


Genetic analysis reveals novel variants for vascular cognitive impairment

Saana Mönkäre^{1,2}  | Liina Kuuluvainen^{3,4} | Johanna Schleutker^{2,5} | Jose Bras^{6,7} |
 Susanna Roine⁸ | Minna Pöyhönen^{1,9} | Rita Guerreiro^{6,7} | Liisa Myllykangas^{9,10}

¹Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland

²Laboratory Division, Department of Medical Genetics, Genomics, Turku University Hospital, Turku, Finland

³Diagnostic Center, Department of Clinical Genetics, Helsinki University Hospital, Helsinki, Finland

⁴Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland

⁵Institute of Biomedicine, University of Turku, Turku, Finland

⁶Center for Neurodegenerative Science, Van Andel Institute, Grand Rapids, Michigan, USA

⁷Division of Psychiatry and Behavioral Medicine, Michigan State University College of Human Medicine, Grand Rapids, Michigan, USA

⁸Neurocenter, Department of Cerebrovascular Diseases, Turku University Hospital, Turku, Finland

⁹HUS Diagnostic Center, Helsinki University Hospital, Helsinki, Finland

¹⁰Department of Pathology, University of Helsinki, Helsinki, Finland

Correspondence

Liisa Myllykangas, Department of Pathology, University of Helsinki, POB 21 00014 Helsinki, Finland.
 Email: liisa.myllykangas@helsinki.fi

Funding information

This study was funded by Academy of Finland (294817, 341007), Liv och Hälsa Foundation, Yrjö Jahnsson Foundation and Tyks Foundation

Abstract

Objectives: The genetic background of vascular cognitive impairment (VCI) is poorly understood compared to other dementia disorders. The aim of the study was to investigate the genetic background of VCI in a well-characterized Finnish cohort.

Materials & Methods: Whole-exome sequencing (WES) was applied in 45 Finnish VCI patients. Copy-number variant (CNV) analysis using a SNP array was performed in 80 VCI patients. This study also examined the prevalence of variants at the miR-29 binding site of *COL4A1* in 73 Finnish VCI patients.

Results: In 40% (18/45) of the cases, WES detected possibly causative variants in genes associated with cerebral small vessel disease (CSVD) or other neurological or stroke-related disorders. These variants included *HTRA1*:c.847G>A p.(Gly283Arg), *TREX1*:c.1079A>G, p.(Tyr360Cys), *COLGALT1*:c.1411C>T, p.(Arg471Trp), *PRNP*:c.713C>T, p.(Pro238Leu), and *MTHFR*:c.1061G>C, p.(Gly354Ala). Additionally, screening of variants in the 3'UTR of *COL4A1* gene in a sub-cohort of 73 VCI patients identified a novel variant c.*36T>A. CNV analysis showed that pathogenic CNVs are uncommon in VCI.

Conclusions: These data support pathogenic roles of variants in *HTRA1*, *TREX1* and in the 3'UTR of *COL4A1* in CSVD and VCI, and suggest that vascular pathogenic mechanisms are linked to neurodegeneration, expanding the understanding of the genetic background of VCI.

KEYWORDS

cerebral small vessel diseases, cerebrovascular disorders, vascular dementia, whole exome sequencing

Rita Guerreiro and Liisa Myllykangas contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Acta Neurologica Scandinavica* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Vascular cognitive impairment (VCI) is a term describing cognitive impairment associated with cerebrovascular disease, ranging from mild cognitive impairment (MCI) to vascular dementia (VaD).¹ In clinical studies, vascular dementia is the second most common cause of dementia.² VCI can be caused by a cerebral event which causes acute significant neurological symptoms such as a stroke or a bleed. However, VCI is often caused by cerebral small vessel disease (CSVD) which usually causes clinically silent cerebral vascular events that are only evident in imaging studies as small subcortical infarcts, lacunes and microbleeds, white matter hyperintensities, perivascular spaces, and brain atrophy.^{1,3} Vascular dementia can often be present with other dementia disorders such as Alzheimer's disease,⁴ making its diagnosis often challenging. The genetic background of VCI is poorly understood compared to other dementia disorders. Several monogenic disorders causing VCI have been described⁵ but many VCI cases seem to be sporadic and affected by environmental and lifestyle risk factors. Recently, variants were discovered at the binding site for miR-29 microRNA located within the 3' untranslated region (UTR) of the *COL4A1* gene in patients with multi-infarct dementia⁶ and pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL).⁷ The miR-29 microRNAs are known regulators of extracellular matrix genes, and the miR-29 family has been implicated in the development of fibrosis in various organs.⁸ Variants in the 3'UTR of *COL4A1* were shown to disrupt the same miR-29 binding site and consequently cause upregulation of *COL4A1*.^{6,7} These inherited CSVDs are distinguished from other autosomal dominant (*COL4A1/2*)-related diseases which are typically caused by pathogenic variants affecting the triple-helical domain of the protein, and which are associated with small vessel disease (SVD) in various organs.⁹

Copy-number variation (CNV) in the human genome is a major cause of human disease. However, most studies of the genetics of CSVD and VCI have examined single nucleotide variants and small insertions or deletions (indels), whereas the role of CNVs in the pathogenesis of VCI remains largely unknown. Some CNVs associated with stroke have been described,¹⁰ but to our knowledge, no studies of CNVs in VCI have been conducted thus far.

