REVIEW ARTICLE

Exploring *HTRA1*-Autosomal Dominant Disease: Literature Review of Clinical Features and Molecular Mechanisms

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Abstract: Heterozygous *HTRA1*-autosomal dominant disease is a gradually recognized hereditary cerebral small vessel disease (cSVD) characterized by debilitating conditions and extensive white matter hyperintensities (WMHs), but doubts remain on the underlying mechanisms of this disease. This review summarizes the clinical, MRI, and molecular genetics features of heterozygous *HTRA1*-autosomal dominant disease in combination with two better-studied hereditary cSVDs. A total of 31 mutations in *HTRA1*-autosomal dominant cases documented between 2020 and 2023 were also reviewed, characterizing the mutation features and clinical manifestations. This review aims to gain better insight into the unique characteristics of the disease and its correlations with other hereditary cSVDs.
Keywords: Heterozygous; *HTRA1;* mutation; CARASIL.

INTRODUCTION

High-temperature requirement A serine peptidase 1 (*HTRA1*) gene was first linked with hereditary cerebral small vessel disease (cSVD) in patients harboring homozygous or compound heterozygous mutations. This condition is clinically recognized as "cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy," or CARASIL. Recent research has indicated the pathogenicity of heterozygous *HTRA1* mutations (Verdura *et al.*, 2015), demonstrating that heterozygous variant carriers can exhibit similar clinical features as CARASIL, albeit milder in severity. Characteristic clinical symptoms and MRI features are present, including stroke, cognitive impairments, gait disturbances, and white matter hyperintensities (WMH) (Uemura *et al.*, 2020). This emerging variant of hereditary arteriopathy has been designated as *HTRA1*-autosomal dominant disease (Mancuso *et al.*, 2020) or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, type 2 (CADASIL2, OMIM 616779).

To date, more than 70 symptomatic patients carrying over 50 mutations have been reported. Since the first documentation of 11 probands in France with heterozygous *HTRA1* mutations predicted to be deleterious (Verdura *et al.*, 2015), a series of familial cases have been explored, including 4 mutations in 6 Japanese patients (Nozaki *et al.*, 2016), 5 mutations in Italian patients (Di Donato *et al.*, 2017), 7 mutations in Taiwanese patients (Lee *et al.*, 2018) and 7 novel mutations in Chinese patients (Zhang *et al.*, 2022). The frequency of heterozygous *HTRA1* carriers among individuals affected by non-*NOTCH3* autosomal dominant cerebral small vessel disease (cSVD) has been

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estimated to range from 3.15% to 5.31% (Di Donato *et al.*, 2017; Lee *et al.*, 2018; Nozaki *et al.*, 2016; Verdura *et al.*, 2015; Zhang *et al.*, 2022). Novel mutations are continuously reported from across the world.

Although the entity of symptomatic *HTRA1* carriers is growing and the range of potentially deleterious mutations is expanding, researchers are still uncertain about the underpinning mechanism of *HTRA1*-autosomal dominant disease. The difference between *HTRA1*-autosomal dominant disease and CARASIL also remained ambiguous. This review generalizes the clinical, MRI, and molecular genetics features of *HTRA1*-autosomal dominant disease in comparisons with other hereditary cSVDs, and summarizes the novel cases reported between 2020 and 2023, in seek of a comprehensive understanding of the disease for future research.

HTRA1 GENE: STRUCTURE AND FUNCTION

HTRA1 gene is located on 10q26.13 and composed of 9 exons. The *HTRA1* serine protease encoded by *HTRA1* consists of 480 amino acids and is arranged into four distinct domains: the insulin-like growth factor binding protein (IGFBP, 33~98 aa), the Kazal-like serine protease inhibitor (99~157 aa), the trypsin-like serine protease domain (204~364 aa), and the PDZ domain (365~467 aa). The serine protease domain can be subdivided into Loop D (LD, 283~291 aa), Loop 3 (L3, 301~314 aa), and other regions, not L3/LD (Uemura *et al.*, 2020). The protein forms a homotrimer, stabilized by the interactions between three amino acids from each monomer: Tyr169, Phe171, and Phe278 (Truebestein *et al.*, 2011). Activation of *HTRA1* protease relies on the signal relay between 3 monomers, with L3/LD playing an important role in the process.

The function of *HTRA1* protease includes interaction with various cellular signaling pathways. Regarding cSVD, the most notable pathway regulated by *HTRA1* protease is the transforming growth factor-β (TGF-β) signaling pathway. *HTRA1* protease cleaves and inhibits TGF-β activation, thereby repressing gene transcription of various downstream genes participating in extracellular matrix protein synthesis (Hara *et al.*, 2009). However, contradictory evidence has emerged suggesting that *HTRA1* protease can increase TGF-β release and signaling (Beaufort *et al.*, 2014). It has been suggested that TGF-β participates in various pathogenic mechanisms in CARASIL (Xu *et al.*, 2023; Yamamoto & Ihara, 2017), while the precise role of TGF-β in heterozygous *HTRA1*-related disease requires further investigation.

FEATURES OF *HTRA1*-AUTOSOMAL DOMINANT DISEASE COMPARED WITH CARASIL

Although *HTRA1*-autosomal dominant disease and CARASIL share a common genetic background and exhibit similar characteristics, differences have been depicted in terms of clinical features, MRI characteristics, mutation site distributions, and histopathological features. Herein, we summarize the major features of *HTRA1*-autosomal dominant disease while indicating its differences with CARASIL, as shown in Table 1. These discrepancies are important for understanding the unique features of *HTRA1*-autosomal dominant disease.

1. CLINICAL FEATURES

Clinical manifestations of *HTRA1*-autosomal dominant disease include stroke, cognitive decline, gait disorders, psychiatric disorders, alopecia, migraine, and spine disorders. The first symptom of onset is mostly stroke (45%) (Xu *et al.*, 2023). Extra-neurological features are less common, with spine disorders present in 47.27% and alopecia in 20% of cases. Clinical manifestations are highly variable, even within families (Kondo *et al.*, 2023). Considering epidemiological features, the ages of onset are mainly around 40~50 years (Uemura *et al.*, 2020; Xu *et al.*, 2023). Most patients present vascular risk factors, with hypertension being the most prevalent (50.91%) (Xu *et al.*, 2023).

Compared with CARASIL, *HTRA1*-autosomal dominant disease demonstrates milder symptoms, showing later age of onset and fewer presentations of extra-neurological symptoms (Di Donato *et al.*, 2017; Uemura *et al.*, 2020; Verdura *et al.*, 2015). Typical symptoms shared by both diseases are stroke, cognitive impairment, gait disturbances, etc. Previous findings showed that *HTRA1*-autosomal dominant disease patients exhibit a lower prevalence of psychiatric disorders (Zhou *et al.*, 2022) and gait disturbances ($p=0.019$) (Uemura *et al.*, 2020; Zhou *et al.*, 2022), but a higher occurrence of stroke ($p=0.089$) (Uemura *et al.*, 2020). Slower progression of cognitive impairment was also noted in *HTRA1*-autosomal dominant disease ($p=0.017$)

	***HTRA1*-AD**	**CARASIL**
Clinical features	Most common: stroke, cognitive decline, gait disorders, psychiatric disorders. Less common: alopecia, and spinal disorders.	
Rare: migraine, encephalopathy.	Most common: gait disorders, stroke, alopecia, spinal disorders, cognitive decline, and psychiatric disorders.	
Severity	Less severe	Severe
Initial symptom	Stroke (45%)	Gait disturbance (most common)
Ages of onset	40~60 yrs	20~40 yrs
Sex correlation	Male predominance	Not pronounce
Vascular risk factors	Mostly present. Hypertension is most prevalent (50.91%)	Common
MRI features		
WMHs	Extensive	Extensive
LIs	Less	More
CMBs	Less	More
"Arc sign"	Rare or none	Common in late-stage
Mutation sites	Linker region or protease domain, particularly L3/LD.	Disperse, infrequent L3/LD involvement
Histopathology features	Intimal proliferation, medial smooth muscle loss, hyaline degeneration, adventitial fibrosis, and internal elastic lamina splitting	Severe presentation of intimal proliferation, medial smooth muscle loss, hyaline degeneration, adventitial fibrosis, and internal elastic lamina splitting

HTRA1-AD: *HTRA1*-related autosomal dominant disease. CARASIL: cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. WMH: white matter hyperintensity. LI: lacunar infarct. CMB: cerebral microbleed. L3: loop 3. LD: loop D.

