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Unraveling the genetic architecture of migraine: exploring the vascular components

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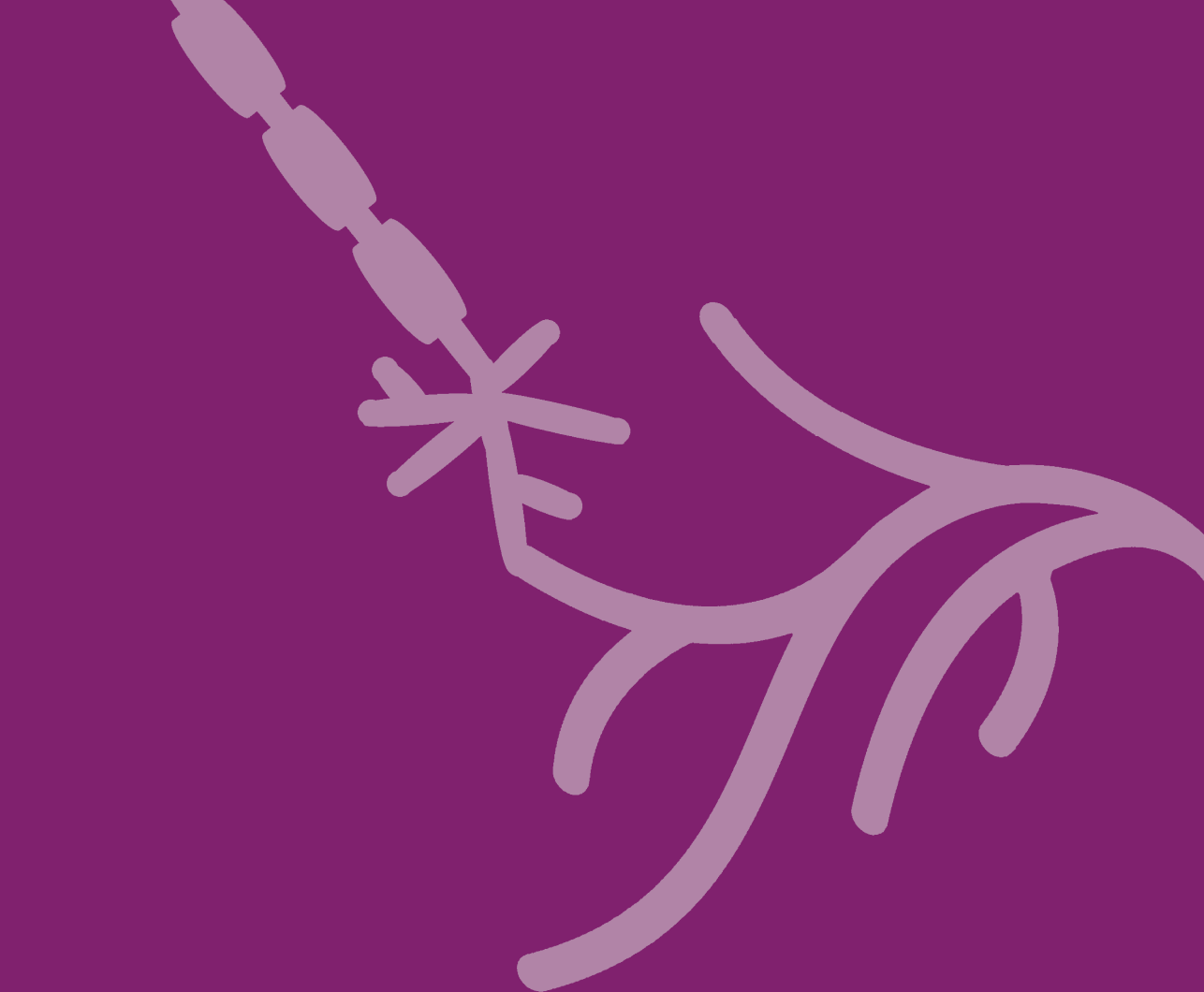
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CHAPTER 7

RVCL-S and CADASIL display distinct impaired vascular function

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Abstract

Objective: We aimed to evaluate the role of endothelial-dependent and endothelial-independent vascular reactivity in Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S) and Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), two cerebral small vessel diseases that are considered models for stroke, vascular dementia and migraine.

Methods: RVCL-S (n=18) and CADASIL (n=23) participants with *TREX1* and *NOTCH3* mutations, respectively, were compared with age-, BMI- and sex-matched controls (n=26). Endothelial function was evaluated by flow-mediated vasodilatation and endothelial-independent vascular reactivity (i.e. vascular smooth muscle cell function) was assessed by dermal blood flow response to capsaicin application.

