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ORIGINAL ARTICLE

Diagnosis of CADASIL disease in normotensive and non-diabetics with lacunar infarct ☆

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Abstract
Background: CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is characterized by recurrent cerebral ischemic episodes of the lacunar subtype usually without traditional vascular risk factors. We investigated the frequency of CADASIL among selected patients with cerebral ischemia of the lacunar subtype.
Methods: We studied patients under 65 years old who presented cerebral ischemia of the lacunar subtype without hypertension, diabetes mellitus or other causes that explained the cerebral ischemia. On the skin biopsies, we performed immunostaining analysis on 5µm frozen sections with monoclonal antibody anti-Notch 3 (1E4). We also performed a genetic analysis of the Notch 3 gene (exons 3,4,5,6,11 and 19).
Results: Of 1.519 patients analyzed, only 57 (3.7%) fulfilled the selection criteria, and 30 of them accepted to participated in the study. We studied 30 patients, mean age was 53 years (range 34 to 65), 50% were men and all patients suffered a lacunar stroke. Immunostaining analysis was positive in two patients (6.6%) and the genetic analysis confirmed a mutation characteristic of CADASIL in exon 4 nt 622C/T (Arg 182 Cys) and 694 T/C (Cys206Arg) respectively.
Conclusions: CADASIL disease was present in 6.6% of patients younger than 65 years with a lacunar stroke and without hypertension or diabetes mellitus. Screening for CADASIL should be considered in these patients.

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PALABRAS CLAVE
CADASIL;
Infarto lacunar;
Rictus;
Gen Notch 3;
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Infarto cerebral

Diagnóstico de la enfermedad de CADASIL en pacientes normotensos y no diabéticos con infarto lacunar

Resumen
Introducción: La enfermedad de CADASIL (*Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy*) se caracteriza por isquemias cerebrales recurrentes de tipo lacunar, habitualmente en pacientes sin factores de riesgo vascular. Analizamos la frecuencia de enfermedad de CADASIL en pacientes con infarto lacunar sin factores de riesgo vascular clásicos.
Métodos: Estudiamos pacientes con un primer infarto lacunar menores de 65 años sin hipertensión, diabetes mellitus u otra causa que justificara la isquemia cerebral. Realizamos estudio inmunohistoquímico de 5µm de espesor sobre biopsia cutánea usando el anticuerpo monoclonal anti-Notch 3 (1E4). Además del estudio inmunohistoquímico se realizó en todos los casos el estudio genético del gen Notch 3 de los exones 3, 4, 5, 6, 11 y 19.
Resultados: De 1.519 pacientes con infarto lacunar, sólo 57 (3,7%) cumplieron los criterios de selección, y 30 de ellos aceptaron participar en el estudio. Analizamos 30 pacientes con edad media de 53 años; el 50% fueron hombres y todos presentaron un primer infarto cerebral tipo lacunar. El estudio inmunohistoquímico y genético confirmó la enfermedad de CADASIL en dos pacientes (6,6%) en el exón 4 nt 622C/T (Arg 182 Cys) y 694 T/C (Cys206Arg) respectivamente.
Conclusiones: Detectamos la enfermedad de CADASIL en un 6,6% de los pacientes menores de 65 años con un primer infarto lacunar sin hipertensión ni diabetes mellitus. El despistaje de esta enfermedad debería de ser considerado en estos casos.

Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a rare hereditary disease characterised by transient ischemic attacks, strokes, vascular dementia, migraine with aura and psychiatric disorders.[1] Although the pathophysiological mechanism is neither an amyloidotic nor an atherosclerotic systemic arteriopathy, CADASIL manifests itself clinically as affecting the central nervous system almost exclusively.

The CADASIL disease is caused by a mutation of the Notch 3 gene. This gene codes a transmembrane receptor with an extracellular domain that contains 34 epidermal growth factor (EGF)-like repeat domains.[2] The mutations described condition the gain or loss of cysteine residue in the EGFs of the extracellular part of the receptor, altering the number of disulfide bridges that bond the cysteine residues among themselves and with it the tri-dimensional configuration of the protein. These changes lead to a disease cluster of the Notch 3 protein in the arterial wall,[2] as well as granular osmiophilic material (GOM) deposits (pathognomonic of the disease).

