Monogenic models of migraine : from clinical phenotypes to pathophysiological mechanisms

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Chapter 9.

Circulating endothelial markers in retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations

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Stroke 2017;48:3301–3307

Abstract

Background and Purpose: Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S) is a monogenic small vessel disease, caused by C-terminal truncating TREX1 mutations, that can be considered a model for stroke and vascular dementia. The pathophysiology of RVCL-S is largely unknown, but systemic endothelial involvement has been suggested, leading to pathology in the brain and other highly vascularized organs. Here, we investigated circulating endothelial markers to confirm endothelial involvement and identify biomarkers for disease activity.

Methods: We measured circulating levels of von Willebrand factor (VWF) antigen, VWF propeptide, and angiopoietin-2 in members of 3 Dutch RVCL-S families and matched unrelated healthy controls. Stratified analyses based on symptomatology and age were performed.

Results: We found elevated levels of VWF antigen, VWF propeptide, and angiopoietin-2 in TREX1 mutation carriers (n=31) compared with family members without a TREX1 mutation (n=33) and unrelated healthy controls (n=31; Kruskal–Wallis test P<0.001 for all comparisons). Effects were most pronounced in mutation carriers with clinical manifestations aged ≥40 years (Mann–Whitney U test P<0.001 for all comparisons). Compared with healthy controls, levels of VWF antigen (P=0.02) and angiopoietin-2 (P=0.04) were also elevated in mutation carriers aged <40 years. All 3 markers showed moderate correlations with markers of kidney and liver disease and inflammation (ie, systemic symptoms of RVCL-S).

Conclusions: Our results confirm an important role of the endothelium in RVCL-S pathophysiology. VWF antigen, VWF propeptide, and angiopoietin-2 might serve as early biomarkers of disease activity. Our findings might also help to understand the pathophysiology of common neurovascular disorders, such as stroke.

Key Words: angiopoietin-2, endothelium, leukoencephalopathies, mutation, von Willebrand factor

Introduction

Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S) is an autosomal dominant cerebral and systemic small vessel disease caused by C-terminal truncating mutations in the *TREX1* (3 prime repair exonuclease 1) gene.[1,2] The disease is characterized by brain white matter lesions, intracerebral pseudotumors, vascular retinopathy, Raynaud phenomenon, migraine, and disease of internal organs, such as kidneys and liver. There is no treatment available to halt or cure the disease. Toward middle-age, symptoms progress rapidly, leading to premature death. It has been hypothesized that small vessels and likely their endothelium, typically in highly vascularized organs, are affected in RVCL-S, as evidenced by ultrastructural pathological studies that revealed thicker endothelial cells with increased vesicles and coarse cytoplasm and thicker multilaminated basement membranes of endothelial cells.[2]

Currently, there is no biomarker for RVCL-S predicting clinical onset or progression of the disease. Increased circulating levels of von Willebrand factor (VWF) were reported in patients with hereditary systemic angiopathy[3] and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[4] which both have clinical features that resemble some of those of RVCL-S. VWF is considered a reliable circulating marker of endothelial dysfunction, and its level is increased after endothelial damage and during acute phase responses.[5] VWF propeptide (VWFpp) is cleaved from immature VWF during post-translational modification. On secretion, mature VWF and VWFpp are released in equimolar amounts into the blood but cleared independently. Mature VWF, measured in blood as VWF antigen (VWF:Ag), has a much longer half-life than VWFpp.[6] Ratios between VWF:Ag and VWFpp can therefore differentiate between chronic and acute endothelial activation.[7] Angiopoietin-2 (Ang-2) is a possible biomarker that has also been associated with diseases with endothelial damage or activation.[8] Like VWF, Ang-2 is stored in Weibel–Palade bodies in endothelium and released after endothelial activation, thereby inducing inflammation.[9,10] Ang-2 promotes the dissociation of pericytes from endothelial cells by negatively interfering with angiopoietin-1–mediated Tie-2 signaling, resulting in destabilization of the capillary network and loss of microvascular integrity.[11,12]

In this study, we investigated circulating levels of VWF and Ang-2 to further confirm endothelial involvement in RVCL-S and to identify biomarkers for disease activity and clinical progression.

