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ORIGINAL ARTICLE

# Genetic analysis reveals novel variants for vascular cognitive impairment

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## 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.

## 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 described[5] 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[6] 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 *(COL4)A1/2*-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 ≤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 |
|---|---|---|---|---|---|---|---|---|---|
| 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.(His727Asp) | 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).

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

[b]Although 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 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 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 disease[28] 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

**TABLE 2** Clinical details associated with the possibly causative sequence variants

| 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.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 dementiaVCI, vascular cognitive impairment.

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

| 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 | Hyperchlolesterolaemia, 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 |

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,

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.

[a]The region between the two duplications in 8p23.1 was not covered in the GSAMD array.

p.(Gly354Ala) had multiple strokes and was diagnosed with VaD. Brain MRI showed white matter disease and multiple infarcts.

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/).

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## SUPPORTING INFORMATION

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

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