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Article Title

Multi-ancestry meta-analysis identifies 2 novel loci associated with ischemic stroke and reveals heterogeneity of effects between sexes and ancestries

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Summary

Cerebrovascular accident (stroke) is the second leading cause of death and disability worldwide. Stroke prevalence varies by sex and ancestry, which could be due to genetic heterogeneity between subgroups. We performed a genome-wide meta-analysis of 16 biobanks across multiple ancestries to study the genetic contributions underlying ischemic stroke (60,176 cases, 1,310,725 controls) as part of the Global Biobank Meta-analysis Initiative (GBMI). Two novel loci associated ischemic stroke with plausible candidate genes, FGF5 and CENPQ/MUT, were identified after replication in four additional datasets. One locus showed significant ancestry heterogeneity (PDE3A) and two loci showed significant sex-heterogeneity (SH3PXD2A and ALDH2). The ALDH2 locus had a male-specific association for stroke in GBMI (P-value males = 1.67e-24, P-value females = 0.126). To test whether we would see a difference in the predictive power of sex-specific polygenic risk scores (PRSs), we compared the C-indexes for sex-specific and sex-combined PRSs in HUNT dataset. A sex-combined PRS was more successful at predicting stroke cases than a sex-specific PRS, most likely due to more stable effect estimates from the sex-combined summary-statistics. These approaches can be applied to further unravel the genetic underpinnings of stroke and other complex diseases.
INTRODUCTION

Cerebrovascular accidents (stroke) are the second leading cause of death and disability worldwide due to brain infarction (ischemic stroke) or intracerebral hemorrhage (Katan and Luft, 2018). The former can be further divided into different subgroups, including cardioembolic, large vessel, and small vessel stroke, and the latter into lobar and non-lobar hemorrhagic stroke. Mapping genetic variants associated with stroke has been more challenging than for other homogenous complex diseases, such as coronary artery disease (Nelson et al., 2017) or type 2 diabetes (Mahajan et al., 2018) given that stroke subgroups have different etiologies (Hankey, 2017) and heritability (Bevan et al., 2012).

Thirty-five loci have been identified using genome-wide association study (GWAS) methods (Malik et al., 2018a; Malik et al., 2018b; Woo et al., 2014) despite the complex phenotypic heterogeneity of stroke. These studies consist of sample sizes up to 900,000 (72,000 cases with all-cause stroke) and show that genetic predisposition varies between subgroups. Most known loci are associated with ischemic stroke, likely due to higher prevalence of that subtype (~80% of the cases), and thus, more power to detect an association (Donkor, 2018). Additionally, stroke prevalence has been shown to differ between populations of different ancestry and sexes (Guzik and Bushnell, 2017), suggesting possible heterogeneity of environmental and/or genetic factors contributing to the risk for stroke.

The predictive power of polygenic risk scores (PRSs) has been evaluated to preemptively identify stroke risk (Abraham et al., 2019; Marston et al., 2021; Rutten-Jacobs et al., 2018). These scores have shown lower predictive power compared to other cardiovascular disease outcomes (Khera et al., 2018; Mars et al., 2020), likely due to phenotypic heterogeneity and limited sample sizes in the discovery cohorts underlying the PRS calculations. Here, we perform
a new GWAS as part of the Global Biobank Meta-analysis Initiative (GBMI) to examine genetic
variants and test whether the observed associations and PRS for ischemic stroke show either
ancestry- or sex-specific effects.

RESULTS

Ischemic stroke locus discovery and ancestry heterogeneity

Following discovery and replication stages, we identified two novel loci associated with
ischemic stroke. We initially assessed association summary statistics from 16 biobanks with
participants from various ancestries (Zhou et al., 2021) (Figure 1, Supplementary Table 1) and
performed replication in four additional biobanks (STAR Methods, Table 1, Figures 2A-B,
Supplementary Figures 1A-B). One variant showed a significant replication P-value
(PRDM8/FGF5 locus, replication P-value < 7.1e-3, Bonferroni correction for 7 tested putative
novel variants) and in the other locus (CENPQ/MUT), the replication results supported the
original discovery results (combining results from discovery excluding BioMe and replication
showed more significant association than original discovery alone). Both loci also showed
suggestive association in a largely independent meta-analysis of stroke (Supplementary Figures
2A-B) with association P-values 4x10^-4 and 2x10^-4 for rs12509595 (PRDM8/FGF5) and
rs2501968 (CENPQ/MUT), respectively (Malik et al., 2018a). Replication results for 7 variants
tested for replication are in Supplementary Table 2.

