The Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) Scale : A Screening Tool to Select Patients for NOTCH3 Gene Analysis

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The Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) Scale

A Screening Tool to Select Patients for *NOTCH3* Gene Analysis

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Background and Purpose—Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) phenotype is highly variable, and, although the full clinical–neuroimaging picture may be suggestive of the disease, no characteristic is pathognomonic. Thus, a genetic test remains the diagnostic gold standard, but because it is costly and time-consuming, a pregenetic screening appears desirable. We aimed at developing the CADASIL scale, a screening tool to be applied in the clinical setting.

Methods—A preliminary scale was created assigning weighted scores to common disease features based on their frequencies obtained in a pooled analysis of selected international CADASIL series. The accuracy of the scale versus the genetic diagnosis was tested with receiver operating characteristic analysis after the application of this scale to 61 CADASIL and 54 *NOTCH3*-negative patients (no pathogenic mutation on exons 2–23 of the *NOTCH3* gene). To improve the scale accuracy, we then developed an ad hoc optimization algorithm to detect the definitive scale. A third group of 39 patients affected by sporadic small-vessel disease was finally included in the algorithm to evaluate the stability of the scale.

Results—The cutoff score of the definitive CADASIL scale had a sensitivity of 96.7% and a specificity of 74.2%. This scale was robust to contamination of patients with sporadic small-vessel disease.

Conclusions—The CADASIL scale is a simple and sufficiently accurate screening tool to select patients with a high probability to be affected by the disease and therefore to be subjected to the genetic testing. **(*Stroke*. 2012;43:2871-2876.)**

Key Words: CADASIL ■ diagnostic methods ■ CADASIL scale ■ lacunar infarcts ■ leukoencephalopathy ■ screening tool

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL; MIM 125310) is an autosomal-dominant small-vessel disease (SVD) caused by highly stereotyped mutations on exons 2 to 23 of the *NOTCH3* gene.[1,2] The disease is characterized by migraine, frequently with aura, transient ischemic attacks and/or strokes, mood disorders, and cognitive decline leading progressively to dementia and disability.[3] However, the clinical picture may be extremely variable and some patients present few disturbances and a milder course even at old age.[4,5] Typical neuroimaging features are severe leukoencephalopathy, frequently involving the temporal pole and the external capsule, lacunar lesions, and microbleeds.[6–11] Also the neuroimaging picture may be variable, particularly in the initial stages.[12]

Overall, the recognition of the disease before the development of the full clinical–neuroimaging picture may be challenging. Moreover, as we recently reported, none of the

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mentioned characteristics is pathognomonic of CADASIL and a considerable number of patients with clinical and neuroimaging features suggestive of CADASIL but without pathogenic mutations (*NOTCH3*-negative patients) is emerging.[13] The genetic analysis is costly and time-consuming because the *NOTCH3* gene is long and mutations can be found in any of the 22 exons codifying for the epidermal growth factors-like repeats.[14] Furthermore, one must consider the ethical implications and psychological burden associated with the request to perform a genetic test for a disease with an autosomal-dominant pattern of inheritance. Skin biopsy, the other diagnostic test for CADASIL, although specific, has a variable sensitivity.[15,16] Thus, a pregenetic screening tool appears needed.

The aim of this study was to develop a simple scale (the CADASIL scale) to be applied in the clinical setting as a screening tool able to predict the genetic diagnosis of CADASIL and to test its accuracy.

Methods

The study was carried out in 3 phases. In the first one, we performed a pooled analysis of selected CADASIL series to derive data to be used for the creation of a preliminary CADASIL scale. In the second phase, we applied this preliminary scale to CADASIL and *NOTCH3*-negative patients already followed in our centers. In the third phase, to further improve the scale accuracy, we conducted an advanced statistical analysis including also a third group of patients affected by sporadic SVD and we obtained the definitive CADASIL scale.

The study was approved by the local ethics committee of the 3 participating centers. All patients gave informed consent for genetic testing and involvement in the study.

