HTRA1-related autosomal dominant cerebral small vessel disease

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Abstract

Background: Homozygous or compound heterozygous mutations in high temperature requirement serine peptidase A1 (*HTRA1*) gene are responsible for cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). Recently, increasing evidence has shown that heterozygous *HTRA1* mutations are also associated with cerebral small vessel disease (CSVD) with an autosomal dominant pattern of inheritance. This study was aimed to analyze the genetic and clinical characteristics of *HTRA1*-related autosomal dominant CSVD.

Methods: We presented three new Chinese cases of familial CSVD with heterozygous *HTRA1* mutations and reviewed all clinical case reports and articles on *HTRA1*-related autosomal dominant CSVD included in PUBMED by the end of March 1, 2020. CARASIL probands with genetic diagnosis reported to date were also reviewed. The genetic and clinical characteristics of *HTRA1*-related autosomal dominant CSVD were summarized and analyzed by comparing with CARASIL.

Results: Forty-four *HTRA1*-related autosomal dominant CSVD probands and 22 CARASIL probands were included. Compared with typical CARASIL, *HTRA1*-related autosomal dominant probands has a higher proportion of vascular risk factors ($P < 0.001$), a later onset age ($P < 0.001$), and a relatively slower clinical progression. Alopecia and spondylosis can be observed, but less than those in the typical CARASIL. Thirty-five heterozygous mutations in *HTRA1* were reported, most of which were missense mutations. Amino acids located close to amino acids 250–300 were most frequently affected, followed by these located near 150~200. While amino acids 250~300 were also the most frequently affected region in CARASIL patients, fewer mutations precede the 200th amino acids were detected, especially in the Kazal-type serine protease domain.

Conclusions: *HTRA1*-related autosomal dominant CSVD is present as a mild phenotype of CARASIL. The trend of regional concentration of mutation sites may be related to the concentration of key sites in these regions which are responsible for pathogenesis of *HTRA1*-related autosomal dominant CSVD.

Keywords: CARASIL; Cerebral small vessel disease; Heterozygous mutation; *HTRA1*

Introduction

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a monogenic cerebral small vessel disease (CSVD) clinically characterized by young-adult-onset non-hypertensive cerebral small vessel arteriopathy with extraneurologic signs including alopecia and spondylosis.[1] In 2009, high temperature requirement serine peptidase A1 gene (*HTRA1)* was first reported as the causative gene of CARASIL.[2] *HTRA1* gene encodes high-temperature requirement protease A1, a serine enzyme that mediates cell signaling and protein degradation, and plays an important role in vascular integrity, skeletal development, and osteogenesis.[3-5] CARASIL is considered to be caused by biallelic mutations of *HTRA1*. Only 19 recessive mutations (17 homozygous, 2 compounds heterozygous)

in the *HTRA1* gene related to CARASIL have been reported worldwide to date.[2,6-19]

Of note, in some heterozygous relatives of CARASIL patients (including p.P285L, p.G295R, p.E277Vfs, p.A321T and p.Q42fs heterozygous mutation carriers), mild to moderate non-hypertensive leukoencephalopathy and spondylosis can be observed.[6,9,10,15] This led to the pathogenic hypothesis of heterozygous *HTRA1* mutations. Verdura *et al*[20] found that 4.97% patients of familial CSVD without *NOTCH3* mutations in France carried *HTRA1* heterozygous mutations, which was much higher than that detected in the healthy controls (0.23%, $P = 4.2 \times 10^{-6}$). Further, *in vitro* activity analysis revealed that these mutations resulted in a significant reduction of HTRA1 protease activity, strongly suggesting causality and pathogenicity.[20] The similarity of histopathological features between the patients with heterozygous *HTRA1*

mutations and CARASIL patients indicated their common pathological mechanisms underlying the development of CSVD.[3]

So far, a growing number of heterozygous *HTRA1* mutations have been detected in CSVD patients with dominant inheritance background in clinical practice, but their clinical and genetic characteristics are still under debate. Here we present three new Chinese CSVD patients with heterozygous *HTRA1* mutations and review 41 cases of *HTRA1*-related autosomal dominant CSVD that previously reported worldwide. By comparing them with typical CARASIL, we aimed to summarize the clinical and genetic characteristics of *HTRA1*-related autosomal dominant CSVD; and clarify the pathogenesis of *HTRA1*-related autosomal dominant CSVD.