Results: Flow-mediated vasodilatation was decreased in RVCL-S participants compared with controls (2.32 ± 3.83 vs. 5.76 ± 3.07 % change in diameter, $p=0.023$), but normal in CADASIL participants. Vascular smooth muscle cell function was reduced in CADASIL participants compared with controls (maximal dermal blood flow increase at 40 minutes after capsaicin: 1.38 ± 0.88 vs. 2.22 ± 1.20 Arbitrary Units, $p=0.010$), but normal in RVCL-S participants.

Conclusions: We identified endothelial dysfunction in RVCL-S and confirmed impaired vascular smooth muscle cell relaxation in CADASIL. Our findings may prove to be biomarkers for disease progression in both monogenic cerebral small vessel diseases and improve mechanistic insight in their pathophysiology. This may help to understand common neurovascular disorders, including stroke, dementia, and migraine.

Introduction

Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S) and Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) are monogenic small vessel diseases with overlapping clinical features, including white matter lesions and stroke. Migraine and depressive symptoms are often presenting symptoms and in many patients apathy and premature cognitive decline are found.¹⁻⁷ Because of clinical similarities these monogenic syndromes may serve as a model for common vascular disorders, including stroke, vascular dementia and migraine.

In CADASIL degeneration of vascular smooth muscle cells (VSMCs) with morphologically normal endothelium is observed.⁵ In contrast, in RVCL-S muscle cell degeneration is minimal but electron microscopy shows irregular thickening of basement membranes, whose formation is dependent on endothelial cells.⁷ ⁸ These morphological abnormalities supposedly affect the dilatation capability of blood vessels, which may lead to hypoxia. Studies in CADASIL showed reduced baseline cerebral blood flow and impaired hemodynamic reserve with an impaired vasodilation response to acetazolamide.⁹⁻¹¹ However, it is unclear whether this reduced vascular reactivity is caused by an endothelium-independent or -dependent mechanism.¹²⁻¹⁶ In RVCL-S, functional vascular properties have not been studied. Furthermore, in RVCL-S there is systemic involvement of highly vascularized organs and in CADASIL involvement of peripheral blood vessels has been demonstrated by electron microscopy and staining of NOTCH3.^{1,5} Therefore, we investigated whether RVCL-S and CADASIL participants have impaired vascular reactivity and whether this is mediated by endothelial dysfunction or by a VSMCs-mediated mechanism in the peripheral blood vessels.

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Methods

Protocol approvals and patient consents

The study was approved by the Ethics Committee of the Leiden University Medical Center (LUMC) and conducted in accordance with the Declaration of Helsinki. All participants were at least eighteen years of age and gave written informed consent.

Participants

RVCL-S and CADASIL participants with a confirmed pathogenic gene mutation in *TREX1* or *NOTCH3*, respectively, were recruited. Controls were recruited through the

families (friends or spouses, not related individuals) and public advertisements. An investigator, different from the operator performing the vascular measurements, obtained medical history and genetic and clinical information. In order to prevent un-blinding, participants were instructed not to reveal their mutation status or medical history to the operator. Patients were age-, BMI- and sex-matched to controls.

Clinical assessment

Participants completed a questionnaire with variables on medical history including migraine, Raynaud's phenomenon, retinopathy and other medical conditions. Additional questions related to current medication, alcohol, caffeine, smoking and drugs intake were asked. During the interview preceding the measurements, diagnoses of migraine and Raynaud's phenomenon were verified, according to established criteria.^{17,18} Medication was subdivided in the following categories: anti-hypertensive, cholesterol-lowering, anti-platelet, anti-coagulant, prophylactic migraine medication, analgesics and oral contraceptive. If possible, participants included in the study, were asked to abstain from medication with vascular (side-)effects for one week prior to the measurements. Participants were instructed to abstain from alcohol and caffeine (for 12 hours) and smoking and food (for 6 hours). This was verified during the interview. Weight and height were measured; BMI calculated.

Biochemical measurements

Laboratory parameters (Table 1) were determined according to standard procedures.

Experimental design

One operator, blinded for the participants' status, performed all vascular measurements. Prior to the vascular measurements, participants rested in supine position for 15 minutes in a room with recorded stable ambient temperature ($22 \pm 1^\circ\text{C}$). Resting blood pressure and pulse rate were recorded at the right upper arm using a validated semi-automated oscillometric device (OMRON 705IT, OMRON Healthcare, Hoofddorp, The Netherlands). Median blood pressure and heart rate of three measurements were analyzed. Subsequently, flow-mediated dilatation (FMD) and capsaicin-induced dermal blood flow (DBF) were measured in a standardized order.

