Mendelian Genes and Risk of Intracerebral Hemorrhage and Small-Vessel Ischemic Stroke in Sporadic Cases

Michael Chong, MSc; Martin O'Donnell, MB BCh; Vincent Thijs, MD; Antonio Dans, MD; Patricio López-Jaramillo, MD; Diego Gómez-Arbeláez, MD; Charles Mondo, MB ChB; Anna Czlonkowska, MD; Marta Skowronska, MD; Shahram Oveisgharan, MD; Salim Yusuf, DPhil; Guillaume Paré, MD

Background and Purpose—Mendelian strokes are rare genetic disorders characterized by early-onset small-vessel stroke. Although extensively studied among families with syndromic features, whether these genes affect risk among sporadic cases is unknown.

Methods—We sequenced 8 genes responsible for Mendelian stroke in a case–control study of sporadic stroke cases (≤70 years). Participants included 1251 primary stroke cases of small-vessel pathology (637 intracerebral hemorrhage and 614 small-vessel ischemic stroke cases) and 1716 controls from the INTERSTROKE study (Study of the Importance of Conventional and Emerging Risk Factors of Stroke in Different Regions and Ethnic Groups of the World).

Results—Overall, the prevalence of canonical disease-causing mutations was 0.56% in cases and 0.23% in controls (odds ratio=1.89; 95% confidence interval, 0.54–7.57; *P*=0.33). CADASIL (Cerebral Autosomal Dominant Arteriopathies with Subcortical Infarcts and Leukoencephalopathies) mutations were more frequent among cases (0.48%) than controls (0.23%) but were not significantly associated with stroke risk (odds ratio=2.03; 95% confidence interval, 0.58–8.02; *P*=0.27). Next, we included all rare nonsynonymous mutations to investigate whether other types of mutations may contribute to stroke risk. Overall, 13.5% of cases and 14.2% of controls were carriers of at least one rare nonsynonymous mutation among the 8 Mendelian stroke genes. Mutation carriers were not at elevated risk of stroke (odds ratio=0.93; 95% confidence interval, 0.75–1.16; *P*=0.55).

Conclusions—In the absence of syndromic features and family history of stroke, screening for Mendelian mutations among small-vessel stroke patients is unlikely to have high diagnostic utility. **(*Stroke*. 2017;48:2263-2265. DOI: 10.1161/STROKEAHA.117.017322.)**

Key Words: CADASIL ■ case–control studies ■ genetics ■ prevalence ■ stroke

Mendelian strokes are a collection of monogenic stroke disorders caused by rare nonsynonymous mutations. They are characterized by substantial risk of early-onset small-vessel stroke, family history of stroke, and other debilitating features and thus are often referred to as familial stroke syndromes.[1] Pathologies affecting the small cerebral arteries include intracerebral hemorrhage (ICH) and small-vessel ischemic stroke (SVIS).[1] CADASIL (Cerebral Autosomal Dominant Arteriopathies with Subcortical Infarcts and Leukoencephalopathies) is the most frequent Mendelian stroke disorder, with an estimated prevalence of 0.001% to 0.002% in the general population[2,3] and 0.40% to 1.25% among SVIS patients.[4,5] Mutation prevalence and penetrance for CADASIL and other Mendelian stroke disorders remain elusive because of a scarcity of case–control studies. To address this issue, we sequenced 8 Mendelian genes in 1251 small-vessel stroke cases (mean age: 55.5 years), consisting of 614 SVIS and 637 ICH cases, in addition to 1716 controls from the INTERSTROKE study (Study of the Importance of Conventional and Emerging Risk Factors of Stroke in Different Regions and Ethnic Groups of the World).[6] Our objective was to assess prevalence and penetrance of Mendelian mutations in sporadic cases, and we hypothesized that rare nonsynonymous mutations in Mendelian stroke genes are important determinants of sporadic stroke.

Methods

The present research investigation is a subproject of INTERSTROKE, a large, global case–control study.[6] Because most Mendelian stroke

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From the Population Health Research Institute, Hamilton, Ontario, Canada (M.C., S.Y., G.P.); HRB Clinical Research Facility, NUI Galway, University Hospital Galway, Ireland (M.O.); Vlaams Instituut voor Biotechnologie, Vesalius Research Center, Department of Neurology, University Hospitals KU Leuven, Belgium (V.T.); Philippine General Hospital, Manila (A.D.); Fundación Oftalmológica de Santander, Medical School, Universidad de Santander, Bucaramanga, Colombia (P.L.-J., D.G.-A.); Division of Cardiology, Uganda Heart Institute, Kampala (C.M.); 2nd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland (A.C., M.S.); Department of Pharmacology, Medical University of Warsaw, Poland (A.C., M.S.); and Iranian Center of Neurological Research, Tehran University of Medical Sciences, Iran (S.O.).

