

Simultaneous Impairment of Intracranial and Peripheral Artery Vasoreactivity in CADASIL Patients

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Key Words

CADASIL · Vasoreactivity · ¹²³I-IMP-SPECT · Reactive hyperemia peripheral arterial tonometry

Abstract

Background: Reduced cerebrovascular reactivity (CVR) is an important step in the pathogenesis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). The present study utilized quantitative single photon emission computed tomography (SPECT) with the autoradiographic (ARG) method and reactive hyperemia peripheral arterial tonometry (RH-PAT) to assess vasoreactivity in intracranial arteries and in peripheral arteries in patients with CADASIL. **Methods:** Quantitative SPECT studies were conducted in eight patients with CADASIL, while RH-PAT analysis was conducted in eight CADASIL patients and in eight age-matched normal subjects. Quantitative SPECT studies with the ARG method were performed at baseline and after administration of acetazolamide. Regional cerebral blood flow (rCBF) values were measured using stereotactic extraction estimation (SEE) methods. The rCBF of CADASIL patients was averaged in the bilateral fron-

tal, temporal, parietal, and occipital lobes as well as in the limbic system, cerebellar hemisphere, whole cerebral cortex and basal ganglia. The CVR index from acetazolamide stress of intracranial arteries was calculated in each area. Vasoreactivity of peripheral arteries was estimated by the reactive hyperemia index (RHI) measured with a PAT device before and after interruption of arterial flow. **Results:** Average RHI after post-deflation was lower in CADASIL patients than in normal subjects. RHI correlated significantly with CVR in all brain areas in CADASIL patients. **Conclusions:** Vasoreactivity is reduced in peripheral arteries and in intracranial arteries in patients with CADASIL.

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Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary microangiopathy due to *Notch3* mutation [1], leading to migraines, recurrent cerebral infarction, and vascular dementia. *Notch3* is mainly expressed in vascular smooth muscle cells (VSMCs) and is preferentially ex-

pressed in small arteries of the adult human brain [2]. Histological examination of systemic arteries from patients with CADASIL has revealed thickening of the arterial wall and degeneration of VSMCs [3]. Therefore, degeneration of VSMCs is thought to represent an important step in the pathogenesis of CADASIL [4]. Early reduced cerebrovascular reactivity (CVR) in intracranial arteries, which may be related to dysfunction of VSMCs, has been demonstrated by various modalities, such as magnetic resonance imaging (MRI) bolus tracking methods [5], single photon emission computed tomography (SPECT) [6], transcranial Doppler (TCD) sonography with acetazolamide [7].

Histologic examination of systemic arteries from transgenic (Tg) mice overexpressing mutant human NOTCH3 has revealed the destruction of VSMCs and the accumulation of granular osmiophilic materials around VSMCs, which is a specific diagnostic feature of CADASIL [8]. Systemic arteries from mutant NOTCH3 Tg mice also have reduced vascular reactivity in response to physiological stimuli [9]. Human pathological studies of skin arteries and cerebral arteries from CADASIL patients have also revealed destruction of VSMCs and the accumulation of granular osmiophilic materials [10]. Although the disease pathology affects mainly the tunica media, endothelial changes and dysfunction have been also reported [11–13]. Moreover, a recent large prospective cohort study showed that atherosclerosis has an impact, although small, on the clinical and brain MRI in CADASIL patients [14]. Ultrasound [12] and laser Doppler techniques [15] have demonstrated that flow-mediated vasodilation (FMD) of the brachial artery is reduced in peripheral vessels in CADASIL patients. Endothelial dysfunction in cerebral vessels has also been shown using the TCD method with L-arginine [13]. However, intracranial and peripheral vasoreactivity have not been compared within the same CADASIL patient, and the relationship between intracranial vasoreactivity and peripheral vasoreactivity in CADASIL patients remains unknown.

Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a novel noninvasive technique to assess peripheral microvascular endothelial function by measuring changes in digital pulse volume during reactive hyperemia [16]. RH-PAT can assess peripheral vasoreactivity more accurately and more easily than FMD [17, 18]. In addition, while brachial artery flow-mediated dilation measures single conduit artery vasodilatation, the PAT hyperemic response largely reflects vasodilatation in digital microvessels.

We hypothesize that the vasoreactivity of intracranial arteries and peripheral arteries are reduced in CADASIL patients. To test this hypothesis, the present study utilized quantitative *N*-isopropyl-*p*-¹²³I-iodoamphetamine (¹²³I-IMP) SPECT with the autoradiographic (ARG) method and RH-PAT to assess vasoreactivity in intracranial arteries and in peripheral arteries in patients with CADASIL.

