Unraveling the genetic architecture of migraine: exploring the vascular components

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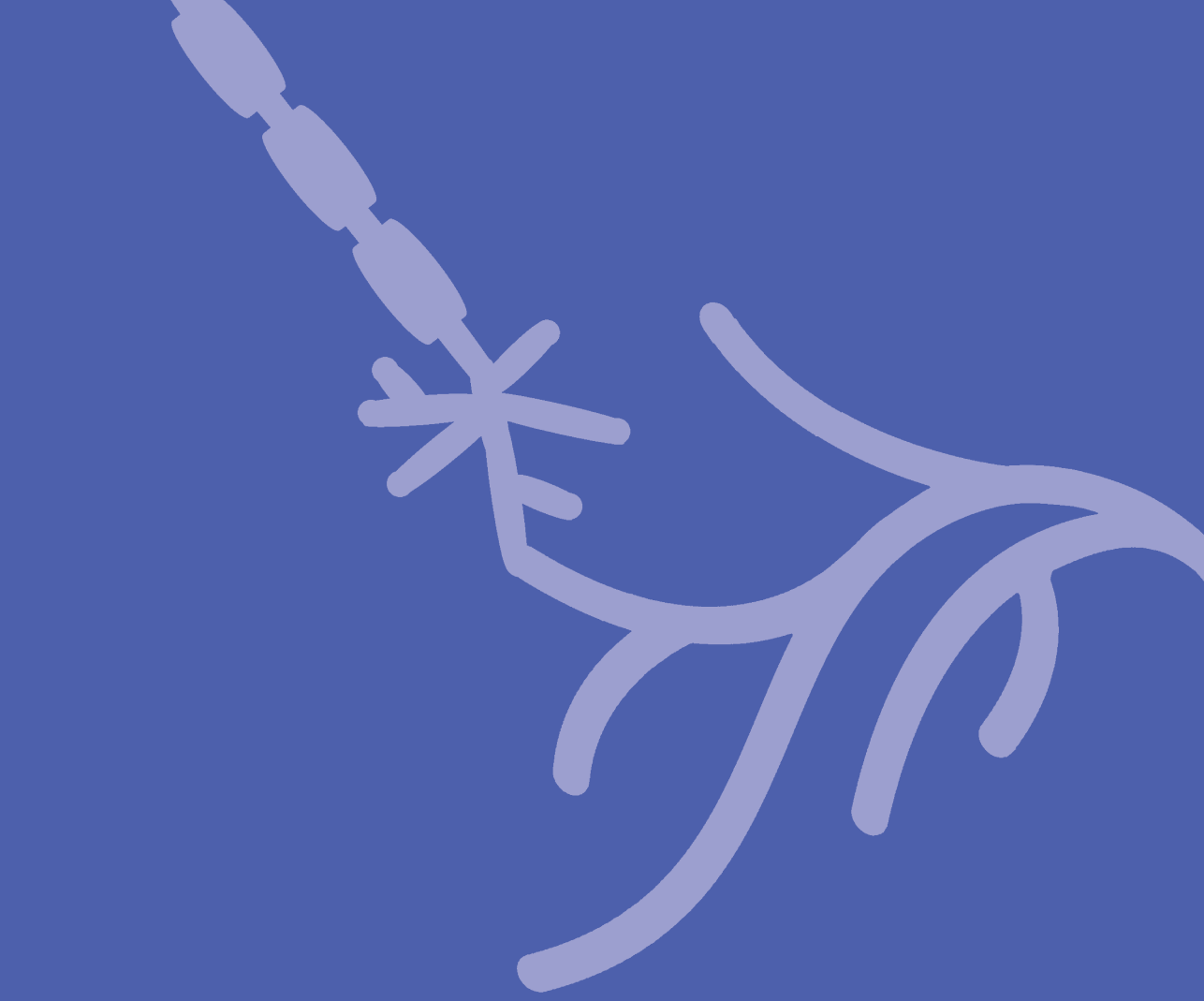

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CHAPTER 12

Neuropsychiatric and MRI findings in TREX1 mutation carriers: lessons from a small vessel disease

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Abstract

Background and Purpose: Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S) is caused by mutations in *TREX1* and a model for (early) stages of vascular dementia. We aimed to investigate the neuropsychiatric status of (pre)symptomatic mutation carriers (MC) and whether MRI characteristics (White Matter Hyperintensities (WMH) and Cerebrovascular Reactivity (CVR) in WM or grey matter (GM)) are related to cognitive impairment, psychiatric morbidity and apathy.

