The CADASIL Scale-J, A Modified Scale to Prioritize Access to Genetic Testing for Japanese CADASIL-Suspected Patients

Takashi Koizumi, MD, Ikuko Mizuta, MD, PhD, Akiko Watanabe-Hosomi, MD, PhD, Mao Mukai, MD, Ai Hamano, MD, Jun Matsuura, MD, Tomoyuki Ohara, MD, PhD, and Toshiki Mizuno, MD, PhD

Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is definitely diagnosed by genetic testing. Such testing involves the analysis of exons 2-24 of *NOTCH3*, which encode the epidermal growth factor-like repeat domain, where CADASIL mutations are localized. We previously reported clinical diagnostic criteria for screening CADASIL-suspected Japanese patients prior to genetic testing. Because of its high sensitivity but low specificity, most patients need to undergo genetic testing. In this study, we aimed to develop the CADASIL scale-J, a modified scale to prioritize access to genetic testing for CADASIL-suspected Japanese patients. *Methods:* We modified the CADASIL scale reported by Pescini et al based on clinical features of 126 CADASIL patients and 53 *NOTCH3*-negative CADASIL-like patients diagnosed up until March 2016 (Phase 1). For validation, we recruited 69 consecutive patients for genetic testing of *NOTCH3* from April 2016 to March 2017 (Phase 2). *Results:* We developed the CADASIL scale-J with a score ranging from 0 to 25 and the cut-off value of 16, using 8 items: hypertension, diabetes, young onset (≤50 years old), pseudobulbar palsy, stroke/TIA, family history, subcortical infarction, and temporal pole lesion. The sensitivity and specificity of the CADASIL scale-J were 78.9% and 85.7%, respectively. In Phase 2, we obtained a positive predictive value of 70.0% and a negative predictive value of 89.2%. In this study, we identified 54 mutations, 7 of which were novel. *Conclusions:* The CADASIL scale-J is helpful to prioritize access to genetic testing for CADASIL-suspected Japanese patients.

Key Words: CADASIL—NOTCH3—genetic testing—clinical score

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, OMIM #125310) is one of the most frequent hereditary cerebral small vessel diseases, manifested by recurrent strokes, migraine, and white matter lesions.[1,2] Typical CADASIL patients are young individuals, despite the absence of traditional cardiovascular risk factors; however, several atypical CADASIL cases, including those involving an elderly onset and asymptomatic disease, solely with magnetic resonance imaging (MRI) findings of white matter lesions have been reported.[3-5] Therefore, for a definite diagnosis of CADASIL, genetic testing of the causative gene *Notch homolog 3 (NOTCH3)* on chromosome 19[2] or pathological detection of granular osmiophilic material in a skin biopsy sample[6,7] is essential.

From the Department of Neurology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan.

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Address correspondence to Toshiki Mizuno, MD, PhD, Department of Neurology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho Kamigyo-ku, Kyoto 602-8566, Japan. E-mails: mizuno@koto.kpu-m.ac.jp, toshimizuno2001@yahoo.co.jp.

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NOTCH3 encodes a transmembranous receptor, and all CADASIL mutations have been identified in exons 2-24 encoding the epidermal growth factor (EGF)-like repeat domain.[1,8] When no mutation is identified at mutational hotspots, exons 3-6, the remaining 19 exons should be analyzed. To perform genetic analysis efficiently, prioritizing access to genetic testing for CADASIL-suspected patients is necessary.

Recently, we reported new diagnostic criteria with high sensitivity, helpful to screen CADASIL-suspected patients to determine who should undergo genetic testing.[4] However, because of their extremely low specificity, the new criteria are not useful to prioritize access for such patients. Previously, Pescini et al proposed the CADASIL scale as a screening tool to determine patients who should receive genetic testing of *NOTCH3*.[9] Patients with a high CADASIL scale score are preferentially tested. The CADASIL scale showed high sensitivity in European studies; however, low sensitivity was reported in Chinese patients,[10] possibly due to ethnic differences in the clinical characteristics of CADASIL.

