p.Arg332Cys Mutation of NOTCH3 Gene in Two Unrelated Japanese Families with CADASIL

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Abstract

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebrovascular disease caused by NOTCH3 mutations, usually localized to exons 3 and 4. This report describes the clinical and neuroradiological findings of 2 subjects of two unrelated Japanese families who shared a common p.Arg332Cys mutation. The subject from family A presented syncope attacks as the sole clinical presentation at the beginning of his disease course. The subject from family B showed recurrent ischemic attacks, followed by a large intracranial hemorrhage. This is the first report to describe the detailed phenotypes of patients with a rare p.Arg332Cys mutation in Japan.

Key words: CADASIL, Notch3, syncope, intracranial hemorrhage


Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebrovascular disease due to mutations of the NOTCH3 gene which has 33 exons (1). Most of these mutations result in gain or loss of cysteine residues in epidermal growth factor (EGF)-like domains in the Notch 3 protein and tend to cluster in exon 3 and 4 coding for the first five EGF-like repeats (1). The clinical spectrum includes recurrent ischemic episodes, cognitive decline, migraine, and psychiatric disorders (2, 3). The pathological feature is a systemic vasculopathy characterized by progressive degeneration of vascular smooth muscle cells, predominantly involving the smooth muscle cells of the small cerebral arteries (4). An ultrastructural examination shows granular osmiophilic material (GOM) located close to the membrane of vascular smooth muscle cells (4, 5). This disease may be suspected based on the clinical symptoms, a positive family history, and a typical magnetic resonance imaging (MRI) with T2-weighted or fluid level attenuated inversion recovery hyperintensity in the anterior temporal pole and the external capsule (6, 7). Here we report the characteristic manifestations of Japanese CADASIL patients with a p.Arg332Cys mutation at exon 6 of the NOTCH3 gene. This is the first report to describe the detailed clinical phenotypes of Japanese CADASIL patients with this rare mutation. Furthermore, this is also the first report indicating that syncope could be the sole presentation with CADASIL.

Case Reports

Case report

This study was approved by the ethics committee of Yamaguchi University Hospital and written informed consent was obtained from all subjects. Two affected families living in well separated areas were identified in Yamaguchi Prefecture in Japan. No relationship could be found between

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these two families. Family A included two affected members (2 men) through two generations and Family B also included two affected members (2 men) (Fig. 1).

**Patient 1**

The proband of family A (patient 1) was a 50-year-old man who had experienced recurrent syncope attacks, initially presenting at 40 years of age. Most of these syncope attacks occurred when the patient stood up after drinking alcohol, and his consciousness recovered almost immediately thereafter without neurological deficits. There were no convulsions or urinary incontinence accompanying these attacks. At 48 years of age, he began to demonstrate memory disturbance. He had diabetes mellitus (DM) without any regular medical treatment and also had smoking and drinking habits that had been ongoing for about 30 years. There was no history of migraine headaches. At admission, his neurological examination was unremarkable except for mild memory disturbance. His performance on the mini-mental status examination (MMSE) was slightly impaired (a score of 26 out of 30). The electrocardiogram (ECG) and echocardiogram were normal. There was no blood pressure decrease with a tilting table test. The electroencephalogram (EEG) was also normal. Brain MR fluid-attenuated inversion recovery (FLAIR) images demonstrated diffuse hyperintensities in the cerebral white matter and basal ganglia, characteristically in the anterior temporal pole and external capsule (Fig. 2A-C). MR cerebral angiography revealed no intracranial arterial stenosis or occlusion. Other than elevated fasting blood sugar (146 mg/dL) and HbA1c (6.2%), the results of the laboratory tests including the coagulation profile and cerebrospinal fluid (CSF) analysis were unremarkable. Based on his brain MRI findings, he was suspected to have CADASIL and therefore underwent genetic testing for the NOTCH3 gene with genetic counseling. A missense mutation c.994C>T in codon 332, thus resulting in the replacement of an arginine residue with a cysteine, was detected (Fig. 3). After instructing him to stay away from alcohol, his syncope attacks significantly decreased in frequency.