Methods: *TREX1* MC (n=29) and matched controls (n=29) underwent a comprehensive set of neuropsychiatric and functional tests, including Cambridge Cognitive Examination-Revised (CAMCOG-R), Mini-Mental State Examination (MMSE), Wechsler Memory Scale (WMS), Word Verbal Learning Test (WVLT), Stroop test, digit symbol substitution test (DSST), Frontal Assessment Battery (FAB), Hospital Anxiety and Depression Scale (HADS), Center for Epidemiological Studies Depression Scale (CES-D), Apathy Evaluation Scale (AES-I), Neuropsychiatric Inventory-Q (NPI-Q), modified Rankin Scale (mRS) and Barthel Index of Activities of Daily Living. The relationship with 3T-MRI findings was assessed. *TREX1* MC were analyzed separately in age groups < 50 and ≥ 50 years.

Results: TREX1 MC showed increased complaints of depression ($p<0.001$), anxiety ($p=0.040$) as well as worse global cognitive functioning ($p=0.008$) and executive functioning ($p=0.022$) compared to controls. MC ≥ 50 years additionally demonstrated worse memory ($p=0.001$) and apathy ($p=0.016$). Young MC demonstrated depressive symptoms ($p=0.047$) which correlated with reduced GM CVR ($p=0.030$). In MC ≥ 50 years decreased CVR correlated with worse global cognitive functioning (CVR in WM and GM respectively: $p=0.003$ and $p=0.008$). Decreased GM CVR was associated with impaired memory ($p<0.001$) and praxis ($p=0.046$), and higher WMH load with worse executive functioning ($p=0.024$).

Conclusions: TREX1 MC exhibited poorer cognitive function, increased functional disability and higher neuropsychiatric morbidity, including depression and anxiety, particularly in older individuals, with these impairments linked to increased WMH volume and reduced CVR.

Introduction

Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S) is an autosomal dominant neurovascular small vessel disease (SVD) caused by C-terminal truncating mutations in *TREX1*.[1,2] The best-known clinical features of RVCL-S are vascular retinopathy and focal and diffuse brain damage with early onset white matter hyperintensities (WMHs), with signs of inflammatory activity with diffusion restriction and gadolinium enhancement.[1] In addition, RVCL-S is a systemic vasculopathy with anemia, Raynaud's phenomenon and liver, thyroid and kidney disease.[1,3]

Additional imaging characteristics include the following findings: on CT brain scans, leukoencephalopathy and focal white matter calcifications and on MRI scans global progressive white matter hyperintensities (WMH) and punctate WMH with nodular enhancement. These might progress into intracerebral mass lesions accompanied by surrounding edema.[1,4] Besides global WMH also other SVD markers, such as subcortical infarcts, lacunes, enlarged perivascular spaces and atrophy can be present in RVCL-S.[1,4] The progression of RVCL-S varies, but symptoms often worsen quickly during middle age, typically resulting in premature death in the fifth decade of life.[1] Cognitive impairment has been shown to manifest as impairment of working memory, processing speed, information progressing and impaired judgement.[1,5] This is based on retrospective evaluation of medical records and a study of 12 RVCL-S patients. There are no studies focusing on the pattern of cognitive decline and psychiatric symptoms in mutation carriers without neurological complaints.[1,6]

Cerebral SVD is characterized by a wide range of focal and global brain changes, which can be made visible on MRI. In SVD there is evidence that WMH and reduced cerebrovascular reactivity (CVR) are associated with cognitive decline and psychiatric disorders.[7-11] For instance, in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), another small vessel disease affecting cognition, a correlation is found between white matter volume and different cognitive domains.[12] WMH have been described in RVCL-S since the disorder was discovered,[1] and more recently it was demonstrated that RVCL-S is associated with reduced CVR.

In the current study, we aimed to assess the neuropsychiatric status of TREX1 MC compared to controls and to determine whether presymptomatic TREX1 mutation carriers already demonstrated neuropsychiatric complaints. In addition, we conducted exploratory analyses to determine whether specific MRI characteristics (WMH volume

and CVR) correlated with neuropsychiatric symptoms to assess whether WMH and CVR could serve as (early) biomarkers for (pre)symptomatic RVCL-S as monogenic model for SVD in general.

Methods

Participants

Participants, both patients and controls, were included from the RVCL-S ID study, a cross-sectional observational study in *TREX1* MC from three large Dutch families.[3] For this study we included known MC and family members at 25% or 50% risk of being a carrier, who all underwent genetic testing for mutations in *TREX1* and a neuropsychiatric evaluation. Family members without a mutation and unrelated controls were included as controls. We matched controls based on age strata (10 years) and education level (according to the Dutch Verhage classification system).[13] Controls were excluded if they had a (history of) psychiatric disorder or neurological brain disorder. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all participants provided written informed consent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

MRI acquisition and post-processing

A uniform MRI protocol was performed on a 3.0T clinical MRI system (Philips Healthcare, Best, The Netherlands) on the same day as the neuropsychiatric evaluation. WMH volume and CVR data are previously reported by Hoogeveen et al.[14]