In the present study, we aimed to develop the CADASIL scale-J, a scale optimized for CADASIL-suspected Japanese patients, by modifying the CADASIL scale by Pescini et al.[9]

Materials and Methods

Participants

All participants were Japanese adults who underwent genetic testing of *NOTCH3* at Kyoto Prefectural University of Medicine (KPUM). To propose the CADASIL scale-J, we recruited 126 CADASIL patients and 53 *NOTCH3*-negative patients genetically diagnosed up until March 2016 (Phase 1). One humdred two of the 126 CADASIL patients and all the 53 *NOTCH3*-negative patients were included in our previous study.[4] *NOTCH-3*-negative patients were suspected for CADASIL because of white matter lesions extending to anterior temporal tip and/or their family history of stroke, but had no mutations in *NOTCH3* exons 2-24. To validate the CADASIL scale-J, we recruited 69 consecutive patients, who underwent genetic testing of *NOTCH3* by us from April 2016 to March 2017 (Phase 2). The negative genetic testing result in Phase 2 means no mutations in *NOTCH3* exons 2-24. Blood samples and clinical information on patients were transferred to KPUM by their physicians. Informed consent was obtained from all participants, and approval for the study was obtained from the ethical committee of KPUM.

Collection of Clinical Information

We collected clinical information including data on the clinical backgrounds (age at onset, sex, family history, and vascular risk factors), neurological symptoms, and MRI findings. The onset of the symptoms and family history included stroke/TIA, cognitive impairment, seizure, and mood disturbance. Neurological symptoms included stroke/TIA, migraine, motor palsy, sensory disturbance, dizziness, Parkinsonism, pseudobulbar palsy, seizure, mood disturbance, and cognitive impairment. MRI studies included T2-weighted imaging, fluid-attenuated inversion recovery imaging, susceptibility-weighted or T2-star-weighted imaging, and MR angiography. MRI acquisitions and imaging parameters were based on each institute standard.

Genetic Testing

Genetic testing of *NOTCH3* was performed as described previously.[4] In brief, *NOTCH3* exons 2-24, which encode the EGF-like repeat (EGFr) domain of the *NOTCH3* receptor, were analyzed by direct sequencing of genomic DNA extracted from the peripheral blood. 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 as pathogenic and/or when it resulted in cysteine-related missense mutation in one of the EGFr.

Application of the CADASIL Scale

The CADASIL scale of Pescini et al[9] was developed as a simple scale to be applied in a clinical setting as a screening tool able to predict the genetic diagnosis of CADASIL. The scale involves the additive score of 12 items (ranging from 0 to 25), whose cut-off score is 15 (Supplemental Fig 1C). Result categories of the CADASIL scale were determined as positive ($\geq$15) or negative ($<$15). When calculating the CADASIL scale score, patients with insufficient clinical information were excluded.

Development of the CADASIL Scale-J in Phase 1

The CADASIL scale-J, a scale suitable for Japanese patients, was developed by modifying the CADASIL scale by Pescini et al[9] based on the difference in clinical features between CADASIL patients and *NOTCH3*-negative patients in Phase 1. We assigned weighted scores as follows: 5 points for items showing both a significant difference on multivariate analysis and greater than or equal to 70% frequency in CADASIL patients; 3 points for items showing a significant difference on multivariate analysis but less than 70% frequency in CADASIL patients; 2 points for items showing a significant difference with a P value $< .01$ on univariate analysis; and 1 point for items showing a significant difference with $.01 < P$ value $< .05$ on univariate analysis (Table 1).

The CADASIL scale-J was applied to the patients with sufficient information (114 CADASIL patients and 49 *NOTCH3*-negative patients). The accuracy of the CADASIL scale-J versus genetic diagnosis was evaluated based on the area under the curve (AUC) value of the receiver operating characteristic (ROC) curve using JMP12 (SAS Institute,

Table 1. *Clinical features of CADASIL patients and* NOTCH3*-negative patients in Phase 1*