First Phase: Pooled Analysis of CADASIL Series and Identification of Variables to be Used in the Scale

We performed a systematic review of the published CADASIL series to estimate the frequency of the typical clinical (transient ischemic attack/stroke, migraine, psychiatric disturbances, and cognitive deficits) and neuroimaging (leukoencephalopathy and subcortical infarcts) features of the disease. We carried out a MEDLINE search from 1990 to 2009 using the key word "CADASIL" and all the published articles were reviewed. To include data in the pooled analysis, studies were selected based on the following criteria: (1) CADASIL diagnosis based on the finding of typical *NOTCH3* gene mutations involving cysteine and/or granular osmiophilic material deposits in skin biopsy on electronic microscopy; (2) availability of the clinical and/or neuroimaging data; (3) sample size ≥10 patients; and (4) English, French, Italian, or Spanish language. In the case of articles from the same group, we selected the one reporting the highest number of patients or the most recent publication. Articles from which it was not possible to extract crude data were excluded. In addition, we included one unpublished series for which data were accessible (M. Viitanen, personal communication).

For the creation of the scale we itemized clinical, neuroimaging, and family history characteristics. We attributed weighted scores to each clinical and neuroimaging feature according to the frequency pointed out in the pooled analysis, considering only patients with clinical or neuroimaging findings typical of the disease: 1 for 0% to 49%, 2 for 50% to 69%, and 3 for ≥70% frequency. Because of the autosomal-dominant pattern of inheritance of the disease, although data concerning family history were not specifically reported in the mentioned series, we added a score also for this variable. Family history was considered positive when at least one typical disturbance of CADASIL (cerebrovascular events, psychiatric and cognitive disturbances, and headache) was present in at least one generation. A higher score was attributed if the disturbances were present in ≥2 generations.

Second Phase: Application of the Scale to CADASIL and *NOTCH3*-Negative Patients

The scale obtained in the first phase was applied to 2 groups of patients filed in our database: (1) patients with a genetic diagnosis of CADASIL; and (2) patients with a phenotype suggestive of CADASIL but in whom the *NOTCH3* gene analysis did not reveal any pathogenic mutation on exons 2 to 23 (*NOTCH3*-negative group).

The genetic analysis was performed at the Department of Neurological, Neurosurgical and Behavioral Sciences, University of Siena as previously reported.[13] In short, total genomic DNA was extracted from peripheral blood leukocytes using standard procedures. Polymerase chain reaction was performed with primers (comprising intron–exon boundaries) specific for exons 2 to 23 of the *NOTCH3* gene. Sequencing was performed using the automated sequencer ABI 3730 (Applied Biosystems, Foster City, CA).

Only probands who had clinical and neuroimaging features suggestive of CADASIL were considered. For all patients, information on clinical data and family history (headache, transient ischemic attack, stroke, psychiatric disorders, and cognitive deficits) in ≥2 generations was collected by means of a structured interview focused on the typical CADASIL features (for definitions, see Pantoni et al[13]). Because of the screening aim of the scale, we used only data available at the time of disease suspicion when blood was withdrawn for genetic test. In 3 patients with CADASIL, family history information was not obtainable.

Regarding neuroimaging data, we used MRI scans performed for clinical purposes before or at the time of blood withdrawal for genetic testing. A neurologist experienced in brain imaging evaluation (L.P.) and blind to the diagnosis reviewed all the available scans and assessed the following parameters: presence of leukoencephalopathy (white matter changes on fluid-attenuated inversion recovery turbo spin echo or T2-weighted turbo spin echo images); leukoencephalopathy uni- or bilaterally extended to the region anterior to the temporal horn (temporal pole)[7]; leukoencephalopathy uni- or bilaterally extended to the external capsule that could be partially or completely involved[7]; presence of lacunar infarcts defined as focal hyperintensities on T2-weighted turbo spin echo images, ≥3 mm in size, and with a corresponding hypointensity on T1-weighted turbo spin echo images.[17] In 2 CADASIL and 2 *NOTCH3*-negative patients, it was not possible to evaluate the external capsule involvement on the available MRI scans.

Differences in the frequency of clinical features, family history, and neuroimaging findings between the 2 groups were assessed by Fisher exact test for categorical variables and *t* test for numeric variables.

For each patient we calculated the total score applying the CADASIL scale previously obtained. Then, we performed a receiver operating characteristic analysis to evaluate the accuracy of this scale in relation to the genetic diagnosis.[18] For each score of the scale we determined sensitivity and specificity to identify a cutoff point able to predict the presence of the disease.