Materials and Methods

Subjects

Eight CADASIL subjects (three males and five females; mean age 54.6 ± 13.1 years, range 42–75 years) from six families were recruited for this study. All CADASIL patients were diagnosed with CADASIL according to clinical symptoms, imaging studies and detection of a *Notch3* mutation. Eight aged-matched normal subjects (five males and three females; mean age 48.9 ± 5.3 years, range 40–57 years) were recruited for PAT analysis. Mini-mental state examination (MMSE) and modified Rankin Scale (mRS) were evaluated in all CADASIL patients.

Image Acquisition and Analysis

MRI was performed at the time of the study using a 1.5T MR instrument (Gyrosan Intera Nova; Philips Medical Systems, Best, The Netherlands). The severity of white-matter lesions (WML) on a fluid-attenuated inversion recovery (FLAIR) image was classified into four different grades, as previously reported [19].

Two quantitative SPECT studies with the ARG method were performed within a 7-day period. The first study was performed as a baseline study, and the second study was performed following acetazolamide injection on a separate day from the first study. For both studies, a 185 MBq dose of ¹²³I-IMP (Nihon Mediphysics, Hyogo, Japan) was injected intravenously with the subject at rest in a sitting position with the eyes open. Measurement of regional cerebral blood flow (rCBF) was performed in accordance with the ARG method, as previously reported [20]. One-point arterial blood sampling was performed from the brachial artery at 10 min after the start of ¹²³I-IMP injection for the assessment of whole blood radioactivity concentration. SPECT examination was started 22 min after the intravenous ¹²³I-IMP injection and was conducted for 16 min using a triple-head gamma camera system (PRISM IRIX; Picker International, Cleveland, Ohio, USA) equipped with low energy, parallel collimators. The SPECT images with a 128 × 128 matrix were reconstructed using ordered-subset expectation maximization (OSEM) reconstructions with four iterations and 12 subsets. Attenuation correction was performed using Chang's method, and scatter was corrected with the triple-energy window method. For the acetazolamide stress study, acetazolamide (1 g) was administered intravenously starting 10 min before the beginning of ¹²³I-IMP injection. For each subject, data obtained from ¹²³I-IMP SPECT images were analyzed with three-dimensional stereotactic surface projections (3D-SSP) using image-analysis software (iSSP version 5, Nihon Mediphysics, Hyogo, Japan), which was modified based on the NEUROSTAT program [21, 22], and data were normalized to the mean global activity. Stereotactic extraction (SEE) estimation was conducted with

Table 1. Clinical data from eight CADASIL patients

Case	Age	Gender	Notch3 mutation	MMSE score	mRS	Severity of MRI WML
1	49	Female	R182C	30	0	C
2	42	Female	R332C	24	3	D
3	68	Female	R141C	20	4	D
4	47	Male	R141C	30	0	D
5	45	Male	C106R	29	0	C
6	67	Female	C106R	22	0	D
7	45	Female	R141C	24	0	C
8	75	Male	R141C	22	4	D

Severity of WML in the FLAIR imaging was classified by Chabriat's method [15].

analysis software (SEE version 2.1, Nihon Mediphysics, Hyogo, Japan) to quantify rCBF in each area. The accuracy of rCBF values obtained by this technique in combination with ARG methods is supported by a previous study, which documented the utility of SEE and 3D-SSP programs with ARG methods to objectively assess the severity of hemodynamic brain ischemia [23]. The whole brain was divided into segments according to SEE methods (level 1; cerebrum, cerebellum, brainstem, level 2; lobe level classification), and the rCBF values (ml/100 g/min) of CADASIL patients were averaged in the bilateral frontal, temporal, parietal, and occipital lobes as well as in the limbic system, cerebellar cortex, whole cerebral cortex and brainstem. CVR index from acetazolamide stress of intracranial arteries was calculated as follows:

$$\text{rCVR (\%)} = \frac{[\text{acetazolamide challenge rCBF} - \text{resting rCBF}]}{\text{resting rCBF}} \times 100.$$