Table 1. Comparisons between *HTRA1*-AD disease and CARASIL features.

(Liu *et al.*, 2020). Ischemic attacks were commonly demonstrated as onset events for *HTRA1*-autosomal dominant disease (59.1%) (Liu *et al.*, 2020). Moreover, migraine and encephalopathy were present in several cases of *HTRA1*-autosomal dominant disease while considered rare in CARASIL patients (Mancuso *et al.*, 2020).

Extra-neurological symptoms also vary between the diseases. Several investigations indicated high occurrences of early-onset alopecia (20%~30%) and spondylosis (70%~100%) (Chen *et al.*, 2022; Lee *et al.*, 2018; Nozaki *et al.*, 2016) in *HTRA1*-autosomal dominant disease, while others reported absence of such symptoms (Di Donato *et al.*, 2017; Verdura *et al.*, 2015). Nonetheless, the overall proportion of extra-neurological features of *HTRA1*-autosomal dominant disease is still lower than CARASIL, which demonstrates alopecia in more than 90% of patients and spinal lesions in almost 100% of patients (Mancuso *et al.*, 2020).

The features of epidemiological distributions and vascular risk factors are also diverse between the two diseases. The average age of onset in *HTRA1*-autosomal dominant disease patients is 51.6~61.3 years, while that in CARASIL patients is around 30 years (Lee *et al.*, 2018; Liu *et al.*, 2020; Uemura *et al.*, 2020). Male predominance is more pronounced in *HTRA1*-autosomal dominant disease compared to CARASIL (Liu *et al.*, 2020; Uemura *et al.*, 2020; Zhou *et al.*, 2022). The prevalence of vascular risk factors, particularly hypertension, is also significantly higher in the former group (Liu *et al.*, 2020; Uemura *et al.*, 2020; Zhou *et al.*, 2022). Conversely, consanguineous marriage backgrounds are more common in CARASIL pedigrees due to the autosomal recessive nature of the disease (Zhou *et al.*, 2022).

2. MRI CHARACTERISTICS

Extensive WMH (98%), mainly located in deep white matter and periventricular regions is a predominant radiological hallmark of *HTRA1*-autosomal dominant disease. The anterior temporal lobe was affected in several cases (Lee *et al.*, 2018; Zhang *et al.*, 2021), while spared in others (Muthusamy *et al.*, 2021). U-fibers remain primarily unaffected (Di Donato *et al.*, 2017; Nozaki *et al.*, 2016). Lacunar infarcts (LIs, 75%) and cerebral microbleeds (CMBs, 55.77%) are less common findings (Xu *et al.*, 2023). Additionally, a characteristic status cribrosum, associated with dilated PVS, has been observed in *HTRA1*-autosomal dominant disease (Nozaki *et al.*, 2016; Verdura *et al.*, 2015).

Both *HTRA1*-autosomal dominant disease and CARASIL present with prevalent MRI findings, including WMHs, LIs, CMBs, brain atrophy, etc. Findings from previous studies illustrated that *HTRA1*-autosomal dominant disease patients tend to exhibit lower proportions of WMHs than CARASIL, although both are highly correlated with WMH (Uemura *et al.*, 2020; Zhou *et al.*, 2022). The "arc sign", which is a signature finding in late-stage CARASIL patients, has not been reported in *HTRA1*-autosomal dominant disease patients (Chen *et al.*, 2022; Kitahara *et al.*, 2022; Nozaki *et al.*, 2015). On the other hand, Corpus callosum involvement was reported in a few *HTRA1*-autosomal dominant disease cases while uncommon in CARASIL (Chen *et al.*, 2022; Wu *et al.*, 2020). Considering the less prevalent features, CMBs were less frequently documented in the *HTRA1* heterozygous family than in CARASIL (Shang *et al.*, 2021).

3. MUTATION SITES

In *HTRA1* heterozygous disease, a missense mutation is the most common pathogenic mutation. Other possible mutation forms include nonsense/frameshift mutation and splice-site mutation. The mutations are mostly clustered in exon 4 (~50%) (He *et al.*, 2023). Missense mutations are commonly localized in the linker region or the protease domain, particularly affecting the L3/LD loops (Xu *et al.*, 2023), whereas stop-gain mutations are more widely distributed across the gene (Coste *et al.*, 2021). One plausible explanation for the aggregation in L3/LD and the linker region is that these regions are significant in enzyme activities. L3/LD is essential for enzyme activation, while the linker region contains two stacking sites (Y169 and F171) crucial for trimerization (Liu *et al.*, 2020). Notably, mutations located in the linker region were associated with later onset of symptoms but a predisposition for stroke occurrence (Shang *et al.*, 2021).

Comparing *HTRA1*-autosomal dominant disease and CARASIL, the mutation distribution patterns are quite diverse. Affected amino acids in *HTRA1*-autosomal dominant disease are mainly located within the L3/LD loops and the linker region (He *et al.*, 2023; Liu *et al.*, 2020; Uemura *et al.*, 2020). In contrast, CARASIL variants are more dispersed in distribution and spread across the protease domain, with relatively infrequent L3/LD involvement (Grigaitė *et al.*, 2021). Considering other domains, the Kazal-like region was involved in some cases of *HTRA1*-autosomal dominant disease but was generally absent in CARASIL patients (Liu *et al.*, 2020; Zhou *et al.*, 2022). The function of the Kazal-like region remained largely unknown, yet some studies suggested that it might play a role in preventing autolysis of the *HTRA1* enzyme(Li *et al.*, 2020; Risør *et al.*, 2014). The divergence in mutation distribution presumably underlies the difference in molecular mechanisms of the two diseases.

4. HISTOPATHOLOGY FEATURES

Histopathological demonstrations of *HTRA1*-autosomal dominant disease include intimal proliferation, medial smooth muscle loss, hyaline degeneration, adventitial fibrosis, and internal elastic lamina splitting (Nozaki *et al.*, 2016). CARASIL demonstrates similar features as *HTRA1*-autosomal dominant disease, yet commonly in a severe form (Lee *et al.*, 2018; Nozaki *et al.*, 2015; Uemura *et al.*, 2020).

Overall, CARASIL and *HTRA1*-autosomal dominant diseases share similarities but also demonstrate important differences in clinical, radiographical, and histopathological characteristics. Comprehensive inspections of individual cases are required for better distinction between the two.

PROPOSED MOLECULAR GENETICS OF *HTRA1*-AUTOSOMAL DOMINANT DISEASE

The molecular genetic mechanism underlying *HTRA1*-autosomal dominant disease and its differences from CARASIL have long been the subject of discussion. Upon the initial identification of heterozygous *HTRA1* mutations, Verdura *et al.* (2015)

proposed 2 possible mechanisms for their pathogenicity: dominant-negative effect and haploinsufficiency. Regarding the molecular mechanisms, researchers have also linked *HTRA1*-autosomal dominant disease with dysfunction of the TGF-β signaling pathway, yet little evidence has been raised and controversy remains. The following paragraphs review the two hypotheses of heterozygous *HTRA1* mutation pathogenicity and the current understanding of the TGF-β pathway involved in hereditary cSVD disease, hoping to provide a deeper insight into the pathogenicity of *HTRA1*-autosomal dominant disease.

1. GENETIC MECHANISM OF *HTRA1*-AUTOSOMAL DOMINANT DISEASE

(1) Mechanism 1: dominant-negative effect

Researchers postulated that in heterozygous mutation carriers, normal enzymatic functions are interrupted when the products of the mutant allele interfere with the products of the wild-type (WT) allele. This mechanism is termed the "dominant-negative effect", as demonstrated in Fig. 1A.

To test the assumed dominant-negative effect, Nozaki *et al.* (2016) designed in vitro functional tests involving co-transfecting cells with WT and mutant plasmids and compared the mixture protease activities with the control group. A positive result would show less than half of protease activity in the mutant group than in the WT group. As predicted, all 4 heterozygous *HTRA1* variants demonstrated dominant-negative effects, while 2 out of 3 CARASIL variants displayed little reduction in protease activities. This result further suggests that heterozygous *HTRA1* variants may have a more crucial impact on protease activity compared with CARASIL. The possible mechanisms with which mutant alleles interfere with WT activities include (1) impairing protease trimerization through damaging the linker region, and (2) disrupting the protease activation cascade by affecting L3/LD, which relays activation signals to other monomers in the trimer.

