Recurrent stroke prediction by applying a stroke polygenic risk score in the Japanese population

Naoki Kojima (MSc)[1], Masaru Koido (PhD)[1], Yunye He (PhD)[1], Yuka Shimmori (MSc)[1], Tsuyoshi Hachiya (PhD)[1], BioBank Japan, Stéphanie Debette (MD, PhD)[2,3], Yoichiro Kamatani (MD, PhD)[1]

1. Laboratory of Complex Trait Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan.
2. Bordeaux Population Health Research Center, University of Bordeaux, Inserm, UMR 1219, Bordeaux, France.
3. Department of Neurology, Institute for Neurodegenerative Diseases, CHU de Bordeaux, Bordeaux, France.

Short title: Recurrent stroke prediction by PRS

Corresponding author:

Yoichiro Kamatani[1]

Address: 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639 Japan

Email: kamatani.yoichiro@edu.k.u-tokyo.ac.jp

Total word count of the manuscript (including Title Page, Abstract, Text, References, Tables, and Figures Legends): 6306

Abstract

Background

Recently, various polygenic risk score (PRS)-based methods were developed to improve stroke prediction. However, current PRSs (including cross-ancestry PRS) poorly predict recurrent stroke. Here, we aimed to determine whether the best PRS for Japanese individuals can also predict stroke recurrence in this population by extensively comparing the methods and maximizing the predictive performance for stroke onset.

Methods

We used data from the BioBank Japan (BBJ) 1st cohort (n=179,938) to derive and optimize the PRSs using a 10-fold cross-validation. We integrated the optimized PRSs for multiple traits, such as vascular risk factors and stroke subtypes to generate a single PRS using the meta-scoring approach (metaGRS). We used an independent BBJ 2nd cohort (n=41,929) as a test sample to evaluate the association of the metaGRS with stroke and recurrent stroke.

Results

We analyzed recurrent stroke cases (n=174) and non-recurrent stroke controls (n=1,153) among subjects within the BBJ 2nd cohort. After adjusting for known risk factors, metaGRS was associated with stroke recurrence (adjusted OR per SD 1.18 [95% CI: 1.00–1.39, p=0.044]), although no significant correlation was observed with the published PRSs. We administered three distinct tests to consider the potential index event bias; however, the outcomes derived from these examinations did not provide any significant indication of the influence of index event bias. The high metaGRS group without a history of hypertension had a higher risk of stroke recurrence than that of the low metaGRS group (adjusted OR 2.24 [95% CI: 1.07–4.66, p=0.032]). However, this association was weak in the hypertension

group (adjusted OR 1.21 [95% CI: 0.69–2.13, p=0.50]).

Conclusions

The metaGRS developed in a Japanese cohort predicted stroke recurrence in an independent cohort of patients. In particular, it predicted an increased risk of recurrence among stroke patients without hypertension. These findings provide clues for additional genetic risk stratification and help in developing personalized strategies for stroke recurrence prevention.

Keywords: recurrent stroke, stroke, polygenic risk score, LDpred2, risk factor, index event bias, hypertension, metaGRS

Non-standard abbreviations and acronyms

PRS	polygenic risk score
P+T	pruning and thresholding
BBJ	BioBank Japan
BBJ1	BBJ 1st cohort
BBJ2	BBJ 2nd cohort
ToMMo	Tohoku Medical Megabank
AIS	all ischemic stroke
IPW	inverse probability weighting
LD	linkage disequilibrium
GWAS	genome-wide association study
PC	principal component
IPW	inverse probability weight
AUC	area under the curve
LAS	large artery stroke
SVS	small vessel stroke
CES	cardioembolic stroke
TIS	transient ischemic attack
HWE	Hardy-Weinberg equilibrium
WGS	whole genome sequencing
MI	myocardial infarction
SAP	stable angina pectoris
AP	unstable angina pectoris
AF	atrial fibrillation
DM	diabetes mellitus

87	SM	smoking
88	BMI	body mass index
89	HE	height
90	SBP	systolic blood pressure
91	DBP	diastolic blood pressure
92	TC	total cholesterol
93	TG	triglyceride
94	HDL	high-density lipoprotein
95	LDL	low-density lipoprotein

96

Introduction

Stroke is a major cause of mortality in Japan, with 56,000 deaths reported in 2020.[1] The conventional risk factors for stroke include hypertension, high waist-to-hip ratio, smoking, cardiac causes, dyslipidemia, and diabetes mellitus.[2] In Japan, the stroke recurrence rate is up to 30–50% during 5-10 years of follow-up after the first stroke.[3,4] Accordingly, it will be medically beneficial to stratify high-risk groups for recurrent stroke among those who have experienced a stroke to potentially generate more intensive secondary prevention strategies than current recommendations.

Genome-wide association studies (GWAS) have identified many disease-susceptibility variants associated with complex traits.[5] A polygenic risk score (PRS) is the weighted summation of the individual genetic effects of these variants. Its weighting strategy varies depending on the construction method; traditionally, only significant variants are used in developing this score. The recently developed PRS methods involve non-significant variants and updated effect weights and consider the linkage disequilibrium (LD) structure. The development of PRS methods has helped stratify high-risk groups for complex traits,[6–11] including stroke.[12]

Polygenic risk scores developed using the 32 genome-wide significant ($p< 5.0\times10^{-8}$) variants or 90 marginally associated ($p<1.0\times10^{-5}$) variants (PRS_{90}) from the MEGASTROKE study[12] are associated with stroke onset in subjects of European ancestry.[12,13] The meta-scoring PRS approach (metaGRS) includes 3.2 million variants by combining PRSs for stroke subtypes, risk factors, and comorbidities by adjusting the effect weight via elastic-net logistic regression; this approach has an improved predictive performance for stroke compared to that of PRS_{90}.[14] MetaGRS can predict stroke incidence independent of environmental factors and could help motivate individuals with high genetic risk to make lifestyle changes for

stroke prevention (although not yet implemented in clinical practice outside a research setting).[15] The PRS shows reduced transferability between populations. Additionally, a PRS developed using various variants derived from Japanese GWAS successfully predicted stroke onset in the Japanese population.[16,17] Most recently, the GIGASTROKE study proposed an integrated PRS approach among PRSs derived from populations of multiple ancestries using the metaGRS framework (iPGS), which showed a better predictive ability than the MEGASTROKE European or East Asian PRS.[18] However, the PRS did not successfully predict stroke recurrence; for example, PRS_{32} and iPGS did not significantly predict stroke recurrence after adjusting for clinical comorbidities, with notably smaller effect sizes than for non-recurrent stroke.[12,18] Furthermore, the potential effect of index event (also known as "collider") bias that may distort the association of PRS was suspected.[19,20]

The optimal method to improve the predictive accuracy of PRS depends on the population- and trait-specific genetic architecture.[21–30] Therefore, we compared different PRS methods and determined whether the best PRS can predict the onset of recurrent stroke in a Japanese population.

Methods

The workflow of this study is shown in Figure 1. This article follows the TRIPOD (Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) reporting guidelines.

Study subjects and quality control

BioBank Japan (BBJ) involves physicians diagnosing all ischemic stroke (AIS) cases at the collaborating hospitals. BBJ was established in 2003 and recruited 267,000 patients from 12 medical institutions (66 hospitals) in two phases.[31–33] The recruited patients had at least one of the 51 primarily multifactorial (common) diseases, which accounted for 440,000 cases. We used BBJ 1st cohort (BBJ1) data to derive PRSs and available independent BBJ 2nd cohort (BBJ2) data to evaluate the performance of PRSs in predicting AIS and recurrent AIS. Recurrent AIS information was unavailable for the BBJ1 data. In BBJ2, any AIS cases (n=1,470), AIS-free controls (n=40,459), recurrent AIS cases (n=174), and non-recurrent AIS controls (n=1,153) were available. The mean duration from the first episode of AIS onset to recurrent AIS onset was 4.88 years. Detailed sample characteristics are listed in Supplementary Methods and Table 1.

This study was approved by the ethics committee of the Institute of Medical Science, the University of Tokyo, Japan. Quality control, pre-phasing, and genotype imputation were conducted using PLINK(v2.0),[34–37] Eagle (v2.4.1), and Minimac4 (v1.0.2), respectively. The detailed processes are presented in the Supplementary Methods.

Constructing PRSs for AIS

Unbiased PRSs were obtained by applying a 10-fold cross-validation to select the model and optimize the parameters.[26,38] Briefly, BBJ1 samples were randomly split into ten equal-sized

subsamples. We retained one subsample for validation and the others for training. We repeated this process 10 times, with each of the ten subsamples used exactly once for validation. A GWAS was conducted on the training set in each iteration, adjusted for age, sex, and the first 10 principal components (PCs) via Firth logistic regression using PLINK (v.2.0).[34]

We obtained the weights of variants for PRS from the GWAS summary statistics of the training set using five PRS methods—P+T (PLINK (v.1.9)[35] for clumping), LDpred2,[39] Lassosum2,[40] (LDpred2 and Lassosum2 by bigsnpr package (v.1.7.2) in R (v.3.5.0)), PRS-CS (v.1.0.0),[41] and PRS-CSx (v.1.0.0).[42] The PRS-CSx integrated BBJ1 with the European stroke GWAS summary statistics (MEGASTROKE; the largest study available at this study design)[43] by learning an optimal linear combination. We used combinations of parameters for P+T (1,224 parameters), LDpred2 (126 parameters), Lassosum2 (200 parameters), PRS-CS (9 parameters), and PRS-CSx (9 parameters), as described in the Supplementary Methods.

Subsequently, the PRSs for the validation sample were calculated using the weights obtained from the training samples. The accuracy for predicting AIS cases was evaluated from Nagelkerke's R^2 (simply "R^2" from this point onwards)[29,44] after adjusting for age, sex, and the first 10 PCs. We calculated the mean R^2 over 10 cross-validation results for each method with each parameter after a 10-fold cross-validation. We chose the method and parameters that maximized incremental R^2 (PRS_{AIS}) among these PRSs.

We further integrated the PRS_{AIS} with PRSs of vascular risk factors, such as stroke subtypes and comorbid diseases presence, using the elastic net framework to construct a metaGRS using the glmnet package (v.4.1.3) in R (v.4.1.0). Nine binary traits and eight quantitative traits of vascular risk factors reported in a previous study[14] are described in the Supplementary Methods. Binary traits were determined by conducting GWAS and attempting to obtain unbiased weights using cross-validations. The effect weights from the derivation

sample every 17 traits were calculated using PRS-CS-auto since it did not require an independent validation sample set for parameter optimization and performed well for various traits.[21,26,39,41,45] Subsequently, we used a validation sample to calculate the weight of AIS and the 17-trait PRSs to predict AIS using elastic-net logistic regression. We conducted a 10-fold cross-validation and used the mean weight for testing.

We used PLINK (v2.0)[34] to calculate the individual PRS by aggregating the effect estimates multiplied by each imputed dosage into a single score per person.

Risk factors

The following seven risk factors that were previously utilized as covariates[12] were used as covariates for testing: hypertension (systolic blood pressure>140 mmHg, diastolic blood pressure>90 mmHg, or hypertension history), hyperlipidemia, diabetes (all types), smoking (current smoker), vascular disease (myocardial infarction, peripheral artery disease, stable angina pectoris, and unstable angina pectoris), congestive heart failure, and atrial fibrillation (including atrial flutter). A sample was considered to have a risk factor status if it had that status at enrollment or from historical records (Tables 1 and S1).

Assessment of the association of PRS with AIS and AIS recurrence

We used a single selected method with optimized parameters and calculated the metaGRS in independent testing sample sets. We used a logistic regression model to assess the association of the PRS using the two case-control settings for AIS (any-AIS versus AIS-free controls) and AIS recurrence (recurrent AIS versus non-recurrent AIS). We also applied two other combinations of case-controls: recurrent AIS versus AIS-free controls and non-recurrent AIS versus AIS-free controls (Figure 1). Furthermore, we examined additional PRS contributions of the seven risk factors to predictive accuracy and discriminative performance using the

values of R^2 and area under the curve (AUC), according to our previous studies.[14,46] Additionally, we evaluated the performance of the following PRSs derived from other milestone studies for stroke prediction: 32 genome-wide significant variants (for any stroke, ischemic stroke, or ischemic stroke subtypes) from the MEGASTROKE cross-ancestry study of 524,354 individuals (PRS_{32}),[43] 89 genome-wide significant variants of 1,614,080 multi-population individuals (PRS_{89}), and 6,010,730 variants of the East Asian PRS developed from 9,809 individuals ($iPGS_{EAS}$),[18] both from the GIGASTROKE study. The PRS calculation process is described in the Supplementary Methods.