Of the 2 confirmed novel associations, one index variant was a common missense variant
in the gene encoding centromeric protein Q -- CENPQ (p.Asp266Gly, rs2501968). However, the
index variant was also associated with CENPQ gene expression (Supplementary Table 3A) in
multiple tissues, with the strongest eQTL associations observed in arterial tissues. The variant
was also a significant eQTL for the MUT gene. Additionally, there were significant splice QTLs for this variant in both CENPQ and MUT genes (Supplementary Table 3B). The lead variant for the other confirmed novel association, PRDM8/FGF5, was intergenic and showed a significant eQTL for FGF5 gene expression in the kidney.

Next, the ancestry specific results of the discovered ischemic stroke associations were examined. We observed one locus showing significant ancestry heterogeneity (P-value < 5.5x10^−3, Bonferroni correction for 9 tests, Table 2). The lead variant, rs12811752, lies in the intron of the PDE3A gene (a cGMP-inhibited cyclic nucleotide phosphodiesterase) and had consistent effects in the European (non-Finnish European [NFE], and Finns [FIN]), East Asian (EAS), and African (AFR) ancestry populations. However, for the admixed American (AMR) individuals the effect size was approximately 4 times larger. Additionally, for the South Asian (SAS) ancestry individuals the direction of effect was opposite (although non-significant) to that observed in the other ancestry groups. In addition to PDE3A, we observed nominally significant ancestry heterogeneity for two loci; CDKN2B and COL4A1.

**Sex-specificity of ischemic stroke associations and polygenic risk score**

In addition to ancestry heterogeneity, we looked for evidence of sex-specific heterogeneity in the associated loci. Two of the nine associated loci, ALDH2 and SH3PXD2A, showed significant sex-heterogeneity (P-value < 5.56x10^−3, Bonferroni correction for 9 tests, Supplementary Table 4, Supplementary Figures 3A-B). The two significant sex-heterogeneity loci have been associated with stroke in previous GWAS (Malik et al., 2018a). The sex-heterogenic effects on ischemic stroke have not been previously reported for SH3PXD2A, but have been indirectly implied for ALDH2 (Millwood et al., 2019). For both loci, the stroke
association was stronger in males than for females (effect sizes for males, -0.160 and -0.062, for females -0.031 and -0.031, for ALDH2 and SH3PXD2A, respectively), and significant only in males (P-values for males 1.67e-24 and 4.84e-8, and for females 0.126 and 0.022, for ALDH2 and SH3PXD2A, respectively). At the ALDH2 locus, the association was East Asian specific, reflecting the absence or very low frequency of this variant in other ancestries in the GBMI meta-analysis. Furthermore, the allele frequency of the lead variant, rs671, was 25.5% for the EAS and ≤0.05% for other ancestries in the publicly available gnomAD database.

We next tested whether a genome-wide polygenic risk score demonstrated sex-differential effects on stroke risk since sex-heterogeneity for multiple lead-variants within the associated loci were observed. Specifically, we created 3 different PRS: for the joint meta-analysis, and for the male- and female-only meta-analyses. The predictive performance of the scores was tested in the HUNT dataset using Cox Proportional Hazards models. The model performance of the joint PRS was low as evidenced by a small change in the model C-index when adding the PRS into the model (C-index for the reference model with age and sex only = 0.858 95% CI [0.849; 0.866], C-index after adding the PRS = 0.860 [0.852; 0.868], Supplementary Figure 4). When using the sex-specific PRS instead of the joint PRS, the performance was slightly attenuated for both males (from 0.850 [0.839; 0.862] to 0.848 [0.836; 0.859]) and females (from 0.868 [0.857; 0.880] to 0.867 [0.856; 0.879]), most likely due to decreased power in the sex-specific meta-analysis compared to the joint meta-analysis. Interestingly, when looking at the predictive performance of age only (the reference model), the C-index for females was notably higher (C-index = 0.867 [0.856; 0.879]) compared to males (C-index = 0.846 [0.834; 0.858]). The incremental increase in C-index when adding the ischemic
stroke PRS to the prediction model was higher in males, however, this difference between the C-index changes between males and females was not statistically significant.

**DISCUSSION**

A genome wide association analysis identified two novel genetic loci associated with stroke, confirmed through replication in four independent datasets. Moreover, we observed significant ancestry heterogeneity in one locus (*PDE3A*) and significant sex heterogeneity in two loci (*ALDH2* and *SH3PXD2A*).

