IMPORTANCE It is uncertain whether typical variants causing monogenic stroke are associated with cerebrovascular disease in the general population and why the phenotype of these variants varies so widely.

OBJECTIVE To determine the frequency of pathogenic variants in the 3 most common monogenic cerebral small vessel diseases (cSVD) and their associations with prevalent and incident stroke and dementia.

DESIGN, SETTING, AND PARTICIPANTS This cohort study is a multicenter population-based study of data from UK Biobank participants recruited in 2006 through 2010, with the latest follow-up in September 2021. A total of 9.2 million individuals aged 40 to 69 years who lived in the United Kingdom were invited to join UK Biobank, of whom 5.5% participated in the baseline assessment. Participants eligible for our study (n = 454,756, excluding 48,569 with incomplete data) had whole-exome sequencing and available data pertaining to lacunar stroke-related diseases, namely stroke, dementia, migraine, and epilepsy.

EXPOSURES NOTCH3, HTRA1, and COL4A1/2 pathogenic variants in monogenic stroke; Framingham cardiovascular risk; and ischemic stroke polygenic risk.

MAIN OUTCOMES AND MEASURES Primary outcomes were prevalent and incident stroke and dementia. Odds ratios (ORs) and hazard ratios (HRs) were adjusted for age, sex, ethnicity, exome sequencing batch, and top 10 genetic principal components.

RESULTS Of the 454,756 participants (208,027 [45.8%] men; mean [SD] age, 56.5 [8.1] years), 973 participants carried NOTCH3 variants, 546 carried HTRA1 variants, and 336 carried COL4A1/2 variants. Variant carriers were at least 66% more likely to have had stroke. NOTCH3 carriers had increased vascular dementia risk (OR, 5.42; 95% CI, 3.11-8.74), HTRA1 carriers an increased all-cause dementia risk (OR, 2.17; 95% CI, 1.28-3.41), and COL4A1/2 carriers an increased intracerebral hemorrhage risk (OR, 3.56; 95% CI, 1.34-7.53). NOTCH3 variants were associated with incident ischemic stroke and vascular dementia. NOTCH3 and HTRA1 variants were associated with magnetic resonance imaging markers of cSVD. Cardiovascular risk burden was associated with increased stroke risk in NOTCH3 and HTRA1 carriers. Variant location was also associated with risk.

CONCLUSIONS AND RELEVANCE In this cohort study, pathogenic variants associated with rare monogenic stroke were more common than expected in the general population and associated with stroke and dementia. Cardiovascular risk burden is associated with the penetrance of such variants. Our results support the hypothesis that cardiovascular risk factor control may improve disease prognosis in individuals with monogenic cSVD variants. This lays the foundation for future studies to evaluate the effect of early identification before symptom onset on mitigating stroke and dementia risk.

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Several monogenic disorders are associated with stroke and vascular dementia. These predominantly cause lacunar stroke. The most common of these, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), is caused by NOTCH3 variants, and the second most frequent, CADASIL2, by autosomal dominant HTRA1 variants. COL4A1/2 variants can cause small vessel stroke and intracerebral hemorrhage.

Monogenic stroke was thought to be rare, with an estimated prevalence of CADASIL of 4 per 100,000 in the United Kingdom. However, recent studies have reported pathogenic variants are more prevalent in the general population, with typical NOTCH3 variants present in 1 in 452 individuals and HTRA1 variants in 1 in 275.

These findings raise 2 important questions. First, are these apparently “asymptomatic” variants associated with stroke and dementia? Second, why do some individuals who have these variants present with severe early-onset stroke and dementia while others remain asymptomatic?

Previously, it was thought that CADASIL was a disease with high penetrance and that most individuals with typical variants would experience early-onset stroke. More recently, the clinical phenotype of CADASIL has been shown to be widely variable, with some having a stroke young while others remaining stroke-free until their 80s. Several factors have been suggested to modulate phenotype, including variant location (variants in epidermal growth factor–like repeats [EGFRs] 1–6 are associated with more severe disease), the presence of cardiovascular risk factors, and modifying genes. The UK Biobank resource includes data on vascular risk factors and imputed genome-wide genotyping, allowing cardiovascular risk factor and polygenic risk scores to be calculated to investigate the importance of such modifying factors.

