Case Report

Histopathologic features of an autopsied patient with cerebral small vessel disease and a heterozygous HTRA1 mutation

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Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a hereditary cerebral small vessel disease (CSVD) caused by homozygous or compound heterozygous mutations of the high temperature requirement A serine peptidase 1 gene (HTRA1). Affected patients suffer from cognitive impairment, recurrent strokes, lumbago and alopecia. Recently, clinical studies have indicated that some patients with heterozygous mutations in HTRA1 may also suffer CSVD. Here, we report the histopathologic features of an autopsied 55-year-old male patient who had shown cognitive impairment and multiple cerebral infarcts, and was found to have a heterozygous missense mutation (p.R302Q) in the HTRA1 gene. Histologically, small vessels in the brain and spinal cord showed intimal proliferation, splitting of the internal elastic lamina, and degeneration of smooth muscle cells in the tunica media. Thus, although less severe, the features were quite similar to those of patients with CARASIL, indicating that patients with heterozygous mutations develop CSVD through underlying pathomechanisms similar to those of CARASIL.

Key words: autopsy, CARASIL, cerebral small vessel disease, HTRA1, TGF-β1.

INTRODUCTION

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is a hereditary cerebral small vessel disease caused by homozygous or compound heterozygous mutations in the high temperature requirement A serine peptidase 1 gene (HTRA1). HTRA1 represses the production of transforming growth factor-β (TGF-β), whereas mutated HTRA1 has decreased protease activity and does not repress the production of TGF-β. As a result, the amount of TGF-β increases, and the subsequent TGF-β signaling may cause cerebral small vessel disease (CSVD). Histopathologically, CARASIL is characterized by distinctive arterial changes and associated leukoencephalopathy.

Recently, clinical studies have indicated that some patients with heterozygous mutations in HTRA1 may also develop CSVD similar to that of patients with CARASIL. In these patients, mutated HTRA1 has decreased protease activity or inhibits wild-type HTRA1 activity. So far, only a single autopsy report of a patient with a heterozygous HTRA1 mutation, p.G283E, has been published, the vessels showing similar histological features of those of CARASIL. However, it has remained unclear whether patients with heterozygous mutations show variability of the changes seen in vessels and whether there is a genotype–phenotype correlation.

Herein, we report in detail the clinicopathological features of an autopsied patient with a heterozygous HTRA1 mutation, p.R302Q, and discuss their significance in relation to the histogenesis of CSVD.

CLINICAL SUMMARY

A 55-year-old Japanese man visited a hospital because of cognitive impairment with episodes of forgetting his own job schedule and becoming lost. At the age of 30 years, he had undergone surgery for lumbar disc hernia. At the age of 47 years, he had developed follicular lymphoma and received chemotherapy and autologous peripheral blood stem cell transplantation, which had successfully achieved
remission. Alopecia was not observed. The patient was a smoker, but no other risk factors for stroke, including hypertension, diabetes mellitus or dyslipidemia, were noted. His parents had not been consanguineous. His father had a history of cerebral hemorrhage in his 50s, and two out of four of his brothers had developed cerebral infarction in their 40s. The Hasegawa dementia scale revised (HDS-R) score was 28/30. Brain magnetic resonance imaging (MRI) fluid-attenuated inversion recovery (FLAIR) images demonstrated hyperintensity in the deep and periventricular white matter (Fig. 1A, B), and T2-star gradient echo imaging demonstrated several foci of microbleeding (Fig. 1C). Clinically, the patient was diagnosed as having vascular dementia, and aspirin was administered. At the age of 59 years, he suddenly developed dysarthria. Neurological examination revealed dysphagia, right facial palsy, left hemiplegia, and eye movement disability. Based on the findings of a CT scan, he was diagnosed as having pontine hemorrhage. In the same year, a genetic analysis of his younger brother, who had developed cognitive impairment and leukoaraiosis at the age of 44 years, revealed a heterozygous missense mutation, p.R302Q, in the HTRA1 gene. Therefore, with informed consent, we further performed genetic analysis of the present patient and found that he harbored the same mutation. Subsequently, he suffered from secondary myelodysplastic syndrome after high-dose chemotherapy with autologous stem cell support for treatment of the malignant lymphoma. He died of bronchial pneumonia at the age of 61 years.