The diagnosis can be carried out with an electronic microscope to detect GOM deposits,[3] with an immunostaining analysis of a skin biopsy with monoclonal antibodies anti-Notch 3 (revealing the disease cluster of this protein in the arterial wall)[4] or through a genetic analysis of the Notch 3 gene.[5] However, diagnosis is difficult due to the phenotypic variability of the disease, even in the same family, as there is no clear genotype-phenotype correlation.

The main clinical manifestation of the disease is recurrent cerebral ischemic episodes of the lacunar subtype, which can be confused with cases of sporadic lacunar ischemia.

Patients with CADASIL present a low prevalence of vascular risk factors, particularly hypertension (5%-25%) and diabetes mellitus (0%-4%),[6-8] compared to patients with sporadic lacunar ischemia (hypertension 40%-83% and diabetes 28%-41%).[9-12] However, approximately 18% of patients with sporadic lacunar ischemia do not present any vascular risk factor or other cause that could justify cerebral ischemia.[9,10] Therefore, in these cases, we should consider differential diagnosis with other diseases such as CADASIL.

Given the low prevalence of vascular risk factors in patients with CADASIL, we assessed the hypothesis that selecting patients with lacunar infarct without vascular risk factors could increase the number of diagnoses of this disease.

Our main aim was to assess the prevalence of CADASIL in patients under 65 years old with a first lacunar infarct without hypertension, diabetes mellitus or any other cause that would justify cerebral ischemia. Then, in second place, depending on the number of cases detected, we would assess clinical and radiological differences among both groups.

Patients and methods

From the records of 2 tertiary hospitals in Barcelona, we retrospectively selected Caucasian patients under 65 years old with a first lacunar infarct without hypertension, diabetes mellitus or any other cause that would justify cerebral ischemia. We considered patients with a classic lacunar syndrome and with a symptomatic cerebral ischemic lesion of less than 1.5cm diameter in the perforating artery area in absence of any other aetiology[13] as having a lacunar infarct.

We included a cranial CT or MRI scan, intracranial angiography (CT angio, MRI angio or cerebral arteriography), carotid ultrasound, ECG, echocardiography and general blood tests with coagulation during hospitalisation within the diagnostic protocol.

Patients with a high embolic heart disease risk,[14] extra- or intracranial arterial stenosis in the lacunar infarct area higher than 50%, or the presence of any other illness that could lead to a lacunar infarct (for example, haematological disease, inflammatory vasculopathy, dysplasia or arterial dissection) were excluded.

The study was approved by the ethics committee at Santa Creu i Sant Pau hospital and informed consent was requested from all patients.

The cases that met with the selection criteria underwent a medical visit where we gathered the following data: new cerebral ischemic episodes after the initial event (transient ischemic attack, lacunar infarct, non-lacunar infarct or cerebral haemorrhage.) We listed previous history of migraine, psychiatric disorders or epileptic fits. A family history of migraine, psychiatric disorders, dementia or cerebral vascular disease among first- and second-degree relatives was also assessed.

All patients had blood extracted during the medical visit for genetic analysis and also underwent a skin biopsy for immunostaining analysis.

A transient ischemic attack was considered as any cerebral focal neurological or retinal deficit, which started suddenly with a presumably vascular cause with a normal cranial MRI.[15] We considered that there was arterial hypertension if the patient received any antihypertensive treatment, or if we detected systolic blood pressure values higher than 140mm Hg and/or diastolic of 90mm Hg in 2 readings outside the acute phase of the cerebral ischemia.[16] A moderate alcohol intake was considered as higher than 40g/day. A diabetic was considered as a patient who received oral anti-diabetic treatment, was on insulin, on a diabetic diet or had fasting blood glucose levels above 126mg/dl.[17]

We regarded there was hypercholesterolemia if the patient followed lipid-lowering treatment because of a history of hypercholesterolemia or if the plasmatic cholesterol reading was higher than 200mg/dl.[18] All patients that had smoked within the last 5 years were considered as smokers.[19] We classified migraine according to the International Headache Society.[20]

Lesion study was done using magnetic resonance

A neuroradiologist blinded to clinical outcomes assessed the images in T2 and FLAIR sequences using the modified Scheltens scale.[21] This scale allowed lesion measurement in the white matter, basal ganglia and infratentorial lesions characteristic of patients with CADASIL.[22]

Skin biopsy immunostaining analysis with monoclonal anti-Notch 3 antibodies (1E4)

We took a 4mm skin punch that was frozen at −80° C for its later analysis. The samples were cut into 5µm sections to carry out the immunostaining analysis.