Endothelium-Dependent FMD

To investigate endothelial vasomotor function using RH-PAT, digital pulse amplitude was measured with a PAT device placed on the tip of each finger (Endo-PAT 2000; Itamar Medical, Caesarea, Israel). After baseline pulse amplitude was measured for 5 min, arterial flow was interrupted for 5 min by placing a cuff on the left proximal forearm at 200 mm Hg occlusion pressure. After releasing the pressure, pulse amplitude was recorded electronically in bilateral fingers and was analyzed with a computerized automated algorithm. Reactive hyperemia index (RHI) of peripheral arteries was calculated as follows:

$$\text{RHI} = \frac{(\text{test/baseline ratio of test arm})}{(\text{test/baseline ratio of control arm})} \times \text{baseline correction factor}.$$

The augmentation index was defined as the ratio of height of the peak above the shoulder of the wave to the pulse pressure. It was calculated automatically by analyzing the waveform of the PAT signal, averaged from multiple signals during the baseline period. Peripheral pulsation was intact, and arteriosclerosis obliterans was excluded in these subjects.

This study protocol was approved by the ethical committee of Kyoto Prefectural University. Written informed consent was obtained from all subjects.

Table 2. rCBFs before and after acetazolamide injection, and rCVRs in the cerebral whole cortex and in each brain area in patients with CADASIL

	rCBF at rest ml/100 g/min	rCBF after ACZ injection ml/100 g/min	rCVR %
Frontal	37.7 ± 6.4	63.8 ± 11.4	74.6 ± 47.6
Temporal	35.4 ± 6.8	61.3 ± 11.1	79.9 ± 52.7
Parietal	41.1 ± 8.1	68.3 ± 12.6	72.1 ± 49.3
Occipital	45.7 ± 10.6	73.3 ± 10.4	69.3 ± 51.7
Limbic	37.0 ± 7.0	61.0 ± 8.9	71.3 ± 46.2
Cerebellar	43.4 ± 12.0	64.3 ± 10.1	56.1 ± 42.1
Whole cortex	38.4 ± 7.0	64.3 ± 10.6	73.1 ± 48.0
Brain stem	34.1 ± 7.4	51.7 ± 7.7	56.9 ± 38.7

Statistical Analysis

Data are expressed as mean ± SD. Regional CBF before and after acetazolamide injections were compared using the paired t test. Regional CBF before or after acetazolamide injection and CVR in each area were compared among CADASIL patients using the analysis of variance (ANOVA) test. RHI was compared between CADASIL and normal subjects using the Mann-Whitney test. Correlations between CVR and RHI were determined by the Pearson test. A p value <0.05 was considered to represent statistical significance. Statistical analyses were performed with Dr. SPSS II for Windows (SPSS Japan Inc., Tokyo, Japan).

Results

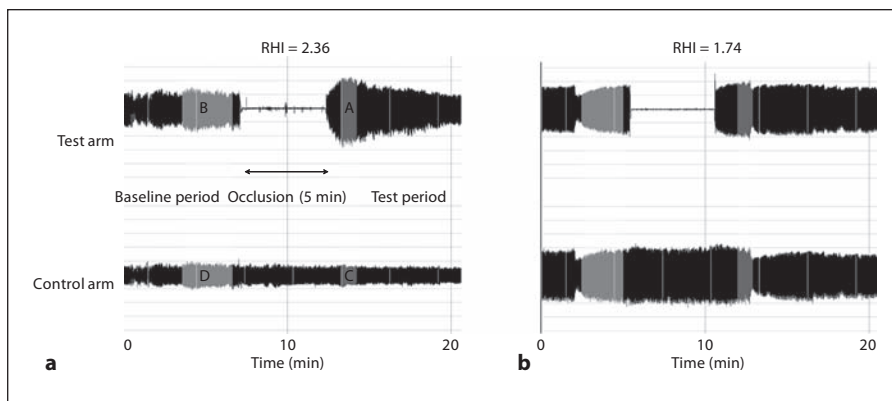
Clinical Data

The clinical characteristics of eight CADASIL patients are summarized in table 1. mRS ranged from 0 to 4 (0 in five patients, 3 in one patient, and 4 in two patients). MMSE score ranged from 20 to 30. Three patients (cases 1, 5, 7) had nodular or moderate periventricular hyperintensities on FLAIR images (score C), while five patients (cases 2, 3, 4, 6, 8) had confluent lesions or severe periventricular hyperintensities (score D). WMLs in the anterior part of the temporal lobes, which are typical MRI findings in CADASIL, were observed in six patients (cases 1, 3, 4, 6, 7, 8).

