Common Variants Within Oxidative Phosphorylation Genes Influence Risk of Ischemic Stroke and Intracerebral Hemorrhage

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Background and Purpose—Previous studies demonstrated association between mitochondrial DNA variants and ischemic stroke (IS). We investigated whether variants within a larger set of oxidative phosphorylation (OXPHOS) genes encoded by both autosomal and mitochondrial DNA were associated with risk of IS and, based on our results, extended our investigation to intracerebral hemorrhage (ICH).

Methods—This association study used a discovery cohort of 1643 individuals, a validation cohort of 2432 individuals for IS, and an extension cohort of 1476 individuals for ICH. Gene-set enrichment analysis was performed on all structural OXPHOS genes, as well as genes contributing to individual respiratory complexes. Gene-sets passing gene-set enrichment analysis were tested by constructing genetic scores using common variants residing within each gene. Associations between each variant and IS that emerged in the discovery cohort were examined in validation and extension cohorts.

Results—IS was associated with genetic risk scores in OXPHOS as a whole (odds ratio [OR], 1.17; P=0.008) and complex I (OR, 1.06; P=0.050). Among IS subtypes, small vessel stroke showed association with OXPHOS (OR, 1.16; P=0.007), complex I (OR, 1.13; P=0.027), and complex IV (OR, 1.14; P=0.018). To further explore this small vessel association, we extended our analysis to ICH, revealing association between deep hemispheric ICH and complex IV (OR, 1.08; P=0.008).

Conclusions—This pathway analysis demonstrates association between common genetic variants within OXPHOS genes and stroke. The associations for small vessel stroke and deep ICH suggest that genetic variation in OXPHOS influences small vessel pathobiology. Further studies are needed to identify culprit genetic variants and assess their functional consequences.

Key Words: genes ● mitochondria ● OXPHOS ● stroke

Despite remarkable advances in prevention, diagnosis, and treatment, stroke remains the second leading cause of death in the world, and a leading cause of disability. Although many modifiable environmental factors contribute to stroke risk, there are ample data demonstrating a genetic risk component as well.1 Recent genome-wide association studies

Received July 30, 2012; final revision received November 9, 2012; accepted December 19, 2012.

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*Stoke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.112.672089

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(GWAS) have demonstrated that common DNA variants influence risk of ischemic stroke (IS). 2-4

A previous study demonstrated that common mitochondrial variants influence risk of IS. 4 The mitochondrial genome is vital to the assembly of the oxidative phosphorylation (OXPHOS) apparatus, but the majority of OXPHOS structural proteins are encoded within the autosomal genome. 5 The OXPHOS apparatus consists of 5 complexes that are necessary to maintain aerobic homeostasis and preserve reduction/oxidation (redox) balance in the cellular environment. Multiple rare disorders are caused by mutations of OXPHOS genes, many of which result in neurodegenerative or stroke-like phenotypes, including seizures, metabolic infarcts, and encephalomyopathies. 6 Additionally, OXPHOS fitness plays a role in the neuronal response to and recovery after oxidative stress. 7

We hypothesized that common genetic variants in OXPHOS genes, both within the autosomal and mitochondrial genome, influence the risk of stroke. To test this hypothesis, we performed a pathway-based genetic association analysis interrogating genetic variants within OXPHOS genes. We initially performed a cumulative test of all common genetic variation within OXPHOS loci by using a gene-set enrichment analysis (GSEA) technique. This allowed us to investigate whether the OXPHOS pathway was enriched for association with stroke risk. On the basis of this analysis, we sought to ascertain and quantify the role of these variants by calculating a genetic risk score from OXPHOS genes in the Massachusetts General Hospital (MGH) IS GWAS. We then replicated this risk score association in a separate data set comprising individuals from the Ischemic Stroke Genetics Study (ISGS) and Siblings with Ischemic Stroke Study (SWISS). We then tested the same risk score in intracerebral hemorrhage (ICH) using individuals from the International Stroke Genetics Consortium ICH GWAS (ISGC ICH).

