Prolonged Cerebral Transit Time in CADASIL
A Transcranial Ultrasound Study

Martin Liebetrau, MD; Jürgen Herzog, MD; Christian U.A. Kloss, MD; Gerhard F. Hamann, MD; Martin Dichgans, MD

Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary angiopathy caused by mutations in *Notch3*. Cerebral microvessels show an accumulation of granular osmophilic material in the vicinity of degenerating vascular smooth muscle cells. In this study, we measured the arteriovenous cerebral transit time (CTT) to identify changes related to the microangiopathy in CADASIL.

Methods—CTT is the time that a contrast agent needs to pass from a cerebral artery to its corresponding vein. CTT was measured in 17 CADASIL individuals (mean age, 50.2±12.3 years) and an equal number of age- and sex-matched control subjects (mean age, 48.9±13.0 years) with transcranial color-coded duplex sonography. The intensity curves were recorded in the P2 segment of the posterior cerebral artery and the vein of Galen after injection of the ultrasound contrast agent Levovist.

Results—CTT was significantly prolonged in individuals with CADASIL (4.4±1.9 seconds) compared with control subjects (1.3±0.5 seconds, \(P<0.0001\)). This difference was also significant when only nondisabled CADASIL individuals (Rankin score=0, \(n=9\)) were analyzed (\(P<0.0001\)). There was a nonsignificant trend for a correlation between Rankin score and CTT (\(r=0.39, P=0.11\)).

Conclusions—The prolonged CTT likely reflects microvascular changes in CADASIL. Measurements of the CTT may be used clinically to disclose small-vessel disease. Studies comparing CADASIL subjects with other patient populations seem warranted to determine possible differences in CTT between different types of small-vessel disease. (Stroke. 2002; 33:509-512.)

Key Words: blood circulation time • dementia, multi-infarct • ultrasonography, Doppler, duplex • ultrasonography, Doppler, transcranial

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited angiopathy secondary to mutations in *Notch3*. Manifestations include ischemic stroke, progressive cognitive deficits, and migraine with aura, as well as psychiatric disturbance. The onset of ischemic symptoms is usually in mid adulthood. Advanced stages of this disease correspond to the clinical syndrome of Binswanger’s encephalopathy.

MRI reveals a microangiopathic pattern of signal abnormalities. The underlying angiopathy is characterized by a unique type of ultrastructural basal membrane deposits and by degeneration of vascular smooth muscle cells, which are the major source of Notch3 expression. In CADASIL, there is an excessive accumulation of the extracellular domain of the Notch3 receptor within arteries, capillaries, and venules at the cell surface of vascular smooth muscle cells.

Consistent with these changes, recent studies have provided evidence for a disturbed vascular reactivity and a reduction in cerebral blood flow (CBF) and cerebral blood volume (CBV). To further evaluate cerebral microcirculation in CADASIL, we determined the arteriovenous cerebral transit time (CTT) using transcranial color-coded duplex sonography (TCCD). The method is based on the assumption that the time required by an ultrasound agent to pass from the cerebral arteries to the veins should be prolonged in patients with small-vessel disorders.

