RESEARCH ARTICLE

Identifying genetic variants associated with the ICD10 (International Classification of Diseases10)-based diagnosis of cerebrovascular disease using a large-scale biomedical database

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Abstract

Objectives

To utilize the UK Biobank to identify genetic variants associated with the ICD10 (International Classification of Diseases10)-based diagnosis of cerebrovascular disease (CeVD).

Background

Cerebrovascular disease occurs because of a complex interplay between vascular, environmental, and genetic factors. It is the second leading cause of disability worldwide. Understanding who may be genetically predisposed to cerebrovascular disease can help guide preventative efforts. Moreover, there is considerable interest in the use of real-world data, such as EHR (electronic health records) to better understand disease mechanisms and to discover new treatment strategies, but whether ICD10-based diagnosis can be used to study CeVD genetics is unknown.

Methods

Using the UK Biobank, we conducted a genome-wide association study (GWAS) where we analyzed the genomes of 11,155 cases and 122,705 controls who were sex, age and ancestry-matched in a 1:11 case: control design. Genetic variants were identified by Plink's firth logistic regression and assessed for association with the ICD10 codes corresponding to CeVD.

Results

We identified two groups of SNPs closely linked to *PITX2* and *LRRTM4* that were significantly associated with CeVD in this study ($p < 5 \times 10^{-8}$) and had a minor allele frequency of $> 0.5\%$.

Competing interests: The authors have declared that no competing interests exist.

Discussion

Disease assignment based on ICD10 codes may underestimate prevalence; however, for CeVD, this does not appear to be the case. Compared to the age- and sex-matched control population, individuals with CeVD were more frequently diagnosed with comorbid conditions, such as hypertension, hyperlipidemia & atrial fibrillation or flutter, confirming their contribution to CeVD. The UK Biobank based ICD10 study identified 2 groups of variants that were associated with CeVD. The association between *PITX2* and CeVD is likely explained by the increased rates of atrial fibrillation and flutter. While the mechanism explaining the relationship between *LRRTM4* and CeVD is unclear, this has been documented in previous studies.

Introduction

Cerebrovascular disease (CeVD), which commonly manifests as a stroke, is one of the leading causes of serious long-term disability and the second leading cause of death worldwide [1]. Although there is a decline in cerebrovascular disease mortality in the United States, cerebrovascular disease continues to be the fourth leading cause of death among women and fifth leading cause of death among men, with an estimated 795,000 new or recurrent strokes occurring each year [2]. What is even more concerning is that there has been a reported 19% increase in the rate of cerebrovascular disease in women between the ages of 35 and 64 [1]. Cerebrovascular disease is the result of a complex interplay between vascular, genetic and environmental factors [3]. Recent studies have demonstrated that traditional risk factors such as diabetes, hyperlipidemia, hypertension, and smoking may only account for a small proportion of variance in atherosclerosis, suggesting that there may be novel non-traditional and genetic risk factors contributing to this process that have yet to be identified [3].

There is considerable interest in the use of real-world data, such as EHR (electronic health records) to better understand disease mechanisms and to discover new treatment strategies [4, 5]. The UK Biobank is a large, ongoing prospective cohort study that recruited 502,682 UK participants between 2006–2010. UK Biobank has compiled extensive health-related records and genetic data from participants [6, 7]. In this manuscript, using ICD-10 diagnostic codes as a diagnosis of CeVD, we conducted a genome-wide association study (GWAS) to identify genetic variants associated with cerebrovascular disease.

Methods

Ethical approval

The current study involved de-identified data obtained from the UK Biobank Resource under Application Number 49852. It has received the proper ethical oversight, including the determination by the University of Maryland, Baltimore Institutional Review Board that the study is not human research (IRB #: HF-00088022).

Study population

We carried out a GWAS using the UK Biobank to assess for statistically significant single nucleotide polymorphisms (SNPs) and the clinical manifestations of cerebrovascular disease at the population level. The UK Biobank recruited 502,682 UK participants between 2006 and

2010. The participants were between the ages of 40 and 69 at the time of recruitment. Extensive health-records were collected from participants, including clinical and genetic data with over 820,000 genotyped SNPs and up to 90 million imputed variants available for most individuals [7].

Genome-wide association study

Using data from the UK Biobank Resource on 487,310 subjects with imputed genotypes, we performed quality control by removing those with genetic relatedness exclusions (Data-Field 22018—UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=22018; 1532 subjects), sex chromosome aneuploidy (Data-Field 22019 –UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=22019; 651 subjects), mismatch between self-reported sex and genetically determined sex (Data-Field 31 –UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=31; Data-Field 22001 –UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=22001; 372 subjects), recommended genomic analysis exclusions (Data-Field 22010—UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=22010; 480 subjects), and outliers for heterozygosity or missing rate (Data-Field 22017 –UKB, https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=22077; 968 subjects).