Flow-mediated dilatation (endothelial function of conduit artery)

FMD of the brachial artery was performed to assess endothelium-dependent vasodilatation according to guidelines¹⁹ using an echo-tracking system (Wall Track System, Pie Medical, Maastricht, The Netherlands). The system consists of an ultrasound device (Esaote AU5, Esaote Biomedical, Genoa, Italy) connected to a

data acquisition and processing unit. Measurements were performed as described earlier.²⁰ In brief, right brachial artery diameter was measured 5-10 cm proximal to the antecubital crease in a longitudinal plane. At baseline, brachial artery diameter and arterial blood velocity profiles were recorded three times; the mean was used for data analysis. After baseline measurements, a cuff (TMC7, D.E. Hokanson, Bellevue, WA, USA), placed around the forearm, was inflated to 220 mm Hg to temporarily occlude the arterial perfusion. The cuff was released after 5 minutes with recording of the peak blood velocity profile within the first 15 seconds. Brachial artery diameter was measured for 4 seconds at 0.5, 0.75, 1, 1.25, 1.5, 2-5 minutes after cuff release. FMD was expressed as maximal absolute and percentage increase in diameter from baseline. The FMD was corrected for hyperemic stimulus using peak shear rate ($=\text{peak flow velocity}/\text{baseline diameter}$) and for blood viscosity using peak shear stress ($=\text{peak shear rate} \times \text{hematocrit}$), by dividing the FMD by the peak shear rate and shear stress, respectively.

After 15 minutes of recovery, 400 μg sublingual nitroglycerin (NTG) was administered as an exogenous NO-donor and every minute for 6 minutes NTG-induced brachial artery dilatation was assessed. Endothelium-independent vasodilatation capacity after administration of NTG was expressed as percentage increase in diameter from baseline. After one week we asked participants whether a migraine attack was provoked by NTG.

Capsaicin-induced dermal blood flow (microcirculation in the skin)

Local application of capsaicin results in binding to the Transient Receptor Potential Vanilloid type I (TRPV1) receptor, which is present at primary sensory neurons ($A\delta$ - and C-fiber nociceptors). TRPV1 receptor binding of capsaicin induces a neurogenic inflammatory response due to predominant release of calcitonin gene-related peptide (CGRP).²¹ CGRP is a very potent vasodilator causing a local increase in DBF, which can be quantified by Laser Doppler Perfusion Imaging (LDPI) (PeriScan PIM II®; Perimed, Järfälla, Sweden). Reproducibility of this test was confirmed earlier.²² After 20 minutes of supine rest the baseline DBF was measured. Participants received single topical doses of 1000 μg per 20 μL capsaicin (in ethanol/polysorbate 20/water) in two 10 mm rubber 'O'-rings on the volar surface of one forearm. In two rings on the opposite arm, vehicle was applied; DBF was measured at 10, 20, 30 and 40 minutes after capsaicin application. Almost half of the CADASIL group was measured on a differently calibrated LDPI scanner, for which a separate control group was recruited. Results are expressed as Arbitrary Units (AU) presented as change versus baseline in DBF after capsaicin application.

Statistical analysis

Continuous variables were described by mean $\pm$ standard deviation (SD) and discrete variables are presented as counts (percentages) unless indicated otherwise. The Shapiro-Wilk test was used to determine if the data was normally distributed. General characteristics of each group were compared using a one-way analysis of variance (ANOVA) for normally distributed continuous variables (Kruskal-Wallis test for non-normally distributed variables) and the Chi-square test for categorical variables. The results from the FMD were compared using the independent sample T-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Mean increase in DBF after capsaicin (40 minutes) and mean area under the curve over 40 minutes (AUC_{0-40}) were compared using the one way analysis of covariance (ANCOVA) to take into account the use of two scanners. For all analyses a two sided p-value of $\alpha < 0.05$ was considered statistical significant. All statistical analyses were performed using SPSS 23.0 software (IBM Corp., Armonk, NY, USA).

Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Participants

Eighteen RVCL-S and 23 CADASIL participants were compared with 26 matched controls. In Table 1 demographic details, clinical symptoms, and laboratory values are summarized. All tests were well-tolerated. No participant reported an NTG provoked migraine attack. Abstaining from alcohol, caffeine, smoking and food was successful, except for smoking in the CADASIL group (78% abstinence; five participants smoked <6 hours prior to measurements), abstinence from alcohol in the RVCL-S group (94% abstinence; one participant used 2 units alcohol <10 hours) and caffeine in the CADASIL group (96% abstinence; one participant used 1 unit coffee <6 hours). Success for abstaining from medication is depicted in Table 1. Not all participants could be included for all measurements. No significant differences for age, sex, BMI and blood pressure were found between included participants and those excluded.

