Reduced Cerebrovascular CO$_2$ Reactivity in CADASIL: A Transcranial Doppler Sonography Study

Thomas Pfefferkorn, MD; Sebastian von Stuckrad-Barre, MD; Jürgen Herzog, MD; Thomas Gasser, MD; Gerhard F. Hamann, MD; Martin Dichgans, MD

**Background and Purpose**—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy (CADASIL) is a hereditary angiopathy caused by mutations in Notch3. Cerebral microvessels show an accumulation of granular osmiophilic material in the vicinity of degenerating vascular smooth muscle cells. To study cerebrovascular function in CADASIL, we performed measurements on cerebral hemodynamics by using transcranial Doppler sonography.

**Methods**—Middle cerebral artery (MCA) mean blood flow velocity (MFV), cerebrovascular CO$_2$ reactivity, and the resistance index were measured by bilateral transcranial Doppler sonography in 29 CADASIL individuals (mean age, 49.0±2.4 years) and an equal number of age- and sex-matched control subjects.

**Results**—Compared with control subjects, CO$_2$ reactivity was reduced in CADASIL (33.4±2.7% versus 45.3±3.0%; *P*<0.01). This difference remained significant when only nondisabled CADASIL individuals (Rankin=0, n=21) were included in the analysis (*P*<0.05). CO$_2$ reactivity was significantly lower in disabled than in nondisabled CADASIL individuals (24.5±2.7% versus 36.8±3.4%; *P*<0.05). MCA MFV was reduced in CADASIL (45.6±2.2 cm/s versus 54.2±2.4 cm/s; *P*<0.05) and correlated negatively with age both in affected individuals (*r* =−0.314; *P*<0.05) and control subjects (*r* =−0.339; *P*<0.05). Resistance index was not significantly altered (59.0±1.0% versus 57.7±1.2%; *P* =0.42).

**Conclusions**—In CADASIL, there is a reduction of both CO$_2$ reactivity and basal MCA MFV. The reduced CO$_2$ reactivity suggests functional impairment of cerebral vasoreactivity probably related to vascular smooth muscle cell dysfunction. The reduction of CO$_2$ reactivity in nondisabled CADASIL individuals suggests an early role of impaired cerebral vasoreactivity in the evolution of the disease. (*Stroke. 2001;32:17-21*.)

**Key Words:** CADASIL ■ carbon dioxide ■ ultrasonography, Doppler, transcranial

The clinical, pathological, and genetic spectrum of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy (CADASIL) has recently been characterized by several comprehensive studies.1–8 Clinical manifestations include recurrent ischemic episodes (TIAs and strokes) (70% to 80%), cognitive deficits (30% to 50%), and migraine (20% to 40%), mostly with aura, as well as psychiatric disorders (20% to 30%) and epileptic seizures (6% to 10%).1–3 Onset of ischemic symptoms is usually in mid adulthood.1–3

MRI reveals a microangiopathic pattern of signal abnormalities: diffuse white matter T2-signal hyperintensities and small cystic lesions compatible with lacunes.4 These changes are caused by a distinctive angiopathy characterized by granular osmiophilic material within the vascular basal membrane, often located in close contact with degenerating smooth muscle cells (VSMC).6–9

The disease is caused by mutations within the Notch3 gene.10 Notch3 codes for a large transmembrane receptor that is physiologically expressed in VSMC. In the brains of CADASIL patients there is a dramatic accumulation of the extracellular domain of Notch3 within arteries, capillaries, and venules. This accumulation takes place at the cell surface of VSMC.11

So far, information on microvascular function and hemodynamic parameters in CADASIL is limited. Using SPECT, Mellies et al12 found a reduction of cerebral blood flow (CBF) that correlated with the amount of MRI white matter abnormalities. Chabriat et al13 used PET to study 2 CADASIL individuals (1 asymptomatic, 1 demented). CBF was reduced both in the symptomatic case and the asymptomatic case, thus suggesting a role of CBF reduction early in the disease.

To investigate microvascular function in CADASIL, we studied 3 hemodynamic parameters by using transcranial Doppler sonography (TCD): CO$_2$ reactivity, middle cerebral artery (MCA) mean blood flow velocity (MFV), and resistance index (RI).

**Subjects and Methods**

Twenty-nine CADASIL individuals (13 men, 16 women; mean age, 49.0±2.4 years; range, 28 to 79 years) and 29 age- and sex-matched control subjects were included. TCD was performed by the same highly experienced operator. CO$_2$ reactivity was calculated as the difference between MFV during hypercapnia (breathing 7% CO$_2$) and hypocapnia (breathing 1% CO$_2$). Resistance index was not significantly altered (59.0±1.0% versus 57.7±1.2%; *P* =0.42). MCA MFV was reduced in CADASIL (45.6±2.2 cm/s versus 54.2±2.4 cm/s; *P*<0.05) and correlated negatively with age both in affected individuals (*r* =−0.314; *P*<0.05) and control subjects (*r* =−0.339; *P*<0.05). Resistance index was not significantly altered (59.0±1.0% versus 57.7±1.2%; *P* =0.42).