Methods

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking Union Medical College Hospital, and written informed consent was received from all the patients or legal guardians.

Cases report

Three cases of *HTRA1*-related autosomal dominant CSVD confirmed at Peking Union Medical College Hospital, were presented. Clinic exome sequencing was performed for the patients through the commercial service provided by Running Gene (Beijing, China). Exonic DNA was captured with the DNA Isolation Kit (Blood DNA Kit V2, CW2553) and sequenced on the Illumina NovaSeq platform (San Diego, CA, USA).

Literature search

We searched with the keywords of high temperature requirement serine peptidase A1, *HTRA1*, heterozygous *HTRA1* mutation, or CARASIL in PubMed (Medline) for articles published in English up to March 1, 2020. The references of included studies were also checked to identify additional researches. All obtained articles were reviewed to identify the cases of *HTRA1*-related autosomal dominant CSVD and gene diagnosis confirmed CARASIL.

Inclusion criteria and data extraction

All case reports and original articles of CSVD related to heterozygous *HTRA1* mutations were included. The full text of these articles was screened and qualified. We recorded the clinical characteristics and genetic test results of each proband from the included articles. For comparison, the genetic and clinical characteristics of all typical CARASIL probands confirmed by genetic examination were also recorded. The age of onset was defined as the age at which clinical symptoms related to CSVD appeared, including stroke or transient ischemic attack (TIA), cognitive decline, gait disorders, psychiatric disorders, *etc*. Cognitive decline at the time of examination was rated as follows: 0 = normal (Mini-mental State Examination

[MMSE] $\geq$ 27, or Montreal Cognitive Assessment [MoCA] $\geq$ 27, or described as "normal" in the article); 1=mild (MMSE 21–26, or MoCA 18–26); 2 = moderate (MMSE 10–20, or MoCA 10–17); 3 = severe (MMSE $\leq$ 9, or MoCA < 10, or described as "severe" or "dementia" in article); 9 = have cognitive impairment but without detailed information. Undescribed information was recorded as not known. We checked the accuracy of data extraction and discussed and resolved any disputes.

Statistical analyses

The clinical characteristics were presented as median (interquartile range), or frequency (percentage). Pearson Chi-square test, Fisher exact test, Mann-Whitney *U* test, and Kruskal-Wallis test were used for categorical variables. All analyses were performed using the statistical software package SPSS version 25.0 (IBM Corp., Armonk, NY, USA). All *P* values were two-tailed and criteria for significance were $P < 0.05$.

Results

Case report

Patient 1

A 40-year-old female presented to our department because of a history of recurrent ischemic stroke attacks since the age of 37. She also complained of progressive memory deterioration and mood disorder. She denied history of lumbar pain, alopecia, and migraine. None of the history of hypertension, diabetes mellitus, and other cardiovascular risk factors was reported. Both her mother and her mother's two siblings suffered a ischemic stroke in their 40s and died in their 50s. On examination, left-side dominant hypermyotonia, brisk tendon reflexes of limbs, and mild spastic gait were observed. Babinski sign was bilaterally positive. The neuropsychological assessment showed impairment in visuospatial/executive functions, attention, and delayed recall ability according to the score (24/30) of MoCA. Blood count and blood chemistry were normal. Both immune and metabolic screening tests were negative. Cerebrospinal fluid analysis were unremarkable.