To explore this further, we analyzed the latest exome sequencing data from UK Biobank. We identified NOTCH3, HTRA1, and COL4A1/2 pathogenic variants that cause the 3 most common monogenic cerebral small vessel diseases (cSVDs). We determined the frequency of such variants and their associations with both prevalent and incident stroke and dementia. We also determined associations with magnetic resonance imaging (MRI) markers of cSVD.

Methods

Study Population

UK Biobank is a prospective study of more than 500,000 participants aged 40 to 69 years recruited across the United Kingdom in 2006 to 2010. Phenotypic data were collected through questionnaires and physical examinations. A subset of 100,000 individuals, selected based on traveling distance from the imaging center, underwent MRI. All MRIs were performed on Siemens Skyra 3.0-T scanners with identical acquisition parameters and quality control. In October 2021, whole-exome sequences of 454,756 participants were released and were assessed for this study. UK Biobank received ethical approval from the National Health Service National Research Ethics Service Northwest (21/NW/0157). Written informed consent was obtained from all participants. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Ascertainment of Pathogenic Variants

Variants in NOTCH3 (chr19:15,159,038–15,200,995, GRCh38), HTRA1 (chr10:122,458,551–122,514,907), COL4A1 (chr13:110,148,963–110,307,157), and COL4A2 (chr13:110,305,812–110,513,209) were extracted in PLINK format. The extracted variants were annotated using the Ensembl Variant Effect Predictor and then filtered by a priori pathogenicity criteria for each gene (as specified below).

For NOTCH3, we identified CADASIL variants that cause the gain or loss of cysteine in 1 of the 34 EGFR domains of the NOTCH3 protein (amino acid position 40–1373). HTRA1 and COL4A1/2 pathogenic variants are not stereotyped in the same way as NOTCH3 variants. Therefore, we performed a systematic review to identify those that had been reported in patients with familial cSVD. We searched PubMed using the terms “(CARASIL OR HTRA1 mutation) AND (COL4A1 mutation) OR COL4A2 mutation) OR COL4A1/2 mutation)” and selected English publications up to February 6, 2022. These variants were classified using the American College of Medical Genetics and Genomics (ACMG) criteria and the Association for Clinical Genomic Science Best Practice Guidelines for Variant Classification in Rare Disease 2020. Only variants classified as pathogenic or likely pathogenic were included in subsequent analyses.

Phenotypic Data Fields

History of vascular risk factors needed to calculate the Framingham cardiovascular risk score (FRS) and parental history of stroke were recorded. History of diseases, including migraine, migraine with aura, any stroke, ischemic stroke, intracerebral hemorrhage, vascular dementia, all-cause dementia, and epilepsy, were determined from self-report and hospital and death records (eTable 1 in the Supplement).

Diagnoses of stroke, ischemic stroke, intracerebral hemorrhage, and vascular dementia after recruitment to UK Biobank were identified as incident cases.

Key Points

**Question** What factors are associated with penetrance of variants in monogenic cerebral small vessel disease (cSVD)?

**Findings** In this population-based cohort study of 454,756 individuals, NOTCH3, HTRA1, and COL4A1/2 variants causing monogenic cSVD were associated with increased stroke and dementia risk. Cardiovascular risk factors were found to be associated with penetrance of these variants.

**Meaning** The results of this cohort study support the hypotheses that cardiovascular risk factor control may improve disease prognosis in individuals with monogenic cSVD variants and that identifying individuals early in life before symptom onset may help reduce stroke and dementia risk.
Brain Imaging Analysis
In the 38,332 participants with both exome sequences and MRI, measures were compared between participants harboring pathogenic variants and controls. Among the sequences acquired were T1-weighted, fluid-attenuated inversion recovery (FLAIR), and diffusion tensor imaging (DTI) images (full acquisition details previously reported, brief details provided in eTable 2 in the Supplement). We used measures for brain and white matter hyperintensities (WMH) volume generated by UK Biobank. Brain volume was estimated from T1-weighted images by SIENAX and normalized for head size. The WMH were quantified on FLAIR images through the brain intensity abnormality classification algorithm and normalized for brain volume. Degree of white matter ultrastructural damage was calculated using software to derive peak width skeletonized mean diffusivity (PSMD), which provides a summary measure from DTI. Additionally, DTI was used to derive structural brain networks via tractography analysis, from which global and local structural efficiency measures were derived in house. Following tractography, an anatomic labeling atlas was used to generate a connectivity matrix (detailed in Shen et al). Global efficiency is estimated by averaging the number of steps it takes to go from any given node to any other; local efficiency is calculated from the efficiency of the connections between a given node and those nodes connected to it. Global network efficiency has been shown to be sensitive to damage in cSVD, correlate with cognitive impairment, and predict future dementia.