Third Phase: Ad Hoc Optimization Algorithm, Creation, and Assessment of Stability of the Definitive CADASIL Scale

To improve the previously developed scale, logistic regression analyses were conducted including in the models the variables selected on the basis of the pooled analysis with the addition of the variable "age at first transient ischemic attack or stroke" that although not present in the pooled analysis turned out to be important in predicting the presence of *NOTCH3* gene mutations. All the regression models were coherent in identifying leukoencephalopathy extended to the external capsule and age at first transient ischemic attack or stroke ≤50 years as the most highly significant variables.

Then, we developed an ad hoc optimization algorithm to identify optimal scores for each variable and the cutoff point that best distinguished CADASIL from *NOTCH3*-negative patients. Only

Table 1. Frequency of Clinical Features in the Series of Patients With CADASIL Used in the Pooled Analysis

CADASIL Series	Migraine	Migraine With Aura	TIA or Stroke	Psychiatric Disturbances	Cognitive Decline/Dementia
Chabriat et al, 1995,[20] No.			38/45	9/45	14/45
Dichgans et al, 1998,[21] No.	39/83	34/39	72/83	31/83	49/83
Desmond et al, 1999,[22] No.	45/105	30/45	71/105	22/105	63/105
Uchino et al, 2002,[23] No.	1/10				5/10
Singhal et al, 2004,[24] No.	92/119		71/119	35/112	22/119
Kim et al, 2006,[25] No.	1/14	1/1	13/14	5/14	7/14
Yin et al, 2009,[26] No.	5/37		35/37		22/37
Lee et al, 2009,[27] No.			15/21	4/21	6/21
M. Viitanen,* No.	56/95		65/102		
Total No. (%)	239/463 (52)	65/85 (76)	380/526 (72)	106/380 (28)	188/434 (43)

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; TIA, transient ischemic attack.
*Personal communication.

integer values between 1 and 5 were used to weigh variables. Each combination of integer weights generated a different scale and, for each scale, a cutoff score was chosen by maximizing the total number of correct classifications. This gave an efficiency frontier of scales. The best cutoff scores were evaluated on the basis of sensitivity and specificity. The Cartesian crossproduct of all the possible weights generated a huge number (>240 million) of scales to be evaluated.

Correlations among variables and estimates of regression coefficients were used to refine the range of integer values assigned to each variable, markedly reducing the number of scales to be evaluated. An ad hoc algorithm was implemented to evaluate all the remaining scales (12 million); the algorithm moved inside them looking for the frontier using a technique similar to those characterizing surface response methodology in experimental design. To evaluate the stability of the scales on the frontier, the algorithm was applied extending the data to a group of patients with sporadic SVD previously enrolled in a multicenter national study[19]; inclusion criteria were age between 65 and 84 years and presence of leukoencephalopathy on brain MRI related to SVD. In these patients, other causes of leukoencephalopathy (infectious, demyelinating, inflammatory, metabolic) were excluded. The scales on the new frontier computed by the algorithm were matched with those previously identified to choose the definitive scale. Wilson score intervals were calculated for both sensitivity and specificity of the definitive scale to provide a confidence level for each estimate.

All the statistical analyses and computing were performed using the packages SPSS (Statistical Package for Social Sciences, Chicago, IL; Version 17.0) and R (R Foundation for Statistical Computing; Version 2.12.2), both for the Windows operating system.

Results

Fifteen CADASIL series were finally implemented in the pooled analysis.[6,7,11,20–30] Nine of these reported data about clinical features accounting for a total of 536 patients (Table 1) and 8 about neuroimaging accounting for 435 patients (Table 2). These series included patients from Europe, North and Central America, and Asia. Based on the obtained frequencies of the most typical clinical and neuroimaging features of the disease, we developed the preliminary CADASIL scale.

We applied the scale on 61 genetically diagnosed patients with CADASIL and 54 *NOTCH3*-negative patients. The mean age at the time of disease suspicion was 57.0±12.3 years in CADASIL and 60.1±13.6 years in *NOTCH3*-negative patients. Table 3 reports the frequency of the clinical, family history, and neuroimaging variables in both groups.