Brain WMH volume was assessed using tools of the FSL software package (Functional MRI of the Brain [FMRIB] Software Library v5, www.fmrib.ox.ac.uk/fsl).[15] T1-weighted (MPRAGE, TR=9.8 ms, TE=4.6 ms, flip angle of 8°, voxel size of 0.86 x 0.85 mm, 130 slices with a thickness of 1.2 mm) and FLAIR (TR=4800 ms, TE=291 ms, flip angle of 90°, voxel size of 1 x 1 mm, 310 slices with a thickness of 1.1 mm) images were used. FLIRT was used as brain extraction tool (FMRIB's linear image registration tool). FLAIR images were registered to the MNI152 standard space using FLIRT. WMH were automatically in a conservative MNI white matter mask, identified using a threshold of three standard deviations above mean FLAIR signal intensity.

CVR to hypercapnia (5% carbon dioxide) was measured using dual-echo arterial spin labelling (ASL) MRI, resulting in both cerebral blood flow (CBF; from the first echo) reactivity and blood oxygen level dependent (BOLD; second echo) reactivity.[14] We

used gray matter CBF CVR since CBF is the physiologically easier to interpret parameter, and white matter BOLD CVR due to the too low SNR of ASL in the white matter. To ensure that our results were not impacted by deciding to use the CBF instead of the more traditionally used BOLD CVR gray matter measurement we evaluated if the results were similar for both measurement methods. For detailed description of this method in this patient population see Hoogeveen et al.[14] In brief, a dual-echo pseudo continuous ASL (pCASL) scan was acquired with the following scan parameters: TR = 4100 ms, TE1/TE2 = 13/35 ms, label duration/post-label delay = 1650/1525 ms, multi-slice single shot EPI with background suppression (inversion pulses at 1680 and 2760 ms), 15 slices, voxel size = 3 × 3 × 7 mm, 75 pairs of label and control images. We further acquired a dual-echo M0 scan (TR= 2000 ms, TE1/TE2= 13/35 ms, resolution of 80 x 80, flip angle 90°, voxel size 3 x 3 mm, 15 slices of 7 mm), and a T1blood scan (TR= 150 ms, TE = 11 ms, resolution 240 x 240, flip angle 95°, voxel size 1 x 1 mm, 1 slice of 2 mm). For pre-processing, extraction, smoothing and transforming we refer to Hoogeveen et al.[14]

Neuropsychiatric assessment

A neuropsychiatric test battery lasting 2 hours was administered by trained examiners supervised by an experienced clinical neuropsychologist (H.A.M.M). Global cognitive functioning was assessed using the Cambridge Cognitive Examination-Revised (CAMCOG-R) and Mini-Mental State Examination (MMSE).[16,17] The CAMCOG provides a total score for global cognitive functioning as well as sub scores for specific cognitive functions, i.e. orientation, language comprehension, language expression, memory, praxis, abstract thinking, perception and attention and calculation.[16] We used the sub scores of the CAMCOG-R memory, praxis, gnosis, language and executive functioning domains for analyses. Moreover, memory was elevated by the Wechsler Memory Scale (WMS) and 15 Word Verbal Learning Test (WVLT).[18] Executive functioning was assessed with the Trail-Making Test (TMT), the colour interference section of the Stroop colour and word test (Stroop), the digit symbol substitution test (DSST) of the Wechsler Adult Intelligence Scale–III and the Frontal Assessment Battery (FAB).[19-22] The raw scores of the neuropsychiatric tests were used for analyses and computed into compound scores. Time to complete TMT part A, Stroop interference task, TMT interference time, and Stroop interference score were multiplied minus 1 so lower scores represented poorer performance. Raw scores of each test were converted to standardized Z-scores ((test score - mean)/SD) and a mean Z-score was calculated across the tests tapping cognitive domains of interest, i.e. global memory, language, praxis, gnosis and executive function. The Z-scores were created using the following tests and/or questionnaires: global cognitive functioning: COMCOG-R, MMSE; memory: COMCOG-memory and -orientation, WVLT (total words imprinted, recalled and recognized) and

subtests WMS; language: CAMCOG-expression and -comprehension; praxis: COMCOG-praxis; gnosis: CAMCOG-perception; executive functioning: time to complete TMT part A and Stroop interference task, TMT interference time, Stroop interference score, FAB total score, DSST score and COMCOG-abstract thinking and attention and calculation).