Methods

Participants

Participants of this study were included from the RVCL-ID study (Identifying Biomarkers and Disease Stages of RVCL-S), a cross-sectional observational study for which *TREX1* mutation carriers were recruited from the Leiden University Medical Center Neurology outpatient clinic or previous studies. All known *TREX1* mutation carriers from the 3 known (unrelated) Dutch RVCL-S families and their first- and second-degree family members (all ≥18 years of age) were invited. As many mutation carriers as possible were included as well as a comparable number of first- and second-degree family members without a *TREX1* mutation. Unrelated healthy individuals were included as a second control group and matched for age and sex with the *TREX1* mutation carriers. Exclusion criteria for this group were hypertension, diabetes mellitus, coronary disease, thrombotic disease, peripheral or other major artery disease, kidney or liver disease, hematologic conditions, systemic lupus erythematosus, Raynaud phenomenon, transient ischemic attack or stroke, migraine or other primary headache syndromes, chronic neurological disorders, or current malignancy. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all participants provided written informed consent. All data are available through the corresponding author on reasonable request.

Demographic and Clinical Characteristics

All participants were interviewed to collect information about medical history and lifestyle habits, such as smoking and alcohol use, that are known to influence the levels of VWF and Ang-2.[13–15] Average systolic and diastolic blood pressures were calculated from blood pressures measured at 2 study visits. Hypertension was defined as (1) use of antihypertensive medication; (2) systolic blood pressure >140 mm Hg; or (3) diastolic blood pressure >90 mm Hg.[16] Height and weight were measured to calculate body mass index. Subjects also underwent an extensive neurological examination and were asked to participate in a brain magnetic resonance imaging scan as part of the study.

Sample Collection and Laboratory Assays

Venous blood samples were collected during morning hours under fasting conditions.

Genomic DNA was extracted from peripheral leucocytes from EDTA blood according to standard protocols. For assessing the presence of the *TREX1* mutation in family members with unknown status, a genetic test was performed in our research laboratory using direct Sanger sequencing, as

described before.[1] Standard assays of total blood count, glucose, hemoglobin A1c, estimated glomerular filtration rate (using the Chronic Kidney Disease Epidemiology Collaboration equation), γ-glutamyl transferase, erythrocyte sedimentation rate, cholesterol spectrum, and ABO blood type, which is of major influence on VWF levels,[17] were performed at the hospital's clinical diagnostic laboratory, immediately after sampling. Urine samples were collected to assess albuminuria.

Blood samples were immediately centrifuged and stored at −80°C. VWF:Ag was measured according to standard diagnostic laboratory protocols in citrated plasma samples by ELISA using rabbit polyclonal antihuman VWF antibodies. VWFpp was measured in microtiter wells that were coated with antibody CLB-Pro 35 (Sanquin, Amsterdam, the Netherlands) overnight at 4°C, blocked with 1% bovine serum albumin at room temperature for 2 hours, and subsequently incubated with diluted citrated plasma samples. Wells were washed, and VWFpp was detected with peroxidase-conjugated antibody CLB-Pro 14.3 coupled to peroxidase (Sanquin). Measurements were performed in duplicate with samples diluted to 2 different concentrations. Normal pooled plasma calibrated against the World Health Organization 6th international standard for factor VIII/VWF (07/316)[18] was used as standard. Ang-2 levels in serum were determined by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer supplied protocol.

Statistical Analysis

Categorical variables were compared using χ2 tests. We compared continuous variables using nonparametric tests (ie, Mann–Whitney *U* tests and Kruskal–Wallis tests). Bivariate correlations were assessed by calculating Spearman rho (ρ) correlation coefficients. Analyses were stratified for age where scatter plots appeared to show different outcomes for different age categories. To adjust for multiple comparisons (n=≈50), $P<0.001$ was considered as statistically significant. All statistics were performed using SPSS 23.0 (IBM Corp, Armonk, NY).

Results

Included Population

A total of 103 members (aged ≥18 years) of the 3 known Dutch RVCL-S families were invited, including 29 known carriers of a *TREX1* mutation (either p.Val235fs or p.Leu287fs), 33 known nonmutation carriers, and 41 family members of unknown genetic status. Twenty-one known *TREX1* mutation carriers were included. Genetic testing in the family members of unknown genetic status identified 10 further *TREX1* mutation carriers, so a total of 31 *TREX1* mutation carriers (13 men, 18 women; median age 52 years) participated in the study (Table 1). After genetic testing, it was clear

Table 5: Demographic and clinical characteristics of RVCL-S family members with and without a *TREX1* mutation.