We used antibody anti-Notch 3 (1E4) in a 1:5 dilution as the primary antibody and immunoreactivity was revealed with an anti-mouse antibody (1:50 dilution) and with 3,3' diaminobenzidine (DAB; Sigma-Aldrich Chemie, Germany). Lack of accumulation in 3 separate vessels was considered as a negative result in the immunostaining analysis.[4] In the cases where an immunostaining analysis was not possible, all the Notch 3 gene was sequenced.

Genetic analysis

The DNA was isolated in the peripheral blood sample using the Miller et al method.[23] We analysed the 6 most prevalent exons reported in literature for CADASIL disease (exons 3, 4, 5, 6, 11 and 19) of the 33 contained in the Notch 3 gene in all patients. Intron sequences adjacent to the genes studied were amplified using CRP.

Definitive diagnosis of CADASIL was considered when the genetic analysis confirmed a characteristic mutation of the disease, independently of the immunostaining analysis. We considered it a negative result when the genetic study was normal (fig. 1).

Results

We selected 1,519 patients with lacunar infarct from our data base. From these, 57 patients presented a first lacunar infarct, were under 65 years old and did not present hypertension or diabetes mellitus or any other cause that would justify cerebral ischemia. Of these 57 patients, only 30 agreed to take part in the study.

The patients presented a mean age of 52.7±7.3 years (range, 34-65) and 50% were male. The mean follow-up time from the first cerebral infarction to the time of the genetic study was 5.1 years (range, 1-17).

As table 1 shows, 2/3 of the patients had at least 1 vascular risk factor: 40% had hypercholesterolemia, 16.6% reported moderate alcohol consumption and 23.3% were smokers.

The lacunar syndrome was pure sensory in 28 patients and motor sensory in 2. All patients underwent a neuroimaging study (93.3% had a MRI and 6.6%, a cranial CT scan.) The modified Scheltens score of the 28 patients with cranial MRI showed a mean score of 12 points (range, 0-58). The score was <5 points in 50%, between 5-20 points in 25%, between 21-40 points in 18% and >41 in 7% of patients.

Twelve patients had cerebral vascular recurrence after the first lacunar infarct; 4 presented a new lacunar infarct, 1 patient a cerebellar infarct and 7 patients suffered from transient ischemic attacks, which were all assessed with a new cranial MRI.

Patients with CADASIL disease

The skin biopsy was not correctly analysed in 6 cases because of technical problems. In these 6 patients, the Notch 3 gene was completely sequenced, with 2 mutations detected, 1 in exon 19 nt 3136 G/C (Ala1020Pro) and another in exon 13 nt 2031C/A (Pro685Thr), but neither of these