Calculation of Framingham Cardiovascular and Polygenic Risk Scores
To compare ischemic stroke risk associated with the NOTCH3 and HTRA1 variants and that of cardiovascular risk factors and common genetic variants, we calculated the FRS and an ischemic stroke polygenic risk score (PRS), respectively.

Statistical Analysis
The effects of NOTCH3, HTRA1, and COL4A1/2 variants on phenotypes were assessed by linear regression for continuous outcomes and logistic regression for binary outcomes. All the variant carriers only had 1 of the prespecified variants in these genes. A Firth correction was applied to logistic regression to reduce rare event bias. All regression models were adjusted for age, sex, ethnicity, exome sequencing batch, and the first 10 principal components of genetic ancestry. The WMH volumes were natural log-transformed for analyses.

Kaplan-Meier analyses and Cox proportional-hazards regression with Firth corrections (using time since recruitment to UK Biobank as the underlying timescale and adjusting for FRS, PRS, and the same covariates included in the above-mentioned regression analyses) were performed to compare the cumulative probability of incident disease in variant groups and in different PRS and FRS tiers. Assumptions of Cox regression were tested based on Schoenfeld residuals, and no violations were observed. When calculating hazard ratios (HRs) for ischemic stroke associated with 1-SD higher FRS and PRS as well as NOTCH3 and HTRA1 variant status, Cox proportional-hazards regression models were limited to participants without history of stroke, coronary heart disease, peripheral vascular disease, or congestive heart failure at recruitment. By dividing the HR associated with the variant status by that associated with 1-SD higher FRS or PRS (on the log scale, assuming a linear association), we estimated the increment of the FRS and PRS in SD that was predicted to be equivalent to the risk associated with the NOTCH3 and HTRA1 variants.

We performed statistical tests for interaction between the FRS or PRS and variant status of each gene; for this, we divided the participants into 2 groups based on FRS or PRS: low (bottom 50%) and high (top 50%) risk. Multiplicative and additive interaction was assessed by analysis of variance and the synergy index, respectively. All statistical analyses were performed using R version 4.0.3 with 2-sided P values and P < .05 considered statistically significant.

Results
Systematic Review and Classification of the HTRA1 and COL4A1/2 Variants
After performing ACMG classification of HTRA1 and COL4A1/2 variants identified in the literature search (eFigure 1 in the Supplement), we found 63 pathogenic and likely pathogenic variants in HTRA1, 131 in COL4A1, and 21 in COL4A2. Although the HTRA1 p.Gln151Lys variant was classified as likely pathogenic, we excluded it from our analyses based on evidence indicating it does not affect protease activity.

Prevalence and Distribution of the NOTCH3, HTRA1, and COL4A1/2 Pathogenic Variants
Of the 454,756 participants (208,027 [45.8%] men; mean [SD] age, 56.5 [8.1] years), 973 were heterozygous NOTCH3 carriers (1 in 467). Ninety-nine unique NOTCH3 variants were identified, of which 54 were previously reported on dbSNP (eFigure 2 and eTable 3 in the Supplement). Variants were predominantly in EGFRs 7 through 34; only 22 participants (2%) had a NOTCH3 variant in EGFRs 1 through 6. The most common NOTCH3 variants were p.Arg1231Cys and p.Cys1222Gly, found in 255 and 212 individuals, respectively.

For HTRA1, 546 heterozygous carriers were found (1 in 832); 18 unique variants were identified (eFigure 3 and eTable 4 in the Supplement). Two-thirds of the identified variants affected the protease domain, and these were carried by 92% of HTRA1 carriers. Notably, p.Arg227Trp was the most common HTRA1 variant, found in 379 individuals.

For COL4A1/2, 336 heterozygous carriers were found (1 in 1353); 11 unique variants were identified (eFigures 4 and 5 and eTable 5 in the Supplement). All the identified variants affected the triple helix region. The most common variant in COL4A1/2 was p.Gly332Arg, found in 174 individuals.