Current anxiety and depression were determined using the Hospital Anxiety and Depression Scale (HADS) and the Center for Epidemiological Studies Depression Scale (CES-D).[23,24] To assess presence of apathy and other psychiatric symptoms, informants were asked to complete the Apathy Evaluation Scale (AES-I) and Neuropsychiatric Inventory-Q (NPI-Q).[25,26] The psychiatric data were also converted to z-scores using the following: global psychiatric functioning: HADS-D, CES-D, HADS-A, AES-I, NPI-Q severity score; depression: HADS-D, CES-D, anxiety: HADS-A, apathy: AES-I. Before calculating the z-scores, time to complete TMT part A, Stroop interference task, TMT interference time, and Stroop interference score were multiplied minus 1 so lower scores represented poorer performance. The degrees of functional disability of all participants was determined by the modified Rankin Scale (mRS) and Barthel Index of Activities of Daily Living (BI).[27,28] mRS scores ≥ 1 and BI scores < 20 were defined as abnormal. Raw MMSE, CAMCOG-R, WMS, HADS, CES-D, BI and MRS data were previously reported by Pelzer et al.[3]

Statistical Analysis

Demographics and general assessments were reported as medians (interquartile range (IQR)) or counts (percentages). Differences in categorical demographics were analysed using Fisher's exact test. Additionally, we evaluated the effect of RVCL-S on different cognitive domains (separately adjusted for psychopathology) and neuropsychiatric symptoms by generalized linear model. We separately adjusted for the effect of neuropsychiatric symptomatology on cognition to insure that the results were not explained by those symptoms.[29] Before assessing the correlation between MRI markers and cognitive performance, neuropsychiatric symptoms, and functional disability, we first examined the strength of the correlations among the MRI markers themselves to determine their potential independent utility. Spearman rank-order correlation correcting for age were used to assess the correlation between WMH volume and CVR in gray and white matter on cognitive performance, neuropsychiatric symptoms and functional disability due to the non-normality of these measurements. *TREX1* MC were also analyzed separately for <50 years of age and ≥50 years based on the age of onset of notable neurological deficits.[1,3] Significance thresholds were set at $p<0.05$. Statistical analyses were performed using SPSS 25 (SPSS inc., IBM, USA).

Results

Demographics

In 29 individuals a *TREX1* mutation was confirmed. These mutation carriers (MC) were matched to 29 controls for age (median age (IQR) 44.0 (27.0-56.0) vs 43.0 (34.5-54.5)) and education (modus both groups: intermediate vocational education). Sex distribution of MC and controls was similar (59% vs 55% female). Despite visual impairments in some MC due to vascular retinopathy, all MC were able to execute the neuropsychiatric tests, and visual acuity did not influence outcomes (data not shown).

Neuropsychiatric and functional assessment

For the raw neuropsychiatric scores see table 1. *TREX1* MC showed worse global cognitive functioning and executive functioning and memory compared with controls (table 2). Across age categories, measures of global and executive functioning and memory were lower for mutation carriers aged ≥ 50 compared with age matched controls, but no differences were found between young MC (< 50 age) and controls (table 3). These findings were independent of depressive symptoms (Supplementary table 1). Overall psychiatric morbidity, depression and anxiety were more often reported in *TREX1* MC (table 2). While most differences appear to be specific to

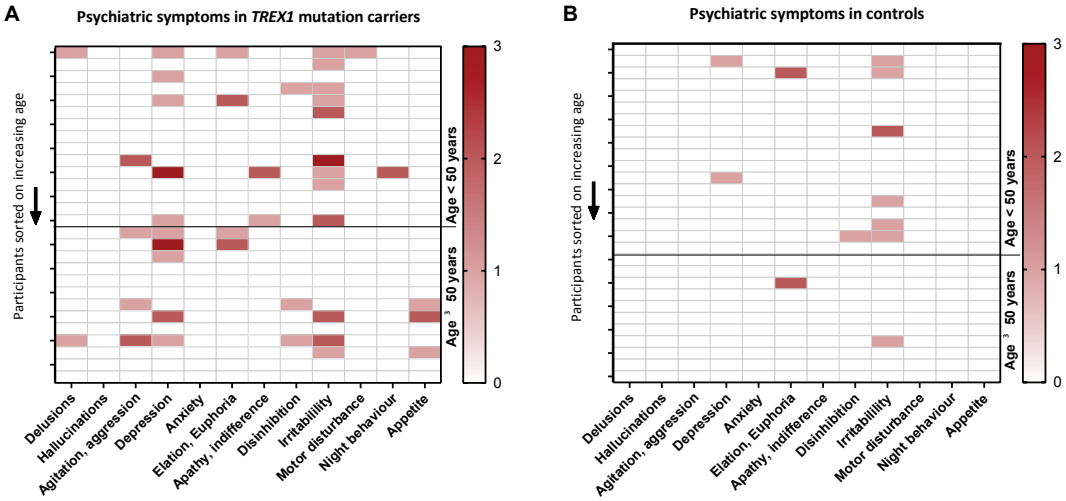

Figure 1. Psychiatric symptoms in *TREX1* mutation carriers and controls.