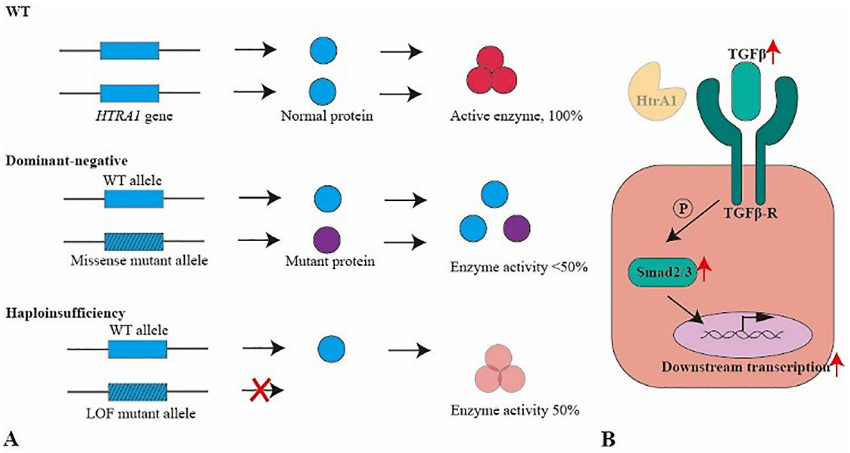

Figure 1. Two possible genetic mechanisms and TGF-β signaling pathway.
A: Genetic mechanisms of dominant negative and haploinsufficiency. WT, wild type; *HTRA1*, high-temperature requirement serine peptidase A1; LOF, loss-of-function; TGFβ, transforming growth factor-β; TGFβ-R, transforming growth factor-β receptor.
B: Effects of decreased *HTRA1* activity on downstream TGF-β signaling.

(2) Mechanism 2: haploinsufficiency

The dominant-negative hypothesis mainly applies to missense mutations. In the cases of nonsense/frameshift mutations and splice-site mutations, the mRNA transcripts are degraded through nonsense-mediated mRNA decay (NMD) (Coste *et al.*, 2021), thus no protein products of the mutant allele are present to affect the WT allele. For these mutations, the pathogenicity was explained by haploinsufficiency, which postulated that half of the protease activity is insufficient for normal cellular functions (Fig. 1A). Moreover, several missense mutations were shown with decreased protease activity but no dominant-negative effect (Lee *et al.*, 2018; Uemura *et al.*, 2019). These mutations were also hypothesized to demonstrate haploinsufficiency effects.

(3) Comparing the pathogenic features of two variant categories

The pathogenicity of dominant-negative variants was assumed to be higher than the loss-of-function variants. Research indicated that patients carrying mutations with dominant-negative effects demonstrate severer and more widespread leukoencephalopathy (Lee *et al.*, 2018). Additionally, the penetrance of stop-gain mutations was comparatively low, thus not all haploinsufficient variants display clinical symptoms (Coste *et al.*, 2021). On the other hand, the disease severity of dominant-negative variants was believed to correlate with residual protease activity (Uemura *et al.*, 2019), signifying the importance of evaluating protease activities in individual mutation cases.

(4) Doubts regarding the hypotheses

Despite substantial evidence supporting the dominant-negative and haploinsufficiency explanation for *HTRA1*-autosomal dominant disease, a few cases challenged these theories. Some mutations capable of disease induction did not exhibit decreased protease activities (Uemura *et al.*, 2019; Verdura *et al.*, 2015), whose pathogenic mechanisms remained unrevealed. Meanwhile, nonsense/frameshift mutations were also associated with a dominant-negative effect, although simultaneously resulting in less protein expression (Lee *et al.*, 2018), casting doubts on the effectiveness of dominant-negative tests. Considering the distinction between heterozygous *HTRA1* variants and CARASIL variants, the difference in protease activities has grown obscure, as several mutations associated with both diseases have been reported (Bekircan-Kurt *et al.*, 2021; Chen *et al.*, 2022; Kondo *et al.*, 2023; Muthusamy *et al.*, 2021). Further investigations are necessary to elucidate the genetic characteristics of *HTRA1*-autosomal dominant disease and its differences with CARASIL.

2. MOLECULAR MECHANISMS REGARDING THE TGF-β PATHWAY

(1) TGF-β pathway and *HTRA1*-autosomal dominant disease

As depicted in Fig. 1B, the downstream pathogenic mechanisms of *HTRA1*-autosomal dominant disease have been related to the TGF-β pathway, similar to CARASIL. *HTRA1* protease encoded by the *HTRA1* gene normally functions as a serine protease that cleaves proTGF-β1 proteins in the endoplasmic reticulum (ER). The cleaved proTGF-β1 is then degraded through the ER-associated protein degradation (ERAD) process, instead of secreting to the extracellular matrix (ECM) and performing biological functions. Decreased *HTRA1* protease activities lead to un-inhibited TGF-β1 secretion, which can result in highly activated TGF-β1 signaling (Shiga *et al.*, 2011). TGF-β signaling pathway plays an important role in cellular differentiation, proliferation, and activation. TGF-β activates downstream proteins through phosphorylation. The canonical TGF-β signaling pathway involves Smads, which can translocate into the cell nucleus and function as transcription factors (Derynck *et al.*, 1998). Non-Smad pathways have also been discovered with parallel functions in targeting gene transcriptions (Zhang, 2017).

Limited information regarding the changes in the TGF-β pathway in *HTRA1*-autosomal dominant disease has been reported. One research detected elevated gene expressions downstream to the TGF-β pathway, although the levels of intermediate TGF-β substrates remained unchanged (Fasano *et al.*, 2020). Another research demonstrated increased intermediate TGF-β substrates in *HTRA1*-autosomal dominant disease patients (Zhuo *et al.*, 2020). These two pieces of research were generally consistent with the hypothesized mechanism of TGF-β pathway dysregulation, yet the discordance in details revealed the complexity of molecular regulations. More effort is required for further analysis of TGF-β pathway involvement in *HTRA1*-autosomal dominant disease.

(2) Current knowledge of TGF-β pathway correlations with other hereditary cSVDs

To achieve a better depiction of the pathogenic features of the TGF-β pathway in hereditary cSVDs, current understandings are summarized regarding the associations between the TGF-β pathway and two important hereditary cerebral small arterial disorders, CARASIL and CADASIL.

In CARASIL patients, elevated levels of TGF-β and downstream gene expressions were detected (Hara *et al.*, 2009). The subsequent elevation of ECM protein production was considered relevant to vascular fibrosis, tunica intima thickening, and various histopathological features in CARASIL

and *HTRA1*-autosomal dominant patients (Tikka *et al.*, 2014). However, contrasting pieces of evidence have been reported in recent years. Beaufort *et al.* (2014) demonstrated that a decreased TGF-β level was found in *HTRA1* mutant cells, which was related to a *HTRA1* protease substrate, latent TGF-β binding protein 1 (LTBP1). LTBP1 interacts with major ECM components and sequestrates TGF-β, preventing its activation. Mutant *HTRA1* protease leads to decreased LTBP1 cleavage, decreased TGF-β release, and reduced downstream signaling. On the other hand, Kato *et al.* (2021) recently demonstrated accumulated TGF-β with no concomitant elevation in downstream substrates or gene expressions related to *HTRA1* mutations. Rather, increased levels of other *HTRA1* protease substrates in ECM, including latent TGF-β binding protein 4 (LTBP4) and fibronectin (FN), were suggested to underly the vascular damage of *HTRA1* mutations. The contrasting results of different studies may partly be due to the different experiment models applied. Nonetheless, considering all information, further investigations are required to clarify the role of the TGF-β signaling pathway in the pathogenic processes of *HTRA1*-related diseases.

TGF-β signaling pathway has also been associated with CADASIL, which is caused by mutations in the *NOTCH3* gene. Although the pathogenic genes of CADASIL and *HTRA1*-related disease are different, similarities have been reported between the two diseases, especially the diffuse WMH distribution in the anterior temporal lobe (Tikka *et al.*, 2014; Zhang *et al.*, 2021). These phenomena may suggest a commonly affected downstream pathway (e.g. the TGF-β pathway), thus requires further investigation. The mutant proteins tend to form deposits of the Notch3 extracellular domain (Notch3-ECD) in the vascular wall, which is considered a vital process of disease onset (Tikka *et al.*, 2014). Research has discovered elevated LTBP1 proteins in Notch3 deposits and simultaneous dysregulation of the TGF-β signaling pathway in CADASIL patients, suggesting the involvement of the TGF-β pathway in the CADASIL pathogenic process (Kast *et al.*, 2014). "Omic" studies have also found alterations in substrate protein levels of TGF-β pathways in CADASIL patients, including *HTRA1* protease and various Smad-binding proteins, further supporting the association (Muiño *et al.*, 2021). Moreover, the low proliferation rate of vascular smooth muscle cells (VSMCs) in CADASIL patients was found to relate to increased TGF-β expression levels (Panahi *et al.*, 2018), indicating the participation of TGF-β pathway in small arterial degeneration in CADASIL, which might be responsible for the similarities of these two hereditary CSVD caused by mutations in different genes.