The MRI scan showed bilateral diffuse white matter abnormalities involving anteroinferior temporal lobes, periventricular areas, basal ganglia, external capsules, and semi-oval center, with multiple lacunar infarctions in the brainstem, bilateral paraventricular, and semi-oval center [Figure 1A]. Only one microbleed in the right basal ganglia was observed on susceptibility weighted images (SWI). Spine MRI showed multilevel degenerative disc disease (C5–6, C6–7, T11–12, L4–5, and L5–S1).

Genetic panel diagnostic, including *NOTCH3*, *HTRA1*, *COL4A1*, *COL4A2*, and *TREX1*, were performed. A heterozygous nucleotidic substitution (c.971A>C) in exon 4 of the *HTRA1* gene resulting in a missense mutation (p.N324T), was detected. According to the standards and guidelines for the interpretation of sequence variants released by American College of Medical Genetics and Genomics (ACMG) in 2015, the rate of this mutation
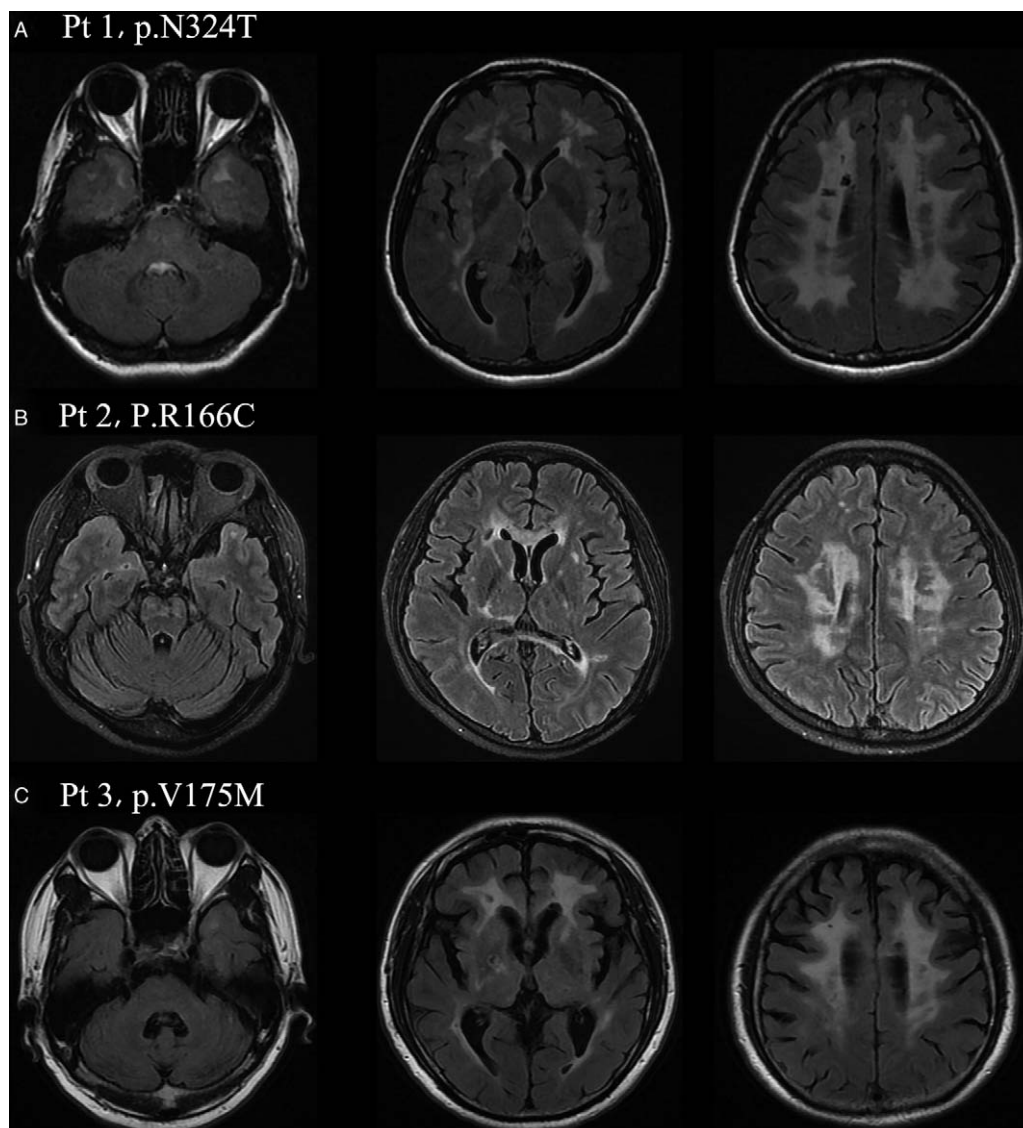
Figure 1: Fluid attenuation inversion recovery images of brain MRI of Patients 1, 2 and 3, to illustrate white matter hyperintensity.