Table 1. Participants demographics

Variable	Controls (n=26)	CADASIL (n=23)	RVCL-S (n=18)	p-value
Age (years)	49.0 ± 9.1	49.5 ± 12.7	50.1 ± 9.6	0.94*
BMI (kg/m ²)	25.2 ± 3.6	26.7 ± 4.3	25.2 ± 3.7	0.17**
Systolic blood pressure (mm Hg)	129.2 ± 20.5	126.2 ± 11.1	132.8 ± 21.7	0.85**
Diastolic blood pressure (mm Hg)	77.8 ± 10.1	75.4 ± 6.7	75.3 ± 8.8	0.41**
Female, n (%)	14 (54)	11 (48)	10 (56)	0.87***
Smoking (Pack years)	3.0 ± 5.0 ^a	13.4 ± 16.0	3.7 ± 5.8	0.029**
Migraine, n (%)	0 (0)	6 (27) ^b	6 (33)	N.A.
Retinopathy, n (%)	0 (0)	0 (0)	9 (56) ^c	N.A.
Raynaud's phenomenon, n (%)	0 (0)	3 (13)	15 (83)	
Chronic medication use, n (%) ^d	5 (19)	17 (74)	12 (67)	N.A.
Oral contraceptive use (% of women)	3 (21)	1 (9)	0 (0)	
Anti-hypertensive drugs	0 (0)	6 (26) (1)	6 (33) (2)	
Cholesterol lowering medication	0 (0)	9 (39) (2)	6 (33) (2)	
Antiplatelet drugs	0 (0)	13 (57) (3)	8 (44) (4)	
Prophylactic migraine medication	0 (0)	1 (4)	0 (0)	
Analgesics (acetaminophen, NSAIDs) ^e	1 (4)	2 (9)	5 (28)	
Other (%) ^f	1 (4) (1)	7 (30) (1)	10 (56) (3)	
Laboratory parameters				
Hb (g/dL)	8.99 ± 0.71	9.15 ± 0.73	8.10 ± 0.65	<0.001*
Ht (%)	0.43 ± 0.03	0.44 ± 0.04	0.40 ± 0.04	0.001*

Hb = hemoglobin; Ht = hematocrit; N.A. = not applicable. Values presented are means ± SD or number (percentage). P-values calculated using * the one-way ANOVA, ** the Kruskal-Wallis test, *** the Chi-square test. ^aFor one participant pack years was unavailable. ^bFor one participant migraine was unclear. ^cRetinopathy status for two participants unknown. ^dNumber of participants that abstained from medication 7 days prior to the measurements are depicted in italic. No participant used oral anticoagulants. ^eAll participants used these painkillers for acute migraine treatment, none of them used acetaminophen or NSAIDs 24 hours prior to measurements. ^fOther medication: glucose lowering drugs, stomach acid inhibitors, alifuzosin, solifenacin, gabapentin, carbamazepine, pyridoxine, folic acid, valproic acid, alfalcidol, allopurinol, dorzolamide/timolol and dexamethasone eye drops, baclofen, antihistamine drugs, ursodeoxycholic acid, lynestrenol, levothyroxine, atovaquon, darbepoetin alfa.

Flow-mediated dilatation

FMD was performed in 18 RVCL-S, 14 CADASIL participants and 18 controls but a valid set could only be obtained in 10 participants of each disease group and in 14 controls. When a valid set could not be obtained this was due to technical difficulties with ECG-gating (6) and movement artefacts (10). RVCL-S participants showed a lower FMD compared with controls (2.32 ± 3.83 vs. 5.76 ± 3.07 , $p=0.023$) (Figure 1).

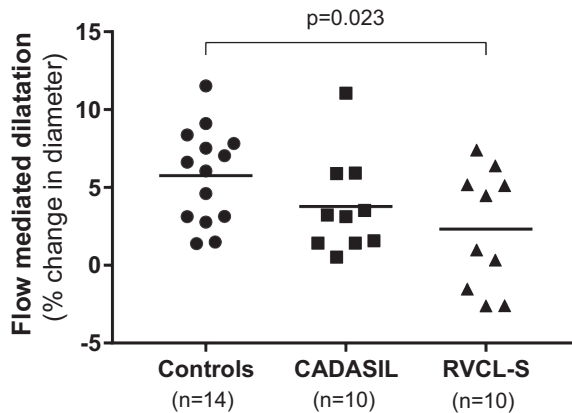


Figure 1. Flow-mediated dilatation expressed as the percentage change in brachial artery diameter after hyperemic stimulus in RVCL-S and CADASIL participants and controls.

