Novel Heterozygous HTRA1 Pathogenic Variant Found in a Chinese Family with Autosomal Dominant Cerebral Small Vessel Disease

Sir,

Cerebral small vessel disease (CSVD) represents a heterogeneous group of disorders leading to stroke and cognitive impairment. Although most of the cases are sporadic, familial monogenic causes have been identified in a growing minority of patients. CARASIL (cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy), linked to high temperature requirement protease A1 (HTRA1) gene mutations is a rare but well-known autosomal recessive CSVD. Recently, heterozygous HTRA1 mutations have been described in patients with autosomal dominant CSVD. Here, we report two autosomal dominant cases in a Chinese family with a novel p.Val279Glu (c.836T > A) mutation in HTRA1 gene.

Cerebral small vessel disease (CSVD) is a heterogeneous group of disorders affecting small arteries, arterioles, veins, and/or capillaries of the brain.[1]It is commonly recognized to be the leading cause of vascular cognitive impairment. Besides the common sporadic forms, mostly related to age and hypertension, a minority of CSVD has a monogenic cause, and most of them have a dominant inheritance pattern.[2]

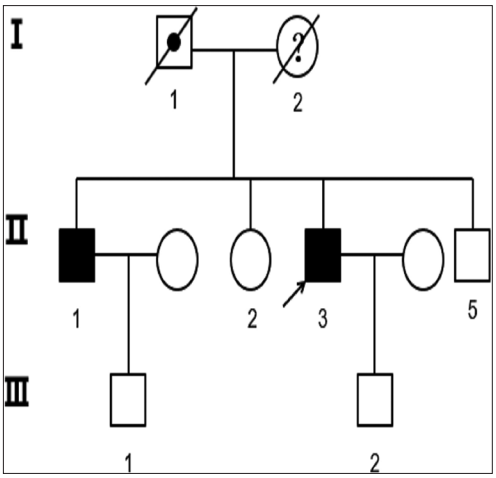

Figure 1: Pedigrees of the probands. Square = male; circle = female; diagonal black line = deceased individual; black filled symbol = clinically and MRI proven affected individual, showing an HTRA1 deleterious variant; empty symbol = clinically healthy and HTRA1 negative relative; black dot = affected individual based on clinical charts; question mark = unknown status

CARASIL is a rare form of autosomal recessive CSVD. Biallelic mutations of the HTRA1 (high temperature requirement protease A1) were the only identified reason for CARASIL.[3] Recently, heterozygous HTRA1 mutations were also reported associated with autosomal dominant CSVD.[4] In this study, two patients from a Chinese family with clinical data suggestive of autosomal dominant CVSD were investigated. We found a novel heterozygous p.Val279Glu (c.836T >A) mutation in HTRA1 gene, with negative NOTCH3 mutations.

The genealogical tree of the family is shown in Figure 1. The proband (II3) is a 51-year-old male who is a government worker with no vascular risk factor, such as hypertension, diabetes mellitus, atrial fibrillation, or smoking. At age 45 he experienced an acute episode of left-sided hemiparesis, which completely resolved in about one week. Since then, he has suffered two more minor ischemic strokes (National Institute of Health stroke scale score $\leq$ 3) at the ages of 49 and 50. Since age 49 he complained of progressive impairment of work performance and recent memory decline, especially the text dictation ability. He did not have alopecia, migraine, spondylopathy, and retinal dysfunctions. Neuropsychological testing revealed mild alterations in processing speed, executive functions, and verbal episodic and visuospatial memory performances suggestive of subcortical cognitive impairment. Mini-Mental State Examination score was 24/30. Montreal Cognitive Assessment was 23/30. Computed tomography angiography of cervical and brain arteries showed no obvious arteriostenosis. Blood cell count, antinuclear antibody, thyroid function, and other fundus examination were normal. Brain magnetic resonance image (MRI)[Figure 2] showed multifocal cerebral infarction and extensive cerebral white matter lesions. Areas of encephalomalacia, likely the sequelae of remote lacunar infarcts, were seen in the right hemisphere.