SPECT Study

Table 2 summarizes rCBFs before and after acetazolamide injection and rCVRs in each brain area in CADASIL patients. The averaged rCBFs before and after acetazolamide injection were comparatively lower in the temporal lobe and limbic system than in the occipital lobe, but the averaged rCBFs before or after acetazol-

Fig. 1. Representative pulse amplitude tracing in CADASIL patients (**b**) and in normal subjects (**a**). The x- and y-axes represent time and pulse volume amplitude, respectively. Sequential pulse amplitude of the bilateral second fingers was recorded before and after interrupting arterial flow for 5 min using a cuff placed on the forearm. Each gray area represents a region of interest for the automated calculation. RHI was calculated as follows: $RHI = (A/B)/(C/D) \times \text{baseline correction factor}$. RHI was lower in CADASIL patients than in normal subjects.



amide injection did not differ significantly among different brain areas. The averaged rCBF values of the whole cerebral cortex were significantly higher after acetazolamide injection ($64.3 \pm 10.6 \text{ ml}/100 \text{ g}/\text{min}$) than before acetazolamide injection ($38.4 \pm 7.0 \text{ ml}/100 \text{ g}/\text{min}$). The rCBF in each cerebral cortex significantly increased after acetazolamide injection, and the minimum value of rCVR was $69.3 \pm 51.7\%$ in the occipital lobe while the maximum value was $79.9 \pm 52.7\%$ in the temporal lobe.

Post-Occlusive Hyperemic Test (Endo-PAT 2000)

Representative pulse amplitude tracing in CADASIL and control patients is illustrated in figure 1. In the control patients, the pulse amplitude rose rapidly after forearm cuff deflation in the hyperemic fingertip, with maximal response occurring in the 60- to 90-s postdeflation interval. In the contralateral arm, only minimal increase or no increase was seen after deflation. Averaged RHI was significantly lower in CADASIL patients (1.49 ± 0.17) than in normal subjects (2.18 ± 0.62 ; $p = 0.01$) (fig. 2; table 3). These results suggest that the postdeflation hyperemic response in peripheral arteries was abnormal in CADASIL patients.

There were no significant differences between the CADASIL patients and normal subjects in terms of age, systolic and diastolic blood pressure, or laboratory test results (table 3). The relationship between CVR from SPECT study and RHI from RH-PAT was assessed (fig. 3) and demonstrated a significant correlation between averaged CVR in the whole cortex and RHI ($r = 0.872$, $p = 0.005$). There was also a significant correlation between CVR in any area of the brain and RHI (frontal, parietal, temporal, and occipital lobes and limbic system, cerebellar cortex and brainstem, $r = 0.823, 0.842, 0.887, 0.896, 0.910, 0.895$,

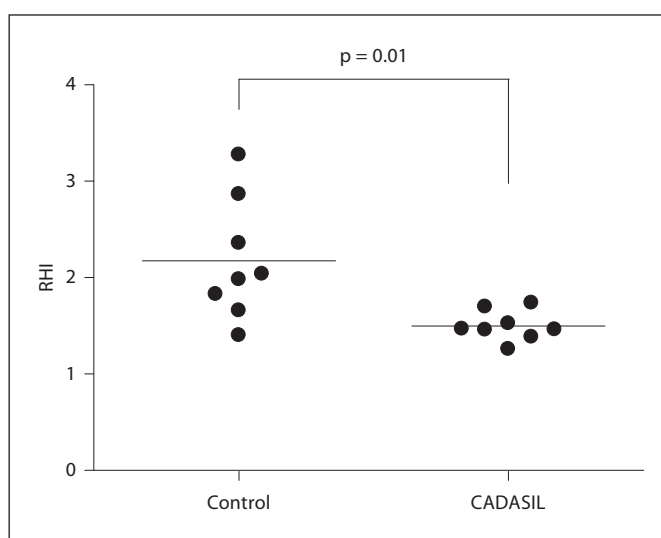


Fig. 2. Scatter plot of RHI in CADASIL patients and in normal subjects. Averaged RHI was significantly lower in eight CADASIL patients than in eight normal subjects (1.49 ± 0.17 vs. 2.18 ± 0.62 , respectively).

Table 3. Clinical characteristics and indices from PAT analysis of CADASIL patients and normal subjects

Characteristic	CADASIL (n = 8)	Control (n = 8)	p value
Age, years	54.6 ± 13.1	48.9 ± 5.3	NS
Men/women	3/5	5/3	NS
Systolic BP, mm Hg	117.6 ± 11.4	120.0 ± 10.1	NS
Diastolic BP, mm Hg	71.6 ± 10.5	80.0 ± 9.5	NS
RHI	1.49 ± 0.17	2.18 ± 0.62	0.01
Augmentation index	8.14 ± 16.21	10.86 ± 25.73	NS

BP = Blood pressure; NS = not significant.