We recently published a whole-exome analysis of Finnish patients with VCI (v.1.0 cohort, $n = 35$).¹¹ The aim of the current study was to further investigate the genetic background of familial VCI by performing a similar analysis in the second part of a Finnish VCI patient population (v.2.0 cohort, $n = 45$) using whole-exome sequencing (WES). Furthermore, CNVs were explored in both datasets, using a genome-wide single nucleotide polymorphism (SNP) array. Additionally, we investigated the prevalence of variants in the 3'UTR of *COL4A1* gene in a cohort of Finnish CSVD patients.

2 | MATERIALS & METHODS

2.1 | Subjects

The study was approved by the Ethical Committee of the Hospital District of Southwest Finland. The approval for the use of patient DNA samples was obtained from the National Supervisory Authority for Welfare and Health (Valvira) and the Hospital District of Southwest Finland. The permit for the access to medical records was obtained from the National Institute for Health and Welfare.

The study subjects were selected from among 326 patients referred for diagnostic testing for *NOTCH3* to the Department of Medical Genetics of Turku University Hospital between the years 2004 and 2018. Patients were screened negative for variants in *NOTCH3* exons 3–8, 11, and 18–20 or in all *NOTCH3* exons. Diagnosis or clinical phenotype of all 326 patients was assessed from medical records. Based on the medical records, 73 patients from the cohort of 326 patients were confirmed to have VCI and were selected for sequence analysis of the miR-29 binding site at the 3'UTR of *COL4A1*. Of these, 45 patients were selected for WES and CNV analysis by applying the following inclusion criteria: (1) presence of VCI with white matter changes in magnetic resonance imaging (MRI) and (2) age at onset up to 75 years, and/or family history of dementia or stroke. The inclusion criteria for the comprehensive genetic analysis were applied to determine the best candidates with adequate clinical information for the investigation of familial VCI. Of the 73 VCI patients, 28 were excluded from WES and CNV analyses. Of these, nine patients were excluded due to age and missing information of family history, unavailability of MRI report or due to low amount of DNA sample. Other reasons for exclusion patients from WES and CNV analyses included suspicion of Alzheimer's disease or mixed dementia, ongoing heavy alcohol consumption, patent foramen ovale detected in etiological examinations, patients with pulmonary embolism, or deficient/contradictory description of the patient's cognitive status in the available medical notes. The workflow of the patient selection and genetic analyses is presented in Figure S1.

2.2 | Whole-exome sequencing (WES)

Details of library preparation and data processing are shown in the Supplement. Variants passing VQSR score and QC filters described by Patel et al.¹² were included in the analyses. Variants located in known genomic duplication regions, synonymous variants, and intronic variants that were not located within splice sites were excluded from analyses. We focused on variants present with an allele frequency $\leq 1\%$ in gnomAD (v3.1.1). First, we analyzed the stroke-gene panels SGP1 and SGP2 compiled by Ilinca et al.¹³ These panels contain 168 genes that are associated with monogenic causes of stroke. To update the