The only protein-altering lead variant resided in *CENPQ*, which has been previously reported to be associated with blood homocysteine levels, a known risk factor for stroke (Paré et al., 2009). *CENPQ* has also been suggested to regulate *MUT* expression, one of the driver genes in the causal relationship between blood homocysteine levels and small vessel stroke (Larsson et al., 2019). The one novel intergenic association was observed near the *FGF5* gene. This locus has been previously associated with blood pressure traits in Chinese individuals with higher body mass index (Li et al., 2015), and the expression of the *FGF5* gene has been shown to be different in hypertensive patients (Ren et al., 2018). Additionally, recent studies have shown that the FGFs could potentially be used to treat stroke in animal models (Dordoe et al., 2021).

Two of the associated loci, rs671 in *ALDH2* and rs7091346 in *SH3PXD2A*, showed significant sex-heterogeneity. The lead variant rs671 is a well-known polymorphism linked to alcohol consumption and hypertension in East Asian individuals (Millwood et al., 2019). The effect of this variant is observed more strongly in men, likely due to cultural and societal differences in patterns of alcohol consumption between sexes. We observed an association with
ischemic stroke for this variant only in males, and more specifically, in those with East Asian ancestry.

The ischemic stroke PRS did not significantly predict stroke in the HUNT dataset when added on top of the age and sex information. Additionally, in an accompanying paper by Wang et al., the performance of the GBMI derived PRS was compared to one derived using the MegaStroke summary statistics (Wang et al., 2021). Wang et al. concluded that the previous meta-analysis, with more cases underlying the summary statistics, performed better for African ancestry individuals whereas the GBMI derived PRS was slightly better for European individuals. Previous PRS studies have shown that a stroke PRS could slightly increase the prediction of future stroke cases. In a recent study, a stroke PRS derived from the MegaStroke summary statistics performed better than any of the individual risk factors for stroke (Abraham et al., 2019). However, they did not evaluate the performance of their score on top of the age and sex as shown here. In our test dataset, we observed a high C-index when using age information only, especially for females (C-index = 0.867 [0.856; 0.879]).

Limitations

The high representation of East Asian individuals was a notable strength of this investigation, likely leading to the discovery of East Asian driven sex-specific effects in two previously published stroke loci, $ALDH2$ and $SH3PX2A$. Our study has several limitations despite the large overall size of the meta-analysis. First, the ischemic stroke phenotype was defined in the GBMI meta-analyses using an EHR-derived phenotype definition only. This approach does not allow for dissection of results by sub-phenotypes, which has previously been done in large stroke meta-analysis efforts (Malik et al., 2018a). Additionally, the number of participating biobanks and total number of individuals for some of the ancestry groups are low.
(FIN, one biobank N = 180,062; SAS, one biobank N = 21,940; AMR, two biobanks N = 15,064), resulting in limited power to fully test for ancestry-specific effects.

**Outlook/Conclusion**

We present 2 novel loci associated with ischemic stroke and show that some stroke loci show sex- and/or ancestry-specific patterns. These findings emphasize the need for more diverse datasets with large enough sample sizes to further understand the genetic predisposition of stroke in different ancestry groups. Finally, we recommend evaluation of polygenic risk scores in males and females separately in different populations, and ideally with stroke subtypes.

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HUNT: IS, BB, AH, MEG, KH

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**Declaration of interests**

NRS is an advisor for Abbott, Philips, and Shockwave and have received honoraria for speaking from Zoll, Cordis. CJW’s spouse works at Regeneron pharmaceuticals.
FIGURES AND FIGURE LEGENDS

Figure 1. Breakdown of stroke meta-analysis ancestries. This figure presents the prevalence and number of cases by cohort participating in the GBMI stroke meta-analysis. BBJ = Biobank Japan, MGB = Mass General Brigham Biobank, UCLA = UCLA Precision Health Biobank, MGI = Michigan Genomics Initiative, BioMe = Mount Sinai BioMe Biobank, HUNT = Trøndelag Health Study, BioVU = Biorepository at Vanderbilt University, CCPM = Colorado Center for Personalized Medicine, QSKIN = Queensland Skin Study, ESTBB = Estonian Biobank, GNH = East London Genes & Health, GS = Generation Scotland, TWB = Taiwan Biobank, UKBB = UK Biobank, AFR = African ancestry, MID = Middle Eastern ancestry, AMR = Admixed American ancestry, EAS = East Asian ancestry, NFE = non-Finnish European ancestry, FIN = Finnish ancestry, SAS = South Asian ancestry.
Figure 2A-B. Discovery and replication results for the two confirmed associations.

Presented are the effect sizes together with their 95% confidence intervals. Panel A shows the association results for rs12509595 (PRDM8/FGF5) and panel B for rs2501968 (CENPQ/MUT).