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Correspondence to Guillaume Paré, MD, Population Health Research Institute, 237 Barton St East, C3-103, Hamilton, Ontario, Canada L8L 2X2. E-mail pareg@mcmaster.ca

disorders are associated with early-onset small-vessel disease,[1] we targeted primary early-onset stroke cases of small-vessel pathogenesis (SVIS or ICH). Among 1951 small-vessel stroke cases that consented to genetic analysis, 1378 were <70 years. Of these 1378 participants, we could match 1251 to one or more controls according to stringent criteria (sex, ethnicity, recruitment center, and age) for a total of 1251 cases and 1716 controls. Exome (n=368) and targeted gene (n=2599) sequencing were performed to detect genetic mutations within 8 Mendelian stroke genes (*APP*, *CECR1*, *COL4A1*, *COL4A2*, *GLA*, *HTRA1*, *NOTCH3*, and *TREX1*). See Methods section in the online-only Data Supplement for explicit gene selection criteria. Research was approved by the Hamilton Integrated Research Ethics Board. Study participant characteristics are available in Table I in the online-only Data Supplement.

Statistical Analysis

Only rare, nonsynonymous mutations were analyzed. Two different variant subsets were examined. First, a subset of mutations with strong priori for clinical pathogenicity, canonical disease-causing mutations, was evaluated, where the genotypic state of the mutation carrier was required to be consistent with the known mode of inheritance (Methods section and Table II in the online-only Data Supplement). Second, we conducted an exploratory analysis of all rare nonsynonymous mutations irrespective of clinical evidence assuming a dominant model because most Mendelian stroke disorders exhibit dominant modes of inheritance (Table II in the online-only Data Supplement).

To evaluate whether mutation burden differed between cases and controls, logistic regression was conducted using R version 3.0.1. Mutation carrier status was grouped per gene, such that rare nonsynonymous mutations within the same gene were consolidated into a single factor. The independent variable was mutation carrier status, the dependent variable was the combined phenotype of all stroke (SVIS+ICH), and covariates included ethnicity, age, and sex. For each association, post hoc power calculations were performed to identify the effect size at which our study was capable of detecting with 95% reliability. Essentially, this upper bound odds ratio can be interpreted as the maximum effect size for an association compatible with observed results.

Results

Canonical Disease-Causing Mutations

Canonical disease-causing mutations follow stereotypical patterns consistent with known pathogenic mutations. Among 2967 INTERSTROKE participants, 11 (0.37%) carried canonical disease-causing mutations including 7 (0.56%) cases and 4 (0.23%) controls (Table III in the online-only Data Supplement). Mutation carriers did not exhibit greater risk for stroke (odds ratio=1.89; 95% confidence interval, 0.54–7.57; *P*=0.33). Mutations were found in *NOTCH3* (10) and *GLA* (1), the causative genes for CADASIL and Fabry disease, respectively. CADASIL mutations were observed in 4 controls (0.23%) and 6 cases (0.48%), including 3 SVIS (0.49%) and 3 (0.47%) ICH cases. CADASIL mutation carrier status was not significantly associated with stroke risk (odds ratio=2.03; 95% confidence interval, 0.58–8.02; *P*=0.27). A single Fabry disease mutation was present in 1 SVIS case (0.10%). Detailed phenotypic and genotypic information for canonical disease-causing mutation carriers is available in Table IV in the online-only Data Supplement.

The prevalence of Mendelian stroke in the general population is uncertain, and the observed genetic prevalence of 0.23% among controls is high. To assess the robustness of this finding, we estimated genetic prevalence in the Exome Aggregate Consortium, an ethnically diverse genetic database of 60 706 individuals.[7] Exome Aggregate Consortium contained 273 (0.45%) mutation carriers, including 204 (0.34%) *NOTCH3*, 63 (0.10%) *COL4A1*, and 6 (0.01%) *TREX1* mutation carriers.

Rare Nonsynonymous Mutations

Although the previous analysis was highly specific only including mutations with high likelihood of being disease-causing, it lacks sensitivity and could preclude novel types of disease-associated mutations. Consequently, we expanded inclusion criteria to encompass all rare nonsynonymous mutations. In total, 412 individuals (13.9%) had at least one rare nonsynonymous mutation, including 169 cases (13.5%) and 243 controls (14.2%; Table V in the online-only Data Supplement). Mutation carriers were not at significantly greater risk of stroke (odds ratio=0.93; 95% confidence interval, 0.75–1.16; *P*=0.55). No associations were observed when stratifying by gene (Figure). Also, stratifying by stroke subtype or applying an earlier age cutoff, deleteriousness prediction algorithm, or recessive model did not reveal significant associations (Tables VI through X in the online-only Data Supplement).

Discussion

Mendelian mutations confer substantial risk to early-onset stroke in families with stroke syndromes, but few studies have formally evaluated mutation penetrance and frequency using a case–control design. Screening 8 Mendelian stroke genes in 2967 individuals, we observed no significant differences between cases and controls despite being sufficiently powered to detect effects larger than 5-fold. Accordingly, rare coding variation within Mendelian stroke genes may have a lesser role in patients without family history of stroke and syndromic features.

