RESEARCH PAPER

Impaired vasoreactivity in mildly disabled CADASIL patients

Jonica Campolo,[1] Renata De Maria,[1] Marina Frontali,[2] Franco Taroni,[3] Domenico Inzitari,[4] Antonio Federico,[5] Silvia Romano,[6] Emanuele Puca,[7] Caterina Mariotti,[3] Chiara Tomasello,[3] Leonardo Pantoni,[4] Francesca Pescini,[4] Maria Teresa Dotti,[5] Maria Laura Stromillo,[5] Nicola De Stefano,[5] Alessandra Tavani,[8] Oberdan Parodi[1]

[1]CNR Clinical Physiology Institute, Cardiovascular Department, Niguarda Ca' Granda Hospital, Milan, Italy
[2]Institute of Translational Pharmacology, CNR, Rome, Italy
[3]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[4]Department of Neurological and Psychiatric Sciences, University of Florence, Florence, Italy
[5]Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Siena, Italy
[6]Center for Experimental Neurological Therapy, S Andrea Hospital, University of Rome, La Sapienza, Rome, Italy
[7]Neurovascular Treatment Unit, University of Rome, La Sapienza, Rome, Italy
[8]Department of Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Correspondence to
Dr J Campolo, CNR Clinical Physiology Institute, Niguarda Hospital, Piazza Ospedale Maggiore 3, 20162 Milan, Italy; jonica.campolo@ospedaleniguarda.it

For the list of study participants see the appendix.

Received 22 March 2011
Revised 30 September 2011
Accepted 10 October 2011
Published Online First
9 November 2011

ABSTRACT

Background and purpose CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare genetic disease caused by *NOTCH3* gene mutations. A dysfunction in vasoreactivity has been proposed as an early event in the pathogenesis of the disease. The aim of this study was to verify whether endothelium dependent and/or independent function is altered in CADASIL patients with respect to controls.

Methods Vasoreactivity was studied by a non-invasive pletismographic method in 49 mildly disabled CADASIL patients (30–65 years, 58% male, Rankin scale ≤2) and 25 controls. Endothelium dependent vasodilatation was assessed by reactive hyperaemia (flow mediated dilation—peripheral arterial tone (FMD-PAT)) and endothelium independent vasoreactivity by glyceryl trinitrate (GTN) administration (GTN-PAT).

Results Patients and controls showed comparable age, gender and cardiovascular risk factor distribution. GTN-PAT values were significantly lower in CADASIL patients (1.54 (1.01 to 2.25)) than in controls (1.89 (1.61 to 2.59); p=0.041). FMD-PAT scores did not differ between patients and controls (1.88 (1.57 to 2.43) vs 2.08 (1.81 to 2.58); p=0.126) but 17 CADASIL patients (35%) had FMD-PAT scores below the fifth percentile of controls. FMD-PAT and GTN-PAT values correlated both in controls (ρ=0.648, p<0.001) and CADASIL patients (ρ=0.563, p<0.001). By multivariable logistic regression for clinical and laboratory variables, only GTN-PAT (OR 0.39, 95% CI 0.15 to 0.97; p=0.044) was independently associated with FMD-PAT below the fifth percentile in CADASIL patients.

Conclusions The impaired vasoreactivity observed in CADASIL patients highlights the fact that both endothelial and smooth muscle functional alterations may already be present in mildly disabled subjects. The improvement in vascular function could be a new target for pharmacological trials in CADASIL patients.

INTRODUCTION

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare genetic disease caused by mutations in the *NOTCH3* gene on the short arm of chromosome 19. The *NOTCH3* gene encodes a cytoplasmic membrane receptor involved in cellular differentiation and cell cycle regulation in neuronal and vascular development during embryogenesis.[1] This receptor protein is predominantly and constitutively expressed in vascular smooth muscle cells (VSMCs) in adult subjects where its role remains to be defined.

Clinical manifestations of CADASIL are migraine with aura, recurrent strokes starting in mid-adulthood and psychiatric disturbances. CADASIL may progress to severe motor disability with pseudobulbar palsy and dementia of the subcortical type.[2 3] This non-atherosclerotic and non-amyloid angiopathy specifically affects capillaries and small sized arteries of the brain but also other organs,[4] indicating that CADASIL is a systemic vascular disease.

Morphological alterations include loss of normal VSMC anchorage to the extracellular matrix and other nearby cells, abnormalities of the cytoskeleton and endothelium,[1] thickened arterial wall with ensuing lumen stenosis,[5] granular osmiophilic material deposits within the media and adventitia,[6] and progressive degeneration and loss of VSMCs.