Mutation carriers (A) demonstrated an overall increase in neuropsychiatric symptoms as assessed with Neuropsychiatric Inventory-Q (ranging 0 to 3) compared to controls (B). Increased color intensity indicates more severe symptoms. In one mutation carrier and one control no informant reported information was available on night behavior.

the elderly, depression already occurred in young MC (table 3). The older patients also suffered from apathy. Moreover, informants indicated more neuropsychiatric symptoms in MC (figure 1). Regarding functional disability, 18% of RVCL-S patients had an abnormal BI score, while 55% had an abnormal mRS score (no controls had abnormal scores) (Fisher exact test, p=0.052 and p<0.001, respectively).

Table 1. Raw scores of cognitive tests and psychiatric surveys.

	RVCL-S n=29	Controls n=29
Global cognitive functioning		
CAMCOG-R, median[IQR]	93.0 [89.0-96.0]	97.0 [91.0-98.5]
MMSE, median[IQR]	29.0 [29.0-30.0]	30.0 [29.5-30.0]
Memory		
CAMCOG-memory, median[IQR]	21.0 [19.0-23.0]	23.0 [20.5-24.0]
CAMCOG-orientation, median[IQR]	10.0 [9.5-10.0]	10.0 [10.0-10.0]
WVLT, median[IQR]	49.0 [44.5-57.5]	50.0 [41.5-57.0]
WMS, median[IQR]	69.0 [60.0-74.3]	71.3 [66.5-76.3]
Language		
CAMCOG-comprehension, median[IQR]	9.0 [9.0-9.0]	9.0 [9.0-9.0]
CAMCOG-expression, median[IQR]	19.0 [17.0-20.0]	20.0 [18.5-20.5]
Gnosis		
CAMCOG-perception, median[IQR]	8.0 [8.0-8.0]	8.0 [8.0-8.0]
Praxis		
CAMCOG-praxis, median[IQR]	12.0 [11.0-12.0]	11.0 [11.0-12.0]
Executive functioning		
CAMCOG-abstract thinking, median[IQR]	7.0 [6.0-8.0]	8.0 [8.0-8.0]
CAMCOG-attention and calculating, median[IQR]	9.0 [8.0-9.0]	9.0 [8.5-9.0]
DSST, median[IQR]	67.0 [52.0-79.0]	83.0 [68.5-91.5]
FAB, median[IQR]	18.0 [17.0-18.0]	18.0 [16.5-18.0]
TMT-A, seconds, median[IQR]	24 [21-34]	20 [18-28]
TMT interference, seconds, median[IQR]	38 [28-55]	28 [16-43]
Stroop time, seconds, median[IQR]	79.5 [72-104]	78 [66-89.5]
Stroop interference, seconds, median[IQR]	37 [29-53]	35 [23-47]
Psychiatric morbidity		
HADS-A, median[IQR]	3.0 [2.0-7.0]	3.0 [1.0-4.0]
HADS-D, median[IQR]	5.0 [1.5-7.0]	1.0 [0.5-3.0]
CES-D, median[IQR]	7.0 [4.0-13.0]	2.0 [0.0-6.5]
AES-I, median[IQR]	27.0 [23.5-33.5]	24.0 [19.5-31.0]
NPI-Q, severity, median[IQR]	1.0 [0.0-4.8]	0.0 [0.0-0.0]

Cambridge Cognitive Examination-Revised (CAMCOG-R), Mini-Mental State Examination (MMSE), Wechsler Memory Scale (WMS), Word Verbal Learning Test (WVLT), Stroop test, digit symbol substitution test (DSST), Frontal Assessment Battery (FAB), Hospital Anxiety and Depression Scale (HADS), Center for Epidemiological Studies Depression Scale (CES-D), Apathy Evaluation Scale (AES-I), Neuropsychiatric Inventory-Q (NPI-Q).

MRI markers and cognitive and functional impairment in *TREX1* mutation carriers

In *TREX1* MC WMH volume showed weak negative correlations with both GM CVR (r_s = -0.37, p= 0.09) and WM (r_s = -0.41, p= 0.06). CVR in both GM and WM also showed a weak correlation with one another (r_s = 0.40, p = 0.07). In older MC (≥ 50 age) impairment of global cognition correlated with lower CVR in GM and WM (r_s = 0.89, p = 0.008 and r_s = 0.87, p = 0.003, respectively) (table 4). Additionally, a correlation was found between lower CVR in GM and worse memory and praxis performance (r_s = 0.96, p < 0.001 and r_s = 0.76, p = 0.046, respectively). Furthermore, a higher WMH volume correlated with worse executive functioning (r_s = -0.64, p = 0.024) and language performance (r_s = -0.74, p = 0.006). Additionally in young MC (< 50 age), a correlation was found with overall neuropsychiatric comorbidity and GM CVR (coefficient: 0.62, p = 0.030) (table 4). Evaluating GM CVR using the BOLD method resulted in similar results (data not shown).

Table 2. Neuropsychiatric assessment of *TREX1* MC compared to controls.