Subjects and Methods
Seventeen CADASIL individuals (8 women, 9 men; mean age, 50.2±12.3 years; range, 34.2 to 71.1 years) and 17 age- and sex-matched control subjects (8 women, 9 men; mean age, 48.9±13.0 years; range, 31.5 to 75.0 years) were enrolled in this study. None of the control subjects had a history of stroke-related deficits. Control subjects were healthy volunteers (\(n=8\)) or patients without disease of the central nervous system (\(n=9\)). CADASIL patients were derived from 15 families. In all CADASIL patients, the diagnosis had been confirmed either by skin biopsy or by demonstration of 1 of the following Notch3 mutations (\(n=14\)): R90C, C117F, R141C, C174Y, C194F, or R182C. Clinical assessment was done on the day of the MR study. Disability was graded by use of the modified Rankin scale. Cognitive perfor-
mance was assessed with the Mini-Mental State Examination (MMSE). 21
Extracranial duplex sonography was performed in all control subjects to exclude carotid artery stenosis. None of the individuals had evidence of heart failure or arrhythmia. All subjects gave informed consent according to the Declaration of Helsinki.
TCCD studies were performed with the Sonos 5500 from Hewlett-Packard with a 4-MHz, phased-array probe through a transtemporal bone window. All subjects had an adequate acoustic bone window for TCCD. The arteriovenous CTT was measured as previously described.14,15 In brief, the thalamus and pineal gland were depicted at the level of the third ventricle. The P2 segment of the posterior cerebral artery (PCA) was found by TCCD to be lateral of the pinealis gland, and the corresponding vein of Galen was found dorsal to the pinealis gland. A bolus of contrast agent (6 mL Levovist [Schering] at 400 mg/mL) was injected intravenously into the cubital vein within 5 seconds with a constant injection speed. Doppler sonographic verification of both vessels was always performed at the end of the examination after the ultrasound contrast agent had been given intravenously. The examination was digitally recorded at 5 images per second.
Data analysis was done offline with a computerized video-imaging system (Optimas, MediaCybernetics). The signal intensity curves were calculated from regions of interest at the P2 segment of the PCA and the vein of Galen. The time from injection of the contrast agent to the beginning of signal intensity increase in the PCA was defined as the wash-in time. CTT was measured as the latency of signal intensity increase between the P2 segment of the PCA and the vein of Galen. This method has previously been shown to be a robust and reproducible method for the assessment of cerebral microcirculation.14
Values are presented as mean±SEM. To test for differences in CTT and wash-in time in CADASIL subjects and control subjects, the Mann-Whitney U test was performed. To test for correlations between CTT and clinical scales, bivariate analysis (Spearman) was performed.

Results
Sixteen CADASIL subjects had developed symptoms consisting of ≥1 of the following manifestations: migraine with aura (n=5), transient ischemic attacks and/or stroke (14 patients), dementia (n=3), or depression (n=4). Their Rankin scores ranged from 0 to 3 (mean, 1.0), and their MMSE examination score was between 13 and 30 (mean, 25.7).
The contrast agent was tolerated in all individuals without any side effects. The wash-in time showed no difference between the CADASIL and control groups (14.1±3.9 versus 14.5±3.4 seconds, P=0.76; the Table) and was not correlated to age in these groups (r=0.15, P=NS in CADASIL subjects; r=0.27, P=NS in control subjects). CTT was significantly prolonged in CADASIL subjects compared with the control group (4.4±1.9 versus 1.3±0.5 seconds, P<0.0001; Figures 1 and 2). This difference was also significant when only those CADASIL subjects who were not disabled (Rankin score=0) and had no cognitive decline (no subjective complaints and

<table>
<thead>
<tr>
<th>CADASIL</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=17)</td>
<td>(n=17)</td>
</tr>
<tr>
<td>Age</td>
<td>50.2±12.3</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>8/9</td>
</tr>
<tr>
<td>Wash-in time, s</td>
<td>14.5±3.4</td>
</tr>
<tr>
<td>CTT, s</td>
<td>4.4±1.9</td>
</tr>
</tbody>
</table>

MMSE≥24) were included (4.0±1.8 versus 1.2±0.5 seconds in age- and sex-matched controls, P<0.0001). There was a nonsignificant trend toward a positive correlation between CTT and Rankin score (r=0.40, P=0.12). No significant correlation was observed between MMSE and CTT (r=−0.08, P=NS). CTT was not correlated to age in CADASIL individuals and control subjects (r=0.27 and 0.29, P=NS).
Discussion
This study shows that the arteriovenous CTT is markedly prolonged in CADASIL subjects. CTT measures the time an indicator substance needs to pass from the cerebral arteries to the corresponding veins. Most of the transit time is for the tagged bolus to pass through arterioles and capillaries. In fact, histopathological and ultrastructural studies in CADASIL have shown occlusive changes in both small arteries and capillaries. Against this background, our finding of a prolonged CTT seems consistent.