Association Between the NOTCH3, HTRA1, and COL4A1/2 Variants and Prevalent Stroke, Vascular Dementia, and Other Clinical Features
The presence of a NOTCH3 variant was associated with at least 2-fold higher odds of any stroke (odds ratio [OR], 2.16; 95% CI, 1.67-2.74; P = 3.2 × 10⁻⁵), ischemic stroke (OR, 2.65; 95% CI,
The presence of an HTRA1 variant was associated with increased risk of any stroke (OR, 1.67; 95% CI, 1.03-2.55; \(P = .04\)), which was accounted for by an increase in intracerebral hemorrhage risk (OR, 3.56; 95% CI, 1.34-7.53; \(P = .01\)), while there was no difference in ischemic stroke risk (OR, 1.16; 95% CI, 0.54-2.15; \(P = .69\)). Among strokes, 0.04% were attributed to the COL4A1/2 variants (Figure 1).

After limiting our analyses to unrelated individuals with adjustment for FRD and PRS, variant carrier prevalence was consistent, and the disease associations, excluding the risk of any stroke associated with COL4A1/2 variants, remained significant (eTables 6-8 in the Supplement).

**Association Between Variants and MRI Features of cSVD**
Magnetic resonance images were available for 92 NOTCH3, 44 HTRA1, and 28 COL4A1/2 carriers. NOTCH3 variants were associated with increased WMH volume (standardized difference \(\beta\), 0.48; 95% CI, 0.32 to 0.64; \(P = 5.5 \times 10^{-5}\)) and white matter ultrastructural damage on PSMD (\(\beta\), 0.64; 95% CI, 0.46 to 0.83; \(P = 8.5 \times 10^{-12}\)), as well as decreased local (\(\beta\), −0.25; 95% CI, −0.45 to −0.05; \(P = .01\)) and global (\(\beta\), −0.27; 95% CI, −0.47 to −0.07; \(P = .01\)) structural network connectivity.

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**Figure 1. Association of the NOTCH3, HTRA1, and COL4A1/2 Variants With Cerebral Small Vessel Disease–Related Diagnoses (N = 454 276)**

<table>
<thead>
<tr>
<th>Source</th>
<th>Variant noncarriers, No. (%)</th>
<th>Variant carriers, No. (%)</th>
<th>Odds ratio (95% CI)</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any stroke</td>
<td>16 468 (3.6)</td>
<td>70 (7.2)</td>
<td>2.16 (1.67-2.74)</td>
<td>NOTCH3, HTRA1, COL4A1/2</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>16 468 (3.6)</td>
<td>35 (6.4)</td>
<td>1.86 (1.30-2.59)</td>
<td></td>
</tr>
<tr>
<td>Intracerebral hemorrhage</td>
<td>9579 (2.1)</td>
<td>50 (5.2)</td>
<td>2.65 (1.96-3.50)</td>
<td></td>
</tr>
<tr>
<td>Family history of stroke</td>
<td>122 741 (27.1)</td>
<td>339 (35.0)</td>
<td>1.50 (1.31-1.71)</td>
<td></td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>122 741 (27.1)</td>
<td>185 (34.0)</td>
<td>1.36 (1.14-1.63)</td>
<td></td>
</tr>
<tr>
<td>All-cause dementia</td>
<td>1526 (3.0)</td>
<td>15 (1.5)</td>
<td>5.42 (3.11-8.74)</td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td>6968 (1.5)</td>
<td>29 (3.0)</td>
<td>2.26 (1.52-3.23)</td>
<td></td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>19092 (4.2)</td>
<td>23 (4.2)</td>
<td>0.99 (0.64-1.47)</td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td>6841 (1.5)</td>
<td>24 (2.5)</td>
<td>1.72 (1.12-2.51)</td>
<td></td>
</tr>
</tbody>
</table>

