, LIs	No	c.971A>C, p. N324T	Exon 4	↓ 30	LP (PS3 +PM2_Supporting +PP3)
Liu[18]	F/37	China	Stroke, cognitive impairment, psychiatric disorders	WMHs, LIs, CMBs	No	c.971A>C, p. N324T	Exon 4	↓ 30	LP (PS3 +PM2_Supporting +PP3)
Verdura[6]	F/66	France	Stroke	WMHs, LIs, CMBs	Hypertension	c.973–1G>A	Intron4	NA	LP (PVS1 +PM2_Supporting)
Zhang[37]	F/55	China	Stroke, cognitive impairment, ICH, migraine, gait disorders	WMHs, LIs, CMBs	Hypertension	c.973–2A>G	Intron4	NA	LP (PVS1 +PM2_Supporting)
Zhang[37]	F/44	China	Stroke, cognitive impairment, ICH, migraine, gait disorders, spine disorders	WMHs, LIs, CMBs, ICH	Hypertension	c.1015G>A, p. V339M	Exon 6	↓ # 37	LP (PS3 +PM2_Supporting +PP3)
Zhang[37]	M/39	China	Stroke, cognitive impairment, alopecia, spine disorders	WMHs, LIs	Smoking	c.1049G>A, p. G350E	Exon 6	↓ # 37	LP (PS3 +PM2_Supporting +PP3)

Note: References cited in the column "HtrA1 protease activity" are those of the authors who have performed relevant validation.

Abbreviations: F, female; M, male; NA, not available; CMBs, cerebral microbleeds; ICH, intracranial hemorrhage; LIs, lacunar infarctions; WMHs, white matter hyperintensities; ↓The decrease in HtrA1 protease activity; #Dominant negative effect; *NMD, nonsense mediated mRNA decay; ACMG, American College of Medical Genetics and Genomics; P, pathogenic; LP, likely pathogenic; TGA, Transient global amnesia.

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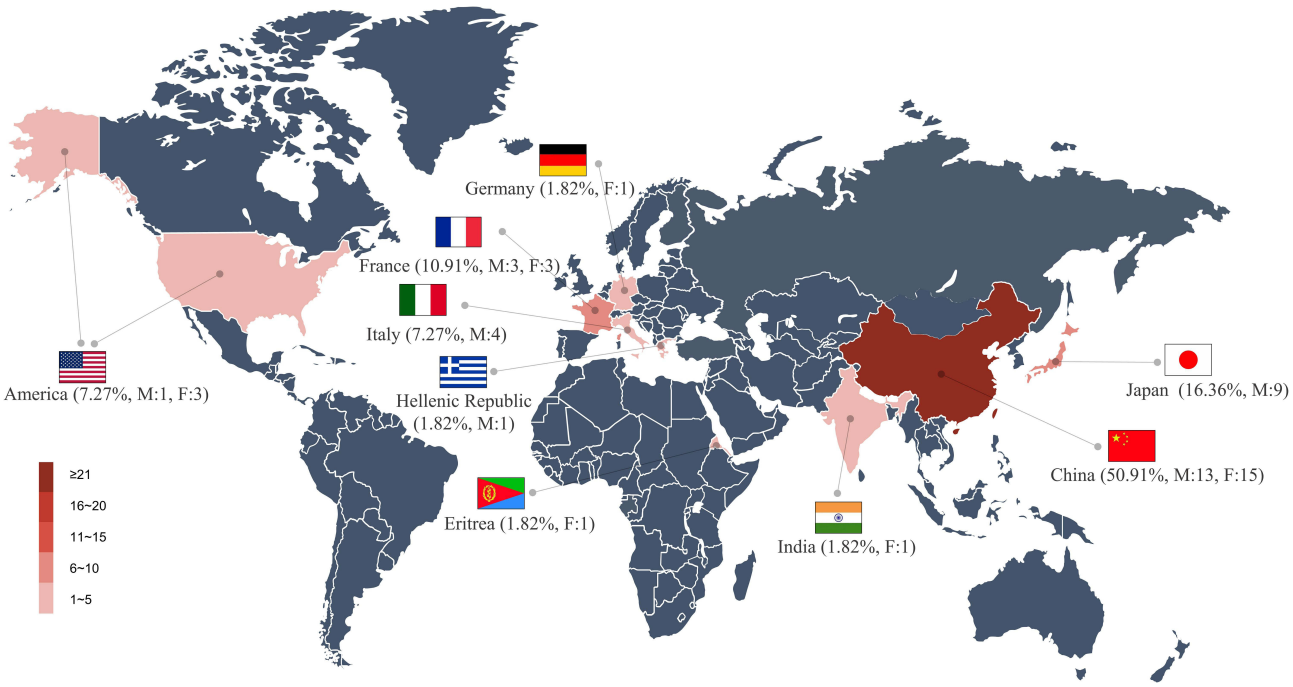