Mutations above the axis were reported only in heterozygous *HTRA1*-autosomal dominant disease, while mutations below the axis were also reported in CARASIL. *, Dominant-negative effect; #, Decreased protease activity. SP, signal peptide domain; IGFBP, insulin-like growth factor binding protein; Kazal, Kazal-type serine protease inhibitor domain; Serine Protease, Trypsin-like serine protein domain; PDZ, PDZ domain; aa, amino acid. The figure is drawn based on Figure 2 in our previous work of Liu *et al.* (2020).

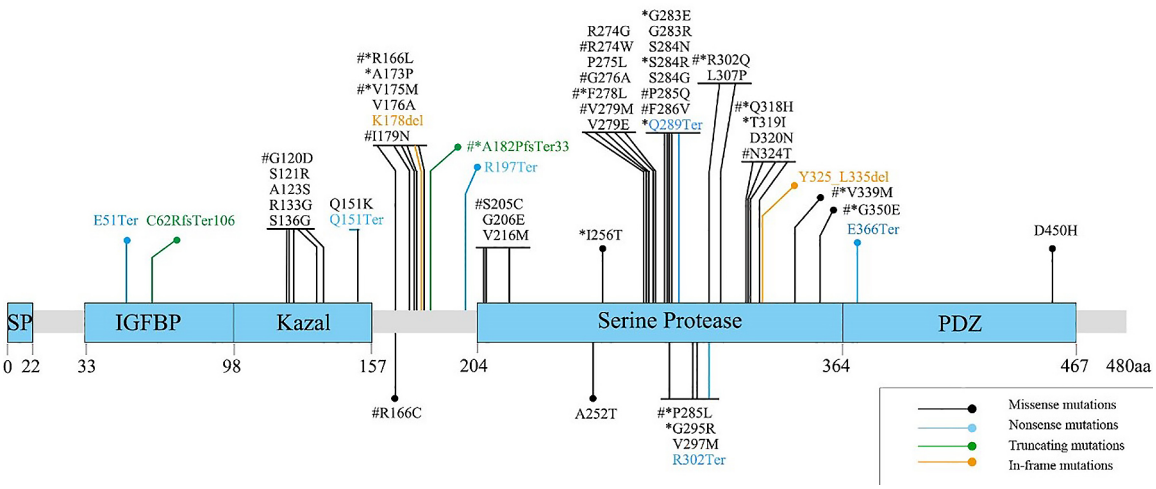

Figure 2. Distribution of 53 heterozygous *HTRA1* mutations.

To sum up, the above-mentioned evidence highlights the important pathologic correlations of TGF-β with hereditary cSVD. TGF-β has also been related to vascular changes in Alzheimer's disease and sporadic cSVDs (Müller *et al.*, 2017), further suggesting the close connections and important roles of the TGF-β signaling pathway in the pathogenesis of cerebral vascular diseases. Therefore, the TGF-β signaling pathway might be a possible promise as a therapeutic target in some hereditary cSVD and deserves further investigation.

NOVEL CASES REPORTED IN 2020 ~ 2023

A comprehensive overview of 31 mutations detected across 43 unrelated families, all reported between 2020 and 2023, is presented in Table 2. These mutations encompassed 19 novel variants that were undocumented before 2020. The majority of mutations are missense mutations (22/31). Of all mutations identified, most are concentrated within exon 4. Regarding protein domains, the region not L3/LD is affected most (15/22), followed by the linker region (3/22), L3 (2/22), and LD (2/22). However, taking all reported mutations into account, the most affected regions are still L3 and LD loops, consistent with previous studies (summarized based on our previous work of Liu *et al.* (2020), as shown in Fig.2). The emerging involvement of not L3/LD regions in heterozygous disease suggests its influences on enzymatic functions, which remained largely unclear. Functional analyses regarding the region are lacking, and several tested mutations showed contradictory results, ranging from no influence on enzyme functions to positive for dominant negative effect (Muthusamy *et al.*, 2021; Nozaki *et al.*, 2016; Yao *et al.*, 2022). Investigation into the pathogenic mechanisms of mutations in regions not L3/LD should be further tested.

All reported variants were classified as "damaging" or "probably damaging" by at least three different *in silico* tools (Table 2). The allele frequencies acquired from The Genome Aggregation Database (gnomAD) were low (<10-4) or not available. Due to the relative absence of functional assessments in recent studies, the pathogenicity of many variants remains unclear, and further analyses are required for better characterization of the mutant allele functions.

Table 3 summarizes the clinical features, MRI characteristics, and risk factors of the reported patients. Clinical presentations mainly included cognitive impairment (37/42) and stroke (30/42), followed by gait disturbances (20/42), psychiatric disorder (9/42), and transient ischemic attack (TIA, 8/42). Extra-neurological features including spinal disorders (14/42) and alopecia (8/42) were also reported. Considering MRI features, WMH was highly involved in almost all patients (41/42), while other features were less prevalent, including cerebral microbleeds (CMB, 23/42), lacunar infarcts (LI, 21/42), and enlarged perivascular space (PVS, 9/42). A few patients presented with atrophy (7/42) and intracranial hemorrhage (ICH, 4/42). Generally, the clinical landscapes and MRI findings were similar to the features reported previously.

The patients' risk factors were diverse, spanning from the absence of risk factors (18/42) to the existence of multiple factors such as hypertension, diabetes mellitus, hypercholesterolemia, and smoking. Hypertension was the most prevalent risk factor, present in 19 out of 42 patients. All these suggested that monogenic cSVD should not be ruled out with the presence of vascular risk factors (Mancuso *et al.*, 2020). Indeed, the onset of symptoms in heterozygous carriers was considered relevant to the presence of vascular risk factors (Zhou *et al.*, 2022). The high frequency of vascular risk factors in this review aligns with previous statements.

DIAGNOSIS AND PROSPECTS OF *HTRA1*-AUTOSOMAL DOMINANT DISEASE

1. Factors influencing disease severity

The severity of *HTRA1*-autosomal dominant disease is influenced by various factors. First, the severity of WMH (evaluated by Fazakas score) was correlated with *in silico* predictions of mutation deleteriousness (evaluated by CADD-score, $p<0.05$) (He *et al.*, 2023). Second, increased phenotype severity was associated with mutations in specific regions (L3/LD and exon 4), smoking, hyperlipidemia, hypertension, and diabetes mellitus (Zhang *et al.*, 2021; Zhou *et al.*, 2022). Last, Kondo *et al.* (2023) considered vascular risk factors as the key determinant of cSVD symptom onset in mutation carriers.

