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ORIGINAL COMMUNICATION

# Primary involvement of neurovascular coupling in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy

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## Abstract

**Background** Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most frequent monogenic cause of cerebral ischemia, but reliable biomarkers to monitor the disease are lacking.

**Aims and objectives** To evaluate cerebral autoregulation (CA), vasoreactivity (VR), and neurovascular coupling (NVC) in CADASIL patients through a battery of dynamic transcranial Doppler tests.

**Methods** We screened our database for all pre-dementia CADASIL cases. We monitored cerebral blood flow velocity (CBFV) with transcranial Doppler, blood pressure, and expiratory carbon dioxide ($CO_2$) non-invasively. CA was assessed by transfer function from the spontaneous oscillations of blood pressure to CBFV, VR with inhalation of $CO_2$ at 5%, and hyperventilation and NVC by the CBFV response to visual stimulation.

**Results** We included 27 CADASIL patients and 20 healthy controls with similar age and sexes. CA and VR were similar between groups. However, NVC was significantly affected in CADASIL patients, with lower magnitudes of CBFV upsurge (overshoot $19 \pm 5$ vs $26 \pm 6$%, $p = 0.013$; gain $12 \pm 7$ vs $17 \pm 5$%, $p = 0.003$) and altered time behavior during visual stimulation (natural frequency $0.18 \pm 0.06$ vs $0.24 \pm 0.06$ Hz, $p = 0.005$; rate time $0.7 \pm 1.7$ vs $2.7 \pm 3.5$ s, $p = 0.025$).

**Conclusion** Our results express a primary and selective involvement of the neurovascular unit in CADASIL rather than a generalized cerebral vasomotor disturbance. Functional cerebrovascular testing could be useful in patient evaluation and monitoring.

**Keywords** Neurovascular coupling · CADASIL · Cerebral blood flow · Vasoreactivity · Small vessel disease · Ultrasound

## Introduction

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most frequent monogenic cause of cerebral ischemia and vascular cognitive deficit, but suitable biomarkers for early management are lacking [4]. CADASIL is caused by mutations in

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the *NOTCH3* gene, which encodes a receptor expressed in pericytes and smooth-muscle cells of cerebral small vessels [4, 10]. Both cells are essential for the regulation of cerebral blood flow [28]. This control encompasses distinct mechanisms, namely, cerebral autoregulation (CA) in response to blood pressure variations, vasoreactivity (VR) to metabolites, and functional hyperemia upholding active neuronal populations, also referred to as neurovascular coupling (NVC) [28].

Microvascular deregulation is an early phenomenon in CADASIL [7, 14] that precedes white matter lesions [14]. This justifies the assessment of cerebrovascular functional status rather than radiological lesions to better monitor the disease activity and progression. The few studies in humans report only opposite or null findings on VR [19, 20, 25] and CA [25]. However, recent data from animal studies focus the primordial impairment on the neurovascular unit [10, 14]. This unit is responsible for NVC and it is composed of astrocytes, pericytes, neurons, and endothelial cells [12]. The first two components were found to be particularly dysfunctional and responsible for the reduced flow activation in cerebral cortex [10, 14].

Transcranial Doppler (TCD) is a non-invasive method used routinely to measure cerebral blood flow velocity (CBFV), but it also yields information about the functional status of downstream vasculature [18, 19, 27]. TCD is a standardized method to measure CA [6], VR [18], and NVC [21]. To date, there are no studies that systematically analyzed the distinct mechanisms of cerebrovascular regulation, particularly NVC.

## Aims

We aimed to study the status of cerebrovascular regulation by dynamic TCD in CADASIL patients.

## Methods

### Population

This study was conducted in Centro Hospitalar Universitário São João (Porto, Portugal). It was approved by the local committee of ethics and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants.