meets two evidences, PM2, and PS3, and finally classified as "likely pathogenic." Since her relatives with a history of early stroke have died, genetic diagnosis cannot be performed. Her father and sister were healthy and did not have the mutation.

Patient 2

This female patient has experienced several ischemic stroke events (manifested as hemiplegia, dysarthria and diplopia) since the age of 35, and gradually developed progressive motor and mental decline within three years, leading to progressive memory deficit, emotional instability, gait disorders, and urinary incontinence.

Cognitive impairment (MMSE 24/30), forced laughter, pseudo-bulbar palsy, and spastic paralysis of both lower limbs were observed during her medical examinantion at the age of 38. Her hair was sparse, and she suffered from chronic lumbar pain for many years. Her father, who had a history of smoking and drinking, suffered a stroke at the

age of 60 and died of stroke complications in his 70s. Her elder brother suffered from cervical disc herniation in his 30s and had a cranial MRI during a physical examination that revealed white matter lesions (image not available).

The brain MRI showed a diffuse leukoencephalopathy, involving the periventricular and deep white matter and corpus callosum, with multiple lacunaes in the deep white and gray matter of both brain hemispheres and brainstem [Figure 1B]. SWI revealed multiple microbleeds in pons, left thalamus, and bilateral parietal lobe. Granular osmiophilic materials (GOMs), the pathologic hallmark of CADASIL, were not found by electron microscopy examination of skin biopsy. Mild stenosis of the cervical canal due to multilevel degenerative changes was observed on cervical spine MRI.

A heterozygous mutation, c.496C>T (P.R166C), was identified in the exon 2 of the *HTRA1* gene. The grade of this mutation, according to ACMG, is "likely pathogenic."

Her mother did not have the mutation, and we could not obtain additional DNA from her brother due to his refusal.

Patient 3

This patient was a 54-year-old man with 4-year history of hypertension and 1-year of diabetes mellitus. At the age of 42, asymptomatic white matter lesions were detected on his cranial MRI. At the age of 49, he began to have repeated ischemic stroke attacks, which manifested as hemiplegia, dysarthria, and visual field defects lasting for several days. Memory loss, emotional disturbance, and urinary incontinence gradually developed at the age of 53. His neurological examination indicated cognitive impairment (MMSE 26/30, MoCA 20/30), involuntary crying, bilateral pyramidal signs, mild hypermyotonia of both lower limbs, and ataxia of left upper extremity. His mother suffered from a stroke at the age of 40, and her nine siblings all died of stroke, but their specific age of onset were unknown. He complained of chronic lumbar pain and progressive alopecia for five years. Spinal MRI was not available. No GOMs was found in skin biopsy. Brain MRI showed progressing diffuse white matter hyperintensity, multiple lacuna infarcts, dilated perivascular spaces, and microbleeds [Figure 1C]. Genetic testing revealed a heterozygous G>A change at nucleotide 523 (p.V175M) in the exon 2 of the *HTRA1* gene. Although the ACMG classification of the variant was "uncertain significance" because co-segregation analysis could not be performed (all symptomatic relatives died), the variant is predicted to be deleterious by four silico tools (Polyphen-2, Scale Invariant Feature Transform [SIFT], Mutation Taster and Mutation Assessor), and is absent in 1000 Genomes and the Genome Aggregation Database.