Reference	Variants	Mutation type	Protease activity (Dominant negative)	Domain	Intron/ exon	Number of patients/ families	GnomAD frequency	PolyPhen2	SIFT	PROVEAN	CADD
W. Chen *et al.* (2022)	c.151G>T # p.E51X	NFM	NA	—	exon1	1/1	NA	NA	NA	NA	Damaging
Muthusamy *et al.* (2021)	c.184_185del # p.C62Rfs*106	NFM	NA	—	exon1	1/1	NA	NA	NA	NA	NA
M. J. Chen *et al.* (2022)	c.472+1G>A # Splicing	Splicing	NA	—	intron1	2/1	NA	NA	NA	NA	Damaging
Liu *et al.* (2020)	c.496C>T p.R166C	Missense	↓ (-) [1]	Linker region	exon2	1/1	NA	Probably damaging	Damaging	Damaging	Damaging
Cao *et al.* (2022)	c.497G>T p.R166L	Missense	↓ (+) [1]	Linker region	exon2	2/1	NA	Probably damaging	Damaging	Damaging	Damaging
Liu *et al.* (2020) Muthusamy *et al.* (2021) Shang *et al.* (2021) Zhang *et al.* (2021) Zhang *et al.* (2022)	c.523G>A p.V175M	Missense	↓ (+) [2]	Linker region	exon2	5/5	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
Grigaite *et al.* (2021)	c.533_535del # p.K178del	Deletion	NA	—	exon2	1/1	NA	NA	NA	NA	NA
M. J. Chen *et al.* (2022)	c.543del p.A182Pfs*33	NFM	↓ (+) [3]	—	exon2	1/1	NA	NA	NA	NA	Damaging
Zhou *et al.* (2022)	c.589C>T p.R197X	NFM	NA	—	exon3	1/1	7.95E-06	NA	NA	NA	Damaging
Zhuo *et al.* (2020)	c.614C>G # p.S205C	Missense	↓ (NA) [4]	Not L3/LD	exon3	2/1	NA	Probably damaging	Damaging	Damaging	Damaging
Kondo *et al.* (2023)	c.754G>A p.A252T	Missense	– (–) 5	Not L3/LD	exon3	4/1	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
Hidalgo Mayoral *et al.* (2022)	c.820C>G # p.R274G	Missense	NA	Not L3/LD	exon4	4/1	NA	Probably damaging	Damaging	Damaging	Damaging
Yao *et al.* (2022)	c.820C>T # p.R274W	Missense	↓ (NA) [6]	Not L3/LD	exon4	1/1	1.42E-05	Probably damaging	Damaging	Damaging	Damaging
Bekircan-Kurt *et al.* (2021) M. J. Chen *et al.* (2022) Zhang *et al.* (2021)	c.824C>T # p.P275L	Missense	NA	Not L3/LD	exon4	8/3	1.20E-05	Probably damaging	Damaging	Damaging	Damaging
Zhang *et al.* (2022)	c.832T>C # p.F278L	Missense	↓ (+) [2]	Not L3/ LD	exon4	2/1	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
Zhang *et al.* (2022)	c.834C>G # p.F278L	Missense	↓ (+) [2]	Not L3/ LD	exon4	1/1	NA	Probably damaging	Damaging	Damaging	Damaging

Reference	Variants	Mutation type	Protease activity (Dominant negative)	Domain	Intron/ exon	Number of patients/ families	GnomAD frequency	PolyPhen2	SIFT	PROVEAN	CADD
Yao *et al.* (2022)	c.835G>A # p.V279M	Missense	↓ (NA) [6]	Not L3/LD	exon4	1/1	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
Wu *et al.* (2020)	c.836T>A # p.V279E	Missense	NA	Not L3/LD	exon4	2/1	NA	Probably damaging	Damaging	Damaging	Damaging
Oluwole *et al.* (2020)	c.847G>A # p.G283R	Missense	NA	LD	exon4	1/1	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
M. J. Chen *et al.* (2022) He *et al.* (2023) Zhang *et al.* (2022)	c.854C>T p.P285L	Missense	↓ (+) [2,5]	LD	exon4	7/3	3.98E-06	Probably damaging	Damaging	Damaging	Damaging
Muthusamy *et al.* (2021) M. J. Chen *et al.* (2022)	c.889G>A p.V297M	Missense	– (–) [5]	Not L3/LD	exon4	2/2	NA	Probably damaging	Damaging	Damaging	Damaging
Ohta *et al.* (2020)	c.904C>T p.R302X	NFM	NA	—	exon4	1/1	1.06E-05	NA	NA	NA	Damaging
Mahale *et al.* (2021) Kitahara *et al.* (2022)	c.905G>A p.R302Q	Missense	↓ (+) [5]	L3	exon4	3/3	NA	Probably damaging	Damaging	Damaging	Damaging
Zhou *et al.* (2022)	c.920T>C # p.L307P	Missense	NA	L3	exon4	2/1	NA	Probably damaging	Damaging	Damaging	Damaging
Yao *et al.* (2022) Zhang *et al.* (2022)	c.954G>C # p.Q318H	Missense	↓ (+) [2,6]	Not L3/LD	exon4	14/2	3.99E-06	Probably damaging	Damaging	Damaging	Damaging
Muthusamy *et al.* (2021)	c.958G>A p.D320N	Missense	NA	Not L3/LD	exon4	1/1	1.60E-05	Probably damaging	Damaging	Damaging	Damaging
Liu *et al.* (2020)	c.971A>C p.N324T	Missense	↓ (–) [3]	Not L3/LD	exon4	1/1	NA	Probably damaging	Tolerable	Damaging	Damaging
Zhang *et al.* (2022)	c.973-2A>G # p.Splicing	Splicing	NA	—	intron4	3/1	3.98E-06	NA	NA	NA	Damaging
Zhang *et al.* (2022)	c.1015G>A # p.V339M	Missense	↓ (+) [2]	Not L3/LD	exon6	1/1	NA	Probably damaging	Damaging	Damaging	Damaging
Zhang *et al.* (2022)	c.1049G>A # p.G350E	Missense	↓ (+) [2]	Not L3/LD	exon6	1/1	NA	Probably damaging	Damaging	Damaging	Damaging
W. Chen *et al.* (2022)	c.1096G>T # p.E366X	NFM	NA	—	exon6	1/1	NA	NA	NA	NA	Damaging

Novel mutation sites.
L3: Loop 3. LD: Loop D. NA: Not available. NFM: Nonsense frameshift mutation.
1 Uemura *et al.* (2019); 2 Zhang *et al.* (2022); 3 Lee *et al.* (2018); 4 Zhuo *et al.* (2020); 5 Nozaki *et al.* (2016); 6 Yao *et al.* (2022).

Table 2. Heterozygous *HTRA1* mutations and pathogenicity predictions.

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Reference	Variants	Significant Clinical Features	MRI	Risk factors
W. Chen *et al.* (2022)	c.151G>T p.E51X	Cognitive impairment	LI, WMHs, atrophy, CMBs	Abs
Muthusamy *et al.* (2021)	c.184_185del p.C62Rfs*106	Cognitive impairment, TIAs, gait disturbances	WMHs	Hypertension, migraine
M. J. Chen *et al.* (2022)	c.472+1G>A Splicing	Alopecia, spinal disorders	WMHs	Hypertension
Liu *et al.* (2020)	c.496C>T p.R166C	Stroke, cognitive impairment, gait disturbances, psychiatric disorders, alopecia, spinal disorders	WMHs, CMBs	Abs
Cao *et al.* (2022)	c.497G>T p.R166L	Cognitive impairment, stroke	LIs, CMBs,	Abs
Liu *et al.* (2020)	c.523G>A p.V175M	Stroke, cognitive impairment, psychiatric disorders, alopecia, spinal disorders	WMHs, LIs, PVS, CMBs	Hypertension, diabetes mellitus
Muthusamy *et al.* (2021)	c.523G>A p.V175M	Stroke, TIAs, gait disturbances, cognitive impairment,	WMHs, ICHs.	Hypertension
Zhang *et al.* (2022)	c.523G>A p.V175M	Stroke, cognitive impairment, gait disturbance, spinal disorders	WMHs, PVS, CMBs, atrophy	Hypertension
Zhang *et al.* (2021)	c.523G>A p.V175M	Stroke	WMHs, LIs, CMBs	Hypertension
Shang *et al.* (2021)	p.V175M	Cognitive impairment, spondylosis	WMHs, CMBs, LIs,	Hypertension
Grigaite *et al.* (2021)	c.533_535del p.K178del	Cognitive impairment, psychiatric symptoms, stroke, TIAs,	Atrophy, WMHs, LIs,	Hypertension, dyslipidemia, smoking
M. J. Chen *et al.* (2022)	c.543del p.A182Pfs*33	Stroke, cognitive impairment, psychiatric symptoms, alopecia	LIs, WMHs, CMBs	Abs
Zhou *et al.* (2022)	c.589C>T p.R197X	Cognitive impairment	WMHs	Abs
Zhuo *et al.* (2020)	c.614C>G p.S205C	Cognitive impairment, stroke	WMHs, Lis	Abs
Kondo *et al.* (2023)	c.754G>A p.A252T	Stroke, cognitive impairment, spinal disorders,	WMHs, LIs, CMBs, atrophy	Hypertension, dyslipidemia, smoking
Hidalgo Mayoral *et al.* (2022)	c.820C>G p.R274G	Stroke, cognitive impairment	WMHs, CMBs,	Hypertension, diabetes mellitus, hypercholesterolemia, smoking
Yao *et al.* (2022)	c.820C>T p.R274W	Stroke/TIA, cognitive decline	WMHs, LIs, CMBs, ICH,	Hypertension
Bekircan-Kurt *et al.* (2021)	c.824C>T p.P275L	Spondylosis, migraine	WMHs, LIs	Abs