Cases were defined using the International Standard Classification of Diseases and Related Health Problems, 10th edition (ICD-10) diagnostic codes related to cerebrovascular disease as primary or secondary diagnosis at the time of this analysis (August 2021). **Table 1** has a list of all ICD-10 codes included as cases in this analysis. The set of selected cases was purged of relatedness by removing one from each related pair in an iterative fashion until no subjects remained. Relatedness was defined as a kinship coefficient greater than 0.44. This treats third-degree relationships, which was defined a kinship coefficient of 0.0625, as related. Kinship coefficients for all subject pairs greater than 0.44 were provided by the UK Biobank as part of the standard set. To select the controls, all cases and related individuals were first removed from the pool. From the reduced pool, we were able to select 11 control subjects for each case, matching for age, sex and ancestry using incremental tolerances. The tolerance for age ranged from 0 (exact match) up to 7 years. Ancestry matching was performed with principal components (PCs) supplied by the UK Biobank. The mathematical distance in a graph plotting the PC1 x PC2 was used to test similarity in ancestry. The “distance” in ancestry tolerance ranged from 2 PC units to a maximum of 80 PC units with PC1 (**S1 Fig**), ranging from 0 to +400 and PC2 ranging from -300 to +100 units. These tolerances were used to identify the 11 matching controls for every case.

The association analysis was carried out using Plink’s firth logistic regression model and adjusted for age, sex and the 5 PCs using data supplied by the UK Biobank Resource [8]. Firth regression was chosen because it has been shown to provide the best combination of control for type 1 error and power for detection of low frequency variants [9, 10]. The cases of CeVD were analyzed with the 40 million imputed genetic variants provided by the UK Biobank with imputation quality scores of greater than 0.70. The covariates included in the analyses were sex, age and principal components 1 through 5. Although pre-calculated PC for the first 40 principal components was supplied by the UK Biobank, our preliminary analysis showed that only the first 5 PCs were considered significant (p value less than 0.05). Therefore, only the first 5 PCs were included in this GWAS.

Identification of variants for the CeVD phenotype

Variants were determined to be of interest if they had a minor allele frequency (MAF) of 0.5% or greater and met the standard threshold for genome-wide significance (p-value of less than 5 x 10^{-8}). Identified variants were assessed for the presence of previously reported phenotypic

Table 1. List of ICD-10 codes included as cerebrovascular disease cases.

ICD-10 code	Diagnosis
G45.0	Vertebro-basilar artery syndrome
G45.1	Carotid artery syndrome (hemispheric)
G45.2	Multiple and bilateral precerebral artery syndromes
G45.3	Amaurosis fugax
G45.4	Transient global amnesia
G45.8	Other transient cerebral ischemic attacks and related syndromes
G45.9	Transient cerebral ischemic attack
G46.0	Middle cerebral artery syndrome
G46.1	Anterior cerebral artery syndrome
G46.2	Posterior cerebral artery syndrome
G46.3	Brain stem stroke syndrome
G46.4	Cerebellar stroke syndrome
G46.5	Pure motor lacunar syndrome
G46.6	Pure sensory lacunar syndrome
G46.7	Other lacunar syndromes
G46.8	Other vascular syndromes of brain in cerebrovascular diseases
I63.0	Cerebral infarction due to thrombosis of precerebral arteries
I63.1	Cerebral infarction due to embolism of precerebral arteries
I63.2	Cerebral infarction due to unspecified occlusion or stenosis of precerebral arteries
I63.3	Cerebral infarction due to thrombosis of cerebral arteries
I63.4	Cerebral infarction due to embolism of cerebral arteries
I63.5	Cerebral infarction due to unspecified occlusion or stenosis of cerebral arteries
I63.6	Cerebral infarction due to cerebral venous thrombosis, nonpyogenic
I63.8	Other cerebral infarction
I63.9	Cerebral infarction, unspecified
I64	Stroke, not specified as hemorrhage or infarction
I65.0	Occlusion and stenosis of vertebral artery
I65.1	Occlusion and stenosis of basilar artery
I65.2	Occlusion and stenosis of carotid artery
I65.3	Occlusion and stenosis of multiple and bilateral precerebral arteries
I65.8	Occlusion and stenosis of other precerebral artery
I65.9	Occlusion and stenosis of unspecified precerebral artery
I66.0	Occlusion and stenosis of middle cerebral artery
I66.1	Occlusion and stenosis of anterior cerebral artery
I66.2	Occlusion and stenosis of posterior cerebral artery
I66.3	Occlusion and stenosis of cerebellar arteries
I66.4	Occlusion and stenosis of multiple and bilateral cerebral arteries
I66.8	Occlusion and stenosis of other cerebral artery
I66.9	Occlusion and stenosis of unspecified cerebral artery
I67.2	Cerebral atherosclerosis
I67.9	Cerebrovascular disease, unspecified
I69.3	Sequelae of cerebral infarction
I69.4	Sequelae of stroke, not specified as hemorrhage or infarction
I69.8	Sequelae of other and unspecified cerebrovascular diseases