We screened all CADASIL patients ≥ 18 years with confirmed *NOTCH3* mutation and followed-up at our center. Exclusion criteria were dementia, absence of temporal bone window for TCD, or (3) significant cervical or intracranial arterial stenosis. Dementia was diagnosed using the cut-off values of Montreal cognitive assessment (MCA) scores stratified by age and level of education [8]. MoCA is particularly sensitive to vascular cognitive impairment [9] and validated in Portuguese population [8]. In addition, cognitive alterations had to be associated with dependence in activities of daily living as reflected by instrumental activities of daily living score ≤ 6 [15]. Twenty healthy controls with similar age and sexes, without vascular risk factors, were selected within center facilities.

### Clinical evaluation

Participants were characterized by age, gender, medical history, and chronic medication. Systolic and diastolic blood pressure was averaged from three measurements in the sitting position with an oscillometric cuff (Omron M6, Japan). Participants underwent cervical and transcranial Doppler ultrasound examinations (Vivid e, GE, UK) to exclude hemodynamically significant cervical or intracranial arterial stenosis. In patient group, MoCA score was evaluated ahead of instrumentation with the participant alone in a quiet room. The score was used to quantify cognitive deficits and to exclude patients with dementia as detailed above. We also assessed the low- and high-density lipoprotein cholesterol.

### Monitoring protocol

Evaluations were carried out in a dim lighted room, ambient temperature, and supine position. Subjects were refrained from caffeine, alcohol, exercise, or vasoactive drugs for at least 12 h before evaluation. CBFV was recorded in the M1 segment of the right middle cerebral artery (MCA) and the P2 segment of left posterior cerebral artery (PCA), with 2-MHz TCD probes secured with a headframe (Doppler BoxX, DWL, Singen, Germany) [3]. Arterial blood pressure (BP) was recorded with Finometer (FMS, Amsterdam, The Netherlands). Heart rate was assessed with three-lead electrocardiogram. End-tidal carbon dioxide ($EtCO_2$) was recorded with capnography by nasal cannula (Respsense Nonin, Amsterdam, The Netherlands). Data were synchronized and digitized at 400 Hz with Powerlab (AD Instruments, Oxford, UK) and stored for offline analysis. After resting for 20 min, a 5-min period of resting data was stored for calculation of CA indexes. Afterwards, VR and NVC protocols were performed.

### CA calculations

For each heart beat, systolic, diastolic, and mean values of CBFV and BP were calculated in the dedicated software. CA was assessed by transfer function parameters coherence, gain, and phase from beat-to-beat spontaneous oscillations of BP to CBFV in compliance with standard recommendations

[6]: interpolation at 10 Hz; window length of 102 s; Hanning anti-leakage; 50% superposition; and triangular filtering. Lower coherence (correlation coefficient), lower gain (damping of BP oscillations), and higher phase (speed of the autoregulatory response) between oscillations of BP and CBFV indicate more effective CA. Values were reported in very low (VLF: 0.02–0.07 Hz), low (LF: 0.07–0.20 Hz), and high (HF: 0.20–0.50 Hz) frequency bands [6].

## Vasoreactivity protocol

Subjects inspired a gas mixture of 5% $CO_2$ + 95% $O_2$ for 2 min to reach a hypercapnia steady-state $EtCO_2$ level of 7–10 mm Hg above baseline after which they breathed air room again and returned to normocapnia. Finally, they hyperventilated to keep $EtCO_2$ 7–10 mm Hg below baseline. VR is calculated as the slope of the relationship between the average values of $EtCO_2$ plotted against those of relative CBFV achieved at three stages (hypocapnia–normocapnia–hypercapnia). It is expressed as % of the mean CBFV per mm Hg $EtCO_2$ [17]. VR was also calculated separately for hypercapnia and hypocapnia.