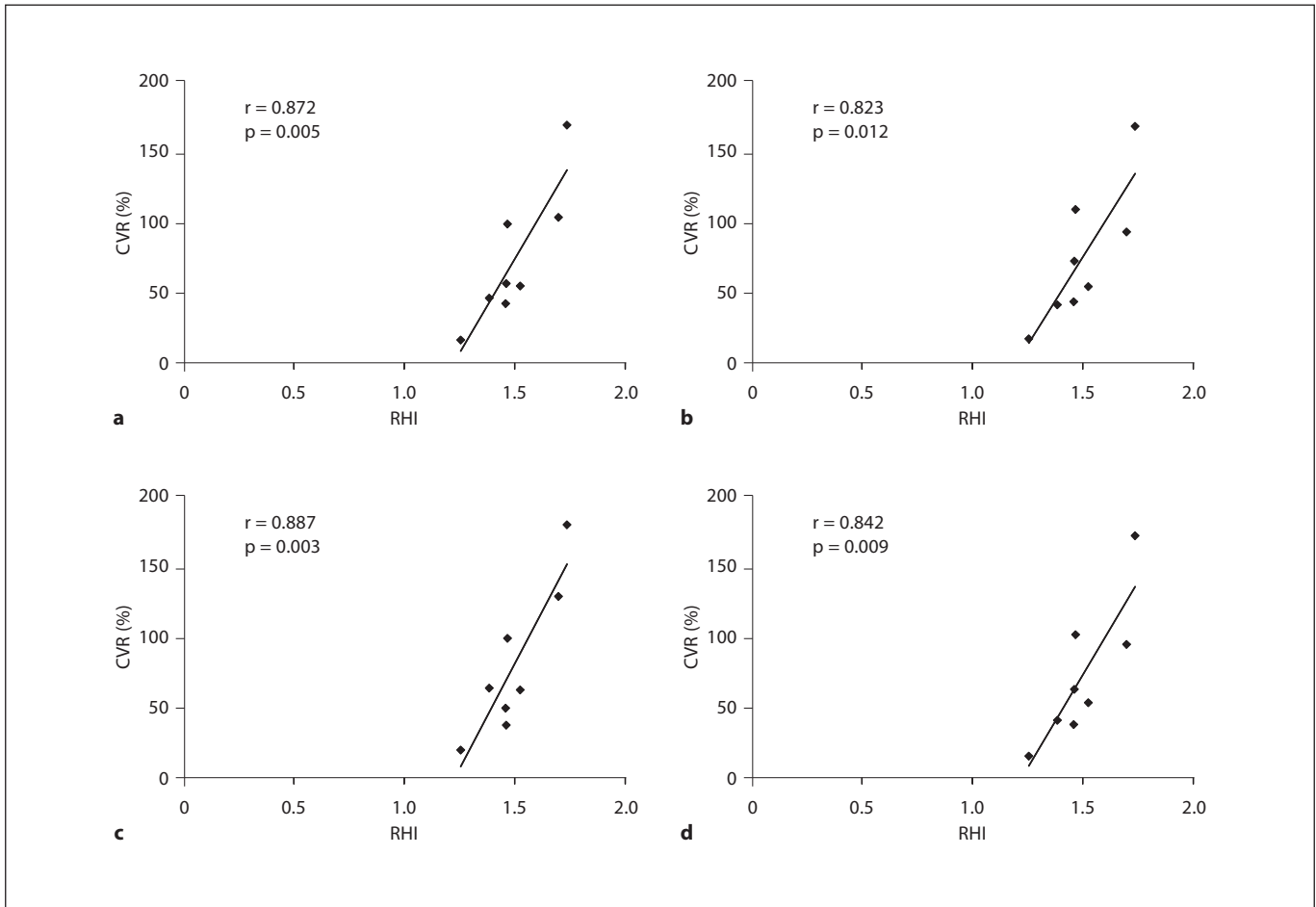


Fig. 3. Correlation between CVR and RHI of the whole cortex (a), frontal cortex (b), temporal cortex (c), and parietal cortex (d). CVRs in the whole cortex and each cortex correlated with RHI.

and 0.901, respectively; $p = 0.012, 0.009, 0.003, 0.003, 0.002, 0.003,$ and 0.002 , respectively). In contrast, there was no correlation between RHI and MMSE, RHI and mRS, CVR and MMSE, CVR and mRS.