The collective prevalence of Mendelian stroke in the general population is unknown, except for CADASIL, which ranges from 0.001% to 0.002%.[2,3] In our study, the genetic prevalence was 0.48% among cases, consistent with other case studies (0.40%–1.25%).[4,5] Although genetic prevalence among cases is at least 200 times higher than clinical prevalence in the general population, this enrichment is greatly attenuated when compared with controls (0.23%) and Exome Aggregate Consortium participants (0.34%). The large disparity between clinical and genetic prevalence could be explained by: (1) underdiagnosis of CADASIL in the general population or (2) CADASIL mutations exhibiting incomplete penetrance and allelic heterogeneity. Both possibilities imply that disease severity varies among mutation carriers. This notion, although controversial, has been bolstered by Rutten et al[8] discovery of 4 CADASIL mutation carriers remaining stroke-free beyond 70 years. Environmental and other genetic factors are known to influence disease manifestation. For example, traditional cardiovascular risk factors (eg, hypertension) remain potent risk factors,[9] and environmental stressors (eg, trauma) often precipitate hemorrhagic stroke in *COL4A1* mutation carriers.[10] Plausibly then, stroke manifestation among Mendelian mutation carriers is not completely penetrant because of disease modifiers.

Our results bear important implications for clinical and research settings. First, in the absence of family history of stroke and syndromic features, screening Mendelian stroke

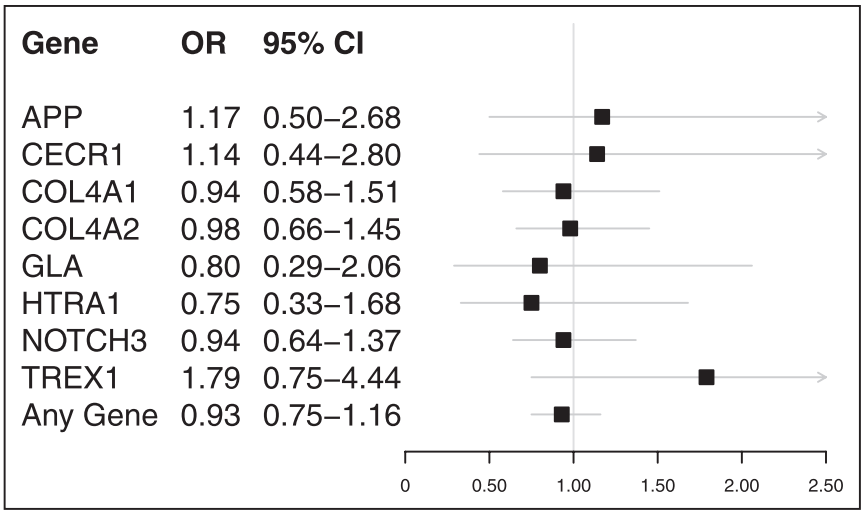

Figure. Association of rare nonsynonymous mutation carrier status with stroke stratified by gene. CI indicates confidence interval; and OR, odds ratio.

genes is not expected to yield a high proportion of mutation-positive cases. Second, given that next-generation sequencing is becoming routinely used for clinical diagnosis, caution is warranted when interpreting the discovery of Mendelian stroke gene mutations. Third, including controls is imperative in studies of rare genetic disorders especially when mutation frequency among disease-free individuals may be unknown.

Our study had several limitations. First, power was limited to detect associations with lowly penetrant mutations though the primary aim was to evaluate clinically relevant high-penetrant mutations. Nonetheless, among genes examined, our capacity to detect mutations was suboptimal for *HTRA1* (Table XI in the online-only Data Supplement). Second, classification of canonical disease-causing mutations depends on the current knowledge of pathogenic mutations, which is subject to change. Third, Mendelian stroke patients could be under-represented among cases because of exclusion of the most severe cases. Specifically, individuals with recurrent strokes or who were unable to communicate because of severe stroke without a valid proxy respondent were excluded. Last, a detailed clinical assessment was not performed for mutation carriers, and therefore, we could not evaluate the presence of intermediate phenotypes (eg, microbleeds) or other secondary features.

Conclusions

In the absence of syndromic features and family history of stroke, rare coding variation within Mendelian stroke genes may play a lesser role in sporadic cases of small-vessel pathogenesis.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Methods

Definition of Stroke Subtypes and other Clinical Features

All strokes were first-ever strokes confirmed by neuroimaging. 94.7%, 3.1%, and 2.2% of strokes were confirmed by CT, MRI, or both methods, respectively. Ischemic stroke subtypes (e.g. SVIS) were determined according to Trial of Org 10172 in Acute Ischemic Stroke Treatment (TOAST) guidelines[1]. ICH was determined by clinical evaluation and neuroimaging. Cardiovascular risk factors were defined in the same manner as described for the main INTERSTROKE study.[2] Hypertension was defined by 1) self-report or 2) having a blood pressure greater than 160/90 mmHg (mean of 3 measurements for cases). Diabetes, migraine, current smoking, high cholesterol, and parental history of stroke were determined by self-report.

