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New diagnostic criteria for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukocencephalopathy in Japan

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ABSTRACT

Purpose: Definite diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukocencephalopathy (CADASIL) is mostly done by identification of *NOTCH3* mutations. We aimed to develop criteria for selecting patients suspected for CADASIL to undergo genetic testing.
Subjects and methods: All subjects were Japanese. We recruited CADASIL patients genetically diagnosed up until 2011 (n = 37, Group 1) or after 2011 (n = 65, Group 2), 67 young stroke patients (≤55 years old), and 53 *NOTCH3*-negative CADASIL-like patients. The members of Japanese research committee for hereditary cerebral small vessel disease discussed and generated the new criteria to maximize positive rate in Group 1 CADASIL patients, followed by validation of sensitivity and specificity.
Results: In Group 1 CADASIL patients, the ages at onset excluding migraine were distributed widely (37–74 years old) and bimodal (<55 and >55 years old). Frequencies of an autosomal dominant family history and vascular risk factor(s) were 73 and 65%, respectively. From these findings, the panel considered appropriate cut-off values and weighting for each item. In CADASIL Group 1 versus young stroke controls, the sensitivity and specificity of the new criteria were 97.3% and 80.6%, respectively. However, in CADASIL Group 2 versus *NOTCH3*-negative controls, the sensitivity and specificity were 96.9% and 7.5%, respectively. Forty mutations of *NOTCH3* distributed in exons 2–8, 11, 14, 18, 19, and 21 were identified in this study. Ten mutations were unreported ones.
Conclusion: We propose the new criteria of high sensitivity, which will help physicians to assess the need for genetic testing.

1. Introduction

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukocencephalopathy (CADASIL, OMIM#125310) is one of the most frequent hereditary small-vessel diseases manifested by recurrent stroke and white matter lesions in young individuals despite the absence of traditional cardiovascular risk factors [1]. Typical CADASIL patients show migraine, progressive white matter lesions, brain ischemic events, neurological symptoms, and mood disturbance in this order, and it frequently leads to subcortical dementia [1]. For a definite diagnosis of CADASIL, genetic testing of the causative gene, *NOTCH3* [2], or pathological detection of granular osmiophilic material (GOM) [3] in a skin biopsy sample is necessary. Genetic testing was previously time-consuming and expensive, and skin biopsy was and still remains invasive. Considering these disadvantages, strict screening of CADASIL-suspected cases was necessary for national survey to detect the accurate incidence of this disease. Conventional clinical criteria for CADASIL, proposed by Davous, focused on the typical clinical features of CADASIL [4]. To differentiate CADASIL from Binswanger disease, a major sporadic small cerebral vessel disease, his criteria excluded cases with severe vascular risk factors, no family history, or an advanced age at onset (>70) [4]. However, several reported CADASIL patients had cardiovascular risk factors [5] or no family history because of de novo mutation [6,7]. In addition, some patients were reported to show their initial ischemic event at later than 70 years old [8–10]. These atypical

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CADASIL cases were excluded by Davous' criteria. Genetic testing has since become faster and less expensive, making it available for not only typical but also atypical cases. Sensitive criteria are thought to be necessary for the screening. The aim of this study is to develop criteria of high sensitivity to help physicians, even if not expertized in CADASIL, for screening suspected CADASIL patients to undergo genetic testing.

2. Methods

This study included development and validation of the new criteria. Data of clinical information and MRI findings were retrospectively collected as many as possible from our database of *NOTCH3* genetic testing in Kyoto Prefectural University of Medicine (KPUM) until 2015, and stroke registry of consecutive patients in KPUM from 2001 to 2010.

2.1. Participants

All subjects were Japanese adults. We set two groups of CADASIL patients. Group 1 consisted of 37 CADASIL patients from 31 families, genetically diagnosed in KPUM up until 2011. Group 2 consisted of 65 CADASIL patients from 58 families genetically diagnosed after we proposed the new criteria in Japanese, between 2011 and 2015. In addition, 53 CADASIL-like patients without mutations in *NOTCH3* exons 2–24 were recruited as *NOTCH3*-negative CADASIL-like controls. Flow of genetic testing is shown in Supplemental Fig. 1. The CADASIL and *NOTCH3*-negative patients were recruited from about 75 institutes in Japan. Their blood samples and clinical information were transferred to KPUM. Informed consent was obtained from all participants, and approval for the study was obtained from the ethical committee of KPUM. We also recruited 67 sporadic young stroke patients (≤55 years old) from our stroke registry of consecutive patients with acute brain infarction from 2001 to 2010.