Figure 1 Geographical and gender distribution of pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers. Symptomatic carrier is prevalent in Asian and European countries, especially in China (50.91%). Symptomatic carrier has gender differences, and it mostly affects males. The color depth represents the number of cases. **Abbreviations**: F, female; M, male.

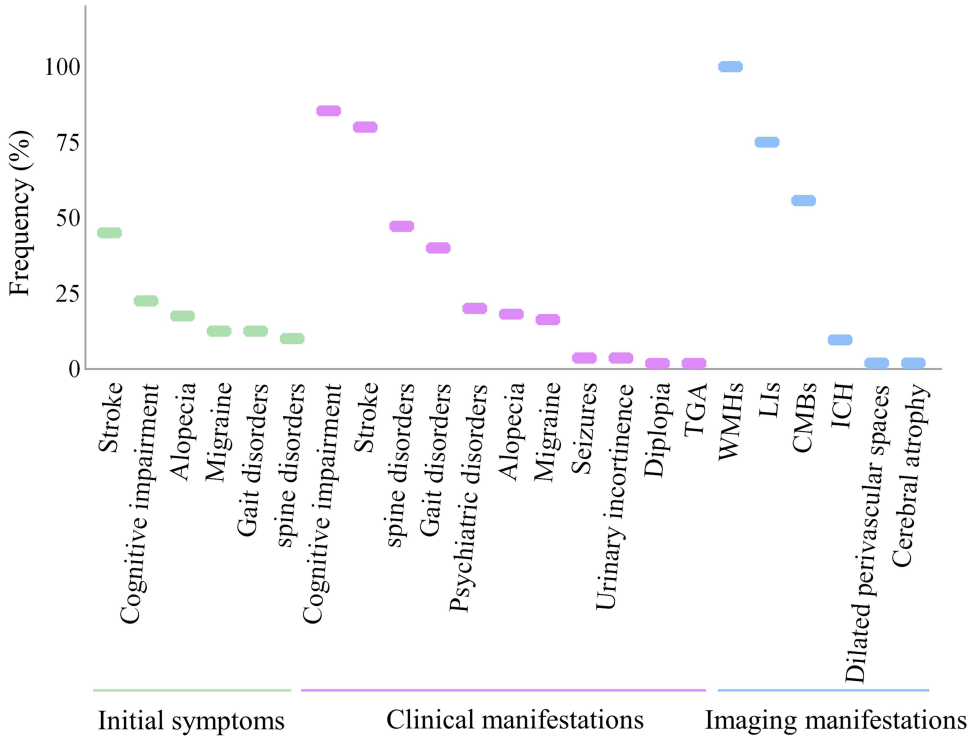

Figure 2 The frequency of initial symptoms, clinical manifestations, and imaging manifestations in pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers. **Abbreviations**: TGA, Transient global amnesia; WMHs, white matter hyperintensities; LIs, lacunar infarctions; CMBs, cerebral microbleeds; ICH, intracranial hemorrhage.

Some patients had gait and psychiatric disorders. The symptoms of symptomatic carriers outside the nervous system are less common than those of CARASIL. Patients with spine disorders and alopecia accounted for 47.27% and 20% of the total number of patients, respectively. Ragno[23] has reported that symptomatic carriers may have cutaneous sensory and autonomic small-fiber neuropathy.

Similar to common hereditary CSVDs such as CADASIL and CARASIL, some pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers do not have traditional vascular risk factors (Table 1). Some patients have hypertension (50.91%) and dyslipidemia (10.91%), and one patient have vitiligo, autoimmune hyperthyroidism.[24] Consensus recommendations of the European Academy of Neurology[25] suggest that even if patients are accompanied by traditional cerebrovascular disease risk factors, the diagnosis of monogenic CSVD should be considered. Ordinal logistic regression analysis was performed to reveal risk factors for the clinical phenotype.[26] Patients with vascular risk factors presented with more severe clinical symptoms.

