



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

## Rare neurovascular genetic and imaging markers across neurodegenerative diseases

### Citation for published version:

ONDRI Investigators, Dilliot, AA, Berberian, SA, Sunderland, KM, Binns, MA, Zimmer, J, Ozzoude, M, Scott, CJM, Gao, F, Lang, AE, Breen, DP, Tartaglia, MC, Tan, B, Swartz, RH, Rogaeva, E, Borrie, M, Finger, E, Fischer, CE, Frank, A, Freedman, M, Kumar, S, Pasternak, S, Pollock, BG, Rajji, TK, Tang-Wai, DF, Abrahao, A, Turnbull, J, Zinman, L, Casaubon, L, Dowlathahi, D, Hassan, A, Mandzia, J, Sahlas, D, Saposnik, G, Grimes, D, Marras, C, Steeves, T, Masellis, M, Farhan, SMK, Bartha, R, Symons, S, Hegele, RA, Black, SE & Ramirez, J 2023, 'Rare neurovascular genetic and imaging markers across neurodegenerative diseases', *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*.  
<https://doi.org/10.1002/alz.13316>

### Digital Object Identifier (DOI):

[10.1002/alz.13316](https://doi.org/10.1002/alz.13316)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Publisher's PDF, also known as Version of record

### Published In:

Alzheimer's & Dementia: The Journal of the Alzheimer's Association

### General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

### Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact [openaccess@ed.ac.uk](mailto:openaccess@ed.ac.uk) providing details, and we will remove access to the work immediately and investigate your claim.



## RESEARCH ARTICLE

## Rare neurovascular genetic and imaging markers across neurodegenerative diseases

Allison A. Dillio<sup>1</sup> | Stephanie A. Berberian<sup>2</sup> | Kelly M. Sunderland<sup>3</sup> |  
 Malcolm A. Binns<sup>3,4</sup> | Julia Zimmer<sup>1</sup> | Miracle Ozzoude<sup>2,5</sup> | Christopher J.M. Scott<sup>2</sup> |  
 Fuqiang Gao<sup>2</sup> | Anthony E. Lang<sup>6</sup> | David P. Breen<sup>7</sup> | Maria C. Tartaglia<sup>6,8</sup> |  
 Brian Tan<sup>3</sup> | Richard H. Swartz<sup>2,9,10</sup> | Ekaterina Rogava<sup>5</sup> | Michael Borrie<sup>11,12</sup> |  
 Elizabeth Finger<sup>11</sup> | Corinne E. Fischer<sup>13</sup> | Andrew Frank<sup>14,15</sup> | Morris Freedman<sup>3,16</sup> |  
 Sanjeev Kumar<sup>17,18</sup> | Stephen Pasternak<sup>19</sup> | Bruce G. Pollock<sup>17,18</sup> |  
 Tarek K. Rajji<sup>17,18,20</sup> | David F. Tang-Wai<sup>9</sup> | Agessandro Abrahao<sup>9</sup> | John Turnbull<sup>21</sup> |  
 Lorne Zinman<sup>9</sup> | Leanne Casaubon<sup>9</sup> | Dar Dowlatshahi<sup>14</sup> | Ayman Hassan<sup>22</sup> |  
 Jennifer Mandzia<sup>11</sup> | Demetrios Sahlas<sup>21</sup> | Gustavo Saposnik<sup>23,24</sup> | David Grimes<sup>14</sup> |  
 Connie Marras<sup>6</sup> | Thomas Steeves<sup>24</sup> | Mario Masellis<sup>2,9</sup> | Sali M.K. Farhan<sup>1</sup> |  
 Robert Bartha<sup>19</sup> | Sean Symons<sup>25</sup> | Robert A. Hegele<sup>19</sup> | Sandra E. Black<sup>2,3,9,10</sup> |  
 Joel Ramirez<sup>2</sup>  | ONDRI Investigators

## Correspondence

Allison A. Dillio, Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, McGill University, Irving Ludmer Building, 1033 Pine Ave W, Montréal, Quebec, H3A 1A1 Canada.  
 Email: [allison.dillio@mcgill.ca](mailto:allison.dillio@mcgill.ca)

Joel Ramirez, LC Campbell Cognitive Neurology, Sunnybrook Research Institute, 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5, Canada.  
 Email: [joel.ramirez1@sunnybrook.ca](mailto:joel.ramirez1@sunnybrook.ca)

## Abstract

**Introduction:** Cerebral small vessel disease (SVD) is common in patients with cognitive impairment and neurodegenerative diseases such as Alzheimer's and Parkinson's. This study investigated the burden of magnetic resonance imaging (MRI)-based markers of SVD in patients with neurodegenerative diseases as a function of rare genetic variant carrier status.

**Methods:** The Ontario Neurodegenerative Disease Research Initiative study included 520 participants, recruited from 14 tertiary care centers, diagnosed with various neurodegenerative diseases and determined the carrier status of rare non-synonymous variants in five genes (*ABCC6*, *COL4A1/COL4A2*, *NOTCH3/HTRA1*).

**Results:** *NOTCH3/HTRA1* were found to significantly influence SVD neuroimaging outcomes; however, the mechanisms by which these variants contribute to disease progression or worsen clinical correlates are not yet understood.

**Discussion:** Further studies are needed to develop genetic and imaging neurovascular markers to enhance our understanding of their potential contribution to neurodegenerative diseases.

## KEYWORDS

HTRA1, neurodegeneration, NOTCH3, ONDRI, small vessel disease, WMH

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Alzheimer's & Dementia* published by Wiley Periodicals LLC on behalf of Alzheimer's Association.

## 1 | INTRODUCTION

Cerebral small vessel disease (SVD) represents a group of pathologies with heterogeneous etiology that affect the small arteries, veins, and capillaries of the brain's vascular system.<sup>1</sup> Notably, SVD most commonly leads to vascular cognitive impairment and dementia in an aging population. Additionally, SVD is associated with several markers that are quantifiable on structural magnetic resonance imaging (MRI), including white matter hyperintensities (WMH), MRI-visible perivascular spaces (PVS), and lacunes.<sup>2</sup> These observable neuroimaging features have been reported in various neurodegenerative diseases, including Alzheimer's disease (AD), mild cognitive impairment (MCI), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and frontotemporal dementia (FTD), as well as in cerebrovascular disease (CVD),<sup>3–10</sup> all of which are represented in the Ontario Neurodegenerative Disease Research Initiative (ONDRI). These diagnoses were selected based on their high prevalence within the aging population as well as the high rates of neuropsychological dysfunction reported in these patient populations. As the cases of dementia increase worldwide, one of the key objectives of ONDRI is to accurately identify the individuals at risk of developing dementia, with and without comorbidities with other neurodegenerative and/or neurovascular disorders, at an earlier stage. Therefore, these diagnoses were selected to align with this objective.

Previously, several rare nonsynonymous variants had been associated with vascular neuroimaging markers in various neurodegenerative diseases via linkage analyses and candidate gene sequencing of patient cohorts.<sup>11–17</sup> Specifically, five genes that were previously associated with SVD and vasculopathy include ATP Binding Cassette Subfamily C Member 6 (*ABCC6*), Collagen Type IV Alpha-I (*COL4A1*), Collagen Type IV Alpha-II (*COL4A2*), High-Temperature Requirement A Serine Peptidase 1 (*HTRA1*), and Notch Receptor 3 (*NOTCH3*). Under normal conditions, *ABCC6* encodes a protein that aids in the transportation of molecules across the cell membrane; however, when mutated, it can cause arterial calcification and pseudoxanthoma elasticum, an autosomal recessive disease primarily affecting the connective tissue in the skin, retina, and cardiovascular system, and more recently the gene has been associated with increased ischemic strokes.<sup>18,19</sup> *COL4A1* and *COL4A2* encode for collagen chain proteins that combine and together play a critical role in supporting the basement membranes of human vasculature.<sup>20</sup> Autosomal dominant *COL4A1* mutations cause *COL4A1*-related SVD, which presents as early strokes and brain cysts,<sup>21</sup> whereas autosomal dominant *COL4A2* mutations cause intracerebral hemorrhage and SVD, which result in neural degeneration.<sup>22</sup> *NOTCH3* encodes a transmembrane protein receptor critical for the survival and function of vascular smooth muscle cells; however, when mutated it is associated with a monogenic form of SVD commonly known as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).<sup>17</sup> Similarly, *HTRA1* encodes a protein involved with cell signaling, musculoskeletal development, vascular maturation, and protein degradation; however, when mutated it is associated with a phenotype similar to CADASIL, known as cerebral autosomal recessive arteriopathy with

