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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].

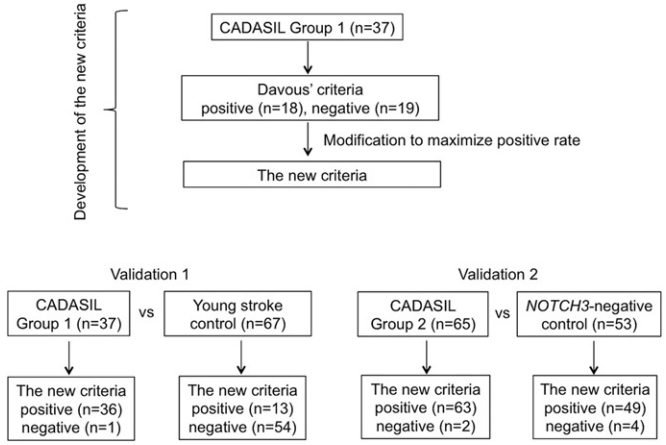

Fig. 1. Flow of participants in development and validation of the new criteria.

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

Table 1
Key items of Davous' CADASIL criteria.

	Probable	Possible	Exclusion
Onset	<50	50–70	>70
Clinical findings (at least two of them)			
	Stroke-like episodes with permanent neurological signs	Stroke-like episodes without permanent signs	
	Migraine	Migraine	
	Major mood disturbance	Minor mood disturbance	
	Subcortical dementia	Global dementia	
Vascular risk factors	No	Mild	Severe
Family history	Autosomal dominant	Unknown or incomplete	No
MRI of white matter	Typical	Atypical	Normal (age>35)

Modified from Davous P, 1998 [4].

the t-test for numeric variables. 95% confidence interval of sensitivity and specificity of the criteria was calculated. We used GraphPad Prism6 for statistic calculation. Values of $P<0.05$ were considered significant.

3. Results

3.1. Overview and flow of participants in this study

Mean ages at assessment, SD, and ranges were 52.7 ± 10.1 (37–74) in CADASIL Group 1, 55.7 ± 10.0 (25–77) in CADASIL Group 2, 45.6 ± 9.4 (21–55) in young stroke controls, and 60.5 ± 11.4 (31–84) in *NOTCH3*-negative controls. Mean ages at assessment in symptomatic (excluding migraine) patients were 54.4 ± 10.0 (Group 1), 56.8 ± 8.3 (Group 2), and 63.1 ± 10.6 (*NOTCH3*-negative). Time latencies from onset (excluding migraine) were 4.7 ± 5.8 (Group 1), 6.7 ± 6.6 (Group 2), 0 (young stroke) and 3.0 ± 5.5 (*NOTCH3*-negative) years. Other basal and clinical information are summarized in Table 3. Flow of the participants in this study is shown in Fig. 1.

3.2. New CADASIL criteria based on the clinical features of Japanese CADASIL patients

As a preliminary analysis, Davous' criteria (Table 1) were applied to CADASIL Group 1 patients. Of the 37 patients, 19 (51.4%) were excluded or unclassified because of an advanced age at onset (>70), no family history, being asymptomatic, or having only one symptom (Table 2). In this study, we did not use the age at onset of migraine because it often occurs markedly earlier than the other symptoms. To maximize positive rate, the expert panel considered appropriate cut-off values and weightings for each item base on clinical features in Group 1 (Table 3). Firstly, the cut-off age at onset for probable CADASIL was raised to 55 years old and the exclusion cut-off, >70, was omitted in the new criteria (Table 4), because the distribution of ages at onset of 28 symptomatic patients in Group 1 was wide (37–74 years old) and bimodal (≤55 and >55 years old) (Fig. 2). Secondly, the item of family history was omitted from possible criteria (Table 4), because six patients (16.2%) were excluded in Davous' criteria due to absence of a family history (Table 2). Thirdly, the item of vascular risk factors was omitted from the new criteria (Table 4) because high frequency (64.9%) of Group 1 had at least one of vascular risk factors (Table 3). Finally, the item of white matter lesions was strongly weighted in possible criteria (Table 4), because all the patients in Group 1, even asymptomatic, had MRI findings of white matter lesions (Table 3).