Table 2. Frequency of Neuroimaging Features in the Series of Patients With CADASIL Used in the Pooled Analysis

CADASIL Series	Presence of LE	LE Extended to Temporal Pole	LE Extended to External Capsule	Presence of Subcortical Infarcts
Chabriat et al, 1998,[6] No.	68/68		36/68	47/68
Auer et al, 2001,[7] No.		26/28	23/28	
van den Boom et al, 2003,[28] No.	40/40	39/40	24/40	32/40
Singhal et al, 2005,[11] No.		90/112	93/112	
Kim et al, 2006,[25] No.	14/14	6/14	14/14	10/14
Choi et al, 2006,[29] No.		4/20	18/20	
Lee et al, 2009,[27] No.	21/21	9/21	20/21	
Viswanathan et al, 2010,[30] No.	134/134			121/132
Total No. (%)	277/277 (100)	174/235 (74)	228/303 (75)	210/254 (83)

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; LE, leukoencephalopathy.

Table 3. Clinical and Neuroimaging Features in CADASIL and *NOTCH3*-Negative Patients

	Patients With CADASIL (n=61)	*NOTCH3*-Negative Patients (n=54)	*P* Value*
Age at disease suspicion, y, mean±SD	57.0±12.3	60.1±13.6	0.205
Age at first stroke/TIA, y, mean±SD	50.7±12.3	58.2±11.2	0.008
Male sex, No. (%)	30/61 (49)	25/54 (46)	0.852
Migraine, No. (%)	30/61 (49)	26/54 (48)	1.000
With aura, No. (%)	15/30 (50)	10/26 (39)	0.430
TIA/stroke, No. (%)	49/61 (80)	29/54 (54)	0.003
Psychiatric disturbances, No. (%)	32/61 (53)	37/54 (69)	0.089
Cognitive deficits/dementia, No. (%)	35/61 (57)	25/54 (46)	0.265
Presence of family history† in at least 1 generation, No. (%)	58/58 (100)	54/54 (100)	1.000
Presence of family history† in at least 2 generations, No. (%)	48/58 (83)	50/54 (93)	0.155
Presence of leukoencephalopathy, No. (%)	61/61 (100)	51/54 (94)	0.100
Extended to temporal poles, No. (%)	47/61 (77)	26/51 (51)	0.005
Extended to external capsule, No. (%)	57/59 (97)	21/49 (43)	0.000
Subcortical infarcts, No. (%)	54/61 (89)	34/54 (63)	0.002

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; TIA, transient ischemic attack.

*Fisher exact test except *t* test for continuous variable (age).

†For at least 1 of the typical disturbances (headache, TIA/stroke, cognitive decline, psychiatric disturbances).

We performed a receiver operating characteristic analysis and found an area under the curve of 0.769 (range, 0.681–0.858) that corresponds to a moderate accuracy.[18] Because the CADASIL scale was primarily developed to be a screening tool, a high sensitivity was to be preferred when selecting a cutoff score able to predict the presence of the disease. However, we found low specificity values when sensitivity was sufficiently high (best identified values were 53.7% and 91.8%, respectively).

Applying the ad hoc optimization algorithm in CADASIL and *NOTCH3*-negative patients, on the obtained efficiency frontier we found 30 different scales with maximum values of sensitivity of 98.4% and specificity of 66.7%. Including in the algorithm also 39 patients with sporadic SVD (mean age, 72.0±5.1; males 57%), we found a new frontier in which the scales that maximize the sensitivity were 10. Because of the few classification errors in the last group of patients, despite a mild reduction of the sensitivity (96.7%), higher specificity (74.2%) was found for these 10 scales (95% Wilson score intervals, 87.6%–99.4% and 63.9%–82.5%, respectively).

All these 10 scales belonged to the set of the 30 scales found on the efficiency frontier previously computed on data from CADASIL and *NOTCH3*-negative patients, showing that the methodology adopted to identify the efficiency frontier was reliable. The obtained 10 scales were overlapping except for values attributed to the presence of leukoencephalopathy (ranging from 1 to 5) and positive family history in at least one generation (ranging from 1 to 2). We chose the scale including the score of 3 for the first variable to balance its weight in comparison with the other variables and the score of 1 for the second variable to attribute different weights to the positivity of family history in one or more generations (Table 4). On the basis of this scale (total score ranging from 0 to 25), the best cutoff score able to select patients with CADASIL suspicion who should undergo the genetic analysis was 14 (scale values >14 are suggestive of CADASIL).