In addition, muscle and skin biopsies of CADASIL patients exhibited endothelial structural changes[7] that may interfere with endothelial nitric oxide synthase activity and nitric oxide bioavailability and cause endothelial dysfunction (ED). ED, found in many cardiovascular (CV) diseases, is a strong predictor of CV adverse events.[8 9]

In CADASIL patients, impaired vasoreactivity has been previously suggested as a functional consequence of vascular wall modifications. Decreased cerebral basal perfusion and haemodynamic reserve in response to acetazolamide[10] or carbon dioxide[11] have been reported. Human small arteries studied ex vivo displayed abnormal reactivity to vasoconstricting agents and normal response to vasodilatory substances.[12] CADASIL patients presented with impaired endothelial dependent function in forearm resistance arteries with respect to healthy non-smoking controls.[13] On the other hand, endothelium independent vasodilation, an index of VSMC function, has been poorly investigated.[13 14]

Measurement of peripheral vasodilator response using a fingertip pulse amplitude tonometry device was recently validated[15] and has been recognised as a valid tool to identify patients with early stage coronary artery disease.[16]

The aim of our study was to verify whether endothelium dependent and independent function,

assessed by fingertip pulse amplitude tonometry, is altered in CADASIL patients with respect to controls of comparable age, gender and CV risk factors.

METHODS

Study population

We studied 49 subjects, aged 30–65 years, with a diagnosis of CADASIL confirmed by the identification of a mutation in the *NOTCH3* gene, in combination with typical neuroimaging abnormalities.[17] We enrolled both patients with overt clinical disease and relatives of patients with established CADASIL who had requested presymptomatic genetic testing. None of the patients had experienced a cerebrovascular accident, myocardial infarction or pulmonary embolism in the 3 months preceding the study. None had evidence of autoimmune disorders, or liver or renal disease. We chose to study only mildly disabled (Rankin Scale score ≤2) subjects and excluded patients with Rankin Scale scores >2 or demented individuals (DSM-IV criteria, with a SIDAM score <33) to ensure informed consent and adequate cooperation.

At enrolment, a complete clinical history was collected, including period of symptom onset, concomitant treatments and CV risk factors, such as hypertension,[18] hypercholesterolaemia,[19] diabetes,[20] moderate intermediate hyperhomocysteinaemia (plasma total homocysteine ≥15 and <100 μmol/l)[21] and smoking habit. A complete neurological examination was performed in all patients. Disability was assessed by the Rankin Scale.

Between January and March 2010, 25 subjects of similar age and sex, with no history, signs or symptoms of cerebrovascular and/or CV disease, who had been referred to our institution for vasoreactivity studies to improve the definition of their cardiovascular risk profile, were enrolled as controls.

The study was approved by the institutional review boards of the participating units. All patients gave written informed consent to participate.

Biochemical measurements

After an overnight fast, an antecubital vein was cannulated and blood was drawn into different prechilled Vacutainer tubes for biochemical determinations. Routine biochemistry was performed by standard laboratory methods. The concentration of plasma total homocysteine was evaluated, according to a method validated in our laboratory,[22] by high performance liquid chromatography after a reducing step with tri-n-butylphosphine, followed by sample derivatisation with ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate, a specific reagent for the sulfhydryl group.

Peripheral vasoreactivity

Endothelium dependent and independent vasodilation was assessed by a non-invasive pletismographic method (Endo-PAT2000, Itamar Medical Ltd, Caesarea, Israel) based on the registration of pulsatile blood volume in the fingertips of both hands. The Endo-peripheral arterial tone (PAT) equipment consists of two finger mounted probes, composed of inflatable latex air cushions within a rigid external case; pulsatile volume changes of the fingertip are sensed by a pressure transducer, located at the end of each probe, and transferred to a personal computer where the signal is band pass filtered (0.3–30 Hz), amplified, displayed and stored.

Endo-PAT studies were performed with the patient in the supine position and both hands on the same level in a comfortable, thermoneutral environment. Systolic and diastolic blood pressures and heart rate were measured before starting the test. A blood pressure cuff was placed on one upper arm (study arm) while the contralateral served as a control (control arm). After a 10 min equilibration period, signal recording started. The blood pressure cuff on the study arm was inflated to 60 mm Hg above systolic pressure for 5 min. The cuff was then deflated to induce reactive hyperaemia where the signal was recorded for 10 min.

After signal recovery to baseline, we assessed the endothelium independent response. Provided that pretest blood pressure was ≥110/70 mm Hg, patients were given two sublingual puffs (300 μg) of glyceryl trinitrate (GTN). Volume changes at the fingertips were then recorded for 10 min.

The PAT score output, an index of endothelial dependent flow mediated dilation (FMD), was automatically calculated as the ratio of the amplitude of the PAT signal averaged post and pre occlusion (FMD-PAT).

Endothelium independent vasodilatation, evoked by GTN, was manually assessed as the mean ratio of PAT signal in control and study arms post and pre drug administration (GTN-PAT) by two independent observers; reading results were then averaged. Intraobserver and interobserver variability of GTN-PAT readings in our laboratory are 1.7% and 1.5%, respectively.