Methods

Subjects

Genetic data and phenotypic information were contributed by the MGH IS GWAS, 6 ISGS, 7 SWISS, 8 and the ISGC ICH GWAS 9 (Table 1). Additional control individuals for the MGH data set were contributed by the MIGen Consortium, a case-control study of genetic risk for myocardial infarction. 10 Hospital-based IS case and control recruitment and phenotype ascertainment were performed according to protocols described previously, and stroke subtypes were assigned by Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria. 9-10 In cases in which IS subtype data were unavailable, individuals were dropped from subtype analyses but were allowed to remain in all-cause IS analyses (n=124 in MGH; n=387 in ISGS/SWISS). Multicenter hospital-based ICH case and control recruitment and phenotype ascertainment were performed according to protocols described previously. 11 Location of ICH was assigned by stroke neurologists based on standard criteria with central adjudication. 11,12 Institutional review boards from all participating centers approved the study, and all participants gave informed consent for data collection, genotyping, and analysis of genetic data.

Genotyping and Imputation

Blood samples from MGH/MIGen were processed and genotyped using the Affymetrix 6.0 platform, whereas ISGS and SWISS were assayed with the Illumina 660 W and 1M platforms according to previously published protocols. 8-10 Blood samples for the ISGC ICH cases and controls were genotyped on Illumina 660 W. 11 For harmonization across platforms, all data sets had additional genotypes imputed using PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and the International HapMap Project phase 3 reference data set (http://www.hapmap.org). Captured mitochondrial variants from the genotyping arrays were extracted, 12 and raw intensity files were inspected visually by C.D.A. and A.B. to confirm accuracy of genotype calls.

Genetic Quality Control

For all analyzed cohorts, quality control of genotyped individuals included gender-sex discordance, filtering for missingness by individual >0.1, missingness by single nucleotide polymorphism (SNP) >0.05, and minor allele frequency (MAF) <0.01. Individuals displaying cryptic relatedness (p≥0.125) and genotypes with significant departure from Hardy–Weinberg equilibrium (P<10E-5) were excluded from analysis. 13 Autosomal imputation was performed using PLINK v1.07 after quality control filtering. Mitochondrial imputation was performed using a haplotype-based approach with reference data sets from GenBank and Mitokor after additional mtDNA-specific quality control measures (Appendix I in the online-only Data Supplement). 14 After imputation, SNPs were excluded with MAF <0.01 or RSQR quality index <0.3.

OXPHOS SNP Selection

Genes encoding proteins directly involved in the OXPHOS respiratory chain were selected based on published criteria from a chemical dissection of mitochondrial function, yielding a total of 95 genes in the autosomal genome and 13 genes in the mitochondrial genome. 4 SNPs falling within these genes ≥100 kilobases and passing quality control filtering were extracted from the MGH and ISGS/SWISS data sets after imputation and included in the final analysis. Subanalyses were performed for genes grouped according to each OXPHOS respiratory complex, classified according to annotation in the Ensembl Genome Browser (http://www.ensembl.org; Tables I and II in the online-only Data Supplement).

Population Structure and Control

Only individuals of European ancestry were analyzed in the present study. Population structures for autosomal and mitochondrial variants were assessed independently because of their significantly different inheritance patterns, using principal component (PC) analysis. 14,15 Autosomal PCs 1 through 5 were extracted for each individual and were added in association testing of autosomal SNPs until no additional reduction in genomic inflation factor could be achieved (PC1–2 in all analyses). Mitochondrial PCs 1 to 10 were extracted for each individual and were similarly added in association testing of mitochondrial SNPs until mitochondrial genomic inflation factor was minimized (PC1–5 in all analyses).

Gene-Set Enrichment Analysis

Testing for cumulative OXPHOS pathway associations with IS risk was performed using the GSEA method, 17 as implemented in the GenGen v.2010Apr29 software package. 18 GSEA was implemented in this study as a preliminary screen of the OXPHOS pathway before generation of genetic scores and as a means to minimize the possibility of any false-positive associations. The GSEA method determines whether variants within a predefined biological pathway contain more associations with the chosen phenotype than would be expected by chance alone. For IS, GSEA testing was performed in the ISGS/SWISS cohort as a preliminary analysis before genetic score generation. GSEA was performed in the ISGS/SWISS replication cohort rather than the MGH/MIGen discovery cohort to prevent any chance enrichment of OXPHOS association in the MGH/MIGen cohort from influencing gene-sets chosen for genetic score testing. A separate GSEA was performed in the ISGC ICH cohort. Results are
Table 1. Study Populations

