Genetic analysis reveals novel variants for vascular cognitive impairment

Saana Mönkäre1,2 | Liina Kuuluvainen3,4 | Johanna Schleutker2,5 | Jose Bras6,7 | Susanna Roine8 | Minna Pöyhönen1,9 | Rita Guerreiro6,7 | Liisa Myllykangas9,10

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

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
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). In clinical studies, vascular dementia is the second most common cause of dementia.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 Vascular dementia can be present with other dementia disorders such as Alzheimer’s disease, 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 described 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 dementia and pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL). 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. Variants in the 3′UTR of COL4A1 were shown to disrupt the same miR-29 binding site and consequently cause upregulation of COL4A1. 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, 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, patient 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 ≤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 stroke-gene panel,
### TABLE 1  Possibly causative variants detected in sequence analyses

| Patient | Gene | Nucleotide change | Amino acid change | Zygocity | RefSeq | Allele frequency (gnomAD total) | Allele frequency (gnomAD Finnish) | CADD Phred | Phenotype MIM number |
|---------|------|-------------------|-------------------|----------|--------|-------------------------------|----------------------------------|-----------|---------------------|
| 1000²   | COL4A1 | c.*36T>A<sup>b</sup> | . | 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 | 0.0001315 | 0 | 33 | 618360 |
| 1007    | UBQLN2 | c.304A>G | p.(Ile102Val) | Het | NM_013444.4 | 0.000008916 | 0.0001623 | 25.6 | 300857 |
| 1015    | C1R | c.716G>A p.(Arg239Gln) | Het | NM_013444.4 | 0.000008916 | 0.0001623 | 25.6 | 130080 |
| 1016    | PRNP | c.713C>T | p.(Pro238Leu) | Het | NM_000311.5 | 0 | 0 | 23.9 | 617440, 123400, 603218, 600072, 606688 |
| 1017    | PCNT | c.2179C>G | p.(His727Asp) | Het | NM_006031.6 | 0 | 0 | 23.0 | 210720 |
| 1017    | VPS13A | c.9257T>C | p.(Gly354Ala) | Hom | NM_001330358.2 | 0 | 0 | 23.5 | 127750, 168601, 605543 |
| 1019    | SMAD4 | c.1060G>A | p.(Val354Met) | Het | NM_005359.5 | 0 | 0 | 26.1 | 175050 |
| 1020    | GRN2A | c.937A>G | p.(Ile313Val) | Het | NM_000833.5 | 0 | 0 | 22.4 | 245570 |
| 1025    | DIAP1 | c.1093T>C | p.(Phe365Leu) | Het | NM_005219.5 | 0.00007229 | 0.0007529 | 25.7 | 613254 |
| 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    | HTR1A | 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    | SNC2 | 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_00129835.2 | 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.(Asp740Asp) | 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).

²Sample 1000 was not exome-sequenced, COL4A1 variant c.*36T>A was detected by sanger sequencing.

³Although the COL4A1 variant c.*36T>A is of interest, pending further evidence and information, we classify it as a variant of unknown significance.
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 CADDISIL 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 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.21

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.24 Variants affecting function in HTRA1 cause cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) 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

| Patient | Gene | Variant | Zygocity | 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.9257C>G, 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.1093C>G, 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.
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, which has been shown to be associated with CSVD and cognitive impairment. The patient carrying the MTHFR variant c.1061G>C,
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  | chr:163104432–165045334                                             | 1,940,903 | PARK2, PACTRG, LOC401282, CAHM, QKI                                         |
| 289     | 48  | M      | Gain  | chr:15:94876580–95356210                                             | 479,631   | MCTP2                                                                         |
| 1005    | 60  | F      | Gain  | chr:7:19035920–20617266                                              | 1,581,347 | HDAC9, TWIST1, FERD3L, TWISTNB, TMEM196, MACC1, ITGB8                      |
| 1010    | 62  | M      | Gain  | chr:3:193061741–193467943                                            | 406,203   | ATP13A5, ATP13A4, OPA1                                                        |
| 1014    | 60  | M      | Gain  | chr:8:6155658–6391302                                                 | 235,645   | ANGPT2, MCHP1, AGPAT5                                                         |
| 1029    | 63  | F      | Loss  | chr:16:15491006–16258173                                             | 767,168   | MPV17L, BMERB1, MARF1, NDE1, MYH11, CEP20, ABCC1, ABCC6                    |
| 1031    | 55  | F      | Loss  | chr:2:133949948–134070417                                             | 120,47    | NCKAP5                                                                        |
| 1033    | 50  | F      | Gain  | chr:1:92293721–92574940                                               | 281,22    | TGFR3, BRDT, EPHX4, BTBD8                                                     |
| 1039    | 27  | M      | Loss  | chr:12:43937166–44009983                                              | 72,818    | ADAMTS20                                                                     |

Note: Genome assembly: GRCh37/hg19.
Abbreviation: AAO, age at onset; bp, base pair.
*The region between the two duplications in 8p23.1 was not covered in the GSAMD array.

Table 3: Rare heterozygous CNVs of interest identified in patients with VCI

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. 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. 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. The finding supports the previous studies of the pathogenic role of variants in this genetic region in CSVD. 

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. MP involved in design of the study and interpretation of the data, and drafted and revised the manuscript. MP involved in design of the study and interpretation of the data, and drafted and revised the manuscript. MP 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
1. Dichgans M, Leys D. Vascular cognitive impairment. Circ Res. 2017;120(3):573-591. doi:10.1161/CIRCRESAHA.116.308426
2. O’Brien JT, Thomas A. Vascular dementia. Lancet. 2015;386(10004):1698-1706. doi:10.1016/S0140-6736(15)00463-8
3. Pasi M, Cordonnier C. Clinical relevance of cerebral small vessel diseases. Stroke. 2020;51(1):47-53. doi:10.1161/STROKEAHA.119.024148
4. Schneider JA, Arvaniatikis 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
5. Marini S, Anderson CD, Rosand J. Genetics of cerebral small vessel disease. Stroke. 2020;51(1):12-20. doi:10.1161/STROKEAHA.119.024151
6. Sitonon 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
7. 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
8. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: genetics, cell biology, and relevance to renal and cardiovascular injury. Physiol Genomics. 2012;44(4):237-244. doi:10.1152/physiogenomics.00141.2011
9. Jeanne M, Gould DB. Genotype-phenotype correlations in pathologies 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
10. 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
11. 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
12. 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
13. 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
14. 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
15. 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
16. 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;5(1):e382. doi:10.1212/NXG.0000000000000382
17. Haras 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
18. 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
19. 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

20. 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/s0043999000124

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, Gaditia 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;146:42-50. doi:10.1111/ane.13613