	CADASIL patients (n = 126)	*NOTCH3*-negative patients (n = 53)	*P* value†	Corrected *P* value‡
Background, n (%)				
Age at onset* ≤50 y	50/126 (39.7)	9/53 (17.0)	0.00030	.097
Sex (male)	60/126 (47.6)	24/53 (45.3)	.96	
Family history*	104/117 (88.9)	34/50 (68.0)	.0017	.008
Vascular risk factors				
Smoking	38/123 (30.9)	24/53 (45.3)	.44	
Hypertension	20/124 (16.1)	31/52 (59.6)	<.001	<.0001
Diabetes	6/123 (4.9)	8/52 (15.4)	.026	.31
Hyperlipidemia	32/122 (26.2)	16/53 (30.2)	.59	
Neurological symptoms, n (%)				
Stroke/TIA	89/126 (70.6)	20/53 (37.7)	<.001	.19
Migraine	54/122 (44.3)	18/52 (34.6)	.23	
Motor palsy	63/125 (50.4)	21/53 (39.6)	.19	
Sensory disturbance	20/121 (16.5)	8/53 (15.1)	.81	
Dizziness	24/123 (19.5)	5/53 (9.4)	.084	
Parkinsonism	16/123 (13.0)	5/53 (9.4)	.49	
Pseudobulbar palsy	32/124 (25.8)	4/53 (7.5)	.0030	.017
Seizure	7/122 (5.7)	4/53 (7.5)	.66	
Mood disturbance	26/122 (21.3)	12/52 (23.1)	.80	
Cognitive impairment	58/124 (46.8)	31/52 (59.6)	.12	
MRI findings, n (%)				
White matter hyperintensity				
Temporal pole	99/125 (79.2)	31/53 (58.5)	.0053	.083
External capsule	70/106 (66.0)	35/53 (66.0)	1.00	
Brain stem	29/52 (55.8)	25/53 (47.2)	.38	
Corpus callosum	2/18 (11.1)	14/52 (26.9)	.24	
Cerebral microbleeds	19/35 (54.3)	21/40 (52.5)	.82	
Subcortical infarcts	109/125 (87.2)	30/53 (56.6)	<.001	.0004
MR angiography: stenosis	16/102 (15.7)	13/47 (27.7)	.093	

*Onset of the symptoms and family history included stroke/TIA, cognitive impairment, seizure, and mood disturbance.
†Univariate logistic regression analysis was employed.
‡Corrected *P* values were calculated by multiple logistic regression analysis.

Cary, North Carolina, USA). Simultaneously, the cut-off value was determined to maximize the Youden index (Sensitivity−[1-Specificity]) by ROC analysis.

Validation of the CADASIL Scale-J in Phase 2

We validated the CADASIL scale-J by applying it to patients in Phase 2. Result categories of the CADASIL scale-J were determined as positive (score equal to or more than the cut-off value) or negative (score lower than the cut-off value). The positive predictive value was calculated as the proportion of patients with *NOTCH3* mutation among those positive using the CADASIL scale-J. The negative predictive value was calculated as the proportion of patients without *NOTCH3* mutation among those negative using the CADASIL scale-J.

Statistics

The frequencies of clinical features variables in CADASIL patients were compared with those of *NOTCH3*-negative patients by logistic regression analysis. The covariate was dichotomized into with or without each clinical item. The outcome variable was dichotomized into CADASIL or *NOTCH3*-negative non-CADASIL diagnosed by genetic testing. After the univariate logistic regression analysis, only the significant variables were included in the multivariate logistic regression analysis. We used JMP12 (SAS Institute) for statistical calculations. Values of $P < .05$ were considered significant.

Results

The CADASIL Scale-J Based on Clinical Features of Japanese CADASIL Patients

The flow of participants is shown in Figure 1A. Mean ages at the assessment of CADASIL patients (n = 126, 111 families) and *NOTCH3*-negative patients (n = 53) were 49.6 ± 9.4 and 60.2 ± 11.9, respectively. Fifty-one out of the 53 *NOTCH3*-negative patients (96%) were tentatively diagnosed as cerebral small vessel disease (CSVD),[11] indicating that they were appropriate for the control in this study (Supplemental Table 1). According to the classification by