This patient was recruited in a genetic study of heterozygous HTRA1 mutations.4

PATHOLOGICAL FINDINGS

At autopsy, the fresh brain weighed 1330 g, and showed no cerebral atrophy. Moderate atherosclerosis of the intracranial large arteries was observed. On multiple coronal sections of the cerebrum, no apparent changes in the white matter were evident (Fig. 2A, B), but slight and focal myelin pallor was seen in the frontal white matter with preservation of U-fibers (Fig. 2C). The corpus callosum was severely atrophic (Fig. 2A, B), and showed linear myelin pallor and macrophage infiltration, indicating funicular degeneration resulting from a small infarct involving the left internal capsule (Fig. 2C). A hemorrhagic infarct involving the pontine tegmentum on the right side (Fig. 2D) was also observed.

Fig. 1  Brain MRI FLAIR images. (A, B) images demonstrating hyperintensity in the deep and periventricular white matter. (C) T2-star gradient echo image showing several foci of microbleeding in the bilateral periventricular white matter (arrows).

Fig. 2  Gross macroscopic findings in the brain. (A, B) A coronal section of the left cerebral hemisphere through the basal ganglia, showing severe atrophy of the corpus callosum, but no other apparent gross abnormalities (A) or myelin pallor (B) in the white matter. (C) A coronal section of the frontal convexity showing mild myelin pallor in the deep white matter. The corpus callosum showing linear myelin pallor (arrowheads). (D) A transverse section of the pons, showing a hemorrhagic infarct involving the tegmentum on the right side. (B, C) Klüver–Barrera stain. Scale bar: 10 mm (B) and 8 mm (C).
Many leptomeningeal arterioles with a diameter of less than 100 μm showed splitting of the internal elastic lamina, proliferation of smooth muscle cells (SMC) and fibrosis of the adventitia. The outer rim of the internal elastic lamina attached to the adventitia. Some arterioles showed lipohyalinosis (Fig. 3C). The lumen of some arterioles was narrow or occlusive with marked fibrosis (Fig. 3D). Arterioles in the cerebral white matter, basal ganglia and brainstem showed similar changes (Fig. 3E, F). Leptomeningeal small arteries with a diameter of 100–500 μm showed proliferation of myointimal cells, multilayered elastic lamina, and marked loss of SMC (Fig. 3G–I), but the lumen was not narrow, rather showing distorted dilation (Fig. 3J). Several thin arteries branching from thick arteries formed aneurysm-like structures (Fig. 3I). Immunohistochemistry with an anti-TGF-β1 antibody (monoclonal, clone TB21; Abcam, Cambridge, UK; diluted 1:200, pretreated by heating) showed marked reactivity in the tunica media of the small arteries (Fig. 3K). On the other hand, leptomeningeal large arteries with a diameter of more than 500 μm showed severe atherosclerosis, although degeneration of the tunica media was not evident. Several foci of microbleeding were observed in the cerebral white matter (Fig. 3L). Electron microscopy examination of the leptomeningeal arterioles revealed dense deposits in the outer layer of the elastic lamina, proliferation of myointimal cells, an increase of extracellular matrix, and loss of SMC in the tunica media (Fig. 4).

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There were no specific features of neurodegeneration, including senile plaques, amyloid angiopathy, neurofibrillary tangles, argyrophilic grains or Lewy bodies.

General autopsy revealed bronchial and organizing pneumonia. Severe atherosclerosis was observed in the aorta and coronary arteries. However, there were no significant pathologic features of small vessels in the visceral organs and skin. There was also no evidence of malignant lymphoma recurrence.