Sample Selection Criteria

From those ~8000 INTERSTROKE samples for which patients consented to genetic analysis, eligible individuals were selected based on the following criteria:

Stroke Cases:

1) Maximum age of 70 years old
2) SVIS or ICH confirmed by neuroimaging.
3) This individual could be matched to a control based upon sex, ethnicity, and recruitment centre.

Stroke-Free Controls:

1) This individual could be matched to a case based upon sex, ethnicity, and recruitment centre.
2) When possible, age-matched a case +- 5 years. If not, this control must be older than the case.

Study Participant Characteristics

Overall, in the present study, there were 2967 participants including 1251 cases and 1716 controls (Table I). Among stroke cases, there were 614 (49.1%) SVIS cases and 637 (50.9%) ICH cases. Cases (mean: 55.5 years) were significantly younger than controls (mean: 60.6 years) ($P<0.001$). Women comprised 36.8% of all study participants. There were 884 (28.4%) Europeans, 731 (24.6%) Latin Americans, 729 (24.6%) East Asians, 418 (14.1%) Africans, 93 (3.1%) Persians, 76 (2.6%) South Asians, 74 (2.5%) Arabs and 4 (0.1%) Native Americans. Hypertension, current smoking status, BMI, ApoB/ApoA1 ratio, and parental stroke were significant risk factors for stroke ($Pall<0.001$).

Candidate Gene List

We searched the OMIM database,[3] with the following key terms: “stroke” OR “intracerebral hemorrhage” OR “ischemic stroke” OR “cerebral aneurysm” OR “arterial thrombosis”. Search results were limited to those with “phenotype description, molecular basis known”, “Mendelian phenotype or locus, molecular basis unknown”, or “other, mainly phenotypes with suspected Mendelian basis”. Genes were excluded if 1) stroke was not the primary feature, 2) cardioembolic stroke, thoracic aortic aneurysm, strokes of large-vessel etiology, or venous thrombosis was the primary phenotypic characteristic, 3) disease-onset

was not adult (neonatal or pediatric), 4) they did not show clear evidence of Mendelian inheritance, or 5) they were encoded in the mitochondrial genome. A more recently discovered stroke gene not yet recognized by the OMIM search, *CECR1*[4], was also added resulting in 8 stroke genes (Table II).

Next-Generation Sequencing of Mendelian Genes

Blood samples for all study participants were collected in EDTA Whole Blood tubes. DNA was extracted using the QIAGEN QIAsymphony DSP DNA Midi kit. DNA was subsequently prepared for either exome sequencing or targeted sequencing of Mendelian genes. Exome sequencing was performed on the Illumina HiSeq with paired-end reads (2 x 100 bp reads). Exonic sequences were captured with the Illumina TrueSeq Exome Enrichment Kit. For targeted sequencing, a custom panel focusing on the exons of Mendelian stroke genes was designed using the Ion Ampliseq Designer. Samples were sequenced on the Ion Proton Sequencer with P1 chips (1 x 125 bp reads) or Ion S5XL Sequencer with 540 chips (1 x 125 bp reads). Importantly, samples belonging to the same case-control grouping were processed with identical sequencing chemistries and platforms in order to mitigate systematic biases which could confound rare variant associations. The average sequencing depth was 58x and 284x for samples that underwent exome and targeted sequencing respectively (Table III). Coverage did not differ significantly between cases and controls for any gene ($P>0.05$).

Variant Calling, Filtering, and *Quality Control*

Generally, we adopted standard variant calling and filtering procedures for all sequencing data. However, the specific variant calling algorithms and filtering criteria differed between exome and targeted sequencing because of the different sequencing technologies utilised. For exome sequencing, sequence reads were mapped to the hg19 reference genome using the Burrows Wheeler Aligner and processed according to the Genome Analysis Tool Kit (GATK) best practices.[5] The GATK Unified Genotyper algorithm was used for variant calling. For targeted sequencing, reads were mapped to the hg19 reference genome using the Torrent Mapping Alignment Program (TMAP) and variants were called via the Torrent Variant Caller (TVC) pipeline as recommended for Ion Sequencing data. For exome sequencing data, variant quality control checks were applied in KGGSEQ[6] using default settings, except for the read depth filter which was increased to require eight reads per call. For targeted sequencing data, the "low stringency" filtering criteria recommended for Ion Torrent sequencing data by Damiati et al (2016) was utilised[7].

