THE ROLE OF CLINICAL AND NEUROIMAGING FEATURES IN THE DIAGNOSIS OF CADASIL

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CONFLICTS OF INTEREST/ DISCLOSURES

The authors report no disclosures relevant to the manuscript.

ABSTRACT

Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common familial cerebral small vessel disease, caused by *NOTCH3* gene mutations. The aim of our study was to identify clinical and neuroradiological features which would be useful in identifying which patients presenting with lacunar stroke and TIA are likely to have CADASIL. *Methods*: Patients with lacunar stroke or TIA, were included in the present study. For each patient demographic and clinical data were collected. MRI images were centrally analysed for the presence of lacunar infarcts, microbleeds, temporal lobe involvement, global atrophy and white matter hyperintensities. *Results*: 128 patients (mean age 56.3±12.4 years) were included. A *NOTCH3* mutation was found in 12.5% of them. A family history of stroke, the presence of dementia and external capsule lesions on MRI were the only features significantly associated with the diagnosis of CADASIL. Although thalamic, temporal pole gliosis and severe white matter hyperintensities were less specific for CADASIL diagnosis, the combination of all these factors together with familial history for stroke result in a higher positive predictive value and specificity. *Conclusions*: A careful familial history collection and neuroradiological assessment can identify patients in whom *NOTCH3* genetic testing has a higher yield.

Key words: CADASIL, NOTCH3 gene, stroke genetics, diagnosis, neuroimaging, monogenic disorders

INTRODUCTION

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy; OMIM#125310) is the most common cause of familial cerebral small vessels disease. [1]. It usually manifests at middle adulthood with a highly variable clinical phenotype including recurrent TIA or ischemic stroke, migraine with aura, cognitive deficits and mood disorders [2,3]. T2-White matter hypertensities (WMHs), with involvement of the external capsules and anterior pole of the temporal lobes and multiple lacunar infarcts have been identified as typical CADASIL neuroimaging findings [4,5]. The disease is due to mutations in the *NOTCH3* gene, occurring in exons 2–24 [6], which encode for epidermal growth factor (EGF)-like repeats [7].

The extremely variable phenotype makes determining which patients presenting with lacunar stroke or small vessel disease should be screened for CADASIL challenging [8-9]. Hence, the identification of reliable clinical and neuroimaging screening criteria would be useful as *NOTCH3* genetic analysis is costly and time consuming [9].

The aim of the present study was to determine which patients presenting with lacunar stroke and TIA are likely to have a diagnosis of CADASIL and to identify clinical and neuroradiological features which could be used as possible markers to decide which patient to refer for genetic screening.

PATIENTS AND METHODS

Patients

We included a series of consecutive patients with lacunar ischemic and/or TIA and/or deep intracerebral heamhorrage (basal ganglia) who had been prospectively recruited from the 18 stroke services participating in the Lombardia GeNetics of Stroke (GENS) project (2009-2016) who had undergone sequencing of the *NOTCH3* gene [10-11; *Supplemental data 1*].

Recruited patients met inclusion criteria suggesting a high likelihood of familial small vessel disease.

A specific clinical algorithm was implemented based on a literature review to identify patients with a higher probability of CADASIL (*Figure 1*) [12,13]. One (1) point score was attributed to every single item (total sum ranging from 0 to 6), and patients with a $\geq$ 2 score were included in the study and addressed to genetic analysis. The full study methodology has been previously reported [10-11].

On a standard pro-forma demographic, clinical and risk factor data were collected as well as specific clinical features commonly associated with CADASIL, including history of recurrent strokes/TIA, migraine with or without aura, dementia, cognitive impairment, and the presence of family history of stroke, psychiatric disturbance, and seizure [11; *Supplemental data 2*].

The study was approved by the local ethical committees of all participating centres, in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. All the subjects gave informed consent for genetic testing and participation in the study.