Table 2. Vascular parameters of the brachial artery

Variable	Controls (n=14)	CADASIL (n=10)	<i>p</i> -value ^a	RVCL-S (n=10)	<i>p</i> -value ^b
Pre-occlusion diameter (μm)	5655 ± 1014	5934 ± 839	0.48*	6438 ± 1103	0.09*
Maximal post-occlusion diameter (μm)	5987 ± 1109	6150 ± 828	0.70*	6602 ± 1252	0.22*
FMD (absolute increase in diameter) (μm)	332 ± 187	216 ± 169	0.13*	165 ± 256	0.08*
Pre-occlusion velocity (cm/s)	11.7 ± 4.1	9.5 ± 3.4	0.20**	13.4 ± 6.3	0.76***
Maximal post-occlusion velocity (cm/s)	82.4 ± 24.9	81.7 ± 20.5	0.94*	88.5 ± 21.4	0.56*
Peak shear rate (s ⁻¹)	140.6 ± 33.7	138.4 ± 35.7	1.00**	141.4 ± 34.6	0.94**
Peak shear rate normalized FMD (*10 ⁻³) (%.s)	4.35 ± 2.31	2.99 ± 2.33	0.20*	1.32 ± 2.91	0.015*
Peak shear stress (s ⁻¹)	61.6 ± 16.2	59.6 ± 14.6	0.78*	56.3 ± 15.3	0.46*
Peak shear stress normalized FMD (*10 ⁻³) (%.s)	10.07 ± 5.73	6.96 ± 5.58	0.23*	3.31 ± 7.24	0.027*
Pre-NTG diameter (μm)	5710 ± 844	6198 ± 1019	0.21*	6190 ± 1034	0.22*
Maximal post-NTG diameter (μm)	6496 ± 899	6947 ± 891	0.18**	6980 ± 1193	0.29**
Post-NTG (% change in diameter)	14.13 ± 6.71	12.72 ± 6.61	0.62*	12.99 ± 7.39	0.70*

NTG = nitroglycerin, FMD = flow-mediated dilatation. Data are presented as mean ± SD. *P*-values calculated using * the independent sample T-test, ** the Mann-Whitney U test. Due to missing peak shear rate and stress the corrected FMD could not be calculated in 2 controls and in 1 participant of each disease group.

^a Difference between controls and CADASIL participants and ^b controls and RVCL-S participants.

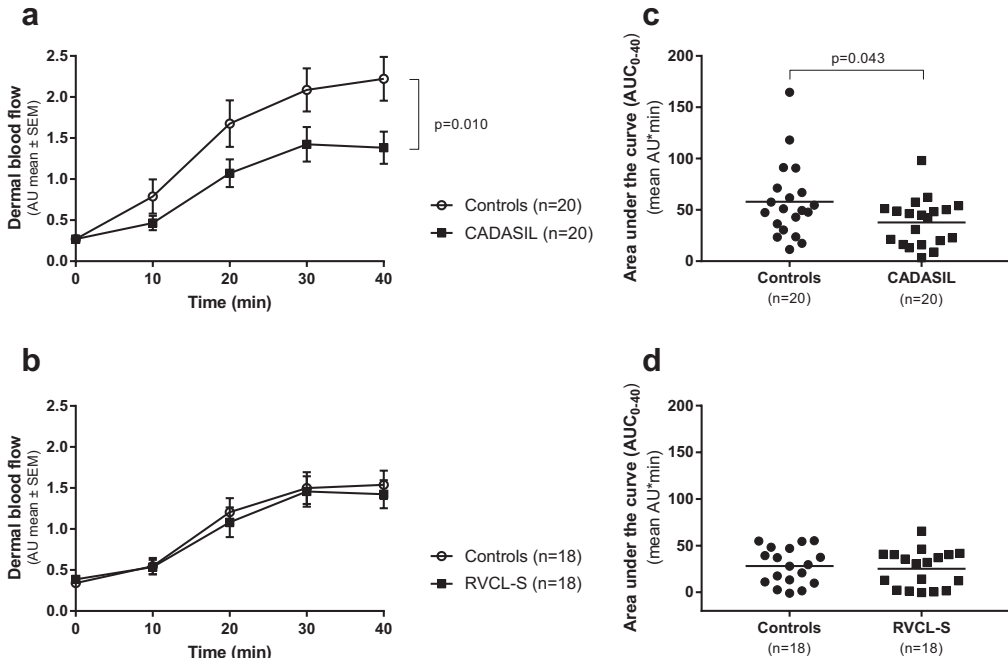


Figure 2. Microvascular reactivity of the skin after topical capsaicin application in CADASIL and RVCL-S participants.

Results shown as mean $\pm$ SEM. DBF increase over 40 minutes after capsaicin application in CADASIL (**A**) and RVCL-S (**B**) participants. Total DBF response over a period of 40 minutes after capsaicin application, displayed as AUC₀₋₄₀ in CADASIL (**C**) and RVCL-S (**D**) participants. The measurements of (**A**) and (**C**) were conducted using two different scanners.

No significant difference in FMD was observed between CADASIL participants and controls (3.77 ± 3.15 vs. 5.76 ± 3.07 , $p=0.14$). The decreased FMD in RVCL-S persisted after correction for peak shear rate ($p=0.015$) and peak shear stress ($p=0.027$) (Table 2). There was no difference between RVCL-S and CADASIL participants and controls in FMD after the admission of nitroglycerin (Table 2).