	RVCL-S families *TREX1* mutation present (n=31)	**RVCL-S families *TREX1* mutation absent** (n=33)	**Unrelated healthy controls** (n=31)	***P* value**
Age, y				
Median (range)	52 (20–64)	45 (23–73)	49 (22–62)	*P*=0.90*
≥40 y, n (%)	19 (61%)	25 (76%)	20 (65%)	
Sex				
Male, n (%)	13 (42%)	14 (42%)	13 (43%)	*P* >0.99†
≥40 y, n (%)	6 (32%)	11 (44%)	6 (30%)	
Pedigree				
A: p.Val235fs, n (%)	20 (65%)	29 (88%)	-	*P* =0.06†
B: p.Val235fs, n (%)	6 (19%)	1 (3%)	-	
C: p.Leu287fs, n (%)	5 (16%)	3 (9%)	-	
Vascular retinopathy‡, n (%)	22 (71%)	0 (0%)	N.A.	-
Features of focal and/or global brain dysfunction, n (%)	12 (39%)	4 (12%)	N.A.	*P* =0.014†
T2 hyperintense white matter lesions on brain MRI||, mL, median (range)	2 (0–35)	1 (0–7)	N.A.	*P* =0.445§
γ-GT, U/L, median (range)	40 (8–448)	25 (9–86)	N.A.	*P* =0.004§
eGFR, mL/min/1.73m^2, median (range)	84 (24–125)	95 (69–132)	94 (84–101)	*P* =0.253§
Albumin (urine), mg/L, median (range)	31 (3–569)	4 (3–33)	N.A.	*P* <0.001§
ESR, mm/hour, median (range)	19 (2–58)	2 (2–31)	N.A.	*P* <0.001§
Anemia, n (%)	10 (32%)	2 (6%)	N.A.	*P* =0.007†
Hypertension, n (%)	13 (42%)	11 (33%)	N.A.	*P* =0.440†
Migraine with/without aura, n (%)	8 (26%)	13 (39%)	N.A.	*P* =0.247†
Raynaud's phenomenon, n (%)	13 (42%)	5 (15%)	N.A.	*P* =0.017†

Controls were selected based on absence of RVCL-S related symptoms, only kidney function was assessed to ensure gadolinium contrast could be safely administered. γ-GT indicates γ-glutamyl transferase; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; MRI, magnetic resonance imaging; N.A., not applicable; and RVCL-S, retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations. *Kruskal–Wallis test. †χ^2 test. ‡All 22 *TREX1* mutation carriers investigated by an ophthalmologist had vascular retinopathy, and the remaining 9 *TREX1* mutation carriers were not investigated. §Mann–Whitney *U* test. ||Brain MRIs were available for n=29 *TREX1* mutation carriers and n=25 family members without *TREX1* mutation.

that 33 nonmutation carriers (14 men, 19 women; median age 45 years) had been included, which was considered sufficient to serve as a control group. In addition, 31 unrelated age- and sex-matched healthy controls were recruited as an extra control group (13 men, 18 women; median age 49 years). The median age and sex distribution did not differ between the 3 groups. Clinical characteristics of all included *TREX1* mutation carriers and the nonmutation carriers, including all symptoms incorporated in the diagnostic criteria of RVCL-S,[2] are summarized in Table 1.

The 33 family members who did not participate in the study were younger (median age 34 years, ranging from 23 to 71 years) and consisted of relatively more male family members (n=25/33; 76% men) than the included population.

Circulating Levels of VWF:Ag, VWFpp, and Ang-2

Median levels of VWF:Ag (2.55 IU/mL) and VWFpp (1.75 IU/mL) were found to be elevated in *TREX1* mutation carriers compared with family members without a *TREX1* mutation (1.02 and 0.93 IU/mL, respectively) and unrelated healthy controls (0.92 and 1.08 IU/mL, respectively; *P*<0.001 for both control groups; Figures 1 and 2; Table 2). VWF:Ag and VWFpp levels were especially elevated in those of ≥40 years of age (medians 2.75 and 1.90 IU/mL, respectively), from which age most symptoms of RVCL-S generally become clinically evident.[2] VWF:Ag levels differed between mutation carriers of <40 years of age and either family members without a *TREX1* mutation or healthy controls (*P*=0.02 for both control groups), but the difference was statistically significant only in *TREX1* mutation carriers aged ≥40 years (*P*<0.001 for both control groups). Levels of VWFpp differed from control groups only for mutation carriers aged ≥40 years (*P*<0.001 for both control groups). Also median Ang-2 levels were markedly increased in *TREX1* mutation carriers (2515 pg/mL) compared with family members without *TREX1* mutation (1530 pg/mL) and unrelated healthy controls (1420 pg/mL; *P*<0.001 for both control groups; Table 2). Again, effects were more pronounced for *TREX1* mutation carriers aged ≥40 years (median 3144 pg/mL) although also increased in *TREX1* mutation carriers aged <40 years (median 1905 pg/mL). No differences were found for levels of VWF:Ag, VWFpp, or Ang-2 when comparing family members without a *TREX1* mutation and unrelated healthy controls, regardless of age.