**Variants With Cerebral Small Vessel Disease–Related Diagnoses (N = 454 276)**

- NOTCH3: OR, 5.42 (95% CI, 3.11-8.74; \(P = 5 \times 10^{-9}\)); intracerebral hemorrhage (OR, 5.42; 95% CI, 3.11-8.74; \(P = 5 \times 10^{-9}\)), and intracerebral hemorrhage risk (OR, 3.56; 95% CI, 1.34-7.53; \(P = .01\)).
- HTRA1: OR, 1.67 (95% CI, 1.03-2.55; \(P = .04\)), which was accounted for by an increase in intracerebral hemorrhage risk (OR, 3.56; 95% CI, 1.34-7.53; \(P = .01\)), while there was no difference in ischemic stroke risk (OR, 1.16; 95% CI, 0.54-2.15; \(P = .69\)). Among strokes, 0.04% were attributed to the COL4A1/2 variants (Figure 1).
- COL4A1/2: OR, 1.07 (95% CI, 0.84-1.36; \(P = .30\)) for any stroke associated with increased odds of any stroke (OR, 1.67; 95% CI, 1.03-2.55; \(P = .04\)), which was accounted for by an increase in intracerebral hemorrhage risk (OR, 3.56; 95% CI, 1.34-7.53; \(P = .01\)).
efficiency (Table 1). They were associated with larger brain volume ($\beta$, 0.24; 95% CI, 0.07 to 0.40; $P$ = .01).

HTRA1 variants were associated with higher WMH volume ($\beta$, 0.55; 95% CI, 0.31 to 0.78; $P$ = 5.6 x 10 $^{-6}$) and DTI-PSMD ($\beta$, 0.68; 95% CI, 0.42-0.95; $P$ = 5.2 x 10 $^{-7}$) and lower local ($\beta$, −0.48; 95% CI, −0.76 to −0.20; $P$ = .001) and global ($\beta$, −0.50; 95% CI, −0.77 to −0.22; $P$ = .0004) structural efficiency (Table 1). They were not associated with brain volume. COL4A1/2 variants were not associated with any of the MRI markers (Table 1).

Table 1. Standardized Effects of the NOTCH3, HTRA1, and COL4A1/2 Variants on Different MRI Markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Variant noncarriers, No.</th>
<th>Variant carriers, No.</th>
<th>Standardized effect (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOTCH3</td>
<td>Brain volume</td>
<td>39 598</td>
<td>92</td>
<td>0.24 (0.07 to 0.40)</td>
</tr>
<tr>
<td></td>
<td>WMH volume</td>
<td>38 295</td>
<td>89</td>
<td>0.48 (0.32 to 0.64)</td>
</tr>
<tr>
<td></td>
<td>PSMD</td>
<td>37 274</td>
<td>87</td>
<td>0.64 (0.46 to 0.83)</td>
</tr>
<tr>
<td></td>
<td>Local efficiency of structural brain network</td>
<td>34 486</td>
<td>84</td>
<td>−0.25 (−0.44 to −0.05)</td>
</tr>
<tr>
<td></td>
<td>Global efficiency of structural brain network</td>
<td>34 486</td>
<td>84</td>
<td>−0.27 (−0.47 to −0.07)</td>
</tr>
<tr>
<td>HTRA1</td>
<td>Brain volume</td>
<td>39 405</td>
<td>44</td>
<td>0.21 (−0.03 to 0.45)</td>
</tr>
<tr>
<td></td>
<td>WMH volume</td>
<td>38 110</td>
<td>42</td>
<td>0.55 (0.31 to 0.78)</td>
</tr>
<tr>
<td></td>
<td>PSMD</td>
<td>37 094</td>
<td>42</td>
<td>0.68 (0.41 to 0.95)</td>
</tr>
<tr>
<td></td>
<td>Local efficiency of structural brain network</td>
<td>34 313</td>
<td>42</td>
<td>−0.48 (−0.76 to −0.20)</td>
</tr>
<tr>
<td></td>
<td>Global efficiency of structural brain network</td>
<td>34 313</td>
<td>42</td>
<td>−0.50 (−0.77 to −0.22)</td>
</tr>
<tr>
<td>COL4A1/2</td>
<td>Brain volume</td>
<td>39 486</td>
<td>24</td>
<td>0.11 (−0.25 to 0.48)</td>
</tr>
<tr>
<td></td>
<td>WMH volume</td>
<td>38 486</td>
<td>24</td>
<td>0.10 (−0.27 to 0.47)</td>
</tr>
<tr>
<td></td>
<td>PSMD</td>
<td>37 486</td>
<td>24</td>
<td>0.06 (−0.29 to 0.41)</td>
</tr>
<tr>
<td></td>
<td>Local efficiency of structural brain network</td>
<td>34 486</td>
<td>24</td>
<td>0.01 (−0.33 to 0.30)</td>
</tr>
<tr>
<td></td>
<td>Global efficiency of structural brain network</td>
<td>34 486</td>
<td>24</td>
<td>0.14 (−0.16 to 0.44)</td>
</tr>
</tbody>
</table>

Abbreviations: MRI, magnetic resonance imaging; PSMD, peak width skeletonized mean diffusivity; WMH, white matter hyperintensities.

variants were associated with incident stroke (HR, 1.80; 95% CI, 1.05-2.86; $P$ = .03) but not with vascular dementia (HR, 3.08; 95% CI, 0.87-7.55; $P$ = .08). For COL4A1/2, variant status was not predictive of incident stroke (HR, 1.03; 95% CI, 0.05-2.34; $P$ = .98).