Variant QC metrics were generated in PicardTools[8], BedTools[9], GATK[5], and SnpSift[10]. Sample QC included checks for ethnicity, sex, cryptic relatedness, and genotypic concordance with exome chip data. A subset of 758 (32.7%) samples was also genotyped on either the Illumina HumanExome Chip V 1-1, Human Core Exome Chip V 1-0, or Infinium Core Exome Chip V 1-0 to assess overall variant calling accuracy. After excluding samples with less than 90% concordance, the average genotypic concordance was 99.4% with no significant differences between cases (99.5%) and controls (99.3%) ($P=0.56$). Note that we report a more stringent measure of genotypic concordance than is traditionally reported by GATK. In our calculations, homozygote reference calls were excluded because they inflate concordance (i.e. genotypic concordance=1-Non-Reference Discrepancy Rate). Other sample quality control checks were performed in PLINK including checks for ethnicity, sex, and cryptic relatedness. Samples failing any quality control check or left without a matching sample were removed. All Sample QC checks were performed using PLINK[11], GCTA[12], GATK[5], or Variant Tools.[13] After removing failing samples, 368 exome sequence and 2599 targeted sequence samples remained.

Variant Annotation, Filtering, & Association Testing

Annovar was used to annotate variant mutation effects based on RefGene transcripts.[13] Only rare non-synonymous mutations within the 8 Mendelian stroke genes (*APP, CECR1, COL4A1, COL4A2, GLA, HTRA1, NOTCH3, TREX1*) were analyzed in the present study. Non-synonymous mutations included any

mutations predicted to change the primary amino acid sequence of the protein, including splice site, missense, nonsense, and insertion/deletion (inframe and frameshift) mutations. Rare mutations were defined as those having a minor allele frequency (MAF) less than 0.01 within INTERSTROKE samples and participants from the NHLBI GO Exome Sequencing Project (ESP), the 1000 Genomes (1KG) project, and the Exome Aggregate Consortium (ExAC). This MAF threshold was applied within each ethnic subdivision of external databases and the predominant ethnic groups in INTERSTROKE (Europeans, Africans, Latin Americans, East Asians). If a variant was common (MAF$\geq$0.01) in even a single ethnic group, then it was excluded. Thus, all remaining variants belong to a globally rare variant set.

Rare non-synonymous mutations within the same gene were treated as a single unit for association testing. Logistic regression was used to evaluate whether the burden of rare non-synonymous mutations differed between cases and controls. A minimum of five mutation carriers was required to perform association testing. The independent variable was mutation carrier status, the dependent variable was the combined phenotype of all stroke (SVIS + ICH), and covariates included age, sex, and ethnicity. Various sensitivity analyses were also performed. We stratified by stroke subtype to ensure that ICH and SVIS specific associations were not missed (Tables V & VI). We applied a more stringent age cut-off of 55 years for cases to ensure that true associations weren't diluted by including relatively older cases more likely attributable to traditional risk factors (Table VII). We also used the "Mendelian Clinically Applicable Pathogenicity Score" (M-CAP)[14], an *in silico* prediction algorithm with 95% sensitivity to detect pathogenic disease-causing Mendelian mutations (Table VIII). Lastly, we adopted a recessive model to ensure that recessive effects were not missed (Table IX).

Definition of "Canonical Disease-Causing" (CDC) mutations

Rare variants were categorized as CDC mutations if they fulfilled one of the following criteria: 1) unambiguously classified as "pathogenic" in ClinVar[15], 2) determined to be disease-causing in large case series, or 3) strongly resembled reported disease-causing mutations from the literature. Specifically, this last criteria was applied to *NOTCH3*, *COL4A1*, and *TREX1* for which disease-causing mutations are known to follow highly stereotypical mutation patterns. Pathogenic *NOTCH3* mutations causing CADASIL involved the gain or loss of a cysteine residue encoded in the first 24 exons[16]; pathogenic *COL4A1* mutations disrupting glycine residues within the triple-helix domain[17]; pathogenic *TREX1* mutations causing RVCL included C-terminal frameshifts beyond the first 242 amino acids[18]. Given the many conflicting interpretations regarding the pathogenicity of missense mutations in *GLA*, only highly disruptive mutation types (i.e. nonsense, splice site, and frameshift) were considered pathogenic for Fabry's disease. It is important to note that the "pathogenic" variant classification did not encompass all loss-of-function mutations (i.e. frameshifts, splice site, nonsense, and stoploss) because for many Mendelian stroke disorders, this is not the underlying mechanism causing disease. Additionally, pathogenic mutation carriers were only counted if genotypes were consistent with the disease's known mode of inheritance. For example, heterozygote carriers of pathogenic mutations for recessive diseases (e.g. CARASIL) were not counted unless a second mutation was identified.