Considering potential index event bias

An index event bias may be induced when the samples are only selected from cases.[47] We evaluated the extent to which the index event bias affected our results since we used case-only samples in this study. The association of PRSs with recurrent AIS was evaluated after adjusting for seven risk factors, in addition to age, sex, and the first 10 PCs. Adjusting for such confounding bias will not be enough to eliminate bias for a recurrence association study.[48] Therefore, we sought to mitigate a potential index event bias by applying three distinct methodologies.[48,49] First, we utilized linear and logistic regression models to assess the relationships between metaGRS and covariates within the any-AIS case (n=1,327) and AIS-free control groups (n=40,459). Initially, we did not adjust for age, sex, the seven risk factors, or the first 10 PCs. Subsequently, we observed the distributions of covariate values across the metaGRS quintiles. We performed statistical tests to detect heterogeneity in the estimates between the prevalent case and control groups, following the methodology of a prior study.[19,50] Second, we refined our analysis by adjusting for associations between metaGRS and AIS recurrence while considering the differential effects of covariates, according to a previous method.[19] Finally, we applied the inverse probability weighted (IPW)

239 approach[47] to comprehensively account for index event bias. Collectively, these analytical
240 approaches were adopted to enhance the validity of our findings.
241
242 **Association of metaGRS and AIS recurrence in patients with/without hypertension**
243 Logistic regression was conducted in subgroups with and without hypertension and metaGRS
244 tertiles among recurrent AIS cases (with hypertension n=107, without hypertension n=67)
245 and non-recurrent AIS controls (with hypertension n=731, without hypertension n=422) to
246 assess the relationship between the PRS and the risk of AIS recurrence. The low metaGRS
247 tertile was set as the reference group and adjusted for age, sex, and the first 10 PCs.
248
249 **Statistical analysis**
250 The mean with standard deviation (SD) or proportion of factors was reported for the baseline
251 characteristics of testing samples. The incremental value (R^2 or AUC) was estimated from
252 the differences between patients with and without PRSs of the fitted values of age, sex, first
253 10 PCs, and seven risk factors[48,57,58] and calculated as the 95% confidence interval. The
254 pROC package (v.1.18.0) in R was used to determine the discriminative ability of the AUC.
255 The IPW package (v.1.2) in R was used for the IPW approach. R (v. 3.5.0) was used to
256 perform logistic regression to calculate R^2, Pearson's correlation coefficient, and linear
257 regression. All statistical tests were two-sided. The significance level was set at $p = 0.05$.
258
259

Results

Derivation of effect weight

The imputed genotype data of 17,621 AIS cases and 162,317 controls without an AIS diagnosis were used for 9,622,629 autosomal variants after implementing quality control of the BBJ1 dataset (Tables S2-S4).

We conducted a 10-fold cross-validation to adjust the parameters and select the best PRS associated with AIS. We performed GWAS 10 times on 90% of the randomly selected BBJ1 dataset (training data). We successfully detected previously reported[43,51] signals in each dataset ($p<5\times10^{-8}$), including *SH3PXD2A*, *CCDC63* (eight times), *CUX2*, and *LINC02356* (every time) (Figure S1, Table S5).

We confirmed some expected characteristics of each PRS method (such as low accuracy) using only genome-wide significant variants (Tables S6–10 and Supplementary Notes). The mean incremental R^2 values of each scoring method with the best-performed parameters were 0.0038 (95% CI: 0.0030–0.0046), 0.00443 (95% CI: 0.0035–0.0054), 0.0039 (95% CI: 0.0030–0.0048), 0.00441 (95% CI: 0.0036–0.0053), and 0.0037 (95% CI: 0.0031–0.0042) for P+T, LDpred2, Lassosum2, PRS-CS, and PRS-CSx, respectively (Table 2). We chose LDpred2 with the parameter set of ρ-value = 0.0056, a heritability-value = $1.0\times h^2_{LDSC}$, where h^2_{LDSC} is the heritability estimate from the constrained LD score regression[52], and a no-sparse model for subsequent analyses, since it showed the best mean incremental R^2 value among the five methods.

We observed an average number of nonzero weights for 8.4 traits after computing the metaGRS via elastic net regularization 10 times (10-fold). The metaGRS weight of AIS was highest (mean=0.123, SD=0.026), followed by diastolic blood pressure (mean=0.039, SD=0.039), atrial fibrillation (mean=0.023, SD=0.024), and myocardial infarction

(mean=0.018, SD=0.025) (Figure S2 and Table S11). Only the triglyceride weights were zero at all 10 measurements among the 18 traits included in the metaGRS calculation. The number of variants used for metaGRS was 1,014,026; a total of 1,011,847 variants (99.8%) remained after matching with the BBJ2 dataset.

Association of metaGRS with AIS cases and recurrent AIS

We used the imputed genotype data of 1,470 AIS cases and 40,459 controls without a diagnosis of AIS for 59,387,070 variants from the BBJ2 dataset to test the association of metaGRS with AIS and AIS recurrence. The AIS case-only sample of the BBJ2 was used to analyze AIS recurrence. Table 1 presents the characteristics of the test samples.

MetaGRS was associated with AIS diagnosis after adjusting for age, sex, first 10 PCs, and seven risk factors (adjusted OR, 1.21 [95% CI: 1.15–1.27, $p=2.89\times10^{-12}$]), as previously reported.[14,18] MetaGRS was also associated with AIS recurrence compared with recurrence-free AIS (adjusted OR 1.18 [95% CI: 1.00–1.39, p=0.044]; Table 3 and Figure S3). MetaGRS showed stronger association when comparing recurrent AIS with AIS-free controls (adjusted OR 1.37 [95% CI: 1.18–1.59, $p=5.35\times10^{-5}$]; Table S12).

The contribution of the metaGRS and traditional risk factors showed an AIS prediction accuracy with an R^2 value of 0.06 and an AUC of 0.689 after constructing the baseline model using age, sex, the first 10 PCs, and seven risk factors. The incremental AUCs were 0.0087 and 0.0123 for AIS and AIS recurrence, respectively when metaGRS was added to the baseline model (Table 3). In our dataset, clinical risk factors (including hypertension) were related to AIS diagnosis but were insignificantly associated with AIS recurrence (Table S1).

We assessed the prediction performance of previously developed PRSs for AIS and AIS recurrence in our dataset. After matching with the BBJ2 dataset (Supplementary Methods), 27, 84, and 5,756,652 variants remained in PRS_{32}, PRS_{89}, and iPGS, respectively. We

confirmed their association with AIS diagnosis; adjusted ORs were 1.11 [95% CI: 1.06–1.17, $p=4.23\times10^{-5}$], 1.08 [95% CI: 1.03–1.14, $p=2.96\times10^{-3}$], and 1.26 [95% CI: 1.20–1.33, $p=1.24\times10^{-17}$] for PRS_{32}, PRS_{89}, and iPGS, respectively (Table 3, Figure S3); however, a significant association was not observed between PRSs and AIS recurrence (p-values of 0.41, 0.054, and 0.37, respectively; Table 3 and Figure S3). Our Japanese optimized metaGRS was the only PRS significantly associated with AIS recurrence in this study.

Analyzing for potential index event bias

We observed the values of covariates at the AIS-free control group and any-AIS case group in each quintile. We did not find any significant heterogeneous relationships between the covariates and the metaGRS in terms of regression estimates in the prevalent case and control samples ($p>0.05$, Table S13).

We used three different variable models—i) age and sex; ii) age, sex, and seven risk factors; and iii) age, sex, the first 10 PCs, and seven risk factors—to determine the association between metaGRS and recurrent AIS; none of these confounders significantly influenced our results (Figure S4).

We compared the association results of IPW adjusted (accounting for index event bias) with those of non-adjusted IPW (accounting for confounding bias). The results remained almost unchanged, but the 95% confidence intervals overlapped (Figure S5). A comparison of the three distinct models did not indicate an effect of index event bias.

Association of metaGRS and AIS recurrence in patients with/without hypertension

We divided the test sample into subgroups according to the presence or absence of a history of hypertension and evaluated the risk effect of the metaGRS tertile. The high metaGRS group without a history of hypertension showed a higher risk effect for AIS recurrence

compared to the low metaGRS group (OR of the high metaGRS group compared to that of the low metaGRS group was 2.24 [95% CI: 1.07–4.66, p=0.032], Figure 2 and Table S14). However, no significant association was observed between the metaGRS and AIS recurrence in the group with a history of hypertension (the OR of the high metaGRS group compared to that of the low metaGRS group was 1.21 [95% CI: 0.69–2.13, p=0.50] (Figure 2 and Table S14).

Discussion

We successfully examined the association between recurrent AIS and our best model PRS (metaGRS using LDpred2); the adjusted OR was 1.18 for each unit of SD increase in PRS. Our metaGRS showed stronger (adjusted OR per SD=1.37) association when comparing recurrent AIS with AIS-free controls. Furthermore, a high PRS was associated with AIS recurrence particularly in groups without a history of hypertension (OR of the top vs. bottom metaGRS tertile=2.24). These results are consistent with the result of a previous study wherein the stroke prediction accuracy of the PRS was high in the group with low CHA2DS2-VASc scores.[12] These results indicate the utility of the PRS in developing more precise strategies to prevent AIS recurrence in individuals with a high PRS who do not have high profiles based on clinical risk factors.

We attempted to mitigate potential index event bias since our purpose was to specifically determine the efficacy of PRS among AIS patients. It is difficult to predict and provide an accurate assessment of recurrent AIS based on genetic predisposition owing to the possible effect of index event bias leading to a distorted association in studies on recurrent stroke.[19,20,53] This study found no evidence of heterogeneous associations between covariates and the metaGRS; we did not find any evidence of a solid collider bias of known variables. By applying IPW, we confirmed that our results support the association between metaGRS and recurrent AIS.

There are three putative reasons our metaGRS could predict AIS recurrence. First, the metaGRS algorithm combines the genetic profiles of related traits and slightly improves the performance, reaching the level of significance. Second, the performances of PRS-CS (incremental R^2=0.00441) and LDpred2 (incremental R^2=0.00443) in our validation analysis were better than those of other traditional PRS methods, such as P+T (incremental R^2=0.0038) . This demonstrated the importance of using shrinkage estimation methods that

consider LD to predict AIS and AIS recurrence. Third, we restricted to use only single matched ancestry throughout.

Nevertheless, our study had several limitations. First, the sample size for recurrent AIS needs to be increased (n=174 at testing), even in the largest hospital-based biobank in Japan. Compared to our metaGRS, iPGS constructed in GIGASTROKE showed a stronger association for AIS and weaker association for AIS recurrence. Although potential discrepancies exist, both PRSs (metaGRS and iPGS) exhibit the same direction of effects and have overlapping confidence intervals (Table 3, Figure S3). Second, despite using as many covariates (age, sex, the first 10 PCs, and seven risk factors (hypertension, hyperlipidemia, diabetes mellitus, smoking, vascular disease, congestive heart failure, and atrial fibrillation)) as possible based on a previous study,[12] other confounders might have affected our results. Finally, there may have been an index event bias that was not fully detected by each method that we implemented; however this risk was minimized using multiple approaches. Further studies using different sample sets (including other ancestry groups) are warranted to confirm the prediction of recurrent stroke using the PRS.

In conclusion, our study indicated that PRS can be applied to predict AIS recurrence in addition to traditional clinical risk factors. This shows the potential utility of PRS in population-based screening and in the clinical setting. Overall, our results indicate that stratifying high-risk groups for recurrent stroke among those who have experienced a stroke could be medically beneficial and help in developing personalized strategies for recurrence prevention. Our results suggest that it might be particularly useful in patients with AIS without hypertension, although this requires confirmation in independent datasets.

Acknowledgments

We want to acknowledge all the participants and investigators of BioBank Japan. Supercomputing resources were provided by the Human Genome Center, Institute of Medical Science, and the University of Tokyo (http://sc.hgc.jp/shirokane.html). The ToMMo summary statistics were derived from jMorp (https://jmorp.megabank.tohoku.ac.jp/gwas-studies/TGA000007). The MEGASTROKE project received funding from sources specified at https://www.megastroke.org/acknowledgements.html

Data availability

The weights of metaGRS derived in this study will be publicly available after acceptance. Genotype datasets were deposited in the National Bioscience Database Center Human Database (BBJ1, Research ID: hum0014; BBJ2, Research ID: hum0311).

Source of Funding

This research was supported by the Ministry of Education, Culture, Sports, Sciences and Technology (MEXT) of the Japanese government and the Japan Agency for Medical Research and Development (AMED) under grant nos. JP18km0605001/ JP23tm0624002 (the BioBank Japan project), JP223fa627011 (Y.K.), and JP23tm0524003 (Y.K.).

Disclosures

Y.K. holds stock of StaGen Co, Ltd.