Discussion

We developed the CADASIL scale, a simple screening tool to be used in the clinical setting for the selection of patients to be subjected to *NOTCH3* gene analysis. The statistical methodology that we applied led us to achieve an enough

Table 4. CADASIL Scale

Migraine	1
Migraine with aura	3
TIA or stroke	1
TIA/stroke onset ≤50 y	2
Psychiatric disturbances	1
Cognitive decline/dementia	3
Leukoencephalopathy	3
Leukoencephalopathy extended to temporal pole	1
Leukoencephalopathy extended to external capsule	5
Subcortical infarcts	2
Family history* in at least 1 generation	1
Family history* in at least 2 generations	2

The total score (ranging from 0 to 25) is obtained by the sum of the score attributed to each variable. A total score ≥15 is predictive of CADASIL diagnosis.

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

*For at least 1 of the typical disturbances (headache, transient ischemic attack/stroke, cognitive decline, psychiatric disturbances).

high accuracy of the scale with optimal sensitivity and specificity values of the cutoff score favoring sensitivity as requested for a screening tool.

Previously, a few attempts of diagnostic strategies for CADASIL have been made,[8,31,32] but they have been seldom used and never validated. Some of the items of the Davous criteria[31] (ie, age, presence or absence of vascular risk factors, neuroimaging findings) that were proposed at a time when the knowledge about the disease was more limited may be questionable on the basis of the currently available data. For the same reason a genetic analysis limited to a few exons in association with clinical and neuroimaging data as proposed by Markus and coworkers[8] seems dated today.[14,33]

In CADASIL, a pregenetic screening is needed because the analysis of the *NOTCH3* gene is costly and time-consuming. Furthermore, one must consider the emotional distress of patients and their relatives related to the performance of a genetic test for an autosomal-dominant disease; the time before the result is achieved can be very long, and the negativity of the test does not exclude the presence of other inherited diseases and therefore is not entirely reassuring for the patients. Thus, a careful selection of patients to be subjected to genetic testing is desirable.

When performing the *NOTCH3* gene analysis, another aspect to consider is that a significant number of patients with a phenotype suggestive of CADASIL but without pathogenic mutations emerges.[13] An accurate selection of patients could allow to limit the number of *NOTCH3*-negative patients and to identify a group that can be more appropriately considered "CADASIL-like" (ie, with a high clinical and neuroimaging suspicion of the disease and no mutation on exons 2–23 of the *NOTCH3* gene). The identification of this group is very important considering that the genetic spectrum of CADASIL could be wider than so far considered. Indeed, possible novel sites and mechanistic classes of *NOTCH3* mutations are arising: a new mutation has been reported on exon 24,[34] and a novel activating mutation on exon 25 has been described in a patient with cerebral SVD but lacking GOM deposits and *NOTCH3* receptor accumulation.[35] Finally, other genes could be involved in causing CADASIL-like hereditary cerebral SVD.