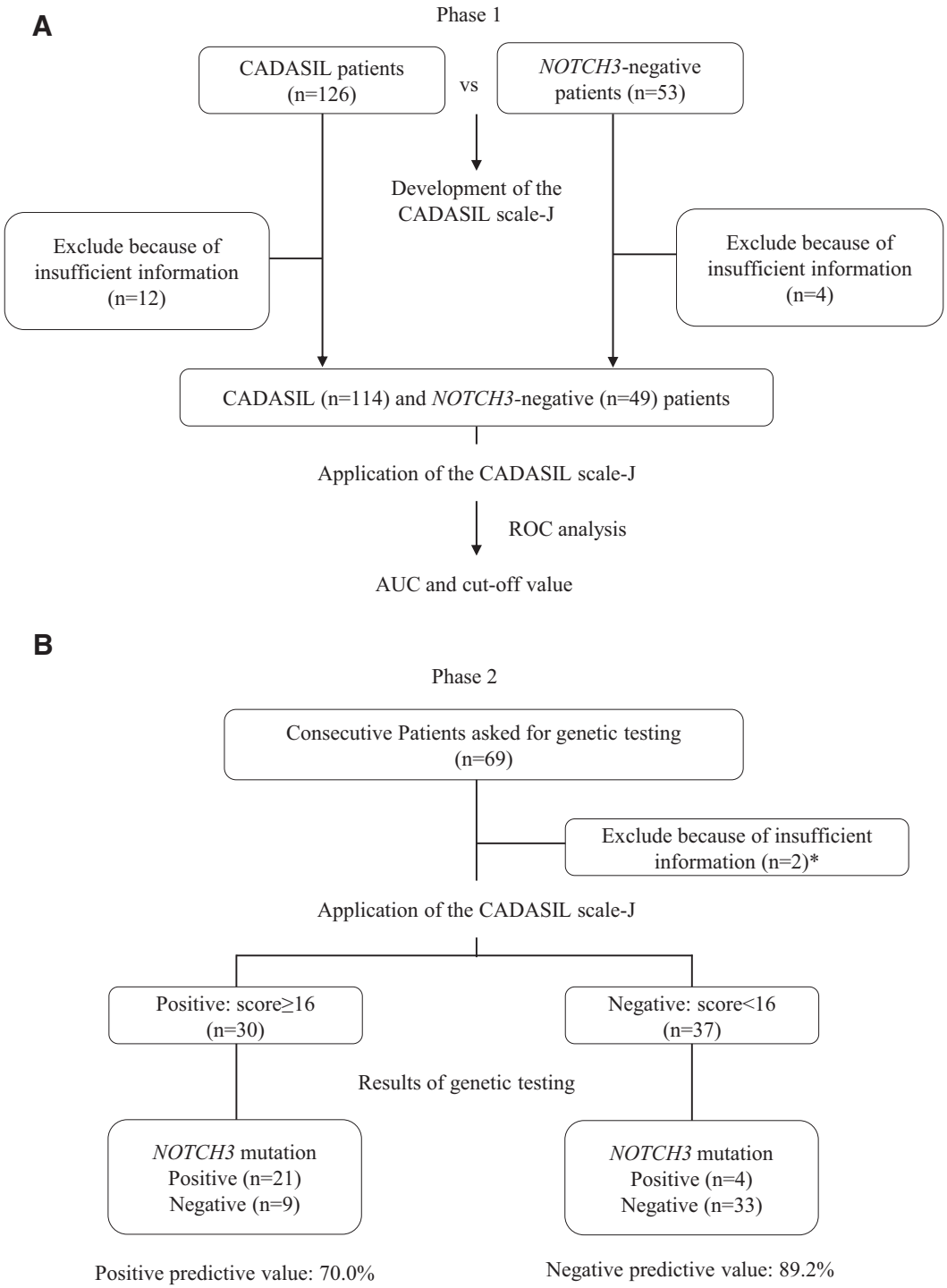

Figure 1. *Flowchart of participants. (A) Development of the CADASIL scale-J in Phase 1. (B) Validation of the CADASIL scale-J in Phase 2. *Two patients excluded because of insufficient information were CADASIL patients.*

Pantoni,[11] they were classified into CSVD type 1 (age-related and vascular risk factor-related, n = 35), CSVD type 2 (cerebral amyloid angiopathy, n = 1), CSVD type 3 (inherited or genetic, n = 8), CSVD type 4 (inflammatory and immunologically mediated, n = 2), and CSVD type 6 (other, n = 6). The remaining 2 were diagnosed as suspected leukodystrophy. Eight patients with CSVD type 3 included 7 patients with family history and without vascular risk factors and 1 diagnosed as mitochondrial encephalopathy.