Supplementary Material

Supplementary Methods

Supplementary Notes

Tables S1–S15

Figures S1–S5

References

1. Ministry of Health, L. and W. Vital Statistics of Japan, https://www.mhlw.go.jp/toukei/saikin/hw/jinkou/kakutei20/index.html. (2020).
2. O'Donnell, M. J. *et al.* Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. *The Lancet* **388**, 761–775 (2016).
3. Hata, J. *et al.* Ten year recurrence after first ever stroke in a Japanese community: The Hisayama study. *J Neurol Neurosurg Psychiatry* **76**, 368–372 (2005).
4. Takashima, N. *et al.* Long-term survival after stroke in 1.4 million japanese population: Shiga stroke and heart attack registry. *J Stroke* **22**, 336–344 (2020).
5. MacArthur, J. *et al.* The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Res* **45**, D896–D901 (2017).
6. Torkamani, A., Wineinger, N. E. & Topol, E. J. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet* **19**, 581–590 (2018).
7. Khera, A. v. *et al.* Whole-Genome Sequencing to Characterize Monogenic and Polygenic Contributions in Patients Hospitalized With Early-Onset Myocardial Infarction. *Circulation* **139**, 1593–1602 (2019).
8. Konuma, T. & Okada, Y. Statistical genetics and polygenic risk score for precision medicine. *Inflamm Regen* **41**, (2021).
9. Levin, M. G. & Rader, D. J. Polygenic Risk Scores and Coronary Artery Disease. *Circulation* 637–640 (2020) doi:10.1161/CIRCULATIONAHA.119.044770.
10. Plagnol, V. Polygenic score development in the era of large-scale biobanks. *Cell Genomics* **2**, 100088 (2022).
11. Khera, A. v. *et al.* Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nature Genetics* vol. 50 1219–1224 Preprint at https://doi.org/10.1038/s41588-018-0183-z (2018).
12. Marston NA *et al.* Clinical Application of A Novel Genetic Risk Score Predicts Ischemic Stroke in Patients With Cardiometabolic Disease. *Circulation. 2021 Feb 2;143(5):470-478. doi: 10.1161/CIRCULATIONAHA.120.051927. Epub 2020 Nov 13. PMID: 33185476; PMCID: PMC7856243.* (2020).
13. Rutten-Jacobs, L. C. A. *et al.* Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: Cohort study of 306 473 UK Biobank participants. *BMJ (Online)* **363**, 1–8 (2018).
14. Abraham, G. *et al.* Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke. *Nat Commun* **10**, 1–10 (2019).
15. Thomas, E. A. *et al.* Polygenic Risk, Midlife Life's Simple 7, and Lifetime Risk of Stroke. *J Am Heart Assoc* **11**, (2022).
16. Hachiya, T. *et al.* Genetic Predisposition to Ischemic Stroke: A Polygenic Risk Score. *Stroke* **48**, 253–258 (2017).
17. Hachiya, T. *et al.* Genome-wide polygenic score and the risk of ischemic stroke in a prospective cohort: The Hisayama study. *Stroke* 759–765 (2020)

doi:10.1161/STROKEAHA.119.027520.

18. Mishra, A. *et al.* Stroke genetics informs drug discovery and risk prediction across ancestries. *Nature* (2022) doi:10.1038/s41586-022-05165-3.
19. Howe, L. J. *et al.* Polygenic risk scores for coronary artery disease and subsequent event risk amongst established cases. *Hum Mol Genet* **29**, 1388–1395 (2020).
20. Dahabreh, I. J. & Kent, D. M. *Index Event Bias as an Explanation for the Paradoxes of Recurrence Risk Research*. https://jamanetwork.com/.
21. Ni, G. *et al.* A Comparison of Ten Polygenic Score Methods for Psychiatric Disorders Applied Across Multiple Cohorts. *Biol Psychiatry* **90**, 611–620 (2021).
22. Hahn, G. *et al.* A Smoothed Version of the Lassosum Penalty for Fitting Integrated Risk Models Using Summary Statistics or Individual-Level Data. *Genes (Basel)* **13**, (2022).
23. Osterman, M. D., Kinzy, T. G. & Bailey, J. N. C. Polygenic Risk Scores. *Curr Protoc* **1**, (2021).
24. Zhao, Z., Song, J., Wang, T. & Lu, Q. Polygenic risk scores: effect estimation and model optimization. *Quantitative Biology* **0**, 0 (2021).
25. Ma, Y. & Zhou, X. Genetic prediction of complex traits with polygenic scores: a statistical review. *Trends in Genetics* **37**, 995–1011 (2021).
26. Pain, O. *et al.* Evaluation of polygenic prediction methodology within a reference-standardized framework. *PLoS Genet* **17**, 1–22 (2021).
27. Zhang, Q., Privé, F., Vilhjálmsson, B. & Speed, D. Improved genetic prediction of complex traits from individual-level data or summary statistics. *Nat Commun* **12**, 1–9 (2021).
28. Yang, S. & Zhou, X. PGS-server: accuracy, robustness and transferability of polygenic score methods for biobank scale studies. *Brief Bioinform* **23**, 1–19 (2022).
29. Wang, Y., Tsuo, K., Kanai, M., Neale, B. M. & Martin, A. R. Challenges and opportunities for developing more generalizable polygenic risk scores. 293–320 (2022).
30. Kulm, S., Marderstein, A., Mezey, J. & Elemento, O. A systematic framework for assessing the clinical impact of polygenic risk scores. *medRxiv preprint doi: https://doi.org/10.1101/2020.04.06.20055574* (2021) doi:10.1101/2020.04.06.20055574.
31. Nagai, A. *et al.* Overview of the BioBank Japan Project: Study design and profile. *J Epidemiol* **27**, S2–S8 (2017).
32. Hirata, M. *et al.* Cross-sectional analysis of BioBank Japan clinical data: A large cohort of 200,000 patients with 47 common diseases. *J Epidemiol* **27**, S9–S21 (2017).
33. Hirata, M. *et al.* Overview of BioBank Japan follow-up data in 32 diseases. *J Epidemiol* **27**, S22–S28 (2017).
34. Chang, C. C. *et al.* Second-generation PLINK: Rising to the challenge of larger and richer datasets. *Gigascience* **4**, 1–16 (2015).
35. Purcell, S. *et al.* PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559–575 (2007).
36. Marees, A. T. *et al.* A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. *Int J Methods Psychiatr Res* **27**, 1–10 (2018).
37. Choi, S. W., Mak, T. S. H. & O'Reilly, P. F. Tutorial: a guide to performing polygenic risk score analyses. *Nat Protoc* **15**, 2759–2772 (2020).
38. Koyama, S. *et al.* Population-specific and trans-ancestry genome-wide analyses identify distinct and shared genetic risk loci for coronary artery disease. *Nat Genet* **52**, 1169–1177 (2020).
39. Privé, F., Arbel, J. & Vilhjálmsson, B. J. LDpred2: Better, faster, stronger.

Bioinformatics **36**, 5424–5431 (2020).

40. Privé, F., Arbel, J., Aschard, H. & Vilhjálmsson, B. J. Identifying and correcting for misspecificationsin GWAS summary statistics and polygenic scores. *Human Genetics and Genomics Advances* 100136 (2022) doi:10.1016/j.xhgg.2022.100136.
41. Ge, T., Chen, C. Y., Ni, Y., Feng, Y. C. A. & Smoller, J. W. Polygenic prediction via Bayesian regression and continuous shrinkage priors. *Nat Commun* **10**, 1–10 (2019).
42. Ruan, Y. *et al.* Improving polygenic prediction in ancestrally diverse populations. *Nat Genet* (2022) doi:10.1038/s41588-022-01054-7.
43. Malik, R. *et al.* Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet* **50**, 524–537 (2018).
44. Nagelkerke N. J. D. A Note on a General Definition of the Coefficient of Determination. *Biometrika* **78**, 691–692 (1991).
45. Wang, Y. *et al.* Global Biobank analyses provide lessons for developing polygenic risk scores across diverse cohorts. *Cell Genomics* **3**, (2023).
46. Hindy, G. *et al.* Genome-wide polygenic score, clinical risk factors, and long-term trajectories of coronary artery disease. *Arterioscler Thromb Vasc Biol* 2738–2746 (2020) doi:10.1161/ATVBAHA.120.314856.
47. Mitchell, R. E. *et al.* Strategies to investigate and mitigate collider bias in genetic and Mendelian randomisation studies of disease progression. *PLoS Genetics* vol. 19 Preprint at https://doi.org/10.1371/journal.pgen.1010596 (2023).
48. Sep, S. J., Van Kuijk, S. M. & Smits, L. J. Index event bias: Problems with eliminating the paradox. *Journal of Stroke and Cerebrovascular Diseases* vol. 23 2464 Preprint at https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.06.025 (2014).
49. Levine, D. A. *et al.* Smoking and mortality in stroke survivors: Can we eliminate the paradox? *Journal of Stroke and Cerebrovascular Diseases* **23**, 1282–1290 (2014).
50. Altman, D. G. & Bland, J. M. Interaction revisited: The difference between two estimates. *BMJ* **326**, 219 (2003).
51. Ishigaki, K. *et al.* Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. *Nat Genet* **52**, 669–679 (2020).
52. Bulik-Sullivan, B. *et al.* LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* **47**, 291–295 (2015).
53. Cho, S. M. J. *et al.* Genetic, sociodemographic, lifestyle, and clinical risk factors of recurrent coronary artery disease events: a population-based cohort study. *Eur Heart J* (2023) doi:10.1093/eurheartj/ehad380.
54. Weale, E. M. Quality control for genome-wide association studies. *Methods Mol Biol* (2010) doi:10.1007/978-1-60327-367-1_19.
55. Akiyama, M. *et al.* Characterizing rare and low-frequency height-associated variants in the Japanese population. *Nat Commun* **10**, (2019).
56. Privé, F., Vilhjálmsson, B. J., Aschard, H. & Blum, M. G. B. Making the Most of Clumping and Thresholding for Polygenic Scores. *Am J Hum Genet* **105**, 1213–1221 (2019).
57. Chagnon, M., O'Loughlin, J., Engert, J. C., Karp, I. & Sylvestre, M. P. Missing single nucleotide polymorphisms in Genetic Risk Scores: A simulation study. *PLoS One* **13**, 1–14 (2018).
58. Goldstein, B. A., Yang, L., Salfati, E. & Assimes, T. L. Contemporary Considerations for Constructing a Genetic Risk Score: An Empirical Approach. *Genet Epidemiol* **39**, 439–445 (2015).
59. Wang, Y. *et al.* Global Biobank analyses provide lessons for developing polygenic risk

scores across diverse cohorts. *Cell Genomics* **3**, (2023).

60. Tadaka, S. *et al.* jMorp updates in 2020: Large enhancement of multi-omics data resources on the general Japanese population. *Nucleic Acids Res* **49**, D536–D544 (2021).
61. Restuadi, R. *et al.* Polygenic risk score analysis for amyotrophic lateral sclerosis leveraging cognitive performance, educational attainment and schizophrenia. *European Journal of Human Genetics* (2021) doi:10.1038/s41431-021-00885-y.
62. Turley, P. *et al.* Multi-trait analysis of genome-wide association summary statistics using MTAG. *Nat Genet* **50**, 229–237 (2018).
63. Maier, R. M. *et al.* Improving genetic prediction by leveraging genetic correlations among human diseases and traits. *Nat Commun* **9**, 1–17 (2018).
64. Ho, W. K. *et al.* European polygenic risk score for prediction of breast cancer shows similar performance in Asian women. *Nat Commun* **11**, 1–11 (2020).
65. Amariuta, T. *et al.* Improving the trans-ancestry portability of polygenic risk scores by prioritizing variants in predicted cell-type-specific regulatory elements. *Nat Genet* **52**, 1346–1354 (2020).

Tables

Table 1. Characteristic of the testing sample (BBJ2)

	Any-AIS versus AIS-free controls				Recurrent AIS versus non-recurrent AIS			
	N sample (%, SD)	OR	95% CI	p-value	N sample (%, SD)	OR	95% CI	p-value
Total participants	41,929 (100%)	-	-	-	1,327 (100%)	-	-	-
AIS / AIS recurrent	1,470 (3.5%)	-	-	-	174 (13.1%)	-	-	-
Age (SD)*	70.0 (SD=12.6)	1.18	[1.06–1.31]	0.002	64.1 (SD=11.5)	0.83	[0.59–1.16]	0.256
Female sex	19,407 (46.3%)	0.48	[0.43–0.54]	<0.001	383 (28.9%)	0.63	[0.42–0.93]	0.019
Hypertension	17,710 (42.2%)	2.27	[2.04–2.53]	<0.001	838 (63.1%)	0.92	[0.66–1.3]	0.673
Hyperlipidemia	11,604 (27.7%)	2.17	[1.95–2.41]	<0.001	603 (45.4%)	0.92	[0.66–1.29]	0.625
Diabetes	3,622 (8.6%)	1.16	[0.97–1.39]	0.089	125 (9.4%)	0.97	[0.52–1.7]	1.000
Smoking**	21,570 (51.9%)	1.43	[1.28–1.59]	<0.001	795 (60.5%)	1.28	[0.90–1.82]	0.179
Vascular disease	3,136 (7.5%)	1.31	[1.08–1.56]	0.005	122 (9.2%)	1.08	[0.59–1.87]	0.778
Heart failure	1,329 (3.2%)	1.27	[0.95–1.66]	0.095	48 (3.6%)	1.79	[0.78–3.75]	0.124
Atrial fibrillation	1,004 (2.4%)	2.22	[1.71–2.84]	<0.001	65 (4.9%)	1.07	[0.46–2.23]	0.850

All risk factor characteristics were derived from history and not at the time of registration. Odds ratio (OR), 95% CI (95% confidence intervals, and p-values were calculated using logistic regression for onset and AIS recurrence (unadjusted for other factors). *Age at AIS