	Beta	Confidence interval	P value*
Cognition			
Global cognitive functioning	-0.65	-1.13:-0.17	**0.008**
Memory	-0.41	-0.91:0.09	0.11
Language	-0.40	-0.90:0.10	0.11
Praxis	0.12	-0.39:0.63	0.65
Gnosis	-0.46	-0.95:0.04	0.07
Executive functioning	-0.57	-1.07:-0.08	**0.022**
Psychiatric morbidity			
Overall psychiatric morbidity	0.81	0.34:1.28	**0.001**
Depression	0.86	0.40:1.32	**<0.001**
Anxiety	0.52	0.02:1.01	**0.040**
Apathy	0.39	-0.11:0.89	0.13

* Analyzed using generalized linear models.

Table 3. Effect of age on neuropsychiatric assessment of *TREX1* MC compared to controls

Age < 50 years	Beta	Confidence interval	P value*
Cognition			
Global cognitive functioning	-0.40	-1.16 - 0.35	0.29
Memory	0.08	-0.62 - 0.78	0.82
Language	-0.22	-0.90 - 0.47	0.53
Praxis	0.14	-0.54 - 0.82	0.69
Gnosis	-0.53	-1.20 - 0.13	0.12
Executive functioning	-0.26	-0.82 - 0.31	0.37
Psychiatric morbidity			
Overall psychiatric morbidity	0.54	-0.09:1.17	0.09
Depression	0.61	0.01:1.21	**0.047**
Anxiety	0.55	-0.16:1.26	0.13
Apathy	0.12	-0.52:0.77	0.71
Age ≥ 50 years	Beta	Confidence interval	
Cognition			
Global cognitive functioning	-1.01	-1.49:-0.53	**<0.001**
Memory	-0.95	-1.52:-0.38	**0.001**
Language	-0.66	-1.39:0.07	0.08
Praxis	0.10	-0.69:0.88	0.81
Gnosis	-0.33	-1.08:0.43	0.40
Executive functioning	-0.81	-1.58:-0.04	**0.040**
Psychiatric morbidity			
Overall psychiatric morbidity	1.11	0.41:1.80	**0.002**
Depression	1.11	0.42:1.80	**0.002**
Anxiety	0.49	-0.18:1.15	0.15
Apathy	0.88	0.17:1.60	**0.016**

* Analyzed using generalized linear models. Age < 50 years *TREX1* mutation carriers (n=15) and controls (n=18). Age ≥ 50 years *TREX1* mutation carriers (n=14) and controls (n=11).

Table 4. **Effect of age on association between MRI parameters and neuropsychiatric outcomes in *TREX1* mutation carriers**

	WMH volume†				CVR WM BOLD‡				CVR GM CBF‡			
	< 50 years		≥ 50 years		< 50 years		≥ 50 years		< 50 years		≥ 50 years	
	Coefficient	P value*	Coefficient	P value*	Coefficient	P value*	Coefficient	P value*	Coefficient	P value*	Coefficient	P value*
Cognition												
Global cognitive functioning	0.18	0.56	0.50	0.25	0.08	0.80	0.87	**0.003**	0.39	0.19	0.89	**0.008**
Memory	0.10	0.75	-0.30	0.36	0.07	0.82	0.66	0.10	0.14	0.65	0.96	**<0.001**
Language	0.45	0.12	-0.74	**0.006**	-0.09	0.76	0.04	0.93	0.47	0.10	0.43	0.33
Praxis	-0.34	0.26	-0.39	0.21	0.17	0.58	0.47	0.28	-0.17	0.57	0.76	**0.046**
Gnosis	-0.12	0.69	0.33	0.29	0.04	0.89	0.40	0.38	0.30	0.32	0.14	0.76
Executive functioning	-0.05	0.87	-0.64	**0.024**	0.09	0.78	0.06	0.90	0.01	0.99	0.30	0.51
Psychiatric morbidity												
Overall psychiatric morbidity	0.21	0.52	-0.12	0.70	0.01	0.97	-0.04	0.94	0.62	**0.030**	-0.18	0.71
Depression	0.44	0.13	-0.24	0.46	-0.22	0.47	-0.46	0.29	0.41	0.17	-0.23	0.62
Anxiety	0.09	0.77	-0.18	0.58	-0.06	0.84	-0.56	0.19	0.15	0.63	-0.35	0.45
Apathy	-0.05	0.86	-0.20	0.54	-0.09	0.78	0.57	0.18	0.17	0.57	0.43	0.33
Functional disability												
mRS	0.30	0.32	0.065	0.84	-0.19	0.54	-0.17	0.71	0.41	0.17	-0.61	0.14
Barthel index	-0.16	0.59	0.06	0.86	0.08	0.81	0.73	0.06	0.41	0.17	-0.61	0.14

†n=27, ‡ n=32, due to disability or contra indications not all patients could be scanned. * Spearman rank-order correlation correcting for age. Blood oxygen level dependent (BOLD), Cerebral blood flow (CBF), Cerebrovascular reactivity (CVR), gray matter (GM), white matter (WM), white matter hyperintensities (WMH).