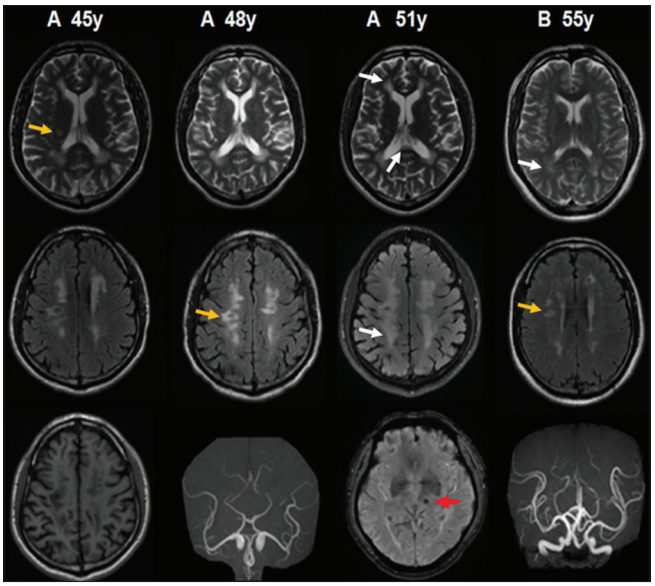

Figure 2: MRI images of proband and II-1. Left three columns: Proband at the age of 45, 48, 51. Right column: II-1 at the age of 55. First line: T2-weighted images. Second line: FLAIR images. Third line: T1-weighted image (A 45y), 3D time of flight image (A 48y, B 55y), gradient-echo image (A 51y). Multifocal cerebral infarction (yellow arrow). White matter hyperintensities (white arrow) involve corona radiata, bilateral periventricular and corpus callosum. Microbleeds (red arrow). No atherostenosis are detected

Diffuse white matter abnormalities were seen in the supratentorial white matter, corpus callosum, and basal ganglia region, but sparing the anteroinferior temporal lobes. Several microbleeds were detected on susceptibility weighted images.

His elder brother (II-1) has been suffered a cerebral lacunar infarction with right-sided hemiparesis two years ago. MRI [Figure 2] also shows several subcortical lacunar infarcts and high intensities in the corona radiate, and bilateral periventricular white matter did not show specific signal abnormalities at the temporal pole or basal ganglia. He has not yet shown signs of dementia. His father (I-1) had a history of recent memory impairment, slowness of mental processes for about five years, and died of cerebral hemorrhage at the age of 72. His head CT reports white matter degeneration in addition to right temporal lobe hemorrhage. Unfortunately, we did not find imaging data. His mother (I-2) died of cardiogenic cerebral embolismat the age of 80 with no obvious cognitive impairment.

Genetic analysis of hundreds of leukoencephalopathy related genes by targeted next-generation sequencing (NGS) in the probrevealed a novel heterozygous missense mutation [Figure 3], p.Val279Glu (c.836T>A), in exon4 of HTRA1, which is located in serine protease domain. In silico analysis with PolyPhen-2 and sorting intolerant from tolerant (SIFT) all revealed the mutation to be deleterious. The mutation was not found in the 1000 Genomes database, ClinVar, and our in-house database (including 300 next-generation

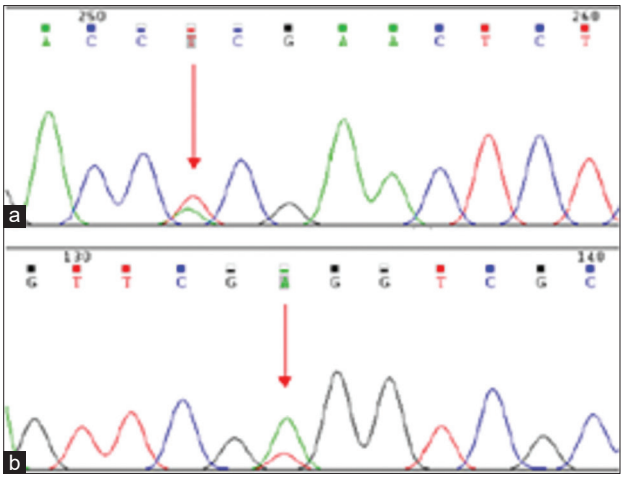

Figure 3: Sequence diagram of heterozygous HTRA1 c. 836T > A mutation in the (a) proband (II-3) and (b) his elder brother (II-1)