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associations using pheweb.org and the Cerebrovascular Disease Knowledge Portal. Pheweb.org is a GWAS dataset for electronic health record-derived disease associations from the white British participants in the UK Biobank. PheWeb utilizes a generalized mixed model association test that uses the saddle point approximation to account for case-control imbalance and imputed using the Haplotype Reference Consortium Panel [11]. The Cerebrovascular Disease Knowledge Portal (cd.hugeamp.org) is an online platform that provides comprehensive quality-assured genetic and phenotypic data on a large number of patients with stroke worldwide. It includes data from up to 18 different datasets [12].

Results

A total of 11,155 individuals (6,777 males and 4,378 females) were identified as CeVD cases at the time of this analysis (January 2021). This represents a prevalence of 22.2 cases per 1,000 people in the study population. Around 60% of the cases were male. The average age of the cases at the time of diagnosis was 63.8 years (+/- 8.3). Additionally, cases tend to have a higher BMI (28.5 vs 27.5, p <0.001) and a greater waist circumference (37.6 inches vs 36.4 inches, p <0.001). Most cases described their ethnic background as British (89.6%) with a smaller proportion reporting their ethnic background as Irish (3.0%) and Indian (1.0%). Demographic characteristics are shown in Table 2. Relevant biometric and biochemical profiles of both the cases and controls can be seen in Table 3. A more expansive list can be seen in S1 Table.

Relevant ICD-10 diagnoses associated with CeVD can be seen in Table 4. A full list of ICD-10 diagnoses associated with CeVD can be seen in S2 Table. Cases were more likely to be diagnosed with chronic renal failure (N18.9: 6% vs 1%, p <0.001) and acute renal failure (N17.9: 10% vs 2%, p <0.001) and had higher levels of urea (35.2 mg/dl vs 33.8 mg/dl, p <0.001) and creatinine (0.79 mg/dl vs 0.75 mg/dl, p <0.001) which is consistent with the increased rate of renal disease in the cases. Additionally, cases had higher rates of chronic ischemic heart disease (I25.9: 18% vs 5%, p <0.001), atherosclerotic heart disease (I25.1: 18% vs 7%, p <0.001), old myocardial infarction (I25.2: 12% vs 3%, p <0.001), left ventricular failure (I50.1: 6% vs 1%, p <0.001), atrial fibrillation and flutter (I48: 23% vs 6%, p <0.001), syncope and collapse (R55: 11% vs 3%, p <0.001), epilepsy (G40.9: 6% vs 1%, p <0.001) and personal use of long-term anticoagulants (Z92.1: 20% vs 4%, p <0.001). Cases were also more likely to have risk factors that are typically associated with cerebrovascular disease including tobacco use (Z72.0: 11% vs 3%, p <0.001), hyperlipidemia (E78.5: 8% vs 2%, p <0.001), primary hypercholesterolemia (E78.0: 41% vs 12%, p <0.001) and essential hypertension (I10: 66% vs 28%, p <0.001). Although the systolic blood pressure was higher in cases (145 mm Hg vs 144 mm Hg, p <0.001), there was no significant difference in the diastolic blood pressure (83 mm Hg in cases

Table 2. Demographic characteristics of individuals with cerebrovascular disease and their respective controls.

	Cases	Controls
Cases (#)	11,155	122,705
Male	60.8%	60.8%
Males, mean age at time of diagnosis (years ± SD)	63.76 ± 8.18	Age matched to cases
Females, mean age at time of diagnosis (years ± SD)	63.86 ± 8.52	Age matched to cases
Ethnic Background		
White British	89.6%	89.8%
White Irish	3.0%	2.5%
Other white background	2.5%	2.9%
Indian	1.0%	1.2%
Pakistani	0.4%	0.4%
African	0.6%	0.6%
Caribbean	0.9%	0.9%

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Table 3. Relevant biometrics and biomarkers of cases and controls.