TABLE 1 Possibly causative variants detected in sequence analyses

Patient	Gene	Nucleotide change	Amino acid change	Zygosity	RefSeq	Allele frequency (gnomAD total)	Allele frequency (gnomAD Finnish)	CADD Phred	Phenotype MIM number
1000 ^a	COL4A1	c.*36T>A ^b	.	Het	NM_001845.6	0	0	.	618564
1001	TUBB2A	c.1309G>A	p.(Glu437Lys)	Het	NM_001069.3	0	0	23.1	615763
1006	COLGALT1	c.1411C>T	p.(Arg471Trp)	Het	NM_024656.3	0.00001315	0	33	618360
1007	UBQLN2	c.304A>G	p.(Ile102Val)	Hem	NM_013444.4	0.000008916	0.0001623	25.6	300857
1015	C1R	c.716G>A	p.(Arg239Gln)	Het	NM_001354346.2	0.000006572	0	26.5	130080
1016	PRNP	c.713C>T	p.(Pro238Leu)	Het	NM_000311.5	0	0	23.9	137440, 123400, 603218, 600072, 606688
1017	PCNT	c.2179C>G	p.(His27Aasp)	Het	NM_006031.6	0	0	23.0	210720
1017	VPS13A	c.9257T>C	p.(Met3086Thr)	Het	NM_033305.3	0	0	21.8	200150
1017	SMAD4	c.1060G>A	p.(Val354Met)	Het	NM_005359.5	0	0	26.1	175050
1019	CHMP2B	c.157G>C	p.(Gly53Arg)	Het	NM_014043.4	0.000006578	0.00009448	28.3	600795
1020	GRIN2A	c.937A>G	p.(Ile313Val)	Het	NM_000833.5	0.000006573	0.00009418	22.4	245570
1025	DIAPH1	c.1093T>C	p.(Phe365Leu)	Het	NM_005219.5	0.00007229	0.0007529	25.7	
1027	TSC2	c.4432G>A	p.(Asp1478Asn)	Het	NM_000548.5	0	0	29.0	613254
1029	MTHFR	c.1061G>C	p.(Gly354Ala)	Hom	NM_001330358.2	0.0001839	0.002541	25.5	236250
1030	HTRA1	c.847G>A	p.(Gly283Arg)	Het	NM_002775.5	0	0	33	600142, 616779
1033	THSD1	c.1619dupT	p.(Met540fs)	Het	NM_018676.4	0	0	.	618734
1033	SNCA	c.370G>T	p.(Ala124Ser)	Het	NM_000345.4	0.00001315	0.0001886	19.55	127750, 168601, 605543
1036	CCM2	c.391G>A	p.(Asp131Asn)	Het	NM_001029835.2	0.0001840	0.0002825	28.2	603284
1038	DNAJC13	c.1036C>G	p.(Leu346Val)	Het	NM_015268.4	0.00001314	0.0001885	21.6	616361
1039	SLC20A2	c.1858C>T	p.(Arg620Trp)	Het	NM_006749.5	0.00002631	0	28.9	213600
1043	NMNAT2	c.427G>A	p.(Val143Met)	Het	NM_015039.4	0	0	21.3	.
1045	POLG	c.2218A>G	p.(Asn740Asp)	Het	NM_002693.3	0.00005260	0	22.6	203700, 613662, 607459, 157640, 258450
1045	TREX1	c.1079A>G	p.(Tyr360Cys)	Het	NM_016381.5	0.0001314	0	24.9	192315

Note: All variants were classified as being of unknown significance based on ACMG guidelines

Abbreviations: Hem, hemizygous; Het, heterozygous; Hom, homozygous; MIM, Mendelian inheritance in Man.

CADD Combined Annotation Dependent Depletion, algorithm for scoring the deleteriousness of variants (≥ 10 = belongs to 10% of the most deleterious variants in the human genome, ≥ 20 = belongs to 1% of the most deleterious variants in the human genome).

^aSample 1000 was not exome-sequenced, COL4A1 variant c.*36T>A was detected by sanger sequencing.

^bAlthough the COL4A1 variant c.*36T>A is of interest, pending further evidence and information, we classify it as a variant of unknown significance.

TABLE 2 Clinical details associated with the possibly causative sequence variants

Patient	Gene	Variant	Zygosity	Gender	AAO	Diagnosis/clinical features
1000	COL4A1	c.*36T>A	Het	M	32	VCI, gait disturbance, mild depression
1001	TUBB2A	c.1309G>A, p.(Glu437Lys)	Het	F	56	VCI, depression, atypical parkinsonism
1006	COLGALT1	c.1411C>T, p.(Arg471Trp)	Het	M	48	VaD
1007	UBQLN2	c.304A>G, p.(Ile102Val)	Hem	M	58	VaD
1015	C1R	c.716G>A, p.(Arg239Gln)	Het	M	66	VaD
1016	PRNP	c.713C>T, p.(Pro238Leu)	Het	M	59	VaD, depression
1017	PCNT	c.2179C>G, p.(His727Asp)	Het	M	66	VaD, depression
1017	VPS13A	c.9257T>C, p.(Met3086Thr)	Het			
1017	SMAD4	c.1060G>A, p.(Val354Met)	Het			
1019	CHMP2B	c.157G>C, p.(Gly53Arg)	Het	F	57	VaD, epilepsy, depression
1020	GRIN2A	c.937A>G, p.(Ile313Val)	Het	F	67	VaD, epilepsy
1025	DIAPH1	c.1093T>C, p.(Phe365Leu)	Het	M	66	VCI
1027	TSC2	c.4432G>A, p.(Asp1478Asn)	Het	F	44	VCI
1029	MTHFR	c.1061G>C, p.(Gly354Ala)	Hom	F	63	VaD
1030	HTRA1	c.847G>A, p.(Gly283Arg)	Het	F	55	VaD
1033	THSD1	c.1619dupT, p.(Met540fs)	Het	F	50	VaD, schizophrenia
1033	SNCA	c.370G>T, p.(Ala124Ser)	Het			
1036	CCM2	c.391G>A, p.(Asp131Asn)	Het	M	64	VCI
1038	DNAJC13	c.1036C>G, p.(Leu346Val)	Het	F	56	VaD, depression
1039	SLC20A2	c.1858C>T, p.(Arg620Trp)	Het	M	27	VaD, epilepsy
1043	NMNAT2	c.427G>A, p.(Val143Met)	Het	M	46	VaD
1045	POLG	c.2218A>G, p.(Asn740Asp)	Het	M	39	VaD
1045	TREX1	c.1079A>G, p.(Tyr360Cys)	Het			

