Two Japanese CADASIL Families with a R141C Mutation in the Notch3 Gene

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Abstract

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare hereditary disease characterized by recurrent transient ischemic attacks (TIA) and strokes, and vascular dementia with Notch3 gene mutations as the cause of the disease. To date, there are only a few Japanese families ever reported with a mutation in the gene. Here, we report two more Japanese CADASIL families carrying a missense mutation in the Notch3 gene (R141C) with a unique lesion in the corpus callosum. This is the first report of two unrelated Japanese CADASIL families with a R141C mutation in the Notch3 gene. Although the disease is very rare among the Japanese population, our result suggests a possible relationship of this particular mutation (R141C) with the lesions of the corpus callosum.

Key words: missense mutation, autosomal dominant, hereditary infarction

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary adult-onset disease first reported in 1993 (1). The disease is characterized by recurrent TIAs and strokes with progressive vascular dementia, migraine with aura, psychiatric disturbances, and pseudobulbar palsy (2-4). In 1996, Joutel et al reported Notch3 as the causative gene for CADASIL, and detected several point mutations (5, 6). However, most mutations identified were from Caucasian families, and so far, only a few families with the gene mutations have been reported among the Japanese population (7-9). We newly identified two Japanese CADASIL families with a missense mutation in the Notch3 gene, causing a substitution of Arg to Cys (R141C). Magnetic resonance (MR) images showed ischemic lesions in the caudal portion of the corpus callosum. The same mutation was already reported in European populations in 1996, but it is the first in the Japanese as well as the characteristic callosal lesion.

Patients and Methods

Family 1

The proband was a 57-year-old woman (Fig. 1A, III-3) with recurrent episodes of migraine without aura from 30 years old. Her ancestors lived near Takahashi-city, a northwest part of Okayama Prefecture. The frequency of her headache was approximately once a year. Although she did not have aura prior to headache, the severe headache sometimes led her to have nausea or even vomiting. In most cases, the headache lasted more than half a day. Her episodic headache continued until age 45. From age 51, she had several episodes of TIA and stroke. The first symptom of her stroke, at age 51, was dysarthria. At age 53, she experienced TIA with dysarthria and right-sided hemiparesis. Since then, she began to feel instability in gait. At age 56, she noticed difficulty in moving her right upper extremity, especially when writing or using an electronic calculator. At the same age, she was diagnosed to be in a depressive state. In her family, her grandfather, father, aunt, and two of her siblings had the same disease (Fig. 1A, I-1, II-2, 5, III-1, and 4). Although bradykinesia and dysarthria were obvious, her mental state was normal. Deep tendon reflexes were increased in all extremities, but no abnormal reflexes were found. None of her family members had known risk factors for ischemic strokes, such as hypertension, diabetes mellitus, and hyperlipidemia.

Family 2

A 55-year-old woman living in Okayama-city, Okayama Prefecture, was the proband (Fig. 1B, III-3). Her first episode

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Received for publication April 12, 2001; Accepted for publication July 6, 2001
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For editorial comment, See p 1075.
New CADASIL Families in Japan

Figure 1. Pedigrees of families 1 (A) and 2 (B). Numbers under each symbol are the ages of the onset. Each arrow indicates the proband.

Table 1. Clinical Features of Two CADASIL Families

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age of onset</th>
<th>Duration of illness</th>
<th>Dementia</th>
<th>Dysarthria</th>
<th>Migraine</th>
<th>Depression</th>
<th>Involvement of the corpus callosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A, III-3)</td>
<td>51</td>
<td>6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (A, III-1)</td>
<td>34</td>
<td>26</td>
<td>+</td>
<td>+</td>
<td>unknown</td>
<td>unknown</td>
<td>not examined</td>
</tr>
<tr>
<td>3 (A, III-4)</td>
<td>43</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4 (B, III-3)</td>
<td>51</td>
<td>4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The number in the parenthesis correlates to each member in Fig. 1. The mean age of onset was 44.8.

of stroke was at age 51 with transient dysarthria and memory disturbance. Both symptoms improved once, whereas she experienced a similar transient loss of recent memories several times since then. At age 53, she went to a hospital for a health check, and there multiple infarctions and leucoaraiosis were detected on her MR images. Her grandfather and mother (Fig. 1B, I-1 and II-1) had similar recurrent episodes of stroke. Although she showed no signs of dementia, she showed hyperreflexia in all extremities. Mild spasticity was observed in her lower extremities. Sensory disturbances, especially superficial touch sense in all extremities, were obvious. She did not have any episodes of migraine in the past. She had never been pointed out to have risk factors for stroke in the past. The clinical features of these two families are summarized in Table 1.

Materials and Methods

MR study

MR images of three patients and two healthy siblings were examined. The patients examined were both probands (Fig. 1A, III-3 and 1B, III-3), and the younger sister of the proband from family 1 (Fig. 1A, III-4). Healthy siblings were older sisters of both probands (Fig. 1A, III-2 and 1B, III-2). T1 and T2 weighed images of at least two directions (axial and sagittal) were analyzed.