<table>
<thead>
<tr>
<th></th>
<th>MGH/MIGen Cases</th>
<th>MGH/MIGen Controls</th>
<th>ISGS/SWISS Cases</th>
<th>ISGS/SWISS Controls</th>
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Sex (% female)

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Age at enrollment, (mean, SD)

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<th>ISGS/SWISS Cases</th>
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<td>66.5 (14.6)</td>
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<td>66.5 (12.6)</td>
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Hypertension, %

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DM 2, %

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Atrial fibrillation, %

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<th>ISGC ICH Cases</th>
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Current smoker, %

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<th>ISGS/SWISS Cases</th>
<th>ISGS/SWISS Controls</th>
<th>ISGC ICH Cases</th>
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<td>0.06</td>
<td>0.17</td>
<td>0.20</td>
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Warfarin use, %

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<th>MGH/MIGen Controls</th>
<th>ISGS/SWISS Cases</th>
<th>ISGS/SWISS Controls</th>
<th>ISGC ICH Cases</th>
<th>ISGC ICH Controls</th>
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<td>...</td>
<td>...</td>
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DM 2 indicates type 2 diabetes mellitus; ICH, intracerebral hemorrhage; ISGS, International Stroke Genetics Consortium; ISGS, Ischemic Stroke Genetics Study; MGH, Massachusetts General Hospital; n, number of cases/controls; SWISS, Siblings with Ischemic Stroke Study; and ... , not applicable.

reported as a permutation-derived empirical $P$ value (100,000 gene-set permutations) for gene-set association with the IS or ICH risk, with the null hypothesis derived from random sampling of an equal number of variants of similar MAFs chosen from genes not within the OXPHOS pathway. Using this technique, the significance threshold for our GSEA was set at empirical $P < 0.05$.

Figure. Flowchart describing the MGH/MIGen discovery, ISGS/SWISS validation, and ISGC ICH extension cohorts. ICH indicates intracerebral hemorrhage; ISGS, Ischemic Stroke Genetics Study; ISGC, International Stroke Genetics Consortium; MGH, Massachusetts General Hospital; OXPHOS, all structural proteins directly contributing to oxidative phosphorylation complex function; SNP, single nucleotide polymorphism; and SWISS, Siblings with Ischemic Stroke Study.
Genetic Score Generation in IS

Combined effects of all autosomal and mitochondrial OXPHOS SNPs were evaluated using a score-based method described previously. Briefly, each OXPHOS SNP was tested for association with all-cause IS risk in the MGH/MIGen discovery case-control data set. The results of this analysis, expressed as a $\beta$-coefficient for the risk allele at each SNP, were then clumped according to linkage disequilibrium using the clump function in PLINK v1.07. Only the SNP with the highest significance value was retained in regions in which linkage disequilibrium was >0.6 between SNPs. No additional pruning or thresholding was performed in an attempt to optimize the SNPs included in the genetic score. These $\beta$-coefficients were then applied to the corresponding OXPHOS SNPs in the ISGS/SWISS validation data set. All subsequent analyses in replication and extension were based on this single set of $\beta$-coefficients from the MGH/MIGen discovery cohort in association with all-cause IS (Table III in the online-only Data Supplement). After $\beta$-coefficient extraction, the MGH/MIGen discovery cohort was not included in any further analysis. A risk score was generated for each individual by summing the $\beta$-coefficients associated with each risk allele present in the individual. Informed by GSEA results, scores were developed for all OXPHOS complexes: complex I and complex IV. Because the risk score distributions failed testing for normality by Shapiro-Wilk, the score was divided into quintiles in an unsupervised fashion using the cut command in STATA v10.0 (http://www.stata.com) for association testing.

Genetic Score Association Testing in IS

The IS risk score quintiles were used as the independent variable in an ordinal logistic regression model for IS risk, using age and sex as unspecified covariates. Results reported represent risk (as expressed by odds ratio [OR]) per unit increase in score quintile. SNPs that were present in the MGH discovery cohort but were absent from the ISGS/SWISS validation cohort were dropped from the analysis (n=30). Additional covariates of hypertension, diabetes mellitus, and atrial fibrillation were also tested. Results of genetic score testing in the replication and extension data sets represent independent tests. Therefore, $P<0.05$ in the replication and extension data sets is considered statistically significant. All regression analyses were performed using STATA v10.0.