Table 2
Evaluation of 37 CADASIL patients in Group 1 based on Davous' conventional criteria.

		Number of patients (%)
Fulfilled the criteria		18 (48.6)
Excluded or unclassified		19 (51.4)
Probable		2 (5.4)
Possible		16 (43.2)
Unclassified because of		
	No clinical finding[a]	4 (10.8)
	Migraine alone	7 (18.9)
	Stroke episode alone	2 (5.4)
Excluded because of		
	Onset > 70	2 (5.4)
	No family history[a]	6 (16.2)

[a] Two patients overlapped in these two categories.

3.3. Validation of the new criteria

Using CADASIL Group 1 and young stroke controls, the sensitivity and specificity of the new criteria were calculated as 97.3 and 80.6%, respectively (Table 5, validation 1). For the second validation, CADASIL Group 2 and *NOTCH3*-negative controls were recruited. There were no significant differences in the frequency of symptoms between CADASIL Groups 1 and Group 2, except for migraine (Table 3). High sensitivity was replicated in Group 2 (96.9%), however, the specificity in *NOTCH3*-negative controls was only 7.5% (Table 5). When excluding asymptomatic patients, increase in sensitivity (100% in both Group 1 and Group 2) and decrease in specificity (0% in *NOTCH3*-negative) were observed (Supplemental Table 1).

3.4. Comparison with Davous' criteria and CADASIL scale

For comparison, we applied the new criteria, Davous' criteria [4] and CADASIL scale proposed by Pescini et al. [11] in CADASIL patients combined (Group 1 and Group 2) and *NOTCH3*-negative controls (Supplemental Table 2). The sensitivity and specificity of Davous' criteria were 52.0% and 66.0%, respectively. Similarly, the sensitivity and specificity of CADASIL scale were 52.1% and 61.7%, respectively. Both Davous' criteria and CADASIL scale showed lower sensitivity but higher specificity compared with the new criteria (97.1% sensitivity and 7.5% specificity).

3.5. NOTCH3 mutations

In the total of 102 CADASIL patients recruited, we identified 40 different missense mutations of *NOTCH3* located in exons 2–8, 11, 14, 18, 19, and 21 (Table 6). All mutations, except for R75P [15], resulted in either the gain or loss of a cysteine residue. Fifteen mutations (C55G, C87F, C93G, C108F, C146W, C174L, C185Y, C212R, C245Y, C260F, C323W, C329Y, C729G, C988F, and C1004G) were not included in a recent review [13]. The case of C174L is reported elsewhere [14]. C146W, C245Y, C260F and C329Y were also reported [9]. Most of the mutations existed in exons 3–6 (77 of 102 patients, 75.5%), particularly exons 3–4 (68 of 102 patients, 66.7%).

4. Discussion

In this study, we propose the new diagnostic criteria of high sensitivity based on Japanese CADASIL patients.

Table 3
Clinical features of Japanese CADASIL patients and controls studied.