Correlations Between Circulating Levels of VWF:Ag, VWFpp, and Ang-2 and Systemic Symptoms of RVCL-S

To assess how the biomarkers relate with symptoms of RVCL-S, correlations with estimated glomerular filtration rate, urine albumin, γ-glutamyl transferase, and erythrocyte sedimentation rate levels were calculated because these hallmarks of systemic disease are expressed as continuous variables. Although correlations were moderate for all groups of participants (Spearman ρ's ≈0.5), correlations were stronger within *TREX1* mutation carriers alone (Spearman ρ's ≈0.6; Tables I and II in the online-only Data Supplement). Correlation between VWF:Ag and VWFpp levels was strong (ρ=0.682; *P*<0.001) in all groups but stronger in *TREX1* mutation carriers alone (ρ=0.770; *P*<0.001). Levels of Ang-2 also correlated with both VWF:Ag (ρ=0.707; *P*<0.001) and VWFpp levels (ρ=0.712; *P*<0.001) in *TREX1* mutation carriers (Tables I and II in the online-only Data Supplement).

The 4 patients with RVCL-S (2 sibling pairs) aged >50 years, in whom VWF:Ag levels were within normal range, were also among the 6 patients with RVCL-S >50 years with the lowest Ang-2 levels. This finding is of clinical relevance because these subjects had relatively mild symptoms compared with other *TREX1* mutation carriers in their age category and may have had less active disease in the past as well.