Some possible limitations of our study need to be discussed. A few may apply to the construction of the scale. We developed a scale based on data derived from a pooled analysis of selected international CADASIL series. Some disease features such as the presence of subcortical lacunar lesions in the anterior temporal lobes were not included because these were reported by only one study.[28] Clinical suspicion of CADASIL might differ according to different combinations of history, clinical, neuroimaging features, and patient age. Age at the time of blood withdrawal for gene analysis was not included among the CADASIL scale but it should be noted that all the variables that could be related with age at the time of genetic test were explicitly introduced in the algorithm for the identification of the optimal score. In particular, because the clinical and radiological manifestations of CADASIL might be to some extent age-dependent, one could argue that the accuracy of the CADASIL scale could be lower in younger patients. Our analyses showed instead that accuracy was even better for the 31 patients <50 years with both sensitivity and specificity equal to 1 (95% Wilson score intervals, 79.1%–100% and 69.9%–100%, respectively). The scale was also developed to be applicable even in patients in whom the family history is negative or not available. The use of a single scale in this sense may be a limitation but it is to be noted that our statistical method allowed us to obtain weighted scores for different variables. Another possible limitation is that the analyses were carried out on patients previously selected on the basis of the local expertise in 3 centers highly specialized in the field of cerebrovascular and metabolic diseases. Centers with different scientific and clinical interest and expertise could have generated case series with some different characteristics. A possible weakness is attributable to the relatively limited number of patients included in the analysis compared with the number of considered predictors. Despite this limitation, the scale demonstrated high stability when the group of patients with sporadic SVD was included in the algorithm. Our results need however to be confirmed and further validated with the application of the scale on larger and different series. A final possible limitation is that we considered *NOTCH3*-negative patients those in whom no pathogenic mutation was found on exons 2 to 23 without analyzing the remaining exons (1 and 24–33). However, this genetic analysis can be currently considered complete because only single cases have been recently reported with pathogenic mutation in exons different from 2 to 23.[34,35] Our scale could be modified if new genetic data emerge and if these data bear clinical implications.

Conclusions

We propose the use of the CADASIL scale as a simple and enough accurate screening tool for clinicians to select patients with high suspicion of CADASIL before genetic testing. This selection might allow identifying patients with CADASIL even in centers with less expertise in this disease and to better characterize a more homogeneous group of *NOTCH3*-negative patients that can be more appropriately considered CADASIL-like and in whom deepening of genetic investigations may be warranted.