Estimation of CDC Mutation Carrier Prevalence in ExAC

Analysis was restricted to those Mendelian genes in which disease-causing mutations follow a highly stereotyped pattern (*NOTCH3, COL4A1, TREX1, GLA*) to avoid ambiguity in pathogenic variant classification. Definition of CDC mutations was identical to that performed for the INTERSTROKE analysis. ExAC release version 0.3 was used for all ExAC carrier/frequency calculations.[19] Heterozygote and homozygote individuals each counted as one. Access to individual level data was not available, and as a result, the "Any Gene" category, representing the aggregate mutation count/frequency across all Mendelian genes, relied upon the assumption that carriers possessed only one mutation each, which is reasonable when mutations are expected to be extremely rare.

Post-Hoc Power Calculations

The aggregate frequency of rare mutations varies substantially by gene, and thus power to detect associations is also variable. For each association test, we conducted post-hoc power calculations estimating the maximum effect size our study was capable of detecting at 95% power and alpha of 0.05. Using control mutation carrier frequencies as the reference, we simulated the power corresponding to a given odds ratio using the "statmod" R package.

Table I. Characteristics of study subjects.

	Case	Control	OR	P-value
N	1251	1716	-	-
Stroke Subtype	-	-	-	-
SVIS, n	614	850	-	-
(%)	(49.1)	(49.5)		
ICH, n	637	866	-	-
(%)	(50.9)	(50.5)		
Women, n	792	1084	-	-
(%)	(26.7)	(36.5)		
Age	55.5	60.6	-	<0.001
(SD)	(10.7)	(11.6)		
Hypertension	765	749	2.03	<0.001
(%)	(61.2)	(25.2)		
Diabetes	219	255	1.22	0.052
(%)	(17.5)	(14.9)		
High Cholesterol	229	355	0.86	0.11
(%)	(18.3)	(20.7)		
Current Smoker	330	250	2.10	<0.001
(%)	(26.4)	(14.6)		
BMI*	27.0	26.3	1.15	<0.001
(SD)	(4.9)	(4.8)		
ApoB/ApoA1*	0.92	0.79	1.60	<0.001
(SD)	(0.33)	(0.29)		
Parental Stroke	246	241	1.50	<0.001
(%)	(19.7)	(14.0)		

SVIS=Small-vessel ischemic stroke. ICH=Intracerebral Hemorrhage. ApoB/ApoA1=Apolipoprotein B / Apolipoprotein A-1 ratio. BMI=Body Mass Index. OR=odds ratio. *odds ratios expressed as change in disease risk per one SD increase.

Table II. Candidate Mendelian stroke genes investigated in this study.

Gene	Disease	Mode of Inheritance	ExAC pLI‡
APP[20]	Cerebral Amyloid Angiopathies	Dominant	0.33
CECR1[4]	Early-onset stroke and cerebral vasculopathy	Recessive	0.00
COL4A1[21]	Porencephaly / ICH	Dominant	1.00
COL4A2[22]	Porencephaly / ICH	Dominant	0.00
GLA[23]	Fabry's Disease*	Dominant/Recessive	0.99
HTRA1[24]	Small vessel disease / CARASIL	Dominant/Recessive	0.02
NOTCH3[16]	CADASIL	Dominant	0.21
TREX1[25]	Retinal vasculopathy with cerebral leukodystrophy	Dominant	0.25

*While Fabry's disease is mainly considered a X-linked recessive disease, stroke is considered a feature of heterozygous carriers.
‡ExAC probability of Loss-of-Function Intolerance

Table III. Pathogenic mutations in INTERSTROKE and ExAC.

Gene	INTERSTROKE Case (%)	INTERSTROKE Control (%)	OR	95% CI	P-value	Upper Bound OR	ExAC Carrier (%)
APP	0 (0)	0 (0)	-	-	-	-	NA
CECR1	0 (0)	0 (0)	-	-	-	-	NA
COL4A1	0 (0)	0 (0)	-	-	-	-	63 (0.10)
COL4A2	0 (0)	0 (0)	-	-	-	-	NA
GLA	1 (0.20)	0 (0)	-	-	-	-	0 (0)
HTRA1	0 (0)	0 (0)	-	-	-	-	NA
NOTCH3	6 (0.48)	4 (0.23)	1.78	0.49-7.25	0.39	4.86	204 (0.34)
TREX1	0 (0)	0 (0)	-	-	-	-	6 (0.01)
Any Gene	**7 (0.56)**	**4 (0.23)**	**1.89**	**0.54-7.57**	**0.33**	**4.76**	**273 (0.45)**

Table IV. Characteristics of CDC mutation carriers.