Abbreviations: AAO, age at onset; F, female; Hem, hemizygous; Het, heterozygous; Hom, homozygous; ICH, intracerebral hemorrhage; M, male; MRI, magnetic resonance imaging; VaD, vascular dementia; VCI, vascular cognitive impairment.

stroke-gene panel, a search for recent publications of genes related to stroke was performed on the PubMed in September 2021. Based on the search results, we updated the panel with seven additional genes (Table S1). Mitochondrial genes were excluded from gene panel analysis. We also did an analysis with the Exomiser software (v.12.1.0)

using X-chromosomal and autosomal (dominant and recessive) inheritance models and CADASIL phenotype (ORPHA:136). The highest ranked variants were evaluated in the Exomiser results. Finally, we also analyzed non-synonymous and splice site variants that were absent from the Genome Aggregation Database (gnomAD) v.3.1.1. In silico

Family history	Affected family members	Migraine	Hypertension	Other risk factors	Other conditions or additional information
Yes	Uncle (cerebral hemorrhage at age 28)	No	No		ICH at age 32, multiple lacunar infarcts and microbleeds in brain MRI
Yes	Grandfather and a brother (atypical parkinsonism)	No	Yes	Hypercholesterolaemia	
Yes	Several relatives with cardiovascular accidents	No	Yes	Hypercholesterolaemia, smoking, obesity, coronary artery disease	Hearing loss, renal failure
n/a		No	Yes	Hypercholesterolaemia	No frontotemporal or hippocampal atrophy in brain MRI
No		No	No	Hypercholesterolaemia, smoking	Macular degeneration
Yes	Mother (dementia, AAO 60 years), son with unknown neurological disease	No	No	Hypercholesterolaemia	Slowly progressive walking difficulty
n/a		Yes	No		Abdominal aortic aneurysm
Yes	Father (died of subarachnoid hemorrhage at young age)	No	Yes		Brain MRI also showed tigroid pattern
n/a		No	No	Diabetes	
Yes	Mother (dementia), father (died of stroke)	No	No	Diabetes, smoking	
No		Yes	No	Smoking	Multiple brain aneurysms
n/a		No	Yes	Hypercholesterolaemia	
Yes	Several relatives with cardiovascular diseases	No	No		
No		No	No		Balance impairment, falls, extrapyramidal symptoms
Yes	Several maternal relatives with dementia	Yes	No	Smoking	Osteoarthritis, lumbar spine degeneration, nerve root compression
Yes	Mother (dementia, AAO 64 years), father (dementia AAO 70 years)	Yes	No	Smoking	
n/a		No	No		
n/a		No	Yes	Hypercholesterolaemia	
Yes	Mother (multiple strokes)	Yes	Yes	Obesity	Mild hypertensive retinopathy

predictions were performed with SIFT, PolyPhen2, MutationTaster, LRT, MutationAssessor, FATHMM, PROVEAN, and CADD. Only variants with a CADD score ≥ 10 were considered potentially pathogenic. Variants were interpreted based on the American College of Medical Genetics and Genomics (ACMG) criteria.¹⁴

2.3 | CNV analysis

Genotyping was performed at the Genotyping laboratory of Institute for Molecular Medicine Finland FIMM Technology Centre, University of Helsinki (56 samples) or at Van Andel Institute (24 samples) using Illumina

Infinium Global Screening Array MD-24 (GSAMD) v.2.0 or v.3.0 (Illumina). The CNVs were called using PennCNV software.¹⁵ See Supplementary Materials for details of data processing. Called CNVs were filtered by the number of consecutive probes ≥ 10 , gene content (at least one gene), and their size ≥ 50 kb. Identified CNVs were evaluated by comparison with Database of Genomic Variants and DECIPHER database, and the clinical significance of CNVs were assessed based on their type, size, and gene content. Detected CNVs were visually confirmed using GenomeStudio.