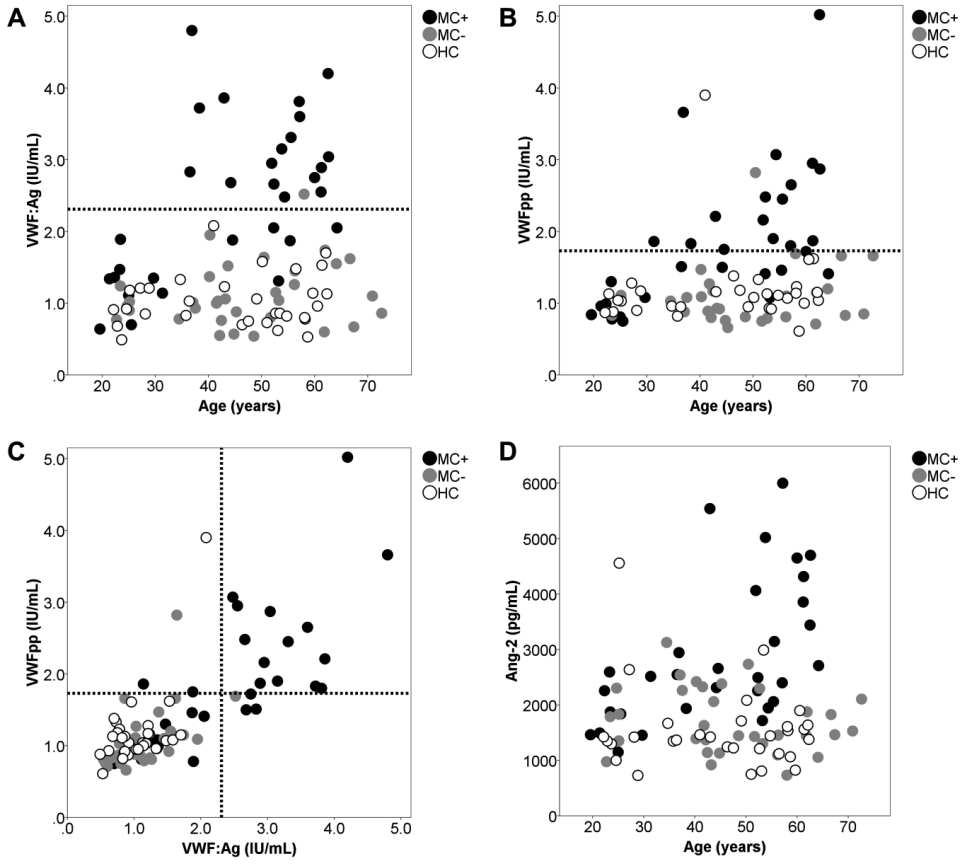

Figure 1. Distribution of circulating levels of von Willebrand factor antigen (VWF:Ag; IU/mL; **A**), von Willebrand factor propeptide (VWFpp; IU/mL; **B**), and angiopoietin-2 (Ang-2; pg/mL; **D**) with age in the 3 groups of participants. **C**, Correlations between VWFpp and VWF:Ag. Black circles/MC+: *TREX1* mutation carriers; gray circles/MC−: family members without a *TREX1* mutation; white circles/HC: unrelated healthy controls.

Table 2: Von Willebrand Factor antigen (VWF:Ag), propeptide (VWFpp) and angiopoietine-2 (Ang-2) circulating levels in RVCL-S.

	RVCL-S families *TREX1* mutation present (n=31)	**RVCL-S families *TREX1* mutation absent** (n=33)	**Unrelated healthy controls** (n=31)	***P* value all groups**	***P* value MC+ vs MC-**	***P* value MC+ vs HC**	***P* value MC- vs HC**
VWF:Ag, IU/mL							
Total, median (IQR)	2.55 (1.36–3.15)	1.02 (0.79–1.41)	0.92 (0.78–1.21)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.47†
<40 y, median (IQR)	1.36 (1.12–2.60)	0.95 (0.81–1.02)	0.92 (0.83–1.21)	*P*=0.02*	*P*=0.02†	*P*=0.02†	*P*=0.87†
≥40 y, median (IQR)	2.75 (2.05–3.31)	1.06 (0.78–1.54)	0.91 (0.76–1.42)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.51†
VWFpp, IU/mL							
Total, median (IQR)	1.75 (1.08–2.45)	0.93 (0.82–1.16)	1.08 (0.95–1.18)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.09†
<40 y, median (IQR)	1.04 (0.82–1.75)	1.04 (0.87–1.09)	0.96 (0.88–1.13)	*P*=0.85*	*P*=0.73†	*P*=0.57†	*P*=0.93†
≥40 y, median (IQR)	1.90 (1.50–2.65)	0.92 (0.81–1.24)	1.14 (1.01–1.31)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.11†
VWFpp/VWF:Ag ratio							
Total, median (IQR)	0.74 (0.63–0.93)	1.02 (0.75–1.24)	1.06 (0.92–1.57)	*P*<0.001*	*P*=0.01†	*P*<0.001†	*P*=0.10†
<40 y, median (IQR)	0.75 (0.58–1.02)	1.10 (0.92–1.20)	0.99 (0.92–1.13)	*P*=0.06*	*P*=0.06†	*P*=0.04†	*P*=0.51†
≥40 y, median (IQR)	0.74 (0.63–0.93)	0.89 (0.72–1.29)	1.23 (0.90–1.58)	*P*=0.002*	*P*=0.04†	*P*<0.001†	*P*=0.05†
Ang-2, pg/mL							
Total, median (IQR)	2515 (1936–3856)	1530 (1327–2280)	1420 (1211–1637)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.09†
<40 y, median (IQR)	1905 (1471–2539)	2047 (1461–2481)	1366 (1298–1668)	*P*=0.12*	*P*=0.88†	*P*=0.04†	*P*=0.16†
≥40 y, median (IQR)	3144 (2312–4649)	1455 (1222–2083)	1433 (1143–1630)	*P*<0.001*	*P*<0.001†	*P*<0.001†	*P*=0.33†

Ang-2 indicates angiopoietine-2; IQR, interquartile range; RVCL-S, retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations; VWF:Ag, Von Willebrand factor antigen; and VWFpp, Von Willebrand factor propeptide.

*Kruskal–Wallis test.

†Mann–Whitney *U* test.