Discussion

This study of TREX1 MC revealed poorer global cognitive function, executive functioning, and memory compared to controls, independent of depressive symptoms. Notably, these impairments were not observed in younger individuals (below 50 years of age). Overall neuropsychiatric morbidity, including also depression and anxiety, was more frequently reported in TREX1 MC, with patients above the age of 50 experiencing more apathy. Depressive symptoms appears to be an early neuropsychiatric sign of RVCL-S. Moreover, impaired cognitive function and depression correlated with increased WMH volume and reduced CVR, but this was not the case in young RVCL-S patients.

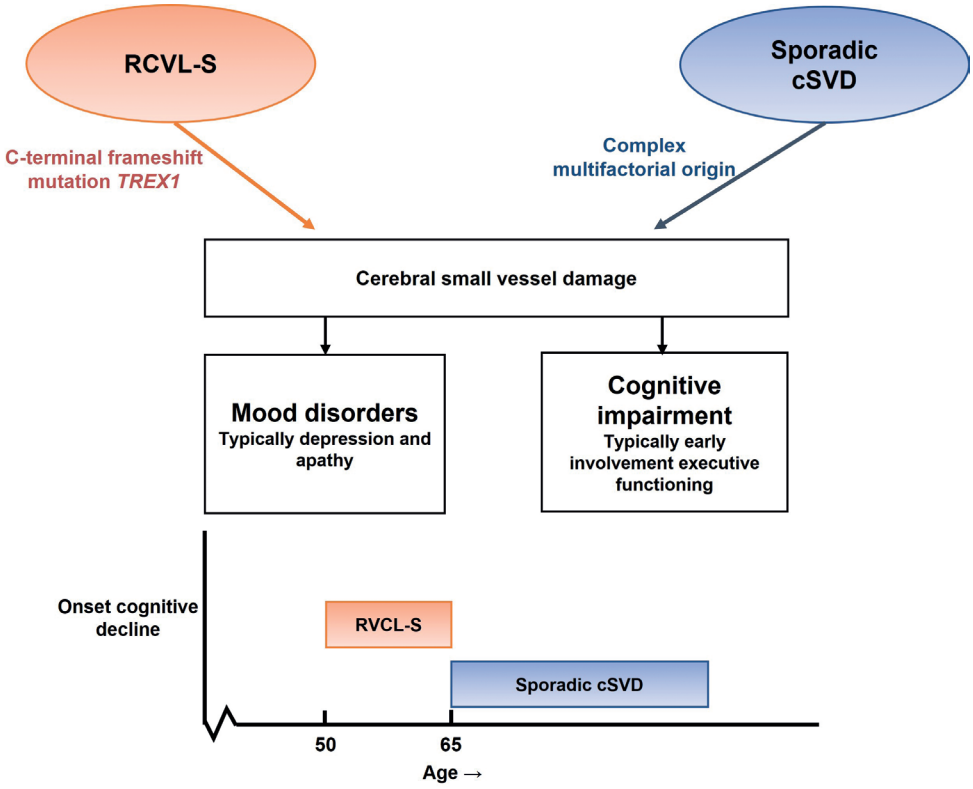

Figure 2. Comparison of cognitive and psychiatric findings in RVCL-S to other cerebral small vessel diseases.

Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S), cerebral small vessel disease (cSVD)

In line with our findings, an earlier small study evaluating some cognitive domains demonstrated impaired working memory and processing speed and increased depression in RVCL-S.[5] Due to the relatively young age at which we assessed the MC, other neurodegenerative or neurovascular causes are unlikely. The decreased executive functioning and memory deficits are in line with findings in sporadic cSVD and other monogenic diseases such as CADASIL (figure 2).[30-32]

Similarly, depressive symptoms in young individuals and apathy at older age are commonly reported features of CADASIL and sporadic cSVD.[33-35] While WMH volume is thought to contribute to cognitive decline and dementia in the general population, as it does in our RVCL-S MC, this association is still debated in CADASIL. In another hereditary small vessel disease, Dutch type cerebral amyloid angiopathy (CAA), as well as sporadic CAA, neuropsychiatric symptoms were also a prevalent finding, particularly apathy and irritability.[36] In the presymptomatic stages, before a first bleeding had occurred, this was already apparent. It should be noted however, that overall this CAA cohort was much older than our RVCL-S cohort. In the CAA patients these symptoms were associated with decreased processing speed and executive function but were not linked to the radiological burden of CAA.