2.2. Development of the new criteria

The new criteria were developed by data-driven, expert-panel consensus approach. Expert panel consisted of members of the Japanese research committee for hereditary cerebral small vessel disease, including OO, TM, HT, TH and MU. The panel members discussed and determined positivity cut-off and weight of items to maximize positive rate, in exploratory manner based on profile of 37 CADASIL patients (Group 1). The committee proposed the new criteria (Table 4) and published them in the Journal of the Society of Japanese Neurology [9].

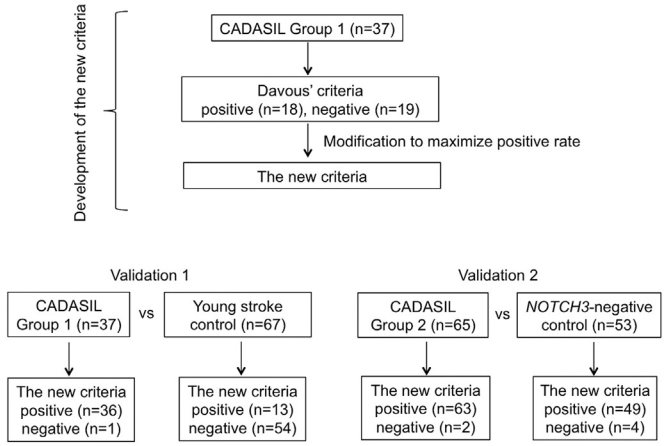

Fig. 1. Flow of participants in development and validation of the new criteria.

2.3. Validation of the new criteria

Definite, probable and possible criteria were defined in the new criteria (Table 4). Definite criteria needed positive genetic or pathological testing result. Assessment of probable/possible criteria was determined by clinical and MRI information in the database. Therefore, the performers were not interfered by results of genetic testing. In this study, result categories of the new criteria were determined as positive (fulfilling possible or probable criteria) or negative (not fulfilling possible criteria). The criteria could be applied even when the participant had missing data. Sensitivity was calculated as ratio of positive patients in CADASIL patients. Specificity was calculated as ratio of negative patients in young stroke controls or *NOTCH3*-negative controls. We performed two validations. Firstly, we validated the new criteria in CADASIL Group 1 versus young stroke controls. Secondly, we validated in CADASIL Group 2 versus *NOTCH3*-negative controls.

2.4. Collecting clinical information

We collected clinical information including the clinical background (gender, age at onset of clinical symptoms, family history of stroke and migraine), cardiovascular risk factors (histories of hypertension, diabetes mellitus, hyperlipidemia, and smoking), neurological symptoms, and the results of imaging studies. All neurological symptoms were documented including paralysis, ataxia, sensory disturbance, vertigo, dizziness, parkinsonism, bulbar palsy, seizure, mood disorder, dementia, and the results of neuropsychological tests. Imaging studies including magnetic resonance imaging (MRI) and magnetic resonance angiography and any therapies were also recorded.

2.5. Genetic testing

For definite diagnosis of CADASIL, we performed genetic testing because of its non-invasiveness and high detection rate compared with skin biopsy. Genomic DNA was extracted from the peripheral blood using QIAamp DNA Blood Mini/Midi kits (QIAGEN). *NOTCH3* exons 2–24, coding for 34 epidermal growth factor (EGF)-like repeats, were amplified by the polymerase chain reaction (PCR), followed by direct sequencing using an ABI3130 capillary sequencer (Applied Biosystems). The sequence data were analyzed with SEQUENCHER (Gene Codes, HITACHI) to screen for mutations. Nucleotide substitutions were confirmed by restriction fragment length polymorphism analysis. We concluded that the variation was pathogenic when it was previously reported in another CADASIL family and/or when it resulted in Cysteine-related missense mutation in an EGF-like repeat (also see genetic criteria in Table 4). Because we selected patients who undergo analysis of exons 2–24 according to MRI finding and family history (Supplemental Figure 1), clinical information were available to assessors of genetic testing.

2.6. Davous' criteria and CADASIL scale

Davous' criteria [4] could be applied for all the participants, even though the participants had missing data. Result categories of Davous' criteria were determined as positive (fulfilling possible or probable criteria) or negative (exclusion or unclassified). The CADASIL scale by Pescini et al. [11] involves the additive score of 12 items (ranging from 0 to 25), whose cutoff score is 15. Result categories of CADASIL scale were determined as positive (≥15) or negative (<15). To calculate the score, patients with sufficient clinical information were extracted.

2.7. Statistics

Differences in the mean age at onset and frequency of clinical features, family history, and neuroimaging findings between the 2 groups were assessed using Fisher's exact test for categorical variables and