### RESEARCH IN CONTEXT

- 1. Systematic Review:** Burden of white matter hyperintensities, perivascular spaces, and lacunes were quantified in a study examining 520 participants diagnosed with Alzheimer's disease, mild cognitive impairment, amyotrophic lateral sclerosis, Parkinson's disease, frontotemporal dementia, or cerebrovascular disease. Carrier status of rare non-synonymous variants in *ABCC6*, *COL4A1*, *COL4A2*, *HTRA1*, and *NOTCH3* was determined for each study participant, and multiple regression models were used to estimate effects across the neuroimaging markers as a function of variant carrier status.
- 2. Interpretation:** We observed a significant influence of rare variants in *HTRA1* and *NOTCH3* on neuroimaging markers of cerebral small vessel disease in a variety of neurodegenerative disease patient cohorts.
- 3. Future directions:** Our study highlights the importance of genetic analysis in understanding the development of vascular injury and the potential contribution to neurodegenerative disease, yet future work must determine the biological mechanisms of these associations and their influence on downstream clinical outcomes.

subcortical infarcts and leukoencephalopathy (CARASIL).<sup>23</sup> Although the compendium of SVD-associated genes continues to grow, these five largely remain a consensus of the most well-established genes of interest.<sup>24</sup>

The primary aim of this study was to examine the burden of MRI-based SVD markers (WMH, PVS, and lacunes) as a function of carrier status of non-synonymous, rare genetic variants of interest in a sample of patients with neurodegenerative diseases that are represented in the ONDRI study. In light of these previous findings in genetics and neuroimaging, we hypothesized that carrying rare genetic variation in SVD-associated genes described would contribute to greater burdens of SVD markers in both neurodegenerative disease and CVD patients.

## 2 | METHODS

### 2.1 | Participants

ONDRI recruited 520 participants from 14 tertiary care centers across Ontario, Canada, each of whom had passed preliminary screening. Every participant was previously diagnosed with one of five neurodegenerative diseases: (1) AD; (2) amnesic MCI; (3) ALS; (4) FTD; (5) Parkinson's disease (PD); or (6) cerebrovascular disease (CVD) with or without cognitive impairment. Detailed inclusion/exclusion criteria, demographics, and cohort characteristics of the ONDRI study were published previously.<sup>25,26</sup> Briefly, the prevailing consensus-based

clinical diagnostic criteria at the time of enrollment for each ONDRI-focused disease were implemented at each tertiary clinic: AD/MCI patients met National Institute on Aging–Alzheimer's Association criteria for probable or possible AD or MCI, where amnesic MCI patients were tracked annually to ensure accurate characterization of the cohort<sup>25</sup>; PD patients met the criteria for idiopathic PD defined by the UK's Parkinson's Disease Society Brain Bank clinical diagnostic criteria; ALS patients met El Escorial World Federation of Neurology diagnostic criteria for possible, probable, or definite familial or sporadic ALS; FTD patients included possible or probable behavioral variants of frontotemporal degeneration, agrammatic/non-fluent and semantic variants of primary progressive aphasia, and possible or probable progressive supranuclear palsy. The CVD cohort comprised of individuals who experienced mild to moderate acute ischemic stroke, transient ischemic attack, or subcortical infarction, at least 3 months prior to enrollment, in compliance with the National Institute of Neurological Disorders and Stroke and the Canadian Stroke Network vascular cognitive impairment harmonization standards. Evidence of stroke required clinical imaging confirmation; however, individuals with large vessel occlusive infarction causing severe neurological deficits were excluded. Additionally, the AD/MCI patients' MRI scans were assessed by a neuroradiologist to exclude individuals with non-AD-related causes for cognitive impairment. Ethics approval was obtained from the Research Ethics Board at each participating site. All participants provided written, informed consent.

## 2.2 | Gene sequencing and variant prioritization

Of the 520 participants recruited by ONDRI, 519 had a blood sample collected, and genomic DNA was extracted from them as previously described.<sup>27</sup> All participant DNA samples were then sequenced with the custom-designed next-generation sequencing (NGS) gene panel, ONDRISeq, which covers the exonic regions of 80 genes, each previously associated with a neurodegenerative disease or SVD phenotype.<sup>28</sup> Briefly, all genomic DNA samples were sequenced using ONDRISeq on the Illumina MiSeq Personal Genome Sequencer (Illumina, San Diego, CA, USA). A custom bioinformatics pipeline was subsequently used to process the FASTQ files and produce a variant calling format (VCF) file and binary alignment map (BAM) file for each participant.<sup>27,29</sup>

Generated VCF files for each ONDRI participant were annotated using VarSeq® (Golden Helix, Bozeman, MT, USA). Annotations included sequence ontologies, minor allele frequencies (MAFs) from the Genome Aggregation Database (gnomAD; version 3.1), in silico prediction scores from Combined Annotation Dependent Depletion (CADD, version 1.3), and ClinVar pathogenicity classifications. The variant data were then filtered to include only those within genes on ONDRISeq that had been previously associated with SVD or neurovasculopathy, namely, *ABCC6*, *COL4A1*, *COL4A2*, *HTRA1*, *NOTCH3*, *SAMHD1*, and *TREX1*. Of the variants in these genes, we identified those considered non-synonymous, including missense variants, frameshift insertions or deletions, non-frameshift insertions or dele-

tions, nonsense variants, and splicing variants. Variants were further prioritized to identify those most likely to be deleterious, first by identifying those considered rare in the general population (MAF < 0.01, gnomAD version 3.1), then by selecting variants with a CADD score  $\geq$  20 (top 1% of deleterious variants in the human genome)<sup>30</sup> or variants that had been classified as pathogenic or likely pathogenic in ClinVar. All prioritized variants are hereafter referred to as "non-synonymous, rare variants of interest." The genes *SAMHD1* and *TREX1* were eliminated from further analysis, as no non-synonymous, rare variants of interest were identified in *SAMHD1* and only two non-synonymous, rare variants of interest were identified in *TREX1*, each carried by only a single ONDRI participant.

Based on the similar molecular pathways and disease associations of the genes under study and to preserve statistical power due to our modest sample size, rare, non-synonymous variants of interest identified in *COL4A1* or *COL4A2* were binned, as were variants of interest identified in *NOTCH3* or *HTRA1*. Participants carrying a non-synonymous, rare variant of interest in at least one of *ABCC6*, *COL4A1/COL4A2*, or *NOTCH3/HTRA1* were considered variant positive, while those not carrying a variant in at least one a gene bin were considered variant negative.

All ONDRI participants were also assessed for the *APOE* genotype using the ONDRISeq data by extracting calls for the *APOE* variants rs429358(CT):p.Cys130Arg and rs7412(CT):p.Arg176Cys and mapping to the respective genotype, as previously described.<sup>31</sup> Any participant carrying at least one copy of either the  $\epsilon$ 2 or  $\epsilon$ 4 genotype were considered *APOE* variant positive, as both variants have been previously shown to increase the presence of SVD markers, including WMH burden.<sup>32–34</sup>

## 2.3 | Neuroimaging

Of the 520 ONDRI study participants, 513 had usable 3 Tesla MRI that included the following sequences: three-dimensional (3D) T1-weighted (T1), interleaved proton density and T2-weighted (T2), and T2-fluid attenuated inversion recovery (FLAIR). Imaging protocol details were published previously.<sup>35</sup> All MRI images were evaluated to ensure excellent imaging quality by a medical biophysicist (R.B.) and for clinical incidental findings by a licensed neuroradiologist (S.S.). Neuroimaging-based markers for cerebral SVD (WMH, PVS, lacunes) were quantified using ONDRI's standardized image-processing pipeline<sup>36</sup> in compliance with the STRIVE criteria.<sup>2</sup> Following initial segmentation, PVS and lacunar counts were generated using a 3D, six-connected voxel connectivity contour segmentation algorithm.<sup>37</sup>

## 2.4 | Statistical analyses

Of the 513 ONDRI participants with usable 3 Tesla MRI and 519 ONDRI participants that underwent genetic testing, 512 had both imaging and genetics data available. However, two participants' imaging segmentation failed quality control of the aforementioned

**TABLE 1** Participants in ONDRI carrying non-synonymous, rare variants of interest in CVD-associated genes.

Cohort	Sample size	ABCC6 variant positive	COL4A1/COL4A2 variant positive	HTRA1/NOTCH3 variant positive
ONDRI	510	27 (5.3%)	28 (5.5%)	42 (8.2%)
AD/MCI	126	5 (4.0%)	8 (6.3%)	8 (6.3%)
ALS	38	1 (2.6%)	2 (5.3%)	3 (7.9%)
FTD	52	1 (1.9%)	3 (5.8%)	3 (5.8%)
PD	139	6 (4.3%)	10 (7.2%)	15 (10.8%)
CVD	155	14 (9.0%)	5 (3.2%)	13 (7.9%)

Abbreviations: AD/MCI, Alzheimer's disease/mild cognitive impairment; ALS, amyotrophic lateral sclerosis; CVD, cerebrovascular disease with or without cognitive impairment; FTD, frontotemporal dementia; ONDRI, Ontario Neurodegenerative Disease Research Initiative; PD, Parkinson's disease.

image-processing pipeline, resulting in a final ONDRI total cohort size of 510 used in our analyses.