Capsaicin-induced dermal blood flow

Capsaicin-induced DBF measurements were performed in 18 RVCL-S participants, 20 CADASIL participants and equal numbers of matching controls. Three participants were excluded because of skin allergy to alcohol containing solutions, colored skin and prophylactic anti-migraine medication (pizotifen). Results are summarized in Figure 2. CADASIL participants compared with controls displayed a lower increase in DBF 40 minutes after capsaicin application (1.38 ± 0.88 vs. 2.22 ± 1.20 AU, $p=0.010$) and a lower AUC₀₋₄₀ (37.8 ± 23.0 vs. 57.9 ± 36.6 AU.min, $p=0.043$). No significant differences were found between RVCL-S participants and controls.

Discussion

This study provides evidence for endothelial dysfunction in RVCL-S patients. In addition, an impaired endothelial-independent vascular reactivity in the microvasculature is confirmed in CADASIL patients.

RVCL-S is caused by heterozygous C-terminal frameshift mutations in the *TREX1* gene⁷, which encodes the major 3'-5' DNA exonuclease.²³ How *TREX1* mutations exactly lead to RVCL-S remains an enigma. *TREX1* mutations that cause RVCL-S preserve the enzymatic function of the protein but alter its intracellular localization because the C-terminus that anchors to the endoplasmic reticulum is absent. RVCL-S mutations are hypothesized to lead to a toxic gain-of-function that primarily affects the microvasculature through a mechanism that remains undetermined. Endothelial dysfunction might be such a mechanism. Mutated *TREX1* protein may *directly* have detrimental effects on endothelial cells and thereby cause further pathological changes causing clinical symptoms. Alternatively, endothelial dysfunction may be a *consequence* of the pathological changes seen in RVCL-S. Regardless, clinical symptoms that occur in RVCL-S patients, i.e. cerebral infarction²⁴, Raynaud's phenomenon²⁵, migraine²⁰ and subcortical cognitive dysfunction (executive function, processing speed)²⁶, all have been linked to endothelial dysfunction. It should be noted that we did not measure endothelial function at the level of resistance arteries. Consequently, we are unable to determine whether endothelial dysfunction in RVCL-S can be extrapolated to small resistance vessels, but this seems likely. Iontophoresis or venous occlusion plethysmography with acetylcholine would be of interest as a follow-up study to further explore this. Only minimal degeneration of VSMCs is seen in RVCL-S, which is in accordance with findings of normal VSMC function in RVCL-S in our study.

CADASIL is caused by heterozygous missense mutations (that change the number of cysteine residues) in the *NOTCH3* gene⁶, which encodes a cell surface receptor that is expressed on VSMCs. *NOTCH3* is involved in a signal transduction pathway critical for development, homeostasis and differentiation of VSMCs.²⁷ A decreased DBF response to capsaicin was observed in CADASIL participants. Since capsaicin activates pre-synaptic TRPV1 receptors on A δ - and C-fiber nociceptors and leads to relaxation of VSMCs of the skin microvasculature upon CGRP release, a decreased DBF could result from defects at different levels in this pathway: i) less sensitivity of TRPV1 receptors to capsaicin; ii) impairment of CGRP release from nociceptors; iii) a decreased expression of functional TRPV1/CGRP-receptors; or iv) a decreased relaxation of VSMCs in response to an endothelium-independent stimulus. Further

studies are required to elucidate the mechanism behind the decreased DBF response observed in CADASIL participants. Given the morphological abnormalities seen in the microvasculature of CADASIL patients, degeneration of VSMCs and a morphologically normal endothelium⁵, a decreased relaxation of VSMCs in response to an endothelium-independent stimulus seems most plausible. Thus, our results suggest impaired endothelium-independent VSMC relaxation in CADASIL, and are in line with previous studies showing: i) impaired microvascular reactivity using other techniques without the involvement of CGRP;¹³⁻¹⁶ ii) reduced baseline cerebral blood flow; and iii) impaired hemodynamic reserve measured as vasodilation response to acetazolamide.⁹⁻¹¹

By measuring FMD at the brachial artery, we did not find evidence for endothelial dysfunction in CADASIL, nor did we find differences in endothelium-independent relaxation of the brachial artery after NTG administration. As such we found no evidence of altered vascular function of conduit vessels, in this case the brachial artery in CADASIL participants. This confirms earlier findings and is consistent with the belief that small arteries are most affected.^{13,14} However, in contrast to our findings one study suggested endothelial dysfunction based on L-arginine-induced vasoreactivity of the middle cerebral artery in CADASIL.¹⁵ There is, however, a methodological concern as that study may be biased by baseline differences between CADASIL and controls. Finally, in the microcirculation, forearm *resistance* arteries, impaired endothelium-dependent vasodilatation was reported.¹⁴ Therefore, although we find no evidence for endothelial dysfunction in conduit arteries, our study is limited by not investigating endothelial function in the microvasculature, as there is evidence for dysfunction of endothelium in the microcirculation of CADASIL patients.¹⁴