Gene	Mutation Effect	Known SNP	Affiliated Disease	Case Status	Sex	Ethnicity	Age	CVD Risk Factors	Parental Stroke	Migraine
GLA	R301X	rs398123224	Fabry's Disease	SVIS	F	European	26	NONE	N	Y
NOTCH3	R133C	rs137852642	CADASIL	Control	M	Latin American	66	HTN, HCHOL	N	Y
NOTCH3	R182C	rs28933697	CADASIL	ICH	F	E. Asian	40	NONE	N	Y
NOTCH3	R544C	rs201118034	CADASIL	Control	M	E. Asian	45	HTN	Y	N
NOTCH3	R587C	Y	CADASIL	Control	F	E. Asian	55	NONE	N	N
NOTCH3	R592C	Y	CADASIL	Control	F	E. Asian	74	HTN	N	N
NOTCH3	R785C	Y	CADASIL	SVIS	M	Persian	65	DBM	N	N
NOTCH3	G881C	N	CADASIL	ICH	M	African	37	NONE	N	N
NOTCH3	R1231C	rs201680145	CADASIL	ICH	M	Persian	52	NONE	N	N
NOTCH3	R1285C	N	CADASIL	SVIS	M	Persian	45	DBM, HCHOL	Y	N

Y=Yes. N=No. ICH=intracerebral hemorrhage. SVIS=small-vessel ischemic stroke. CADASIL=Cerebral Autosomal Dominant Arteriopathies with Subcortical Infarcts and Leucoencephalopathies. HTN=hypertension. DBM=diabetes mellitus. HCHOL=high cholesterol. SMOKE=current smoker. OB=obesity.

Table V. Association between all stroke and rare non-synonymous mutation carrier status.

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	11 (0.88)	14 (0.82)	1.17	0.71	0.50-2.68	2.64
CECR1	8 (0.64)	12 (0.70)	1.14	0.77	0.44-2.80	2.73
COL4A1	30 (2.40)	49 (2.86)	0.94	0.81	0.58-1.51	1.75
COL4A2	49 (3.92)	66 (3.85)	0.98	0.93	0.66-1.45	1.66
GLA	7 (0.56)	12 (0.70)	0.81	0.65	0.29-2.06	2.86
HTRA1	10 (0.80)	16 (0.93)	0.75	0.50	0.32-1.68	2.50
NOTCH3	51 (4.08)	71 (4.14)	0.94	0.74	0.64-1.37	1.60
TREX1	13 (1.04)	9 (0.52)	1.79	0.19	0.75-4.44	3.10
Any Gene	**169 (13.5)**	**243 (14.2%)**	**0.93**	**0.55**	**0.75-1.16**	**1.29**

Table VI. Association between ICH and rare non-synonymous mutation carrier status.

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	9 (1.41)	10 (1.15)	1.36	0.53	0.51-3.58	2.86
CECR1	5 (0.78)	3 (0.35)	3.03	0.13	0.73-15.02	5.59
COL4A1	17 (2.67)	22 (2.54)	1.23	0.55	0.62-2.37	2.19
COL4A2	24 (3.77)	36 (4.16)	0.88	0.66	0.50-1.53	1.97
GLA	4 (0.63)	9 (1.04)	0.60	0.41	0.16-1.90	3.21
HTRA1	6 (0.94)	6 (0.69)	1.08	0.89	0.33-3.56	3.81
NOTCH3	26 (4.08)	36 (4.16)	0.94	0.82	0.55-1.60	1.91
TREX1	8 (1.26)	2 (0.23)	4.73	0.06	1.01-32.05	7.31
Any Gene	**91 (14.29)**	**120 (13.86)**	**1.02**	**0.90**	**0.75-1.38**	**1.40**

Table VII. Association between SVIS and rare non-synonymous mutation carrier status.

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	2 (0.33)	4 (0.47)	0.76	0.75	0.10-3.99	4.75
CECR1	3 (0.49)	9 (1.06)	0.55	0.38	0.12-1.87	3.21
COL4A1	13 (2.12)	27 (3.18)	0.72	0.35	0.35-1.40	2.08
COL4A2	25 (4.07)	30 (3.53)	1.10	0.74	0.63-1.90	2.01
GLA	3 (0.49)	3(0.35)	1.48	0.65	0.25-8.51	5.84
HTRA1	4 (0.65)	10 (1.18)	0.52	0.28	0.14-1.62	3.05
NOTCH3	25 (4.07)	35 (4.12)	0.93	0.80	0.54-1.60	1.90
TREX1	5 (0.81)	7 (0.82)	0.94	0.91	0.27-3.02	3.73
Any Gene	**78 (12.70)**	**123 (14.47)**	**0.85**	**0.31**	**0.62-1.16**	**1.44**

Table VIII. Association between stroke and rare non-synonymous mutation carrier status for cases less than 55 years old.

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	5 (1.01)	5 (0.86)	1.14	0.84	0.31-4.15	4.28
CECR1	1 (0.20)	1 (0.17)	-	-	-	-
COL4A1	13 (2.63)	15 (2.59)	0.94	0.88	0.44-2.02	2.54
COL4A2	16 (3.02)	29 (0.05)	0.63	0.16	0.33-1.18	2.00
GLA	3 (0.61)	3 (0.52)	1.20	0.83	0.22-6.60	5.57
HTRA1	7 (1.41)	7 (1.21)	1.17	0.77	0.40-3.45	3.66
NOTCH3	19 (3.84)	27 (4.67)	0.84	0.57	0.45-1.53	2.02
TREX1	7 (1.41)	2 (0.34)	4.42	0.07	1.06-29.85	7.62
Any Gene	**68 (13.74)**	**88 (15.17)**	**0.89**	**0.51**	**0.63-1.26**	**1.53**

Table IX. Association between stroke and mutations predicted to be deleterious by M-CAP[14].