Neuroimaging analysis

All participants underwent 1.5T MRI scans which included DWI, FLAIR and T2*gradient-echo in axial planes, and T2 (+-PD) in axial or coronal plans. MRIs acquired in the different centres were centrally evaluated by a neuroradiologist with long-term experience in small vessel disease imaging (P.V.; IRCCS C. Mondino National Neurological Institute, Pavia, Italy). Visual assessment was performed blinded to clinical and demographic data as well as genetic analysis results. The STandards for ReportIng Vascular changes on Neuroimaging (STRIVE) guidelines, recognising standards developed by rigorous expert consensus, were applied to assess multifocal vascular lesions [14]. The ratings included the presence and site of lacunar infarcts, the presence of microbleeds, the overall severity of white matter hyperintensities (WMHs) according to the simplified Fazekas scale (range: 0-3), and the presence and the degree of WMH in the anterior temporal poles and external capsule [15]. The global atrophy and medial temporal lobe atrophy

were rated by applying the Pasquier scale (range: 0-3) [16] and the Scheltens scale (range: 0-4) [17], respectively (*Figure 2*).

Genetic analysis

Blood samples for DNA analysis were collected, after informed consent. Total genomic DNA was extracted from peripheral blood leukocytes. Sequencing of all exons of the *NOTCH3* gene encoding for the epidermal growth factor (EGF)-like repeats in which pathogenic CADASIL mutations occur was performed on an automated sequencing system (Applied Biosystems 3730 DNA Analyzer) using the BigDye™ Terminator Cycle Sequencing Kit Version 1.1 (Applied Biosystems). The nucleotide position of mutation present in the coding regions refers to the mRNA sequence (NM_000435).

Statistical analysis

Given the small subgroups sample size, Fisher exact test was applied for categorical variables to evaluate associations between genetic results and algorithm variables, and differences in clinical and neuroimaging features between *NOTCH3* gene positive and negative patients. Non-parametric Kruskal-Wallis test was applied to evaluate associations between genetic results and continuous variables. Two separate multiple logistic regression analysis models (Firth method) adjusted for age at disease onset and sex were applied to assess the independence of single predictive clinical and neuroradiological factors, respectively, which had been found to be significant on univariate analysis. Genetic test result was used as dependent variable for each of single feature.

The accuracy of each variable or combination of them in predicting genetic diagnosis was expressed using the area under curve of the Receiver Operating Characteristic (ROC-AUC).

Tests were considered significant if the p value was less than 0.05. All analyses were calculated using STATA 8.0 (StataCorp LP, College Station, Texas, US).

RESULTS

A total of 128 patients with a suspicion of CADASIL were recruited. Disease age of onset was 55 ± 13.2 years, and 64 (50%) were females. The presenting symptom was stroke in 83 (64.8%) cases, which was ischemic in 89% and haemorrhagic in 11% of subjects and TIA in 45 (35.2%) patients.

Genetic analysis revealed *NOTCH3* gene mutations in 16 (12.5%) of the patients. All mutations were already described missense changes, abolishing or introducing a cysteine residue. Details of mutation site and phenotype of positive *NOTCH3* patients are reported in *Table 1*.

Clinical and risk factors differences between *NOTCH3* positive and negative cases are shown in *Table 2*. Family history of stroke (93.7% *vs.* 47.3%, p=0.003) and dementia (43.7% *vs.* 16.9%, p=0.021) were the only significant clinical features differentiating *NOTCH3* positive from negative patients.

Cerebral MRI scans were available for central assessment in 101 (78.9%) subjects. Neuroimaging analysis (*Table 3*) showed that both the presence and the number of thalamic (92.8% *vs.* 49.4%%; p=0.003; 1.92±2.09 *vs*. 066±0.56; p=0.002, respectively) and external capsule lacunes (92.8% *vs.* 50.5%; p=0.003; 0.93±.27; p=0.003) were significantly associated with a CADASIL diagnosis. Temporal lobe white matter hyperintensities (50% *vs.* 17.2%; p=0.012) and WMHs (Fazekas 3 = 64.2% *vs.* 29.8%%; p=0.031) were also significantly more frequent in *NOTCH3* positive than negative patients. No other neuroradiological feature, including temporo-mesial and global cortical atrophy, was associated with CADASIL.

Multiple logistic regression analysis corrected for age at onset and sex with genetic test as the dependent variable, confirmed the association between family history of stroke (OR 6.27; 95%CI 1.33-29.40; p=0.020) and of dementia (OR 3.19;95%CI 1.00-10.17; p=0.050) and external capsule lacunes (OR 10.99; 95%CI 1.06-114.4; p=0.045) with the positive genetic test (*Supplemental table – Supplemental data 3*).