Extension of Genetic Score Analysis to ICH

The same $\beta$-coefficients of association with IS from the MGH/MIGen discovery cohort were applied to individuals within the ISGC ICH cohort, again resulting in a risk score for each individual. Risk score quintiles were then used as the independent variable in a logistic regression for ICH risk, using age, sex, hypertension, and warfarin exposure as unspecified covariates. For ICH, scores were developed only for all OXPHOS and complex IV. Separate analyses were performed for deep and lobar ICH subtypes. Cerebellar and multicompartment ICH were included in the all ICH analysis but were dropped from deep and lobar analyses.

Post Hoc Power Calculation

Power for discovery of association between individual variants within OXPHOS genes and IS was computed using the Genetic Power Calculator, with calculated ORs of 1.10, 1.20, and 1.40 and MAF of 0.10, 0.20, and 0.30. For this analysis, $\alpha$ was set at 6x10E−5 (842 independent tests for autosomal and mitochondrial variants within OXPHOS genes).

Power to detect an association between the genetic risk score and stroke phenotypes was computed using the expected proportion of variance explained, assuming that the overall information content of the score would account for 0.5%, 1%, or 5% of variance between cases and controls. Power calculations were performed for IS, IS subtypes, ICH, and ICH deep, and lobar subgroups.

Results

Genotyping Quality Control and Imputation Results

Implementation of quality control and imputation methods left 1843 individuals and 707 autosomal SNPs in the MGH/MIGen cohort, 2632 individuals and 677 autosomal SNPs in the ISGS/SWISS cohort, and 1837 individuals and 707 autosomal SNPs in the ISGC ICH cohort (Figure 1); 135 mitochondrial SNPs were retained in all 3 cohorts after extraction and haplotype-based imputation (Appendix II in the online-only Data Supplement).

GSEA in IS

GSEA was performed using the ISGS/SWISS cohort, testing the OXPHOS gene-set 100 000 times against randomly assigned gene-sets of equal size (Table 2). GSEA testing for the full OXPHOS gene-set and those within each respiratory complex demonstrated associations between IS and the full OXPHOS gene-set ($P=0.012$), as well as OXPHOS complexes I and IV ($P=0.024$ in both). Among IS subtypes, GSEA revealed significant association between small vessel (SV) stroke and OXPHOS complexes I and IV ($P=0.008$ and $P=0.005$, respectively), although there was only a trend toward association between SV stroke and the full OXPHOS gene-set ($P=0.091$).

GSEA in ICH

As with our IS analysis, GSEA was performed in the ISGC ICH cohort to determine whether OXPHOS gene-sets were enriched for association with ICH risk (Table 2). On the basis of our

<table>
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<tr>
<th>Table 2. Gene-Set Enrichment Analysis (P Values)</th>
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<tr>
<td>Cases/Controls</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>All ischemic stroke</td>
</tr>
<tr>
<td>CE</td>
</tr>
<tr>
<td>LA</td>
</tr>
<tr>
<td>SV</td>
</tr>
<tr>
<td>All ICH</td>
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<tr>
<td>Lobar ICH</td>
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<td>Deep ICH</td>
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Association between gene-sets and ischemic stroke (Ischemic Stroke Genetics Study/Siblings with Ischemic Stroke Study) and ICH (International Stroke Genetics Consortium ICH), with $P$ values reported from 100 000 permutations against the null. CE indicates cardioembolic stroke; ICH, intracerebral hemorrhage; LA, large artery stroke; SV, small vessel stroke; OXPHOS, all structural proteins directly contributing to oxidative phosphorylation complex function; and ..., analysis not performed.
results from IS GSEA, only the full OXPHOS, complex I, and complex IV gene-sets were carried over for testing in ICH. An association was found between all ICH and complex IV ($P=0.035$). After restricting cases to deep and lobar subgroups, deep ICH retained association with complex IV ($P=0.008$).

### Genetic Score Analysis in IS

On the basis of GSEA results, only SNPs in the full OXPHOS gene-set as well as complexes I and IV were used to calculate genetic scores. Similarly, only all-cause IS and the SV subtype were carried forward for genetic score analysis (Table 3). Application of β-coefficient–based scores in the ISGS/SWISS cohort demonstrated associations between a score comprising the full OXPHOS gene-set and IS (OR, 1.17; 95% confidence interval [CI], 1.03–1.33). This full OXPHOS score was also associated with the SV stroke subtype (OR, 1.16; 95% CI, 1.04–1.29). Of note, these genetic score results were largely driven by autosomal variants, with results deviating <20% when mitochondrial variants were excluded (Table VI in the online-only Data Supplement).