Multiple regression models were used to estimate effects across the three neuroimaging-based markers of SVD (PVS, lacunes, and WMH) as a function of carrier status of rare, non-synonymous variants of interest in each of the gene bins (*ABCC6*, *COL4A1/COL4A2*, and *HTRA1/NOTCH3*) and of disease cohort (AD/MCI, ALS, FTD, PD, CVD), while also accounting for interactions between the gene bins and disease cohort.

The individual coefficient estimates were used to determine the extent to which predictor variables contributed to each neuroimaging metric. We applied negative binomial generalized linear models for the outcome variables that were counts, namely, PVS counts and lacune counts, whereas we applied Gamma log distribution generalized linear models for the continuous variable of WMH volume. All multiple regression models were adjusted for age, sex, supratentorial total intracranial volume (head size), smoking history, and *APOE* carrier status.

To maximize statistical power based on our modest sample sizes, participants diagnosed with AD and participants diagnosed with MCI were binned into a single AD/MCI cohort. Participant disease cohorts were transformed using a weighted effect coding with the *wec* R package version 0.4-1,<sup>38</sup> which adjusts the point of reference for the dataset to be the sample mean of all participants, regardless of cohort. However, one cohort needed to be excluded from the model coding to avoid statistical redundancy. As the ALS cohort was reported to have the least amount of SVD pathology anecdotally, it was chosen as the remainder cohort. The model was rerun using FTD as the omitted cohort to obtain coefficient estimates for the ALS cohort that could be included in the visualizations of the results.<sup>39</sup>

To adjust for multiple comparisons, corrected *p* values were calculated using the Benjamini–Hochberg false discovery rate (FDR) method.<sup>40</sup> Significance for the multiple regression models was then measured at an alpha level of 0.05.

Statistical estimates were calculated using R statistical software version 3.6.0<sup>41</sup> in R Studio 1.1.463, and data visualization was performed using the *ggplot2* R package version 3.3.s.<sup>42</sup>

### 3 | RESULTS

#### 3.1 | Study participants and variant prioritization

In total, after quality control evaluation, genomic and neuroimaging data were available for 510 ONDRI participants (AD/MCI = 126, ALS = 38, FTD = 52, PD = 139, CVD = 155), of which 104 carried a non-synonymous, rare variant of interest in at least one of the genes: *ABCC6*, *COL4A1*, *COL4A2*, *HTRA2*, *NOTCH3* (Table 1). Of the genes examined in this study, 14 unique variants were found in *ABCC6*, 11 unique variants were found in *COL4A1*, nine unique variants were found in *COL4A2*, six unique variants were found in *HTRA1*, and 21 unique variants were found in *NOTCH3* (Supplemental Table S1). All variants identified were of heterozygous zygosity. *HTRA1/NOTCH3* non-synonymous, rare variants of interest were found at the greatest frequency in the full ONDRI cohort, with the highest proportion of carriers in the PD cohort. Four participants were found to carry rare variants in multiple gene groupings, including two participants diagnosed with CVD carrying rare variants in *ABCC6* and *NOTCH3*, one participant diagnosed with PD carrying rare variants in *ABCC6* and *HTRA1*, and one participant diagnosed with FTD carrying rare variants in *COL4A2* and *NOTCH3*. Demographic and whole-brain volumetric comparisons of the ONDRI participants carrying a non-synonymous, rare variant of interest in a SVD-associated gene to non-carriers are displayed in Table 2.

Using multiple regression models, we assessed the contribution of variant carrier status in *ABCC6*, *COL4A1/COL4A2*, or *NOTCH3/HTRA1*; disease cohort; and the two predictor variables to the neuroimaging volumetrics PVS count, lacune count, and WMH volume. Including an interaction term between disease cohort and variant carrier status within the model allowed us to determine the influence of carrying a non-synonymous, rare variant of interest on participant neuroimaging volumetrics in each individual ONDRI disease cohort. Supplementary Figure S1 displays the magnitude of difference in the neuroimaging volumetrics of participants carrying non-synonymous, rare variants of interest in *ABCC6*, *COL4A1/COL4A2*, and *NOTCH3/HTRA1* in comparison to non-carriers across the full ONDRI cohort, as well as in each

**TABLE 2** Demographics and whole-brain volumetrics from magnetic resonance imaging comparison of ONDRI participants carrying and not carrying non-synonymous, rare variants of interest in a CVD-associated gene.

Descriptor	Total cohort	Variant carriers	Variant non-carriers	Effect size
Sample size	510	95	415	
<b>Demographics</b>				
Sex (male:female)	341:169	61:34	280:135	2.7e-2
Age (yr)	68.6 (7.7)	69.0 (7.2)	68.6 (7.9)	4.0e-4
Education (yr)	14.9 (2.9)	15.0 (2.8)	14.9 (3.0)	4.0e-4
MoCA	24.4 (3.4)	24.9 (3.2)	24.3 (3.4)	4.1e-3
Modified Rankin score	1.4 (0.9)	1.3 (0.8)	1.5 (0.9)	4.9e-3
<b>Whole-brain volumetrics</b>				
Supratentorial intracranial volume (cc)	1.3e6 (1.4e5)	1.2e6 (1.5e5)	1.3e6 (1.4e5)	3.0e-4
Normal-appearing white matter (cc)	4.1e5 (6.6e4)	4.0e5 (6.7e4)	4.1e5 (6.6e4)	2.2e-3
Normal-appearing gray matter (cc)	5.5e5 (5.5e4)	5.4e5 (6.0e4)	5.5e5 (5.4e4)	2.0e-4
Sulcal cerebrospinal fluid (cc)	2.5e5 (6.0e4)	2.5e5 (6.3e4)	2.5e5 (6.0e4)	2.0e-5
Ventricular cerebrospinal fluid (cc)	4.1e4 (2.3e4)	4.0e4 (2.2e4)	4.1e4 (2.3e4)	0.0
Periventricular WMH (mm <sup>3</sup> )	5.6e3 (8.4e3)	7.5e3 (1.1e4)	5.2e3 (7.5e3)	1.2e-2
Deep WMH (mm <sup>3</sup> )	6.5e2 (9.8e2)	7.3e2 (1.0e3)	6.3e2 (9.7e2)	1.4e-3
<b>APOE ε2 or ε4 carriers</b>	<b>222</b>	<b>44</b>	<b>178</b>	<b>2.7e-2</b>

Note: Values in table represent mean (standard deviation). Effect sizes were calculated using eta squared of an ANOVA model. A large effect size is defined as >0.8, whereas a small effect size is defined as <0.2. All effect sizes reported were considered small.

Abbreviations: APOE, apolipoprotein E; MoCA, Montreal Cognitive Assessment; WMH, white matter hyperintensities; yr, years.

individual disease cohort. To best illustrate these comparisons, disease effects were removed.

### 3.2 | Associations between *ABCC6*, *COL4A1*, and *COL4A2* and SVD burden

Carrying a non-synonymous, rare variant of interest in *ABCC6* or *COL4A1/COL4A2* was not found to significantly influence ONDRI participants' PVS counts, lacune counts, or WMH volume across the full ONDRI cohort (Supplemental Tables S2 and S3). Similarly, carrier statuses of rare variants of interest in *ABCC6* or *COL4A1/COL4A2* were not significantly associated with SVD markers in either the individual neurodegenerative disease cohorts or the CVD cohort.

### 3.3 | Associations between *HTRA1* or *NOTCH3* and SVD burden

In contrast, carrying a non-synonymous, rare variant of interest in *NOTCH3/HTRA1* was found to significantly influence brain imaging outcomes both across the full ONDRI cohort and within specific diagnostic cohorts (Table 3). More specifically, carriers of non-synonymous, rare variants of interest in *NOTCH3/HTRA1* displayed significantly higher WMH volumes, agnostic of participant diagnosis ( $p = 4.92e-03$ , FDR:  $p = 1.82e-02$ ; Figure 1A). Further, in participants diagnosed with CVD, carrying a rare variant of interest in *NOTCH3/HTRA1* was

associated with significantly increased WMH volume, although the result was no longer significant following multiple testing corrections ( $p = 4.94e-02$ , FDR:  $p = 1.30e-01$ ; Figure 1B). No associations of interest were observed between *NOTCH3/HTRA1* variant status and WMH volume in any of the individual neurodegenerative disease cohorts.