Biometric/Biomarker	Cases (N = 11,155)	Controls (N = 122,705)	P Value	Clinical Significance
BMI (kg/m^2)	28.5	27.5	<0.001	N
Waist Circumference (in)	37.6	36.4	<0.001	N
Weight (lbs)	180	176	<0.001	N
Systolic blood pressure (mmHg)	145	144	<0.001	N
Diastolic blood pressure (mmHg)	83.0	82.9	NS	N
Mean arterial blood pressure (mmHg)	103	103	<0.001	N
Pulse Pressure (mmHg)	60.3	59.1	<0.001	N
Pulse Rate (bpm)	70.6	69.3	<0.001	N
Apolipoprotein A (mg/dL)	148	153	<0.001	N
Apolipoprotein B (mg/dL)	96.7	103	<0.001	N
Total Cholesterol (mg/dL)	202	218	<0.001	M
C Reactive Protein (mg/dL)	99.5	74.4	<0.001	M
HDL (mg/dL)	51.8	54.9	<0.001	N
LDL (mg/dL)	125	137	<0.001	M
Lipoprotein A (mg/dL)	22.8	21.1	<0.001	N
Triglycerides (mg/dL)	167	161	<0.001	N
Glucose (mg/dL)	98.3	93.4	<0.001	N
Hemoglobin A1c (%)	5.7	5.5	<0.001	N
Hemoglobin (g/dL)	14.3	14.4	<0.001	N
Hematocrit (percent)	41.5	41.8	<0.001	N
Mean Corpuscular Volume (fL)	91.8	91.4	<0.001	N
White Blood Cell Count (1x 10^9/L)	7.4	6.9	<0.001	N
Platelet Count (1 x 10^9/L)	251	246	<0.001	N
Blood Urea Nitrogen (mg/dL)	35.2	33.8	<0.001	N
Creatinine (mg/dL)	0.79	0.75	<0.001	N
Cystatin C (mg/L)	1.02	0.94	<0.001	N
Phosphate (mg/dl)	3.56	3.56	<0.05	N
Uric Acid (mg/dL)	5.66	5.46	<0.001	N
Microalbumin–Urine (mg/L)	59.6	31.0	<0.001	M
Creatinine–Urine (mmol/L)	9710	9150	<0.001	L

NS: Not significant, p >0.05; N: No clinical significance; L: Low clinical significance; M: Medium clinical significance; H: High clinical significance

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vs 82.9 in controls, p > 0.05). Finally, cases had a higher rate of type 2 diabetes mellitus (20% vs 7%, p <0.001) which could also be seen through cases having a higher hemoglobin A1c level (5.7% vs 5.5%, p <0.001) and higher serum glucose levels (98.3 mg/dl vs 93.4 mg/dl, p <0.001).

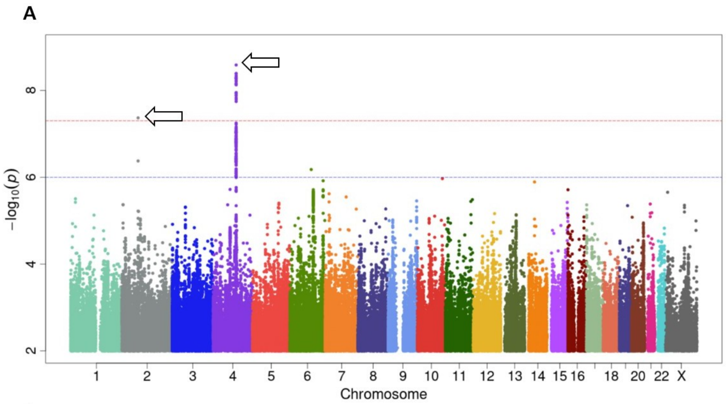
While cases were more likely to be diagnosed with hyperlipidemia and primary hypercholesterolemia, they had lower low-density lipoprotein (LDL) (125 mg/dl vs 137 mg/dl, p <0.001) and cholesterol levels (202 mg/dl vs 218 mg/dl, p <0.001) but higher triglycerides (167 mg/dl vs 161 mg/dl, p <0.001). Additionally, high-density lipoprotein (HDL) levels were lower in the cases (51.8 mg/dl vs 54.9 mg/dl, p <0.001). Interestingly, cases also had higher levels of C-reactive protein (CRP) (99.5 mg/dl vs 74.4 mg/dl, p <0.001).

GWAS identified two group of SNPs with a MAF of ≥ 0.5% that were associated with cerebrovascular disease with a p-value of less than 5 x 10^{-8} (**Figs 1 and 2**). First, a group of 23 SNPS in close to proximity to sequences encoding *PITX2* (Paired like homeodomain 2, a gene that encodes the RIEG/PITX homeobox family) were found to be associated with CeVD (**S3**

Table 4. Relevant ICD10 diagnoses associated with cerebrovascular disease cases.