In analysis of our complex I score, IS (OR, 1.06; 95% CI, 1.00–1.12) and the SV stroke subtype (OR, 1.13; 95% CI, 1.01–1.26) demonstrated significant association. For our complex IV score, there was a trending association for all-cause stroke and a significant association for SV stroke (OR, 1.14; 95% CI, 1.02–1.27).

Regression analyses for IS were performed with and without the inclusion of vascular risk factors as covariates in logistic regression (hypertension, diabetes mellitus, and atrial fibrillation). These regressors did not demonstrate significant association with the genetic scores and did not alter the results of the regression analysis ($P=NS$; data not shown).

### Extension of Genetic Score Analysis to ICH

We constructed genetic scores in the ISGC ICH cohort based on β-coefficients from the MGH/MIGen IS cohort (Table 3). This analysis revealed association between a genetic score from complex IV genes and all ICH (OR, 1.08; 95% CI, 1.01–1.17; $P=0.039$), as well as deep ICH (OR, 1.14; 95% CI, 1.03–1.25; $P=0.008$).

### Post Hoc Power Calculation

Power calculations for discovery of individual OXPHOS genetic variants in association with IS revealed a maximum power of 73% to detect variants conferring an OR of 1.4 in association with IS risk at an MAF of 0.30 (Table III in the online-only Data Supplement). SNPs conferring lower OR and lower MAF substantially limited study power to detect individual variants, as did restriction of samples to the SV subtype. We performed power calculations for our genetic score analyses based on percentage of variance explained by the genetic score, ranging from 0.5% to 5%. The genetic score analysis was powered at 21%, to explain 0.5% of variance in IS risk for all-cause strokes and 3% to explain 0.5% of variance in the SV subtype. In application of this genetic score to ICH, power was 16%, to explain 0.5% of variance in all ICH and 8%, to explain 0.5% of variance in deep ICH (Table IV in the online-only Data Supplement). As a reference, percentages of variance explained in our logistic regression models incorporating genetic scores ranged from 0.5% to 1% in most analyses.

### Discussion

Our pathway-based analysis demonstrates that common genetic variants in OXPHOS genes are associated with risk of both IS and ICH. These associations are robust, having passed GSEA and replication in independent cohorts. Stratifying IS and ICH by subtype and OXPHOS genes by mitochondrial complex, we reveal associations for complexes I and IV in SV stroke and complex IV in deep ICH. These subanalyses retain significance, despite a substantial restriction in sample size and SNP counts.

Although ample evidence exists for rare mutations leading to severe OXPHOS dysfunction in a variety of familial mitochondrial syndromes with stroke phenotypes, our analysis provides evidence of a role for common genetic variants within OXPHOS in sporadic IS and ICH. These results contribute to a growing body of evidence linking OXPHOS genetic and functional variation to common neurological diseases, including Alzheimer disease, amyotrophic lateral sclerosis, and Parkinson disease, to name a few.20–23

Our subanalyses restricted to variants within complexes I and IV genes reveal additional parallels to rare mitochondrial syndromes. Mutations within complex I account for up to one third of the known respiratory chain diseases and represent a major determinant of the redox state of the cell.24–25 Complex IV is the final electron donor in the pathway, receiving electrons from cytochrome C and passing them to oxygen. Complex IV dysfunction, in addition to causing early life mitochondrial diseases such as Leigh disease and encephalomyopathies,
has also been implicated in neurodegenerative diseases.20 Although complex I is much larger than complex IV (50 versus 23 gene products), our demonstrated positive associations for both complexes suggest that statistical power alone did not determine our results, and the correlations with existing knowledge of mitochondrial disease supports a possible role for these complexes in sporadic human disease. Neither complex I nor complex IV dysfunction has effective treatments, although administration of cofactors has been reported to improve function in some instances.26

Both SV stroke and deep ICH result from disease of cerebral SVs and share common risk factors, such as diabetes mellitus and hypertension.27,28 Our findings suggest a possible shared genetic contribution to SV pathobiology underlying SV stroke and deep ICH, which could be mediated through disruption in oxidative function at the tissue level or through modification of upstream systemic or endothelial risk factors shared by the 2 diseases. We previously reported an association between mitochondrial common variants and white matter hyperintensity volume,4 a phenotype to which SV stroke and deep ICH have been linked.29,30 These new data provide additional support for the role of energy metabolism in SV disease. However, given that OXPHOS dysfunction can result in numerous physiological derangements, including ATP depletion, reactive oxygen species generation, defects in cell signaling, and alteration in apoptotic thresholds, our demonstrated associations cannot directly inform the underlying pathobiology of this SV link. Functional studies to identify the mechanisms of bioenergetic dysfunction will be needed to build on these results.