## Neurovascular-coupling protocol

NVC was assessed by a visual paradigm that consisted of ten cycles, each with a resting phase of 20 s (eyes closed) and a stimulating phase with a flickering checkerboard at 10 Hz for 40 s [23]. All cycles were synchronized and averaged. Visual stimulation typically evokes a rapid increase in CBFV which overshoots ~ 10 s and then rapidly stabilizes at a steady-state level (example at Fig. 1) [23]. NVC response can be quantified in two ways. First, we obtained the maximum CBFV change to calculate the overshoot parameter as $\frac{\text{maximum CBFV}-\text{baseline CBFV}}{\text{baseline CBFV}} \times 100\%$; systolic and mean CBFV were used [18]. Second, systolic CBFV curve was modeled to describe the dynamics of NVC response in time according to the second-order linear equation $G(s) = \frac{K\times(1+\mathrm{Tv}s)}{\frac{s2}{\omega 2}+2\xi*\frac{s}{\omega}+1}$, where "$K$" stands for "gain", "Tv" for "rate time", "$\omega$" for "natural frequency", and "$\xi$" for "attenuation" [23]. Gain describes the relative CBFV difference between baseline/rest stage and steady-state level during visual stimulation. Rate time indicates the initial steepness of the CBFV increase, natural frequency represents oscillatory properties of the system, and attenuation describes dampening and tonus features, such as the elastic properties of the wall vessel [22].

## Magnetic resonance imaging data

We collected the cerebral magnetic resonance exams (all 1.5 or 3 T machines with 5-mm thickness slices), performed no more than 1.5 years before the TCD monitoring, and analyzed them on a computer screen to quantify the degree of white matter changes (WMC) burden in T2-weighted fluid-attenuated inversion recovery sequence. We used the age-related WMC (ARWMC) scale by which WMC severity is graded from 0 to 3 points in five regions of each hemisphere separately (frontal lobe, parieto-occipital area, temporal lobe, brain stem/cerebellum, and basal ganglia), so that total score ranges from 0 to 30 [26].

## Statistics

Normality of the variables was determined by Shapiro–Wilk test. Baseline characteristics of CADASIL and control groups were compared with Student's $T$ test and $\chi^2$ test. Repeated-measures ANOVA was used to compare the cerebral hemodynamic data by group (CADASIL vs healthy controls) and arterial territory (PCA vs MCA). For NVC parameters (only PCA), we used Student's $T$ test. To test the influence of selected factors on NVC parameters within CADASIL group, we dichotomized it according to the presence of any vascular risk factor (hypertension, diabetes mellitus, or smoking; dyslipidemia was not considered, because almost all were on statins), history of previous stroke/transient ischemic attack (TIA), or by the median value of

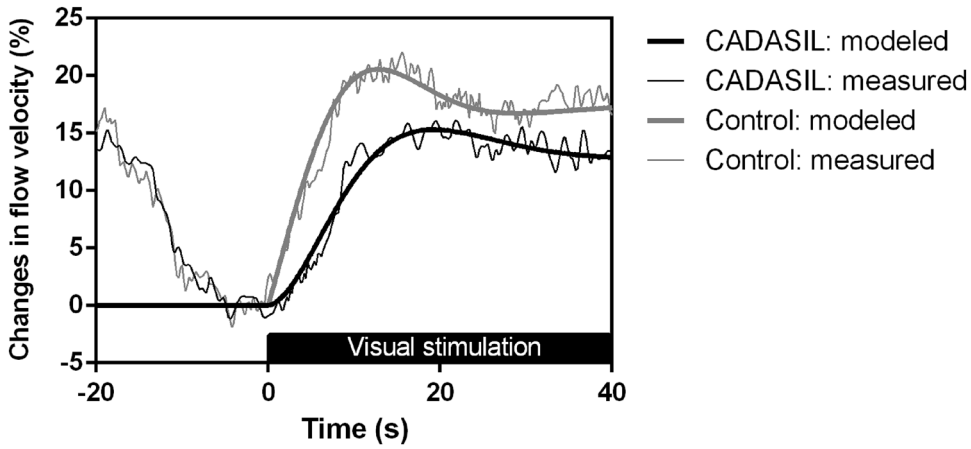


**Fig. 1** Group-averaged evoked systolic CBFV responses during visual stimulation with flickering checkerboard. Healthy controls are represented by grey lines and CADASIL patients by black lines. Thin lines represent measured responses, and thick lines are modeled blood flow data of the second-order linear system

continuous variables (age, ARWHC, and MoCA). We also explored the association between cerebrovascular parameters and regional ARWMH scores related to the monitored vessels (right frontal and left parieto-occipital areas). Subgroups were compared with Student's *T* test.

