LETTER TO THE EDITOR

A Novel Mutation in the *HTRA1* Gene Identified in Chinese CARASIL Pedigree

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Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is an inherited autosomal recessive vascular diseases characterized by stroke onset in nonhypertensive young adults, progressive motor and cognitive impairment, alopecia, and spondylosis [1–4]. The brain magnetic resonance imaging (MRI) shows diffuse leukoencephalopathy and multiple subcortical infarcts [1–3]. Histopathology of brain is characterized by intense arteriosclerosis without the deposition of granular osmiophilic materials (GOMs) [1–4]. The reports on CARASIL are rare. Up to now, there are only about 50 reported cases, and most of them came from Japan except for a Chinese pedigree [5] and a Caucasian [6]. In 2009, Japanese scholars found that CARASIL was associated with mutations in the *HTRA1* gene encoding HtrA serine protease 1 [7]. To date, four missense mutations and two nonsense mutations were identified in *HTRA1* gene. We herein present a Chinese pedigree of patients with CARASIL and identified a novel mutation in their *HTRA1* gene.

The proband (IV2, Figure 1A) was a 27-year-old woman who had experienced severe pain in her right foot and lumbar from age 25 when she was pregnant for about 6 months. She was diagnosed with prolapse of lumbar intervertebral disk (L5/S1) through lumbar computed tomography examination after cesarean section. Despite a lumbar intervertebral disk operation, her pain continued. Subsequently, she developed right limb disability, accompanied with dizziness, poor memory, and forced laughter half a year ago. General physical examination showed no hypertension or other abnormalities except mild alopecia (Figure 1B). Neurological examination showed intelligence decline, mild dyslexia, spastic gait, and increased tender reflexes of the lower extremities with bilateral positive Babinski sign. Laboratory tests involving hemograms, biochemical analysis and cerebral spinal fluid analysis showed normal values. The electromyographic result showed no abnormalities. Lumbar MRI revealed multiple-level narrowing of the lumbar vertebral disks. Electroencephalography

showed diffuse slow waves. Brain MRI demonstrated diffuse leukoencephalopathy that was hyperintense on T2-weighted and T2-Flair (Figure 1C). Scattered multiple lacunar infarcts and encephalomalacia foci were seen in the periventricular white matter and centrum semiovale. Skin and sural nerve biopsy (Figure 1D) showed concentric thickening of the vascular wall, narrowing of the lumen, and mild fibrous proliferation of the intima. There were no amyloid, periodic acid Schiff granular deposition, and ultrastructural GOMs on the vascular wall.

The other patient was proband's younger brother (IV3, Figure 1A). He had been afflicted with lumbodysnia for 5 years and left limb disability for half a year, accompanied with poor memory, forced laughter, mental dullness. No other abnormalities except his baldness (Figure 1B) were elicited on the general physical examination, which were more severe than the proband. Neurological examination showed intelligence decline and positive Babinski and Chaddock signs on the left lower extremities. Brain MRI demonstrated diffuse subcortical leukoencephalopathy.

The pedigree included five generations of a family with two members affected by the disease (Figure 1A). The parents of the proband (III1,2) were first cousins and both died in their 50s, accompanied with emotional instability. Physical examinations of other family members showed no abnormalities.

We identified a homozygous T to C missense mutation (c.1091T>C) in exon 6 in the *HTRA1* gene of the two patients through direct sequencing (Figure 2A). Both proband's parents and daughter had the heterozygous c.1091 T>C mutation. We collected 100 healthy people as control for the sequencing of the exon 6 and did not find the same mutation. As this mutation is not described in SNP databases, we believe that it is not a gene polymorphism. To exclude cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, we sequenced the exons 1–33 of *NOTCH3* gene on patients and found no mutation. Informed consent was obtained from all subjects,

