

[CASE REPORT]

Cerebral Small Vessel Disease Related to a Heterozygous Nonsense Mutation in *HTRA1*

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Abstract:

Homozygous or compound heterozygous mutations in the high-temperature requirement A serine protease 1 gene (*HTRA1*) cause cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy, a very rare hereditary cerebral small-vessel disease (SVD). Recently, the relationship between some heterozygous *HTRA1* mutations, most of which are missense, and the occurrence of cerebral SVD has been reported. We herein report a patient with cerebral SVD carrying a heterozygous nonsense p.R302X mutation in *HTRA1*. This patient had a family history of cerebral infarction. This report suggests that a heterozygous p.R302X mutation in *HTRA1* causes an autosomal dominant cerebral SVD.

Key words: CARASIL, *HTRA1*, heterozygote, nonsense mutation, small vessel disease

(Intern Med 59: 1309-1313, 2020)

(DOI: 10.2169/internalmedicine.4041-19)

Introduction

Cerebral small-vessel disease (SVD) refers to a group of heterogeneous disorders that affect the small arteries, arterioles, capillaries, and venules in the brain, in which small blood vessel injuries often lead to stroke and vascular dementia. The major prevailing risk factors for cerebral SVD are hypertension, dyslipidemia, and smoking; however, approximately 5% of cerebral SVD cases are hereditary.

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a very rare, monogenic form of hereditary cerebral SVD that was originally identified in the Japanese population (1). Both early-onset spondylosis deformans and early-onset alopecia are thought to be characteristic extra-neurological features of CARASIL (1). As the eponym suggests, its clinical manifestations, such as early-onset subcortical infarcts and cognitive decline, resemble those of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is a more common monogenic cerebral SVD caused by heterozygous mutations in the *NOTCH3*

gene.

Migraine is often the first clinical symptom of CADASIL. Unlike CADASIL, CARASIL is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the high-temperature requirement serine protease 1 gene (*HTRA1*) (2). Interestingly, heterozygous mutations in *HTRA1* have recently been identified in patients with familial cerebral SVD (3-5). The accumulation of such cases suggests that some heterozygous *HTRA1* mutations are associated with autosomal dominant cerebral SVD.

We herein report a man with late-adult onset cerebral SVD carrying a heterozygous nonsense p.R302X mutation in *HTRA1*. The patient's deceased father also showed dysarthria and spastic gait and had been diagnosed with cerebral infarction in his early 50s. Since the clinical manifestations of these two individuals were very similar, the heterozygous p.R302X mutation in *HTRA1* might result in autosomal dominant cerebral SVD.

Case Report

A 56-year-old Japanese man was referred to the neuro-

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Received: October 10, 2019; Accepted: January 6, 2020; Advance Publication by J-STAGE: February 26, 2020

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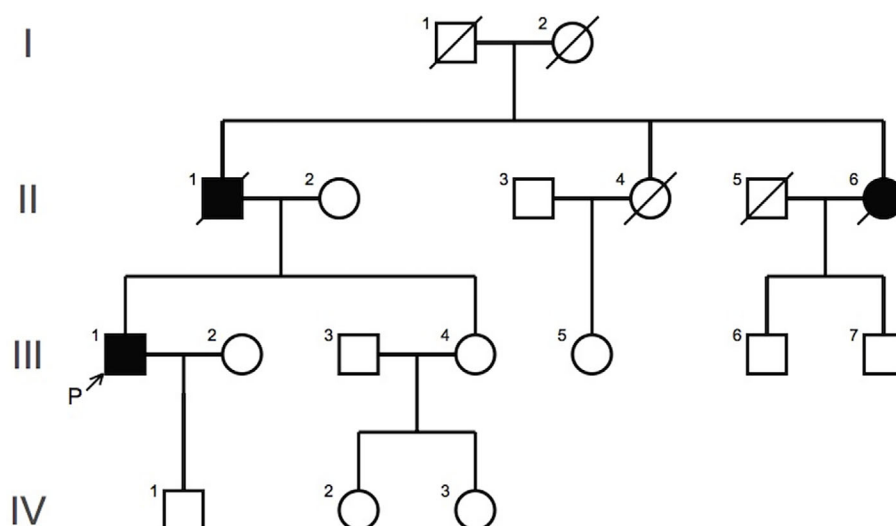