## Results

We screened 49 CADASIL patients and excluded 18 (death $n=4$; dementia $n=11$; and refused to participate $n=3$). We further excluded four patients, because they fulfilled the criteria for dementia after clinical interview. For the final analysis, we included 27 CADASIL patients concerning 15 pedigrees with mutations in the *NOTCH3* gene-affecting cysteine residues [c.1672C > T (p.Arg558Cys) $n=17$; c.3084G > T (p.Trp1028Cys) $n=4$; c.1258G > T (p.Gly420Cys) $n=3$; c.1819C > T (p.Arg607Cys) $n=2$; c.657_660delTTAC (p.Tyr220Thrfs*15) $n=1$].

There were no significant differences in baseline characteristics between CADASIL and healthy controls (Table 1). There was a previous of ischemic stroke/TIA in $n=12$ (44%) that occurred with a median of 3 years (range 1–9) before the TCD monitoring. Clinically, $n=3$ were partial anterior circulation syndromes (with aphasia) and $n=9$ were of lacunar syndromes (3 pure sensitive; 1 pure motor; 5 sensitive motor). Nevertheless, all strokes had lacunar infarcts at MRI and evidence of small vessel disease.

In Table 2, we compare the results of TCD dynamic tests between CADASIL patients and controls. CBFV, CA, and VR indexes did not differed between groups, either in PCA or MCA territories. However, NVC parameters differ significantly between groups. CADASIL patients showed smaller increase of CBFV (overshoot 19 ± 5 vs 26 ± 6%, $p=0.013$; gain 12 ± 7 vs 17 ± 5%, $p=0.003$) and altered hemodynamics (natural frequency 0.18 ± 0.06 vs 0.24 ± 0.06 Hz, $p=0.005$; rate time 0.7 ± 1.7 vs 2.7 ± 3.5 s, $p=0.025$) during visual stimulation. These differences in magnitude and time behavior of NVC to visual stimulus are depicted in Fig. 1.

In CADASIL patients, subgroup analysis showed that age, presence of a vascular risk factor, previous stroke/TIA, WMC burden, and MoCA scores did not affect NVC results (table of Online Resource 1). In addition, CA, VR, and NVC parameters were not related to the regional WMC burden in the territory of the monitored vessels (Online Resource 2).