sequencing samples). The proband's elder brother (II-1) also carried the mutation. The rest family members (II-2, II-4, II-5, III-1) without any neurological symptoms were negative for this variant. Therefore, we believe this HTRA1 variant as a pathogenic mutation in the family.

HTRA1 in the etiology of CSVD was first described in Japanese consanguineous families of CARASIL,[4] based on the disorder's recessive inheritance and resemblance to cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Recent studies[5,6] demonstrated that heterozygous HTRA1 mutations were also associated with autosomal dominant hereditary CSVD. Compare to CADASIL and CARASIL, these studies found in heterozygous HTRA1, the clinical symptoms (mostly subcortical ischemic events and cognitive decline) is milder, the age of onset is older (usually in the sixth decade), the history of illness is long-lasting, extra-neurological signs and migraine are lacking. Brain MRI showed confluent white matter hyper-intensities on MRI T2-weighted and FLAIR images, spared anterior temporal regions and U fibers. However, the hyperintensities of white matter on MRI were less severe than in CADASIL or CARASIL. We reported two cases with the same heterozygous HTRA1 mutation, which segregates as an autosomal dominant disorder. The phenotype and MRI features of our cases are consistent with previous reports. Of note, we found corpus callosum involvement in the proband's brain MRI, which is a finding rarely observed in CADASIL, CARASIL, and sporadic CSVD. Donato *et al*. [6] had also reported one heterozygous HTRA1 mutation case with leukoencephalopathy involving corpus callosum. At the same time, we exhibit the changing of the proband's brain MRI from the first stroke, and we found the white matter hyper-intensities kept stable in the first three years, but increased when he had the symptoms of dementia, which provide a reference for future similar cases.

The HTRA1 gene located on chromosome 10q (10q25.3-q26.2) consists of 9 exons. HTRA1 has an N-terminal insulin-like growth factor–binding protein domain, a trypsin-like serine protease domain, a Kazal-type serine protease inhibitor domain, and a C-terminal PDZ domain.[7]*HTRA1* is a gene that encodes a serine protease, which regulates insulin-like growth factor by cleaving the IGR binding protein. Nozaki *et al*. demonstrated that HTRA1 with heterozygote mutations have dominant-negative effects on wild-type HTRA1 protease activity due to its inability to form stable trimmers.[8]Truebestein *et al*.[9] found the trimerization of HTRA1 is mainly mediated by N-terminal residues of the protease domain: Tyr169, Phe171, and Phe278 of each monomer undergo ring stacking interactions to stabilize the trimer. The mutation V279G found in our patients is located near Phe278, which is a core residue for trimer formation.[9]A similar mechanism was observed in the dominant-negative effect exerted by the p.G278R receptor activator of nuclear factor kappa-B ligand (RANKL) variant. The G278R RANKL monomers failed to assemble into homotrimers and interact with wild-type RANKL, resulting in a dominant-negative effect on trimerization.[10] We speculate that the p.V279G mutation might interfere with the trimerization of wild-type HTRA 1in a similar manner.

We report two autosomal dominant CSVD cases in a Chinese family with a novel heterozygous p.Val279Glu (c.836T>A) mutation in HTRA1 gene. Therefore, we suggest performing the screening of HTRA1 in all patients with a familial CSVD when the extent of microvascular lesions on MRI contrasts with the paucity of vascular risk factors.

Declaration of patient consent

The Medical Ethics Committee of Taizhou Hospital approved the study and all participants provided written informed consent. In the form the patients have given their consent for their images and other clinical information to be reported in the journal.

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Conflicts of interest

There are no conflicts of interest.

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