To develop the CADASIL scale-J, we compared the clinical features of CADASIL and *NOTCH3*-negative patients in Phase 1 (Table 1). In univariate analysis, significant differences were noted in 8 items: ages at first onset of symptom, family history, hypertension, diabetes, stroke/TIA, pseudobulbar

palsy, white matter hyperintensity on MRI in the temporal pole, and subcortical infarcts (Table 1). After multivariate logistic regression analysis of these 8 items, significant differences remained in the family history, hypertension, pseudobulbar palsy, and subcortical infarcts. Considering the frequencies of these significant items (see Materials and Methods section), we assigned a weighted score of 5 for hypertension, subcortical infarcts, and family history, 3 for pseudobulbar palsy, 2 for leukoencephalopathy at temporal lobe, age at first onset less than or equal to 50, stroke/TIA, and 1 for diabetes to the CADASIL scale-J (Fig 2A). The total score ranged from 0 to 25. Modified points are shown by comparing the previously reported CADASIL scale and the CADASIL scale-J (Supplemental Fig 1C). Then, we extracted 114 CADASIL patients and 49 *NOTCH3*-negative patients with sufficient clinical information and calculated the score for each patient. Scores ranged from 8 to 25 with a maximum of 20 in CADASIL patients, whereas they ranged from 0 to 20 with a maximum of 13 in *NOTCH3*-negative patients (Fig 2B). To evaluate the accuracy of this scale, we performed ROC analysis and obtained an AUC of .89 and a cut-off value of 16 (Supplemental Fig 1A). Using this cut-off value, the sensitivity and specificity of the CADASIL scale-J in Phase 1 were 78.9% and 85.7%, respectively (Table 2).

Validation of the CADASIL Scale-J in Phase 2

The flow of participants is shown in Figure 1B. Two patients, both of whom were positive for genetic testing, were excluded because of insufficient information. The CADASIL scale-J scores were greater than or equal to 16 in 30 patients, 21 of whom had *NOTCH3* mutations. This yielded a positive predictive value of 70.0% (21/30). On the other hand, the CADASIL scale-J scores were less than 16 in 37 patients, 33 of whom did not have *NOTCH3* mutations. This yielded a negative predictive value of 89.2% (33 of 37). The overall sensitivity and specificity in Phase 2 were 84.0% and 78.6%, respectively (Table 2).

A

CADASIL scale-J

• Without hypertension	5
• Subcortical infarcts	5
• Family history*	5
• Pseudobulbar palsy	3
• Leukoencephalopathy at temporal pole	2
• Age at first onset* ≤50 y	2
• Stroke / TIA	2
• Without diabetes	1