Literature search

From a literature search performed up to March 1, 2020 in the PUBMED database, 41 probands of *HTRA1*-related autosomal dominant CSVD were included from 10 case reports and four original articles (Supplementary Table 1, http://links.lww.com/CM9/A366),[20-33] with 22 probands of gene confirmed CARASIL included from 14 case reports and one original article (Supplementary Table 2, http://links.lww.com/CM9/A366).[2,6-19]

All probands were *NOTCH3* negative (exons 2–24), except for the one with p.S284N.[26] In this patient, a heterozygous *NOTCH3* variant, c.4039G>C (p.G1347R) was detected. Although this variant is rare and with a score of 0.989 in polyphen-2, indicating probably damaging, it does not lead to a numerical cysteine alteration, which is considered to be a highly distinctive characteristic of the typical pathogenic mutation of CADASIL.[34,35] Combined with CARASIL-like symptoms include alopecia and spondylosis, the symptom of this patient was considered to be associated with *HTRA1* heterozygous mutation, he was therefore included in our analysis.

Clinical manifestations

Clinical data of 44 unrelated probands with *HTRA1* heterozygous mutations and 22 typical CARASIL probands were listed in Supplementary Tables 1, http://links.lww.com/CM9/A366 and 2, http://links.lww.com/CM9/A366, and their main clinical features were summarized in Table 1. Compared with typical CARASIL, *HTRA1* heterozygous mutations were associated with a higher proportion of male probands ($P = 0.012$) and a higher proportion of vascular risk factors ($P < 0.001$). The median age at which clinical symptoms related to CSVD occurred, reported in 40 patients, was 53.5 years (range from 29 to 77), which was significantly later than that of CARASIL (median age 28 years, $P < 0.001$). For probands with *HTRA1* heterozygous mutations, the ischemic event was a common initial symptom of CSVD (59.1%, 26/44). No obvious attack of acute cerebrovascular disease was recorded in 25% of patients (11/44), who underwent neurological examination for chronic progressive symptoms. Four patients received neurological examination due to non-specific symptoms (p.S284R at the age of 49 and p.R133G at the age of 58 because of headache, p.A123S at the age of 50 owing to

Table 1: Clinical characteristics of probands with CSVD related to heterozygous *HTRA1* mutations and typical CARASIL probands.

Characteristics	Heterozygous *HTRA1* (n = 44)	CARASIL (n = 22)	P
Male	32 (72.7)	9 (40.9)	0.012[*]
Vascular risk factors	27 (61.4)	1 (6.3)	<0.001[*]
Age of onset (years)	53.5 (48.0, 61.5)	28.0 (25.0, 32.0)	<0.001[*]
Age at diagnosis (years)	59.5 (50. 3, 65.0)	33.0 (27.8, 44.0)	<0.001[*]
Cognitive impairment at the time of diagnosis[†]			0.017[*]
0	12	3	
1	6	1	
2	6	0	
3	3	8	
Alopecia[†]	10	19	<0.001[*]
Spondylosis[†]	22	21	<0.001[*]

[*]Significance test between two groups, using the Pearson Chi-square test, Fisher's exact test, Mann-Whitney *U* test, and Kruskal-Wallis test. [†]Some clinical data of patients, such as vascular risk factors, were not provided in literatures. Data are shown as median (Q1, Q3), *n* or *n* (%). The degree of cognitive impairment: 0 = normal (MMSE≥27, or MoCA≥27, or described as "normal" in the article); 1 = mild (MMSE 21–26, or MoCA 18–26); 2=moderate (MMSE 10–20, or MoCA 10–17); 3 = severe (MMSE≤9, or MoCA<10, or described as "severe" or "dementia" in article). CARASIL: cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; CSVD: cerebral small vessel disease; HTRA1: high temperature requirement serine peptidase A1; MMSE: Mini-mental State Examination; MoCA: Montreal Cognitive Assessment.