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control subjects (13 men, 16 women; mean age, 48.8±3.0 years; range, 20 to 84 years) were enrolled into this study. CADASIL individuals were derived from 21 families. In all CADASIL cases, the diagnosis had been confirmed by the identification of a mutation in the Notch3 gene (n=24) or by skin biopsy (n=14).17,18,24,25 All CADASIL individuals received a clinical examination at the time of the Doppler study. Demented individuals (satisfying the DSM-IV criteria and having a SIDAM score of <3316) were excluded from the study to ensure informed consent and adequate cooperation during the TCD examination. Extracranial duplex sonography was performed in all subjects to exclude carotid stenosis.

TCD studies were performed with the MultiDop X4 from DWL. The method and its modification have been described previously.17,18 Briefly, while the patients were sitting in a comfortable position, 2-MHz probes were bilaterally fixed over the temporal bone window where highest intensity and velocity of the MCA signal in a depth of 45 to 55 mm had been obtained.

Bilateral MCA MFV was continuously monitored and digitally recorded for later off-line analysis. Patient breathing was through a plastic mouthpiece; a nose clamp kept the nostrils closed. A valve mechanism allowed for prompt switching from room air to carbogene (5% CO\textsubscript{2}, 95% O\textsubscript{2}). The patients started by breathing room air for 30 to 60 seconds until a steady state in MCA MFV was obtained. They were then asked to hyperventilate for 30 to 60 seconds. Thereafter, patients were allowed to breathe normally for another minute. In the next step, patients were ventilated with carbogene for at least 1 minute until MFV remained stable. Finally, patients breathed room air again until MFV had normalized.

For off-line analysis, MCA MFV was deduced from the recorded Doppler curves at the following time points: resting (nornocapnia), during hyperventilation after a steady state in flow velocity had been obtained (hypocapnia), and after at least 1 minute of carbogene ventilation when flow velocity had reached stable values (hypercapnia).

CO\textsubscript{2} reactivity was calculated as the percent change of MCA MFV in hypercapnia compared with normocapnia (CO\textsubscript{2} reactivity\(=\)(MFV\textsubscript{hypercapnia}−MFV\textsubscript{normocapnia})×100%/MFV\textsubscript{normocapnia}). The RI\textsuperscript{19} (expressed as percent) was calculated during resting (normocapnia) as the difference between the systolic flow velocity (FV) and diastolic FV divided by the systolic FV (RI\(=\)(FV\textsubscript{systolic}−FV\textsubscript{diastolic})×100%/FV\textsubscript{systolic}).

For further analysis, the measured values for the left and right MCA were averaged for each individual patient. In case of insufficient signal quality on one side, only the sufficient signal from the contralateral side was used for further analysis.

Values are presented as mean±SEM. To test for differences in CO\textsubscript{2} reactivity, MCA MFV, and RI between CADASIL subjects and control subjects, MANOVA with Rankin score, age, and sex as covariates was performed. To test for correlations between hemodynamic parameters and age, bivariate analysis (Pearson) was performed.

**Results**

Of the 29 CADASIL individuals, 27 had at least 1 of the classic manifestations of CADASIL. Two individuals were asymptomatic. The majority (21 of 29) was nondisabled (Rankin scale score=0). Only two individuals were severely disabled (Rankin=4). Bilateral sufficient TCD signal quality was obtained in 25 CADASIL individuals and 27 control subjects. No significant right-to-left differences were detected for any of the measured flow velocity values. In the remaining 6 subjects with only unilateral TCD detection, signal quality was sufficient for off-line analysis. Individual curves for a patient and a control subject are presented in Figure 1.

CO\textsubscript{2} reactivity was significantly lower in patients compared with control subjects (33.4±2.7% versus 45.3±3.0%; \(P<0.01\); Figure 2). This difference remained significant when including only the 21 nondisabled CADASIL individuals (CO\textsubscript{2} reactivity: 36.8±3.4%\(; \ P<0.05\); Figure 2). CO\textsubscript{2} reactivity was significantly higher in the 21 nondisabled CADASIL individuals (Rankin=0) than in the 8 disabled patients (Rankin>0) (36.8±3.4% versus 24.5±2.7%; \(P<0.05\); Figure 2). CO\textsubscript{2} reactivity was not correlated to age in CADASIL individuals (\(r=-0.118\); \(P=0.541\)) and control subjects (\(r=-0.195\); \(P=0.310\)).

MCA MFV during normocapnia was significantly lower in CADASIL individuals compared with control subjects (45.6±2.2 cm/s versus 54.2±2.4 cm/s; \(P<0.05\); Figure 3).
MCA MFV was negatively correlated to age in CADASIL individuals and control subjects (patients: $r = -0.314$, $P<0.05$; control subjects: $r = -0.339$, $P<0.05$).