Figure 1. Genealogical tree of the patient. Square, man; circle, woman; diagonal black line, deceased; black-filled symbol, affected individual (with cerebral infarction); empty symbol, unaffected individual: P (arrow), proband. Consanguineous marriage was not observed in this family.

ogy department of our hospital to investigate the cause of his cerebral SVD. He had experienced his first symptoms of mild left facial palsy, mild paresis of the left leg, and numbness in the left hand at 49 years old. Five months after the occurrence of the initial symptoms, he visited a clinic in the city where he lived. Because magnetic resonance imaging (MRI) of his brain revealed multiple lacunae, antiplatelet therapy was started. However, he stopped going to the clinic about five years later. At 55 years old, he visited the neurosurgery department of a general hospital because of his progressive gait disturbance and was diagnosed with cerebral SVD based on brain MRI findings. The patient had a family history of cerebral infarction (Fig. 1). His father (II-1) who experienced dysarthria and spastic gait in his early 50s and gradually became bradykinetic, had been diagnosed with cerebral infarction and died at 67 years old. According to the patient's own account, his father showed kyphosis in his later years but did not have evident cognitive impairment, alopecia, or migraine. However, his father's clinical records and neuroimaging data had already been disposed of since the legal storage period had long passed. The patient's aunt (II-6) had also died of cerebral infarction in her 70s, but there was no evidence to associate the pathogenesis of her illness with any genetic factors, as the detailed symptoms and clinical course were unknown.

Since the patient's attending doctor in the general hospital suspected a hereditary form of cerebral SVD based on his clinical symptoms, brain MRI findings, and family history of ischemic stroke, the patient was referred to our hospital for more precise examinations, including a genetic analysis.

When the patient visited our hospital, a neurological examination revealed spastic paraplegia, mild left hemiparesis, including central-type left facial palsy, numbness in the left hand, and mild dysarthria. He could walk slowly on flat ground with a cane but easily fell down without it. He could

climb steps very slowly while holding on to the handrail. He did not have alopecia, spondylosis deformans, or migraine. His scores on the Mini-Mental State Examination and Frontal Assessment Battery were 29 out of 30 and 12 out of 18, respectively. His blood sugar levels and HbA1c values were normal, as were his plasma antithrombin, protein C, and protein S activities. His anti-cardiolipin- β_2 -glycoprotein I complex antibody and lupus anticoagulant tests were negative. The patient had smoked approximately 20 cigarettes per day until the first cerebral infarction episode. He started drinking at the age of 20. In his younger days, he consumed the alcohol equivalent of nearly 80 mg of ethanol per day at most but consumed 1 drink approximately once every 10 days after the first onset of cerebral infarction. He had no history of hypertension or dyslipidemia. The patient's brain MRI findings revealed diffuse leukoencephalopathy involving the periventricular area and extending into the deep white matter with multiple lacunar infarcts (Fig. 2A, B). MRI also showed hyperintense lesions in the pons, with fluid-attenuated inversion recovery and T2-weighted images showing bilateral middle cerebellar peduncles. Although the signal intensities were rather weak in this patient, the MRI findings in the pons and middle cerebellar peduncles resembled characteristic features of advanced CARASIL, termed the "arc sign" by Nozaki et al. (6) (Fig. 2C, D). Cerebral microbleeds were not found by T2*-weighted MRI of his brain.

After obtaining his written informed consent, we analyzed all exons of 24 hereditary vasculopathy-related genes, including *NOTCH3* and *HTRA1*, by next-generation sequencing. The gene analysis was approved by the institutional ethics committee of the National Hospital Organization Niigata National Hospital. We detected a heterozygous C to T single nucleotide substitution at c.904 (NM_002775.4:c.904C>T, dbSNP ID rs113993970) in *HTRA1* that changed an arginine