Clinicoradiographic characteristics of pathogenic and likely pathogenic symptomatic HTRA1 variant carriers may overlap with sporadic CSVD.[27] Presently, there are no specific imaging signs for symptomatic carriers. White matter hyperintensities (WMHs, 100%), lacunar infarctions (LIs, 75%), and cerebral microbleeds (CMBs, 55.77%) were the most common imaging manifestations (Figure 2). Rare genetic variants of the *HTRA1* gene and HtrA1 protease play an important role in the burden of WMH in the general population.[28] Loss-of-function variants in *HTRA1* gene associated with an increased WMH volume. Domain-specific burden tests revealed that the association with WMH volume was restricted to variants in the protease domain. The WMH volume was brought forward by 11 years in carriers of a rare protease domain variant. With the development of brain imaging genomics,[29] quantitative analysis of the location and number of WMHs, LIs, and CMBs; brain volume; cerebral blood flow; and tissue metabolism capacity in symptomatic carriers has become feasible.

Most pathogenic and likely pathogenic heterozygous *HTRA1* gene variants of symptomatic carriers were missense variants, and some of them were nonsense variants,[30–35] frameshift variants,[27,30,36] and splice site variants.[6,36,37] The variant sites of the heterozygous *HTRA1* gene were mostly located in exon 4 (50.91%), and the trypsin-like serine protease domain was the most common domain in HtrA1 protease (61.82%). Moreover, we found two variant site aggregation regions (166–182 aa and 276–302 aa). The former was located in the linker region of the trypsin-like serine protease domain while the latter was located in Loop D and Loop 3, which activate the serine protease activity (Figure 3). The severity of the clinical manifestations of symptomatic carriers was related to the location of the *HTRA1* gene variant. When the variant was located in the loop 3/loop D domain or exon 4, the patient had a more severe clinical phenotype.[26]

According to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines,[14] we conducted pathogenicity analysis on the collected proband data (Table 1). The variants of uncertain significance (VUS) were presented in Supplement Table 1. Not all rare heterozygous *HTRA1* missense variants are pathogenic or likely pathogenic,[25] and heterozygous *HTRA1* nonsense or frameshift variants are pathogenic or likely pathogenic for the moment.[38] Among them, 72.37% of the variants were pathogenic or likely pathogenic, and 27.63% of the variants of uncertain significance. Among probands rated as variants of indeterminate significance, the judgment of pathogenicity was often affected by lack of evidence such as *de novo* data (with or without paternity and maternity confirmed) —PS2/PM6, and functional data —PS3/PM1/PP2. When considering a patient's diagnosis of heterozygous *HTRA1* gene variants of symptomatic carriers or other hereditary diseases, clinicians need to pay attention to *de novo* data and functional data, which may affect the pathogenicity analysis.

Possible Pathogenic Mechanism

The molecular mechanism of heterozygous *HTRA1* gene variant is not completely clear,[21] and the molecular pathogenesis caused by variants at different sites is different.[39] Presently, it is mainly focused on preliminary studies on the effect of HtrA1 protease activity and downstream TGF-β1 signaling pathway (Figure 4). The HtrA1 protease is composed of trimers, and each adjacent HtrA1 subunit activates each other through the linker region, the sensor domains of Loop 3 and Loop D.[40] Variant sites p.R166C,[41] p.R166L,[41] p.A173P,[41] p.G283E,[20] p.G295R,[41] and p.T319I[20] hinder the formation of stable trimers by HtrA1, thus, affecting the activation cascade. It has been suggested that if the variant is located in the key structures of HtrA1 protease, such as the linker region, Loop 3, or Loop D, the communication