2.4 | Sanger sequencing

In the 73 DNA samples extracted from peripheral blood, the miR-29 microRNA binding site in the 3'UTR of *COL4A1* was Sanger sequenced as previously described.¹¹

3 | RESULTS

Characteristics of the initial cohort of 326 patients studied is summarized in Table S2. The study cohort v.2.0 for both WES and CNV analysis was comprised of 45 Finnish CSVD patients. Based on medical records, 48% (22/45) of these were identified as familial cases. The age of onset varied (27–74), and 78% (35/45) had hypertension or other risk factors for VCI. Clinical characteristics of the patients studied (v.2.0 cohort) are summarized in Table S3.

3.1 | Sequence analyses

In 18 patients, WES identified possibly causative variants for VCI. All variants were classified as being of unknown significance based on ACMG guidelines (Table 1 and Table S4). This study also detected several rare candidate variants and variants in genes associated with other conditions related to the phenotypes of the patients. A complete overview of the other variants detected by WES is presented in Table S5 in the Supplement.

WES detected three variants in other known CSVD-related genes; previously reported variants in *HTRA1* and *TREX1* and a novel variant in *COLGALT1* (Table 1). A heterozygous *HTRA1* variant c.847G>A, p.(Gly283Arg) detected in a female patient has previously been reported in a patient with CSVD.¹⁶ Variants affecting function in *HTRA1* cause cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)¹⁷ and autosomal dominant CSVD (*HTRA1*-CSVD) characterized by milder clinical features of CARASIL.^{18–20} The age at onset of the patient carrying the *HTRA1* variant c.847G>A was 55 years, and she had no vascular risk factors (Table 2).

Furthermore, this study identified a heterozygous *TREX1* variant c.1079A>G, p.(Tyr360Cys), which has previously been reported in patients with systemic lupus erythematosus (SLE)^{21,22} or early-onset CSVD.²³ Variants affecting function in *TREX1* are linked to retinal vasculopathy with cerebral leukoencephalopathy and

systemic manifestations.²⁴ The patient carrying the *TREX1* variant c.1079A>G, p.(Tyr360Cys) had their first stroke at age 39 (Table 2), and brain MRI showed multiple microhaemorrhages and lacunar infarcts. Mild hypertensive retinopathy was also observed. The patient was diagnosed with severe amyloid angiopathy, and he also started suffering from migraines without aura and was later diagnosed with cognitive impairment. The patient also carried a heterozygous variant c.2218A>G, p.(Asn740Asp) in the *POLG* gene. Pathogenic variants in *POLG* are associated with a spectrum of mitochondrial disorders.²⁵ The phenotype of the patient was not suggestive for *POLG*-related disorder; therefore, the detected variant may not be clinically relevant.

This study also identified a heterozygous variant c.1411C>T, p.(Arg471Trp) in *COLGALT1*, which has recently been linked to autosomal recessive CSVD with infantile/toddler onset.²⁶ A male patient carrying the variant had several risk factors (Table 2). He had the first transient ischemic attack at age 48 years and developed early-onset VaD. MRI showed severe white matter changes and multiple lacunar infarcts.

This study also detected several novel variants in genes associated with neurodegenerative or other neurological or stroke-related disorders (Table 1, Table 2, and Table S5). A heterozygous variant c.713C>T, p.(Pro238Leu) in the *PRNP* gene was identified in a male patient. Pathogenic variants in *PRNP* cause autosomal dominant genetic prion diseases with a wide range of phenotypic variability.²⁷ The variant has not been reported before, but a different variant affecting the same amino acid residue of *PRNP* has been described in a patient with suspected Creutzfeldt-Jakob disease²⁸ and has been proposed to cause neurodegeneration.²⁹ The patient carrying the *PRNP* variant c.713C>T had an age at onset of 59 years, and his phenotype included depression, slowly progressive walking difficulty, and cognitive impairment (Table 2). Brain MRI showed changes corresponding to CSVD: periventricular white matter lesions and multiple infarcts. Some of the clinical features of the patient could be compatible with genetic Creutzfeldt-Jakob disease; therefore, the novel *PRNP* variant detected in the patient was considered potentially causative for the patient's phenotype. However, the significance of this novel variant will remain unclear until more evidence supporting its pathogenicity is reported. Other novel variants of unknown clinical significance detected in neurodegeneration-related genes included *CHMP2B*, *DNAJC13*, *SLC20A2*, *SNCA*, *UBQLN2*, and *VPS13A* (Table 2). This study also identified novel variants in stroke-related genes, such as *THSD1*, *PCNT*, and *CCM2* (Table 2). See Table S4 for additional information on the variants.