Brain magnetic resonance

MR imaging scans were performed for the diagnosis of white matter lesions and cerebral infarctions on 1.5 T scanners. T1 weighted images and fluid attenuated inversion recovery (FLAIR) weighted images were obtained in the axial plane. MR images were examined to differentiate between white matter lesions, characterised by isointense signals on T1 weighted images and hyperintense signals on T2 weighted and FLAIR images, and cerebral infarction, characterised by hypointense signals on T1 weighted images and hyperintense signals on T2 weighted and FLAIR images.

In a subgroup of 33 subjects, white matter lesions were classified by one independent observer as periventricular hyperintensities (PVH), which adjoined the lateral ventricle, and deep white matter hyperintensities (DWMH), located in the deep white matter, apart from the lateral ventricle. PVH and DWMH scores were evaluated according to the scale of Fazekas[23]: score of PVH=0 (absence), 1 (caps or pencil thin lining), 2 (smooth halo) or 3 (irregular PVH extending into the deep white matter); score of DWMH=0 (absence), 1 (punctuate foci), 2 (beginning confluence of foci) or 3 (large confluent areas).

Statistical analysis

Data are presented as median and IQR (I–III) or frequency (%). Between group differences were tested by the Student's t test for continuous variables, and the χ^2 test or Fisher's exact test for categorical variables. The Mann–Whitney and Kruskall–Wallis tests were used for skewed variables. Correlations were calculated using the Spearman rank correlation test. Non-parametric Spearman's rank correlation coefficients are displayed as 'ρ'. The z statistic was used to compare the correlation coefficients for FMD-PAT and GTN-PAT in controls and CADASIL patients.

The association between altered FMD-PAT (below the fifth percentile of the control population) in CADASIL patients and clinical or laboratory parameters was assessed by univariate logistic regression; significant variables ($p<0.10$) were then entered into a multivariate logistic regression model to identify those independently associated with altered FMD-PAT.

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS Inc) release V.17.0 for Windows. The level of significance was set at $p\leq0.05$.

RESULTS

CADASIL population

Among the 49 enrolled subjects, 34 (74%) had overt clinical disease: 22 (45%) had suffered a previous cerebrovascular event, transient ischaemic attack (n=11) or stroke (n=11); eight (16%) subjects had a history of migraine without other manifestation while four (8%) presented with psychiatric symptoms. The median period since onset of symptoms was 8 (3–13) years. Fifteen subjects (31%) were asymptomatic mutation carriers.

NOTCH3 gene mutations were all missense, involving a Cys residue, and were distributed between exon 2 and exon 22 (figure 1), with the greatest frequency in exon 4. Overall, 29 subjects had mutations in exons 2–4 and 12 in exons 19–22.

Most patients had diffuse and severe white matter hyperintensities on MR scans, as classified by the Fazekas scale (table 1). Both PVH (grade 3, 55% vs 64%, p=0.268) and DWMH (grade 3, 46% vs 64%, p=0.166) distribution overlapped between asymptomatic and symptomatic patients.

Comparison between CADASIL patients and controls

Clinical and biochemical characteristics of the study groups are presented in table 2. CADASIL patients and controls had a similar age and gender distribution and prevalence of CV risk factors. Controls had significantly higher glucose and total cholesterol levels than patients but similar low density lipoprotein cholesterol concentrations. Antihypertensive therapy was balanced between groups, while a higher proportion of CADASIL subjects were on statins.

Systolic blood pressure levels before the vasoreactivity study were slightly, but significantly, lower in CADASIL patients than in controls. After GTN, systolic blood pressure decreased significantly more in controls (−10 (−19; −10) mm Hg) than in CADASIL patients (−5 (−10; 0) mm Hg; p<0.001).

CADASIL patients showed a significantly lower vasodilatory response to GTN than controls (1.54 (1.01–2.25) vs 1.89 (1.61–2.59); p=0.041). Conversely, median FMD-PAT values did not differ between groups (1.88 (1.57–2.43) vs 2.08 (1.81–2.58); p=0.126); however, 17 CADASIL patients (35%) had values below the fifth percentile of the FMD-PAT score control distribution (figure 2); the finding did not appear to be influenced by statin administration.

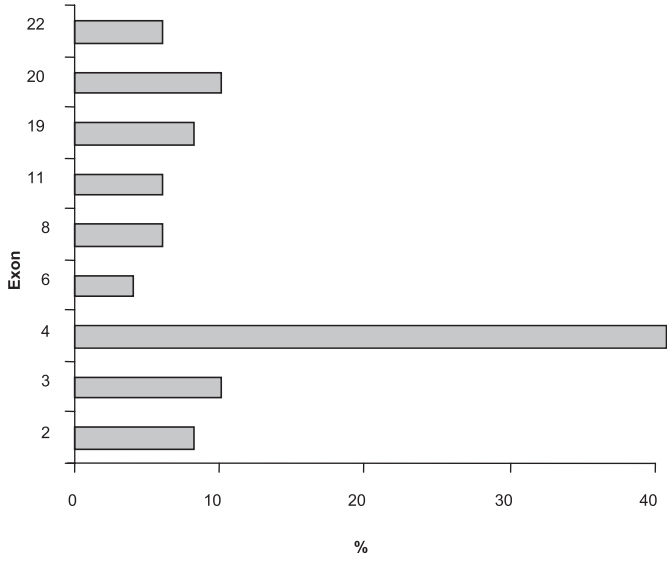

Figure 1 Distribution of NOTCH3 mutations in the study population.