**Table 1** Baseline characteristics of CADASIL patients and healthy controls

| Participant characteristics | CADASIL ($n=27$) | Controls ($n=20$) | $p$ value[a] |
|---|---|---|---|
| Male, *n* (%) | 11 (30) | 6 (29) | 0.777 |
| Age, years, (mean ± SD) | 57 ± 13 | 59 ± 16 | 0.536 |
| Hypertension, *n* (%) | 18 (58) | | |
| Diabetes Mellitus, *n* (%) | 4 (15) | | |
| Dyslipidemia, *n* (%) | 24 (89) | | |
| Tobacco, *n* (%) | 1 (3) | | |
| Previous stroke/TIA, *n* (%) | 12 (44) | | |
| Migraine without aura, *n* (%) | 8 (29) | | |
| Migraine with aura, *n* (%) | 3 (11) | | |
| MoCA score, (mean ± SD) | 22 ± 9 | | |
| Depression, *n* (%) | 19 (70) | | |
| Chronic medication, *n* (%) | | | |
| Antiplatelets | 16 (60) | | |
| Statins | 24 (89) | | |
| Antihypertensives | 18 (58) | | |
| Laboratorial results, (mean ± SD) | | | |
| LDL cholesterol, (mg/dL) | 98 ± 33 | | |
| HDL cholesterol, (mg/dL) | 51 ± 13 | | |
| Systemic hemodynamics, (mean ± SD) | | | |
| Systolic BP, (mm Hg) | 123 ± 13 | 117 ± 11 | 0.170 |
| Mean BP, (mm Hg) | 88 ± 20 | 87 ± 9 | 0.892 |
| Diastolic BP, (mm Hg) | 77 ± 9 | 72 ± 9 | 0.091 |
| Heart rate, (bpm) | 69 ± 12 | 72 ± 8 | 0.307 |
| BP spectral power, (mm $Hg^2$) | 4.8 ± 4.4 | 6.5 ± 3.9 | 0.243 |
| $EtCO_2$, (mm Hg) | 36 ± 5 | 37 ± 7 | 0.754 |

*BP* blood pressure, *CADASIL* cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, $EtCO_2$ end-tidal carbon dioxide, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *MoCA* montreal cognitive assessment, *SD* standard deviation, *TIA* transitory ischemic attack

[a]$p$ value of Student's $T$ test or $\chi^2$ test comparing means and proportions, respectively

## Discussion

We compared 27 CADASIL patients to healthy controls and found that NVC was profoundly impaired with reduced magnitude of CBFV increase and altered time behavior during visual stimulation independently of age, vascular risk factors, and white matter lesions. Other regulatory mechanisms, CA and VR, remained preserved.

Our findings indicate a disturbed NVC in the visual cortex of CADASIL patients. There was a decreased peak and gain in CBFV increase during visual stimulation which reflects a less robust functional hyperemia [2, 3, 16]. There were not only magnitude differences, but also changes in time dynamics. The rate time, that is, the initial speed of flow velocity adaptation, was significantly lower in CADASIL leading to an approximately 2-s delayed hemodynamic adaptation [13]. In addition, the lower natural frequency suggests loss of elastic properties of the dysfunctional microvasculature downstream PCA [3, 16]. A disturbed NVC is also known to be present in patients with vascular dementia [16] and migraine attacks [24] which are hallmarks of CADASIL.

**Table 2** Comparison of cerebrovascular regulatory parameters of cerebral autoregulation, vasoreactivity, and neurovascular coupling between CADASIL and healthy control groups