A possible metabolic cause for VCI was observed in a female patient who carried a novel homozygous variant c.1061G>C, p.(Gly354Ala) in *MTHFR*. Pathogenic variants in *MTHFR* cause homocystinuria due to methylenetetrahydrofolate reductase deficiency,³⁰ which has been shown to be associated with CSVD and cognitive impairment.^{31,32} The patient carrying the *MTHFR* variant c.1061G>C, p.(Gly354Ala) had multiple strokes and was diagnosed with VaD. Brain MRI showed white matter disease and multiple infarcts.

TABLE 3 Rare heterozygous CNVs of interest identified in patients with VCI

Patient	AAO	Gender	Type	CNV	Size (bp)	Genes involved
236	64	M	Gain	chr6:163104432-165045334	1,940,903	PARK2, PACRG, LOC401282, CAHM, QKI
289	48	M	Gain	chr15:94876580-95356210	479,631	MCTP2
1005	60	F	Gain	chr7:19035920-20617266	1,581,347	HDAC9, TWIST1, FERD3L, TWISTNB, TMEM196, MACC1, ITGB8
1010	62	M	Gain	chr3:193061741-193467943	406,203	ATP13A5, ATP13A4, OPA1
1014	60	M	Gain	chr8:6155658-6391302 ^a	235,645	ANGPT2, MCPH1, AGPAT5
				chr8:6412551-6574830 ^a	162,28	
1029	63	F	Loss	chr16:15491006-16258173	767,168	MPV17L, BMERB1, MARF1, NDE1, MYH11, CEP20, ABCC1, ABCC6
1031	55	F	Loss	chr2:133949948-134070417	120,47	NCKAP5
1033	50	F	Gain	chr1:92293721-92574940	281,22	TGFBR3, BRDT, EPHX4, BTBD8
1039	27	M	Loss	chr12:43937166-44009983	72,818	ADAMTS20

Note: Genome assembly: GRCh37/hg19.

Abbreviation: AAO, age at onset; bp, base pair.

^aThe region between the two duplications in 8p23.1 was not covered in the GSAMD array.

Sanger sequencing of the miR-29 binding site in the 3'UTR of COL4A1 in 73 VCI patients revealed a novel heterozygous variant c.*36T>A in a patient not included in the WES analysis (Table 1 and Figure S2). Variants in the 3'UTR of COL4A1 cause pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL) and Swedish hereditary multi-infarct dementia (hMID), which share similar clinical and pathological features.^{6,7} The patient carrying the COL4A1 variant c.*36T>A did not have any vascular risk factors (Table 2). He had intracerebral hemorrhage at 32 years old, and an MRI performed later in life showed confluent white matter lesions, multiple lacunar infarcts, and microbleeds (Table 2). At the age of 47, cognitive impairment, gait disturbance, and mild depression were observed.

3.2 | CNV analysis

CNV analysis of 80 VCI patients (v.1.0 and v.2.0 cohorts) identified nine rare autosomal CNVs that were all classified as being of unknown significance (Table 3). Two of the CNVs were detected in patients for whom WES analysis did not reveal any potential cause of disease (patients 236, 289) in our previous study.¹¹ Three CNVs were detected in patients who had possibly causative variants identified by WES (patients 1029, 1033, and 1039) in this study. CNVs classified as likely benign or benign in the analysis are presented in Table S6.

4 | DISCUSSION

Genetic causes for many familial VCI cases remain unclear. Our previous study examined 35 Finnish VCI patients (v. 1.0 cohort) in an attempt to identify novel gene variants underlying VCI. In the present

study, we continued the research of the genetics of VCI by studying the second part of the Finnish cohort with well-characterized clinical features (v. 2.0 cohort) using WES. The present study resulted in identification of several variants possibly affecting function in genes linked to CSVD, stroke, or other neurological conditions, which is in line with our previous study.¹¹ We also performed CNV analysis using a SNP microarray on all the exome-sequenced patients (v. 1.0 and v. 2.0 cohorts). In this analysis, nine patients (11%) had a rare CNV, which were of unknown significance. These results indicate that CNVs are not a common cause of VCI. However, although GSAMD is useful for screening CNVs, high-resolution microarrays may be better for the detection of smaller CNVs, which may have been missed in our analysis. In this study, we also screened 73 VCI patients (from v. 2.0 cohort) for variants in the miR-29 microRNA-binding site at the 3'UTR of COL4A1 and identified a new heterozygous variant, c.*36T>A. The finding supports the previous studies of the pathogenic role of variants in this genetic region in CSVD.^{6,7}