epilepsy, p.R166C at the age of 31 because of a sudden episode of vertigo), and were suspected based on both MRI abnormalities and family history. Except for the patients with p.R166C who had mild cognitive decline, the other three patients were asymptomatic. At the time of diagnosis, patients of *HTRA1*-related autosomal dominant CSVD were with milder cognitive impairment, although they were older than patients of CARASIL. In terms of extraneuro-logic signs, the proportion of alopecia and spondylosis in heterozygous *HTRA1* patients was lower than that in CARASIL (23.3% *vs*. 86.4% for alopecia; 52.4% *vs*. 100% for spondylosis).

HTRA1 gene mutation

The heterozygous *HTRA1* mutations reported to date were summarized in Figure 2. A total of 35 mutations were reported in 44 probands, including 29 missense mutations, four nonsense mutations, one inframe mutation, and one truncating mutation. The most affected HTRA1 protein domain was the trypsin-like serine protease domain (19/35, 54.3%), followed by the Kazal-type serine protease domain (7/35, 20%). Among 37 patients with 29 missense mutations, R166 was the most frequently involved site (4/37), followed by S284 (3/37), P285 (3/37), and R302 (3/37). Some centralized trends were observed in the location distribution of missense mutations. Amino acids located near amino acids 250~300 were the most frequently involved, followed by those located near 150–200 [Figure 2].

Homozygous and compounds heterozygous *HTRA1* mutations in CARASIL reported to date were also summarized in Figure 2. A total of 19 mutations were reported in 22 probands, including 17 homozygous

mutations (9 missense mutations, 4 nonsense mutations, 4 truncating mutations), and two compounds heterozy-gous mutations. Among them, four mutations—p.R302Ter, p.G295R, p.P285L, and p.R166C—were also reported as heterozygous in *HTRA1*-related autosomal dominant CSVD. The most affected HTRA1 protein domain in CARASIL was the trypsin-like serine protease domain (11/19, 57.9%), followed by the PDZ domain (2/19, 10.5%). Of note, although amino acids 250–300 were also the most frequently affected region in CARASIL patients, there were fewer mutations precede the 200th amino acids, especially in the Kazal-type serine protease domain, where no mutations have been reported yet.

Discussion

In this study, we found that the clinical symptoms of CSVD related to heterozygous *HTRA1* mutations overlapped with typical CARASIL, but the manifestations were milder than CARASIL. The known heterozygous mutation spectrum of *HTRA1* showed a relatively high incidence of missense mutations near two regions (amino acids 250–300 and 150~200), of which amino acids 250–300 were also the most frequently affected region in CARASIL patients. However, mutations precede the 200th amino acids were less detected in CARASIL patients, no mutations in the Kazal-type serine protease domain have been reported yet.