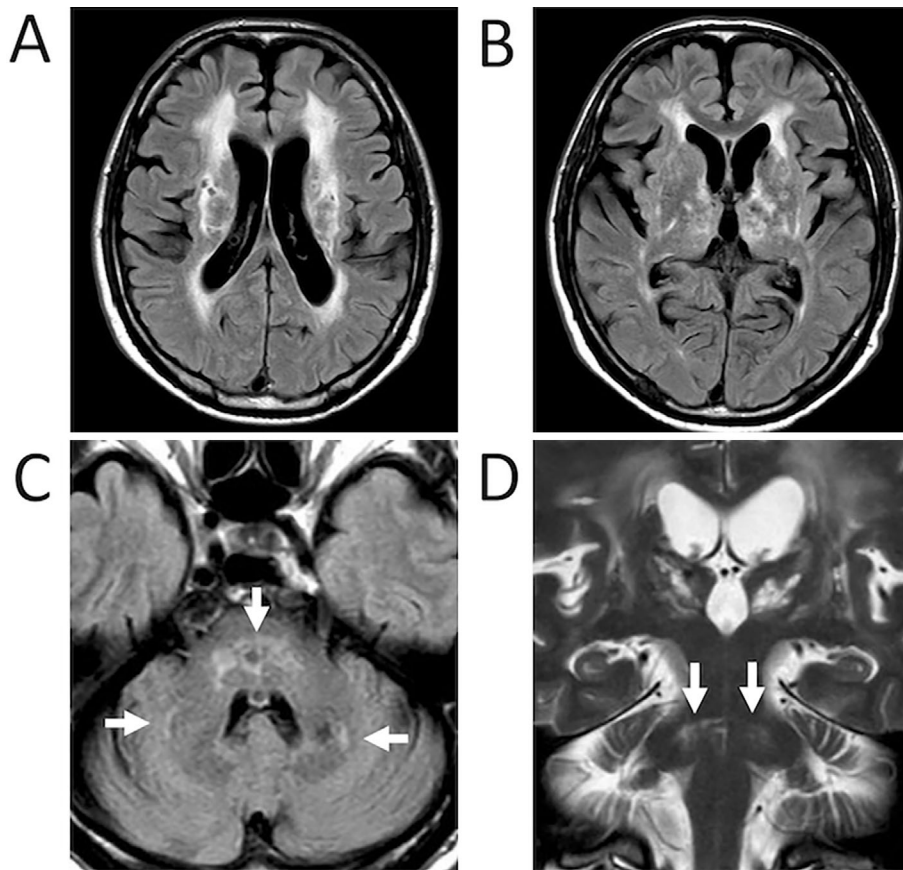


Figure 2. Brain magnetic resonance imaging (MRI) findings of the patient. (A) Axial view of a fluid-attenuated inversion recovery (FLAIR) image showing diffuse leukoencephalopathy involving the periventricular region and extending into the deep white matter. (B) Axial view of a FLAIR image showing hyperintense lesions in the bilateral external and internal capsules of the basal ganglia with multiple lacunar infarcts in the thalami. (C) Axial view of a FLAIR image at the pons level showing hyperintense lesions in the pons and bilateral middle cerebellar peduncles (arrows). Although the signal intensities were weak in this patient, these findings resemble the characteristic features of advanced cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), termed the “arc sign” by Nozaki et al. (6). (D) Coronal view of a T2-weighted image showing multiple hyperintense lesions in the bilateral thalami and belt-like hyperintense signals in the bilateral middle cerebellar peduncles (arrows).

codon (CGA) into a stop codon (TGA) at amino acid position 302 (p.R302X) of the protein (Fig. 3A). In addition to the nonsense mutation in *HTRA1*, this patient was heterozygous for a known pathogenic frame-shift mutation p.V848CfsX83 (NM_001171.5:c.2542delG, dbSNP ID rs67867306) in the ATP-binding cassette subfamily C member 6 gene (*ABCC6*) (Fig. 3B). Alteration to the *ABCC6* gene cause pseudoxanthoma elasticum (PXE), which is a hereditary metabolic disorder with autosomal recessive inheritance. The phenotypic expression of PXE is characterized by the fragmentation of elastic fibers and ectopic mineralization in the skin, eye, and cardiovascular system.

Based on the results of the *ABCC6* gene analysis, the patient underwent dermatological, ophthalmic, and radiological examinations. As a result, neither characteristic cutaneous lesions, such as small yellowish papules or plaques typically located on the neck and axilla, nor characteristic ophthalmic lesions, such as angioid streaks in the ocular fundus, were

found. Calcification of the medium- and small-sized arteries, which are elementary vascular changes in PXE, was not observed by X-ray examinations. No pathogenic variants were found in *NOTCH3*, the gene responsible for CADASIL.