B

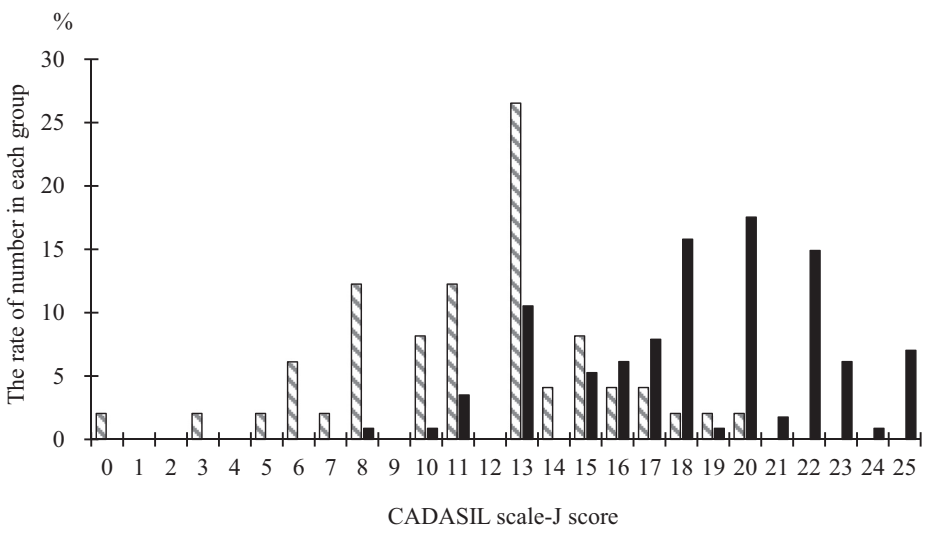

Figure 2. *The CADASIL scale-J. (A) The score for each item. The total score ranges from 0 to 25. The cut-off value is 16 points. *Family history and onset of the symptoms included stroke/TIA, cognitive impairment, seizure, and mood disturbance, but excluded migraine. (B) Distribution of the CADASIL scale-J score of CADASIL patients (black) and* NOTCH3 *negative patients (striped).*

Table 2. *Sensitivity and specificity of the CADASIL scale-J in Phases 1 and 2*

	Phase 1		Phase 2	
	CADASIL patients	*NOTCH3*-negative patients	CADASIL patients	*NOTCH3*- negative patients
Positive (score ≥16) (n)	90	7	21	9
Negative (score <16) (n)	24	42	4	33
Sensitivity (%)	78.9		84.0	
Specificity (%)	85.7		78.6	

Sensitivity was calculated as the positive (score ≥16) rate in CADASIL patients. Specificity was calculated as the negative (score <16) rate in *NOTCH3*-negative patients.

The clinical characteristics of the CADASIL patients and *NOTCH3*-negative patients in Phase 2 are shown in Supplemental Table 2. Significantly different items between CADASIL patients and *NOTCH3*-negative patients in Phase 2 were similar to those in Phase 1, except for the absence of significant differences in family history and diabetes in Phase 2 (Supplemental Table 2).

Comparison between the CADASIL Scale and the CADASIL Scale-J

In our patients, the diagnostic accuracy of the CADASIL scale-J was higher compared with that of the CADASIL scale of Pescini et al.[9] ROC analysis of the CADASIL scale in Phase 1 showed an AUC of .61, which was lower than that of the CADASIL scale-J at .89, and an optimal cut-off value of 16 (Supplemental Fig 1A and B). Both scales showed high specificity (78.6%-85.7%) in Phase 1 and Phase 2. However, sensitivities of the CADASIL scale-J (78.9% in Phase 1 and 84.0% in Phase 2) were apparently higher than those of the CADASIL scale (40.6% in Phase 1 and 48.0% in Phase 2) (Table 2 and Supplemental Table 3).

NOTCH3 Mutations

In the total of 153 CADASIL patients in Phases 1 and 2, including those with insufficient information to calculate the scores, we identified 54 pathogenic mutations of *NOTCH3* located in exons 2-8, 11, 14, and 18-21 (Table 3). Seven mutations: p.C55R, p.C419R, p.C435Y, p.C606S, p.C1015W, p.C1022G, and p.S1067C, were novel ones. The patients with p.C323W were reported elsewhere.[12] One of the patients with p.R544C was homozygous and recently reported elsewhere by us.[13] Most of the mutations existed in exons 3-6 (107 of the 153 patients, 69.9%), particularly exons 3-4 (96 of the 153 patients, 62.7%).

Discussion

We developed the CADASIL scale-J, which can effectively discriminate between CADASIL patients and *NOTCH3*-negative CADASIL-like patients among Japanese patients (Table 2, Fig 1B, and Supplemental Fig 1A). The ROC analysis of our scale in Phase 1 yielded an AUC of .89 showing marked accuracy.[14] Sensitivity and specificity were high in both Phase 1 (78.9% and 85.7%, respectively) and Phase 2 (84.0% and 78.6%, respectively). Also, the positive predictive value was high at 70.0% and the negative predictive value was high at 89.2% in Phase 2.