Interestingly, the directions of association of non-synonymous, rare variant of interest carrier status in *NOTCH3/HTRA1* were not uniform across the disease cohorts with respect to lacune count and PVS count. Although variant status was not associated with lacune counts or PVS counts agnostic of disease cohort compared to variant non-carriers (Figures 2A and 3A, respectively), variant carriers diagnosed with CVD displayed a significantly greater number of lacunes ( $p = 9.93e-03$ , FDR:  $p = 3.29e-02$ ; Figure 2B) and PVS ( $p = 5.93e-04$ , FDR:  $p = 2.77e-03$ ; Figure 3B). Similarly, *NOTCH3/HTRA1* variant carriers diagnosed with AD/MCI displayed significantly greater number of lacunes than non-carriers, but the result was not significant following multiple testing corrections ( $p = 4.35e-02$ , FDR:  $p = 1.25e-01$ ; Figure 2B). In contrast, *NOTCH3/HTRA1* variant carriers diagnosed with PD displayed significantly fewer lacunes than non-carriers ( $p = 1.38e-02$ , FDR:  $p = 4.45e-02$ ; Figure 2B), while carriers diagnosed with FTD displayed significantly fewer PVS, but again this result was not significant following multiple testing corrections ( $p = 2.35e-02$ , FDR:  $p = 7.04e-02$ ; Figure 3B). No additional significant associations were identified between non-synonymous, rare variant of interest carrier status in *NOTCH3/HTRA1* and brain volumetric outcomes in any of the individual disease cohorts.

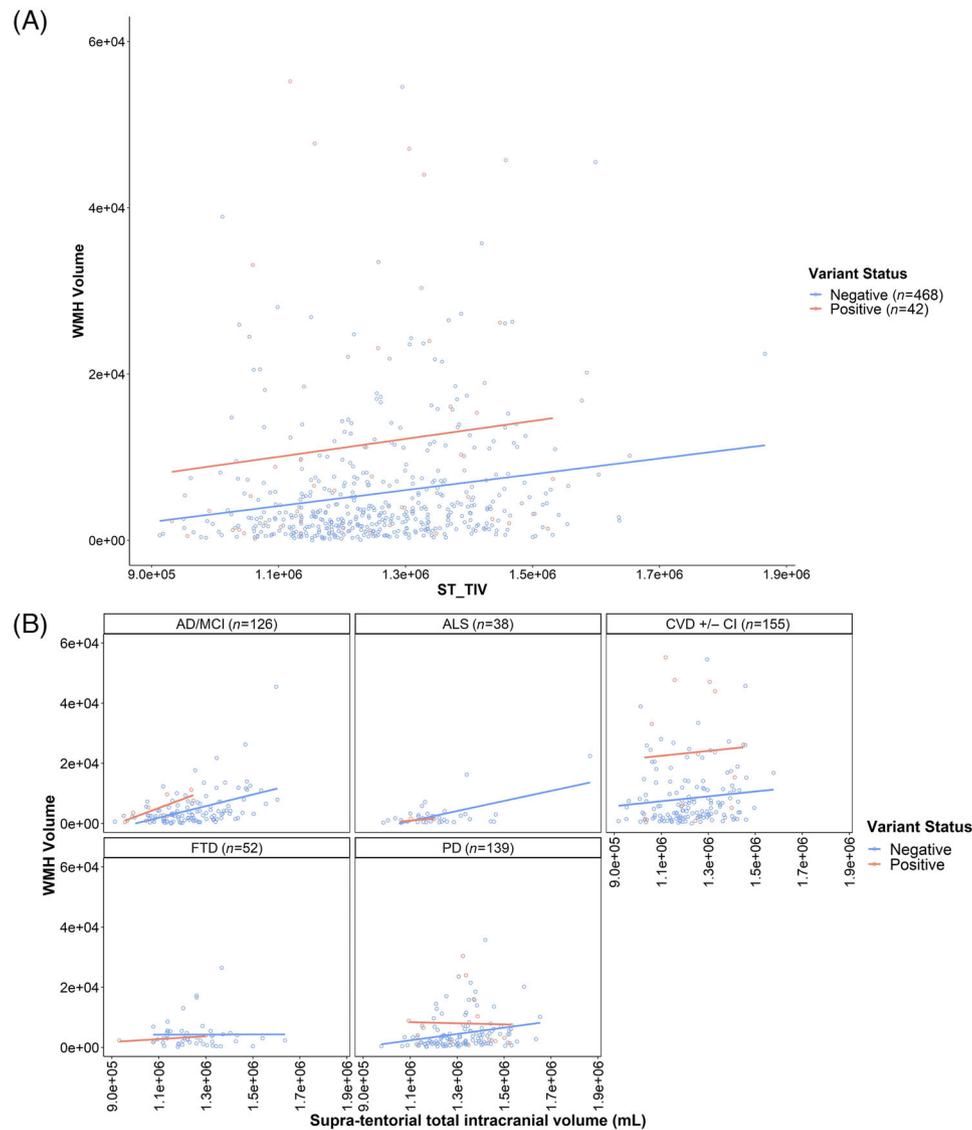
**TABLE 3** Results of multiple regression analyses used to analyze the contribution of variant carrier status in *NOTCH3/HTRA1* and disease cohort to neuroimaging metrics of PVS count, lacune count, and WMH volume.

	Lacune count				PVS count				WMH volume			
	Estimate	Standard deviation	P value	FDR p value	Estimate	Standard deviation	P value	FDR p value	Estimate	Standard deviation	P value	FDR p value
Intercept	-3.9	0.9	8.5e-06	7.7e-05	6.6e-02	0.5	8.9e-01	9.6e-01	1.6	0.6	4.9e-03	1.8e-02
<i>NOTCH3/HTRA1</i> Pos	-4.1e-02	0.3	8.9e-01	9.6e-01	0.2	0.1	9.3e-02	2.1e-01	0.5	0.2	4.3e-04	2.1e-03
AD/MCI	-0.2	0.1	1.5e-01	3.2e-01	9.3e-02	6.7e-02	1.7e-01	3.5e-01	-0.3	8.3e-02	1.2e-04	8.2e-04
<i>NOTCH3/HTRA1</i> Pos x AD/MCI	1.0	0.5	4.4e-02	1.3e-01	-0.3	0.3	3.2e-01	5.4e-01	-8.6e-02	0.3	8.0e-01	8.9e-01
CVD	0.3	0.1	6.4e-03	2.4e-02	0.2	5.8e-02	4.4e-03	1.7e-02	0.6	7.2e-02	4.5e-14	9.4e-13
<i>NOTCH3/HTRA1</i> Pos x CVD	1.0	0.4	9.9e-03	3.3e-02	0.7	0.2	5.9e-04	2.8e-03	0.5	0.2	4.9e-02	1.3e-01
FTD	-8.6e-02	0.2	6.9e-01	8.4e-01	-3.1e-02	0.1	7.8e-01	9.1e-01	-0.1	0.1	4.3e-01	6.2e-01
<i>NOTCH3/HTRA1</i> Pos x FTD	-0.9	1.2	4.5e-01	6.3e-01	-1.2	0.5	2.4e-02	7.0e-02	-0.6	0.6	3.2e-01	5.2e-01
PD	-0.4	0.1	4.3e-03	1.7e-02	-0.1	6.6e-02	9.4e-02	2.1e-01	-0.2	8.1e-02	1.3e-02	4.0e-02
<i>NOTCH3/HTRA1</i> Pos x PD	-1.1	0.4	1.4e-02	4.5e-02	-0.2	0.2	3.0e-01	5.2e-01	0.1	0.2	6.1e-01	8.0e-01
ALS	0.9	0.2	-	-	-0.5	0.1	-	-	-0.3	0.2	-	-
<i>NOTCH3/HTRA1</i> Pos x ALS	-0.5	0.9	-	-	-6.9e-02	0.5	-	-	-0.7	0.6	-	-

Note: Participant disease cohorts were transformed using weighted effect coding using the ALS cohort as the cohort that was not coded in the model. The model was rerun using the FTD cohort as the excluded model to obtain coefficient estimates for the ALS cohort, as displayed below the line. For the outcome variables that were counts, namely PVS counts and lacune counts, negative binomial generalized linear models were applied. For the continuous variable of WMH volume, Gamma log distribution generalized linear models were applied.

Significance for the multiple regression models was measured at an alpha level of 0.05, and significant results are indicated in bold.

Abbreviations: AD/MCI, Alzheimer's disease and mild cognitive impairment; ALS, amyotrophic lateral sclerosis; CVD, cerebrovascular disease with or without cognitive impairment; Dev., deviation; FTD, frontotemporal dementia; PD, Parkinson's disease; Pos, variant positive; PVS, MRI-visible perivascular spaces; WMH, white matter hyperintensities.