Reference	Variants	Significant Clinical Features	MRI	Risk factors
Zhang *et al.* (2021)	c.824C>T p.P275L	Stroke, cognitive impairment, psychiatric symptoms	WMHs	Smoking
M. J. Chen *et al.* (2022)	c.824C>T p.P275L	Stroke, alopecia, spinal disorders, cognitive impairment	WMHs, CMBs	Abs
Zhang *et al.* (2022)	c.832T>C p.F278L	Stroke, cognitive impairment, spinal disorders	WMHs, PVS	Hypertension
Zhang *et al.* (2022)	c.834C>G p.F278L	Stroke, cognitive impairment, gait disturbance, spinal disorders	WMHs, PVS, CMBs, atrophy	Abs
Yao *et al.* (2022)	c.835G>A p.V279M	Stroke/TIA, cognitive decline	WMHs, LIs, CMBs	Abs
Wu *et al.* (2020)	c.836T>A p.V279E	Stroke, cognitive impairment,	LIs, WMHs, CMBs	Abs
Oluwole *et al.* (2020)	c.847G>A p.G283R	Gait disturbances, cognitive impairment	WMHs, LIs	Abs
Zhang *et al.* (2022)	c.854C>T p.P285L	Stroke, cognitive impairment, gait disturbance, spinal disorders	WMHs, PVS	Abs
M. J. Chen *et al.* (2022)	c.854C>T p.P285L	TIAs, cognitive impairment, gait disturbance, alopecia	WMHs, CMBs	Hypertension
He *et al.* (2023)	c.854C>T p.P285L	Migraine, alopecia, cognitive impairment	LIs, WMHs, ICH	Abs
Muthusamy *et al.* (2021)	c.889G>A p.V297M	Cognitive impairment, stroke, gait disturbances	LIs, ICHs, WMHs	Hypertension, hyperlipidemia, smoking
M. J. Chen *et al.* (2022)	c.889G>A p.V297M	Stroke, cognitive impairment, gait disturbance, psychiatric symptoms	WMHs, LIs	Hypertension
Ohta *et al.* (2020)	c.904C>T p.R302X	Stroke, gait disturbance	WMHs, LIs,	Smoking
Mahale *et al.* (2021)	c.905G>A p.R302Q	Psychiatric symptoms, cognitive impairment, gait disturbances,	WMHs, CMBs,	Abs
Kitahara *et al.* (2022)	c.905G>A p.R302Q	Psychiatric symptoms, gait disturbance, spondylosis, cognitive impairment	WMHs, CMBs	Smoking
Zhou *et al.* (2022)	c.920T>C p.L307P	Stroke, gait disturbance, spinal disorders, cognitive impairment	WMHs, CMBs	Hyperlipidemia
Yao *et al.* (2022)	c.954G>C p.Q318H	Stroke, TIA, spinal disorders, cognitive impairment,	WMHs, LIs, CMBs	Abs
Zhang *et al.* (2022)	c.954G>C p.Q318H	Stroke, spinal disorders, cognitive impairment, gait disturbance	WMHs, PVS	Smoking, hypertension

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Reference	Variants	Significant Clinical Features	MRI	Risk factors
Muthusamy *et al.* (2021)	c.958G>A p.D320N	TIAs, cognitive impairment, migraine, gait disturbances	WMHs, ICHs.	Hypertension, diabetes mellitus
Liu *et al.* (2020)	c.971A>C p.N324T	Stroke, cognitive impairment, psychiatric disorders, gait disturbances, spondylosis	WMHs, LIs, CMB	Abs
Zhang *et al.* (2022)	c.973-2A>G p.Splicing	Stroke, cognitive impairment, migraine, gait disturbance	WMHs, PVS, atrophy	Hypertension, diabetes mellitus
Zhang *et al.* (2022)	c.1015G>A p.V339M	Stroke, cognitive impairment, gait disturbance, spinal disorders, cerebral hemorrhage, migraine	WMHs, PVS, CMBs, atrophy	Hypertension
Zhang *et al.* (2022)	c.1049G>A p.G350E	Stroke, cognitive impairment, alopecia, spinal disorders	WMHs, PVS	Smoking
W. Chen *et al.* (2022)	c.1096G>T p.E366X	Stroke	LI, WMH, CMBs	Abs

Abs: absence. CMB: cerebral microbleed. ICH: intracranial hemorrhage. LI: lacunar infarct.
PVS: enlarged perivascular space. TIA: transient ischemic attack. WMH: white matter hyperintensity.

Table 3. Heterozygous *HTRA1* carriers manifestations.

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2. Diagnosis and Management

Clinical diagnosis of *HTRA1*-autosomal dominant disease is challenging due to its late age of onset, symptoms overlapping with other cSVDs, and the prevalence of vascular risk factors in patients (Grigaitė *et al.*, 2021). Of note, hereditary cSVDs should not be excluded even when patients are present with vascular risk factors and no significant familial history (Mancuso *et al.*, 2020). The American College of Medical Genetics and Genomics (ACMG) guidelines should serve as a crucial reference for assessing the pathogenicity of mutations (Xu *et al.*, 2023). While effective treatments are currently unavailable, the management of vascular risk factors holds potential significance in preventing the onset and progression of the disease in heterozygous mutation carriers (Grigaitė *et al.*, 2021). Additionally, the TGF-β pathway might provide a potential target for treatment developments, which requires further research.

3. Prospects

Since its initial report in 2015, *HTRA1*-autosomal dominant disease has undergone extensive investigation. However, the validity of this disease is still a topic under debate. Some scientists contend that heterozygous *HTRA1* mutations are only risk factors for cSVD rather than determinant factors for a novel disease (Zhou *et al.*, 2022). Future research is needed to address the problem.

The reliability of functional analysis is also considered uncertain. Variations in control group designs in dominant-negative tests existed across different studies. Nozaki *et al.* (2016) employed WT/S328A as the control group, taking into consideration the autolysis activity of WT proteases. However, other studies utilized WT/- as control groups (Lee *et al.*, 2018; Zhang *et al.*, 2022). This raises the question of whether results obtained through different research methods are directly comparable.

Despite the prevailing doubts and inconsistencies, a new possibility for laboratory analysis has emerged. Qian *et al.* (2023) established the first induced pluripotent stem cell (iPSC) line derived from an *HTRA1* symptomatic carrier, opening up fresh opportunities for more precise and authentic examinations of disease mechanisms *in vitro*.

CONCLUSION

HTRA1-autosomal dominant disease is a hereditary cSVD characterized by stroke, cognitive decline, and WMHs. Differentiation between *HTRA1*-autosomal dominant disease and CARASIL is important for accurate clinical diagnosis and progress prediction. The similarities of genetic and molecular features among various hereditary cSVDs suggest an underlying common pathogenic process. TGF-β signaling pathway might be a promising therapeutic target. While uncertainties and inconsistencies persist, the continual progress in clinical understanding and laboratory techniques are promising for improvement in the understanding of this disease in the foreseeable future.

Conflict of Interest Disclosures

The authors have no conflicts of interest to declare.

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Author Contributions

Y.L. drafted the manuscript and performed the literature search. Y. Z. revised the manuscript. M.Y. supervised the research and revised the manuscript.

List of Abbreviations

cSVD: cerebral small vessel disease.
HTRA1: high-temperature requirement serine peptidase A1.
CARASIL: cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy.
CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.
IGFBP: insulin-like growth factor binding protein
TGF-β: transforming growth factor-β.
NMD: nonsense-mediated mRNA decay.
gnomAD: The Genome Aggregation Database.
TIA: transient ischemic attack.
WMH: white matter hyperintensity.
CMB: cerebral microbleed.
LI: lacunar infarcts.
PVS: perivascular space.

ICH: intracranial hemorrhage.
iPSC: induced pluripotent stem cell.
ER: endoplasmic reticulum.
ERAD: ER-associated protein degradation.
ECM: extracellular matrix.
LTBP: latent TGF-β binding protein.
FN: fibronectin.
Notch3-ECD: Notch3 extracellular domain.
VSMC: vascular smooth muscle cells.