Discussion

We encountered a Japanese man with late-adult onset cerebral SVD harboring both a heterozygous p.R302X mutation in *HTRA1* and a heterozygous p.V848CfsX83 mutation in *ABCC6*. The patient’s deceased father had also shown spastic gait and dysarthria in his early 50s and been diagnosed with cerebral infarction. Given the similarities of the clinical manifestations between the index patient and his father, we suspected a hereditary form of cerebral SVD. Interestingly, the brain MRI findings of the patient appeared to be consistent with CARASIL, although the severities of signal changes were somewhat mild for the typical findings of

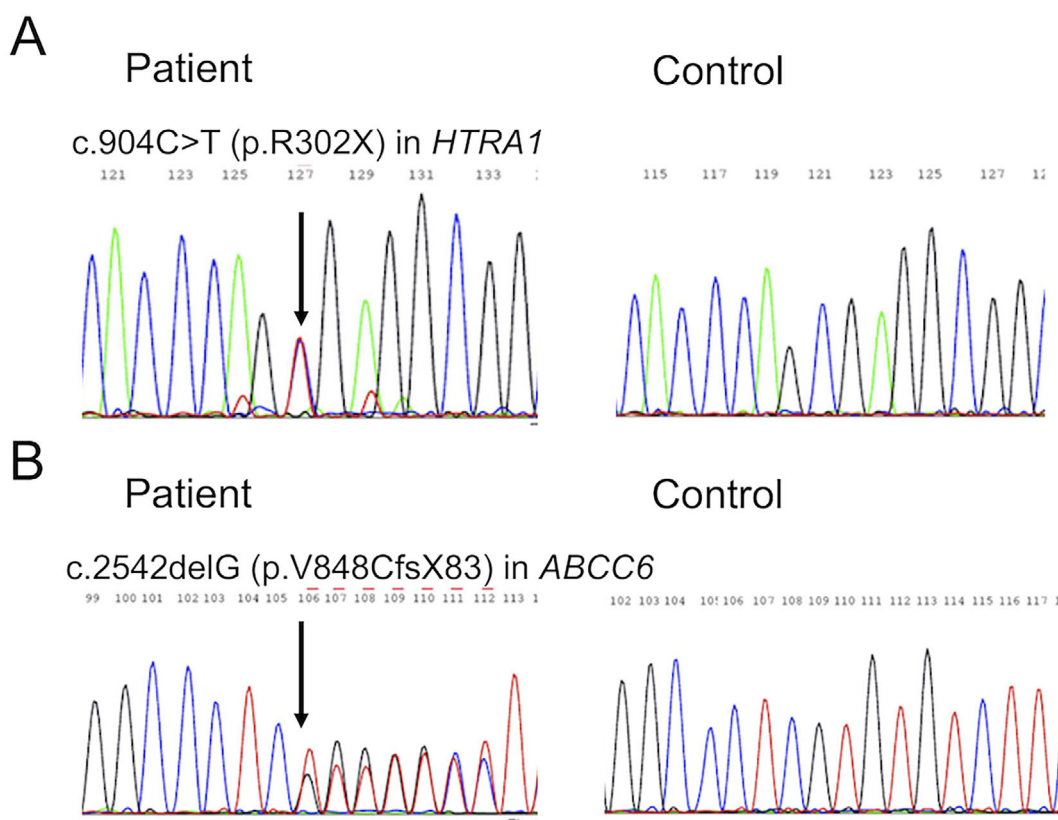


Figure 3. Results of a genetic analysis of *HTRA1* and *ABCC6* in the patient. (A) An electropherogram of exon 4 of the patient's *HTRA1* gene. A heterozygous C>T substitution was confirmed at position c.904 (arrow), changing an arginine codon (CGA) into a stop codon (TGA) at amino acid position 302 (p.R302X). (B) An electropherogram of exon 19 of the patient's *ABCC6* gene. A heterozygous single nucleotide deletion c.2542delG was confirmed (arrow), inducing an amino acid change and frame-shift (p.V848CfsX83).

this condition.

Notably, the patient showed no clinical symptoms of PXE. Along with abnormalities in elastic fibers, the ectopic calcification of medium- and small-sized arteries causes vascular manifestations, including hypertension, coronary artery disease, intermittent claudication, and stroke, in patients with PXE (7, 8). The distribution of arterial calcification, especially in the lower extremities, can be found by standard X-ray in PXE (7); however, we failed to detect any unusual calcification of the arteries in the present patient. Although the allele frequency of p.V848CfsX83 mutations in *ABCC6* in the Japanese population is not very low (0.015 in 3.5 KJPN and 0.039 in HGVD), cerebral SVD is less frequently described in association with PXE than *HTRA1* mutations. Furthermore, there is no clear evidence that heterozygous carriers of pathogenic mutations in *ABCC6* develop vascular manifestations. We therefore presumed that p.R302X in *HTRA1* was primarily related to the occurrence of cerebral SVD in this patient, although the effect of the co-existing *ABCC6* mutation could not be completely ruled out.