ICD10 Diagnosis	Cases (N = 11,155)	Controls (N = 122,705)	P Value
Peripheral vascular disease; unspecified (I73.9)	6%	1%	<0.001
Unstable angina (I20.0)	6%	2%	<0.001
Presence of aortocoronary bypass graft (Z95.1)	7%	2%	<0.001
Other forms of chronic ischemic heart disease (I25.8)	9%	2%	<0.001
Presence of coronary angioplasty implant and graft (Z95.5)	7%	3%	<0.001
Old myocardial infarction (I25.2)	12%	3%	<0.001
Chronic ischemic heart disease; unspecified (I25.9)	18%	5%	<0.001
Angina pectoris; unspecified (I20.9)	17%	6%	<0.001
Atherosclerotic heart disease (I25.1)	18%	7%	<0.001
Left ventricular failure (I50.1)	6%	1%	<0.001
Disorientation; unspecified (R41.0)	6%	1%	<0.001
Tendency to fall; not elsewhere classified (R29.6)	6%	1%	<0.001
Chronic renal failure (N18.9)	6%	1%	<0.001
Acute renal failure; unspecified (N17.9)	10%	2%	<0.001
Malaise and fatigue (R53)	5%	1%	<0.001
Epilepsy; unspecified (G40.9)	6%	1%	<0.001
Hyperlipidemia; unspecified (E78.5)	8%	2%	<0.001
Pleural effusion; not elsewhere classified (J90)	6%	1%	<0.001
Dizziness and giddiness (R42)	7%	1%	<0.001
Lobar pneumonia; unspecified (J18.1)	8%	2%	<0.001
Dyspnea (R06.0)	7%	2%	<0.001
Personal history of diseases of the nervous system and sense organs (Z86.6)	26%	2%	<0.001
Family history of ischemic heart disease and other diseases of the circulatory system (Z82.4)	11%	4%	<0.001
Iron deficiency anemia; unspecified (D50.9)	5%	2%	<0.001
Chronic obstructive pulmonary disease; unspecified (J44.9)	10%	3%	<0.001
Headache (R51)	9%	2%	<0.001
Dysphagia (R13)	6%	2%	<0.001
Tobacco use (Z72.0)	11%	3%	<0.001
Other chest pain (R07.3)	7%	2%	<0.001
Other specified abnormal findings of blood chemistry (R79.8)	7%	3%	<0.001
Alcohol use (Z72.1)	7%	2%	<0.001
Retention of urine (R33)	7%	3%	<0.001
Syncope and collapse (R55)	11%	3%	<0.001
Personal history of long-term (current) use of anticoagulants (Z92.1)	20%	4%	<0.001
Harmful use (F17.1)	11%	3%	<0.001
Anemia; unspecified (D64.9)	10%	3%	<0.001
Nausea and vomiting (R11)	10%	3%	<0.001
Depressive episode; unspecified (F32.9)	11%	3%	<0.001
Obesity; unspecified (E66.9)	8%	4%	<0.001
Personal history of diseases of the circulatory system (Z86.7)	47%	4%	<0.001
Atrial fibrillation and flutter (I48)	23%	6%	<0.001
Unknown and unspecified causes of morbidity (R69)	8%	4%	<0.001
Hypothyroidism; unspecified (E03.9)	8%	4%	<0.001
Chest pain; unspecified (R07.4)	17%	7%	<0.001
Non-insulin-dependent DM Without complications (E11.9)	20%	7%	<0.001
Pure hypercholesterolemia (E78.0)	41%	12%	<0.001
Essential (primary) hypertension (I10)	66%	28%	<0.001

https://doi.org/10.1371/journal.pone.0273217.t004



B

Gene	rs Number	Chromosome	Position	Variant Type	Odds Ratio	95% CI	P value	Minor Allele Frequency
PITX2	rs61411276	4	111,697,815	Intergenic	1.11	1.07 – 1.15	2.6 x 10^{-9}	20.2%
LRRTM4	rs1922809	2	77,687,101	Intronic	1.08	1.05 – 1.11	4.3 x 10^{-8}	42.7%

Fig 1. Genome-wide association study analysis for variants associated with cerebrovascular disease in the UK Biobank. (A) Manhattan plot of GWAS results (MAF > 0.5%) for CeVD identified 2 groups of SNPs that were significantly associated with CeVD **(B)** SNPs with the highest statistical significance from each of the two groups associated with CeVD. For the Manhattan plot, significance is displayed on the y-axis as -$\log_{10}$ of the *p*-value, with results ordered along the x-axis by chromosome (each colored bar represents a different chromosome). Arrows represents SNP associated with a genome-wide significance of 5 x 10^{-8}. OR: Odds ratio; *PITX2*: Paired like homeodomain 2; *LRRTM4*: leucine rich repeat transmembrane neuronal 4; CI: Confidence Interval.