GBMI = Global Biobank Meta-analysis Initiative, BioMe = Mount Sinai BioMe Biobank, TOPMed = Trans-Omics for Precision Medicine Program, PMBB = Penn Medicine Biobank, NFE = Non-Finnish European ancestry, AFR = African ancestry, CanPath = Canadian Partnership for Tomorrow’s Health, MVP = Million Veteran Program, EUR = European ancestry, HIS = Hispanic/Latino ancestry, ASN = Asian ancestry
**TABLES WITH TITLES AND LEGENDS**

**Table 1. Two newly identified and replicated loci associated with ischemic stroke.** For both variants the effect sizes are reported for the minor allele noted also in the legend after the rsID. The discovery results are presented from the leave-BioMe-out summary statistics due to BioME being part of the TOPMed results as well.

| Dataset (N)                  | rs12509595_T (PRDM8/FGF5) | rs2501968_C (CENPQ/MUT) |
|------------------------------|---------------------------|-------------------------|
|                              | Beta     | SE       | P-value     | Beta     | SE       | P-value     |
| GBMI discovery excluding BioMe (1,284,232) | 0.047    | 0.0073   | 1.22e-10    | -0.046   | 0.0069   | 1.65e-11    |
| CanPath NFE (7,260)          | 0.250    | 0.190    | 0.189       | -0.119   | 0.173    | 0.493       |
| Penn Medicine NFE (26,407)   | 0.025    | 0.048    | 0.601       | -0.083   | 0.043    | 0.057       |
| Penn Medicine AFR (6,376)    | -0.138   | 0.138    | 0.318       | -0.048   | 0.081    | 0.553       |
| TOPMed ALL (32,732)          | 0.019    | 0.028    | 0.499       | -0.061   | 0.024    | 0.013       |
| MVP EUR (450,717)            | 0.035    | 0.011    | 0.002       | -0.015   | 0.010    | 0.146       |
| MVP AFR (119,811)            | 0.002    | 0.031    | 0.956       | -0.012   | 0.017    | 0.498       |
| MVP HIS (51,036)             | 0.061    | 0.038    | 0.111       | 0.027    | 0.035    | 0.430       |
| MVP ASN (8,196)              | -0.038   | 0.106    | 0.718       | -0.061   | 0.099    | 0.540       |
| Combined Replication (702,535) | 0.031  | 0.0092   | 8.09e-4     | -0.020   | 0.0077   | 0.010       |
| Combined discovery + replication (1,986,767) | 0.041  | 0.0057   | 1.05e-12    | -0.035   | 0.0051   | 1.56e-11    |

N = Number of samples; SE = Standard error of the effect size; GBMI = Global Biobank Meta-analysis Initiative, CanPath = Canadian Partnership for Tomorrow’s Health, TOPMed = Trans-Omics for Precision Medicine Program, MVP = Million Veteran Program, NFE = Non-Finnish European, AFR = African, EUR = European, HIS = Hispanic, ASN = Asian.
Table 2. Ancestry specific effect sizes from the GBMI meta-analysis for the significant lead variants. For all variants the effect sizes are reported for the minor allele.

| Variant (Locus) | Effect size (SE) NFE | Effect size (SE) FIN | Effect size (SE) SAS | Effect size (SE) EAS | Effect size (SE) AMR | Effect size (SE) AFR | Cochran’s Q-statistic for ancestry | Ancestry heterogeneity P-value |
|----------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------------------|--------------------------------|
| **Novel variants** | | | | | | | | |
| rs12509595 (PRDM8/FGF5) | 0.027 (0.014) | 0.037 (0.014) | 0.100 (0.101) | 0.066 (0.011) | 0.142 (0.096) | 0.036 (0.089) | 6.80 | 0.236 |
| rs2501968 (CENPQ/MUT) | -0.042 (0.013) | -0.042 (0.013) | -0.043 (0.104) | -0.051 (0.011) | 0.010 (0.064) | -0.082 (0.051) | 1.75 | 0.883 |
| **Previously published variants** | | | | | | | | |
| rs1275985 (CIB4/KCNK3) | -0.033 (0.013) | -0.032 (0.013) | -0.001 (0.108) | -0.068 (0.013) | -0.055 (0.066) | -0.025 (0.060) | 5.40 | 0.369 |
| rs1333047 (CDKN2B-AS1/DMRTA1) | 0.057 (0.013) | 0.091 (0.013) | 0.129 (0.093) | 0.037 (0.011) | 0.111 (0.065) | 0.136 (0.087) | 11.89 | 0.036 |
| rs7091346 (SH3PXD2A) | -0.030 (0.013) | NA | -0.130 (0.091) | -0.056 (0.011) | -0.007 (0.064) | -0.134 (0.062) | 5.48 | 0.241 |
| rs1281752 (PDE3A) | 0.038 (0.013) | 0.057 (0.013) | -0.065 (0.090) | 0.054 (0.011) | 0.282 (0.041) | 0.040 (0.074) | 34.44 | 1.94e-6 |
| rs671 (ALDH2) | -1.040 (3.815) | NA | NA | -0.107 (0.012) | NA | NA | 0.060 | 0.807 |
| rs4773140 (COL4A1) | 0.072 (0.016) | 0.007 (0.015) | -0.056 (0.107) | 0.066 (0.012) | -0.078 (0.102) | -0.015 (0.090) | 14.41 | 0.013 |
| rs11880613 (DNM2) | -0.054 (0.017) | -0.038 (0.016) | 0.002 (0.152) | -0.058 (0.014) | -0.047 (0.101) | -0.055 (0.134) | 1.065 | 0.957 |