| | CADASIL ($n=27$) | | Controls ($n=20$) | | $p$ value[b] |
|---|---|---|---|---|---|
| | MCA | PCA | MCA | PCA | |
| Cerebral hemodynamics | | | | | |
| Systolic CBFV, (cm/s) | 76 ± 21 | 39 ± 9 | 75 ± 21 | 32 ± 13 | 0.073 |
| Mean CBFV, (cm/s) | 51 ± 15 | 26 ± 7 | 51 ± 17 | 28 ± 9 | 0.135 |
| Diastolic CBFV, (cm/s) | 35 ± 11 | 17 ± 5 | 35 ± 13 | 18 ± 5 | 0.222 |
| MFV spectral power $(cm/s)^2$ | 3.4 ± 2.7 | 2.7 ± 2.3 | 3.2 ± 2.2 | 2.1 ± 1.3 | 0.221 |
| Cerebral autoregulation | | | | | |
| VLF Coherence, (a. u) | 0.42 ± 0.18 | 0.39 ± 0.17 | 0.38 ± 0.16 | 0.36 ± 0.08 | 0.409 |
| LF Coherence, (a. u) | 0.50 ± 0.20 | 0.44 ± 0.20 | 0.59 ± 0.19 | 0.57 ± 0.15 | 0.250 |
| HF Coherence, (a. u) | 0.52 ± 0.17 | 0.43 ± 0.16 | 0.50 ± 0.15 | 0.46 ± 0.18 | 0.121 |
| VLF gain, (%/mm Hg) | 1.13 ± 0.48 | 1.41 ± 0.65 | 0.87 ± 0.39 | 1.01 ± 0.46 | 0.312 |
| LF gain, (%/mm Hg) | 1.35 ± 0.47 | 1.68 ± 0.67 | 1.40 ± 0.47 | 1.68 ± 0.50 | 0.841 |
| HF gain, %/mm Hg | 1.54 ± 0.48 | 1.76 ± 0.56 | 1.73 ± 0.44 | 2.18 ± 0.72 | 0.422 |
| VLF phase, (radians) | 0.92 ± 0.46 | 0.84 ± 0.53 | 1.13 ± 0.27 | 1.12 ± 0.37 | 0.820 |
| LF phase, (radians) | 0.66 ± 0.33 | 0.78 ± 0.35 | 0.75 ± 0.28 | 0.79 ± 0.24 | 0.351 |
| HF phase, (radians) | 0.01 ± 0.27 | 0.02 ± 0.24 | 0.04 ± 0.15 | 0.08 ± 0.15 | 0.709 |
| Cerebral vasoreactivity | | | | | |
| VR–$CO_2$, (%/mm Hg $CO_2$) | 1.7 ± 0.7 | 0.8 ± 0.4 | 1.7 ± 0.5 | 1.0 ± 0.5 | 0.514 |
| VR (hypercapnia only) | 4.1 ± 1.5 | 4.2 ± 2.1 | 4.2 ± 1.6 | 3.6 ± 1.6 | 0.270 |
| VR (hypocapnia only) | 3.8 ± 1.9 | 3.6 ± 1.8 | 3.4 ± 1.0 | 3.6 ± 0.9 | 0.518 |
| Neurovascular coupling | | | | | |
| Overshoot[a] systolic CBFV, (%) | | 19 ± 5 | | 26 ± 6 | 0.013 |
| Overshoot mean CBFV, (%) | | 23 ± 8 | | 32 ± 9 | < 0.001 |
| Modeled parameters | | | | | |
| Gain, (%) | | 12 ± 7 | | 17 ± 5 | 0.003 |
| Natural frequency, (Hz) | | 0.18 ± 0.06 | | 0.24 ± 0.06 | 0.005 |
| Attenuation, (a.u) | | 0.4 ± 0.2 | | 0.5 ± 0.3 | 0.290 |
| Rate time, (s) | | 0.7 ± 1.7 | | 2.7 ± 3.5 | 0.025 |

*a.u.* arbitrary units, *CADASIL* cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, *CBFV* cerebral blood flow velocity, *HF* high frequency (0.2–0.5 Hz), *Hz* Hertz, *LF* low frequency (0.07–0.2 Hz), *MCA* middle cerebral artery, *PCA* posterior cerebral artery, *s* seconds, *SD* standard deviation, *VLF* very low frequency (0.03–0.07 Hz). All values are in means ± SD

[a]Maximal CBFV increase during visual stimulation

[b]$p$ value of repeated-measures ANOVA for the interaction between group variable (CADASIL vs heathy controls) and arterial territory (MCA vs PCA). For NVC, $p$ values were obtained by Student's $T$ test

Interestingly, a similar dysfunctional NVC pattern with less robust gain and reduced oscillatory properties occurs in Fabry disease, another prototype of genetic cerebral small vessel disease characterized by vessel wall-abnormal accumulation, resembling CADASIL pathophysiology [3]. Furthermore, amyloid deposition also causes NVC impairment [2]. Our results are aligned with the recent data from animal studies with CADASIL models, which report primordial deregulation at the neurovascular unit [10, 14]. This is particularly conspicuous in pericytes and astrocytes [10, 14], causing decreased focal flow response to cortical stimulation [14]. At the molecular level, there is a potassium channelopathy-like defect causing microvasculature to vasodilate inadequately during neuronal activity [7]. Therefore, granular osmophilic deposition causes small vessels to be sluggish in response stimuli prior to the development of major obstruction and irreversible ischemic lesion. This early dysfunction could explain the reduced and slow CBFV response during NVC before the onset of dementia in our cohort of CADASIL patients.