RI showed no significant difference between CADASIL individuals and control subjects (59.0±1.0% versus 57.7±1.2%; $P=0.42$; Figure 3). RI was positively correlated to age in CADASIL individuals ($r=0.552$; $P<0.005$) and control subjects ($r=0.359$, $P<0.05$).

**Discussion**

This TCD study provides evidence for altered cerebral hemodynamics in CADASIL. Changes consist of a reduced cerebral vasoreactivity and a reduction of MCA blood flow velocity. These findings are in good agreement with a recent MRI bolus tracking study that found a reduction of cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral vasoreactivity to acetazolamide within the altered white matter of CADASIL individuals.

In our study, cerebral vasoreactivity was tested as the vascular response to inhaled CO$_2$. CO$_2$ is a strong stimulus for vasodilation caused by VSMC relaxation. The exact mechanisms by which CO$_2$ induces VSMC relaxation are incompletely understood. However, evidence exists that CO$_2$-induced vasodilation is predominantly mediated by a decrease in extracellular pH. Lowering of the extracellular pH has been shown to cause VSMC membrane hyperpolarization, activation of potassium channels, and inactivation of Ca$^{2+}$ channels. These events may result in a reduction of the intracellular Ca$^{2+}$ level and a consecutive decrease of vascular tone. Interestingly, CO$_2$-induced vasodilation does not depend on an intact endothelial layer, as demonstrated in experiments with injured pial endothelium, and denuded cerebral arteries, and isolated cerebral VSMC cultures, thus underlining the pivotal role of VSMC in CO$_2$-induced cerebral vasodilation.

Against this background, the observed impairment of CO$_2$-induced vasodilation may indicate a dysfunction of VSMC in CADASIL. This is in accordance with the histopathological and ultrastructural abnormalities seen in small arteries from CADASIL individuals: degenerating VSMC and osmiophilic granular deposits within a thickened vascular basal membrane. Also, it has recently been shown that the Notch3 gene product is almost exclusively expressed in VSMC and that the extracellular domain of this protein accumulates at the cell surface of VSMC in CADASIL brains.

It is important to note that the impairment of vasoreactivity in our CADASIL subjects did not depend on the presence of disability. This observation is in accordance with biopsy findings that have shown ultrastructural VSMC changes in presymptomatic CADASIL individuals and suggests an early role of VSMC dysfunction in the evolution of the disease.

Clinicoradiologic correlations in our study were limited to physical disability. The exclusion of demented CADASIL individuals did not allow the investigation of correlations between cognitive status and hemodynamic parameters. Future studies on a larger number of individuals covering the whole clinical spectrum of CADASIL may clarify whether such correlations exist. Although we found significant group differences in CO$_2$ reactivity and MCA MFV between CADASIL subjects and control subjects, there was a considerable overlap of individual data obtained in the two groups. Thus, these measurements do not allow us to differentiate CADASIL cases from control subjects on an individual level.

Decreased CO$_2$ reactivity has been reported previously in other forms of cerebral microangiopathy. TCD studies in patients with subcortical vascular encephalopathy revealed reduced cerebrovascular reactivity to apnoe. Through ionization of different intracranial arteries of patients with subcortical vascular encephalopathy, other TCD studies demonstrated diminished blood flow velocities and elevated pulsatility indexes. In our study, MCA MFV was similarly decreased, whereas the RI, a marker equivalent to pulsatility index, was not significantly altered. However, these findings are not sufficient to define a specific hemodynamic pattern for CADASIL. Also, measurements on resistance and pulsatility indexes must be interpreted with particular caution when studying small-vessel disease. Studies directly comparing CADASIL patients with other patient populations may be warranted to determine possible differences in cerebral hemodynamics. Such studies may include additional TCD techniques such as measurements on low-frequency spontaneous oscillations, cerebral transit time, and acetazolamide challenge.
Assuming a constant diameter of the MCA, the MFV reduction in our CADASIL cases may essentially reflect reduced CBF. In fact, a reduction of CBF has been suggested recently by SPECT, PET, and MRI studies in CADASIL. It seems reasonable to assume that the changes in MFV and CBF are related to the morphological alterations within small blood vessels, in particular small arteries and capillaries. Possible mechanisms by which these alterations might cause a reduction in MFV and CBF include narrowing of the vessel lumen and a reduced overall density of the vascular network. This would fit with the known reduction of CBV in white matter abnormalities of CADASIL individuals. Again, it must be mentioned that CBF reduction is not specific for CADASIL but has also been demonstrated in patients with lacunar infarction and leukoaraiosis of other origins. Therefore, additional studies on brain structure, brain metabolism, cerebral hemodynamics, and blood vessel morphology are necessary to elucidate the mechanisms leading to reduced MFV, CBF, and CBV in CADASIL.

From our study, we conclude that cerebral vasoreactivity is decreased in CADASIL patients. This functional impairment is readily seen at an early clinical stage. Associated with well-defined morphological changes affecting the cerebral VSMC, decreased cerebral vasoreactivity may be a key element in disease evolution.

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References


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