Table 1 Distribution of periventricular hyperintensity and deep white matter hyperintensity scores in a subgroup of CADASIL patients

	No (%)
PVH score (n=33)	
≤1	7 (21)
2	6 (18)
3	20 (61)
DWMH score (n=33)	
≤1	4 (12)
2	10 (30)
3	19 (58)

CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; DWMH, deep white matter hyperintensities; PVH, periventricular hyperintenties.

FMD-PAT and GTN-PAT values correlated significantly (figure 3) both in controls (ρ=0.648, p<0.001) and CADASIL patients (ρ=0.563, p<0.001).

Predictors of abnormal vasoreactivity in CADASIL patients

Duration of symptoms did not correlate either with FMD-PAT (ρ=0.029) or with GTN-PAT (ρ=−0.058). Vasoreactivity did not differ between the two largest groups of patients classified according to the mutation site (exons 2–4, n=29 vs exons 19–22, n=12): FMD-PAT and GTN-PAT were 1.80 (1.68–2.43) and 1.56 (1.03–2.20) in the former vs 1.95 (1.46–2.49) and 1.35 (0.98–2.02) in the latter group (p=0.641 and p=0.403, respectively). Furthermore, vasoreactivity parameters were not influenced by white matter changes expressed by PVH and DWMH scores, as depicted in figure 4 (NS for all comparisons).

We tested by univariate logistic regression clinical, functional and laboratory candidate predictors of abnormal endothelium dependent vasodilation (table 3). Significant variables (p<0.10) were then entered into a multivariate logistic regression model: only lower GTN-PAT values were independently associated with abnormal FMD-PAT (OR 0.39, 95% CI 0.15 to 0.97; p=0.044).

At the 1 year follow-up, no impact of altered vasoreactivity on phenotypic manifestations of the disease was apparent. Overall disease progression in terms of disability was modest: the Rankin Scale progressed only in the four patients who had grade 2 at baseline to grade 3 (p=0.180). One patient developed a cerebrovascular event with documented ischaemic lesions on brain MR imaging 12 days after the ENDOPAT study where his FMD-PAT was 2.48.

DISCUSSION

We investigated endothelium dependent and independent vasoreactivity in a large series of CADASIL patients who were compared with healthy controls stratified by the presence of CV risk factors. To our knowledge, this is the first time such a comparison has been conducted. The main finding of the present study was that endothelium independent vasoreactivity was significantly altered in patients with respect to controls, a finding that supports the pathophysiological role of VSMC dysfunction in CADASIL. Moreover, despite similar median FMD-PAT scores between patients and controls, one-third of our CADASIL subjects at an early stage of clinical impairment showed an altered endothelium dependent vasodilation, below the fifth percentile of normal.

The rationale for the study of both endothelium dependent and independent vasodilation in CADASIL rests on the molecular mechanisms of the disease whereby the *NOTCH3* gene

Table 2 Clinical characteristics of the study subjects

	Controls (n=25)	CADASIL (n=49)	p Value
Age (years)	46 (38–51)	46 (40–51)	0.985*
Male gender	14 (56)	27 (55)	1.000
CV risk factors (any)	13 (52)	20 (41)	0.460
Smoking habit	5 (20)	13 (27)	0.582
Hypertension	7 (28)	8 (16)	0.359
Hypercholesterolaemia	5 (20)	8 (16)	0.752
Hyperhomocysteinaemia >15 μmol/l	1 (4)	2 (4)	1.000
Laboratory findings			
Total cholesterol (mmol/l)	5.74 (5.17–6.38)	5.30 (4.42–5.76)	0.013*
LDL cholesterol (mmol/l)	3.51 (3.02–3.98)	3.39 (2.35–3.98)	0.145*
Triglycerides (mmol/l)	0.99 (0.69–1.56)	1.15 (0.69–1.70)	0.671†
Fasting glucose (mmol/l)	5.22 (4.61–5.44)	4.89 (4.33–5.28)	0.047*
Plasma homocysteine (μmol/l)	8.34 (7.53–9.69)	8.39 (7.13–10.40)	0.904†
Creatinine clearance (ml/min)	116 (92–131)	111 (96–132)	0.900†
Folate (nmol/l)	12.5 (10.1–21.0)	15.2 (10.8–23.7)	0.314†
Vitamin B_{12} (pmol/l)	321 (259–399)	308 (225–384)	0.741*
Drug therapy			
RAS inhibitors	3 (12)	6 (12)	1.000
β-blockers	2 (8)	2 (4)	0.600
Statins	1 (4)	7 (14)	0.253
Vital signs at PAT study			
Systolic blood pressure (mm Hg)	130 (128–140)	120 (110–125)	<0.001†
Diastolic blood pressure (mm Hg)	85 (80–90)	80 (70–85)	0.012†
Heart rate (beats/min)	65 (60–71)	68 (62–76)	0.144*
Vasoreactivity			
FMD-PAT	2.08 (1.81–2.58)	1.88 (1.57–2.43)	0.126†
GTN-PAT	1.89 (1.61–2.59)	1.54 (1.01–2.25)	0.041†

