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Severe Cerebral Small Vessel Disease Caused by the Uniallelic p.A252T Variant of *HTRA1*

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Abstract

Objective

To investigate the clinical effect of a heterozygous missense variant of *HTRA1* on cerebral small vessel disease (CSVD) in a large Japanese family with a p.A252T variant.

Methods

We performed clinical, laboratory, radiologic, and genetic evaluations of members of a previously reported family with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL).

Results

Two family members were previously reported patients with CARASIL. Among 6 uniallelic p.A252T carriers, 2 had neurologic symptoms with brain MRI abnormalities, 2 showed CSVD on the MRI only, and the other 2 were unaffected. Clinical phenotypes of 2 heterozygous patients were comparable with those of patients with CARASIL, whereas the other 3 heterozygous patients had developed milder and later-onset CSVD. One heterozygous carrier was asymptomatic.

Discussion

Previous studies have suggested that uniallelic p.A252T causes disease. However, our study revealed that patients with uniallelic p.A252T can have severe and young-onset CSVD. The clinical manifestations of uniallelic variant carriers were highly variable, even within the same family. Male and atherosclerotic risk factors were considered to be additional factors in the severity of neurologic symptoms in uniallelic p.A252T carriers, suggesting that strict control of vascular risk factors can prevent vascular events in uniallelic *HTRA1* carriers.

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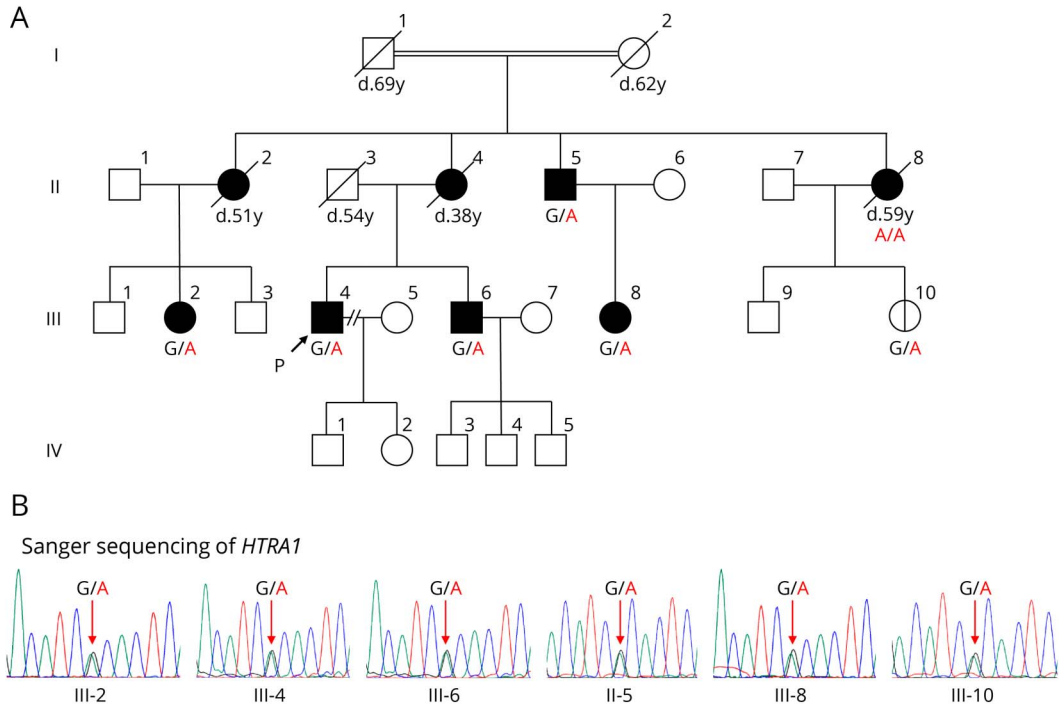
Table Clinical and Neuroimaging Features of the *HTRA1* Variant Carriers