Diagnosing of VCI and distinguishing it from other forms of dementia may be challenging. As in our previous study, most of the subjects from our initial NOTCH3-negative cohort were later diagnosed with another disease than CSVD such as Alzheimer's disease, multiple sclerosis, or FTD-ALS (Table S2) indicating the importance of thorough clinical characterization of this kind of study cohorts. Some of the CSVD patients possibly represent sporadic cases or cases with *de novo* mutations, as only half of the cohort was recorded to have positive family history. The amount of available clinical data varied between patients, which may have caused bias in the selection and categorization of study subjects, mainly regarding cases where clinical information was limited. Furthermore, we were not able to analyze the segregation of the detected variants or CNVs, because samples from family members of the patients were not available. Due to the small cohort size, we focused the analysis on relatively rare variants, possibly missing risk variants that are more frequent in the population. Many of the

patients carried variants in more than one gene, which may indicate an oligogenic or polygenic basis of disease. Environmental risk factors and vascular risk factors also have an impact on the pathogenesis of VCI, which may partially explain the cases that remained negative in our analysis. Indeed, multifactorial VCI influenced by several genetic risk variants together with environmental factors is considered much more common than monogenic conditions causing VCI, but identifying those risk variants requires very large sample sizes. Nevertheless, a significant portion of VCI cases seems to run in families and studying these cases may provide novel insights into molecular processes underlying CSVD and VCI.

In conclusion, the present data on the genetic background of VCI provide further evidence for the view that vascular pathology may be associated with neurodegeneration in VCI. Our findings also support the pathogenic roles of variants in *HTRA1*, *TREX1* and in the 3'UTR of *COL4A1* in CSVD. Other CSVD/dementia-linked genes besides *NOTCH3* are worth examining when diagnosing VCI and CSVD. In addition, our results also indicate that CNVs are not a common cause of VCI. Further research is needed to determine the pathogenicity of the detected variants.

CONFLICT OF INTEREST

None of the authors has any conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

SM involved in design of the study, clinical data and sample collection, WES and CNV analyses, Sanger sequencing, interpretation of the data, and preparation of the first draft of the study, and revised the manuscript. LK involved in design of the study and interpretation of the data, and drafted and revised the manuscript. JS involved in clinical data and sample collection, and drafted and revised the manuscript. JB drafted and revised the manuscript. SR involved in clinical data collection, and drafted and revised the manuscript. MP involved in design of the study and interpretation of the data, and drafted and revised the manuscript. RG and LM involved in design and supervision of the study and interpretation of the data, and drafted and revised the manuscript.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ane.13613>.

DATA AVAILABILITY STATEMENT

Additional data are available from the corresponding author upon reasonable request. The variants reported here are submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>).