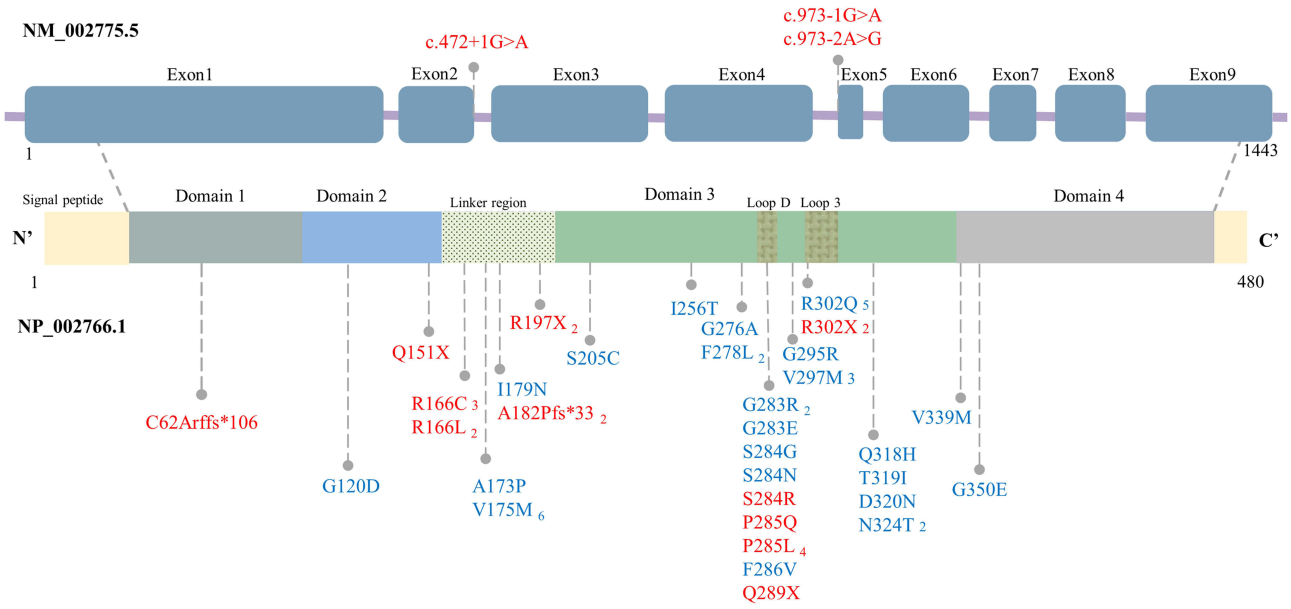

Figure 3 Variant sites of pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers. In NP_002766.1, Domains 1–4 are insulin-like growth factor binding protein, Kazal-type serine protease inhibitor, trypsin-like serine protease, and PDZ domains, respectively. The corner numbers indicate the number of probands at the particular site. According to ACMG guidelines, red represents a pathogenicity rating of pathogenic, and blue represents a pathogenicity rating of likely pathogenic.