Patient (pedigree number)	Pt. 1 (II-2)[a]	Pt. 2 (II-4)	Pt. 3 (II-8)[a]	Pt. 4 (II-5)	Pt.5 (III-4)	Pt. 6 (III-6)	Pt. 7 (III-2)	Pt. 8 (III-8)	Pt. 9(III-10)
Sex	Female	Female	Female	Male	Male	Male	Female	Female	Female
Age at examination (y)	51	38	48	70	49	47	52	39	39
Age at onset (y)	34	28	38	62	37	38	50	—[b]	—[b]
Disease duration (y)	17	10	10	8	12	9	2	—[b]	—[b]
Hypertension	(−)	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(−)
Diabetes	NA	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)
Dyslipidemia	NA	(−)	(−)	(+)	(+)	(+)	(+)	(−)	(−)
Smoke	NA	NA	NA	(+)	(+)	(−)	(−)	(−)	(−)
Initial symptom	Gait disturbance	Dysarthria	Diplopia	Dysarthria	Gait disturbance	Headache	(−)	—[b]	—[b]
Alopecia of younger onset	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
Lumbago/spondylosis	NA	NA	(+)	(+)	(+)	(+)	(+)	(+)	(−)
Walking ability	Bedridden	Bedridden	Wheelchair	Unaided	Wheelchair	Unaided	Unaided	Unaided	Unaided
Pyramidal sign	(+)	(+)	(+)	(−)	(+)	(+)	(−)	(−)	(−)
Dementia	(+)	(+)	(−)	(−)	(+)	(+)	(−)	(−)	(−)
Acute stroke	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(−)
Brain MRI findings									
Periventricular WMHs	NA	NA	(+)	(+)	(+)	(+)	(+)	(−)	(−)
External Capsule	NA	NA	(+)	(+)	(−)	(+)	(+)	(−)	(−)
Temporal Pole	NA	NA	(+)	(−)	(−)	(−)	(−)	(−)	(−)
Abnormal signals of the brainstem	NA	NA	(+)	(+)	(+)	(+)	(+)	(−)	(−)
Lacunar infarctions	NA	NA	NA	(+)	(+)	(+)	(−)	(−)	(−)
Microbleeds	NA	NA	NA	(+)	(+)	(+)	(−)	(−)	(−)
Atrophy of the cerebrum	NA	NA	(−)	(+)	(+)	(+)	(−)	(−)	(−)
brainstem	NA	NA	(−)	(−)	(+)	(−)	(−)	(−)	(−)
***HTRA1* (c.754G>A) genotype**	Unexamined	Unexamined	Homozygote	Heterozygote	Heterozygote	Heterozygote	Heterozygote	Heterozygote	Heterozygote

Abbreviations: NA = information not available; Pt = patient; WMHs = white matter hyperintensities.
[a] Described based on data from the literature.
[b] These patients had no neurologic symptoms or brain imaging abnormalities and had never visited a neurology clinic before we performed a medical survey on the family members of the siblings (V-4 and V-6).

Figure 1 Pedigree and Sanger Sequencing of *HTRA1*



(A) The numbers below the symbols indicate the age in years (y) at death (d) of deceased individuals indicated by a strikethrough. The proband and the younger brother are indicated by arrows. II-8 had been genetically tested and confirmed to have an *HTRA1* homozygous variant in a previous report.[1] Filled symbols indicate that the patient had leukoencephalopathy, lumbago, or spondylosis. A symbol with a secant line indicates an asymptomatic patient. (B) Sanger sequencing of *HTRA1*.

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is an autosomal recessive cerebral small vessel disease (CSVD) caused by biallelic variants in *HTRA1*,[1] whereas *HTRA1* uniallelic variants cause hereditary CSVD with a milder form and later-onset, HTRA1-linked autosomal dominant CSVD (AD-CSVD).[2] Here, we report a Japanese family with the *HTRA1* p.A252T variant (NM_002775.5:c.754G>A), which showed marked intrafamilial phenotypic variation.