Association Between Variant Carriers and Incident Stroke and Vascular Dementia

During follow-up for a median (IQR) duration of 12.6 years (11.8-13.2), NOTCH3 variants were associated with incident stroke (HR, 2.60; 95% CI, 1.87-3.50; $P$ = 2.2 x 10 $^{-7}$) and vascular dementia (HR, 5.74; 95% CI, 3.02-9.77; $P$ = 4.4 x 10 $^{-7}$) (Figure 2). HTRA1 variants were associated with incident stroke (HR, 1.80; 95% CI, 1.05-2.86; $P$ = .03) but not with vascular dementia (HR, 3.08; 95% CI, 0.87-7.55; $P$ = .08). For COL4A1/2, variant status was not predictive of incident stroke (HR, 1.03; 95% CI, 0.05-2.34; $P$ = .98).
Effect of Modulating Factors on Phenotype
Cardiovascular Risk Profile
We calculated the effect of vascular risk factors on ischemic stroke risk in individuals with and without variants. This analysis was limited to NOTCH3 and HTRA1 because significant associations with ischemic stroke had only been found for these variants.

Cardiovascular risk factor burden, as assessed by the FRS, increased ischemic stroke risk (HR for 1-SD higher FRS, 2.06; 95% CI, 2.01-2.12; P < .001). Having a higher FRS was associated with increased stroke risk in both variant carriers and non-carriers, although statistical significance was only seen in non-carriers (HR, 1.56; 95% CI, 1.47-1.65; P < .001). No evidence for multiplicative interaction was observed. However, there was an additive interaction between FRS and NOTCH3 and HTRA1 carrier status (NOTCH3 synergy index [SI], 1.66; 95% CI, 1.14-2.43; P < .001; HTRA1 SI, 1.60; 95% CI, 1.13-2.26; P < .001). We calculated that NOTCH3 variants conferred the same risk as a 1.32-SD increase in FRS and HTRA1 variants a 0.81-SD increase. These placed NOTCH3 and HTRA1 variant carriers as having a cardiovascular risk equivalent to the upper 9.3% and 20.9% of the population, respectively.

Polygenic Risk
Common variants, as assessed by PRS, increased stroke risk in all participants (HR for 1-SD higher PRS, 1.23; 95% CI, 1.20-1.26; P < .001). However, this appeared to only affect non-carriers of NOTCH3 and HTRA1 variants (Figure 3). Unlike the FRS, there was no additive interaction between PRS and NOTCH3 and HTRA1 carrier status (NOTCH3 SI, 0.96; 95% CI, 0.48-1.94; P = .18; HTRA1 SI, 0.74; 95% CI, 0.25-2.25; P = .28). Also, additive interaction but not multiplicative interaction was found between the categorical FRS and PRS groupings (SI, 1.29; 95% CI, 1.24-1.34; P < .001). We calculated NOTCH3 variants conferred the same risk as a 4.62-SD increase in PRS and HTRA1 variants a 2.85-SD increase. NOTCH3 and HTRA1 variant carriers were associated with the same ischemic stroke risk as belonging to the upper 0.1% and 0.2% of the population, respectively.

Location of Variants
Twenty-two individuals had a NOTCH3 EGFR 1-6 variant. Compared with other NOTCH3 carriers, EGFRs 1-6 carriers had increased risk of migraine (OR, 6.61; 95% CI, 2.14-17.89; P = .002), any stroke (OR, 14.01; 95% CI, 5.34-36.56; P = 6.6 × 10^{-7}), ischemic stroke (OR, 13.78; 95% CI, 4.92-36.73; P = 5.7 × 10^{-6}), vascular dementia (OR, 82.68; 95% CI, 22.49-358.03; P = 4.9 × 10^{-10}), and all-cause dementia (OR, 46.19; 95% CI, 14.24-162.62; P = 3.0 × 10^{-6}) but not of epilepsy (OR, 2.17; 95% CI, 0.23-9.46; P = .42) (Table 2).