Our results are in contrast to a recent MRI bolus tracking study that found no significant difference in mean transit time between CADASIL patients and control subjects. However, in that study, the mean transit time was derived from the ratio of 2 calculated parameters (CBV and CBF), whereas in the present study, CTT was determined by direct measurements. There are some methodological limitations to the MRI technique, particularly at the cortical level.

Using SPECT, PET, and MRI, recent authors have shown a reduction in CBF in CADASIL. In line with these findings, transcranial Doppler sonography revealed a diminished mean flow velocity in the middle cerebral artery. It seems reasonable that changes in mean flow velocity, CBF, and CBV are related to alterations of the microvasculature such as rarefaction of the microvascular tree as suspected and CBV are related to alterations of the microvasculature seems reasonable that changes in mean flow velocity, CBF, and CBV are related to alterations of the microvasculature such as rarefaction of the microvascular tree as suspected from the histopathologic data. However, measurements directly comparing mean flow velocity, CBF, CBV, and CTT in single individuals are required to further settle the relation between these different variables. Of note, there was no evidence of large-vessel disease in this and previous hemodynamic studies in CADASIL, thus excluding stenosis of proximal arteries as a cause of the observed hemodynamic change.

We found CTT to be significantly prolonged not only in the overall CADASIL group but also in nondisabled mutation carriers. This observation agrees with biopsy findings that have shown vascular abnormalities in symptomatic CADASIL individuals and suggests an early role of microvascular alterations in the evolution of the disease.

In this study, no correlation was found between CTT and cognitive performance. Obviously, this finding might be connected to the fact that measurements were done within the territory of the PCA. Cognitive dysfunction in small-vessel disease has been related to disturbances in fronto-temporal circuits. Consequently, most of the microvascular changes relevant to cognitive impairment may have escaped our analysis. In addition, the MMSE might be insensitive to slight cognitive changes.

Using the same method, Puls et al found weak correlations between CTT and cognitive performance in patients with small-vessel disease. However, those patients had been selected on the basis of specific clinical and MRI findings. Also, there are differences in the distribution of lesions between CADASIL and sporadic small-vessel disease; in CADASIL, there is a stronger involvement of fronto-temporal and temporopolar brain regions, whereas the sporadic cases show more ubiquitous changes of the small vessels. Studies directly comparing CTT in posterior and anterior parts of the circulation or comparing CTT in CADASIL subjects with cases of sporadic small-vessel disease may evaluate the clinical importance of regional variations. Alternatively, our failure to detect a correlation between CTT and cognitive performance might be due to the small number of subjects investigated. Conceivably, this may have influenced the results.

The CTTs found in our control subjects were shorter than those reported by Puls et al. There are several explanations for this difference, including different equipment, different injection techniques, and the use of a higher dose of the contrast agent, which may have resulted in a steeper increase in the intensity curve—and hence earlier detection—in the low-flow venous system. This clearly indicates the requirement for a control group. We found little overlap between CTT in CADASIL patients and control subjects. This finding shows that transcerebral Doppler sonography may in fact be used clinically to disclose small-vessel disease. Studies directly comparing CADASIL patients with other patient populations seem warranted to determine possible variations in CTT between different types of small-vessel disease. Other possible applications include serial measurements to evaluate the course of the disease process that are either natural or modified by therapeutic interventions.

Acknowledgments
This study was supported by DFG grant Di722/3-1. We are grateful to all the individuals who participated in this study.

References


Prolonged Cerebral Transit Time in CADASIL: A Transcranial Ultrasound Study
Martin Liebetrau, Jürgen Herzog, Christian U.A. Kloss, Gerhard F. Hamann and Martin Dichgans

Stroke. 2002;33:509-512
doi: 10.1161/hs0202.102949

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/33/2/509

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/