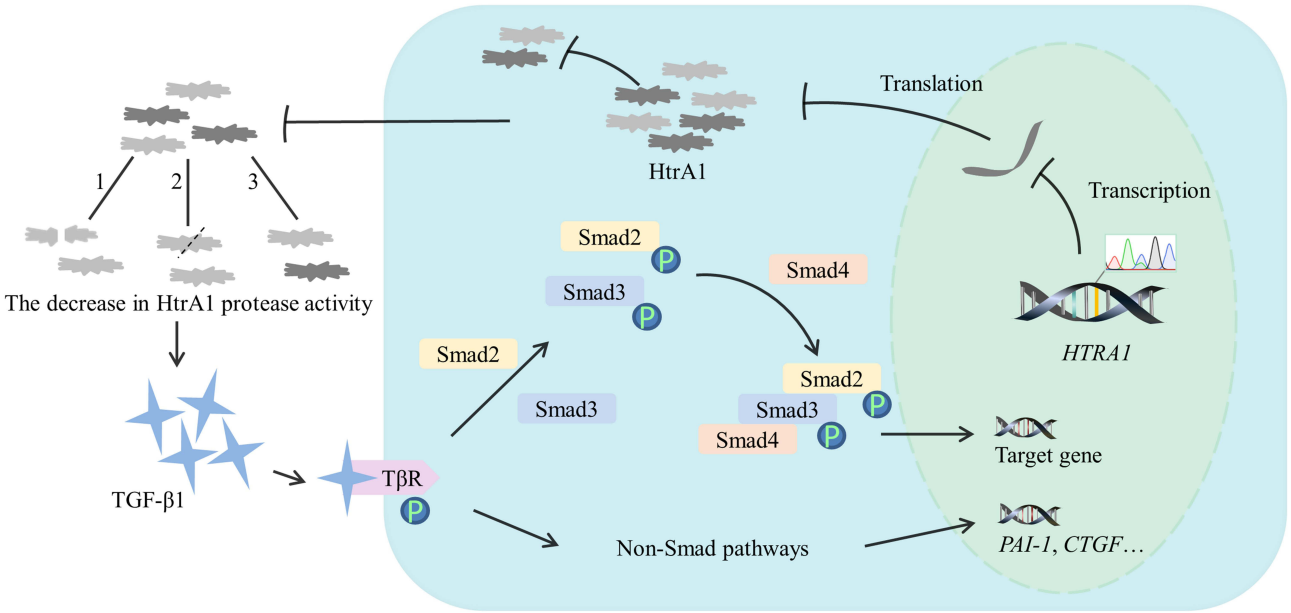

Figure 4 Possible pathogenic mechanism of heterozygous *HTRA1* gene variant. The decrease in HtrA1 protease activity has three pathogenic mechanisms (1–3). 1: variant HtrA1 enzyme is unable to form stable trimers, and it has a dominant negative effect to inhibit wild HtrA1 activity; 2: variant HtrA1 enzyme affects the communication between its subunits, and it has a dominant negative effect on inhibiting wild HtrA1 activity; 3: variant HtrA1 enzyme shows a decrease in its own activity, and it has no dominant negative effect on wild HtrA1.
Abbreviations: CTGF, connective tissue growth factor; PAI-1, plasminogen activator inhibitor-1.

between HtrA1 subunits can be affected.[17] It also has a dominant negative effect, which reduces the activity of HtrA1. For example, variants in p.P285L and p.R302Q cause damage to the HtrA1 enzyme activation cascade.[20] However, the heterozygous *HTRA1* gene variant site located outside the linker region and the Loop 3/Loop D domain require a careful assessment of pathogenicity.

Moreover, mutant HtrA1 inhibited the activity of wild HtrA1, and it had a dominant negative effect, which was defined as the mutated allele being a loss of function mutant that interfered with the normal function of the remaining wild-type allele, leading to a further decrease in enzyme activity. As for heterozygous *HTRA1* gene variants, missense variants in p.R166L,[6,41] p.A173P,[6,41] p.V175M,[37] p.I256T,[30] p.F278L,[37] p.G283E,[20,41] p.S284R,[6,41] p.P285L,[20,37] p.G295R,[41] p. R302Q,[20] p.Q318H,[37] p.T319I,[20] p.V339M[37] and p.G350E[37] have dominant negative effects. However, missense variants in p.G120D,[30] p.R166C,[41] p.I179N,[30] p.S205C,[42] p.G276A,[30] p.P285Q,[6,41] p.F286V,[6,41] p.V297M[20] and p.N324T[30] do not show a dominant negative effect. Nonsense variant site p.Q289X may be submitted to nonsense mediated mRNA decay (NMD).[30] Frame shift variant site p.A182Pfs*33 caused premature termination codons (PTC), and the pathogenesis may be submitted to NMD.[30] Nonsense variant site p.R302X was also related to NMD.[41] In summary, the decrease in HtrA1 protease activity is currently the most important explanation for the genetic pathogenic mechanism of symptomatic heterozygous *HTRA1* variant carriers.

HtrA1 protease is involved in the transduction of TGF-β1 signaling pathway, which can inhibit the expression of TGF-β1.[43] A recent study demonstrated that in HTRA1-/- mouse model, the decreased HtrA1 enzymatic activity can lead to the accumulation of matrisome proteins such as latent TGF-β binding protein 4 (LTBP-4) and hub protein fibronectin (FN), which are substrates of HtrA1. Candesartan treatment alleviated matrisome protein accumulation, and may be a potential treatment for CARASIL.[44] The p.S205C variant site can upregulate the expression of TGF-β1, Smad2/3, Smad4, phosphorylated Smad2/3, and Smad4.[42] The abnormal signal transduction of the TGF-β1/Smads pathway may be a potential molecular pathogenic mechanism, suggesting that TGF-β1 antagonists can be used for the treatment of symptomatic heterozygous *HTRA1* variant carriers.[42] In addition to the TGF-β1/Smad signaling pathway, a high expression of *CTGF* and *PAI-1* genes was observed in patients with p.E42fs and p.A321T variants.[39] It is suggested that the TGF-β1/non-Smad pathway may also be involved in the molecular pathogenesis of symptomatic heterozygous *HTRA1* variant carriers. Increased expression of TGF-β signaling pathway is a common cause of increased *Fn1* mRNA expression and vascular intimal thickening.[45] However, a recent study analyzed various signaling pathways regulated by TGF-β and found that TGF-β is deposited in HTRA1-/- mice, but *Fn1* mRNA expression has not been significantly increased or decreased, suggesting that TGF-β signaling pathway may be less effective in CARASIL.[44]