Case Presentation

Patient 1 (II-2, Figure 1A, Table) developed knee pain at 34 years and had a stroke episode at 37 years. Neurologic examination at 40 years showed cognitive decline, emotional instability, pseudobulbar palsy, urinary incontinence, and bilateral pyramidal signs. She became bedridden and died at 51 years. Based on both clinical and postmortem pathologic findings, we diagnosed patient 1 as likely CARASIL.[1,3]

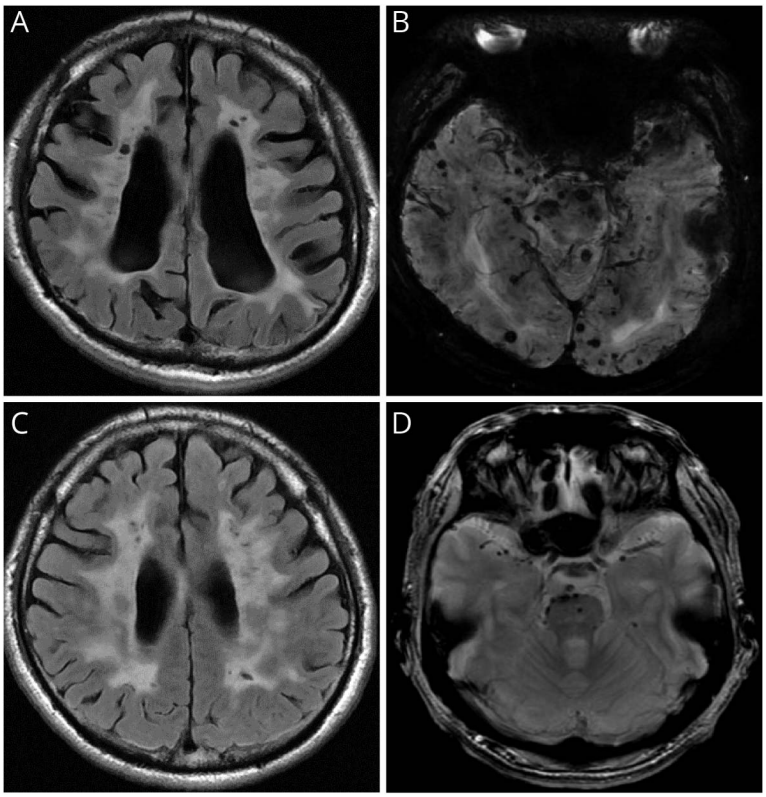
Patient 2 (II-4) first noticed dysarthria, followed by recurrent episodes of transient hemiparesis at 28 years. Neurologic examination at 29 years revealed cognitive decline, rigidity, bradykinesia, and pyramidal tract signs. At 30 years, she developed emotional instability and pseudobulbar palsy. She died at 38 years.

Patient 3 (II-8) had genetically confirmed CARASIL and carried homozygous *HTRA1* p.A252T.[1,3] She developed lumbago and knee pain as a teenager. At 38 years, she had recurrent episodes of stroke and showed dysarthria, hemiparesis, and gait disturbance. She became a wheelchair user because of spastic paresis and cerebellar ataxia at 48 years.

Patient 4 (II-5) was a 70-year-old man who carried heterozygous p.A252T with multiple atherosclerotic risks (Figure 1B). He developed lacunar infarction at 62 years. He was diagnosed with hypertension and diabetes mellitus and treated with cilostazol, pitavastatin, amlodipine, and sitagliptin. At 70 years, he showed neither cognitive dysfunction nor gait disturbance, although MRI showed cerebral atrophy, white matter hyperintensities (WMHs), and microbleeds (eFigure A–D, links.lww.com/NXG/A568).

Patient 5 (III-4) was a 49-year-old man with heterozygous p.A252T. At 37 years, he developed gait disturbance, and MRI showed acute lacunar infarction in the left radiate crown with multiple previous infarctions. He was a smoker but had no other atherosclerosis risks. He repeatedly developed cerebrovascular events, and his gait and cognitive functions deteriorated progressively, despite dual antiplatelet therapy. At 43 years, he was diagnosed with hyperlipidemia and started statin therapy that reduced the frequency of stroke attacks. In serial MRI analyses, WMHs had expanded from the center of