Data are presented as number (frequency %) or median (IQR).
p Values are by χ^2 or Fisher's exact test and the *Student's t test or †Mann–Whitney test.
CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CV, cardiovascular; FMD, flow mediated dilation; GTN, glyceryl trinitrate; LDL, low density lipoprotein; PAT, peripheral arterial tonometry; RAS, renin–angiotensin system.

encoded receptor is constitutively expressed in VSMC, and morphological abnormalities in these patients are observed both in vascular smooth muscle and endothelial cells.

Nitric oxide, generated from the oxidation of L-arginine by endothelial nitric oxide synthase, is a key molecular mediator of normal endothelial function. The reduction in nitric oxide bioactivity, through an increase in vascular reactive oxygen species, contributes to impaired vascular relaxation, platelet aggregation and enhanced leucocyte adhesion to the endothelium.[24] ED is the earliest phenotypic change in the vasculature following exposure to atherothrombotic risk factors: hypertension, hypercholesterolaemia, smoking, diabetes and hyperhomocysteinaemia induce ED in the absence of established atherothrombotic disease.

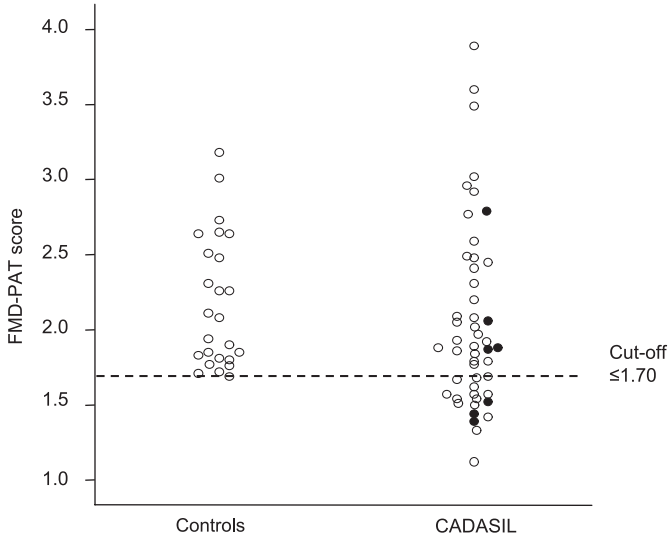

Figure 2 Distribution of flow mediated dilation–peripheral arterial tone (FMD-PAT) in controls and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) patients. Each subject is represented by an open circle; CADASIL patients on statins are represented by filled circles.

Peripheral ED is associated with an increased risk of cardiac and cerebrovascular events.[9 25] Transient ischaemic attacks and ischaemic strokes are the most frequent manifestations of CADASIL and occur in 60–85% of patients.[2 26] ED may therefore have a crucial role in the pathogenesis and progression of disease.

Several pieces of indirect evidence of ED involvement in CADASIL are available. Intravenous infusion of L-arginine, the substrate for endothelial nitric oxide synthase, significantly enhanced vasoreactivity in CADASIL patients compared with controls.[27] Compared with controls, CADASIL patients showed higher plasma levels of asymmetric dimethylarginine, a biological ED marker,[28] and lower concentrations of endothelial progenitor cells that participate in the maintenance of endothelial structure and function.[29]

Stenborg *et al*[13] observed a reduction in hyperaemic maximal blood flow velocity in the brachial artery of CADASIL patients compared with healthy, non-smoking controls without regular medications.