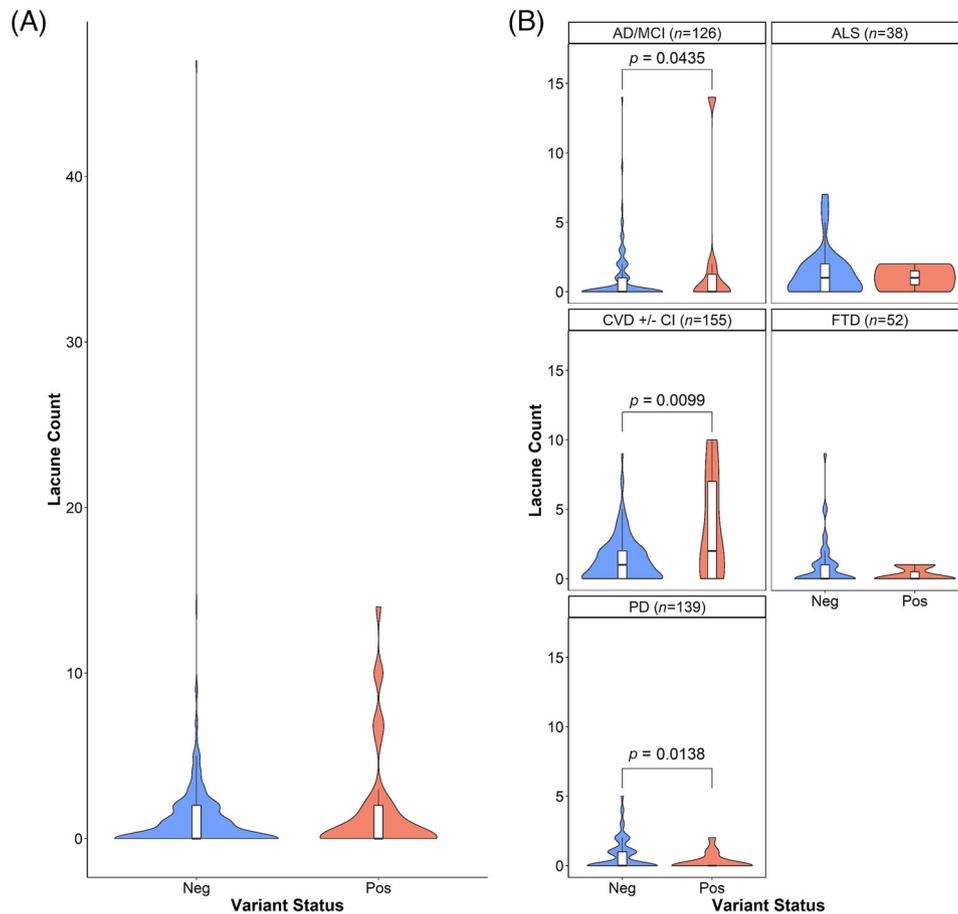
**FIGURE 1** White matter hyperintensity (WMH) volume in the ONDRI participants carrying *NOTCH3/HTRA1* rare, non-synonymous variants compared to variant negative participants. (A) WMH volumes compared to head size volume in ONDRI participants that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* and those that did not. (B) WMH volumes compared to head size volume of participants within each disease cohort of ONDRI that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* compared to those that did not. *P* values are presented for the interaction term of disease status and variant status from the multivariate regression analysis that modeled WMH volume as a function *NOTCH3* or *HTRA1* variant carrier status, disease cohort, and interactions between the two predictor variables, as well as adjustment for head size, age, smoking history, and *APOE*  $\epsilon$ 2 or  $\epsilon$ 4 variant status. To avoid statistical redundancy, the ALS cohort was not coded in the regression model. ST\_TIV, supratentorial total intracranial volume.

## 4 | DISCUSSION

Here we investigated the burden of MRI-based SVD markers, including lacunes, PVS, and WMH, in neurodegenerative disease patients carrying non-synonymous, rare variants in genes previously associated with cerebrovascular neuroimaging features – *ABCC6*, *COL4A1*, *COL4A2*, *HTRA1*, and *NOTCH3*. It is well established that neurodegenerative diseases, which are characterized by neuronal cell loss, display strong genetic components likely driving disease pathology.<sup>43,44</sup> However, in recent years it has become increasingly more accepted that the course and manifestation of neurodegenerative diseases are also influenced

by concurrent/comorbid cerebrovascular injury. In some cases, SVD may drive a neurodegenerative phenotype, as is the case with vascular dementia, whereas in other cases SVD can exist as a concurrent pathology or act as one of many contributors to disease presentation, such as in AD and PD.<sup>45–47</sup> In either scenario, it must be recognized that there may be unique genetic factors that can drive SVD markers, which therefore may also have relevance in neurodegenerative disease risk or features.

We determined that across all neurodegenerative disease and CVD patients, non-synonymous, rare variant carrier status in *ABCC6* or *COL4A1/COL4A2* did not significantly influence lacune count, PVS

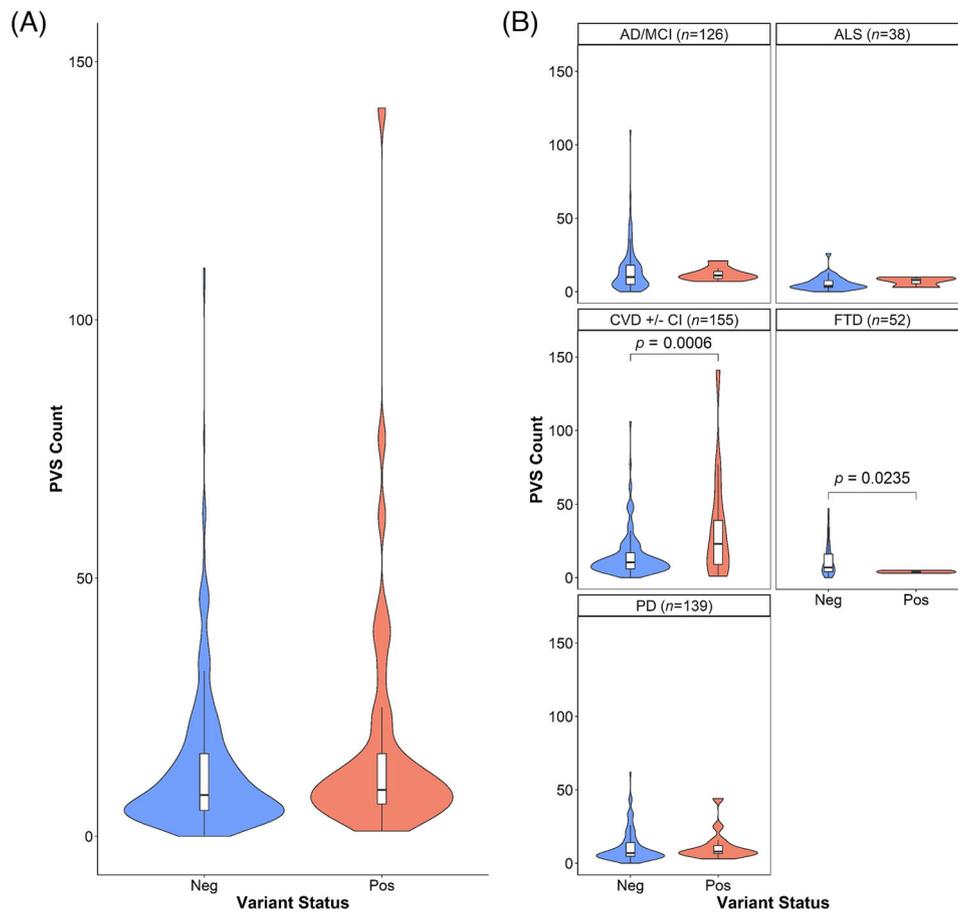


**FIGURE 2** Lacune counts in ONDRI participants carrying *NOTCH3/HTRA1* rare, non-synonymous variants compared to variant negative participants. (A) Distribution of lacune counts in ONDRI participants that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* compared to those that did not. (B) Distribution of lacune counts in each of the participants within each disease cohort of ONDRI that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* compared to those that did not. *P* values are presented for the interaction term of disease status and variant status from the multivariate regression analysis that modeled lacune count as a function of *NOTCH3* or *HTRA1* variant carrier status, disease cohort, and interactions between the two predictor variables, as well as adjustment for head size, age, smoking history, and *APOE*  $\epsilon 2$  or  $\epsilon 4$  variant status. To avoid statistical redundancy, the ALS cohort was not coded in the regression model. Indicated *P* values are uncorrected for multiple testing. Neg, variant negative; Pos, variant positive.

count, or WMH volume. In contrast, across all neurodegenerative diseases, non-synonymous, rare variant carrier status in *NOTCH3/HTRA1* was associated with higher WMH volumes. Yet the influence of variants in these genes had varying effects across the neurodegenerative diagnoses with respect to lacune and PVS counts. While carriers of *NOTCH3/HTRA1* variants diagnosed with CVD displayed a greater number of lacunes and PVS than non-carriers, carriers diagnosed with PD displayed significantly fewer lacunes than non-carriers, which is discussed further in what follows. Altogether, our results suggest that genetic influence on SVD markers in neurodegenerative disease patients is not only gene-specific but potentially disease-specific as well.