Individuals with the heterozygous nonsense mutation p.R302X in *HTRA1* were thought to be asymptomatic (2); however, Tateoka et al. reported the first manifested case of cerebral SVD with a heterozygous p.R302X mutation in

HTRA1 in 2016 (9). They reported a 63-year-old man who had recurrent lacunar infarctions at least 6 times over 4 years despite antiplatelet therapy. As with our patient, the patient did not have alopecia, spondylosis deformans, or migraine. Brain MRI of the patient revealed lacunar infarcts, diffuse leukoencephalopathy, and multiple microbleeds. In contrast to our case, his family history of ischemic stroke was unremarkable. To our knowledge, this is the second report of a patient with cerebral SVD harboring a heterozygous p.R302X mutation in *HTRA1*.

HTRA1 is a serine protease that represses the inhibition of TGF- β family signaling. Increased TGF- β family signaling caused by a complete loss or the marked reduction of the *HTRA1* protease activity is thought to contribute to the pathogenesis of CARASIL (2). Very recently, heterozygous *HTRA1* mutations were identified in patients with adult-onset cerebral SVD. To date, 30 heterozygous mutations in *HTRA1* have been found in patients with sporadic or familial cerebral SVD. These mutations were all missense except for two nonsense mutations (p.R302X, p.Q289X) and one frame-shift mutation (p.A182PfsX33) (5). All of these mutant proteins were shown or expected to have reduced protease activity. The clinical phenotypes of heterozygous carriers of these *HTRA1* mutations tend to be milder than those

of patients with classical CARASIL, probably due to differences in residual protease activity. The molecular mechanism by which these mutations cause cerebral SVD is of great interest; a dominant-negative effect is a convincing explanation, since homotrimer formation is required to activate the adjacent HTRA1 proteins (10). Indeed, several studies have experimentally demonstrated that certain missense mutant HTRA1 proteins show a marked decrease in protease activities as well as reduced wild-type HTRA1 activity, presumably due to interference in the trimer-dependent activation cascade of the enzyme. Recently, two different mechanisms were proposed to underlie the dominant-negative effects of *HTRA1* mutations (4, 11): the inhibition of trimer formation, and the inhibition of the wild-type HTRA1 activity, observed in several missense mutations located in the sensor domain of loop 3 (L3) or the activation domain of loop D (LD) of HTRA1. These two domains play an essential role in the normal protease activity of HTRA1 (10, 12). Among the previously reported missense mutations, p.R302Q, which replaces the evolutionarily well-conserved arginine with glutamine, is the only mutation located in L3. It was experimentally demonstrated that p.R302Q markedly decreased the protease activity, maintained the ability to form trimers, and inhibited the protease activity of wild-type HTRA1 protein (dominant-negative effect) (4). These results have important implications for the pathogenesis of the p.R302X mutation.

p.R302X was shown to result in the complete loss of protease activity (11); however, its ability to form trimers or express a dominant-negative effect has not yet been determined. In addition, Lee et al. showed that p.Q289X and p.A182PfsX33 induced the complete loss of protease activity and dominant-negative effects, although the exact mechanisms were not elucidated (5). These studies suggest that a reduction in the protease activity is prerequisite for a heterozygous mutation in *HTRA1* to induce cerebral SVD and that the dominant-negative effect is a probable mechanism underlying the pathogenesis of the HTRA1-related cerebral SVD.

However, some mutations identified in manifested carriers did not show dominant-negative effects (4, 5, 11). In these cases, haploinsufficiency, which often results in reduced penetrance and variable expressivity, may explain the occurrence of cerebral SVD.

Conclusions

We encountered a patient carrying a heterozygous nonsense p.R302X mutation who presented with cerebral SVD.

The deceased father of the patient had also shown dysarthria and spastic gait in his early 50s and been diagnosed with cerebral infarction. This report indicates that *HTRA1*-related cerebral SVD should be considered, especially when a patient has a family history of SVD.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

The authors thank the patient who provided the data for this report.

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