Our CADASIL patients were similar in terms of risk factors and history of vascular disease to other affected series that

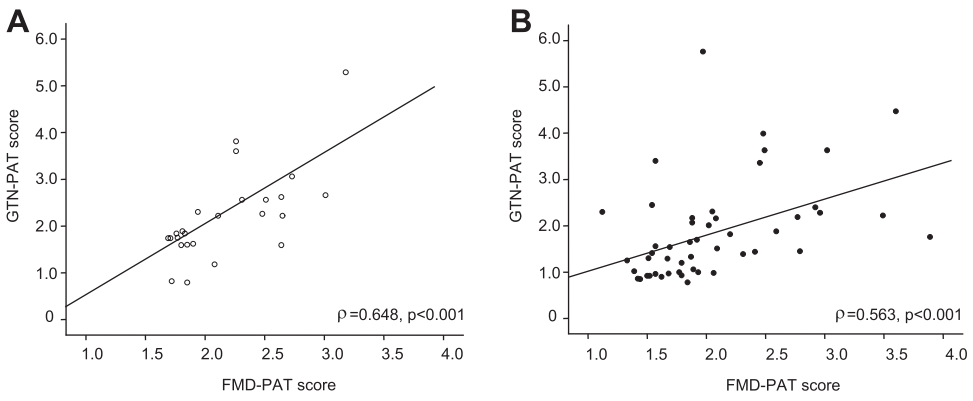

Figure 3 Non-parametric correlation between endothelium dependent and independent vasodilation expressed by flow mediated dilation—peripheral arterial tone (FMD-PAT) and glyceryl trinitrate (GTN)-PAT scores, respectively, in controls (A) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) patients (B). Although the correlation appears closer among controls, no difference was found between the coefficients of the two groups (z statistic=0.519, p=0.603).

detailed prevalence and characteristics of variables related to vascular function.[30–32] To account for the detrimental effect of CV risk factors on endothelial function, unlike previous studies, we enrolled control subjects with a similar prevalence of CV risk factors in comparison with CADASIL patients. This aspect might explain why individual FMD-PAT scores did not differ between groups.

Endothelium independent vasoreactivity has been poorly investigated in CADASIL to date. GTN acts as an exogenous nitric oxide donor directly on VSMCs, to induce endothelium independent vasodilation. Consistent with a primary involvement of VSMCs in CADASIL, our patients showed, despite relative preservation of endothelial function, a significantly lower GTN response than controls. Several lines of evidence support the notion that the early and progressive loss of VSMCs could result in premature vessel wall weakness,[6] as confirmed by ultrastructural studies.[7] In agreement with this pathophysiological substrate, we demonstrated VSMC functional impairment, as expressed by altered endothelium independent vasodilation, in the largest series so far reported and in comparison with a control population balanced by major CV risk factors. Gobron *et al*[14] did not observe any difference between 23 CADASIL patients and 23 matched controls without CV risk factors, in terms of changes in brachial artery diameter after sublingual GTN administration or in cutaneous blood flow after the iontophoretic delivery of sodium nitroprusside. Stenborg *et al*[13] found no significant differences in forearm blood flow after infusion of sodium nitroprusside between 10 CADASIL patients and 20 age and gender matched, healthy, non-smoking control subjects. These unexpected findings were probably due to the relatively limited cohort size and should be viewed with caution. In contrast, alterations in endothelium independent vasoreactivity found in our large CADASIL series are consistent with early structural changes in VSMCs and underscore the systemic nature of the disease. Accordingly, a relevant feature of our study is assessment of vasoreactivity at the level of the peripheral vascular beds where the peculiar and ubiquitous involvement of the microvasculature typical of CADASIL may better be detected.[33]