588 case-control was that at recruitment, while age at recurrent AIS case-control was that at first
589 incidence. The numbers indicate the median threshold age. ** The total number of missing
590 values of smoking was 389 for all case-control and 12 for recurrent AIS case-control
591 samples. Abbreviations: BBJ2, BioBank 2nd cohort; AIS, all ischemic stroke; SD = standard
592 deviation
593

Table 2. Polygenic risk score performance at validation

Method	Best parameters	Mean number of variants	Best mean incremental Nagelkerke R^2	Standard deviation	95% confidence interval
P+T	Clumping R^2=0.95, Clumping kb=526, Imputation R^2=0.8, p-value threshold=1	3,144,737	0.0038	0.0012	0.0030–0.0046
LDpred2	ρ value=0.0056, heritability value x 1.0, no_sparse	898,456	0.00443	0.0013	0.0035–0.0054
Lassosum2	S=0.9, lambda=0.00388	282,520	0.0039	0.0013	0.0030–0.0048
PRS-CS	Phi=1.00E-04	985,439	0.00441	0.0012	0.0036–0.0053
PRS-CSx	Phi=1.00E-05	1,016,745	0.0037	0.0008	0.0031–0.0042

598 **Table 3. Polygenic risk score performance at testing**

599

PRS Method	Association tests	OR per SD [95% CI]	p-value	Incre-mental AUC	Incremental Nagelkerke R^2
MetaGRS	AIS	1.21[1.15–1.27]	2.89E-12	0.0087	0.0044
	Recurrence	1.18[1.00–1.39]	0.044	0.0123	0.0057
MEGASTROKE 27 SNVs	AIS	1.11[1.06–1.17]	4.23E-05	0.0033	0.0015
	Recurrence	1.07[0.91–1.25]	0.41	0.0032	0.0009
GIGASTROKE 84 SNVs	AIS	1.08[1.03–1.14]	2.96E-03	0.0022	0.0008
	Recurrence	1.17[1.00–1.38]	0.054	0.0125	0.0052
GIGASTROKE iPGS	AIS	1.26[1.20–1.33]	1.24E-17	0.0130	0.0066
	Recurrence	1.08[0.91–1.27]	0.37	0.0044	0.0011

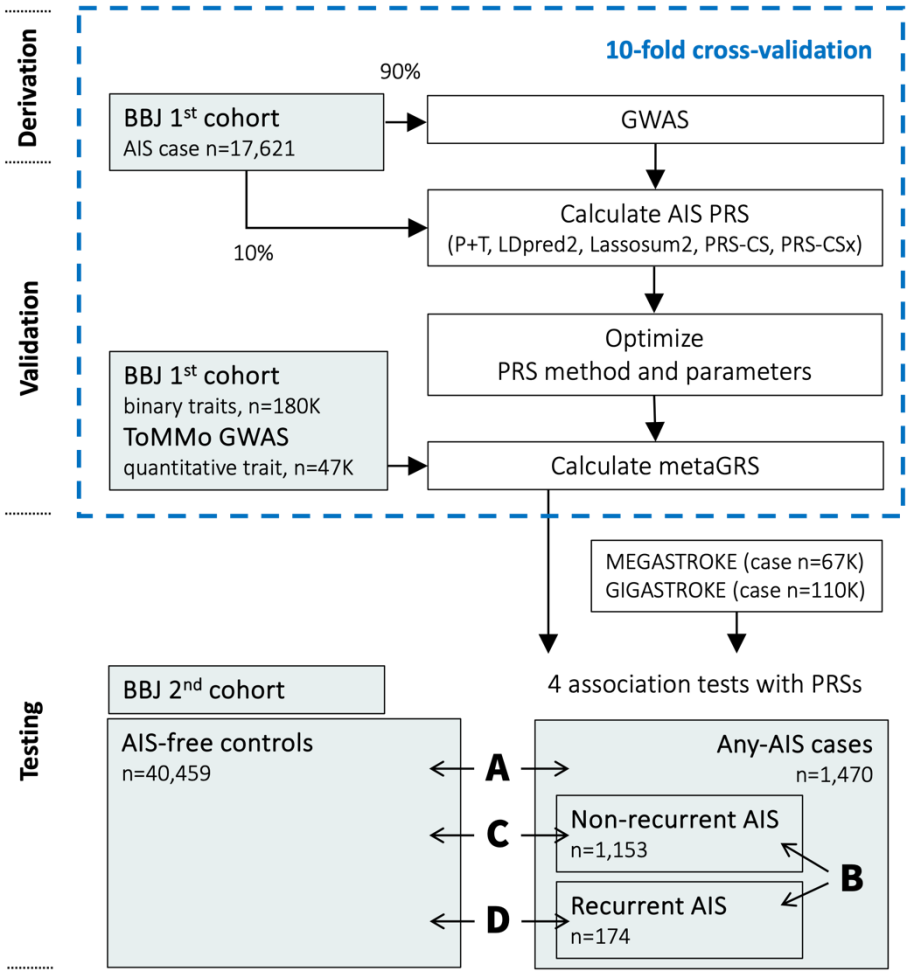
600

601 Polygenic risk score performance was evaluated using an independent testing set for AIS and
602 recurrent AIS. We showed two main association tests; AIS (any-AIS cases vs. AIS-free
603 controls) and AIS recurrence (recurrent AIS vs. non-recurrent AIS). Incremental AUC and R^2
604 are the differences in the values when fitting with/without PRS, along with age, sex, the first
605 10 PCs, and seven risk factors. Abbreviations: AIS, all ischemic strokes; OR, odds ratio;
606 AUC, area under the curve; PC, principal components; PRS, polygenic risk score; SD =
607 standard deviation

608

609 **Figure Legends**

610 **Figure 1. Workflow**



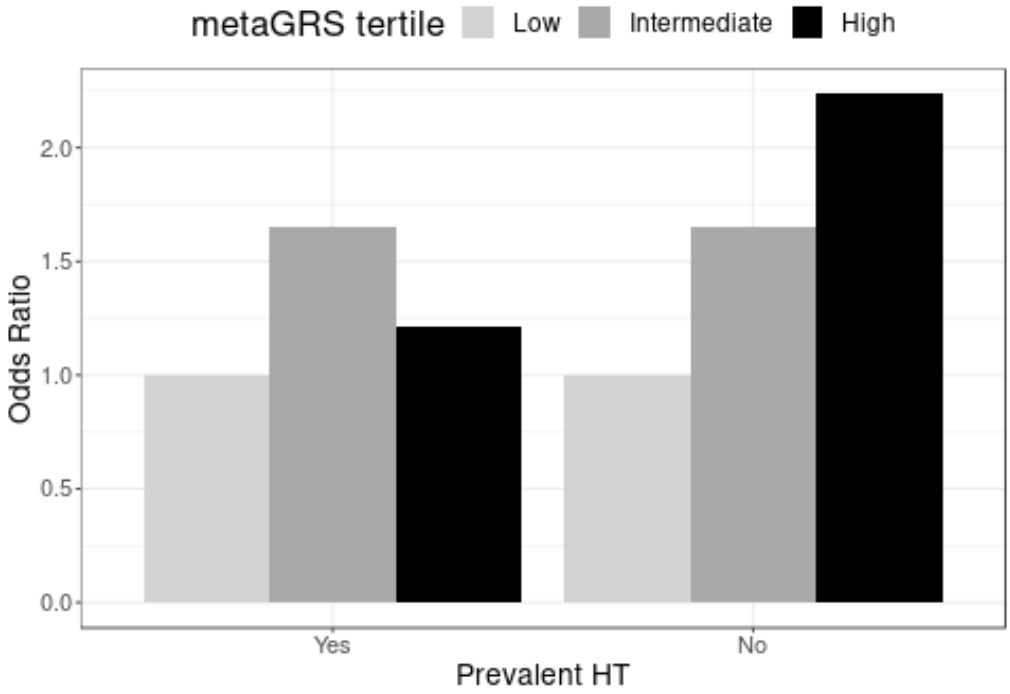
611

612 MEGASTROKE AIS summary statistics of European (EUR) studies were only used for
613 PRS-CSx. 1000 Genomes Project super population samples (EAS or EUR) were used for the
614 LD reference panel. Abbreviations: GWAS = genome-wide association study, PRS =
615 polygenic risk score, P+T = pruning and thresholding, OR = odds ratio, AIS = all ischemic
616 stroke, ToMMo = Tohoku Medical Megabank; LD, linkage disequilibrium.

617 We used a logistic regression model to assess the association of the PRS using the two case-
618 control settings for AIS (**A**: any-AIS vs. AIS-free controls) and AIS recurrence (**B**: recurrent
619 AIS vs. non-recurrent AIS). We also applied two other combinations of case-controls (**C**:
620 recurrent versus AIS-free controls and **D**: non-recurrent versus AIS-free controls).

621

622 **Figure 2. Odds ratio of metaGRS tertiles with/without a history of hypertension**



623

624

625 Association of AIS recurrence and meta-GRS tertiles with or without a history of
626 hypertension (HT) in the testing sample, with reference to the low metaGRS tertiles.

627

628

629

630

631

632

633

SUPPLEMENTARY MATERIALS

Recurrent stroke prediction by applying a stroke polygenic risk score in the Japanese population

Supplementary Methods

Samples

BioBank Japan (BBJ) collected DNA, serum, and medical records (clinical information) with consent from patients. The BBJ 1st cohort (BBJ1) dataset contained all ischemic stroke (AIS, n=17,621) cases, including large artery stroke (LAS, n=981), small vessel stroke (SVS, n=3,108), and cardioembolic stroke (CES, n=608) cases. The patients without AIS were included as controls (n=162,317).

Testing involved the use of data from part of the BBJ 2nd cohort (BBJ2) dataset, which contains information about AIS cases (n=1,470), including LAS (n=268), SVS (n=508), CES (n=122), and transient ischemic attack (TIA, n=105). All patients without AIS were included as controls (n=40,459). Among these cases, recurrent ischemic stroke (n=187) was used as a case of recurrent AIS, which included LAS (n=40), SVS (n=57), CES (n=11), and transient ischemic attack (TIA, n=20). Samples with information on the first onset date and follow-up of under 30 days were excluded; AIS cases with recurrence (n=174) were set as the case group, and AIS cases without recurrence (n=1,153) remained in the control group in the testing sample.

Quality control and imputation process of BBJ1 data

We removed variants with call rates <0.99, samples with call rates <0.98, non-East Asian samples, and sex-discordant samples. We used 939 samples whose genotypes were analyzed using whole-genome sequencing (WGS); we added an additional quality control based on the concordance rate between the genotyping array and WGS. We excluded variants with concordance rates <99.5% or non-reference discordance rates ≥0.5% and Hardy-Weinberg equilibrium (HWE) (p<1e-6). The 10 principal components (PCs) were calculated by

668 applying additional quality control (QC) to the BBJ1 genotyped data. We removed samples
669 from close relatives (King's cutoff >0.0884), and 24 long LD regions,[54] including the MHC
670 region (chr6, position 25,000,000-35,000,000), and MAF<0.01. We pruned (PLINK2
671 parameters: --indep-pairwise 200 50 0.20) and finally used 92,231–92,303 variants to
672 conduct projection and calculate the first 10 PCs depending on a 10-fold sample set of the
673 90% BBJ1 dataset.
674 Subsequently, the datasets were phased (Eagle v2.4.1) and imputed (Minimac4 v1.0.2) using
675 the developed panel.[55] We further conducted quality control to remove variants with minor
676 allele counts <10, close relatives (King cutoff >0.0884), and imputation r-square <0.8.
677

678 **Quality control and imputation process for BBJ2 data**

679 We removed samples with no age/sex information, sex discrepancy, call rates <0.98,
680 heterozygosity rates with SD >4 or <- 4, from duplicate or twins (pi-hat >= 0.75), and from
681 non-East Asian subjects. We then removed variants with a call rate <0.99, duplicate SNPs,
682 heterozygous count <5, HWE (p<1e-6), and allele frequency discrepancies (gap from 1000
683 genomes EAS >0.16). A total of 41,929 samples and 525,239 variants were analyzed.
684 We applied additional quality control to the BBJ2 genotyped data to calculate the 10 PCs. We
685 removed variants in 24 long LD regions,[54] pruned them (PLINK2 parameters: --indep-
686 pairwise 200 50 0.05), extracted close relatives (King cut-off >0.0884), and used 69,068
687 variants to calculate the first 10 PCs.
688 We removed variants of the imputation r-squared <0.3 after phasing (Eagle v2.4.1) and
689 imputing (Minimac4 v1.0.2) the developed panel.[55] We used a lower r-square threshold for
690 BBJ2 to reduce the number of variants unmatched with BBJ1.
691

692 **Polygenic risk score parameters at the derivation**

Pruning and threshold used a total of 1,224 parameter combinations of three stricter imputation r-squared score thresholds {0.8, 0.9, and 0.95}, four base sizes of the clumping window {50, 100, 200, and 500}, six squared correlations of clumping {0.01, 0.05, 0.2, 0.5, 0.8, and 0.95}, and 17 p-value threshold {1e-8, 3e-8, 1e-7, 3e-7, 1e-6, 3e-6, 1e-5, 3e-5, 1e-4, 3e-4, 1e-3, 3e-3, 0.01, 0.03, 0.1, 0.3, and 1}. We used clumping windows and divided the base size by the squared correlation of clumping.[56–59] The following default parameters were used for LDpred2: three heritability {0.7, 1, and 1.4}×h^2_{LDSC}, where h^2_{LDSC} is the heritability estimate from the constrained LD score regression,[52] 21 ρ estimates {equally spaced on a log scale between 1e-5 and 1}, and sparse or not. The following default parameters were used for Lassosum2: 10 values of s {0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0} and 20 values of λ {equally spaced on a log scale between 0.1 and 0.001}. Regarding PRS-CS and PRS-CSx, we used slightly more extended parameters: phi {1e-7, 1e-6, 1e-5, 1e-4, 1e-3, 0.01, 0.1, 1, and auto}, than the default parameters {1e-4, 1e-3, 0.01, 0.1, 1, and auto}, because the optimized parameter in the initial trials using our dataset was the smallest (phi=1e-4) in the default range. Other parameters were set to default values (a=1, b=0.5).[41,42]

Linkage disequilibrium (LD) reference

We used the EAS superpopulation of the 1000 Genomes panel (n=504) as a LD reference for P+T clumping. For Ldpred2, Lassosum2, PRS-CS, and PRS-CSx models, we restricted our use of external LD reference panels to the HapMap 3 variants, which were also constructed from 1000 Genomes EAS. HapMap 3 variant restriction resulted in 898,456, 898,456, 985,440, and 1,076,835 variants of Ldpred2, Lassosum2, PRS-CS, and PRS-CSx, respectively. To use PRS-CSx for the EUR, we used the EUR superpopulation of the 1000 Genomes panel and its HapMap3 variants (1,016,745 variants).