The CADASIL scale-J exhibited a higher diagnostic accuracy compared with the CADASIL scale (Supplemental Fig 1B). This may be at least partially due to ethnic differences in the clinical characteristics of CADASIL. The CADASIL scale of Pescini et al is based on data reported on 536 CADASIL patients, most of whom were Caucasians. Although the items of migraine and leukoencephalopathy extending to the external capsule were highly weighted in the CADASIL scale of Pescini et al, they were not specific to Japanese CADASIL patients in our study (Table 1, Supplemental Fig 1C). Because the frequency of migraine, mood disturbance, cognitive impairment, or external capsule lesions was not significantly different between CADASIL and *NOTCH3*-negative patients, we omitted these items in the CADASIL scale-J (Table 1, Supplemental Fig 1C). Leukoencephalopathy extending to the temporal pole was more specific to CADASIL rather than the external capsule in Japanese patients. In addition, items of the CADASIL scale did not include hypertension or pseudobulbar palsy, both of which showed a clearly significant difference between CADASIL patients and *NOTCH3* negative patients in this study. Our findings suggest that it is necessary to develop a new scale suitable for Japanese patients, in agreement with a previous study in a Chinese population. Similar to our findings, Liu et al reported a higher rate of temporal pole involvement, but not external capsule involvement in CADASIL compared with non-CADASIL Chinese patients.[10] They also noted a significant difference in the rate of hypertension but no significant difference in that of migraine between CADASIL and non-CADASIL patients.[10]

By applying the CADASIL scale-J to consecutive patients (Phase 2), we obtained a positive predictive value of 70.0%, being higher than the positive rate of genetic testing of 39.1% (27 of 69). We also obtained a negative predictive value of 89.1%, being higher than the negative rate of genetic testing of 59.4% (42 of 69), among CADASIL-suspected patients (Fig 1B). Four CADASIL patients who failed to exceed the cut-off score of 16 were asymptomatic or older-onset with multiple vascular risk factors.

Table 3. *Summary of* NOTCH3 *mutations identified in 153 Japanese CADASIL patients*

Amino acid change	Nucleotide change	Exon	EGF-like repeat	Number of individuals
p.C43F	c.128G > T	2	1	1
p.R54C	c.160C > T	2	1	2
p.C55G	c.163T > G	2	1	2
p.C55R*	c.163T > C	2	1	1
p.C65S	c.194G > C	2	1	3
p.C65Y	c.194G > A	2	1	1
p.W71C	c.213G > T	3	1	1
p.R75P	c.224G > C	3	1	14
p.C76Y	c.227G > A	3	1	2
p.C87F	c.260G > T	3	2	1
p.R90C	c.268C > T	3	2	2
p.C93G	c.277T > G	3	2	2
p.C93Y	c.278G > A	3	2	2
p.C106R	c.316T > C	3	2	2
p.C108F	c.323G > T	3	2	2
p.R110C	c.328C > T	3	2	2
p.R133C	c.397C >T	4	3	8
p.R141C	c.421C > T	4	3	17
p.C146W	c.438C > G	4	3	1
p.R153C	c.457C > T	4	3	8
p.R169C	c.505C > T	4	4	6
p.C174L	c.521_522delinsTG	4	4	2
p.S180C	c.539C > G	4	4	6
p.R182C	c.544C > T	4	4	13
p.C185Y	c.554G > A	4	4	2
p.C194Y	c.581G > A	4	4	1
p.C212R	c.634T > C	4	5	2
p.C233S	c.697T > A	5	5	1
p.C245Y	c.734G > A	5	6	1
p.C260F	c.779G > T	5	6	2
p.C323W	c.969C > G	6	8	2
p.C329Y	c.986G > A	6	8	1
p.R332C	c.994C > T	6	8	4
p.G382C	c.1144G > T	7	9	1
p.C388Y	c.1163G > A	7	9	3
p.S396C	c.1187C > G	7	10	1
p.C419R*	c.1255T > C	8	10	3
p.C435Y*	c.1304G > A	8	11	1
p.C455R	c.1363T > C	8	11	1
p.C457S	c.1370G > C	8	11	1
p.C542R	c.1624T > C	11	13	3
p.C542Y	c.1625G > A	11	13	1
p.R544C	c.1630C > T	11	13-14	2
p.C606S*	c.1817G > C	11	15	1
p.R607C	c.1819C > T	11	15	7
p.C729G	c.2185T > G	14	18	1
p.R985C	c.2953C > T	18	25	1
p.C988F	c.2963G > T	18	25	1
p.C1004G	c.3010T > G	19	26	3
p.C1015W*	c.3045C > G	19	26	1
p.Y1021C	c.3062A > G	19	26	1
p.C1022G*	c.3064T > G	19	26	1
p.S1067C*	c.3200C > G	20	27	1
p.R1143C	c.3427C > T	21	29	1
Total				153