SE = Standard error of the effect size, NFE = non-Finnish European ancestry, FIN = Finnish ancestry, SAS = South Asian ancestry, EAS = East Asian ancestry, AMR = Admixed American ancestry, AFR = African ancestry, NA = Not available
STAR METHODS

Multi-Ancestry Meta-Analysis
The GBMI ischemic stroke meta-analysis was conducted from genome-wide association results of 16 biobanks using inverse variance weighted meta-analysis. The overall stroke dataset has 1.9% of African/African American (AFR), 19.6% of East Asian (EAS), 75.8% of European (non-Finnish European: NFE and Finns: FIN), 1.1% of Latino or admixed American (AMR), and 1.6% of South Asian (SAS) ancestry (Figure 1). A detailed description of the meta-analysis methods can be found here (Zhou et al., 2021). The GBMI stroke phenotype was defined using PheCode 433.21 (Cerebral artery occlusion, with cerebral infarction).

Polygenic Risk Scores
For the sex-specificity testing, we calculated 3 PRSs using summary statistics from the overall population (N = 1,370,901; 4.3% cases), female only population (N = 601,704; 3.8% cases), and male only population (N = 498,162; 6.1% cases) using PRS-CS (Ge et al., 2019) with a LD reference panel based on combined 1000 Genomes and UK Biobank. The summary statistics used for the PRS calculation excluded the PRS test cohort, HUNT.

Longitudinal PRS methods in HUNT
HUNT (Krokstad et al., 2013) is a population-based dataset with longitudinal hospital registries linked to the whole genome data (Brumpton et al., 2021). For the PRS prediction in HUNT, we defined IS with ICD9-codes 434 and 436, and ICD10-codes I63 and I64, resulting in 4,256 ischemic stroke cases (285 prevalent, 3,971 incident, 62,375 non-cases). Individuals with no prevalent cardiovascular disease events and full baseline information (non-missing lipid measurements, smoking, anthropometric measures and blood pressure medication information)
were included in the survival analysis and the time of event was recorded based on the first appearance of the above-mentioned ICD-codes from both hospital and death registry. The final number of cases in the Cox Proportional Hazards model was 1,796 for males (28,216 non-cases) and 1,858 for females (32,724 non-cases). The survival was modeled with follow-up time as a time-scale with HUNT collection (HUNT2 or HUNT3) as a covariate to count for the possible periodic bias. Individuals that diseased or had a non-ischemic stroke during the follow-up were censored. The PRSs were adjusted with the first ten genetic PCs (for males and females separately for the sex-specific analysis) to remove possible population stratification and the resulting residuals were inverse normalized. All longitudinal analyses were performed using R v4.1.2.

**Replication cohorts**

Replication of all seven novel lead variants were requested from two additional GBMI cohorts, Penn Medicine Biobank (PMBB) and Canadian Partnership for Tomorrow’s Health (CanPath). Analyses in these two cohorts were performed using the standard GBMI analysis pipeline (Zhou et al., 2021). Furthermore, we received replication results from Million Veteran Program (MVP) and Trans-Omics for Precision Medicine (TOPMed) Program. The MVP (Hunter-Zinck et al., 2020; Klarin et al., 2018) analysis was performed using plink2a (Chang et al., 2015) and with the EHR based stroke phenotype and covariates defined (Klarin et al., 2018). Analysis was completed within each HARE-defined ancestry group (Fang et al., 2019). The TOPMed results have been previously published and the analysis details can be found from the original publication (Hu et al., 2021). As TOPMed includes one overlapping biobank with GBMI
(BioMe), the GBMI results used to combine discovery and replication excluded the BioMe results.
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