APOE allele status has been demonstrated to affect the risk and severity of lobar ICH, presumably attributable to a strong relationship between cerebral amyloid angiopathy and the lobar ICH subtype.11,11 The present study suggests a relationship between OXPHOS variants and deep ICH only, contributing to growing evidence that deep and lobar ICH represent genetically distinct entities. Genetic approaches seem to be useful tools to explore the differences between these ICH subtypes and hopefully can lead to a more comprehensive understanding of the pathogenesis of these similar but unique disease subtypes.

Limitations render our results preliminary. The magnitude of effect sizes for OXPHOS genetic scores in stroke risk in our analyses are small but are inline with the results from other GWAS efforts in ischemic and hemorrhagic stroke.2,4,8,11,13 Our GSEA did not find associations for the large artery or cardioembolic stroke subtypes in IS or the lobar subtype in ICH. Given our power calculations, it is possible that the restriction in sample size for subtype-stratified analyses led to a false-negative for these subtypes. Therefore, we cannot definitively demonstrate that the effect of OXPHOS genetic variants on ischemic or hemorrhagic stroke is isolated to SV ischemic or deep ICH subtypes. Many subjects in the MGH/MIGen and ISGS/SWISS data sets were used in previous study of mitochondrial variants in IS, although the method of analysis differed between these studies.4 These data sets theoretically could be particularly enriched with OXPHOS associations, although the positive extension to the ISGC ICH cohort would not be predicted if this were the case.

Genetic score analysis, although effective in aggregating signals to detect association, cannot identify individual causative variants. As a result, we are unable to determine the particular genetic loci conferring risk in the present study. GWAS platform-based SNPs were used in this analysis, which are not highly enriched for functional variants likely to cause missense, nonsense, or splice-site mutations. It is possible that other common or rare genetic variants in the OXPHOS pathway lie in linkage disequilibrium with our included variants, exerting a more substantial effect in affected individuals. Given the small aggregate effect sizes of the genetic scores in our analysis, prohibitively large sample sizes would be required to achieve sufficient power to detect individual OXPHOS variants. The significance thresholds in the current study were set according to established techniques in GSEA and genetic score analysis and can be considered robust because of the use of permutation in the case of GSEA and the use of separate discovery and replication cohorts in the case of the genetic score analysis. Because the majority of genes encoding OXPHOS proteins are autosomal, we cannot determine whether the low-risk contribution (≤20%) of mitochondrial variants to the risk scores for IS and ICH in our analysis is attributable to an imbalance in SNP contributions to the genetic score or a true difference in risk proportion. Finally, we cannot determine whether the involvement of the OXPHOS pathway in IS and ICH is mediated at the brain tissue level or possibly through modification of systemic vascular or metabolic risk factors. Follow-up analyses will be required to address OXPHOS function in different tissue types.

Conclusion
Through a pathway-based analysis, we have demonstrated that genetic variation within genes involved in the OXPHOS apparatus associates with IS risk, particularly the SV stroke subtype. Extension to ICH reveals retained association with OXPHOS complex IV in deep ICH. Further studies will be necessary to clarify the functional impact of these variants on OXPHOS function.

Acknowledgments
This work used samples and clinical data from the National Institutes of Neurological Disorders and Stroke Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds). This study used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health (NIH), Bethesda, MD (http://biowulf.nih.gov). The project described was supported in part by a grant from the National Institute of Neurological Disorders and Stroke (NINDS) and National Institute on Minority Health and Health Disparities (NS U54NS057405). The content is solely the responsibility of the authors and does not necessarily represent the official views of NINDS or NIH.