Interestingly, TGF-β signaling was found to be associated with some clinical manifestations of *HTRA1*-related CSVD. Bone morphogenetic protein (BMP), a member of the TGF-β family, is involved in the maintenance of hair follicle stem cells.[46,47] When the secretion of BMP is increased or its function is hyperactive, it will inhibit the activity of hair follicle stem cells, thereby promoting alopecia. TGF-β may also promote the production of IL-17 by cooperating with IL-6, aggravate the inflammatory response, stimulate the increase of NO secretion, and eventually lead to migraine.[48,49] TGF-β receptors in astrocytes can be activated by binding to serum albumin, increasing the level of downstream p-Smad1/5/8, and participating in the occurrence of epilepsy.[50]

Pathology and Pathophysiology

The pathology and pathophysiology of CSVD related to heterozygote *HTRA1* variant (symptomatic carrier) is overlapped by that to homozygote *HTRA1* variant (CARASIL). Autopsy study of a heterozygous *HTRA1* variant case (p.G283E) revealed cerebral small arteries showed intimal proliferation, splitting of the internal elastic lamina, hyaline degeneration of media, and diffuse and focally intensive myelin pallor in the white matter.[20] Another autopsy study of a patient with heterozygous *HTRA1* variant (p.R302Q) found that internal elastic lamina splitting, myointimal cell proliferation, smooth muscle cell (SMC) loss, intima and adventitia fibrosis, lipohyalinosis, lumen narrow or occlusive in arterioles smaller than 100 μm.[51] 100–500 μm arteries revealed lumen distorted dilation, aneurysm-like structures, and tunica media TGF-β1 high expression.[51] Pathological observation of frontal white matter suggested focal myelin pallor with preserved U-fibers.[51] Cerebrovascular pathophysiological regulations were secondary to the vascular structure pathological changes. Disruption of the autoregulatory mechanism of cerebral blood flow may ultimately lead to white matter ischemia.[13,52] Animal study also confirmed that cerebral blood flow decreased in HTRA1 -/- mice.[44]

Conclusion and Perspectives

Symptomatic *HTRA1* variant carriers is a monogenic CSVD, and the penetrance appears to be low. According to ACMG standards and guidelines, There are mostly pathogenic or likely pathogenic variants, and a little variants of uncertain significance. Through a literature review, we summarized the following characteristics of pathogenic and likely pathogenic symptomatic *HTRA1* variant carriers: (1) To date, the majority of reported symptomatic *HTRA1* carriers are in European and Asian countries, particularly in China which was found to have the highest number of reported cases. (2) The age of first onset was mostly concentrated in the fourth and fifth decades. The clinical manifestations and imaging signs were similar to those of sporadic CSVDs, and there was no specificity. Diagnosis mainly depends on genetic testing. (3) The heterozygous *HTRA1* variants were mostly missense variants. The variant sites were mostly concentrated in Exon 4, which is located in the trypsin-like serine protease domain of HtrA1 protease. The two variant sites, 166–182 aa and 274–302 aa, were the most concentrated. (4) The molecular pathogenesis caused by variants at different sites is different, and the decrease in *HtrA1* protease activity is currently the most important explanation for the genetic pathogenesis.

However, the correlation between the genotype and clinical phenotype still needs to be further confirmed. In the future, imaging genomic methods can be used to find characteristic images of the disease and to differentiate it from other common monogenic CSVDs, such as CADASIL and CARASIL. Additionally, studies should focus on the genetic testing of patients with sporadic CSVDs to further enrich the spectrum of heterozygous variants in the *HTRA1* gene. The pathogenic mechanism of symptomatic carrier has not been fully clarified yet, and the study of HtrA1 protease activity is currently a hot topic. The downstream TGF-β1 signaling pathway transduction may be taken into consideration for future study.

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Disclosure

The authors report no conflicts of interest in this work.

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