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	11 (0.88)	10 (0.58)	1.70	0.25	0.69-4.26	3.06
CECR1	6 (0.48)	12 (0.70)	0.86	0.77	0.30-2.24	2.81
COL4A1	29 (2.32)	49 (2.89)	0.91	0.68	0.56-1.45	1.78
COL4A2	47 (3.78)	65 (3.79)	0.95	0.79	0.64-1.40	1.65
GLA	7 (0.56)	12 (0.70)	0.80	0.65	0.29-2.06	2.93
HTRA1	7 (0.56)	8 (0.47)	1.29	0.63	0.45-3.63	3.45
NOTCH3	46 (3.68)	65 (3.79)	0.92	0.68	0.62-1.37	1.64
TREX1	7 (0.56)	3 (0.17)	2.90	0.13	0.78-13.83	5.72
Any Gene	**153 (12.23)**	**219 (12.76)**	**0.95**	**0.67**	**0.76-1.19**	**1.32**

Table X. Association between stroke and rare non-synonymous mutation carrier status assuming a recessive model.

Gene	Case Carrier (%)	Control Carrier (%)	OR	P-value	95% CI	Upper Bound OR
APP	0	0	-	-	-	-
CECR1	0	0	-	-	-	-
COL4A1	1 (0.08)	1 (0.06)	-	-	-	-
COL4A2	1 (0.08)	3 (0.17)	-	-	-	-
GLA	3 (0.24)	5 (0.29)	1.06	0.93	0.22-4.37	4.26
HTRA1	0	0	-	-	-	-
NOTCH3	2 (0.16)	4 (0.23)	0.69	0.69	0.09-3.85	4.93
TREX1	0	0	-	-	-	-
Any Gene	**7 (0.56)**	**13 (0.76)**	**0.93**	**0.88**	**0.34-2.32**	**2.67**

Table XI. Coverage metrics for Mendelian genes.

	Exome Seq – Illumina HiSeq (N=368)			**Target Seq – Ion Torrent Proton (N=1114)**			**Target Seq – Ion Torrent S5XL (N=835)**		
Gene	Mean Depth (SD)	% Targets > 8x (SD)	% Targets > 20x (SD)	Mean Depth (SD)	% Targets > 8x (SD)	% Targets > 20x (SD)	Mean Depth (SD)	% Targets > 8x (SD)	% Targets > 20x (SD)
APP	32.8 (8.6)	85.7 (2.9)	69.3 (13.4)	300 (137.3)	88.7 (1.4)	87.6 (2.8)	207.3 (182.5)	91.0 (3.3)	88.6 (0.3)
CECR1	34.7 (9.1)	99.5 (1.6)	81.3 (15.5)	562.2 (222.5)	99.8 (0.9)	99.4 (1.5)	273.6 (238.7)	99.9 (0.5)	99.7 (1.1)
COL4A1	50.6 (11.1)	94.3 (3.3)	79.9 (8.7)	387.9 (165.7)	97.2 (1.7)	95.5 (2.7)	225.8 (192.3)	97.5 (1.3)	95.6 (3.6)
COL4A2	60.0 (13.0)	96.4 (2.0)	87.6 (5.4)	424.5 (203.9)	98.9 (1.6)	97.2 (2.5)	265.1 (226.0)	98.7 (1.6)	95.6 (3.1)
GLA	19.7 (7.7)	89.8 (13.2)	42.9 (29.6)	236.4 (125.6)	99.8 (2.1)	99.3 (3.7)	119.2 (123.3)	99.7 (2.2)	95.8 (11.5)
HTRA1	34.5 (8.5)	66.3 (2.1)	56.3 (6.0)	291 (160.1)	72.4 (2.1)	71.9 (2.5)	205.0 (177.3)	74.7 (0.5)	74.2 (1.4)
NOTCH3	73.3 (16.5)	89.5 (2.2)	82.6 (4.4)	264.6 (141.7)	78.4 (3.7)	74.8 (5.2)	201.2 (167.3)	86.4 (1.2)	83.9 (3.3)
TREX1	156.7 (33.8)	93.4 (1.1)	90.7 (1.3)	449 (255.9)	88.5 (3.3)	86.3 (5.6)	245.8 (211.3)	89.0 (0.8)	88.0 (1.7)
ALL	**57.8 (43.5)**	**89.4 (10.3)**	**73.8 (16.6)**	**364.5 (111.1)**	**90.5 (10.5)**	**89.0 (10.9)**	**217.9 (48.5)**	**92.1 (8.8)**	**90.2 (8.3)**

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