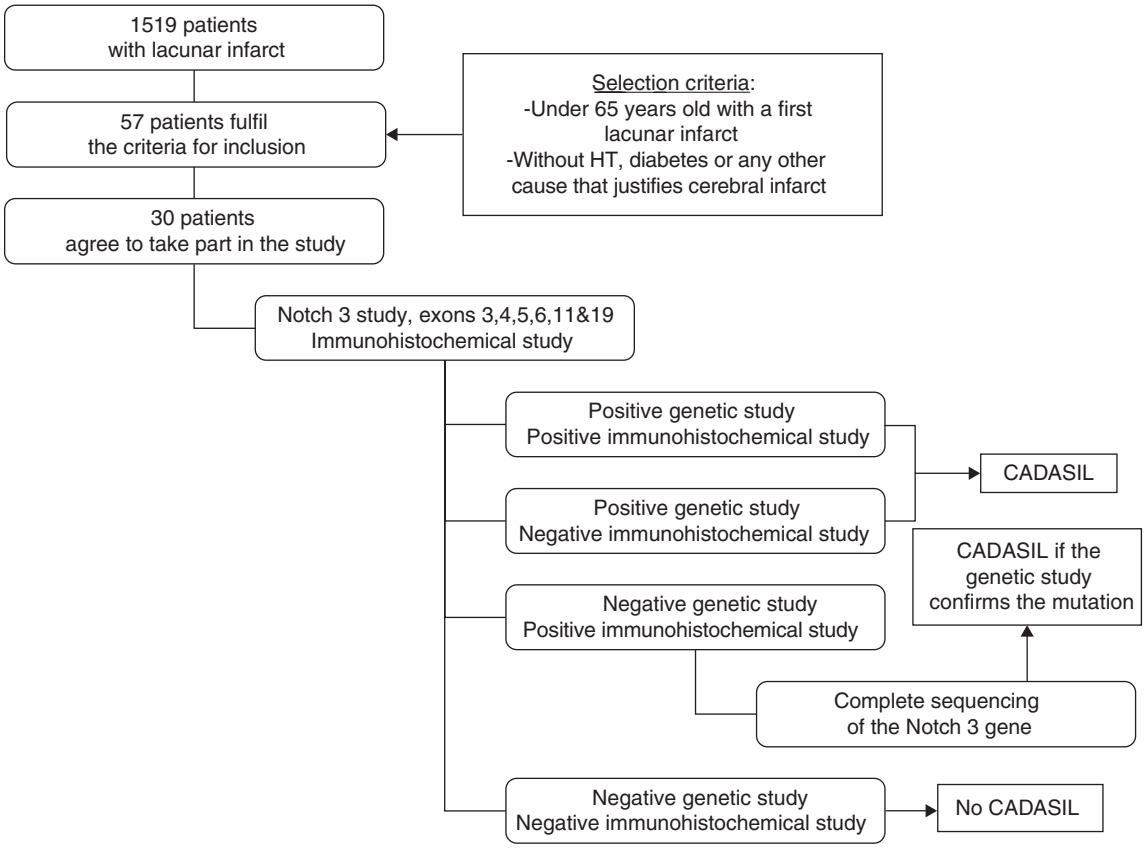

Figure 1 Diagnostic flow chart used in the study.

affected the cysteine residue, and were therefore not considered as a cause for CADASIL.

The immunostaining and genetic analysis were positive in 2 patients (6.6%), with a mutation in exon 4; nt 622C/T (Arg 182 Cys) and 694 T/C (Cys206Arg) respectively. The mutations and polymorphisms detected in the study are shown in table 2.

The overall score on the modified Scheltens scale was greater in patients with CADASIL than in those patients with sporadic lacunar infarct (53 vs 9 points). The 2 patients with CADASIL showed white matter involvement in the anterior temporal pole, a finding that was absent in the rest of the patients. Basal ganglia involvement was greater in patients with CADASIL (mean 21.5 vs 1.5 points), and no patient with sporadic lacunar infarct scored more than 20 points in this area.

The distribution of the vascular risk factors, cerebral vascular recurrence and family history of migraine, seizures, dementia or vascular cerebral disease was similar in both groups (table 1).

Discussion

In our study, we detected that 6.6% of patients under 65 years old with a first lacunar infarct without hypertension or diabetes mellitus presented CADASIL disease.

Although we detected 4 mutations of the Notch 3 gene, only 2 caused CADASIL disease (Cys 206Arg and Arg 182 Cys). The other 2 mutations had not been described previously (Pro 685Thr and Ala1020Pro) and did not affect any cysteine residue; for this reason, we cannot specify their physiological role, as they could be mutations of CADASIL that do not affect cysteine residue.[24]

Lacunar infarcts represent between 11% and 20% of all cerebral infracts.[25,26] The first studies in the area suggested that the pathophysiological mechanism of the lacunar infarct was micro-atheromatosis or lipohyalinosis of the cerebral perforating arteries due to hypertension. However, later pathological studies showed that some patients with lacunar infarct did not present these findings in the perforating arteries and that the lacunar infarct could be due to other aetiological mechanisms.[27]

Table 1 Demographic characteristics, vascular risk factors and family history in patients with CADASIL and with sporadic lacunar infarct