718 **Genome wide association study summary statistics for metaGRS construction**

719 MetaGRS was developed following a previous study[14] and included nine binary and eight
720 quantitative traits. The nine binary traits were SVS, LAS, CES, myocardial infarction (MI),
721 stable angina pectoris (SAP), unstable angina pectoris (AP), atrial fibrillation (AF), diabetes
722 (DM), and ever smoking (SM) from the BBJ1 dataset. Subsequently, we conducted GWAS.
723 The number of cases and controls is listed in Table S15. Summary statistics were obtained
724 from the jMorp website (https://jmorp.megabank.tohoku.ac.jp) and were used for eight
725 quantitative traits—body mass index (BMI), height (HE), systolic blood pressure (SBP),
726 diastolic blood pressure (DBP), total cholesterol (TC), triglyceride (TG), high-density
727 lipoprotein (HDL), and low-density lipoprotein (LDL) from the Tohoku Medical Megabank
728 Project (ToMMo; 22,033 to 47,056 samples).[60]

729

730 **Polygenic risk score calculation from other milestone studies**

731 We obtained effect weights from the PGS catalog (PGS000665 and PGS002725 of
732 https://www.pgscatalog.org for PRS_{32} and $iPGS_{EAS}$, respectively) and for PRS_{89} from the
733 supplementary tables.[18] We used variants that matched with the BBJ2 dataset (imputation r-
734 squared > 0.3). The unmatched variants in PRS_{32} and PRS_{89} included proxy variants that
735 showed the highest r-squared values with the index variants only from an r-squared value
736 greater than 0.3. The r-squared values were calculated using the plink --r2 command[35] with
737 the 1000 Genome EAS as a reference.

738

Supplementary Notes

Characteristics of the PRS methods during validation

The following characteristics of each method were observed while testing several parameters: the best mean incremental R^2 of 0.0038 was observed for the P + T method when we liberalized the p-value threshold to 1 and clumped r^2 >0.8, whereas it was below 0.001 when we used a low p-value threshold ($<10^{-8}$, Table S5). For LDpred2, ρ values higher than 0.001 showed good performance (mean incremental R^2 was 0.0038 for ρ values >=0.001 compared to 0.0016 for ρ values <0.001), while heritability estimates and sparse parameters made less difference (Table S6). For Lassosum2, the closer the value of parameter "s" is to 1, the higher the prediction accuracy, but it wasn't the case at exactly 1. Larger lambda parameters correlate with greater accuracy (mean incremental R^2 was between 0.0025 to 0.0039). However, the accuracy sharply decreases (mean incremental R^2<0.001) if the value is too small (<0.01). The prediction performance was maximized when the parameters were s=0.9 and lambda=0.0038 (Table S7). High and low phi values resulted in low performance for PRS-CS (mean incremental R^2 was 0.0041 for phi=10^{-3}, 10^{-4}, and 10^{-5} compared to 0.0029 for other phi parameters; Table S8). Meanwhile, PRS-CSx was relatively consistent regardless of the phi values (mean incremental R^2 was 0.0032 for phi=10^{-3}, 10^{-4}, and 10^{-5} compared to 0.0028 for other phi parameters; Table S9).

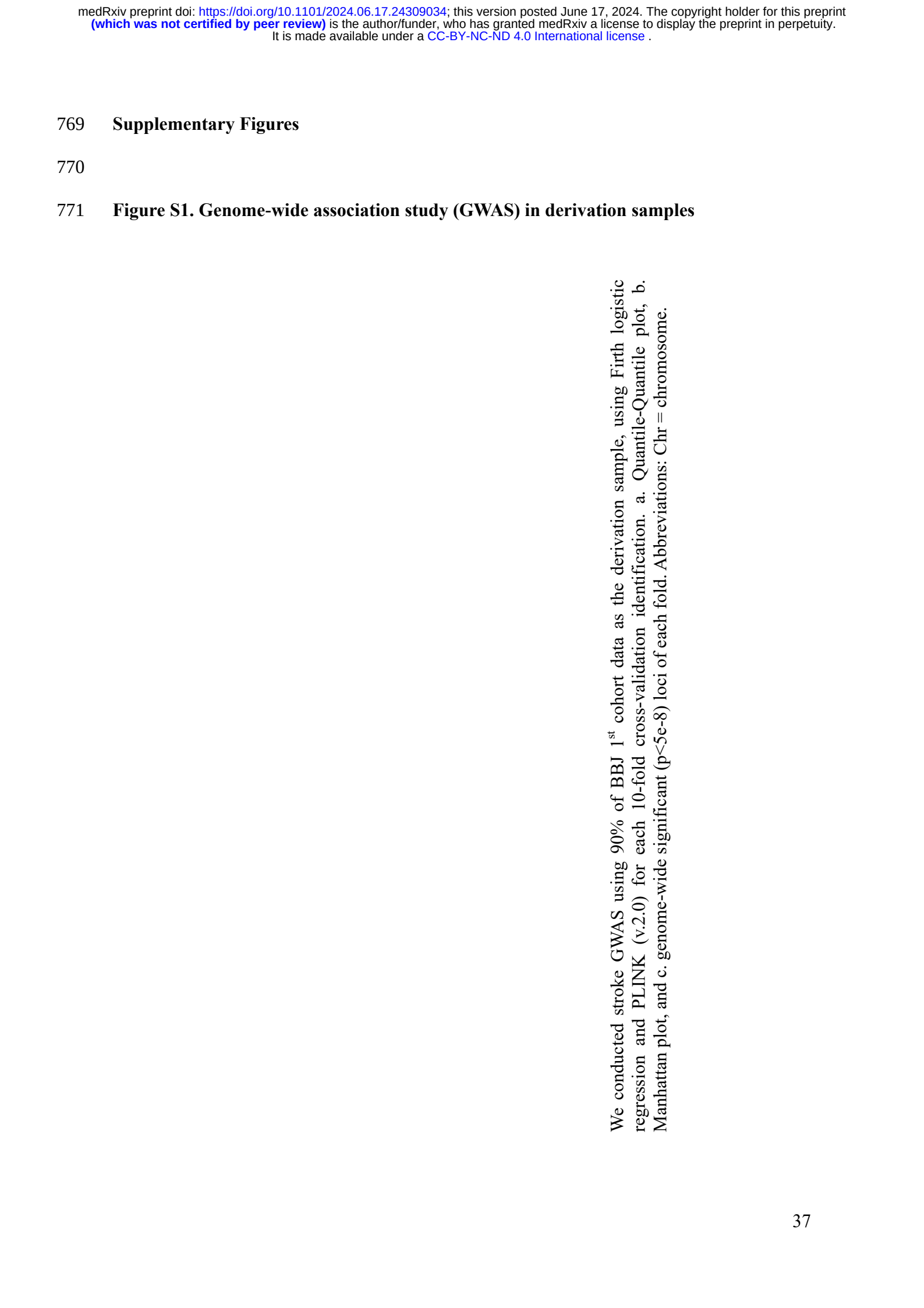
The performance of the PRS-CS (R^2=0.00441) was comparable to that of LDpred2 (R^2=0.00443) in the validation analysis. Low validation predictability (incremental R^2 < 0.001) was observed when we restricted the p-value threshold in the P+T method to genome-wide significance ($p<5\times10^{-8}$). PRS-CSx improves cross-population polygenic prediction by integrating GWAS summary statistics from other populations;[61–65] however, we could not reproduce this result in our current study using PRS-CSx. Our results demonstrate the

764 importance of using Bayesian methods of high-dimensional techniques in variable selection
765 and shrinkage estimation considering LD (such as LDpred2 and PRS-CS) to predict AIS and
766 recurrent AIS.
767
768