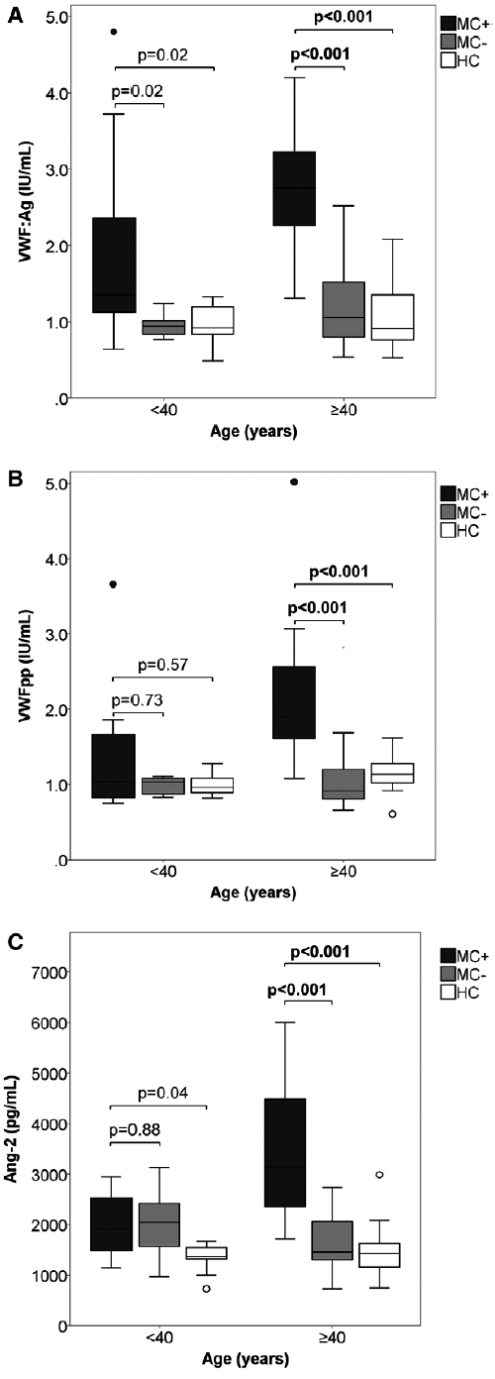

Figure 2. Box plots of circulating levels of von Willebrand factor antigen (VWF:Ag; IU/mL; **A**), von Willebrand factor propeptide (VWFpp; IU/mL; **B**), and angiopoietin-2 (Ang-2; pg/mL; **C**) in the 3 groups of participants divided in age groups of <40 and ≥40 years. Boxes show interquartile ranges (IQR; 25% percentile, median, and 75% percentile); top and bottom whiskers indicate maximum and minimum values, respectively. White circles indicate mild outliers (>1.5×IQR), gray asterisks indicate extreme outliers (>3×IQR). Dark gray/MC+: *TREX1* mutation carriers; light gray/ MC−: family members without *TREX1* mutation; white/HC: unrelated healthy controls.

Vascular Risk Factors Associated With Circulating Levels of VWF and Ang-2

As seen in previous studies,[17] type O blood type was associated with lower VWF levels (data not shown). Blood types (A, B, AB, and O) were equally distributed among all 3 groups of participants (data not shown). Other factors that may have influenced VWF and Ang-2 levels include age, sex, diabetes mellitus, hypertension, hypercholesterolemia, smoking, and alcohol use.[13–15] There were no significant differences for any of these factors between groups (data not shown). Besides endothelial cells, platelets may also be a source of VWF:Ag and VWFpp. There was no difference in platelet counts between mutation and nonmutation carriers (medians 212.0×10^9/L and 223.0×10^9/L, respectively, P=0.08); these counts were not available for the unrelated healthy controls.

Discussion

Levels of circulating endothelial markers VWF:Ag, VWFpp, and Ang-2 were higher in *TREX1* mutation carriers compared with either family members without a mutation or unrelated healthy controls. The fact that the levels of both VWF:Ag and VWFpp are increased in RVCL-S —with VWF:Ag slightly more elevated as shown by the decreased VWFpp/VWF:Ag ratio—suggests a chronic endothelial activation in patients with RVCL-S.[7] By demonstrating similar effects for Ang-2, we confirmed endothelial involvement in RVCL-S. Notably, levels of these markers were mostly increased from ≈40 years onwards when clinical symptoms are known to clinically manifest.[2] We did not find clear linear correlations between the endothelial markers and the patients' age, but ≈40 years, a threshold seems to be surpassed, after which levels vary within the same range until final stages of the disease. We hypothesize that the markers indicate disease activity (associated with clinical exacerbations) and not accumulated damage because of the disease. The levels of all 3 markers correlated with systemic symptoms of RVCL-S, including kidney and liver disease and increased erythrocyte sedimentation rate, indicating that the markers are increased in symptomatic patients but do not show strong correlations, which would be the case if the levels of the endothelial markers linearly increased with the progression of symptoms. Interestingly, levels of VWF:Ag and Ang-2 were also increased in *TREX1* mutation carriers aged <40 years albeit less pronounced. In these younger individuals, organ function is mostly unaffected, and vascular damage is thus expected to be less. The markers therefore seem to truly indicate (early) disease activity rather than secondary vascular damage.