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Table). Of this group, the intergenic SNP rs61411276 (mean allele frequency of 20.2%) was found to be most significantly associated with the outcome, with an odds ratio (OR) of 1.11, 95% confidence interval of 1.07 to 1.15, and a p-value of 2.59 x 10^{-9}. A second SNP (rs1922809) in close proximity to sequences encoding *LRRTM4* (leucine rich repeat transmembrane neuronal 4) was identified. The intronic SNP rs1922809 had an OR of 1.08, 95% confidence interval of 1.05 to 1.11, and a p-value of 4.29 x 10^{-8}. Data from the PheWeb

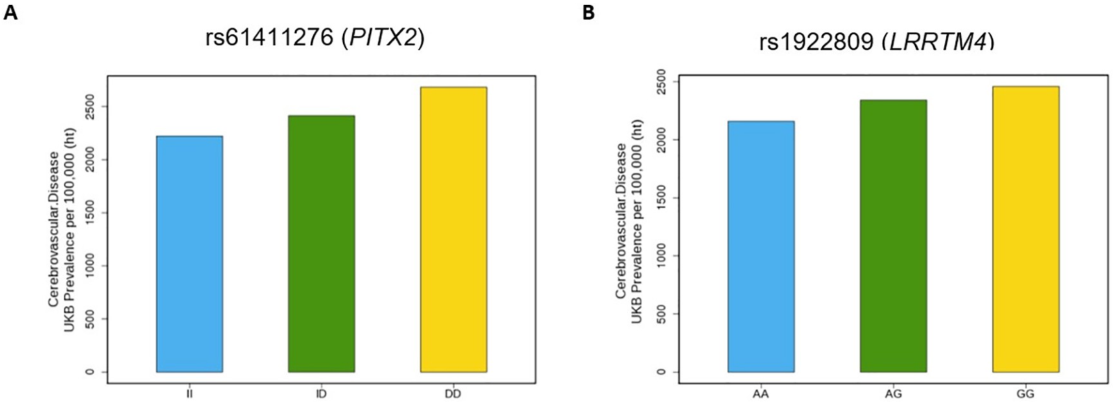

Fig 2. Single nucleotide polymorphisms associated with cerebrovascular disease in the UK Biobank. (A) Prevalence of CeVD increases with PITX2 variant rs61411276 status (2,222 per 100,000 for wild type; 2,414 per 100,000 for heterozygotes; 2,682 per 100,000 for homozygotes; p-value 2.6 x 10^{-9}; OR 1.1 per D allele). (B) Prevalence of CeVD increases with LRRTM4 variant rs1922809 status (2,159 per 100,000 for wild type; 2,341 per 100,000 for heterozygotes; 2,458 per 100,000 for homozygotes; p-value 4.3 x 10^{-8}; OR 1.08 per G allele). OR: Odds ratio; PITX2: Paired like homeodomain 2; LRRTM4: leucine rich repeat transmembrane neuronal 4.

https://doi.org/10.1371/journal.pone.0273217.g002 database was available for rs1922809 and showed that this SNP was associated with cerebrovascular disease. Data from the Cerebrovascular Disease Knowledge Portal was available for both SNPs. While the association between rs61411276 and ischemic stroke was not significant (OR = 1.04, p = 0.09327 with an effective sample size of 93,661), there was a significant association between rs1922809 and ischemic stroke (OR = 1.04, p = 0.0284 with an effective sample size of 513,323) and brain microbleeds (mixed or strictly deep, OR = 1.09, p = 0.0318 with an effective sample size of 4,848).

Discussion

Through this ICD-10 based study, we identified two groups of SNPs near *PITX2* and *LRRTM4* that were significantly associated with CeVD. There has been a growing body of literature to suggest that although traditional risk factors such as smoking, diabetes and hypertension do increase the risk of CeVD, CeVD also has a genetic component that has yet to be fully understood [3]. With that in mind, although we matched for age, sex and ancestry, the cases in our study did have higher rates of traditional risk factors including smoking, diabetes mellitus, atrial fibrillation and flutter, hypertension and were more likely to have other atherosclerotic conditions such as atherosclerotic heart disease and peripheral vascular disease.