The HTRA1 p.Arg227Trp variant was found in 379 individuals. Compared with other HTRA1 carriers, p.Arg227Trp carriers had reduced risk of any stroke (OR, 0.39; 95% CI, 0.20-0.79; P = .01) and ischemic stroke (OR, 0.28; 95% CI, 0.11-0.65; P = .003). They had similar risk of any migraine (OR, 0.92; 95% CI, 0.39-2.35; P = .85) but decreased risk of migraine with aura (OR, 0.17; 95% CI, 0.02-0.90; P = .04) (Table 2).

The COL4A1 p.Gly332Arg variant was found in 172 individuals. Compared with other COL4A1/2 carriers, p.Gly332Arg carriers did not differ in their risk of any of the clinical outcomes assessed (Table 2).

Discussion
In more than 450 000 individuals, we demonstrated that NOTCH3, HTRA1, and COL4A1/2 variants identical to those causing monogenic cSVD are much more frequent than expected in the general population based on the frequency of clinical disease caused by these variants. This is consistent with reports from various population databases demonstrating that such variants occur in 2 to 3 individuals per 1000. Our results extend previous work by showing association of such variants with disease. We demonstrated that NOTCH3 and HTRA1 variants were associated with increased risk of ischemic stroke and vascular dementia. Although COL4A1/2 variants were associated with increased risk of any stroke, this was accounted for by a marked increase in intracerebral hemorrhage risk, and no significant difference in ischemic stroke risk was found.

An important question is why many individuals with these variants remain asymptomatic. Our results suggest that cardiovascular risk factors and variant within the gene affect penetrance. For NOTCH3 and HTRA1, cardiovascular risk factors, as assessed by the FRS, increased ischemic stroke risk in variant carriers, and there was a statistical interaction between variant status and FRS. This is consistent with previous cross-sectional data from patients with symptomatic CADASIL, in which smoking and hypertension were associated with increased stroke risk. We demonstrated that variant location is associated with disease severity. Previous studies have shown that NOTCH3 EGFR 1-6 variants are associated with more severe disease, and our data confirmed this with markedly increased stroke and dementia risk in carriers of EGFR 1-6 variants. We extended this finding to show similar effects in HTRA1 where the p.Arg227Trp variant was found to have lower stroke risk.

In contrast, genetic propensity to common ischemic stroke, as assessed by PRS, was only associated with increased risk in individuals without NOTCH3 or HTRA1 variants. It is possible that the PRS may mask variant-level epistasis with constituting variants and/or other common yet unidentified genetic variants. Common genetic variants have been shown to affect the penetrance of monogenic conditions. In addition, in symptomatic CADASIL cases, WMH volume was demonstrated to have significant heritability when controlling for NOTCH3 variants, suggesting the existence of modifier genes.

Our MRI analysis showed that NOTCH3 and HTRA1 variants were associated with increased WMH volume, reduced white matter integrity as assessed by PSDM, and disruption of structural brain networks as assessed by local and global efficiency. Structural network measures have been shown to mediate the effect of other cSVD pathologies (eg, WMH and lacunar infarcts) on cognition. Surprisingly, NOTCH3 variants were
Figure 3. Interplay of Monogenic, Cardiovascular, and Polygenic Risk for Ischemic Stroke

### Cardiovascular risk factors for NOTCH3 carriers

<table>
<thead>
<tr>
<th>FRS status</th>
<th>NOTCH3 status</th>
<th>Ischemic stroke, No. (%)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Noncarrier</td>
<td>1939 (0.9)</td>
<td>[Reference]</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>14 (2.7)</td>
<td>3.37 (1.91-5.42)</td>
</tr>
<tr>
<td>High</td>
<td>Noncarrier</td>
<td>5373 (2.7)</td>
<td>3.31 (3.14-3.48)</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>25 (6.2)</td>
<td>7.73 (5.09-11.16)</td>
</tr>
</tbody>
</table>