REFERENCES

[1] Beaufort, N., Scharrer, E., Kremmer, E., Lux, V., Ehrmann, M., Huber, R., Houlden, H., Wer-ring, D., Haffner, C., & Dichgans, M. (2014). Cerebral small vessel disease-related protease *HTRA1* processes latent TGF-β binding protein 1 and facilitates TGF-β signaling. *Proc Natl Acad Sci U S A, 111*(46), 16496-16501. https://doi.org/10.1073/pnas.1418087111

[2] Bekircan-Kurt, C. E., Çetinkaya, A., Goc-men, R., Koşukcu, C., Soylemezoglu, F., Ar-sava, E. M., Tuncer, A., Erdem-Ozdamar, S., Akarsu, N. A., & Topcuoglu, M. A. (2021). One Disease with two Faces: Semidominant Inheritance of a Novel *HTRA1* Mutation in a Consanguineous Family. *J Stroke Cerebrovasc Dis, 30*(9), 105997. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.105997

[3] Chen, M. J., Zhang, Y., Luo, W. J., Dong, H. L., Wei, Q., Zhang, J., Ruan, Q. Q., Ni, W., & Li, H. F. (2022). Identified novel heterozygous *HTRA1* pathogenic variants in Chinese patients with *HTRA1*-associated dominant cerebral small vessel disease. *Front Genet, 13*, 909131. https://doi.org/10.3389/fgene.2022.909131

[4] Coste, T., Hervé, D., Neau, J. P., Jouvent, E., Ba, F., Bergametti, F., Lamy, M., Cogez, J., De-rache, N., Schneckenburger, R., Grelet, M., Gollion, C., Lanotte, L., Lauer, V., Layet, V., Urbanczyk, C., Didic, M., Raynouard, I., Dela-val, L., … Tournier-Lasserve, E. (2021). Heterozygous *HTRA1* nonsense or frameshift mutations are pathogenic. *Brain, 144*(9), 2616-2624. https://doi.org/10.1093/brain/awab271

[5] Derynck, R., Zhang, Y., & Feng, X. H. (1998). Smads: transcriptional activators of TGF-beta responses. *Cell, 95*(6), 737-740. https://doi.org/10.1016/s0092-8674(00)81696-7

[6] Di Donato, I., Bianchi, S., Gallus, G. N., Cerase, A., Taglia, I., Pescini, F., Nannucci, S., Battisti, C., Inzitari, D., Pantoni, L., Zini, A., Federico, A., & Dotti, M. T. (2017). Heterozygous mutations of *HTRA1* gene in patients with familial cerebral small vessel disease. *CNS Neurosci Ther, 23*(9), 759-765. https://doi.org/10.1111/cns.12722

[7] Fasano, A., Formichi, P., Taglia, I., Bianchi, S., Di Donato, I., Battisti, C., Federico, A., & Dot-ti, M. T. (2020). *HTRA1* expression profile and activity on TGF-β signaling in *HTRA1* mutation carriers. *J Cell Physiol, 235*(10), 7120-7127. https://doi.org/10.1002/jcp.29609

[8] Grigaitė, J., Šiaurytė, K., Audronytė, E., Pre-ikšaitienė, E., Burnytė, B., Pranckevičienė, E., Ekkert, A., Utkus, A., & Jatužis, D. (2021). Novel In-Frame Deletion in *HTRA1* Gene, Responsible for Stroke at a Young Age and Dementia-A Case Study. *Genes (Basel), 12*(12). https://doi.org/10.3390/genes12121955

[9] Hara, K., Shiga, A., Fukutake, T., Nozaki, H., Miyashita, A., Yokoseki, A., Kawata, H., Koya-ma, A., Arima, K., Takahashi, T., Ikeda, M., Shiota, H., Tamura, M., Shimoe, Y., Hirayama, M., Arisato, T., Yanagawa, S., Tanaka, A., Na-kano, I., … Onodera, O. (2009). Association of *HTRA1* mutations and familial ischemic cerebral small-vessel disease. *N Engl J Med, 360*(17), 1729-1739. https://doi.org/10.1056/NEJMoa0801560

[10] He, Z., Wang, L., Zhang, Y., Yin, C., & Niu, Y. (2023). Clinical features and pathogenicity assessment in patients with *HTRA1*-autosomal dominant disease. *Neurol Sci, 44*(2), 639-647. https://doi.org/10.1007/s10072-022-06454-5

[11] Kast, J., Hanecker, P., Beaufort, N., Giese, A., Joutel, A., Dichgans, M., Opherk, C., & Haffner, C. (2014). Sequestration of latent TGF-β binding protein 1 into CADASIL-related Notch3-ECD deposits. *Acta Neuropathol Commun, 2*, 96. https://doi.org/10.1186/s40478-014-0096-8

[12] Kato, T., Manabe, R. I., Igarashi, H., Ka-metani, F., Hirokawa, S., Sekine, Y., Fujita, N., Saito, S., Kawashima, Y., Hatano, Y., Ando, S., Nozaki, H., Sugai, A., Uemura, M., Fukunaga, M., Sato, T., Koyama, A., Saito, R., Sugie, A., … Onodera, O. (2021). Candesartan prevents arteriopathy progression in cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy model. *J Clin Invest, 131*(22). https://doi.org/10.1172/jci140555

[13] Kitahara, S., Tsuboguchi, S., Uemura, M., Nozaki, H., Kanazawa, M., & Onode-ra, O. (2022). Patients with heterozygous

HTRA1-related cerebral small vessel disease misdiagnosed with other diseases: Two case reports. *Clin Neurol Neurosurg, 223*, 107502. https://doi.org/10.1016/j.clineuro.2022.107502

[14] Kondo, Y., Yoshinaga, T., Nakamura, K., Yamaguchi, T., Ishikawa, M., Kosho, T., & Sekijima, Y. (2023). Severe Cerebral Small Vessel Disease Caused by the Uniallelic p.A252T Variant of *HTRA1*. *Neurol Genet, 9*(1), e200047. https://doi.org/10.1212/nxg.0000000000200047

[15] Lee, Y. C., Chung, C. P., Chao, N. C., Fuh, J. L., Chang, F. C., Soong, B. W., & Liao, Y. C. (2018). Characterization of Heterozygous *HTRA1* Mutations in Taiwanese Patients With Cerebral Small Vessel Disease. *Stroke, 49*(7), 1593-1601. https://doi.org/10.1161/strokeaha.118.021283

[16] Li, Y., Yuan, J., Rothzerg, E., Wu, X., Xu, H., Zhu, S., & Xu, J. (2020). Molecular structure and the role of high-temperature requirement protein 1 in skeletal disorders and cancers. *Cell Prolif, 53*(2), e12746. https://doi.org/10.1111/cpr.12746

[17] Liu, J. Y., Zhu, Y. C., Zhou, L. X., Wei, Y. P., Mao, C. H., Cui, L. Y., Peng, B., & Yao, M. (2020). *HTRA1*-related autosomal dominant cerebral small vessel disease. *Chin Med J (Engl), 134*(2), 178-184. https://doi.org/10.1097/cm9.0000000000001176

[18] Mancuso, M., Arnold, M., Bersano, A., Burlina, A., Chabriat, H., Debette, S., Enzinger, C., Federico, A., Filla, A., Finsterer, J., Hunt, D., Lesnik Oberstein, S., Tournier-Lasserve, E., & Markus, H. S. (2020). Monogenic cerebral small-vessel diseases: diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. *Eur J Neurol, 27*(6), 909-927. https://doi.org/10.1111/ene.14183

[19] Muiño, E., Fernández-Cadenas, I., & Arboix, A. (2021). Contribution of "Omic" Studies to the Understanding of Cadasil. A Systematic Review. *Int J Mol Sci, 22*(14). https://doi.org/10.3390/ijms22147357

[20] Müller, K., Courtois, G., Ursini, M. V., & Schwaninger, M. (2017). New Insight Into the Pathogenesis of Cerebral Small-Vessel Diseases. *Stroke, 48*(2), 520-527. https://doi.org/10.1161/strokeaha.116.012888

[21] Muthusamy, K., Ferrer, A., Klee, E. W., Wierenga, K. J., & Gavrilova, R. H. (2021). Clinicoradiographic and genetic features of cerebral small vessel disease indicate variability in mode of inheritance for monoallelic *HTRA1* variants. *Mol Genet Genomic Med, 9*(10), e1799. https://doi.org/10.1002/mgg3.1799