	NOTCH3-positive CADASIL patients			Controls		P-value		
	"Group 1 (n = 37)"	"Group 2 (n = 65)"	"Groups 1 and 2 (n = 102)"	Young stroke (n = 67)	*NOTCH3*-negative (n = 53)	Group 1 vs. 2	Group 1 vs. Young stroke	Groups 1 and 2 vs. *NOTCH3*-negative
Age at onset[a]	49.7 ± 10.9 (n = 28)	50.2 ± 9.4 (n = 57)	50.0±9.8 (n = 85)	45.6±9.4 (n = 67)	60.2 ± 11.9 (n = 42)	0.8463	0.0680	<0.0001
Male/female	21/16 (56.8)	31/34 (47.7)	52/50 (51.0)	46/21 (68.7)	24/29 (45.3)	0.4154	0.2855	0.6116
Subcortical dementia	19/37 (51.4)	28/64 (43.8)	47/101 (46.5)	2/67 (3.0)	31/51 (60.8)	0.5360	<0.0001	0.1222
Pyramidal sign	22/37 (59.5)	33/65 (50.8)	55/102 (53.9)	35/67 (52.2)	21/53 (39.6)	0.4172	0.5402	0.1271
Pseudobulbar palsy	8/37 (21.6)	21/64 (32.8)	29/101 (28.7)	19/67 (28.4)	4/53 (7.5)	0.2617	0.4934	0.0019
Stroke-like episode	23/37 (62.2)	51/65 (78.5)	74/102 (72.5)	67/67 (100)	22/53 (41.5)	0.1059	<0.0001	0.0002
Mood disorder	6/37 (16.2)	13/62 (21.0)	19/99 (19.2)	4/67 (6.0)	12/51 (23.5)	0.6095	0.1609	0.5312
Migraine	20/34 (58.8)	23/65 (35.4)	43/99 (43.4)	3/66 (4.5)	18/52 (34.6)	0.0332	<0.0001	0.3830
Vascular risk factor(s)	24/37 (64.9)	39/64 (60.9)	63/101 (62.4)	55/67 (82.1)	42/53 (79.2)	0.8316	0.0583	0.0446
Autosomal dominant Family history	27/37 (73.0)	46/65 (70.8)	73/102 (71.6)	15/67 (22.4)	33/53 (62.3)	1.0000	<0.0001	0.2760
Abnormal deep WML[b]	37/37 (100)	65/65 (100)	102/102 (100)	13/67 (19.4)	53/53 (100)	1.0000	<0.0001	1.0000
WML[b] extending to anterior temporal pole	24/36 (66.7)	53/64 (82.8)	77/100 (77.0)	1/67 (1.5)	31/53 (58.5)	0.0844	<0.0001	0.0247
WML[b] extending to external capsule	7/19 (63.2)	40/64 (62.5)	52/83 (62.7)	0/67 (0)	35/53 (66.0)	1.0000	<0.0001	0.7178

Mean ± SD of age at onset, the number of observed/studied (%), and number of males/females (% of males) are shown.
For P-value calculation, the t-test (age at onset) or Fisher's exact test was employed.
[a] Onset of the symptoms excluding migraine
[b] WML: white matter lesion. Deep WML is considered abnormal if its Fazekas grade [12] is 2 or more.

Six out of 102 CADASIL patients, and five out of 53 *NOTCH3*-negative CADASIL-like patients were asymptomatic. As the number of MRI units per million of the population is the largest in Japan among OECD countries [16], MRI is performed more frequently and sometimes leads to the incidental finding of abnormal white matter lesions by brain check-up or routine examination in Japan. When excluding these asymptomatic patients, sensitivity and specificity of the new criteria were 100% and 0%, respectively (Supplemental Table 1). Therefore, the new criteria avoid missing suspected CADASIL patients before genetic testing, if they are symptomatic.

We also evaluated our patients using the CADASIL scale. The sensitivity was 52.1% (Supplemental Table 2), being similar to a previous study reporting a sensitivity of 64.1% in Chinese patients [17]. The low sensitivity may be due to the weighting of each item. Although the highest score, five, is assigned for the item of "leukoencephalopathy extended to external capsule", the frequency of external capsule lesions in our CADASIL patients was 62.7%, that is, 37.3% of our patients lost a score of five (Table 3). In addition, although the second-highest score, three, is assigned to "migraine with aura", few of our patients with migraine had aura.

The new criteria, because of their low specificity, cannot distinguish CADASIL from *NOTCH3-negative* CADASIL-like patients. On the other hand, both Davous' criteria and the CADASIL score, because of their higher specificities than the new criteria, are thought to be helpful to prioritize patients for genetic testing. Genetic testing is essential for an accurate diagnosis for CADASIL, and also for differential diagnosis for other hereditary small vascular diseases. In Japan, cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL, OMIM#600142) caused by the mutation of *HTRA1* should be differentiated from CADASIL [18]. CARASIL shows a similar phenotype except for alopecia and lumbago. Recently,

Table 4
New diagnostic criteria for CADASIL in Japan.