In our neurodegenerative disease cohort, rare genetic variants in *HTRA1* or *NOTCH3* exerted the greatest influence on markers of SVD. As previously described, heterozygous pathogenic variants in *NOTCH3* are causative for CADASIL, while homozygous pathogenic variants in *HTRA1* are causative for CARASIL, with both phenotypes

sharing similar features, such as recurrent ischemic attacks, migraines with aura, cognitive decline and dementia, and hallmark WMH.<sup>17,16,48</sup> Within our cohort, there were more rare, non-synonymous variants reported in *NOTCH3* than in *HTRA1* ( $n = 21$  and  $n = 6$ , respectively), and indeed, all *HTRA1* variants reported were in the heterozygous state. Yet, heterozygous variants in *HTRA1* had been previously associated with cerebral SVD but of lesser severity than CARASIL, accounting for their inclusion in our analyses.<sup>49–51</sup> Similarly, many of the variants in *NOTCH3* that we identified were not considered typical of CADASIL pathogenicity, as they were not cysteine-modifying or located within an epidermal growth factor-like repeat; however, previous studies suggested that these atypical *NOTCH3* variants might contribute to a CADASIL phenotype of milder severity or to more generalized SVD.<sup>52–54</sup> Therefore, while we are not suggesting that the *HTRA1* or *NOTCH3* rare variants identified herein are causing CARASIL or CADASIL, respectively, as confirmed by clinical assessment of our study patients, our results do suggest that the



**FIGURE 3** Enlarged PVS counts in ONDRI participants carrying *NOTCH3/HTRA1* rare, non-synonymous variants compared to variant negative participants. (A) Distribution of PVS counts in ONDRI participants that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* compared to those that did not. (B) Distribution of PVS counts in each of the participants within each disease cohort of ONDRI that carried a rare, non-synonymous variant in either *NOTCH3* or *HTRA1* compared to those that did not. *P* values are presented for the interaction term of disease status and variant status from the multivariate regression analysis that modeled PVS count as a function *NOTCH3* or *HTRA1* variant carrier status, disease cohort, and interactions between the two predictor variables, as well as adjustment for head size, age, smoking history, and *APOE*  $\epsilon 2$  or  $\epsilon 4$  variant status. To avoid statistical redundancy, the ALS cohort was not coded in the regression model. Indicated *P* values are uncorrected for multiple testing. Neg, variant negative; Pos, variant positive.

identified non-synonymous, rare variants identified may contribute to cerebrovascular injury in subjects, potentially acting as a contributing factor to neurodegenerative disease risk or modifying clinical presentation and progression.

Specifically, we observed an association between *NOTCH3/HTRA1* non-synonymous, rare variant carrier status and greater WMH volume across the entire neurodegenerative disease cohort. The association was unsurprising based on the striking hallmark of WMH in both CADASIL and CARASIL. Additionally, a recent analysis of >200,000 participants who self-reported as healthy from the UK Biobank identified a significant association between *NOTCH3* genetic variation and increased WMH volume, as well as ultrastructural damage to white matter.<sup>55</sup> Notably, an association was also observed between *HTRA1* or *NOTCH3* variants and increased WMH volume in the CVD cohort; however, this result did not pass multiple testing corrections, and therefore, we believe it is unlikely for it to fully account for the association observed across the entire cohort and that the variation is still

influencing SVD markers across the neurodegenerative diseases as well. What remains to be determined is how the influence of *HTRA1* or *NOTCH3* variants on WMH volume may contribute to neurodegenerative disease risk, presentation, and progression, particularly due to the complex relationship between cerebrovascular injury and neurodegeneration.

In contrast to the WMH analysis, *NOTCH3/HTRA1* variant carrier status had variable effects on lacune and PVS count across the neurodegenerative disease cohorts. Interestingly, only the CVD cohort displayed an association between genetic variation in *NOTCH3/HTRA1* and PVS count, demonstrating a significant positive association, suggesting that the genes have an influence on PVS only in individuals with a classical CVD phenotype.

As described, the PD cohort displayed the highest proportion of *HTRA1/NOTCH3* variant carriers. In fact, a previous gene-based rare variant association analysis by our group identified a significant enrichment of *NOTCH3* rare variants in the PD cohort,<sup>29</sup> which led to an initial

investigation of the contribution of these variants to SVD features in only the PD patients.<sup>56</sup> Using a Bayesian approach, we identified a doubling of WMH volume in PD patients carrying *NOTCH3* variants compared to variant negative patients. Additionally, *NOTCH3* rare variation was associated with significantly increased lacune volumes. Yet, we did not observe an association between *HTRA1/NOTCH3* variant status and lacune count in the PD patients in the current analysis that passed multiple testing corrections. These seemingly contradictory findings highlight a current gap in the clinical-scientific literature regarding the wide variation in definition of lacunes and the differences between lacunar counts versus volumes, size criterion, etiology of infarction, and future cavitation.<sup>57,58</sup> Given these issues and the difficulty of assessing the precise etiological origins (eg, lipohyalinosis vs ostium/branch disease), the ONDRI study's neuroimaging group implemented a multimodal imaging-based criterion for the segmentation of subcortical lacunar infarcts, defined as regions that are hypointense on T1, hyperintense on T2, and appearing with a hypointense central core with a hyperintense rim on T2 FLAIR.<sup>36</sup> They are differentiated from PVS, which are isointense to gray matter on proton density, tend to be smaller in diameter, and are more linear depending on the orientation of the vasculature.<sup>2</sup> Together with our previous results, this suggests that, although the variant carriers do not have a greater number of lacune counts than non-carriers, the lacunar volumes that are present in the carriers tend to be much more substantial in size, potentially resulting in greater clinical consequences.

Rare genetic variants in *ABCC6*, *COL4A1*, and *COL4A2* did not account for SVD markers in neurodegenerative disease patients in our study. Of note, only heterozygous variants were observed in the studied genes across our participants. Typically, a homozygous variant is required to cause the phenotype associated with *ABCC6*, namely, pseudoxanthoma elasticum, although there have been reports of heterozygous variants in the gene causing a milder vascular disease.<sup>59,60</sup> Yet these cases are rather uncommon, and, due to both the subtlety of the influence of heterozygous *ABCC6* variation and our modest sample sizes, we might not have been able to detect their phenotypic influence. Further, pathogenic variants within *COL4A1* and *COL4A2* are most associated with hemorrhagic stroke<sup>61–64</sup>; however, individuals in the ONDRI cohort were documented to have experienced mild to moderate ischemic stroke, and those with hemorrhagic stroke were excluded. Again, this likely limited the number of variant carriers within our study and, therefore, the ability to detect associations between the collagen chain genes and markers of SVD.

In addition to the limitations imposed by our modest sample sizes described earlier, which might have increased the incidence of results that did not survive multiple testing corrections, the presented work was also inherently limited by focusing upon rare variation, which naturally resulted in fewer variant positive patients to analyze. However, it is generally accepted that variants that are less common in the general population will have a greater likelihood of higher phenotypic effects. Further, our study lacked a normative sample to determine whether the observed associations were specific to neurodegenerative and CVD cohorts, or whether similar results would have been observed in a

healthy elderly population. Finally, we did not further analyze the effect of the SVD-associated genes on clinical outcomes or co-morbid vascular risk, again due to the limited power within our study. These analyses will be important for future investigations using larger sample sizes.

The mechanisms by which rare variants in SVD-associated genes – such as *HTRA1* and *NOTCH3* – may contribute to cerebrovascular injury in neurodegenerative disease patients and how that injury may influence progression of neurodegeneration remain to be determined. Yet, our findings highlight the importance of comprehensive genetic study in understanding the development of SVD markers and the potential contribution to neurodegenerative disease, particularly in light of recent literature highlighting the value of clinical genetic testing in cases of cerebral SVD and neurological phenotypes that present with features of SVD. Our results suggest that variants in these genes indeed alter the burden of MRI-based SVD markers in a variety of neurodegenerative disease patients and demonstrate that potential downstream effects on clinical correlates, pathogenesis, and disease progression must be further explored.