**DISCUSSION**

In patients with CARASIL, cerebral small vessels show intimal thickening, multilayering and splitting of the elastic lamina, loss of SMC and fibrosis of the adventitia. In the present patient with a heterozygous HTRA1 mutation, the histopathologic features of the small vessels (Fig. 3) were quite similar to those of CARASIL, but apparently less severe. On the other hand, in non-hereditary CSVD, including Binswanger’s disease, loss of SMC is also a feature, but multilayering and splitting of the internal elastic lamina is uncommon. Thus, involvement of the intimal elastic lamina may be characteristic of CSVD with homozygous and heterozygous HTRA1 mutations. This alteration may lead to fragility of the vascular wall. Indeed, in the present patient, several small vessels showed distorted dilation (Fig. 3J) or aneurysm-like alteration (Fig. 3I), similar to those seen in patients with CARASIL. Moreover, we identified an electron-dense substance (Fig. 4C) that might have been degenerated or abnormally aggregated elastin, the main protein component of the elastic lamina, presumably being associated with its fragility. The tunica media of small vessels showed marked expression of TGF-β1 (Fig. 3K), suggesting that the mutant HTRA1 fails to repress TGF-β1 production.

These vascular changes were observed in intracranial arterioles and arteries less than 500 μm in diameter, and also in several arteries in the subarachnoid space of the spinal cord, but not in any vessels in the visceral organs. The changes were more remarkable in the subarachnoid space than in the CNS parenchyma, indicating that the former may precede the latter.

In the present patient, there was no apparent change of small vessels in the visceral organs and skin. In contrast, patients with CARASIL show marked narrowing of the vascular lumen, atherosclerosis-like intimal thickening and arteriolosclerosis in the arteries of visceral organs and skin. However, these histological characteristics of the extracranial vessels appear to be quite different from those of intracranial small vessels. Furthermore, it seems unlikely that these features are clearly distinct from athero- and arteriolosclerosis.

Leukoencephalopathy and infarction are common in both hereditary and non-hereditary CSVD. It has been recognized that abnormality of small vessels may disturb the autoregulatory mechanisms for cerebral blood flow, resulting in leukoencephalopathy and ischemic changes in the white matter. In the present patient, the distribution of abnormal small vessels in the cerebral white matter corresponded well to the areas of myelin pallor, and the number of arteries with luminal narrowing was small. These findings seem to be consistent with the hemodynamic theory.

To our knowledge, this is only the second autopsy report of a patient with CSVD and heterozygous HTRA1 mutation. The first such patient reported, who had a p. G283E HTRA1 mutation, showed severe vascular changes
and leukoencephalopathy. In comparison with that patient, the present one had apparently mild vascular changes and leukoencephalopathy. It has been shown that the protease activity can differ according to the mutation locus in HTRA1 and that the activity might correlate with the severity of the vascular changes and leukoencephalopathy. It has been reported that the R302Q HTRA1 mutant, as was seen in the present patient, has markedly reduced protease activity, and exerts a dominant-negative effect on wild-type HTRA1 protease activity. Therefore, in the present patient, although a severe pathologic phenotype was expected, this was not the case. This discrepancy may have affected the duration of the illness. Most autopsied patients with CARASIL died over 10 years after disease onset. On the other hand, the disease duration in the present patient was 6 years, suggesting that death occurred during disease progression. Therefore, further clinicopathologic studies are needed to clarify the severity of the vascular pathology in patients with heterozygous mutations.

The present patient developed cognitive impairment at the age of 55 years, and subsequently suffered from strokes. Therefore, it seems unlikely that the cognitive impairment was induced by cerebral infarction. It seems more likely that leukoencephalopathy, although mild in severity, may have been responsible for the cognitive impairment in this case.

The clinicopathologic features of the present patient suggest that patients with heterozygous HTRA1 mutations develop CSVD through underlying pathomechanisms similar to those of CARASIL. Further pathologic studies will be needed to clarify the significance of the vascular changes in the histogenesis of leukoencephalopathy in patients with heterozygous HTRA1 mutations.

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The authors have no conflicts of interest to declare.

REFERENCES