The strengths of our study are: i) assessment of vascular reactivity with complementary measurements of endothelial and smooth muscle cell function; ii) investigation of an unique sample of the small vessel disorders RVCL-S and CADASIL that can be considered monogenic models for vascular cerebral disorders in general; iii) use of well-validated techniques^{20,22}; and iv) performance of the investigations by one person who was blinded for the health status of the study participants. There are also limitations to our study. Because CADASIL and RVCL-S are rare diseases, sample sizes are relatively small. As a consequence we chose to focus on demonstrating a difference in response to capsaicin or nitroglycerin. Now that a difference in capsaicin-induced dermal blood flow has been demonstrated in CADASIL, it may be interesting to investigate whether there are differences in the dose-response relationship between CADASIL patients and controls. Additionally, not all measurements could be performed in all participants, but there were no differences between included and

excluded participants. Furthermore, due to the different scanners used in the FMD measurements we used two different control groups for the FMD measurements. We did however correct for the effect of the two scanners used. Whereas the effect of hematocrit was corrected via peak shear stress, this was not done for hemoglobin. Hemoglobin absorbs NO, a lower hemoglobin results in an increased FMD.²⁸ However, RVCL-S participants display slightly lower hemoglobin and thus correction would lead to an even lower FMD in RVCL-S. The slightly (not significant) reduced FMD observed in CADASIL might be the result of the mismatch in cigarette pack years, although only two heavy smokers dramatically influenced the number of pack years. For clinical reasons not all participants were permitted to stop their medication.

The increased prevalence of migraine in both CADASIL and RVCL-S might be related to the altered vascular properties found in the present and previous studies. Vascular dysfunction such as endothelial dysfunction²⁰ and increased risk for ischemic brain lesions has been linked with migraine, making this hypothesis more amendable.²⁹⁻³¹ In CADASIL altered vasoreactivity of the microcirculation may induce ischemia which may trigger cortical spreading depression (CSD), the underlying mechanism of the migraine aura.³² In line with this hypothesis, transgenic CADASIL *Notch3* mice show a decreased threshold for CSD.³³ In contrast to CADASIL, RVCL-S patients seem to have mainly migraine without aura. An increased susceptibility for endothelial dysfunction supposedly contributes to the development of migraine without aura in RVCL-S. No migraine attacks were induced by NTG administration, possibly, due to the lower dose nitroglycerin used or sublingual administration.³⁴

Future studies need to confirm our findings of endothelial dysfunction in RVCL-S and investigate endothelial dysfunction in microvasculature to further unravel pathophysiological mechanisms behind the distinct vascular impairment in RVCL-S and CADASIL and determine if our findings can be translated to the cerebral circulation. Additionally, further studies may link these vascular functional markers to disease progression and to the individual symptoms of these syndromes, such as stroke, dementia and migraine.