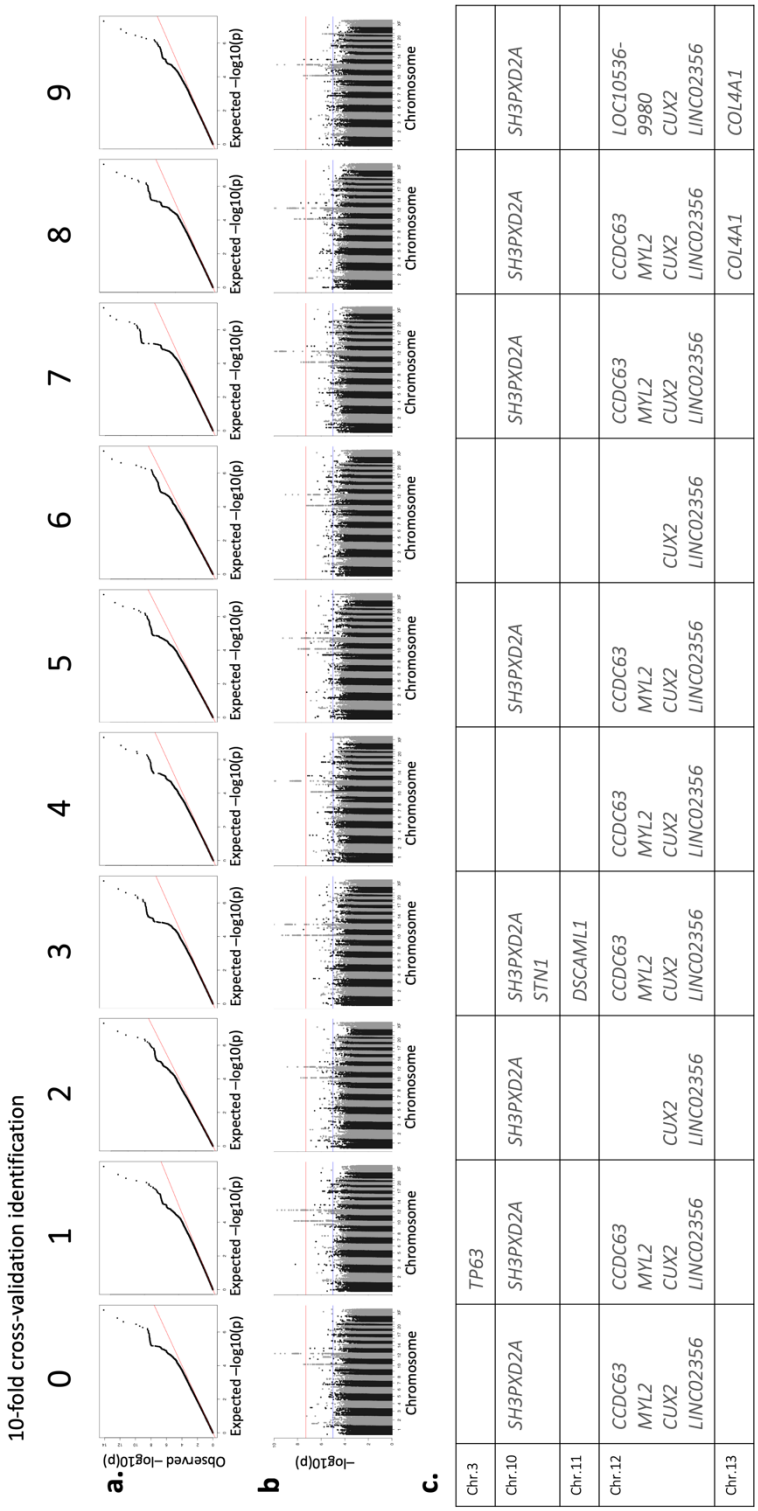
769 **Supplementary Figures**

770

771 **Figure S1. Genome-wide association study (GWAS) in derivation samples**

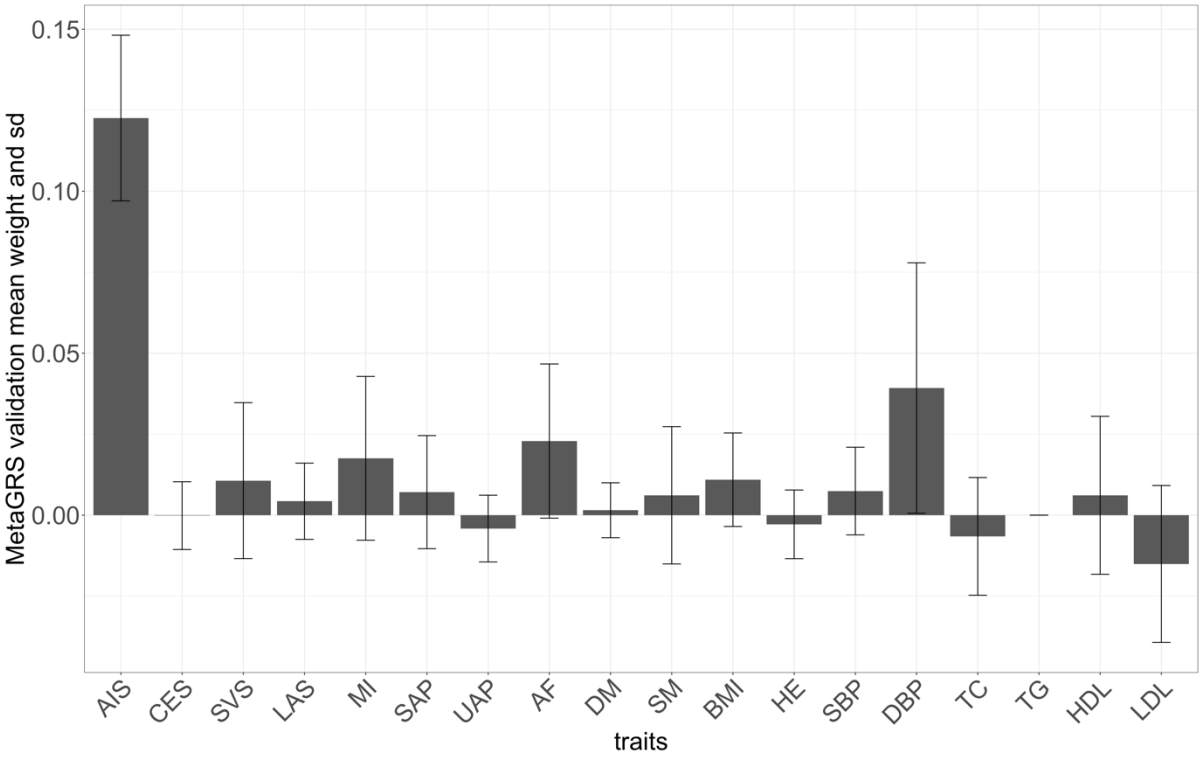


We conducted stroke GWAS using 90% of BBJ 1st cohort data as the derivation sample, using Firth logistic regression and PLINK (v.2.0) for each 10-fold cross-validation identification. a. Quantile-Quantile plot, b. Manhattan plot, and c. genome-wide significant (p<5e-8) loci of each fold. Abbreviations: Chr = chromosome.



c.

Chr.3		*TP63*								
Chr.10	*SH3PXD2A*	*SH3PXD2A*	*SH3PXD2A*	*SH3PXD2A* *STN1*		*SH3PXD2A*		*SH3PXD2A*	*SH3PXD2A*	*SH3PXD2A*
Chr.11			*DSCAML1*							
Chr.12	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*CCDC63* *MYL2* *CUX2* *LINC02356*	*LOC10536-9980* *CUX2* *LINC02356*
Chr.13									*COL4A1*	*COL4A1*



774

775

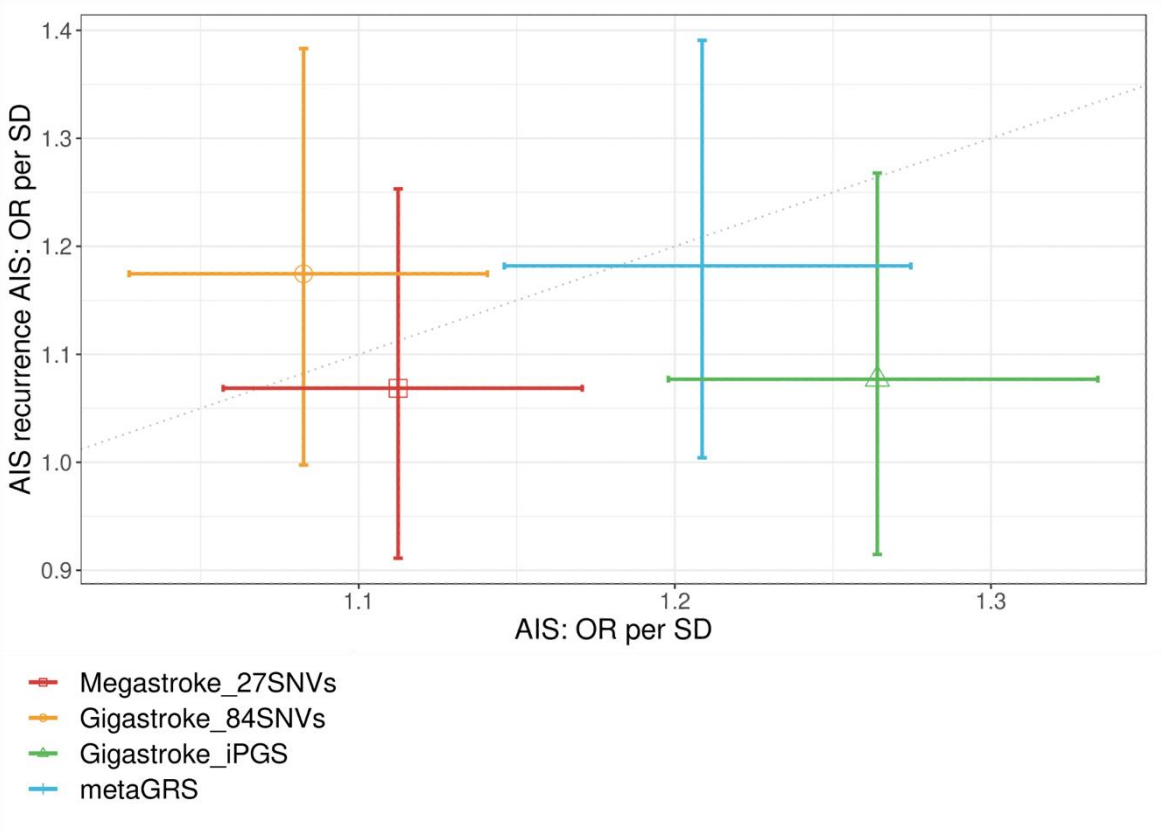
776 **Figure S2. Elastic net weight**

777 The mean weight of 10-fold elastic net regression as determined by "glmnet" in the
778 validation sample. The X-axis shows the AS and the 17 binary and quantitative traits. Error
779 bars represent standard deviations.

780 Abbreviations: AIS, all ischemic stroke; SVS, small vessel stroke; LAS, large artery stroke;
781 CES, cardioembolic stroke; MI, myocardial infarction; SAP, stable angina pectoris; UAP,
782 unstable angina pectoris; AF, atrial fibrillation; DM, diabetes; SM, smoking; BMI, body mass
783 index; HE, height; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total
784 cholesterol; TG, triglyceride; HCL, high-density lipoprotein; LDL, low-density lipoprotein;
785 SD, standard deviation.

786

787



788

789

790 **Figure S3. Odds ratio per SD to predict AIS and AIS recurrence**

791 Odds ratio per standard deviation by metaGRS and three publicly available PRS in the
792 independent test set of AIS (case=1, 470, control=40,459) and AIS recurrence case-control
793 set (case=174, control=1,153). Age, sex, the first 10 PCs, and seven risk factors were
794 adjusted. Error bars represent 95% confidence intervals. The dotted line represents the point
795 at which the ORs per SD of AIS and AIS recurrence were equal. Abbreviations: OR, odds
796 ratio; AIS, all ischemic stroke; PRS, polygenic risk score; PC, principal component; SD,
797 standard deviation.

798

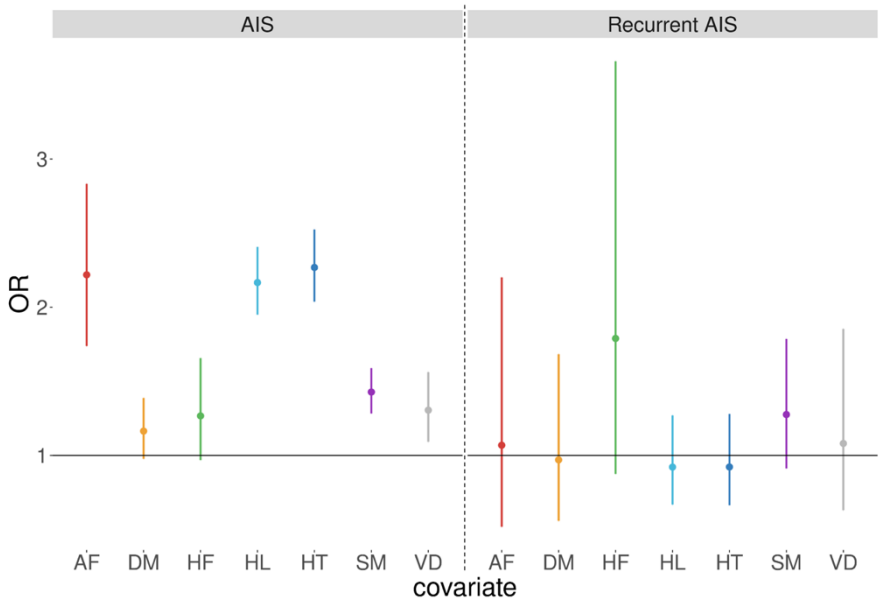


Figure S4. Association between seven comorbidities and AIS/AIS recurrence

The Y-axis shows the odds ratio per standard deviation (SD) and 95% confidence interval (CI) of the seven risk factors (six clinical comorbidities and smoking) in the BBJ 2nd cohort. In our dataset, we used HT defined as SBP>140 mmHg, DBP>90 mmHg, or hypertension history, DM inclusive of type 1 diabetes and other diabetes, SM as a current smoker at the time of registration, VD representing myocardial infarction, arteriosclerosis obliterans, stable angina pectoris, unstable angina pectoris, and AF inclusive of atrial flutter. We used the status at the time of registration and history of these comorbidities.

Abbreviations: HT, hypertension; HL, hyperlipidemia; DM, diabetes; SM, smoking; VD, vascular disease; HF, congestive heart failure; AF, atrial fibrillation.

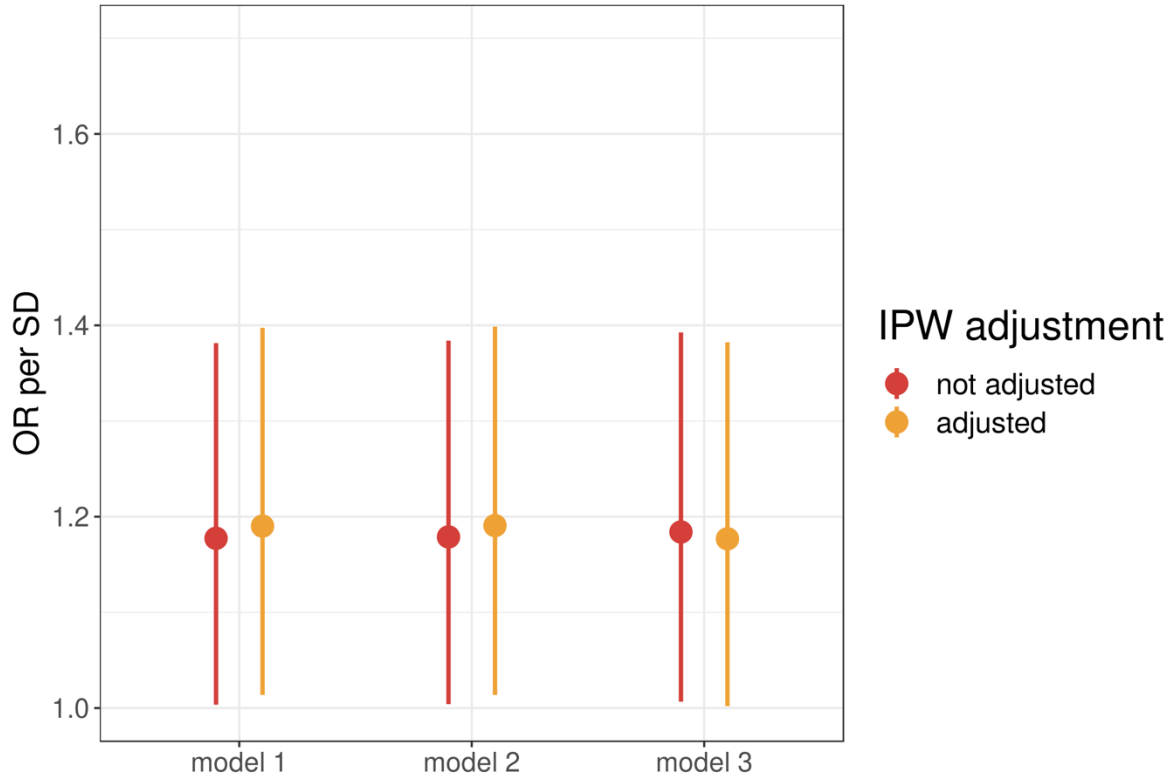


Figure S5. Association between metaGRS and AIS recurrence in patients with or without IPW adjustment

The y-axis shows the odds ratio per SD and the 95% confidence interval for predicting recurrent AIS. Model 1: age and sex; model 2: age, sex, and seven risk factors; model 3: age, sex, the first 10 principal components (PCs), and seven risk factors. The color represents whether the inverse probability weight (IPW) is adjusted. We applied logistic regression using variables as covariates when the IPW was not adjusted. We replaced 14 missing data on the smoking status to mean values.