Clinical criteria
#1 Age at onset (clinical symptoms #2 or white matter lesions) ≤55 years old.
#2 At least two of the following clinical findings:
a. Either of subcortical dementia, long tract signs, or pseudobulbar palsy.
b. Stroke-like episode with a focal neurological deficit.
c. Mood disorder.
d. Migraine.
#3 Autosomal dominant inheritance.
#4 White matter lesions involving the anterior temporal pole by MRI or CT.
#5 Exclusion of leukodystrophy (Adrenoleukodystrophy, metachromatic leukodystrophy, etc.).
Genetic criteria
NOTCH3 mutations localize in exons 2–24 and result in the gain or loss of cysteine residues in the epidermal growth factor-like repeat domain. Cysteine-sparing variants should be carefully evaluated by skin biopsy and segregation studies
Pathological criteria
The pathological hallmark of CADASIL is granular osmiophilic material (GOM) detected by electron microscopy. Immunostaining of NOTCH3 extracellular domain is also useful.
Definite
CADASIL is definite when the individual fulfills
(1) White matter lesions by MRI or CT.
(2) Clinical criteria #5
(3) Genetic criteria and/or pathological criteria
Probable
CADASIL is probable when the individual fulfills clinical criteria #1–#5.
Possible
CADASIL is possible when the individual has abnormal white matter lesions (Fazekas grade ≥2) and fulfills either of
(1) ≤55 years old
(2) At least one of the symptoms in clinical criteria #2
Fazekas grade was reported elsewhere [12].

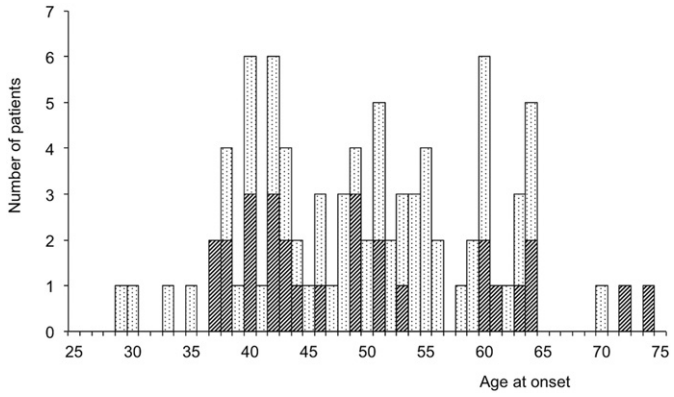

Fig. 2. Distribution of the age at onset of clinical symptoms excluding migraine in 28 symptomatic patients in Group 1 (diagonal lines) and 57 symptomatic patients in Group 2 (dotted).

Table 5
Validations of the new criteria.

	Validation 1		Validation 2	
	CADASIL Group 1 (n = 37)	Young stroke controls (n = 67)	CADASIL Group 2 (n = 65)	*NOTCH3*-negative controls (n = 53)
Positive	36	13	63	49
Negative	1	54	2	4
Sensitivity	97.3 (85.8–99.9)		96.9 (89.3–99.6)	
Specificity	80.6 (69.1–89.2)		7.5 (2.1–18.2)	

Sensitivity and specificity (%) with 95% confidence interval (95% CI, in parenthesis) are shown. Sensitivity was calculated as the positive (probable, possible, or predictive) rate in the cases. Specificity was calculated as the negative (unclassified, excluded or unclassified, or not predictive) rate in controls.

mild-phenotype CARASIL patients were reported, showing only cerebral white matter changes without alopecia or lumbago. Heterozygous mutation of *HTRA1* could also induce cerebral small vessel disease [19]. It has been suggested that only genetic diagnosis can differentiate CADASIL from CARASIL.

The characteristics and distribution of the mutations in the 102 CADASIL patients are in agreement with those in previous reports [13,20]. All of the CADASIL mutations that have been reported localize within exons 2–24, and most of them result in the gain or loss of cysteine in the EGF repeat domain. Eleven patients with R75P, an atypical cysteine-sparing mutation, were included in our subjects. Patients with R75P mutations were previously reported by us, and they were confirmed by the detection of granular osmiophilic material in skin biopsies [15], and also reported from Korea [21]. The frequency of white matter lesions in the anterior temporal lobe was lower and the age at onset tended to be older in patients with R75P than in those with other mutations [22]. However, when excluding eleven patients with R75P, the sensitivities of the new criteria, Davous' criteria and the CADASIL scale were 96.7, 52.7 and 50.0%, respectively, being similar to those in all 102 cases (Supplemental Table 2).