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References

1. Stam AH, Kothari PH, Shaikh A, et al. Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations. *Brain*. 2016;139:2909-22.
2. Grand MG, Kaine J, Fulling K, et al. Cerebroretinal vasculopathy. A new hereditary syndrome. *Ophthalmology*. 1988;95:649-59.
3. Jen J, Cohen AH, Yue Q, et al. Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS). *Neurology*. 1997;49:1322-30.
4. Terwindt GM, Haan J, Ophoff RA, et al. Clinical and genetic analysis of a large Dutch family with autosomal dominant vascular retinopathy, migraine and Raynaud's phenomenon. *Brain*. 1998;121:303-16.
5. Chabriat H., Joutel A, Dichgans M, Tournier-Lasserre E, Boussier MG. Cadasil. *Lancet Neurol*. 2009;8:643-53.
6. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996;383:707-10.
7. Richards A, van den Maagdenberg AM, Jen JC, et al. C-terminal truncations in human 3'-5' DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy. *Nat Genet*. 2007;39:1068-70.
8. Davis GE, Norden PR, Bowers WL. Molecular control of capillary morphogenesis and maturation by recognition and remodeling of the extracellular matrix: functional roles of endothelial cells and pericytes in health and disease. *Connect Tissue Res*. 2015;56:392-402.
9. Chabriat H, Pappata S, Ostergaard L, et al. Cerebral hemodynamics in CADASIL before and after acetazolamide challenge assessed with MRI bolus tracking. *Stroke*. 2000;31:1904-12.
10. Lacombe P, Oligo C, Domenga V, Tournier-Lasserre E, Joutel A. Impaired cerebral vasoreactivity in a transgenic mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy arteriopathy. *Stroke*. 2005;36:1053-58.
11. Liem MK, Lesnik Oberstein SA, Haan J, et al. Cerebrovascular reactivity is a main determinant of white matter hyperintensity progression in CADASIL. *AJNR Am J Neuroradiol*. 2009;30:1244-47.
12. Pfefferkorn T, von Stuckrad-Barre S, Herzog J, Gasser T, Hamann GF, Dichgans M. Reduced cerebrovascular CO(2) reactivity in CADASIL: A transcranial Doppler sonography study. *Stroke*. 2009;32:17-21.
13. Gobron C, Vahedi K, Vicaut E, et al. Characteristic features of in vivo skin microvascular reactivity in CADASIL. *J Cereb Blood Flow Metab*. 2007;27:250-7.
14. Stenborg A, Kalimo H, Viitanen M, Terent A, Lind L. Impaired endothelial function of forearm resistance arteries in CADASIL patients. *Stroke*. 2007;38:2692-7.
15. Peters N, Freilinger T, Opherk C, Pfefferkorn T, Dichgans M. Enhanced L-arginine-induced vasoreactivity suggests endothelial dysfunction in CADASIL. *J Neurol*. 2008;255:1203-8.
16. Campolo J, De Maria R, Frontali M, et al. Impaired vasoreactivity in mildly disabled CADASIL patients. *J Neurol Neurosurg Psychiatry*. 2012;83:268-74.
17. Headache classification subcommittee of the international headache society. The international classification of headache disorders. 3rd edition (beta version). *Cephalalgia*. 2013;33:629-808.
18. Miller D, Waters DD, Warnica W, Szlachet J, Kreeft J, Theroux P. Is variant angina the coronary manifestation of a generalized vasospastic disorder? *N Engl J Med*. 1981;304:763-6.
19. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257-65.
20. Vanmolkot FH, van Bortel HLM, de Hoon JN. Altered arterial function in migraine of recent onset. *Neurology*. 2007;68:1563-70.
21. Van der Schueren BJ, Rogiers A, Vanmolkot FH, et al. Calcitonin gene-related peptide8-37 antagonizes capsaicin-induced vasodilation in the skin: evaluation of a human in vivo pharmacodynamic model. *J Pharmacol Exp Ther*. 2008;325:248-55.
22. Van der Schueren BJ, de Hoon JN, Vanmolkot FH, et al. Reproducibility of the capsaicin-induced dermal blood flow response as assessed by laser Doppler perfusion imaging. *Br J Clin Pharmacol*. 2007;64:580-90.

23. Volkman HE, Stetson DB. The enemy within: endogenous retroelements and autoimmune disease. *Nat.Immunol.* 2014;15:415-22.
24. Stevenson SF, Doubal FN, Shuler K, Wardlaw JM. A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. *Stroke.* 2010;41:434-42.
25. Herrick AL. Pathogenesis of Raynaud's phenomenon. *Rheumatology (Oxford).* 2005;44:587-96.
26. Heringa SM, van den Berg E, Reijmer YD, et al. Markers of low-grade inflammation and endothelial dysfunction are related to reduced information processing speed and executive functioning in an older population-the Hoorn Study. *Psychoneuroendocrinology.* 2014;40:108-18.
27. Joutel A, Andreux F, Gaulis S, et al. The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients. *J Clin Invest.* 2000;105:597-605.
28. Meyer C, Heiss C, Drexhage C, et al. Hemodialysis-induced release of hemoglobin limits nitric oxide bioavailability and impairs vascular function. *J Am Coll Cardiol.* 2010;55:454-9.
29. Kruit MC, van Buchem MA, Hofman PA, et al. Migraine as a risk factor for subclinical brain lesions. *Jama.* 2004;291:427-34.
30. Kurth T, Chabriat H, Bousser MG. Migraine and stroke: a complex association with clinical implications. *Lancet Neurol.* 2012;11:92-100.
31. Palm-Meinders IH, Koppen H, Terwindt GM, et al. Structural brain changes in migraine. *Jama.* 2012;308:1889-97.
32. Lauritzen M. Pathophysiology of the migraine aura: the spreading depression theory. *Brain.* 1994;117:199-210.
33. Eikermann-Haerter K, Yuzawa I, Dilekoz E, Joutel A, Moskowitz MA, Ayata C. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome mutations increase susceptibility to spreading depression. *Ann Neurol.* 2011;69:413-8.
34. Sances G, Tassorelli C, Pucci E, Ghiotto N, Sandrini G, Nappi G. Reliability of the nitroglycerin provocative test in the diagnosis of neurovascular headaches. *Cephalalgia.* 2004 Feb;24(2):110-9.