Supplementary Tables

In separate spreadsheets S1-15

828 **Consortium authors**

829

830 **BioBank Japan**

831 Koichi Matsuda[1,2], Yuji Yamanashi[3], Yoichi Furukawa[4], Takayuki Morisaki[5], Yoshinori
832 Murakami[6], Yoichiro Kamatani[2,7], Kaori Muto[8], Akiko Nagai[8], Wataru Obara[9], Ken Yamaji[10],
833 Kazuhisa Takahashi[11], Satoshi Asai[12,13], Yasuo Takahashi[13], Takao Suzuki[14], Nobuaki
834 Sinozaki[14], Hiroki Yamaguchi[15], Shiro Minami[16], Shigeo Murayama[17], Kozo Yoshimori[18],
835 Satoshi Nagayama[19], Daisuke Obata[20], Masahiko Higashiyama[21], Akihide Masumoto[22],
836 Yukihiro Koretsune[23]

837

838 [1]Laboratory of Genome Technology, Human Genome Center, Institute of Medical Science,
839 The University of Tokyo, Tokyo, Japan.
840 [2]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The
841 University of Tokyo, Tokyo, Japan.
842 [3]Division of Genetics, The Institute of Medical Science, The University of Tokyo, Tokyo,
843 Japan.
844 [4]Division of Clinical Genome Research, Institute of Medical Science, The University of
845 Tokyo, Tokyo, Japan.
846 [5]Division of Molecular Pathology IMSUT Hospital, Department of Internal Medicine Project
847 Division of Genomic Medicine and Disease Prevention The Institute of Medical Science The
848 University of Tokyo, Tokyo, Japan.
849 [6]Department of Cancer Biology, Institute of Medical Science, The University of Tokyo,
850 Tokyo, Japan.
851 [7]Laboratory of Complex Trait Genomics, Graduate School of Frontier Sciences, The
852 University of Tokyo, Tokyo, Japan.

[8]Department of Public Policy, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

[9]Department of Urology, Iwate Medical University, Iwate, Japan.

[10]Department of Internal Medicine and Rheumatology, Juntendo University Graduate School of Medicine, Tokyo, Japan.

[11]Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan.

[12]Division of Pharmacology, Department of Biomedical Science, Nihon University School of Medicine, Tokyo, Japan.

[13]Division of Genomic Epidemiology and Clinical Trials, Clinical Trials Research Center, Nihon University. School of Medicine, Tokyo, Japan.

[14]Tokushukai Group, Tokyo, Japan.

[15]Hiroki Yamaguchi. Department of Hematology, Nippon Medical School, Tokyo, Japan.

[16]Department of Bioregulation, Nippon Medical School, Kawasaki, Japan.

[17]Shigeo Murayama. Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan.

[18]Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan.

[19]The Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan.

[20]Center for Clinical Research and Advanced Medicine, Shiga University of Medical Science, Shiga, Japan.

[21]Masahiko Higashiyama. Department of General Thoracic Surgery, Osaka International Cancer Institute, Osaka, Japan.

[22]Iizuka Hospital, Fukuoka, Japan.

[23]National Hospital Organization Osaka National Hospital, Osaka, Japan.

878

879

880

881 **MEGASTROKE CONSORTIUM**

882

883 Rainer Malik[1], Ganesh Chauhan[2], Matthew Traylor[3], Muralidharan
884 Sargurupremraj[4,5], Yukinori Okada[6,7,8], Aniket Mishra[4,5], Loes Rutten-Jacobs[3],
885 Anne-Katrin Giese[9], Sander W van der Laan[10], Solveig Gretarsdottir[11], Christopher
886 D Anderson[12,13,14], Michael Chong[15], Hieab HH Adams[16,17], Tetsuro Ago[18], Peter
887 Almgren[19], Philippe Amouyel[20,21], Hakan Ay[13,22], Traci M Bartz[23], Oscar R
888 Benavente[24], Steve Bevan[25], Giorgio B Boncoraglio[26], Robert D Brown, Jr.[27],
889 Adam S Butterworth[28,29], Caty Carrera[30,31], Cara L Carty[32,33], Daniel I
890 Chasman[34,35], Wei-Min Chen[36], John W Cole[37], Adolfo Correa[38], Ioana
891 Cotlarciuc[39], Carlos Cruchaga[40,41], John Danesh[28,42,43,44], Paul IW de Bakker[45,46],
892 Anita L DeStefano[47,48], Marcel den Hoed[49], Qing Duan[50], Stefan T Engelter[51,52],
893 Guido J Falcone[53,54], Rebecca F Gottesman[55], Raji P Grewal[56], Vilmundur
894 Gudnason[57,58], Stefan Gustafsson[59], Jeffrey Haessler[60], Tamara B Harris[61], Ahamad
895 Hassan[62], Aki S Havulinna[63,64], Susan R Heckbert[65], Elizabeth G Holliday[66,67],
896 George Howard[68], Fang-Chi Hsu[69], Hyacinth I Hyacinth[70], M Arfan Ikram[16], Erik
897 Ingelsson[71,72], Marguerite R Irvin[73], Xueqiu Jian[74], Jordi Jiménez-Conde[75], Julie A
898 Johnson[76,77], J Wouter Jukema[78], Masahiro Kanai[6,7,79], Keith L Keene[80,81], Brett M
899 Kissela[82], Dawn O Kleindorfer[82], Charles Kooperberg[60], Michiaki Kubo[83], Leslie A
900 Lange[84], Carl D Langefeld[85], Claudia Langenberg[86], Lenore J Launer[87], Jin-Moo
901 Lee[88], Robin Lemmens[89,90], Didier Leys[91], Cathryn M Lewis[92,93], Wei-Yu Lin[28,94],
902 Arne G Lindgren[95,96], Erik Lorentzen[97], Patrik K Magnusson[98], Jane Maguire[99],
903 Ani Manichaikul[36], Patrick F McArdle[100], James F Meschia[101], Braxton D
904 Mitchell[100,102], Thomas H Mosley[103,104], Michael A Nalls[105,106], Toshiharu
905 Ninomiya[107], Martin J O'Donnell[15,108], Bruce M Psaty[109,110,111,112], Sara L Pulit[45,113],
906 Kristiina Rannikmäe[114,115], Alexander P Reiner[65,116], Kathryn M Rexrode[117],
907 Kenneth Rice[118], Stephen S Rich[36], Paul M Ridker[34,35], Natalia S Rost[9,13], Peter M
908 Rothwell[119], Jerome I Rotter[120,121], Tatjana Rundek[122], Ralph L Sacco[122], Saori
909 Sakaue[7,123], Michele M Sale[124], Veikko Salomaa[63], Bishwa R Sapkota[125], Reinhold
910 Schmidt[126], Carsten O Schmidt[127], Ulf Schminke[128], Pankaj Sharma[39], Agnieszka
911 Slowik[129], Cathie LM Sudlow[114,115], Christian Tanislav[130], Turgut Tatlisumak[131,132],
912 Kent D Taylor[120,121], Vincent NS Thijs[133,134], Gudmar Thorleifsson[11], Unnur
913 Thorsteinsdottir[11], Steffen Tiedt[1], Stella Trompet[135], Christophe Tzourio[5,136,137],
914 Cornelia M van Duijn[138,139], Matthew Walters[140], Nicholas J Wareham[86], Sylvia
915 Wassertheil-Smoller[141], James G Wilson[142], Kerri L Wiggins[109], Qiong Yang[47],
916 Salim Yusuf[15], Najaf Amin[16], Hugo S Aparicio[48,185], Donna K Arnett[186], John
917 Attia[187], Alexa S Beiser[47,48], Claudine Berr[188], Julie E Buring[34,35], Mariana
918 Bustamante[189], Valeria Caso[190], Yu-Ching Cheng[191], Seung Hoan Choi[48,192],
919 Ayesha Chowhan[48,185], Natalia Cullell[31], Jean-François Dartigues[193,194], Hossein
920 Delavaran[95,96], Pilar Delgado[195], Marcus Dörr[196,197], Gunnar Engström[19], Ian
921 Ford[198], Wander S Gurpreet[199], Anders Hamsten [200,201], Laura Heitsch[202], Atsushi
922 Hozawa[203], Laura Ibanez[204], Andreea Ilinca[95,96], Martin Ingelsson[205], Motoki
923 Iwasaki[206], Rebecca D Jackson[207], Katarina Jood[208], Pekka Jousilahti[63], Sara
924 Kaffashian[4,5], Lalit Kalra[209], Masahiro Kamouchi[210], Takanari Kitazono[211], Olafur
925 Kjartansson[212], Manja Kloss[213], Peter J Koudstaal[214], Jerzy Krupinski[215], Daniel L
926 Labovitz[216], Cathy C Laurie[118], Christopher R Levi[217], Linxin Li[218], Lars Lind[219],

Cecilia M Lindgren[220,221], Vasileios Lioutas[48,222], Yong Mei Liu[223], Oscar L Lopez[224], Hirata Makoto[225], Nicolas Martinez-Majander[172], Koichi Matsuda[225], Naoko Minegishi[203], Joan Montaner[226], Andrew P Morris[227,228], Elena Muiño[31], Martina Müller-Nurasyid[229,230,231], Bo Norrving[95,96], Soichi Ogishima[203], Eugenio A Parati[232], Leema Reddy Peddareddygari[56], Nancy L Pedersen[98,233], Joanna Pera[129], Markus Perola[63,234], Alessandro Pezzini[235], Silvana Pileggi[236], Raquel Rabionet[237], Iolanda Riba-Llena[30], Marta Ribasés[238], Jose R Romero[48,185], Jaume Roquer[239,240], Anthony G Rudd[241,242], Antti-Pekka Sarin[243,244], Ralhan Sarju[199], Chloe Sarnowski[47,48], Makoto Sasaki[245], Claudia L Satizabal[48,185], Mamoru Satoh[245], Naveed Sattar[246], Norie Sawada[206], Gerli Sibolt[172], Ásgeir Sigurdsson[247], Albert Smith[248], Kenji Sobue[245], Carolina Soriano-Tárraga[240], Tara Stanne[249], O Colin Stine[250], David J Stott[251], Konstantin Strauch[229,252], Takako Takai[203], Hideo Tanaka[253,254], Kozo Tanno[245], Alexander Teumer[255], Liisa Tomppo[172], Nuria P Torres-Aguila[31], Emmanuel Touze[256,257], Shoichiro Tsugane[206], Andre G Uitterlinden[258], Einar M Valdimarsson[259], Sven J van der Lee[16], Henry Völzke[255], Kenji Wakai[253], David Weir[260], Stephen R Williams[261], Charles DA Wolfe[241,242], Quenna Wong[118], Huichun Xu[191], Taiki Yamaji[206], Dharambir K Sanghera[125,169,170], Olle Melander[19], Christina Jern[171], Daniel Strbian[172,173], Israel Fernandez-Cadenas[31,30], W T Longstreth, Jr[65,174], Arndt Rolfs[175], Jun Hata[107], Daniel Woo[82], Jonathan Rosand[12,13,14], Guillaume Pare[15], Jemma C Hopewell[176], Danish Saleheen[177], Kari Stefansson[11,178], Bradford B Worrall[179], Steven J Kittner[37], Sudha Seshadri[48,180], Myriam Fornage[74,181], Hugh S Markus[3], Joanna MM Howson[28], Yoichiro Kamatani[6,182], Stephanie Debette[4,5], Martin Dichgans[1,183,184]