**Patient 2**

The proband of family B (patient 2) was a 46-year-old man. At 35 years of age, he experienced the sudden onset of dysarthria and right hemiparesis, which thereafter was completely resolved within several months. Three years later, he manifested sudden left hemiparesis, followed by a partial recovery. At 40 years of age, he presented with a worsening of dysarthria and gait disturbance and began to take 200 mg ticlopidine daily. Because ticlopidine tablets are 100 mg each and the usual dosage is fixed at 200-300 mg daily for the treatment and prevention of the cerebral infarction in Japan, he was therefore administered 200 mg ticlopidine daily. Thereafter, he experienced two additional episodes of left hemiparesis with incomplete recovery at ages of 45 and 46. He was referred to our department for hospitalization. He did not have any episodes of migraine in the past. He did not have any vascular risk factors other than smoking. A neurological examination revealed left hemiparesis, dysarthria, spasticity in his extremities, diffuse brisk deep tendon reflexes, and bilateral Babinski sign. Mini-mental status examination was moderately impaired (a score of 20 out of 30). FLAIR MRI showed multiple hyperintensity lesions in periventricular white matter, basal ganglia, external capsule and anterior temporal horn (Fig. 2D-F). Serological tests for collagen disease including anti-phospholipid syndrome were unremarkable and serum protein C, S, and homocysteine, and CSF contents were all normal. The ECG findings were normal but cardiac echograms showed a patent foramen ovale. Since CADASIL was suspected based on the characteristic brain MRI findings and his family history, a skin biopsy was performed, but no GOM was detected. He was moved to another hospital for rehabilitation with successive ticlopidine therapy. Six months after discharge, he was admitted again to the hospital via the emergency department with a sudden deterioration of mental status and generalized convulsions. An immediate CT scan revealed a 7.5×6.5 cm
intracranial hemorrhage (ICH) involving the right putamen (Fig. 4), which was surgically removed. This second histological examination using a small brain sample obtained during surgery revealed GOM near the basement membrane of a smooth muscle cell surrounding a small cerebral artery (Fig. 5). Extensive analyses for NOTCH3 mutations disclosed that he had the p.Arg332Cys CADASIL mutation (Fig. 3).

**Genetic analysis**

Genomic DNA was extracted from peripheral blood leukocytes using a standard method. Exon 2-6, 10, 11, 14, 18, 19, 23, which have been reported as pathological mutation sites until 2003 (8), were screened. These exons of NOTCH3 gene were amplified by published primers (9). The sense and antisense primer of Exon 6 were GTGGCTGGAC TGTGCATCTGTG and ACCATCCATGGCTCCCTGCAG, respectively. The polymerase chain reaction (PCR) products were sequenced using BigDye Terminator v. 3.1 (Applied Biosystems, Foster City, CA, USA) and loaded onto a ABI 3,100-Avant Genetic Analyzer (Applied Biosystems). The initial limited scanning of exons 3, 4, and 5 showed no mutations on each patient. Subsequently, analyses of exons 2, 6, 10, 11, 14, 18, 19, and 23 were performed.

**Morphological study**

A punch biopsy was taken from the upper limb of patient 2 on his first admission to our hospital. The second biopsy of patient 2 was performed from the cerebral white matter during the operation for evacuation of a hematoma from the right putamen on his second admission. For the electron microscopic studies, the specimens were fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide, embedded in Epon and processed according to the standard techniques.

**Discussion**

CADASIL patients harboring the p.Arg332Cys mutation of NOTCH3 were initially reported in Italian populations (10), followed by British (7), Dutch (11), German (12), and Taiwanese (13, 14) families. Although only one Japanese language review reported this mutation with CADASIL in Japan (24), the clinical information of the pa-
patients in that study was not described. Therefore, this is the first paper presenting the detailed phenotypes of the patients harboring this rare mutation in Japan. Detailed clinical presentations of cases with the p.Arg332Cys CADASIL mutation could be obtained from only Italian and Taiwanese families (10, 13, 14). All of the affected individuals of these families presented recurrent ischemic episodes (10, 13, 14), thus representing the most frequent presentation with CADASIL (3).