Our study includes several limitations. In the view of generalizability of the new criteria, the most notable limitation is that all patients were Japanese. To see difference between Japanese CADASIL patients and non-Japanese ones, we compared our study with several recent non-Japanese ones [5,11,23–28]. We found little difference between Japanese CADASIL patients and non-Japanese ones in vascular risk factors prevalence. Hypertension prevalence in Japanese CADASIL (16.8%) was slightly less than other reports (20–35.6%), however, prevalence of other factors were in line with other studies: smoking, dyslipidemia, and diabetes mellitus in Japanese CADASIL were 40, 27.7, and 5%, respectively, and range of other studies were 15.6–52% (smoking), 23–68.6% (dyslipidemia), and 3–12.7% (diabetes mellitus). We also found that mean age at onset in Japanese CADASIL (50.0 years old) was within the range of non-Japanese studies (33.6–51.2). Wide distribution of age at onset in Japanese CADASIL (29–74) was also reported in another report (15–84) [24]. These suggest that the new criteria are also applicable to non-Japanese populations.

Another limitation is sampling bias. CADASIL patients and non-*NOTCH3* patients were asked for genetic testing from various regions in Japan but not nationwide. Young stroke patients were all recruited from a single institute. To confirm high sensitivity of the new criteria, validation at other institutes is necessary.

There is also limitation concerning to genetic testing. As shown in flow of genetic testing (Supplemental Fig. 1), we selected patients to undergo full analysis of exons 2–24. This may result in extremely low specificity in *NOTCH3*-negarive controls. In addition, genetic testing to exclude CADASIL was not performed in young stroke patients, except for one patient with white matter lesion extended to anterior temporal pole. Because prevalence of CADASIL in Japanese stroke patients was reported as 0.05% [29], possibility that 67 young stoke patients include CADASIL patients is thought to be very rare.

In conclusion, the new diagnostic criteria for CADASIL showed high sensitivity but low specificity. They will help physicians, even not expertized in CADASIL, to select suspected CADASIL patients to undergo genetic testing easily.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jns.2017.08.009.

Table 6
Summary of *NOTCH3* mutations identified in 102 Japanese CADASIL patients.

Amino acid change	Nucleotide change	Exon	EGF repeat	Number of individuals
C55G[a]	c.163T>G	2	1	2
C65Y	c.194G>A	2	1	1
C65S	c.194G>C	2	1	2
R75P	c.224G>C	3	1	11
C87F[a]	c.260G>T	3	2	1
R90C	c.268C>T	3	2	2
C93G[a]	c.277T>G	3	2	2
C93Y	c.278G>A	3	2	2
C106R	c.316T>C	3	2	2
C108F[a]	c.323G>T	3	2	2
R110C	c.328C>T	3	2	2
R133C	c.397C>T	4	3	5
R141C	c.421C>T	4	3	13
C146W	c.438C>G	4	3	1
R153C	c.457C>T	4	3	4
R169C	c.505C>T	4	4	4
C174L	c.521_522delinsTG	4	4	2
S180C	c.539C>G	4	4	5
R182C	c.544C>T	4	4	7
C185Y[a]	c.554G>A	4	4	1
C194Y	c.581G>A	4	4	1
C212R[a]	c.634T>C	4	5	1
C233S	c.697T>A	5	5	1
C245Y	c.734G>A	5	6	1
C260F	c.779G>T	5	6	1
C323W[a]	c.969C>G	6	8	2
C329Y	c.986G>A	6	8	1
R332C	c.994C>T	6	8	3
G382C	c.1144G>T	7	9	1
C388Y	c.1163G>A	7	9	3
S396C	c.1187C>G	7	10	1
C455R	c.1363T>C	8	11	1
C542R	c.1624T>C	11	13	3
C542Y	c.1625G>A	11	13	1
R544C	c.1630C>T	11	13–14	1
R607C	c.1819C>T	11	15	5
C729G[a]	c.2185T>G	14	18	1
C988F[a]	c.2963G>T	18	25	1
C1004G[a]	c.3010T>G	19	26	1
R1143C	c.1142C>T	21	29	1

[a] Novel mutations not included in the recent review by Rutten et al. [13] or Leiden Open Variation Database (http://databases.lovd.nl/shared/genes/NOTCH3). C175L was reported elsewhere by Suda et al. [14]. C146W, C245Y, C260F and C329Y were reported by Mizuno [9].

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Disclosures

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