The present study supports that this autosomal dominant CSVD manifests as a milder and late-onset phenotype of typical CARASIL. First, compared with the typical CARASIL, the mean age of onset in heterozygous *HTRA1* related CSVD was more than 20 years older. Second, heterozygous *HTRA1* mutation carriers have higher
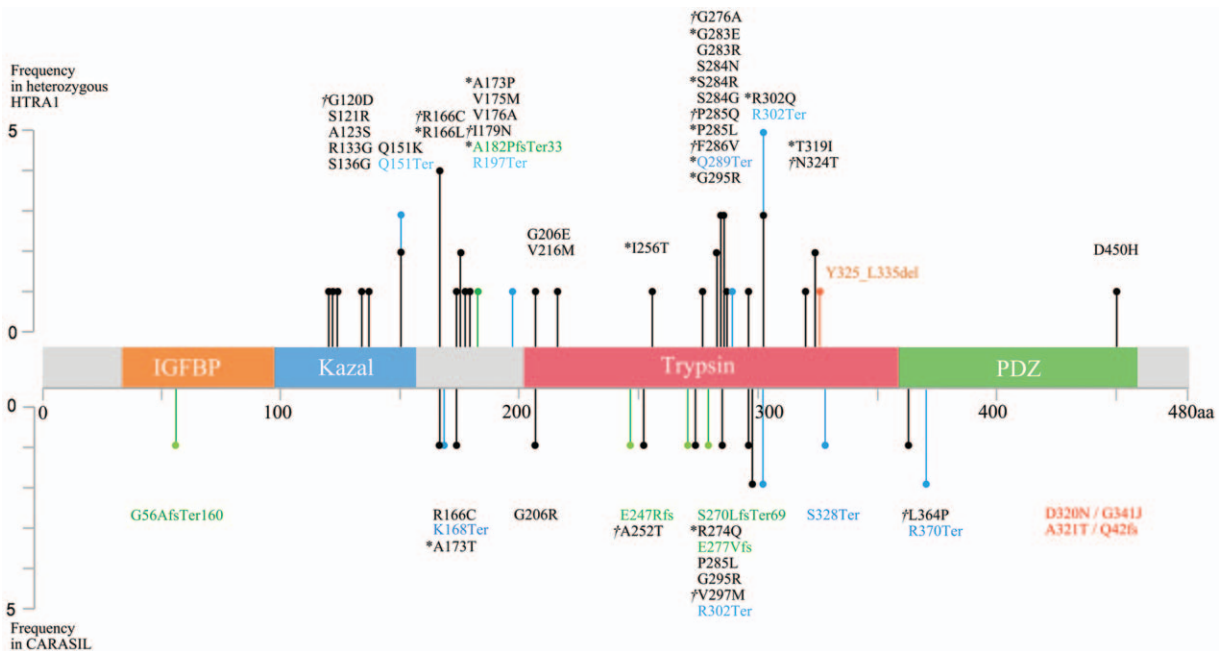

Figure 2: Distribution and frequency of heterozygous *HTRA1* mutations in 44 probands and homozygous *HTRA1* mutations in 22 CARASIL probands. The black bar stands for missense mutations. Blue bar, nonsense mutation. Orange bar, inframe mutation. The green bar, truncating mutation. The asterisk indicates that the mutation was reported to have a dominant-negative effect in previous studies. Mark "†" represents that the mutation can lead to a decrease of protease activity, but dominant-negative effect was not observed in previous studies. Compounds heterozygous *HTRA1* mutations in CARASIL patients are not located in the diagram and are listed separately with red font. *HTRA1*: high temperature requirement serine peptidase A1; CARASIL: cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy; aa: amino acid.

average cognitive scores than CARASIL patients at the time of diagnosis. Third, extraneurological signs (early-onset spondylosis and alopecia) are usually lacking. Except for family history (exist in 83.7% of probands), the clinical characteristics of heterozygous *HTRA1*-related CSVD are similar to sporadic CSVD. However, the familial nature of this disease may be ignored due to its late onset and a high proportion of vascular risk factors.[20] Therefore, screening of *HTRA1* should be considered in CSVD patients of unknown etiology, irrespective of whether they are familial aggregative or not.