As with all GWAS studies, although we were able to demonstrate a statistically significant association between the novel loci identified in this manuscript and the development of CeVD, this does not imply causation. However, the findings of this study do have a biological plausibility that can be explained. For instance, the *PITX2* gene encodes a protein that regulates right-left differentiation of the embryonic heart and expressed in the adult left atrium. A deficiency in *PITX2* can result in electrical and structural remodeling in murine models and this may predispose patients to atrial fibrillation, a known risk factor for thromboembolic stroke [13]. Additionally, our findings are consistent with other studies that have described the association between *PITX2* and cardioembolic stroke [14]. In our analysis, cases had a significantly higher rate of atrial fibrillation and flutter (I48) and were more likely to be on anticoagulation as compared to cases. Interestingly, in both the cases and controls, the proportion of participants with atrial fibrillation increases with progression from wild-type to homozygotes for rs61411276 (Cases: II: 20%, ID: 26%, DD: 36%, $p < 0.001$; Controls: II: 5%, ID: 7%, DD: 10%, $p < 0.001$). However, there was no significant difference in the rate of atherosclerotic heart disease (I25.1) when comparing wild-type, heterozygotes and homozygotes in both cases and controls ($p > 0.05$). This would suggest that the increased rate of CeVD with rs61411276 is likely secondary to atrial fibrillation/flutter as opposed to atherosclerosis.

Also, the SNP rs1922809 closely linked to *LRRTM4* was associated with an increased risk of CeVD in our study. While the mechanism that can explain this association is not clear, this finding is consistent with the Cerebrovascular Disease Knowledge Portal. As seen in the Cerebrovascular Disease Knowledge Portal, rs1922809 was significantly associated with ischemic strokes and microbleeds in populations other than the UK Biobank [12]. *LRRTM4* is expressed in the central nervous system and the structure and expression profile of LRRTM4 mRNAs suggest that it may have a role and maintenance of the nervous system [15]. Additionally, it has been identified as a possible marker of cognitive impairment in one study and has been shown to play a role in synapse function [16, 17]. We performed a post-hoc power calculation on the signal for rs1922809 to assess the probability of detecting the OR that we found in our analysis. Using the power calculator on the University of Michigan website [18] we determined that we had 80% power to detect an OR of 1.10 and 30% power to detect an OR of 1.08.

There are limitations to this study that need to be highlighted. Firstly, although we were able to demonstrate a statistically significant association, this does not imply causality and the findings of the study will need to be verified in future analyses, ideally in a more diverse patient population. Due to the high prevalence of traditional risk factors associated with CeVD, it was not feasible to only include patients without traditional risk factors as this would have significantly reduced the power of this analysis. Additionally, as compared to a study that is dedicated specifically to studying CeVD, this analysis utilized data from the UK Biobank and identified cases using ICD10 codes. ICD10 based studies are limited by the fact that many diseases and medical diagnoses are often underdiagnosed. However, that does not seem to be the case here as the prevalence of cerebrovascular disease in our study group is similar to prevalences reported in recent literature [19]. In this study, controls are selected randomly from a pool of those who do not carry the ICD10 diagnosis, not those in whom CeVD has been specifically ruled out. However, ICD10 based studies also offer the opportunity to study a wide array of diseases and medical conditions, both common and rare, using an already available data source. One method of improving the accuracy of successfully differentiating between cases and controls is to incorporate imaging data, such as magnetic resonance imaging or angiography, into the analysis. Although this may limit the number of patients included in the study, it would more accurately distinguish between cases and controls. Ultimately, ICD10 based studies can help direct research to potential variants that were associated with CeVD so that future studies can then be designed to further explore and validate these findings in other cohorts and if valid, potentially be incorporated into a genetic risk score to better identify high risk patients.

Conclusions

In this ICD-10 based study, we have identified two groups of SNPs (*PITX2* and *LRRTM4*) that were significantly associated with the diagnosis of CeVD in the UK Biobank ($p < 5 \times 10^{-8}$). The SNP (rs61411276) closely linked to *PITX2* gene was associated with an increased risk of atrial fibrillation and flutter which can explain the association between this gene and the increased CeVD risk. Although the mechanism is unclear, *LRRTM4* gene has been associated with cerebrovascular disease in past studies. While ICD10 based studies do have their limitations, they provide the opportunity to study a wide array of diseases and identify potential variants associated with medical conditions (both rare and common) that can then be validated and explored in further studies.