	Patients with sporadic lacunar infarct N=28	Patients with CADASIL N=2
Gender female (n)	13	2
Mean age (years)	52.3	58
Vascular risk factors (n)	18	2
Hypercholesterolemia	10	2
Alcohol >40 g/day	5	0
Smoking	7	0
Vascular recurrence (n)	12	2
Stroke	8	2
Transient ischemic attack	4	1
Haemorrhage	0	0
Migraine (n)	12	1
Seizures (n)	4	1
Psychiatric illness	14	1
(N) Family history of:		
Migraine	8	1
Seizures	3	0
Psychiatric illness	11	2
Cerebral ischemia.	15	2
Dementia	9	1

Table 2 Mutations and polymorphisms detected in the study

	Nucleotide	Amino acid
Mutations		
Exon 4	622C/T	Arg182Cys
Exon 4	694T/C	Cys206Arg
Exon 13	2031C/A	Pro685Thr
Exon 19	3136G/C	Ala1020Pro
Polymorphisms		
Exon 3	379C/T	
Exon 4	682A/G	
Exon 16	2614C/T	
Exon 17	2818A/G	
Intron 7	15G/A	
Intron 17	21G/A	

A recent study assessed the differential characteristics between patients with lacunar infarct with and without hypertension.[28] The study showed a significant increase in males who were over 85 years old and with diabetes mellitus in the group without hypertension, without any significant differences in the clinical subtype of lacunar infarct, radiological lesion distribution or other vascular risk factors. Therefore, the most common characteristics in patients with lacunar infarct without hypertension do not seem to correspond to the CADASIL patient profile.

Given that between 25% and 40% of lacunar infarct patients did not present hypertension,[29-31] and that in 18% in of lacunar infarct patients there was no vascular risk factor that could justify cerebral ischemia,[10] all other physiopathological mechanisms should be excluded in these cases.

CADASIL is characterised by recurrent lacunar-type cerebral ischemic episodes in young patients, who usually did not present classic vascular risk factors.

A previous study assessed the profitability of screening for CADASIL in patients with lacunar infarct with and without leukoaraiosis.[32] The study showed that 0.05% of patients with lacunar infarct presented CADASIL. However, when the results were analysed selecting patients under 65 years old and with leukoaraiosis, the prevalence increased up to 2% and up to 11% when those under 45 years old were selected. However, this study did not exclude patients according to the presence of vascular risk factors.

Our study is the first to carry out CADASIL screening in patients with lacunar infarct, selected by age, hypertension and diabetes mellitus, and we have detected a prevalence in the disease greater than that published in previous studies when patients are selected by age under 65 years old and with leukoaraiosis (6% vs 2%).[32]

Although immunostaining analysis has not been approved for CADASIL diagnosid,[33] we included it in this study because the mutation spectrum of the disease in the Spanish population is unknown and genetic study of the complete gene is too expensive. When we incorporated the immunostaining analysis, we confirmed the deposit of Notch protein 3 on the vessel walls before locating the gene. In these cases, if the genetic study of the 6 exons had not shown any alteration, the gene would have been completely sequenced (a situation not presented in the study). In contrast, if a negative genetic study had been obtained with an absence of deposits in the immunostaining analysis, this would have allowed us not to have to sequence the whole gene.

Our study presented different limitations: a) the patients were retrospectively selected from a data base, and only half of the patients that met the selection criteria took part in the study, which could be a source of confusion; b) we did not completely sequence the gene in all patients and we might consequently have missed the mutation in some cases; c) CADASIL can present in diabetic patients or those with hypertension, so excluding these patients from the study means we might have excluded a patient who had CADASIL; d) immunostaining analysis is a new method for diagnosing this disease that has not yet been approved; and e) the number of patients studied is small, which does not allow us to assess clinical differences between both groups and limits the generalisation of our results.

To conclude, 6.6% of patients with lacunar infarct who were under 65 years old without hypertension, diabetes mellitus or any other cause that could explain the cerebral infarction presented CADASIL; consequently, in our opinion, screening for this disease should be considered in these cases. Future studies with larger samples could confirm our results, as well as assess the physiological role of the new mutations detected in the study.

Conflict of interest

The authors have no conflict of interest to declare.

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