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References

1. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996;383:707–710.
2. Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssière C, et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet*. 1997;350:1511–1515.
3. Opherk C, Peters N, Herzog J, Luedtke R, Dichgans M. Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain*. 2004;127:2533–2539.
4. Mourad A, Levasseur M, Bousser MG, Chabriat H. CADASIL with minimal symptoms after 60 years. *Rev Neurol*. 2006;162:827–831.
5. Pescini F, Bianchi S, Salvadori E, Poggesi A, Dotti MT, Federico A, et al. A pathogenic mutation on exon 21 of the NOTCH3 gene causing CADASIL in an octogenarian paucisymptomatic patient. *J Neurol Sci*. 2008;267:170–173.
6. Chabriat H, Levy C, Taillia H, Iba-Zizen MT, Vahedi K, Joutel A, et al. Patterns of MRI lesions in CADASIL. *Neurology*. 1998;51:452–457.
7. Auer DP, Pütz B, Gössl C, Elbel G, Gasser T, Dichgans M. Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. *Radiology*. 2001;218:443–451.
8. Markus HS, Martin RJ, Simpson MA, Dong YB, Ali N, Crosby AH, Powell JF. Diagnostic strategies in CADASIL. *Neurology*. 2002;59:1134–1138.
9. Dichgans M, Holtmannspötter M, Herzog J, Peters N, Bergmann M, Yousry TA. Cerebral microbleeds in CADASIL: a gradient-echo magnetic resonance imaging and autopsy study. *Stroke*. 2002;33:67–71.
10. van Den Boom R, Lesnik Oberstein SA, van Duinen SG, Bornebroek M, Ferrari MD, Haan J, et al. Subcortical lacunar lesions: an MR imaging finding in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Radiology*. 2002;224:791–796.
11. Singhal S, Rich P, Markus HS. The spatial distribution of MR imaging abnormalities in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and their relationship to age and clinical features. *AJNR Am J Neuroradiol*. 2005;26:2481–2487.
12. Stromillo ML, Dotti MT, Battaglini M, Mortilla M, Bianchi S, Plewnia K, et al. Structural and metabolic brain abnormalities in preclinical cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy. *J Neurol Neurosurg Psychiatry*. 2009;80:41–47.
13. Pantoni L, Pescini F, Nannucci S, Sarti C, Bianchi S, Dotti MT, et al. Comparison of clinical, familial, and MRI features of CADASIL and NOTCH3-negative patients. *Neurology*. 2010;74:57–63.
14. Dotti MT, Federico A, Mazzei R, Bianchi S, Scali O, Conforti FL, et al. The spectrum of Notch3 mutations in 28 Italian CADASIL families. *J Neurol Neurosurg Psychiatry*. 2005;76:736–738.
15. Malandrini A, Gaudiano C, Gambelli S, Berti G, Serni G, Bianchi S, et al. Diagnostic value of ultrastructural skin biopsy studies in CADASIL. *Neurology*. 2007;68:1430–1432.
16. Tikka S, Mykkänen K, Ruchoux MM, Bergholm R, Junna M, Pöyhönen M, et al. Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain*. 2009;132:933–939.
17. Gouw AA, van der Flier WM, Pantoni L, Inzitari D, Erkinjuntti T, Wahlund LO, et al. On the etiology of incident brain lacunes: longitudinal observations from the LADIS study. *Stroke*. 2008;39:3083–3085.
18. Swets JA. Measuring the accuracy of diagnostic systems. *Science*. 1988;240:1285–1293.
19. Pescini F, Cesari F, Giusti B, Sarti C, Zicari E, Bianchi S, et al. Bone marrow-derived progenitor cells in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Stroke*. 2010;41:218–223.
20. Chabriat H, Vahedi K, Iba-Zizen MT, Joutel A, Nibbio A, Nagy TG, et al. Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Lancet*. 1995;346:934–939.
21. Dichgans M, Mayer M, Uttner I, Brüning R, Müller-Höcker J, Rungger G, et al. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol*. 1998;44:731–739.
22. Desmond DW, Moroney JT, Lynch T, Chan S, Chin SS, Mohr JP. The natural history of CADASIL: a pooled analysis of previously published cases. *Stroke*. 1999;30:1230–1233.
23. Uchino M, Hirano T, Uyama E, Hashimoto Y. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and CADASIL-like disorders in Japan. *Ann N Y Acad Sci*. 2002;977:273–278.
24. Singhal S, Bevan S, Barrick T, Rich P, Markus HS. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. *Brain*. 2004;127:2031–2038.
25. Kim Y, Choi EJ, Choi CG, Kim G, Choi JH, Yoo HW, et al. Characteristics of CADASIL in Korea: a novel cysteine-sparing Notch3 mutation. *Neurology*. 2006;66:1511–1516.
26. Yin XZ, Ding MP, Zhang BR, Liu JR, Zhang L, Wang PZ, et al. Report of two Chinese families and a review of Mainland Chinese CADASIL patients. *J Neurol Sci*. 2009;279:88–92.
27. Lee YC, Liu CS, Chang MH, Lin KP, Fuh JL, Lu YC, et al. Population-specific spectrum of NOTCH3 mutations, MRI features and founder effect of CADASIL in Chinese. *J Neurol*. 2009;256:249–255.
28. van den Boom R, Lesnik Oberstein SA, Ferrari MD, Haan J, van Buchem MA. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: MR imaging findings at different ages—III–6th decades. *Radiology*. 2003;229:683–690.
29. Choi JC, Kang SY, Kang JH, Park JK. Intracerebral hemorrhages in CADASIL. *Neurology*. 2006;67:2042–2044.
30. Viswanathan A, Godin O, Jouvent E, O'Sullivan M, Gschwendtner A, Peters N, et al. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. *Neurobiol Aging*. 2010;31:1629–1636.
31. Davous P. CADASIL: a review with proposed diagnostic criteria. *Eur J Neurol*. 1998;5:219–233.
32. Kotorii S, Takahashi K, Kamimura K, Nishio T, Arima K, Yamada H, et al. Mutations of the notch3 gene in non-Caucasian patients with suspected CADASIL syndrome. *Dement Geriatr Cogn Disord*. 2001;12:185–193.
33. Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG. CADASIL. *Lancet Neurol*. 2009;8:643–653.
34. Valenti R, Bianchi S, Pescini F, D'Eramo C, Inzitari D, Dotti MT, et al. First report of a pathogenic mutation on exon 24 of the NOTCH3 gene in a CADASIL family. *J Neurol*. 2011;258:1632–1636.
35. Fouillade C, Chabriat H, Riant F, Mine M, Arnoud M, Magy L, et al. Activating NOTCH3 mutation in a patient with small-vessel-disease of the brain. *Hum Mutat*. 2008;29:452.

SUPPLEMENTAL MATERIAL

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