Figure 2 MRI of Symptomatic *HTRA1* Variant Carriers



(A and B) Patient 5 (III-4) at 49 years of age. (C and D) Patient 6 (III-6) at 47 years of age. Fluid-attenuated inversion recovery images (A and C) showed periventricular to subcortical white matter hyperintensities, lacunar infarctions, and cerebral atrophy. Susceptibility-weighted imaging (B) and T2*(D) showed microbleeds in both the cerebral cortex and the brainstem surface.

the semicircle to the periventricular area of lateral ventricles, and progressive diffuse brain atrophy and microbleeds were evident (Figure 2, A and B; eFigure E, F, links.lww.com/NXG/A568). Intensive screening tests showed no typical causes or risk factors known to be associated with ischemic stroke in young adults, such as vasculitis, antiphospholipid antibody syndrome, protein C/S deficiency, and Fabry disease.

Patient 6 (III-6) was a 47-year-old man with heterozygous p.A252T. He developed lumbago in his twenties. At 39 years, he developed headaches, and MRI showed multiple cerebral infarctions. Neurologic findings were normal. He had no risk of atherosclerosis. He was treated with antiplatelet therapy but had repeated cerebrovascular events. At 43 years, he was diagnosed with hyperlipidemia and hypertension and treated with pitavastatin and amlodipine. At 48 years, he developed cognitive decline and parkinsonism. On the basis of the Wechsler Adult Intelligence Scale 4th edition, he had a full-scale IQ of 78, verbal comprehension index of 96, perceptual reasoning index of 76, working memory index of 91, and processing speed index of 66. Serial MRI analyses showed WMHs around the lateral ventricles that gradually fused and extended subcortically (Figure 2C). Abnormal intensity was also observed in the external capsule and brainstem (eFigure G, H, links.lww.com/NXG/A568). Microbleeds were prominent on the brainstem surface and increased over time (Figure 2D). We also ruled out disease-causative variants of *NOTCH3*, *CTSA*, *GLA*, *COL4A1*, and *TREX1*.

Patient 7 (III-2) was a 52-year-old woman with heterozygous p.A252T. She developed lumbago at 38 years. She had no medical history of stroke but had hypertension and hyperlipidemia. MRI showed asymptomatic WMHs.

Patient 8 (III-8) was a 39-year-old woman with heterozygous p.A252T. She developed lumbago in her twenties but had no other clinical symptoms or brain imaging abnormalities.

Patient 9 (III-10) was a 39-year-old unaffected woman with heterozygous p.A252T.

Discussion

Among previously reported CARASIL cases, family members who carried heterozygous variants were reported to be asymptomatic.[1] Recently, patients with isolated and hereditary CSVD who carried uniallelic *HTRA1* variants have been reported worldwide. Missense variants of HTRA1-linked AD-CSVD are frequently located in the linker region or L3/LD domain, both of which are critical sites for HTRA1 activation. Moreover, patients with symptomatic CSVD with uniallelic null variants have been reported. Thus, residual protease activity of HTRA1 is

considered to be a risk factor associated with the clinical phenotype of HTRA1-linked CSVD.[4,5] Uniallelic p.A252T has not been considered to cause disease because p.A252T is distant from the L3/LD domain and has relatively preserved residual protease activity with no dominant-negative effect.[6] However, our study revealed that patients with uniallelic p.A252T can have severe and young-onset CSVD.

The most important finding of this study is the large heterogeneity of clinical manifestations within a single family. Similar intrafamilial variations in HTRA1-linked AD-CSVD have been reported,[7,8] and sex (male) and atherosclerosis risk factors, including hypertension and hyperlipidemia, are considered to be related to CSVD development.[4] These findings suggest that strict control of vascular risk factors can prevent vascular events in uniallelic *HTRA1* carriers. Among uniallelic p.A252T carriers, the clinical phenotypes of patients 4 and 5 were comparable with those of a family member with the biallelic variant, whereas those of other heterozygous members were milder or asymptomatic. The current study suggests that heterozygous status of p.A252T and other variants with relatively preserved residual protease activity can be at risk, and other variables including vascular risk factors have a much stronger effect than currently believed.

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