Figure 1 The clinical, radiological, and pathological features of the patients with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). **(A)** The pedigree chart of the family with CARASIL. Arrow, the proband; solid symbols, patients; squares, men; circles, women; oblique, dead siblings. **(B)** The upper picture showed mild hair loss in the proband, and the lower showed severe baldness in her younger brother. **(C)** Brain MRI revealed remarkable cerebral white matter lesions and multiple lacunar infarcts (the upper were the proband; the lower were her younger brother). **(D)** Skin and sural nerve biopsy of the proband showed concentric thickening of the vascular wall, narrowing of the lumen (a, HE stain, bar = 25 μ m), and mild fibrous proliferation of the intima (b, Masson stain, bar = 25 μ m). There was no amyloid deposition on the vascular wall (c, Congo red stain, bar = 25 μ m) and no ultrastructural granular osmiophilic material (GOMs) on the basement of smooth muscle cells (d, Electron microscopy, bar = 1 μ m).

Figure 2 The gene sequencing result of the family with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). **(A)** The two patients had a novel homozygous mutation at 364 position in exon 6. The parents and proband's daughter had heterozygous T/C, and the healthy controls had T/T. **(B)** The mutation at 364 revealing a substitution of leucine to proline is located in a highly conserved amino acid residue region (shaded in gray).

and the study was approved by the Ethics Committee on human experimentation of our institution.

The diagnosis of CARASIL in our cases was confirmed clinically, histopathologically, radiologically, and genetically. This mutation is first identified in Chinese CARASIL patients. Different to other four known missense mutations (G297A, V252T, V295M, and R274Q) [6–8], it is a novel missense mutation (c. 1091T>C) in *HTRA1* gene, which results in the substitution of leucine to proline

(p.L364P). It locates in the trypsin-like serine protease domain, a highly conserved region, as was verified by homological analysis of HtrA1 (Figure 2B). This mutation might lead to the change in HtrA1 protease activity and further contribute to the pathogenesis of CARASIL [9,10].

Much work is needed to further verify the pathogenicity of the novel mutation in *HTRA1* gene. At present, there is no effective treatment for patients with CARASIL. The identification and over-

all understanding of the causative gene may hold out the promise of definite diagnosis of CARASIL and potential target gene therapy in the future.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Maeda S, Nakayama H, Isaka K, Aihara Y, Nemoto S. Familial unusual encephalopathy of Binswanger's type without hypertension. *Folia Psychiatr Neurol Jpn* 1976;**30**: 165–177.
2. Fukutake T, Hirayama K. Familial young-adult-onset arteriosclerotic leukoencephalopathy with alopecia and lumbago without arterial hypertension. *Eur Neurol* 1995;**35**: 69–79.
3. Fukutake T. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL): From discovery to gene identification. *J Stroke Cerebrovasc Dis* 2011;**20**: 85–93.
4. Arima K, Yanagawa S, Ito N, et al. Cerebral arterial pathology of CADASIL and CARASIL (Maeda syndrome). *Neuropathology* 2003;**23**: 327–334.
5. Zheng DM, Xu FF, Gao Y, Zhang H, Han SC, Bi GR. A Chinese pedigree of cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL): Clinical and radiological features. *J Clin Neurosci* 2009;**16**: 847–849.
6. Mendioroz M, Fernández-Cadenas I, Del Río-Espinola A, et al. A missense *HTRA1* mutation expands CARASIL syndrome to the Caucasian population. *Neurology* 2010;**75**: 2033–2035.
7. Hara K, Shiga A, Fukutake T, et al. Association of *HTRA1* mutations and familial ischemic cerebral small-vessel disease. *N Engl J Med* 2009;**360**: 1729–1739.
8. Nishimoto Y, Shibata M, Nihonmatsu M, et al. A novel mutation in the *HTRA1* gene causes CARASIL without alopecia. *Neurology* 2011;**76**: 1353–1355.
9. Singh N, Kuppli RR, Bose K. The structural basis of mode of activation and functional diversity: A case study with HtrA family of serine proteases. *Arch Biochem Biophys* 2011;**516**: 85–96.
10. Shiga A, Nozaki H, Nishizawa M, Onodera O. Molecular pathogenesis of cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy. *Brain Nerve* 2010;**62**: 595–599.