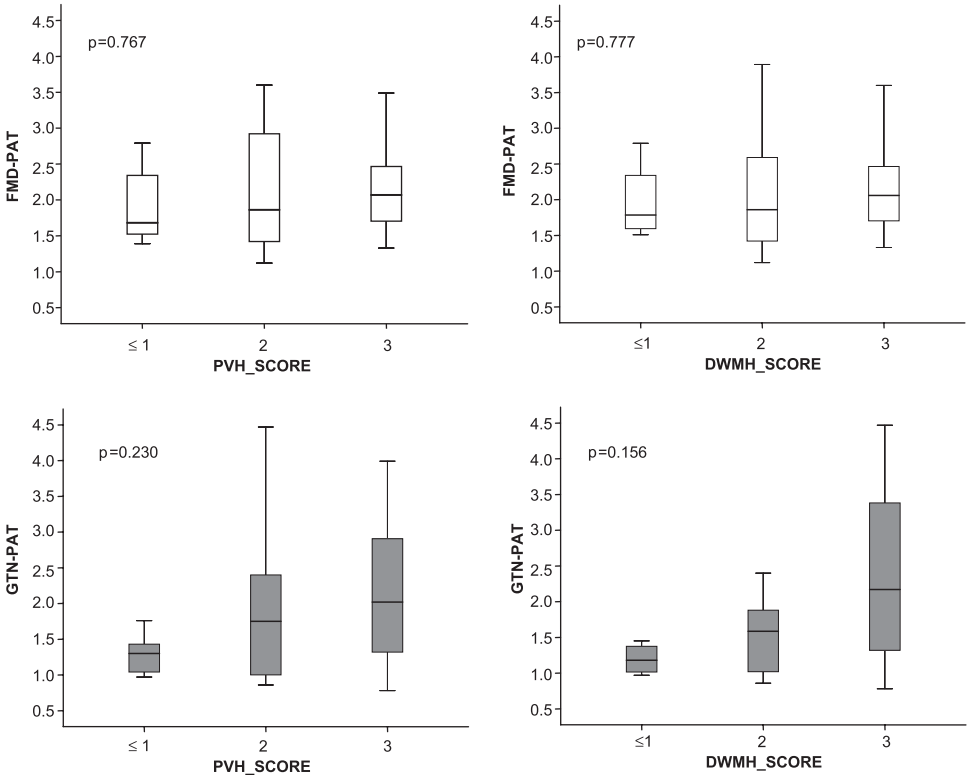

Figure 4 Relationship between white matter hyperintensities and endothelium dependent (flow mediated dilation—peripheral arterial tone (FMD-PAT), upper panels) and endothelium independent (glyceryl trinitrate (GTN)-PAT, lower panels) vasodilation. Differences among severity grades were not significant for all comparisons (Kruskall—Wallis test). DWMH, deep white matter hyperintensities; PVH, periventricular hyperintensities.

Table 3 Univariate predictors of abnormal flow mediated dilation—peripheral arterial tone in CADASIL patients

	p Value	OR (95% CI)
Age (per year)	0.128	1.07 (0.98 to 1.16)
Male vs female gender	0.825	0.88 (0.27 to 2.85)
Presence vs absence of clinical symptoms	0.160	2.80 (0.66 to 3.60)
Onset of clinical symptoms (years)	0.816	1.01 (0.94 to 1.09)
Previous cerebrovascular event vs no event	0.703	0.79 (0.24 to 2.61)
Rankin scale (0 vs >0)	0.300	1.91 (0.56 to 6.48)
PVH score (n=33)		
≤1 (reference)	0.323	1 (—)
2	0.396	0.37 (0.39 to 3.60)
3	0.133	0.25 (0.04 to 1.52)
DWMH score (n=33)		
≤1 (reference)	0.579	1 (—)
2	0.733	0.66 (0.06 to 6.87)
3	0.361	0.36 (0.04 to 3.25)
Cardiovascular risk factors		
Smoking habit (yes vs no)	0.740	1.25 (0.34 to 4.66)
Hypertension (yes vs no)	0.084	4.03 (0.83 to 19.59)
Hypercholesterolaemia (yes vs no)	0.327	2.15 (0.46 to 9.99)
Hyperhomocysteinaemia >15 μmol/l (yes vs no)	0.648	1.94 (0.11 to 33.05)
Laboratory findings		
Total cholesterol (per mmol/l)	0.506	1.01 (0.99 to 1.02)
LDL cholesterol (per mmol/l)	0.260	1.01 (0.99 to 1.03)
Triglycerides (per mmol/l)	0.576	1.00 (0.99 to 1.01)
Fasting glucose (per mmol/l)	0.097	1.06 (0.99 to 1.12)
Plasma homocysteine (per μmol/l)	0.285	1.13 (0.90 to 1.41)
Creatinine clearance (per ml/min)	0.212	1.01 (0.99 to 1.03)
Folate (per nmol/l)	0.382	1.01 (0.99 to 1.02)
Vitamin B_{12} (per pmol/l)	0.805	1.00 (1.00 to 1.01)
Drug therapy		
RAS inhibitors (yes vs no)	0.407	2.07 (0.37 to 11.60)
β-blockers (yes vs no)	0.648	1.94 (0.11 to 33.05)
Statins (yes vs no)	0.626	1.50 (0.29 to 7.65)
Vital signs at PAT study		
Systolic blood pressure (per mm Hg)	0.572	1.01 (0.97 to 1.06)
Diastolic blood pressure (per mm Hg)	0.323	1.05 (0.96 to 1.15)
Heart rate (per beats/mim)	0.767	0.99 (0.92 to 1.07)
GTN-PAT (per unit increment)	0.039	0.37 (0.14 to 0.95)

CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; DWMH, deep white matter hyperintensities; FMD, flow mediated dilation; GTN, glyceryl trinitrate; LDL, low density lipoprotein; PAT, peripheral arterial tonometry; PVH, periventricular hyperintenties; RAS, renin—angiotensin system.