The ROC-AUC curve analysis showed that the predictive diagnostic accuracy was significantly improved by using a combination of multiple factors. The AUC was 0.6728 (95%CI 0.56-0.78) and 0.7085 (95%CI 0.63-0.79) for a family history of stroke and capsule external lacunes alone respectively, whereas increases to 0.826 (95%CI 0.69-0.96) when these factors are combined together and with the presence thalamic lacunes, temporal lobe WMH and severe WMHs (Fazekas grade 3) (*Figure 3*).

DISCUSSION

We developed a new pre-genetic algorithm to select patients with cerebrovascular diseases in whom there is a yield sufficient to merit testing [10-11]. The application of this clinical algorithm allowed the selection of a patient population highly suspected for CADASIL, which was genetically confirmed in a relatively high proportion of cases (12.5%).

There have been some previous attempts of identifying specific clinical and neuroradiological features distinguishing *NOTCH3* positive from negative patients. These studies, showed that several MRI characteristics, such as temporal lobe lesions, external capsule lacunes, severe WMHs as well as the family history, were more frequent in positive patients [9,18-22]. Some specific algorithms supporting the identification of clinically suspected CADASIL have been proposed so far but the reliability is still debated since in most cases they were elaborated from heterogeneous and retrospectively collected populations [12-22]. A recent systematic screening of 994 lacunar stroke subjects with stroke onset by age 70 years detected CADASIL mutations in only 0.5-1.5% of the patients [18] making the use of better screening strategies, as we have developed in this analysis, clinically important.

Although the strength of our study is that the screening strategy was firstly applied in a prospectively collected and well phenotyped series of lacunar stroke or TIA patients, some possible limitations of our work should be highlighted. Firstly, the study was conducted on a relative small sample size and the algorithm has not been validated by systematically performing the genetic tests

in a pool of randomly selected stroke cases [11]. Thus, the reliability of our algorithm should be assessed on wider series. Secondly, our algorithm has been developed from international literature, [12,13] and not from a systematic statistical analysis.

Our study may provide clinically useful information in the selection of patients presenting with lacunar stroke for CADASIL mutation testing. We confirmed that a family history of stroke and dementia as well as the presence of external capsule lacunes are strongly associated with a diagnosis of CADASIL. [9,11,23-25]. Conversely, we found that the presence of severe WMHs, thalamic lacunes, and temporal lobe WMHs may be less useful. However, since the combination of the overall factors together with familial history for stroke likely result in higher positive predictive values and specificity, these neuroradiological aspects should be considered to identify patients yield of genetic testing.

We did not find any correlation between diagnosis of CADASIL and temporo-mesial and cortical atrophy This finding, may reflect the subcortical nature of the disease or may be explained by the relative small proportion of patients with cognitive impairment in the *NOTCH3* positive patients (43.7% *versus* 27.5%) or by the relative short patient disease history (mean 1 year). In fact, a relationship between brain atrophy, clinical severity and cognitive decline has been observed, and age was found as the main risk factor for longitudinal brain volume loss [26-29].

Our results support the idea that the application of a pre-genetic screening selection taking into consideration both familial and neuroradiological data is likely to represent a promising strategy for CADASIL diagnosis.

However, the increasing application of rapid and cost effective high-throughput technologies such as Next generation sequencing in monogenic diseases will have a major impact in CADASIL diagnosis by assessing whether a systematic genetic screening is preferable to a clinical pre-genetic screening approach.

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FIGURE LEGEND

Figure 1. The Lombardia GENS Algorithm.

Figure 2. Neuroradiological Items evaluated

Figure 3. ROC curves of different variable combination in relation to the genetic diagnosis of CADASIL. Line 1: familial history of stroke; Line 2: familial history of dementia; Line 3: thalamic lacunes; Line 4: external capsule lacunes; Line 5: temporal lobe white matter hyperitensities; Line 6: familial history of stroke and temporal lobe white matter hyperintensities; Line 7: familial history of stroke and external capsule lacunes; Line 8: familial history of stroke, temporal pole hyperitensithies and external capsule lacunes; Line 9: familial history of stroke, temporal pole hyperintensities, external capsule lacunes and severe white matter hyperintensities (Fazekas 3).