The involvement of the anterior temporal pole and external capsule has been reported to be characteristic of CADASIL (7). In particular the moderate to severe involvement of the anterior temporal pole on MRI has a sensitivity of 89% and specificity of 86% for the diagnosis of CADASIL (7). These two important findings were identified on brain MRI in both subjects who had the p.Arg332Cys CADASIL mutation, thus leading us to perform the extensive genetic tests for CADASIL in these cases.

Evidence from various sources suggests that the genotype-phenotype correlations in CADASIL are weak (15). In line with these previous investigations, the phenotypes of the two subjects presented here which have the common Arg332Cys mutation were quite different.

Notably, patient 1 presented recurrent episodes of loss of consciousness. This symptom was diagnosed as “syncope”, not an epilepsy attack for the following three reasons. First, he often manifested these episodes when he stood up after drinking alcohol. Second, his EEG was normal. Third, these attacks decreased significantly after he reduced his alcohol intake. Narkiewicz et al (16) reported that short-term alcohol

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**Figure 4.** Brain CT of patient 2 on the second admission. CT showed a right putaminal intracerebral hemorrhage with a prominent mass effect.

**Figure 5.** Electron microscopy of a brain vessel. Granular osmiophilic electron-dense materials (arrows) are present near the basement membrane of a smooth muscle cell. Image b shows the enlargement picture of image a. (bar = 10 μm (a), 1 μm (b))
intake causes orthostatic hypotension because of impairment in the vasoconstrictor response to orthostatic stress and it can induce syncopal events. The dysregulation of cerebral perfusion due to the insufficiency of constriction of small cerebral arteries caused by the degeneration of smooth muscle cells, particularly after the consumption of alcohol, might have caused syncope in patient 1. Of course, careful considerations should be made prior to concluding whether the CADASIL vasculopathy by itself directly results in syncopal events. However, clinicians need to carefully monitor CADAIL patients who have drinking habits or have some other causes of orthostatic hypotension, because these patients might present an attack of syncope. Although it is widely accepted that some CADASIL patients could manifest epileptic seizures due to vascular symptomatic epilepsy (3, 17), there has so far been no report of CADASIL patients who showed only syncope as the sole phenotype.

On the other hand, patient 2 presented with recurrent ischemic attacks, which is the most frequent initial presentation of symptomatic CADASIL individuals (3). Although GOM was not detected in a skin biopsy specimen obtained from this patient, the results of a brain biopsy were positive. Recently Tikka et al demonstrated the high sensitivity of GOM detection in the skin biopsy of genetically verified CADASIL (18). They estimated the sensitivity of the skin biopsy to be more than 90%. But this report also indicated that in some cases a repeat skin biopsy was needed to detect GOM. Therefore, if GOM is not found in the first skin biopsy in the subjects showing typical clinical presentations with CADASIL, then additional biopsies should be considered.

The patient 2 received antiplatelet agents and unfortunately, he developed a large intracerebral hemorrhage (ICH) during the disease process. While CADASIL is considered to be a primarily ischemic form of cerebral vascular disorder, microbleeds have recently been reported in 31-69% of symptomatic CADASIL individuals (3). Although GOM was not detected in a skin biopsy specimen obtained from this patient, the results of a brain biopsy were positive. Recently Tikka et al demonstrated the high sensitivity of GOM detection in the skin biopsy of genetically verified CADASIL (18). They estimated the sensitivity of the skin biopsy to be more than 90%. But this report also indicated that in some cases a repeat skin biopsy was needed to detect GOM. Therefore, if GOM is not found in the first skin biopsy in the subjects showing typical clinical presentations with CADASIL, then additional biopsies should be considered.

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In conclusion, this is the first report of the detailed clinical phenotypes of Japanese CADASIL families with the p.Arg332Cys mutation of NOTCH3 gene, which is rare in Japan. Comprehensive genetic screening for NOTCH3 mutations is definitely important in Japan, as well as in the other parts of the world, when a patient shows recurrent syncope attacks as the presenting sole symptom and characteristic MRI findings, then CADASIL should be included in the differential diagnosis.

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

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