Supporting information

S1 Table. Full biometrics and biomarkers of cases and controls.
(DOCX)

S2 Table. ICD10 codes associated with cerebrovascular disease in the UK Biobank.
(DOCX)

S3 Table. Single nucleotide polymorphisms associated with cerebrovascular disease in this genome-wise association study.
(DOCX)

S1 Fig. Principal components (PCs) by ethnicity for UK Biobank participants. When selecting controls for comparison with cases, control subjects were selected from subjects within 80 units on the PC1 vs. PC2 graph. The size of 80 units is illustrated with the red boxes around subjects who are primarily European, Chinese, or African Ethnicity based on the PC1 and PC2 eigenvalues provided by the UK Biobank.
(TIF)

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References

1. Tong X, Yang Q, Ritchey MD, George MG, Jackson SL, Gillespie C, et al. The Burden of Cerebrovascular Disease in the United States. Prev Chronic Dis. 2019; 16:E52. https://doi.org/10.5888/pcd16.180411 PMID: 31022369
2. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation. 2017; 135(10):e146–e603. https://doi.org/10.1161/CIR.0000000000000485 PMID: 28122885
3. Della-Morte D, Pacifici F, Rundek T. Genetic susceptibility to cerebrovascular disease. Curr Opin Lipidol. 2016; 27(2):187–95. https://doi.org/10.1097/MOL.0000000000000275 PMID: 26959706
4. Casey JA, Schwartz BS, Stewart WF, Adler NE. Using Electronic Health Records for Population Health Research: A Review of Methods and Applications. Annu Rev Public Health. 2016; 37:61–81. https://doi.org/10.1146/annurev-publhealth-032315-021353 PMID: 26667605
5. Abul-Husn NS, Kenny EE. Personalized Medicine and the Power of Electronic Health Records. Cell. 2019; 177(1):58–69. https://doi.org/10.1016/j.cell.2019.02.039 PMID: 30901549
6. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018; 562(7726):203–9. https://doi.org/10.1038/s41586-018-0579-z PMID: 30305743
7. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015; 12(3):e1001779. https://doi.org/10.1371/journal.pmed.1001779 PMID: 25826379
8. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015; 4:7. https://doi.org/10.1186/s13742-015-0047-8 PMID: 25722852
9. Ma C, Blackwell T, Boehnke M, Scott LJ. Recommended joint and meta-analysis strategies for case-control association testing of single low-count variants. Genet Epidemiol. 2013; 37(6):539–50. https://doi.org/10.1002/gepi.21742 PMID: 23788246
10. Wang X. Firth logistic regression for rare variant association tests. Front Genet. 2014; 5:187. https://doi.org/10.3389/fgene.2014.00187 PMID: 24995013
11. Initiative MG. UKBiobank ICD PheWeb 2017 Available from: https://pheweb.org/UKB-SAIGE/about.

12. Cerebrovascular Disease Knowledge Portal Available from: https://cd.hugeamp.org/about.html.

13. Syeda F, Kirchhof P, Fabritz L. PITX2-dependent gene regulation in atrial fibrillation and rhythm control. J Physiol. 2017; 595(12):4019–26. https://doi.org/10.1113/JP273123 PMID: 28217939

14. Hauer AJ, Pulit SL, van den Berg LH, de Bakker PIW, Veldink JH, Ruigrok YM. A replication study of genetic risk loci for ischemic stroke in a Dutch population: a case-control study. Sci Rep. 2017; 7 (1):12175. https://doi.org/10.1038/s41598-017-07404-4 PMID: 28939865

15. Laurén J, Airaksinen MS, Saarma M, Timmusk T. A novel gene family encoding leucine-rich repeat transmembrane proteins differentially expressed in the nervous system. Genomics. 2003; 81(4):411–21. https://doi.org/10.1016/s0888-7543(03)00030-2 PMID: 12676565

16. Chen YC, Liu YL, Tsai SJ, Kuo PH, Huang SS, Lee YS. LRRTM4 and PCSK5 Genetic Polymorphisms as Markers for Cognitive Impairment in A Hypotensive Aging Population: A Genome-Wide Association Study in Taiwan. J Clin Med. 2019; 8(8).

17. Sinha R, Siddiqui TJ, Padmanabhan N, Wallin J, Zhang C, Karimi B, et al. LRRTM4: A Novel Regulator of Presynaptic Inhibition and Ribbon Synapse Arrangements of Retinal Bipolar Cells. Neuron. 2020; 105(6):1007–17.e5. https://doi.org/10.1016/j.neuron.2019.12.028 PMID: 31974009

18. Johnson J, Abecasis C. GAS Power Calculator: University of Michigan; 2017 Available from: http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html.

19. Stroke Statistics: Stroke Association; Available from: https://www.stroke.org.uk/what-is-stroke/stroke-statistics#Stroke%20prevalence%20in%20England.