The loss of systemic arteriolar wall tone caused by VSMC degeneration may lead to failure of cerebral autoregulation with chronic hypoperfusion or abrupt lack of perfusion. The low blood pressure values found in our CADASIL patients during day measurements compared with controls are consistent with previous reports.[34] A functional failure of brain structures and connections controlling circadian blood pressure variations, secondary to white matter damage, may lead to hypotension that was also found to correlate with global cognitive deterioration. Decreasing or loss of cerebral vasoreactivity is considered the pathophysiological basis for the haemodynamic changes leading to subcortical chronic hypoperfusion and ischaemic lacunar lesions.[10 35]

We found no correlation between specific mutations or mutation site and vasoreactivity. The phenotypic variability of CADASIL despite the highly stereotyped nature of *Notch3* mutations is well known. The dominant manifestations may vary in different families, and the clinical picture and functional course may also differ among individuals of the same family. Someone with the mutation may remain asymptomatic for long periods, even with well defined lesions on MR imaging, while others exhibit major symptoms, such as transient ischaemic attacks or lacunar infarcts, migraine with aura, depression or anxiety, or slow evolution of cognitive impairment. Potential factors accounting for this variability include environmental influences and additional genetic factors, such as specific functional polymorphisms.[36–38] In line with these previous findings, the lack of correlation between mutation site and vasoreactivity in our series is not surprising.

The white matter lesion load has been suggested to influence the nature and severity of clinical manifestations. Conversely, the present study confirms previous observations by our group,[17] as even asymptomatic subjects showed advanced white matter changes in a proportion comparable with subjects with overt disease.

The influence of conventional cardiovascular risk factors on disease progression was examined by Singhal *et al*[32] who found an association between smoking and an earlier age of onset for stroke/transient ischaemic attack. However, these findings await confirmation. To date, older age is the only firmly established risk factor for disease progression in CADASIL, leaving a large proportion of the phenotypic variance unexplained.

Clinical implications

Our findings may be of interest for the management of CADASIL patients in clinical practice. As our controls were balanced by type and overall CV risk factor burden, the finding of similar between group FMD-PAT scores points to the importance of intensive risk factor treatment in CADASIL. All patients should stop smoking, and blood pressure and low density lipoprotein cholesterol levels close to those recommended for secondary prevention should be targeted, even in subjects with no clinical manifestations of the disease.

The prognostic value of altered endothelium independent vasoreactivity in CADASIL patients is as yet unclear and needs to be determined in longitudinal studies. Drugs directed towards improving vasoreactivity and preserving the endothelium, by ameliorating global vascular function, might positively impact on outcome. Novel therapeutic options targeted to improve endothelial and VSMCs function need to be assessed in ad hoc clinical trials.

Some limitations of our study deserve consideration. This is a cross sectional investigation and no inferences can be made on the prognostic role of observed abnormalities in CADASIL patients. The 1 year follow-up findings did not seem to suggest an impact of altered vasoreactivity on short term disease progression Although we observed an imbalance in drug treatment between cases and controls, no net impact of statins on vasoreactivity was found.

CONCLUSION

The impaired vasoreactivity observed in our CADASIL patients highlights the fact that both endothelial and smooth muscle functional alterations may already be present in mildly disabled subjects. The detrimental impact of reduced endothelium dependent and independent vasodilation in the evolution of CADASIL should be ascertained in large prospective cohort studies. Improvement of vascular function by increasing nitric oxide bioavailability could be a new target for pharmacological trials in the CADASIL population.

Acknowledgements The authors thank Elisabetta Spagnolo for excellent secretarial assistance.

Funding The study was supported by grant FARM659PTX from the Italian Medicine Agency (AIFA).

Competing interests None.

Ethics approval The study was approved by the institutional review boards of the participating units.

Contributors All the authors have give their final approval to the manuscript and had sufficient access to the data to verify the manuscript's scientific integrity. Conception and design of the research: JC, RDM, OP; data acquisition: JC, MF, SR, EP, CM, CT, FP, MTD, MLS, NDS; analysis and interpretation of the data: JC, RDM, OP; statistical analysis: JC, RDM; funding and supervision: JC, RDM, MF, FT, DI, AF, LP, OP; drafting of the manuscript: JC, RDM; critical revision: JC, RDM, MF, FT, DI, AF, CM, LPL, FP, MTD, AT, OP.

Provenance and peer review Not commissioned; externally peer reviewed.

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APPENDIX

Study participants

CNR Clinical Physiology Institute, Milan: Raffaele Caruso, Marina Parolini, Valentina Sedda, Anna Maria Tonini, Chiara Boroni, Stefania Zuddas. *Institute of Translational Pharmacology, CNR, Rome*: Liana Veneziano. *Department of Neurological Sciences First* and *Second Medical School University of Rome La Sapienza, Rome*: Ada Francia, Francesco Orzi; *Department of Neurological and Psychiatric Sciences, University of Florence*: Raffaella Valenti, Cristina Sarti. *Neuroradiology Unit, Careggi University Hospital, Florence, Italy*: Marco Moretti, Stefano Chiti, Giampaolo Giordano. *Department of Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri, Milan*: Claudio Pelucchi.

Impaired vasoreactivity in mildly disabled CADASIL patients

Jonica Campolo, Renata De Maria, Marina Frontali, et al.

J Neurol Neurosurg Psychiatry 2012 83: 268-274 originally published online November 9, 2011
doi: 10.1136/jnnp-2011-300080