[22] Nozaki, H., Kato, T., Nihonmatsu, M., Saito, Y., Mizuta, I., Noda, T., Koike, R., Miyazaki, K., Kaito, M., Ito, S., Makino, M., Koyama, A., Shiga, A., Uemura, M., Sekine, Y., Murakami, A., Moritani, S., Hara, K., Yokoseki, A., … Onodera, O. (2016). Distinct molecular mechanisms of *HTRA1* mutants in manifesting heterozygotes with CARASIL. *Neurology, 86*(21), 1964-1974. https://doi.org/10.1212/wnl.0000000000002694

[23] Nozaki, H., Sekine, Y., Fukutake, T., Nishimoto, Y., Shimoe, Y., Shirata, A., Yanagawa, S., Hirayama, M., Tamura, M., Nishizawa, M., & Onodera, O. (2015). Characteristic features and progression of abnormalities on MRI for CARASIL. *Neurology, 85*(5), 459-463. https://doi.org/10.1212/wnl.0000000000001803

[24] Panahi, M., Yousefi Mesri, N., Samuelsson, E. B., Coupland, K. G., Forsell, C., Graff, C., Tikka, S., Winblad, B., Viitanen, M., Karlström, H., Sundström, E., & Behbahani, H. (2018). Differences in proliferation rate between CADASIL and control vascular smooth muscle cells are related to increased TGFβ expression. *J Cell Mol Med, 22*(6), 3016-3024. https://doi.org/10.1111/jcmm.13534

[25] Qian, E., Uemura, M., Kobayashi, H., Nakamura, S., Ozawa, F., Yoshimatsu, S., Ishikawa, M., Onodera, O., Morimoto, S., & Okano, H. (2023). A human induced pluripotent stem cell model from a patient with hereditary cerebral small vessel disease carrying a heterozygous R302Q mutation in *HTRA1*. *Inflamm Regen, 43*(1), 23. https://doi.org/10.1186/s41232-023-00273-7

[26] Risør, M. W., Poulsen, E. T., Thomsen, L. R., Dyrlund, T. F., Nielsen, T. A., Nielsen, N. C., Sanggaard, K. W., & Enghild, J. J. (2014). The autolysis of human *HTRA1* is governed by the redox state of its N-terminal domain. *Biochemistry, 53*(23), 3851-3857. https://doi.org/10.1021/bi401633w

[27] Shang, T., Pinho, M., Ray, D., & Khera, A. (2021). Two Unique Mutations in *HTRA1*-Related Cerebral Small Vessel Disease in North America and Africa and Literature Review. *J Stroke Cerebrovasc Dis, 30*(11), 106029. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106029

[28] Shiga, A., Nozaki, H., Yokoseki, A., Nihonmatsu, M., Kawata, H., Kato, T., Koyama, A., Arima, K., Ikeda, M., Katada, S., Toyoshima, Y., Takahashi, H., Tanaka, A., Nakano, I., Ikeuchi,

T., Nishizawa, M., & Onodera, O. (2011). Cerebral small-vessel disease protein *HTRA1* controls the amount of TGF-β1 via cleavage of proTGF-β1. *Hum Mol Genet, 20*(9), 1800-1810. https://doi.org/10.1093/hmg/ddr063

[29] Tikka, S., Baumann, M., Siitonen, M., Pasanen, P., Pöyhönen, M., Myllykangas, L., Viitanen, M., Fukutake, T., Cognat, E., Joutel, A., & Kalimo, H. (2014). CADASIL and CARASIL. *Brain Pathol, 24*(5), 525-544. https://doi.org/10.1111/bpa.12181

[30] Truebestein, L., Tennstaedt, A., Mönig, T., Krojer, T., Canellas, F., Kaiser, M., Clausen, T., & Ehrmann, M. (2011). Substrate-induced remodeling of the active site regulates human *HTRA1* activity. *Nat Struct Mol Biol, 18*(3), 386-388. https://doi.org/10.1038/nsmb.2013

[31] Uemura, M., Nozaki, H., Kato, T., Koyama, A., Sakai, N., Ando, S., Kanazawa, M., Hishikawa, N., Nishimoto, Y., Polavarapu, K., Nalini, A., Hanazono, A., Kuzume, D., Shindo, A., El-Ghanem, M., Abe, A., Sato, A., Yoshida, M., Ikeuchi, T., … Onodera, O. (2020). *HTRA1*-Related Cerebral Small Vessel Disease: A Review of the Literature. *Front Neurol, 11,* 545. https://doi.org/10.3389/fneur.2020.00545

[32] Uemura, M., Nozaki, H., Koyama, A., Sakai, N., Ando, S., Kanazawa, M., Kato, T., & Onodera, O. (2019). *HTRA1* Mutations Identified in Symptomatic Carriers Have the Property of Interfering the Trimer-Dependent Activation Cascade. *Front Neurol, 10,* 693. https://doi.org/10.3389/fneur.2019.00693

[33] Verdura, E., Hervé, D., Scharrer, E., Amador Mdel, M., Guyant-Maréchal, L., Philippi, A., Corlobé, A., Bergametti, F., Gazal, S., Prieto-Morin, C., Beaufort, N., Le Bail, B., Viakhireva, I., Dichgans, M., Chabriat, H., Haffner, C., & Tournier-Lasserve, E. (2015). Heterozygous *HTRA1* mutations are associated with autosomal dominant cerebral small vessel disease. *Brain, 138*(Pt 8), 2347-2358. https://doi.org/10.1093/brain/awv155

[34] Wu, C., Chen, L., & Ke, S. (2020). Novel Heterozygous *HTRA1* Pathogenic Variant Found in a Chinese Family with Autosomal Dominant Cerebral Small Vessel Disease. *Ann Indian Acad Neurol, 23*(6), 832-835. https://doi.org/10.4103/aian.AIAN_74_20

[35] Xu, S. Y., Li, H. J., Li, S., Ren, Q. Q., Liang, J. L., & Li, C. X. (2023). Heterozygous Pathogenic and Likely Pathogenic Symptomatic *HTRA1* Variant Carriers in Cerebral Small Vessel Disease. *Int J Gen Med, 16*, 1149-1162. https://doi.org/10.2147/ijgm.S404813

[36] Yamamoto, Y., & Ihara, M. (2017). Disruption of transforming growth factor-β superfamily signaling: A shared mechanism underlying hereditary cerebral small vessel disease. *Neurochem Int, 107,* 211-218. https://doi.org/10.1016/j.neuint.2016.12.003

[37] Yao, T., Zhu, J., Wu, X., Li, X., Fu, Y., Wang, Y., Wang, Z., Xu, F., Lai, H., He, A., Teng, L., Wang, C., & Song, H. (2022). Heterozygous *HTRA1* Mutations Cause Cerebral Small Vessel Diseases: Genetic, Clinical, and Pathologic Findings From 3 Chinese Pedigrees. *Neurol Genet, 8*(6), e200044. https://doi.org/10.1212/nxg.0000000000200044

[38] Zhang, C., Zheng, H., Li, X., Li, S., Li, W., Wang, Z., Niu, S., Wang, X., & Zhang, Z. (2022). Novel mutations in *HTRA1*-related cerebral small vessel disease and comparison with CADASIL. *Ann Clin Transl Neurol, 9*(10), 1586-1595. https://doi.org/10.1002/acn3.51654

[39] Zhang, H., Qin, X., Shi, Y., Gao, X., Wang, F., Wang, H., Shang, J., Zhao, J., Zhang, J., & Shao, F. (2021). Genotype-phenotype correlations of heterozygous *HTRA1*-related cerebral small vessel disease: case report and systematic review. *Neurogenetics, 22*(3), 187-194. https://doi.org/10.1007/s10048-021-00646-5

[40] Zhang, Y. E. (2017). Non-Smad Signaling Pathways of the TGF-β Family. *Cold Spring Harb Perspect Biol, 9*(2). https://doi.org/10.1101/cshperspect.a022129

[41] Zhou, H., Jiao, B., Ouyang, Z., Wu, Q., Shen, L., & Fang, L. (2022). Report of two pedigrees with heterozygous *HTRA1* variants-related cerebral small vessel disease and literature review. *Mol Genet Genomic Med, 10*(10), e2032. https://doi.org/10.1002/mgg3.2032

[42] Zhuo, Z. L., Cong, L., Zhang, J., & Zhao, X. T. (2020). A novel heterozygous *HTRA1* mutation is associated with autosomal dominant hereditary cerebral small vessel disease. *Mol Genet Genomic Med, 8*(6), e1111. https://doi.org/10.1002/mgg3.1111