A higher grade of white matter lesions does not justify our results, since there was no significant association between NVC parameters and global or regional WMC burden (Online Resources 1 and 2, respectively). More refined methods of assessing cerebral white matter disease (e.g., diffusion tensor imaging) could have been more sensitive to this analysis.

Neural loss may contribute to the impaired NVC in our CADASIL patients, because this cells also participate in the neurovascular unit and there is evidence of the same

disturbances occurring in neurodegenerative disorders such as Alzheimer's disease [11]. Nevertheless, the pathophysiological paradigm of this group of disorders, once thought to be purely neuronal, has been recently changing with the cumulative data, showing microvascular dysfunction in the pre-clinical stage [11]. Subgroup analysis showed no association between NVC parameters and vascular risk factors or previous stroke/TIA (Online Resource 1). However, these comorbidities were highly prevalent in CADASIL group and we cannot exclude some contribution to the findings. Moreover, hypertension and diabetes are significant factors for lacunar stroke recurrence [1], which is frequent in CADASIL.

We found no significant differences in VR and CA, which show that the impaired vascular response during NVC is selective in CADASIL and not a generalized vasomotor dysfunction. Previous studies present divergent results. One study, including 29 CADASIL patients, used TCD to show that these patients have lower VR–$CO_2$ when compared to controls [20]. A recent exploratory study measured VR–$CO_2$ with TCD, yet without controls, and found no relationship with MRI vascular lesions [19]. This latter study also reported that VR measured with arterial spin-labeling MRI, but failed technically in 9 out of 22, which exemplifies the difficulties of this approach. Still, other investigators concluded that there was no impaired $CO_2$–VR when compared to controls [25]. The discrepancies shown in the literature could be due to disease's heterogeneity or methodological differences. For example, Pfefferkorn et al. [20] showed that VR–$CO_2$ tended to approximate the values of healthy controls if only the non-disabled CADASIL patients were selected, as we did. It is possible that, with further diseases' progression, other mechanisms of cerebrovascular regulation are lost besides NVC. The only study concerning CA showed no significant impairment [25]. In our study, we confirmed that CA seems to be preserved in CADASIL with currently standardized methods [6].

Concerning the fact that CADASIL is a rare disease, the small number of enrolled subjects underpowered the statistical analysis of our results particularly regarding the subgroups.

In addition, considering the fact that CADASIL affects heterogeneously the small vessels in brain [4], MRI would be the logical choice for studying cerebral microvasculature because of its spatial resolution. However, VR protocols are more prone to failure [19], expensive, and not standardized as TCD [18]. Functional MRI can assess NVC through the magnitude of the blood-oxygen-level-dependent signal, a similar hemodynamic surrogate for neuronal activity on which functional TCD is based [5]. Cheema et al. [5] studied five CADASIL patients with functional MRI and found that visual cortex response to the stimulus was unchanged or higher than controls. The small number of cases or disease's heterogeneity could explain the inconsistence in results. Moreover, our cohort has more vascular risk factors which could contribute to NVC impairment. TCD has the advantage of extraordinary time resolution (~5 ms) for studying the time behavior of CBFV activation in downstream cortical microvasculature (Fig. 1). Although the cognitive performance of CADASIL patients in this study was low, considering the MoCA scores, these main within normal range as validated in the Portuguese population [8, 9].

## Conclusion

Our results express a primary and selective involvement of the neurovascular unit in CADASIL rather than a generalized vasomotor disturbance. Functional cerebrovascular testing could be useful in patient evaluation and monitoring.

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### Compliance with ethical standards

**Conflicts of interest** The authors declare that there is no conflict of interest.

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