Gene analysis

Gene analysis of the four patients (Fig. 1A, III-1, 3, 4, and 1B, III-3) and two unaffected members (Fig. 1A, II-3 and 1B, III-2) were performed. Genomic DNA was isolated from blood leucocytes using a standard procedure after informed consent was obtained. Exons 3 to 5 of the Notch3 gene were amplified by polymerase chain reaction (PCR). The sense and antisense primer were 20-mer, 5'-CTGCCCAACCAAGCCATCTC-3' and 5'-CTCTCGCTGTCCAGCCATT-3', respectively. PCR was performed in a 50-μl reaction mixture containing 1 μg of genomic DNA, 1 U of Taq polymerase, and 10 pg of each primer. After an incubation at 95°C for 5 minutes, steps of 95°C for 60 seconds, 57°C for 60 seconds, 72°C for 120 seconds were repeated for 35 cycles, followed by an incubation at 72°C for 5 minutes. An aliquot of the PCR product was reacted with Dye Terminator Kit (Amersham Pharmacia Biotech, Piscataway, NJ), and the reaction product was analyzed using an automated DNA sequencer (Long Read Tower, Amersham Pharmacia Biotech, Piscataway, NJ).

Internal Medicine Vol. 40, No. 11 (November 2001)
Multiple infarctions and diffuse hyperintensity were observed in the basal ganglia and white matter (A, C and D). Ischemic lesions were detected also in the corpus callosum especially in the caudal portion (B).
Results

MR study

MR images of the proband from family 1 showed diffuse leukoencephalopathy and multiple small infarcts in the subcortical areas, brain stem, and cerebellum (Fig. 2A, B). As a unique finding of the patient, ischemic lesions in the corpus callosum were detected especially in the caudal portion (Fig. 2B, arrows). The same findings, including the lesions in the corpus callosum, were also observed in her affected sister (Fig. 1A, III-4). Her healthy sister (Fig. 1A, III-2) did not show any ischemic nor other organic lesions. MR images of the proband from family 2 also showed leukoencephalopathy and lacunar infarctions in the subcortical areas (Fig. 2C, D), although the ischemic lesions in the corpus callosum were not detected. MR images of her healthy sister showed only a few lacunar infarctions in the basal ganglia.

Gene analysis

Sequence analysis of the proband from family 1 showed a heterozygous mutation of a normal sequence and a transition of C to T, which resulted in the substitution of Arg to Cys (R141C) in exon 4 of the Notch3 gene (Fig. 3A). The mutation was also detected in other patients in family 1 (Fig. 1A, III-1 and 4). We detected the same missense mutation from the proband from family 2 (Fig. 3B). However, none of the healthy members in either family (Fig. 1A, II-3, and Fig. 1B, III-2) had the mutation (Fig. 3C). Further, 52 unrelated normal Japanese controls showed no mutation.

Discussion

CADASIL is a hereditary adult-onset disease first reported by Baudrimont et al in 1993 (1). In 1996, several mutations in the Notch3 gene were detected as the cause of the disease (5). Notch encodes a transmembrane receptor with the epidermal growth factor-like (EGF-like) repeats as the extracellular domain. It is essential for proper embryonic development in species ranging from insects to mammals (10). All of the mutations identified in 1996 exist in EGF-like repeats, creating or deleting a cysteine residue in the extracellular domain of the Notch3 protein (6). The R141C mutation is one of the most frequently observed mutations (approximately 15%) in Caucasian CADASIL families. Although the mechanism of Notch3 mutations causing CADASIL phenotype is not still well known, mutated EGF-like domain with the aberrant cystein residue may cause impairment of interaction with the ligands, that finally results in abnormal Notch signaling (11, 12). CADASIL is a disease that has been reported primarily from Caucasian pedigrees, and to our knowledge, there are only a few Japanese CADASIL families with mutations ever reported (7–9). We identified a novel missense mutation of R141C in Notch3, which is the first in the Japanese CADASIL families. We could not find any relation between these two families living in different cities in Okayama Prefecture. However, considering that they are so far the only two families with R141C mutation in Japanese populations, it is possible that they have the same ancestor in common.

We applied the diagnostic criteria proposed by Davous (13) to our CADASIL patients, and diagnosed them as ‘definite CADASIL’, since all patients satisfied all the criteria. However, as compared with the previously reported 102 Caucasian patients (2), some interesting differences were found in the
present families. Although the mean age of onset was almost the same (44.8 and 46.1 years old in Japanese and Caucasian, respectively), the frequency of migraine was much higher in Caucasian than in Japanese (38% and 25%, respectively). On the other hand, dementia and dysarthria were observed at a higher frequency in Japanese patients (50% and 100%, respectively) than in Caucasian (28% and 41%, respectively). MR images showed ischemic lesions in the corpus callosum in two patients especially in the caudal part (Fig. 1A, III-3 and 4). The corpus callosum was normal in the proband of the family 2. This may be because the duration of her illness (4 years) was shorter than those of other patients (6–26 years). The involvement of the corpus callosum in CADASIL was seen in 4/5 patients (80%) in a previous report (14). In the present cases, we detected lesions in the corpus callosum with a prevalence of 67% (2/3 patients). The corpus callosum receives blood flow through the pericallosal artery, thus the caudal lesion suggests ischemic damage in a more peripheral territory of the corpus callosum. Lesions of the corpus callosum are generally very rare in ischemic diseases. The callosal lesions, especially in the posterior portion, suggest not only the usefulness in diagnosing CADASIL, but also an impairment of the smaller arteries in the disease. Our results suggest that CADASIL should be recognized as one of the important causes in familial cases of multiple infarctions and/or vascular dementia without any known risk factors, especially when there are ischemic lesions in the corpus callosum.

References