### Cardiovascular risk factors for HTRA1 carriers

<table>
<thead>
<tr>
<th>FRS status</th>
<th>HTRA1 status</th>
<th>Ischemic stroke, No. (%)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Noncarrier</td>
<td>1963 (0.9)</td>
<td>[Reference]</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>4 (1.6)</td>
<td>2.06 (0.69-4.59)</td>
</tr>
<tr>
<td>High</td>
<td>Noncarrier</td>
<td>5373 (2.7)</td>
<td>3.27 (3.11-3.45)</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>11 (4.7)</td>
<td>5.99 (3.16-10.17)</td>
</tr>
</tbody>
</table>

### Polygenic risk score for NOTCH3 carriers

<table>
<thead>
<tr>
<th>PRS status</th>
<th>NOTCH3 status</th>
<th>Ischemic stroke, No. (%)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Noncarrier</td>
<td>3131 (1.5)</td>
<td>[Reference]</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>18 (4.4)</td>
<td>3.11 (1.90-4.76)</td>
</tr>
<tr>
<td>High</td>
<td>Noncarrier</td>
<td>4181 (2.0)</td>
<td>1.30 (1.24-1.36)</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>21 (4.2)</td>
<td>2.99 (1.89-4.45)</td>
</tr>
</tbody>
</table>

### Polygenic risk score for HTRA1 carriers

<table>
<thead>
<tr>
<th>PRS status</th>
<th>HTRA1 status</th>
<th>Ischemic stroke, No. (%)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Noncarrier</td>
<td>3155 (1.5)</td>
<td>[Reference]</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>8 (3.4)</td>
<td>2.22 (1.04-4.07)</td>
</tr>
<tr>
<td>High</td>
<td>Noncarrier</td>
<td>4181 (2.0)</td>
<td>1.29 (1.23-1.36)</td>
</tr>
<tr>
<td></td>
<td>Carrier</td>
<td>7 (3.0)</td>
<td>2.04 (0.90-3.86)</td>
</tr>
</tbody>
</table>

A. Risk of ischemic stroke by NOTCH3 status and Framingham cardiovascular risk score (FRS) strata. For a multiplicative interaction with the categorical low and high FRS groupings, \( P = .07 \); for the additive interaction with categorical FRS, assessed using the synergy index, \( P < .001 \). B. Risk of ischemic stroke by HTRA1 status and FRS strata. For a multiplicative interaction with the categorical FRS groupings, \( P = .85 \); for the additive interaction with categorical FRS, assessed using the synergy index, \( P < .001 \). C. Risk of ischemic stroke by NOTCH3 status and polygenic risk score (PRS) strata. For a multiplicative interaction with the categorical low and high PRS groupings, \( P = .97 \); for the additive interaction with categorical PRS, assessed using the synergy index, \( P = .18 \). D. Risk of ischemic stroke by HTRA1 status and PRS strata. For a multiplicative interaction with the categorical PRS groupings, \( P = .29 \); for the additive interaction with categorical PRS, assessed using the synergy index, \( P = .28 \).
associated with increased brain volume. The reason for this is unclear because patients with severe CADASIL have reduced brain volume. It is possible that pathological changes in earlier disease stages are associated with inflammatory or edematous changes that could increase brain volume, although further research is required.

Strengths and Limitations

Strengths of our study include that we used the largest cohort to date and extended previous work to analyze associations with all 3 common monogenic cSVDs. Furthermore, the prospective nature of UK Biobank allowed us to compare risk of incident stroke conferred by variants to that from a risk prediction score used in clinics.

Our study also has limitations. The study sample was large but not necessarily representative of the wider UK population, and frequency of monogenic stroke variants may differ between ethnic groups, although this should not affect inferences in this study. Second, we were unable to examine associations of variants directly with lacunar stroke because these data were not available. Also, diseases like dementia can sometimes be misclassified or underrepresented by the codes in health records.

Conclusions

This cohort study demonstrated in more than 450,000 individuals that relatively common variants in genes causing monogenic cSVD are associated with increased risk of both stroke and dementia. This increase in risk is primarily via ischemic stroke for NOTCH3 and HTRA1 and via intracerebral hemorrhage for COL4A1/2. Our results show that factors contributing to the variation in penetrance include variant location within the gene and conventional vascular risk factors. Our results imply that intensive vascular risk factor control is likely to improve disease prognosis in individuals with these genetic variants and that identifying individuals early before onset of symptoms and covert MRI changes of cSVD might help reduce stroke and dementia risk.
REFERENCES