Both VWF:Ag and VWFpp levels did not correlate with platelet counts, which suggests that the increased circulating levels of VWF:Ag and VWFpp mainly originate from endothelium and that release from platelets did not contribute much to the observed differences. This hypothesis is supported by the additional finding of increased levels of Ang-2, which also originates from Weibel–Palade bodies in the endothelium and not platelets.[10]

The systemic symptoms of RVCL-S include liver disease.[2] VWF is mainly metabolized by the liver, and VWF:Ag values of similar magnitude or higher were found in patients with liver cirrhosis.[19] Therefore, theoretically, the decreased VWFpp/VWF:Ag ratio in *TREX1* mutation carriers could have been because of decreased clearance of VWF:Ag, but liver disease was generally mild.[7] Moreover, the clearly increased VWFpp levels indicate that the increased VWF:Ag levels are not caused by decreased clearance alone and that endothelial activation is involved.[7]

Diabetes mellitus is also associated with increased levels of both VWF and Ang-2,[15,20] and VWF is considered a predictor of (cardio)vascular events in patients.[21] Similarly, Ang-2 has been associated with diabetic retinopathy[22] and nephropathy[23,24] but also with myocardial infarction.[25] Recently, an increase in VWF (Ang-2 was not investigated), although less pronounced, was reported for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[4] an autosomal dominant small vessel disease caused by mutations in the *NOTCH3* gene with expression in smooth muscle cells.[26] All these results suggest that VWF and Ang-2 are general markers of (micro)vascular damage and are not specific to RVCL-S. Vice versa, RVCL-S may serve as a model to study neurovascular disease, such as stroke and vascular dementia, and systemic small vessel diseases.

A limitation of our study is the relatively small sample size. For such a rare disease, however, the sample sizes can be considered large and enabled finding significant differences. A strength of our study is that we were able to exclude other factors that might have influenced VWF and Ang-2 levels, such as sex, age, blood pressure, diabetes mellitus, hypercholesterolemia, smoking and alcohol use, ABO blood type, and platelet count.[13,15] In addition, the control group of family members without a *TREX1* mutation is of great significance for the interpretation of our results. First, the lifestyle habits and physical environment of these subjects are likely comparable to those of the patients with RVCL-S. Second, the fact that levels of VWF and Ang-2 were not increased in the nonmutation carrier control group supports a causal relationship with *TREX1* mutations specifically, instead of other (non)genetic factors shared by members of the RVCL-S families. Third, in contrast to the unrelated healthy controls, vascular disease, such as hypertension or coronary disease, occurred in the group without a *TREX1* mutation. This shows that effects in patients with RVCL-S cannot be explained by common vascular disease unrelated to *TREX1* mutations.

Besides biomarkers of disease activity, VWF and Ang-2 may also be therapeutic targets in RVCL-S. Anti-VWF agents have been developed although for clinical purposes (eg, in thrombotic thrombocytopenic purpura) somewhat dissimilar to RVCL-S.[27] Counteracting effects of Ang-2 has been studied extensively in diseases (eg, diabetes mellitus) that bare more resemblance to RVCL-S.[8] Either a chimeric form of its antagonist angiopoietin-1, which has anti-inflammatory effects through the Tie-2 receptor,[28] other Tie-2 agonists,[29] blockers of Ang-2, or inhibition of Ang-2 signaling all have shown promising results that may be of use in treating RVCL-S.[8]

Conclusions

In conclusion, circulating levels of the endothelial markers VWF:Ag, VWFpp, and Ang-2 are elevated in patients with RVCL-S. These results confirm that the endothelium plays an important role in RVCL-S pathophysiology and that VWF:Ag and Ang-2 may serve as early biomarkers of disease activity. Future studies have to show if these markers also predict clinical progression of RVCL-S and may constitute therapeutic targets. This may be of importance not only for the rare disorder RVCL-S but also for systemic disorders with underlying endotheliopathy, such as stroke, vascular dementia, migraine, Raynaud phenomenon, and internal organ disorders.

Acknowledgments: We thank R.J. Dirven (Department of Internal Medicine, Section Thrombosis and Haemostasis and Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands) and C.J.J. van Rijn (Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands) for their technical assistance. We thank Prof Dr J.J. Voorberg (Sanquin, Amsterdam, the Netherlands) for providing the antibodies CLB-Pro 35 and CLB-Pro 14.3 for measurement of von Willebrand factor propeptide.