While cognitive decline was not observed in young RVCL-S MC, potential subtle declines may have been missed due to the limited number of young participants. In contrast, younger patients did show higher levels of depression. Only six of the young MC were aware of their diagnosis, suggesting that depressive symptoms may be an early indicator of neuropsychiatric involvement in the disease beyond just awareness of their condition. The association between depression and cSVD is well-established, but in typical cSVD,[37,38] distinguishing depression as an early sign or consequence of mild neurocognitive dysfunction may be challenging due to the absence of a clear pre-symptomatic phase. Depression commonly coexists with neurological conditions like stroke and Alzheimer's disease, as well as disorders linked to vascular endothelial dysfunction such as cardiovascular disease and migraine. Recognizing depression as a potential sign of neurovascular involvement in the etiology of cSVD is gaining recognition.[39-42]

Whether WMH and CVR will be useful as a biomarker in the clinic requires further study. Given that RVCL-S is a systemic disorder, a comprehensive approach combining biomarkers such as MRI, ophthalmological assessments, and serum (or CSF) biomarkers may yield valuable prognostic insights for RVCL-S. Non-RVCL-S specific biomarkers may also be used to monitor disease progression, such as serum neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) that are associated with decline in cognitive functioning in RVCL-S.[43]

We suggest that RVCL-S is a good model to study one potential important aspect of SVD, the endothelium. Neuropathological studies have identified multilaminated basement membranes on ultrastructural images and endothelial markers were shown to be elevated in serum of patients.[1,44] Furthermore, functional studies demonstrated decreased flow-mediated dilation of the brachial artery (considered an *in vivo* marker of endothelial dysfunction) and reduced CVR (as surrogate measure of endothelial function[45]).[46,47] Interestingly, MRI features of SVD may be a reflection of poor cerebral blood flow regulation, as was also demonstrated in patients with minor ischemic stroke.[48] Further exploration of microvascular dysfunction may uncover new mechanisms and intervention targets to reduce SVD lesion development, cognitive decline, and stroke. Together, this indicates the importance of studying endothelial dysfunction in RVCL-S and common SVD and highlights possible avenues of treatment. RVCL-S is an ideal model for such studies, as there is one cause and a clear pre-symptomatic stage. Furthermore, while the exact mechanism of how aberrant TREX1 leads to a microvascular disease is still under research, their appears to be a clear role for DNA damage and premature senescence pathways.[49] These interesting mechanisms deserve further consideration.

There are limitations to our study to be considered: (i) as RVCL-S is a rare disorder, our sample size is relatively small and as such we could not evaluate all SVD markers; (ii) some MRI data are missing due to patients having contraindications for MRI. This may have led to an underestimation of effect; (iii) the cross-sectional nature of the study prevents statements being made concerning decline and causality, however, this is the largest study in RVCL-S describing cognitive and neuropsychiatric functioning and the first to evaluate association with MRI markers. Strengths of our study include: (i) performing MRI and neuropsychiatric evaluations on the same day; (ii) excluding confounding neurodegenerative or other vascular factors due to the relatively young age of participants; (iii) using an extensive neuropsychiatric test battery to assess various cognitive domains; (iv) the availability of matched patients and controls based on age and education; and (v) separately evaluating the impact of psychiatric symptoms on cognition. It should be noted that the significance level was kept at 0.05 with no correction for multiple comparisons, as is consistent with an exploratory study.

Conclusions

RVCL-S TREX1 mutation carriers exhibit poorer cognitive function and higher neuropsychiatric morbidity, including depression and apathy, particularly above the age of 50. These impairments are linked to increased WMH volume and reduced CVR, though these do not appear as early signs. Importantly, depressive symptoms can be an early indicator of RVCL-S.

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Supplementary

Supplementary table 1. Cognition adjusted for psychopathology.

All mutation carriers	Beta	Confidence interval	
Global cognitive functioning	-0.65	-1.19:-0.12	**0.017**
Memory	-0.41	-0.96:0.14	0.14
Language	-0.46	-1.02:0.09	0.10
Praxis	0.22	-0.34:0.78	0.45
Gnosis	-0.37	-0.92:0.18	0.19
Executive functioning	-0.51	-1.11:-0.01	**0.049**
Age < 50 years	**Beta**	**Confidence interval**	
Global cognitive functioning	-0.40	-1.21:0.42	0.34
Memory	0.02	-0.73:0.77	0.95
Language	-0.31	-1.03:0.41	0.40
Praxis	0.08	-0.64:0.81	0.82
Gnosis	-0.62	-1.33:0.08	0.08
Executive functioning	-0.29	-0.90:0.33	0.36
Age ≥ 50 years	**Beta**	**Confidence interval**	
Global cognitive functioning	-1.06	-1.64:-0.50	**<0.001**
Memory	-0.95	-1.62:-0.28	**0.006**
Language	-0.61	-1.47:0.25	0.16
Praxis	0.53	-0.34:1.40	0.23
Gnosis	0.19	-0.68:1.00	0.63
Executive functioning	-0.85	-1.71:-0.06	0.068

* Analyzed using generalized linear models.