## AFFILIATIONS

<sup>1</sup>Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, McGill University, Montréal, Quebec, Canada

<sup>2</sup>Dr. Sandra Black Centre for Brain Resilience and Recovery, LC Campbell Cognitive Neurology, Hurvitz Brain Sciences Program, Sunnybrook Research Institute, University of Toronto, Toronto, Ontario, Canada

<sup>3</sup>Rotman Research Institute, Baycrest Health Sciences, Toronto, Ontario, Canada

<sup>4</sup>Division of Biostatistics, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

<sup>5</sup>Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada

<sup>6</sup>Edmond J. Safra Program in Parkinson's Disease and the Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, Toronto, Ontario, Canada

<sup>7</sup>Centre for Clinical Brain Sciences, University of Edinburgh; Anne Rowling Regenerative Neurology Clinic, University of Edinburgh; Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK

<sup>8</sup>Division of Neurology, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada

<sup>9</sup>Department of Medicine (Neurology), Sunnybrook Health Sciences and University of Toronto, Ontario, Canada

<sup>10</sup>Heart and Stroke Foundation Canadian Partnership for Stroke Recovery, Hurvitz Brain Sciences Research Program, Sunnybrook Research Institute, Toronto, Ontario, Canada

<sup>11</sup>Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

<sup>12</sup>St. Joseph's Healthcare Centre, London, Ontario, Canada

<sup>13</sup>Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, Ontario, Canada

<sup>14</sup>Department of Medicine (Neurology), University of Ottawa Brain and Mind Research Institute and Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

<sup>15</sup>Bruyère Research Institute, Ottawa, Ontario, Canada

<sup>16</sup>Department of Medicine (Neurology), Baycrest Health Sciences, Mt. Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

<sup>17</sup>Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

<sup>18</sup>Adult Neurodevelopment and Geriatric Psychiatry, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

<sup>19</sup>Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

<sup>20</sup>Toronto Dementia Research Alliance, University of Toronto, Toronto, Ontario, Canada

<sup>21</sup>Division of Neurology, Department of Medicine, Hamilton Health Sciences, McMaster University, Hamilton, Canada

<sup>22</sup>Thunder Bay Regional Health Research Institute, Thunder Bay, Ontario, Canada

<sup>23</sup>Li Ka Shing Knowledge Institute, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada

<sup>24</sup>Division of Neurology, Department of Medicine, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada

<sup>25</sup>Department of Medical Imaging, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada

## ACKNOWLEDGMENTS

We thank the ONDRI study participants and their respective caregivers for their time and significant contribution to our study. Thank you to the ONDRI investigators ([www.ONDRI.ca/people](http://www.ONDRI.ca/people)) – the governing, executive, steering, publication, recruiting, assessment, and project management teams. The authors and investigators would like to acknowledge ONDRI's project management team, past and present: Susan Boyd, Lisa Desalaiz, Catarina Downey, Stephanie Hetherington, Heather Hink, Ruth Kruger, Sean Lucas, Donna McBain, Andrea Richter, Michelle Vanderspank, Joanna White, Ashley Wilcox, and the coordinators at each site. Thank you to the L.C. Campbell Foundation and the brainlab.ca neuroimaging analysis team. M.F. received funding support from the Saul A. Silverman Family Foundation as a Canadian International Scientific Exchange Program and the Morris Kerzner Memorial Fund. J.R. acknowledges support from the Ontario Brain Institute, the Sandra Black Centre for Brain Resilience & Recovery, the Weston-Selfridges UK Brain Institute, and Fondation Leducq Transatlantic Network of Excellence. A.A.D. is supported by the Canadian Institute of Health Research Banting Postdoctoral Fellowship program. This research was conducted with the support of the OBI, an independent not-for-profit corporation funded partially by the Ontario government. The opinions, results, and conclusions are those of the authors, and no endorsement by the OBI is intended or should be inferred. Matching funds were provided by participant hospital and research foundations, including the Baycrest Foundation, Bruyere Research Institute, Centre for Addiction and Mental Health Foundation, London Health Sciences Foundation, McMaster University Faculty of Health Sciences, Ottawa Brain and Mind Research Institute, Queen's University Faculty of Health Sciences, the Thunder Bay Regional Health Sciences Centre, the University of Ottawa Faculty of Medicine, and the Windsor/Essex County ALS Association. The Temerty Family Foundation provided the major infrastructure matching funds.

## CONFLICT OF INTEREST STATEMENT

The authors report no competing interests. Author disclosures are available in the [supporting information](#).

## CONSENT STATEMENT

All study participants provided written, informed consent.

## ORCID

Joel Ramirez  <https://orcid.org/0000-0002-5582-1776>

## REFERENCES

- Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010;9(1474-4465):689-701. Electronic.
- Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol*. 2013;12(8):822-838.
- Cai Z, Wang C, He W, et al. Cerebral small vessel disease and Alzheimer's disease. *Clin Interv Aging*. 2015;10:1695-1704. doi:10.2147/CIA.S90871
- Yoshita M, Fletcher E, Harvey D, et al. Extent and distribution of white matter hyperintensities in normal aging, MCI, and AD. *Neurology*. 2006;67(12):2192-2198.
- Ramirez J, Berezuk C, McNeely AA, Scott CJM, Gao F, Black SE. Visible Virchow-Robin spaces on magnetic resonance imaging of Alzheimer's disease patients and normal elderly from the Sunnybrook dementia study. *J Alzheimer's Dis*. 2015;43(2):415-424. doi:10.3233/JAD-132528
- Wirth AM, Johannesen S, Khomenko A, et al. Value of fluid-attenuated inversion recovery MRI data analyzed by the lesion segmentation toolbox in amyotrophic lateral sclerosis. *J Magn Reson Imaging*. 2019;50(2):552-559. doi:10.1002/jmri.26577
- Chen H, Wan H, Zhang M, et al. Cerebral small vessel disease may worsen motor function, cognition, and mood in Parkinson's disease. *Parkinsonism Relat Disord*. 2021;83:86-92. doi:10.1016/j.parkreldis.2020.12.025
- Patankar TF, Mitra D, Varma A, Snowden J, Neary D, Jackson A. Dilatation of the Virchow-Robin space is a sensitive indicator of cerebral microvascular disease: study in elderly patients with dementia. *AJNR Am J Neuroradiol*. 2005;26(0195-6108):1512-1520. Print.
- Desmarais P, Gao AF, Lanctôt K, et al. White matter hyperintensities in autopsy-confirmed frontotemporal lobar degeneration and Alzheimer's disease. *Alzheimers Res Ther*. 2021;13(1):129. doi:10.1186/s13195-021-00869-6
- Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for health-care professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42(9):2672-2713. doi:10.1161/STR.0b013e3182299496
- Le Saux O, Urban Z, Tschuch C, et al. Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. *Nat Genet*. 2000;25(2):223-227. doi:10.1038/76102
- Bergen AAB, Plomp AS, Schuurman EJ, et al. Mutations in ABCC6 cause pseudoxanthoma elasticum. *Nat Genet*. 2000;25(2):228-231. doi:10.1038/76109
- Gould DB, Phalan FC, Breedveld GJ, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science*. 2005;308(5725):1167-1171. doi:10.1126/SCIENCE.1109418
- Yoneda Y, Haginoya K, Arai H, et al. De novo and inherited mutations in COL4A2, encoding the type IV collagen  $\alpha 2$  chain cause porencephaly. *Am J Hum Genet*. 2012;90(1):86-90. doi:10.1016/J.AJHG.2011.11.016
- Weng YC, Sonni A, Labelle-Dumais C, et al. COL4A1 mutations in patients with sporadic late-onset intracerebral hemorrhage. *Ann Neurol*. 2012;71(4):470-477. doi:10.1002/ANA.22682
- 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/NEJM0A0801560

17. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996;383(0028-0836):707-710. Print.
18. Chassaing N, Martin L, Calvas P, Le Bert M, Hovnanian A. Pseudoxanthoma elasticum: a clinical, pathophysiological and genetic update including 11 novel ABCC6 mutations. *J Med Genet*. 2005;42(12):881-892. doi:10.1136/jmg.2004.030171
19. De Vilder EYG, Cardoen S, Hosen MJ, et al. Pathogenic variants in the ABCC6 gene are associated with an increased risk for ischemic stroke. *Brain Pathology*. 2018;28(6):822-831. doi:10.1111/bpa.12620
20. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. *Microsc Res Tech*. 2008;71(5):357-370. doi:10.1002/jemt.20564
21. Alamowitch S, Plaisier E, Favrole P, et al. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. *Neurology*. 2009;73(22):1873-1882. doi:10.1212/WNL.0b013e3181c3fd12
22. Verbeek E, Meuwissen MEC, Verheijen FW, et al. COL4A2 mutation associated with familial porencephaly and small-vessel disease. *Eur J Hum Genet*. 2012;20(8):844-851. doi:10.1038/ejhg.2012.20
23. Fukutake T. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL): from discovery to gene identification. *J Stroke Cerebrovasc Dis*. 2011;20(2):85-93. doi:10.1016/j.jstrokecerebrovasdis.2010.11.008
24. Manini A, Pantoni L. Genetic causes of cerebral small vessel diseases: a practical guide for neurologists. *Neurology*. 2023;100(16). doi:10.1212/WNL.0000000000201720
25. Farhan SMK, Bartha R, Black SE, et al. The Ontario neurodegenerative disease research initiative (ONDRI). *Can J Neurol Sci*. 2017;44(2):196-202. doi:10.1017/cjn.2016.415
26. Sunderland KM, Beaton D, Arnott SR, et al. Characteristics of the Ontario neurodegenerative disease research initiative cohort. *Alzheimer's Dis*. 2023;19(1):226-243. doi:10.1002/alz.12632. Published online March 30.
27. Dillioott AA, Farhan SMK, Ghani M, et al. Targeted next-generation sequencing and bioinformatics pipeline to evaluate genetic determinants of constitutional disease. *J Vis Exp*. 2018;134(e57266):1-10. doi:10.3791/57266
28. Farhan SMK, Dillioott AA, Ghani M, et al. The ONDRISeg panel: custom-designed next-generation sequencing of genes related to neurodegeneration. *NPJ Genom Med*. 2016;1(1):16032. doi:10.1038/npjgenmed.2016.32
29. Dillioott AA, Abdelhady A, Sunderland KM, et al. Contribution of rare variant associations to neurodegenerative disease presentation. *NPJ Genom Med*. 2021;6(1):80. doi:10.1038/s41525-021-00243-3
30. Kircher M, Witten DM, Jain P, O'roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46(3):310-315. doi:10.1038/NG.2892
31. Dillioott AA, Evans EC, Farhan SMK, et al. Genetic variation in the Ontario neurodegenerative disease research initiative. *Can J Neurol Sci*. 2019;46(5):491-498. doi:10.1017/cjn.2019.228
32. Gesierich B, Opherck C, Rosand J, et al. APOE ε2 is associated with white matter hyperintensity volume in CADASIL. *J Cereb Blood Flow Metab*. 2016;36(1):199-203. doi:10.1038/JCBFM.2015.85
33. Lyall DM, Cox SR, Lyall LM, et al. Association between APOE ε4 and white matter hyperintensity volume, but not total brain volume or white matter integrity. *Brain Imaging Behav*. 2020;14(5):1468-1476. doi:10.1007/S11682-019-00069-9
34. Mirza SS, Saeed U, Knight J, et al. APOE ε4, white matter hyperintensities, and cognition in Alzheimer and Lewy body dementia. *Neurology*. 2019;93(19):e1807-e1819. doi:10.1212/WNL.0000000000008377
35. Scott CJM, Arnott SR, Chemparathy A, et al. An overview of the quality assurance and quality control of magnetic resonance imaging data for the Ontario Neurodegenerative Disease Research Initiative (ONDRI): pipeline development and neuroinformatics. *bioRxiv*. 2020. Published online.
36. Ramirez J, Holmes MF, Scott CJM, et al. Ontario neurodegenerative disease research initiative (ONDRI): structural MRI methods & outcome measures. *Front Neurol*. 2020;11:847. Published online.
37. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006;31(1053-8119):1116-1128. Print.
38. Nieuwenhuis R, te Grotenhuis M, Pelzer B. Weighted effect coding for observational data with wec. *R Journal*. 2017;9(1):477-485.
39. te Grotenhuis M, Pelzer B, Eisinga R, Nieuwenhuis R, Schmidt-Catran A, Konig R. When size matters: advantages of weighted effect coding in observational studies. *Int J Public Health*. 2017;62(1):163-167. doi:10.1007/S00038-016-0901-1/TABLES/2
40. Yekutieli D, Benjamini Y. Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. *J Stat Plan Inference*. 1999;82(1-2):171-196. doi:10.1016/s0378-3758(99)00041-5
41. R Core Team. R: A Language for Statistical Computing. 2013
42. Wickham H, ggplot2. ggplot2. Published online 2009. doi:10.1007/978-0-387-98141-3
43. Bertram L, Tanzi RE. The genetic epidemiology of neurodegenerative disease. *J Clin Invest*. 2005;115(6):1449-1457. doi:10.1172/JCI24761
44. Pihlström L, Wiethoff S, Houlden H. Genetics of neurodegenerative diseases: an overview. *Handb Clin Neurol*. 2017;145:309-323. doi:10.1016/B978-0-12-802395-2.00022-5
45. 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):2197-2204.
46. Seidel GA, Giovannetti T, Libon DJ. Cerebrovascular disease and cognition in older adults. *Curr Top Behav Neurosci*. 2012;10:213-241.. Accessed October 24, 2015. <http://www.ncbi.nlm.nih.gov/pubmed/21773915>
47. Kummer BR, Diaz I, Wu X, et al. Associations between cerebrovascular risk factors and Parkinson disease. *Ann Neurol*. 2019;86(4):572-581. doi:10.1002/ANA.25564
48. Tikka S, Baumann M, Siitonen M, et al. CADASIL and CARASIL. *Brain Pathol*. 2014;24(5):525-544. doi:10.1111/BPA.12181
49. Mendioroz M, Fernández-Cadenas I, Del Río-Espinola A, et al. A missense HTRA1 mutation expands CARASIL syndrome to the Caucasian population. *Neurology*. 2010;75(22):2033-2035. doi:10.1212/WNL.0B013E3181FF96AC
50. Chen Y, He Z, Meng S, Li L, Yang H, Zhang X. A novel mutation of the high-temperature requirement A serine peptidase 1 (HTRA1) gene in a Chinese family with cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL). *J Int Med Res*. 2013;41(5):1445-1455. doi:10.1177/0300060513480926
51. Bianchi S, Di Palma C, Gallus GN, et al. Two novel HTRA1 mutations in a European CARASIL patient. *Neurology*. 2014;82(10):898-900. doi:10.1212/WNL.0000000000000202
52. Ungaro C, Mazzei R, Conforti FL, et al. CADASIL: extended polymorphisms and mutational analysis of the NOTCH3 gene. *J Neurosci Res*. 2009;87(5):1162-1167. doi:10.1002/jnr.21935
53. Ragno M, Berbellini A, Cacchiò G, et al. Parkinsonism is a late, not rare, feature of CADASIL a study on Italian patients carrying the R1006C mutation. *Stroke*. 2013;44(4):1147-1149. doi:10.1161/STROKEAHA.111.000458
54. Muiño E, Gallego-Fabrega C, Cullell N, et al. Systematic review of cysteine-sparing NOTCH3 missense mutations in patients with clinical suspicion of CADASIL. *Int J Mol Sci*. 2017;18(9):1964. doi:10.3390/ijms18091964
55. Cho BPH, Nannoni S, Harshfield EL, et al. NOTCH3 variants are more common than expected in the general population and associated with stroke and vascular dementia: an analysis of 200 000 participants. *J Neurol Neurosurg Psychiatry*. 2021;92(7):694-701. doi:10.1136/JNNP-2020-325838

56. Ramirez J, Dillioth AA, Binns MA, et al. Parkinson's disease, NOTCH3 genetic variants, and white matter hyperintensities. *Mov Disord* 2020;35(11):2090-2095. doi:10.1002/mds.28171
57. Potter GM, Marlborough FJ, Wardlaw JM. Wide variation in definition, detection, and description of lacunar lesions on imaging. *Stroke*. 2011;42(2):359-366. doi:10.1161/STROKEAHA.110.594754
58. Potter GM, Doubal FN, Jackson CA, et al. Counting cavitating lacunes underestimates the burden of lacunar infarction. *Stroke*. 2010;41(2):267-272. doi:10.1161/STROKEAHA.109.566307
59. Nollet L, Campens L, De Zaeytijd J, et al. Clinical and subclinical findings in heterozygous ABCC6 carriers: results from a Belgian cohort and clinical practice guidelines. *J Med Genet*. 2022;59(5). doi:10.1136/JMEDGENET-2020-107565
60. Hornstrup LS, Tybjærg-Hansen A, Haase CL, et al. Heterozygosity for R1141X in ABCC6 and risk of ischemic vascular disease. *Circ Cardiovasc Genet*. 2011;4(5):534-541. doi:10.1161/CIRCGENETICS.110.958801
61. Gould DB, Phalan FC, van Mil SE, et al. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. *NEngl J Med*. 2006;354(14):1489-1496.
62. Lemmens R, Maugeri A, Niessen HWM, et al. Novel COL4A1 mutations cause cerebral small vessel disease by haploinsufficiency. *Hum Mol Genet*. 2013;22(2):391-397. doi:10.1093/HMG/DDS436
63. Jeanne M, Labelle-Dumais C, Jorgensen J, et al. COL4A2 mutations impair COL4A1 and COL4A2 secretion and cause hemorrhagic stroke. *Am J Hum Genet*. 2012;90(1):91-101. doi:10.1016/J.AJHG.2011.11.022
64. Meuwissen MEC, Halley DJJ, Smit LS, et al. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. *Genet Med*. 2015;17(11):843-853. doi:10.1038/GIM.2014.210

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Dillioth AA, Berberian SA, Sunderland KM, et al. Rare neurovascular genetic and imaging markers across neurodegenerative diseases. *Alzheimer's Dement*. 2023;1-13. <https://doi.org/10.1002/alz.13316>