Sources of Funding: This work was supported by grants of the Netherlands Organization for Scientific Research (NWO; VIDI no. 91711319 to Dr Terwindt), the Center for Medical Systems Biology (CMSB) established in the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (NGI/NWO; CMSB no. 050-060-409 to Dr van den Maagdenberg), and the European Community (EC; FP7-EUROHEADPAIN no. 602633 to Drs van den Maagdenberg, Ferrari, and Terwindt and FP7-NIMBL no. 241779 to Dr van den Maagdenberg). They had no role in the design or conduct of the study.

Disclosures: Dr van Zonneveld reports grants from the Dutch Heart Foundation, Netherlands Organization for Scientific Research (NWO), the Dutch Kidney Foundation, and the European Foundation for the Study of Diabetes. Dr Ferrari reports grants and consultancy or industry support from Medtronic and independent support from the European Community, NWO, National Institute of Health, and the Dutch Heart Foundation. Dr van den Maagdenberg reports independent support from NWO and the European Community. Dr Eikenboom reports independent support from Landsteiner Foundation for Blood Transfusion Research and research support from CSL Behring and honorarium for educational activities from Roche. Dr Terwindt reports independent support from NWO, European Community, the Dutch Heart Foundation, and the Dutch Brain Foundation. The other authors report no conflicts.

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SUPPLEMENTAL MATERIAL

Pelzer *et al.*, Circulating endothelial markers in retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations

Supplemental Table I: Spearman's correlation coefficients for total population.

	VWF:Ag (IU/mL)		**VWFpp** (IU/mL)		**Ang-2** (pg/mL)	
	Spearman's rho	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**
VWF:Ag (IU/mL)	-	-	0.682	p<0.001	0.583	**p<0.001**
VWFpp (IU/mL)	0.682	**p<0.001**	-	-	0.465	**p<0.001**
Ang-2 (pg/mL)	0.583	**p<0.001**	0.465	p<0.001	-	-
eGFR (mL/min/1.73m^2)	-0.355	**p<0.001**	-0.489	p<0.001	-0.282	p=0.006
Albumin (urine) (mg/L)	0.482	**p<0.001**	0.450	p<0.001	0.495	**p<0.001**
γ-GT (U/L)	0.512	**p<0.001**	0.519	p<0.001	0.596	**p<0.001**
ESR (mm/hour)	0.599	**p<0.001**	0.534	p<0.001	0.571	**p<0.001**

VWF:Ag= von Willebrand Factor antigen, VWFpp= von Willebrand Factor propetide, Ang-2= Angiopoietin-2, eGFR= estimated glomerular filtration rate (using the Chronic Kidney Disease Epidemiology Collaboration/ CKD-EPI equation), γ-GT= γ-glutamyl transferase, ESR= erythrocyte sedimentation rate.

Supplemental Table II: Spearman's correlation coefficients for RVCL-S patients.

	VWF:Ag (IU/mL)		**VWFpp** (IU/mL)		**Ang-2** (pg/mL)	
	Spearman's rho	**p-value**	**Spearman's rho**	**p-value**	**Spearman's rho**	**p-value**
VWF:Ag (IU/mL)	-	-	0.770	p<0.001	0.707	**p<0.001**
VWFpp (IU/mL)	0.770	**p<0.001**	-	-	0.712	**p<0.001**
Ang-2 (pg/mL)	0.707	**p<0.001**	0.712	p<0.001	-	-
eGFR (mL/min/1.73m^2)	-0.614	**p<0.001**	-0.668	p<0.001	-0.794	**p<0.001**
Albumin (urine) (mg/L)	0.474	**p=0.007**	0.450	p=0.011	0.365	p=0.043
γ-GT (U/L)	0.632	**p<0.001**	0.659	p<0.001	0.768	**p<0.001**
ESR (mm/hour)	0.600	**p<0.001**	0.461	p=0.009	0.625	**p<0.001**

VWF:Ag= von Willebrand Factor antigen, VWFpp= von Willebrand Factor propetide, Ang-2= Angiopoietin-2, eGFR= estimated glomerular filtration rate (using the Chronic Kidney Disease Epidemiology Collaboration/ CKD-EPI equation), γ-GT= γ-glutamyl transferase, ESR= erythrocyte sedimentation rate