CASE REPORT

CADASIL with a Novel Mutation in Exon 7 of NOTCH3 (C388Y)

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Abstract

We report a 38-year-old Japanese woman who had cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) with a novel mutation (TGT to TAT) at nucleotide position 1241 (C388Y) in exon 7 of the Notch3 gene (NOTCH3). Immunostaining of a skin biopsy with a Notch3 monoclonal antibody is a beneficial method for the screening of CADASIL, particularly in the case of rare mutations outside the mutation hotspots in NOTCH3 as shown in this patient.

Key words: CADASIL, NOTCH3, mutation, exon 7

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant disorder, characterized by early-onset stroke, progressive cognitive decline, psychiatric disturbance, and migraine (1-3). An antemortem diagnostic method for CADASIL is electron microscopic examination of skin for granular osmiophilic materials (GOM) or genetic analysis for mutations in the Notch3 gene (NOTCH3) (1, 4-7). However, the microscopic examination of GOM is hampered by false-negative results (1, 4, 5), and NOTCH3 screening outside hotspots of the mutations is a time- and cost-consuming method (2, 6). Immunostaining with a NOTCH3 monoclonal antibody has been identified to be a highly sensitive and specific technique for the diagnosis of CADASIL (2). In the present study, we report an asymptomatic patient with CADASIL with a novel mutation of NOTCH3, which was first diagnosed by the immunostaining of skin biopsy.

Patient and Methods

Case report

A 38-year-old Japanese woman, who suffered from a mild headache following a cold, visited a hospital. The patient’s father had presented with left hemiparesis since the fourth decade and died at age 65. Her elder sister (45 years old) had no headache or neurological symptoms. She showed no abnormalities in the physical, neurological or psychological examinations. Brain MR T2-weighted and fluid-attenuated inversion recovery images demonstrated diffuse hyperintensities in the cerebral white matter and basal ganglia, characteristically in the anterior temporal pole and the external capsule (Fig. 1A). A cerebral angiography and laboratory data including cerebrospinal fluid showed no abnormalities. She was suspected of having CADASIL.

Skin biopsy

A skin biopsy of the left upper arm was performed. Paraffin sections from the skin were stained with hematoxylin-eosin (HE) and periodic acid Schiff (PAS) stains. Immunohistochemistry was performed on 8 μm paraffin sections with an anti-Notch3 murine monoclonal antibody, 1E4 (1:5 dilution), raised against epidermal growth factor-like (EGF-like) repeat17-21 (2). Electron microscopic examinations were performed, especially for GOM of the skin vessels.

NOTCH3 gene analysis

DNA was extracted from the blood after informed written consent had been given. Direct sequencing was performed for all 23 exons encoding 34 EGF-like repeats of Notch3
protein (Fig. 2) as previously described (2).

**Results**

**Skin biopsy**

Light microscopic examination showed no abnormalities with HE or PAS stains. In the electron microscopic study of the skin vessels, we initially failed to find GOM. Notch3 immunohistochemistry of the biopsied skin demonstrated positive staining in some vessels (Fig. 1B), though skin vessels from normal controls showed negative results (data not shown). After the positive result of Notch3 immunohistochemistry, we intensively reexamined the skin vessels electron microscopically, and could finally observe some GOM along the basal lamina of the vascular smooth muscle cells (Fig. 1C).

**NOTCH3 gene analysis**

The initial limited scanning of exons 3 and 4, i.e., the hotspots of CADAIL mutations (2), showed no mutations. Subsequently, a full sequencing of all 23 exons of NOTCH3 revealed a novel mutation in exon 7 (G1241A) (Fig. 3), which lead to an amino acid substitution at position 388 (C388Y) in the 9th EGF-like repeat of Notch3 (Fig. 2). This type of mutation was not observed in a panel of 200 control chromosomes.

**Discussion**

We report a CADASIL patient with a novel mutation of NOTCH3. Almost 90% of the mutations of NOTCH3 are detected within exons 2-6 (8), and previous studies have revealed only a few mutations in exon 7 (8-10). This is the first case in Japan with a mutation of exon 7 (11-21).

This patient presented with no neurological abnormalities except for a transient and nonspecific headache. However, the brain MR findings showed diffuse hyperintensities in the white matter and basal ganglia, particularly in the anterior temporal pole and the external capsule. The involvement of the anterior temporal pole and external capsule has been reported to be characteristic of CADASIL (1); particularly, moderate and severe involvement of the anterior temporal pole on MRI have a sensitivity of 89% and specificity of 86% for diagnosis of CADASIL (1). Therefore we clinically suspected that this patient suffered from CADASIL and that the patient’s father had been similarly affected.

As a diagnostic procedure for CADASIL, electron microscopic examinations for GOM of skin vessels frequently show false-negative results (1, 4, 5) as the initial electron microscopic examination did in our patient. Since clinical manifestations have no significant correlation to the NOTCH
Figure 2. A schematic of the 33 exons of the Notch3 gene (NOTCH3) (A) and the structure of a transmembrane receptor Notch3 (B) with locations of identified CADASIL mutations. NOTCH3 includes 33 exons encoding 2,321 amino acids of the Notch3 protein (A). The full-length Notch3 receptor contains an extracellular domain with 34 tandemly arranged epidermal growth factor (EGF)-like repeat domains, three cysteine-rich Notch/Lin-12 repeats, one transmembrane region, and an intracellular domain containing six ankyrin repeats (B). Exons 1 through 23 code 34 EGF-like repeat domains (A, B). Small closed circles show the previously reported CADASIL mutation sites, and each large closed circle is equal to 10 small ones (A). The CADASIL mutation sites are located at the regions encoding EGF-like repeat domains of the Notch3 protein (B) and show the strong clustering in exons 4 and 3 (A). This patient shows a G1241A mutation in exon 7 (open circle, arrow) outside the hotspots of CADASIL mutations (A), which leads to the replacement of a cysteine by a tyrosine at position 388 in the 9th EGF-like repeat domain (arrowhead) (B).

The NOTCH3 mutation found in the present patient results in a loss of a cysteine residue (cysteine to tyrosine). Although the mutation of this patient is novel, loss or gain of a cysteine residue is a common change in most of the previously reported mutations (2, 7). The gain or loss of a cysteine residue results in an odd number of cysteine residues, which will lead to abnormal accumulations of the Notch3 protein and cause CADASIL through an unknown mechanism (2, 22). It remains unclear why CADASIL mutations of NOTCH3 strongly cluster in exons 4 and 3 in Caucasian (1, 2, 6-8) and non-Caucasian families including Japanese (11-21, 23-30). In Japanese, only one family has previously shown a mutation of exon 5 (11). In non-Caucasian patients other than Japanese, the mutations in exons 6 (23), 11 (24, 25), and 18 (24) have been rarely found outside the hotspots of the NOTCH3 mutations. Earlier studies of non-Caucasian CADASIL patients have been fewer than those of Caucasians. Further studies with non-Caucasian CADASIL patients may disclose non-hotspot mutations as found in the present patient.

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序列分析

图3. NOTCH3基因分析。从该患者（下）和一个对照个体（上）的Notch3基因序列（NOTCH3）表明在第1241位点存在一个核苷酸的异位突变（G→A）（箭头）。

引用文献


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