*Novel mutations not reported by Rutten et al (2014), Mizuta et al (2017), Yeung et al (2018), the Leiden Open Variation Database, or references therein.

We calculated a positive rate and an average score using the CADASIL scale-J for 7 genotypes, each of which included more than 5 patients (Supplemental Table 4). The low positive rate was 63.6% for p.R75P and 61.5% for p.R182C. The p.R75P subgroup showed the lowest average score of the CADASIL scale-J at 15.5 ± 3.8, which was lower than the cut-off value at 16. The p.R75P mutation is an atypical cysteine-sparing but already pathologically confirmed one mainly reported from Japan and Korea.[15,16] The low positive rate and the low average score in p.R75P subgroup are thought to be due to a lower frequency of temporal pole lesions and older age at onset compared with other genotypes, as reported previously.[17] The average score of the CADASIL scale-J in p.R182C subgroup at 17.2 ± 4.3 was the second lowest among the genotypes studied, but exceeded cut-off score of 16, suggesting another reason for the lowest sensitivity at 61.5% in p.R182C. We next compared the distribution of individual scores among p.R75P, p.R182C, and p.R141C, and found the widest distribution in p.R182C (Supplemental Fig 2). We, therefore, think that especially low sensitivity in p.R182C subgroup was mainly due to a wide variety of scores among the individuals.

Because Rutten et al recently reported that *NOTCH3* EGFr 1-6 pathogenic variants are associated with a more severe phenotype compared with EGFr 7-34 pathogenic variants,[18] we also compared the CADASIL scale-J positive rate between EGFr 1-6 and 7-34 subgroups. The positive rates for EGFr 1-6 group and 7-34 groups were similar, 79.8% and 80.0%, respectively (Supplemental Table 4). The average score of the CADASIL scale-J for EGFr 1-6 group and 7-34 groups also were similar, 18.3 ± 3.3 and 18.9 ± 4.1, respectively (Supplemental Table 4).

One limitation of this study was that it was performed in a single center. To confirm and improve the accuracy of the CADASIL scale-J, replication analysis in multiple centers is necessary. Another limitation was that patients with insufficient information were not included in this study. Finally, the possibility of hereditary small vessel diseases other than CADASIL in *NOTCH3*-negative patients was not excluded from this study. When we compared clinical features of 16 *NOTCH3*-negative patients with the high score (≥16) to those of 139 CADASIL patients, no item of clinical features showed a significant difference between them (Supplemental Table 5). This suggested that patients with CADASIL-like disease caused by other than *NOTCH3* may be included in *NOTCH3*-negative patients with high score. To clarify how many patients with other known hereditary CSVD were included in *NOTCH3*-negative controls, further genetic testing of the causative genes including *HTRA1*,[19-21] *COL4A1*,[22-24] *TREX1*,[25,26] and *CTSA*[27] for cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy, pontine autosomal dominant microangiopathy with leukoencephalopathy, retinal vasculopathy and cerebral leukodystrophy, and cathepsin A-related arteriopathy with strokes and leukoencephalopathy, respectively, is necessary.

In conclusion, we established the CADASIL scale-J for Japanese patients. It is useful to prioritize access to genetic testing among CADASIL-suspected Japanese patients and helpful for researchers to perform genetic testing efficiently.

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Author Contributions

T.K., A.W-H., I.M., and T.M. designed the study; T.K., M. M., A.H., A.W-H., I.M., J.M., T.O., and T.M. collected data and analyzed; T.K., I.M., and T.M. wrote the paper.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2019.03.026.

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