The molecular mechanisms underlying heterozygous *HTRA1* associated CSVD are incompletely understood. The present study illustrated that the known spectrum of heterozygous *HTRA1* mutation and CARASIL mutation only partially overlapped. Most heterozygous *HTRA1* mutation carriers in the CARASIL family were previously reported as asymptomatic, which led to the hypothesis that the *HTRA1* mutations identified in symptomatic heterozygous patients have unique molecular characteristics. It has been suggested that those heterozygous missense mutations which have dominant-negative effects are pathogenic.[29] The dominant-negative effect refers to the phenomenon that allele mutation causes physiological dysfunction of the mutant protein itself and affects the function of normal allele product (wild-type protein). The functional HTRA1 exists as homotrimers, with each of the monomers plays an important role in the activation process. The substrate combines with the sensor domain of loop 3 (L3) of a monomer, activates the activation domain of loop D (LD) of the adjacent HTRA1 monomer to complete the activation process. [36,37] When the mutation removes the functional domain but retains the trimerization domain of the monomer, the mutant monomer can inhibit the activity of wild-type HTRA1 by interfering with the normal structure or activation of the trimer, thus leading to a dominant-negative effect.[38] However, subsequent studies have found that the dominant-negative effect may not be indispensable for every heterozygous *HTRA1* missense mutation to induce CSVD. There is no significant inhibition of wild-type HTRA1 protease activity was found in some mutations, such as p.G276A, p.G120D, and p.I179N, *etc*.[28] Symptomatic CSVD was also observed in heterozygous carriers with nonsense *HTRA1* mutation. Tateoka *et al* speculated that the nonsense mutations resulted in the loss of protein synthesis encoded by the mutant allele through nonsense-mediated mRNA decay, leading to haploinsufficiency.[21] Therefore, it is speculated that the impaired HTRA1 protease activity might be associated with an increased risk of CSVD.[38] Although there is no sufficient evidence indicating a linear relationship between protease activity and CSVD severity, Lee *et al*[28] observed that patients carrying mutations with the dominant-negative effect have a more severe and widespread leukoencephalopathy than those harboring mutations without the dominant-negative effect, suggesting that the degree of impairment of protease activity may be affecting the penetrance of the disease. This may also provide a possible explanation for the clinical heterogeneity of individuals harboring *HTRA1* variants, which have very broad phenotypes, ranging from a severe, early-onset CARASIL phenotype to non-penetrance.

The concentrated trend of missense heterozygous *HTRA1* mutations near amino acids 250 to 300 and 150 to 200 might indicate the critical regions associated with higher penetrance of the disease. One possible explanation is that the involvement of these two regions is related to the occurrence of dominant-negative effect. The key sites of trimer activation, L3 and LD, are composed of amino acid 301 to 314 and amino acid 283 to 291, respectively[36,39]; F278 is one of the key sites for stable trimer formation. This evidences may partly explain the high frequency of pathogenic heterozygous *HTRA1* mutations near amino acids 250 to 300. There are also some explanations for the high mutation frequency near amino acids 150 to 200. Y169 and F171 are also known as stacking sites crucial for trimerization. It was speculated that some mutations close to these sites, such as p.V176A, p.R166L, and p.A173P, may be pathogenic due to a failure formation of the stable trimer.[20,29] It is worth noting that no mutation in the Kazal-type serine protease domain has been reported in CARASIL patients yet. The previous finding from highly sensitive enzymatic assays and binding studies showed that the N-terminal domain, including IGFBP and Kazal-like domain, had no significant impact on HTRA1 protease activity. [40] In contrast, the Kazal-type serine protease domain is susceptible involved in heterozygous *HTRA1* associated CSVD in the present study. These findings may suggest the presence of crucial sites responsible for trimer formation and activation in this region. The molecular mechanism for *HTRA1*-related autosomal dominant CSVD should be further evaluated.

In conclusion, the present study shows that heterozygous *HTRA1* mutations may be cause of small vessel disease. This autosomal dominant disease is present as a mild phenotype of typical CARASIL. Amino acids 250~300 and 150~200 might be the hot spots for pathogenic heterozygous *HTRA1* mutations. Further study in expansion of the *HTRA1* heterozygous mutation spectrum and evaluation of the pathogenic characteristics of heterozygous *HTRA1* mutations are needed, in order to improve genetic diagnosis of hereditary CSVD.

Conflicts of interest

None.

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