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

A Screening Tool to Select Patients for NOTCH3 Gene Analysis

Francesca Pescini, MD, PhD; Serena Nannucci, MD; Bruno Bertaccini, PhD; Emilia Salvadori, PhD; Silvia Bianchi, BSc, PhD; Michele Ragno, MD; Cristina Sarti, MD, PhD; Raffaella Valentini, MD; Enza Zicari, MD, PhD; Marco Moretti, MD; Stefano Chiti, RT(MR), MSc; Maria Laura Stromillo, MD; Nicola De Stefano, MD; Maria Teresa Dotti, MD; Antonio Federico, MD; Domenico Inzitari, MD; Leonardo Pantoni, MD, PhD

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;43:00-00.)

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...
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. 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. 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. 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. Vitane, 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. 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 al10). 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 neuroradiologist 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); leukoencephalopathy uni- or bilaterally extended to the external capsule that could be partially or completely involved; 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.11 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. 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
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 (approximately 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; 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. 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.
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. 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\(\pm\)12.3; 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

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<tr>
<th>Table 3. Clinical and Neuroimaging Features in CADASIL and NOTCH3-Negative Patients</th>
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<tr>
<td>Patients With CADASIL (n=61)</td>
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<tr>
<td>Age at disease suspicion, y, mean±SD</td>
</tr>
<tr>
<td>Age at first stroke/TIA, y, mean±SD</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
</tr>
<tr>
<td>Migraine, No. (%)</td>
</tr>
<tr>
<td>With aura, No. (%)</td>
</tr>
<tr>
<td>TIA/stroke, No. (%)</td>
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<tr>
<td>Psychiatric disturbances, No. (%)</td>
</tr>
<tr>
<td>Cognitive deficits/dementia, No. (%)</td>
</tr>
<tr>
<td>Presence of family history† in at least 1 generation, No. (%)</td>
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<tr>
<td>Presence of family history† in at least 2 generations, No. (%)</td>
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<tr>
<td>Presence of leukoencephalopathy, No. (%)</td>
</tr>
<tr>
<td>Extended to temporal poles, No. (%)</td>
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<tr>
<td>Extended to external capsule, No. (%)</td>
</tr>
<tr>
<td>Subcortical infarcts, No. (%)</td>
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</table>

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).

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<th>Table 4. CADASIL Scale</th>
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<tr>
<td>Migraine</td>
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<tr>
<td>Migraine with aura</td>
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<tr>
<td>TIA or stroke</td>
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<tr>
<td>TIA/stroke onset ≤50 y</td>
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<tr>
<td>Psychiatric disturbances</td>
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<tr>
<td>Cognitive decline/dementia</td>
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<tr>
<td>Leukoencephalopathy</td>
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<tr>
<td>Leukoencephalopathy extended to temporal pole</td>
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<tr>
<td>Leukoencephalopathy extended to external capsule</td>
</tr>
<tr>
<td>Subcortical infarcts</td>
</tr>
<tr>
<td>Family history† in at least 1 generation</td>
</tr>
<tr>
<td>Family history† in at least 2 generations</td>
</tr>
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</table>

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, but they have been seldom used and never validated. Some of the items of the Davous criteria (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 seems dated today.

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. 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, 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. 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. 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. 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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Disclosures

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References

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SUPPLEMENTAL MATERIAL

Study co-investigators.

Laura Ciolli, Alessandra Del Bene, Anna Poggesi (Department of Neurological and Psychiatric Sciences, University of Florence, Italy), Mario Mascalchi (Radiodiagnostic Section, Department of Clinical Physiopathology, University of Florence, Florence, Italy), Anna Maria Basile, Lucia Nardetto, Marco Spinazzi, Federica Squarzanti (Department of Neurosciences, University of Padova, Italy); Enrico Adriano, Maurizio Balestrino, Carlo Gandolfo, (Department of Neuroscience, Ophthalmology and Genetics, University of Genova, Italy); Davide Gadda, Andrea Ginestrioni, Gian Paolo Giordano, Graziella La Villa, Cesare Pandolfo, Diana Petacchi, Vania Scardigli, Paolo Simonelli (Department of Radiology, Neuroradiology Unit, Careggi University Hospital, Florence, Italy); Gabriella Cacchiò (Division of Neurology, C & G Mazzoni Hospital, Ascoli Piceno, Italy).