C. Anderson (coleader), A. Biffi (coleader), and J. Rosand were responsible for manuscript preparation. Data acquisition was performed by C. Anderson, N. Rost, A. Ayres, K. Schwab, A. Viswanathan, M. Nalls, W. Devan, V. Valant, B. Hansen, and A. Biffi. Manuscript revision was completed by N. Rost, M. Nalls, O. Ross, R. Saxena, J. Meschia, W. Devan, V. Valant, J. Rosand, B. Worrall, T. Brott, D. Brown, B. Hansen, J. Broderick, B. Norrving, A. Viswanathan, S. Silliman, D. Tirschwell, A. Lindgren, A. Slowik, R. Schmidt, M. Selim, J. Roquer, J. Montaner, A. Singleton, S. Greenberg, C. Kidwell, D. Woo, C. Anderson, A. Biffi, and M. Nalls conducted data analysis. Study

Sources of Funding

Massachusetts General Hospital/MIGen: These studies were funded by the American Heart Association/Bugher Foundation Centers for Stroke Prevention Research (0775010 N), the National Institutes of Health (NIH)—National Institute for Neurological Disorders and Stroke (NINDS; R01 NS059727, U01 NS069208), The Keane Genetics Fund, and the Deane Institute for Integrative Research in Atrial Fibrillation and Stroke. The MIGen study was funded by the US NIH and National Heart, Lung, and Blood Institute STAMPEDE genomics research program (R01 HL087767) and a grant from the National Center for Research Resources. The Broad Institute Center for Genotyping and Analysis is supported by grant U54 RR020278 from the National Center for Research resources. C.D.A., A.B., and N.S.R. were supported in part by the American Heart Association/ BUGher Foundation Centers for Stroke Prevention Research, and C.D.A. was supported by the American Brain Foundation.

Ischemic Stroke Genetics Study/Siblings with Ischemic Stroke Study: These studies were funded by NIH-NINDS (R01 NS42733, R01 NS39987), the Intramural Research Program of NIH-National Institute on Aging (NIA; Z01 AG000954-06), and by the Marriot Disease Risk and Regenerative Medicine Initiative Award in Individualized Medicine and the Marriot Mitochondrial Fund. The inclusion of BLSA samples was supported in part by the Intramural Research Program of NIH-NIA (Z01 AG000015-50). O.A.R. was supported by the American Heart Association, James and Esther King Biomedical Research Program, the Florida Department of Health, and the Myron and Jane Hanley Award in Stroke Research.

International Stroke Genetics Consortium: The Differences in the Imaging of Primary Hemorrhage based on Ethnicity or Race (DECIPHER) project was supported by Award Number U54NS057405 from the NIH-NINDS and National Institute on Minority Health and Health Disparities (NIMHD) (U54NS057405). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health (DECIPHER).

Genetic Risk Factors for Hemorrhagic Stroke (GERHES) study was supported by NIH-NINDS (R01 NS36905 and NS30678), and by the Greater Cincinnati Foundation Grant (Cincinnati Stroke Registry). The Massachusetts General Hospital Intracerebral Hemorrhage Stroke Genome-Wide Association study was funded by NIH-NINDS (R23NS40269, 5K23NS059774, R01NS059727, and 5R01NS042147), the Keane Stroke Genetics Research Fund, the Edward and Maybeth Sonn Research Fund, by the University of Michigan General Clinical Research Center (M01 RR00042), and by a grant from the National Center for Research Resources. The Hospital del Mar ICH (HM-ICH) study was funded by the Instituto de Salud Carlos III, Spanish Research Networks Red HERACLES (R0600009) FEDER. The Jagiellonian University Hemorrhagic Stroke Study (JUHSS) was supported by a grant funded by the Polish Ministry of Education (NN402083934). The Lund Stroke Register (LSR) was funded by Lund University, Region Skåne, King Gustaf V’s and Queen Victoria’s Foundation, and the Swedish Medical Research Council (K2010-61X-20378-04-3). Biobank services and genotyping were done at Region Skåne Competence Center (RSKC Malmö), Skåne University Hospital, Malmö, Sweden. Controls from the Medical University of Graz (MUG-ICH) study were from the Austrian Stroke Prevention Study, which is a population-based study funded by the Austrian Science Fund grant numbers P20545-P05 and P13180; the Medical University of Graz supports the databank of the Austrian Stroke Prevention Study.

Disclosures

None.

References

19. Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003;19:149–150.