ORCID

Saana Mönkäre  <https://orcid.org/0000-0001-7703-8101>

REFERENCES

- Dichgans M, Leys D. Vascular cognitive impairment. *Circ Res*. 2017;120(3):573-591. doi:10.1161/CIRCRESAHA.116.308426
- O'Brien JT, Thomas A. Vascular dementia. *Lancet*. 2015;386(10004):1698-1706. doi:10.1016/S0140-6736(15)00463-8
- Pasi M, Cordonnier C. Clinical relevance of cerebral small vessel diseases. *Stroke*. 2020;51(1):47-53. doi:10.1161/STROKEAHA.119.024148
- Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*. 2007;69(24):2197. doi:10.1212/01.wnl.0000271090.28148.24
- Marini S, Anderson CD, Rosand J. Genetics of cerebral small vessel disease. *Stroke*. 2020;51(1):12-20. doi:10.1161/STROKEAHA.119.024151
- Siitonen M, Börjesson-Hanson A, Pöyhönen M, et al. Multi-infarct dementia of Swedish type is caused by a 3'UTR mutation of *COL4A1*. *Brain*. 2017;140(5):e29. doi:10.1093/brain/awx062
- Verdura E, Herve D, Bergametti F, et al. Disruption of a miR-29 binding site leading to *COL4A1* upregulation causes pontine autosomal dominant microangiopathy with leukoencephalopathy. *Ann Neurol*. 2016;80(5):741-753. doi:10.1002/ana.24782
- Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol Genomics*. 2012;44(4):237-244. doi:10.1152/physiolgenomics.00141.2011
- Jeanne M, Gould DB. Genotype-phenotype correlations in pathology caused by collagen type IV alpha 1 and 2 mutations. *Matrix Biol*. 2017;57-58:29-44. doi:10.1016/j.matbio.2016.10.003
- Grond-Ginsbach C, Erhart P, Chen B, Kloss M, Engelter ST, Cole JW. Copy number variation and risk of stroke. *Stroke*. 2018;49(10):2549-2554. doi:10.1161/STROKEAHA.118.020371
- Mönkäre S, Kuuluvainen L, Kun-Rodrigues C, et al. Whole-exome sequencing of Finnish patients with vascular cognitive impairment. *Eur J Hum Genet*. 2021;29:663-671. doi:10.1038/s41431-020-00775-9
- Patel ZH, Kottyan LC, Lazaro S, et al. The struggle to find reliable results in exome sequencing data: filtering out Mendelian errors. *Front Genet*. 2014;5:16. doi:10.3389/fgene.2014.00016
- Ilinca A, Samuelsson S, Piccinelli P, Soller M, Kristoffersson U, Lindgren AG. A stroke gene panel for whole-exome sequencing. *Eur J Hum Genet*. 2019;27(2):317-324. doi:10.1038/s41431-018-0274-4
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30
- Wang K, Li M, Hadley D, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. *Genome Res*. 2007;17(11):1665-1674. doi:10.1101/gr.6861907
- Oluwole OJ, Ibrahim H, Garozzo D, et al. Cerebral small vessel disease due to a unique heterozygous *HTRA1* mutation in an African man. *Neurol Genet*. 2019;6(1):e382. doi:10.1212/NXG.000000000000382
- Hara K, Shiga A, Fukutake T, et al. Association of *HTRA1* mutations and familial ischemic cerebral small-vessel disease. *N Engl J Med*. 2009;360(17):1729-1739. doi:10.1056/NEJMoa0801560
- Verdura E, Herve D, Scharrer E, et al. Heterozygous *HTRA1* mutations are associated with autosomal dominant cerebral small vessel disease. *Brain*. 2015;138(Pt 8):2347-2358. doi:10.1093/brain/awv155
- Nozaki H, Kato T, Nihonmatsu M, et al. Distinct molecular mechanisms of *HTRA1* mutants in manifesting heterozygotes with *CARASIL*. *Neurology*. 2016;86(21):1964-1974. doi:10.1212/WNL.0000000000002694
- Uemura M, Nozaki H, Kato T, et al. *HTRA1*-related cerebral small vessel disease: a review of the literature. *Front Neurol*. 2020;1:545. doi:10.3389/fneur.2020.00545

21. Lee-Kirsch MA, Gong M, Chowdhury D, et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat Genet.* 2007;39(9):1065-1067. doi:10.1038/ng2091
22. Namjou B, Kothari PH, Kelly JA, et al. Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. *Genes Immun.* 2011;12(4):270-279. doi:10.1038/gene.2010.73
23. Pelzer N, de Vries B, Boon EMJ, et al. Heterozygous TREX1 mutations in early-onset cerebrovascular disease. *J Neurol.* 2013;260(8):2188-2190. doi:10.1007/s00415-013-7050-8
24. Stam AH, Kothari PH, Shaikh A, et al. Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations. *Brain.* 2016;139(11):2909-2922. doi:10.1093/brain/aww217
25. Rahman S, Copeland WC. POLG-related disorders and their neurological manifestations. *Nat Rev Neurol.* 2019;15(1):40-52. doi:10.1038/s41582-018-0101-0
26. Miyatake S, Schneeberger S, Koyama N, et al. Biallelic COLGALT1 variants are associated with cerebral small vessel disease. *Ann Neurol.* 2018;84(6):843-853. doi:10.1002/ana.25367
27. Schmitz M, Dittmar K, Llorens F, et al. Hereditary human prion diseases: an update. *Mol Neurobiol.* 2017;54(6):4138-4149. doi:10.1007/s12035-016-9918-y
28. Windl O, Giese A, Schulz-Schaeffer W, et al. Molecular genetics of human prion diseases in Germany. *Hum Genet.* 1999;105(3):244-252. doi:10.1007/s004399900124
29. Guizzunti G, Zurzolo C. The fate of PrP GPI-anchor signal peptide is modulated by P238S pathogenic mutation. *Traffic.* 2014;15(1):78-93. doi:10.1111/tra.12126
30. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. *Am J Hum Genet.* 1991;48(3):536-545.
31. Froese DS, Huemer M, Suormala T, et al. Mutation update and review of severe methylenetetrahydrofolate reductase deficiency. *Hum Mutat.* 2016;37(5):427-438. doi:10.1002/humu.22970
32. Cajavilca CE, Gadhia RR, Román GC. MTHFR gene mutations correlate with white matter disease burden and predict cerebrovascular disease and dementia. *Brain Sci.* 2019;9(9):211. doi:10.3390/brainsci9090211

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mönkäre S, Kuuluvainen L, Schleutker J, et al. Genetic analysis reveals novel variants for vascular cognitive impairment. *Acta Neurol Scand.* 2022;00:1-9. doi:[10.1111/ane.13613](https://doi.org/10.1111/ane.13613)