Discussion

The present study used IMP-ARG and RH-PAT to demonstrate that vasoreactivity was reduced in both peripheral arteries and in intracranial arteries in patients with CADASIL. Among the various findings, this study showed that the hyperemic response after postdeflation was lower in CADASIL patients than in normal control subjects. Previous studies have documented the utility of Endo-PAT in the assessment of peripheral vasomotor

function [16]. Digital hyperemic response as measured by Endo-PAT may be induced by production of nitric oxide (NO) during occlusion of peripheral artery, since previous studies demonstrated that an endothelial NO synthase inhibitor blunted the increase in digital pulse volume amplitude during reactive hyperemia [24]. The reduction of average RH-PAT index in patients with coronary endothelial dysfunction [25] and the strong relationship between the PAT ratio and multiple vascular risk factors [26] suggests that RH-PAT can accurately detect endothelial dysfunction and assess peripheral vasomotor function. In CADASIL patients, altered endothelial-dependent vasodilatation in cerebral and forearm arteries and higher levels of asymmetrical dimethylarginine, a NO endogenous inhibitor, have been found [12, 13, 27]. These data, in combination with other studies show-

ing a pathological change in VSMCs and endothelial cells in patients with CADASIL, suggest that the reduction of the reactive hyperemic response may be mediated by abnormalities in NO-induced relaxation of VSMCs in patients with CADASIL.

Previous reports using venous occlusive plethysmography demonstrated a reduction of forearm blood flow in CADASIL patients, possibly because of impaired endothelium-dependent vasodilation in resistance arteries. By contrast, the FMD and the pulse wave method did not show any reduction in endothelium-dependent vasodilation in conduit arteries [12]. Further, Gobron et al. [15] reported no alteration in the post-occlusive hyperemic response of CADASIL patients when using a laser Doppler flow meter attached to the finger. However, the delayed response of time to peak and whole duration time in the post-occlusive hyperemia was much larger in CADASIL patients than in normal control subjects. In comparison to ultrasound or laser Doppler techniques, RH-PAT has the advantage of being able to evaluate vascular tone in the peripheral arterial beds quantitatively and accurately after occlusive hyperemia, because the PAT biosensor imparts a uniform sub-diastolic pressure field and because simultaneous recording can exclude the systemic vascular response. Indeed, use of this technique in the present study allowed the characterization of abnormalities in peripheral vasoreactivity in CADASIL patients.

Brulin et al. [28] showed that marked destruction of smooth muscle cells in skin vessels and in brain vessels in patients with CADASIL resulted in a decrease of vessel wall thickness and loss of extracellular matrix area, thereby contributing to vessel wall weakness and hypotonicity of the central and peripheral arteries. These and other findings support the notion that there is dysfunction of arterial tone in the central and peripheral arteries in humans as well as in Tg animals with CADASIL [9, 29]. Indeed, the significant correlation between CVR and RH-

PAT in the present study suggested dysfunction in both the cerebral and peripheral arteries in CADASIL patients and that assessment of central vascular function may be more easily (although indirectly) assessed through characterization of peripheral vascular function in patients with CADASIL.

The present study has several limitations. First, the number of CADASIL patients included in the present study was small, which precluded comprehensive statistical analysis. Second, the spatial resolution and quantification of rCBF using the SPECT scanner was low when compared with positron emission tomography (PET), particularly when employed in the white matter. To improve quantitative evaluation in SPECT study, the present study applied IMP-SPECT with the ARG and the SEE method. Previous studies have demonstrated that rCBF measurements with the IMP-SPECT method correlated well with that obtained by PET [20]. Further, the SEE method of quantifying rCBF within different brain regions can obviate the technical issues or bias associated with arbitrary selection of a specific brain area [21–23]. Third, different methods were used to evaluate vascular reactivity in intracranial arteries and in peripheral arteries. Specifically, peripheral vasoreactivity was evaluated with RH-PAT induced by hyperemia, while intracranial vasoreactivity was evaluated by SPECT with an acetazolamide challenge. Still, previous studies have reported that the average RH-PAT index significantly correlated with the coronary blood flow response to acetylcholine [25, 30], which suggests that Endo-PAT can accurately assess vasoreactivity regardless of the vascular beds or specific stimulus. In conclusion, the present study demonstrated that the vasoreactivity of peripheral and intracranial arteries is reduced in patients with CADASIL. The correlation between the reduction in peripheral and central vasoreactivity suggests that RH-PAT may be a useful non-invasive strategy to predict intracranial vasoreactivity in patients with CADASIL.

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