[1]Institute for Stroke and Dementia Research (ISD), University Hospital, LMU Munich, Munich, Germany
[2]Centre for Brain Research, Indian Institute of Science, Bangalore, India
[3]Stroke Research Group, Division of Clinical Neurosciences, University of Cambridge, UK
[4]INSERM U1219 Bordeaux Population Health Research Center, Bordeaux, France
[5]University of Bordeaux, Bordeaux, France
[6]Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[7]Department of Statistical Genetics, Osaka University Graduate School of Medicine, Osaka, Japan
[8]Laboratory of Statistical Immunology, Immunology Frontier Research Center (WPI-IFReC), Osaka University, Suita, Japan.
[9]Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[10]Laboratory of Experimental Cardiology, Division of Heart and Lungs, University Medical Center Utrecht, University of Utrecht, Utrecht,Netherlands
[11]deCODE genetics/AMGEN inc, Reykjavik, Iceland
[12]Center for Genomic Medicine, Massachusetts General Hospital (MGH), Boston, MA, USA
[13]J. Philip Kistler Stroke Research Center, Department of Neurology, MGH, Boston, MA, USA
[14]Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA
[15]Population Health Research Institute, McMaster University, Hamilton, Canada

[16]Department of Epidemiology, Erasmus University Medical Center, Rotterdam, Netherlands
[17]Department of Radiology and Nuclear Medicine, Erasmus University Medical Center, Rotterdam, Netherlands
[18]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[19]Department of Clinical Sciences, Lund University, Malmö, Sweden
[20]Univ. Lille, Inserm, Institut Pasteur de Lille, LabEx DISTALZ-UMR1167, Risk factors and molecular determinants of aging-related diseases, F-59000 Lille, France
[21]Centre Hosp. Univ Lille, Epidemiology and Public Health Department, F-59000 Lille, France
[22]AA Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[23]Cardiovascular Health Research Unit, Departments of Biostatistics and Medicine, University of Washington, Seattle, WA, USA
[24]Division of Neurology, Faculty of Medicine, Brain Research Center, University of British Columbia, Vancouver, Canada
[25]School of Life Science, University of Lincoln, Lincoln, UK
[26]Department of Cerebrovascular Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milano, Italy
[27]Department of Neurology, Mayo Clinic Rochester, Rochester, MN, USA
[28]MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK
[29]The National Institute for Health Research Blood and Transplant Research Unit in Donor Health and Genomics, University of Cambridge, UK
[30]Neurovascular Research Laboratory, Vall d'Hebron Institut of Research, Neurology and Medicine Departments-Universitat Autònoma de Barcelona, Vall d'Hebrón Hospital, Barcelona, Spain
[31]Stroke Pharmacogenomics and Genetics, Fundacio Docència i Recerca MutuaTerrassa, Terrassa, Spain
[32]Children's Research Institute, Children's National Medical Center, Washington, DC, USA
[33]Center for Translational Science, George Washington University, Washington, DC, USA
[34]Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA, USA
[35]Harvard Medical School, Boston, MA, USA
[36]Center for Public Health Genomics, Department of Public Health Sciences, University of Virginia, Charlottesville, VA, USA
[37]Department of Neurology, University of Maryland School of Medicine and Baltimore VAMC, Baltimore, MD, USA
[38]Departments of Medicine, Pediatrics and Population Health Science, University of Mississippi Medical Center, Jackson, MS, USA
[39]Institute of Cardiovascular Research, Royal Holloway University of London, UK & Ashford and St Peters Hospital, Surrey UK
[40]Department of Psychiatry,The Hope Center Program on Protein Aggregation and Neurodegeneration (HPAN),Washington University, School of Medicine, St. Louis, MO, USA

[41]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA
[42]NIHR Blood and Transplant Research Unit in Donor Health and Genomics, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK
[43]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
[44]British Heart Foundation, Cambridge Centre of Excellence, Department of Medicine, University of Cambridge, Cambridge, UK
[45]Department of Medical Genetics, University Medical Center Utrecht, Utrecht, Netherlands
[46]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands
[47]Boston University School of Public Health, Boston, MA, USA
[48]Framingham Heart Study, Framingham, MA, USA
[49]Department of Immunology, Genetics and Pathology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[50]Department of Genetics, University of North Carolina, Chapel Hill, NC, USA
[51]Department of Neurology and Stroke Center, Basel University Hospital, Switzerland
[52]Neurorehabilitation Unit, University and University Center for Medicine of Aging and Rehabilitation Basel, Felix Platter Hospital, Basel, Switzerland
[53]Department of Neurology, Yale University School of Medicine, New Haven, CT, USA
[54]Program in Medical and Population Genetics, The Broad Institute of Harvard and MIT, Cambridge, MA, USA
[55]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[56]Neuroscience Institute, SF Medical Center, Trenton, NJ, USA
[57]Icelandic Heart Association Research Institute, Kopavogur, Iceland
[58]University of Iceland, Faculty of Medicine, Reykjavik, Iceland
[59]Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[60]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[61]Laboratory of Epidemiology and Population Science, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA
[62]Department of Neurology, Leeds General Infirmary, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[63]National Institute for Health and Welfare, Helsinki, Finland
[64]FIMM - Institute for Molecular Medicine Finland, Helsinki, Finland
[65]Department of Epidemiology, University of Washington, Seattle, WA, USA
[66]Public Health Stream, Hunter Medical Research Institute, New Lambton, Australia
[67]Faculty of Health and Medicine, University of Newcastle, Newcastle, Australia
[68]School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA
[69]Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA

[70]Aflac Cancer and Blood Disorder Center, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA
[71]Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, CA, USA
[72]Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[73]Epidemiology, School of Public Health, University of Alabama at Birmingham, USA
[74]Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX, USA
[75]Neurovascular Research Group (NEUVAS), Neurology Department, Institut Hospital del Mar d'Investigació Mèdica, Universitat Autònoma de Barcelona, Barcelona, Spain
[76]Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics, University of Florida, College of Pharmacy, Gainesville, FL, USA
[77]Division of Cardiovascular Medicine, College of Medicine, University of Florida, Gainesville, FL, USA
[78]Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands
[79]Program in Bioinformatics and Integrative Genomics, Harvard Medical School, Boston, MA, USA
[80]Department of Biology, East Carolina University, Greenville, NC, USA
[81]Center for Health Disparities, East Carolina University, Greenville, NC, USA
[82]University of Cincinnati College of Medicine, Cincinnati, OH, USA
[83]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[84]Department of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA
[85]Center for Public Health Genomics and Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA
[86]MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, UK
[87]Intramural Research Program, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA
[88]Department of Neurology, Radiology, and Biomedical Engineering, Washington University School of Medicine, St. Louis, MO, USA
[89]KU Leuven – University of Leuven, Department of Neurosciences, Experimental Neurology, Leuven, Belgium
[90]VIB Center for Brain & Disease Research, University Hospitals Leuven, Department of Neurology, Leuven, Belgium
[91]Univ.-Lille, INSERM U 1171. CHU Lille. Lille, France
[92]Department of Medical and Molecular Genetics, King's College London, London, UK
[93]SGDP Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
[94]Northern Institute for Cancer Research, Paul O'Gorman Building, Newcastle University, Newcastle, UK
[95]Department of Clinical Sciences Lund, Neurology, Lund University, Lund, Sweden

[96]Department of Neurology and Rehabilitation Medicine, Skåne University Hospital, Lund, Sweden
[97]Bioinformatics Core Facility, University of Gothenburg, Gothenburg, Sweden
[98]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[99]University of Technology Sydney, Faculty of Health, Ultimo, Australia
[100]Department of Medicine, University of Maryland School of Medicine, MD, USA
[101]Department of Neurology, Mayo Clinic, Jacksonville, FL, USA
[102]Geriatrics Research and Education Clinical Center, Baltimore Veterans Administration Medical Center, Baltimore, MD, USA
[103]Division of Geriatrics, School of Medicine, University of Mississippi Medical Center, Jackson, MS, USA
[104]Memory Impairment and Neurodegenerative Dementia Center, University of Mississippi Medical Center, Jackson, MS, USA
[105]Laboratory of Neurogenetics, National Institute on Aging, National institutes of Health, Bethesda, MD, USA
[106]Data Tecnica International, Glen Echo MD, USA
[107]Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[108]Clinical Research Facility, Department of Medicine, NUI Galway, Galway, Ireland
[109]Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA
[110]Department of Epidemiology, University of Washington, Seattle, WA, USA
[111]Department of Health Services, University of Washington, Seattle, WA, USA
[112]Kaiser Permanente Washington Health Research Institute, Seattle, WA, USA
[113]Brain Center Rudolf Magnus, Department of Neurology, University Medical Center Utrecht, Utrecht, The Netherlands
[114]Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, UK
[115]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[116]Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA, USA
[117]Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA
[118]Department of Biostatistics, University of Washington, Seattle, WA, USA
[119]Nuffield Department of Clinical Neurosciences, University of Oxford, UK
[120]Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA
[121]Division of Genomic Outcomes, Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA, USA
[122]Department of Neurology, Miller School of Medicine, University of Miami, Miami, FL, USA
[123]Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan
[124]Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA
[125]Department of Pediatrics, College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[126]Department of Neurology, Medical University of Graz, Graz, Austria

[127]University Medicine Greifswald, Institute for Community Medicine, SHIP-KEF, Greifswald, Germany
[128]University Medicine Greifswald, Department of Neurology, Greifswald, Germany
[129]Department of Neurology, Jagiellonian University, Krakow, Poland
[130]Department of Neurology, Justus Liebig University, Giessen, Germany
[131]Department of Clinical Neurosciences/Neurology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden
[132]Sahlgrenska University Hospital, Gothenburg, Sweden
[133]Stroke Division, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, Australia
[134]Austin Health, Department of Neurology, Heidelberg, Australia
[135]Department of Internal Medicine, Section Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands
[136]INSERM U1219, Bordeaux, France
[137]Department of Public Health, Bordeaux University Hospital, Bordeaux, France
[138]Genetic Epidemiology Unit, Department of Epidemiology, Erasmus University Medical Center Rotterdam, Netherlands
[139]Center for Medical Systems Biology, Leiden, Netherlands
[140]School of Medicine, Dentistry and Nursing at the University of Glasgow, Glasgow, UK
[141]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, NY, USA
[142]Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA
[143]A full list of members and affiliations appears in the Supplementary Note
[144]Department of Human Genetics, McGill University, Montreal, Canada
[145]Department of Pathophysiology, Institute of Biomedicine and Translation Medicine, University of Tartu, Tartu, Estonia
[146]Department of Cardiac Surgery, Tartu University Hospital, Tartu, Estonia
[147]Clinical Gene Networks AB, Stockholm, Sweden
[148]Department of Genetics and Genomic Sciences, The Icahn Institute for Genomics and Multiscale Biology Icahn School of Medicine at Mount Sinai, New York, NY , USA
[149]Department of Pathophysiology, Institute of Biomedicine and Translation Medicine, University of Tartu, Biomeedikum, Tartu, Estonia
[150]Integrated Cardio Metabolic Centre, Department of Medicine, Karolinska Institutet, Karolinska Universitetssjukhuset, Huddinge, Sweden.
[151]Clinical Gene Networks AB, Stockholm, Sweden
[152]Sorbonne Universités, UPMC Univ. Paris 06, INSERM, UMR_S 1166, Team Genomics & Pathophysiology of Cardiovascular Diseases, Paris, France
[153]ICAN Institute for Cardiometabolism and Nutrition, Paris, France
[154]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA
[155]Group Health Research Institute, Group Health Cooperative, Seattle, WA, USA
[156]Seattle Epidemiologic Research and Information Center, VA Office of Research and Development, Seattle, WA, USA
[157]Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA, USA

[158]Department of Medical Research, Bærum Hospital, Vestre Viken Hospital Trust, Gjettum, Norway
[159]Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore
[160]National Heart and Lung Institute, Imperial College London, London, UK
[161]Department of Gene Diagnostics and Therapeutics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan
[162]Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA
[163]Department of Cardiology, University Medical Center Groningen, University of Groningen, Netherlands
[164]MRC-PHE Centre for Environment and Health, School of Public Health, Department of Epidemiology and Biostatistics, Imperial College London, London, UK
[165]Department of Epidemiology and Biostatistics, Imperial College London, London, UK
[166]Department of Cardiology, Ealing Hospital NHS Trust, Southall, UK
[167]National Heart, Lung and Blood Research Institute, Division of Intramural Research, Population Sciences Branch, Framingham, MA, USA
[168]A full list of members and affiliations appears at the end of the manuscript
[169]Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[170]Oklahoma Center for Neuroscience, Oklahoma City, OK, USA
[171]Department of Pathology and Genetics, Institute of Biomedicine, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden
[172]Department of Neurology, Helsinki University Hospital, Helsinki, Finland
[173]Clinical Neurosciences, Neurology, University of Helsinki, Helsinki, Finland
[174]Department of Neurology, University of Washington, Seattle, WA, USA
[175]Albrecht Kossel Institute, University Clinic of Rostock, Rostock, Germany
[176]Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK
[177]Department of Genetics, Perelman School of Medicine, University of Pennsylvania, PA, USA
[178]Faculty of Medicine, University of Iceland, Reykjavik, Iceland
[179]Departments of Neurology and Public Health Sciences, University of Virginia School of Medicine, Charlottesville, VA, USA
[180]Department of Neurology, Boston University School of Medicine, Boston, MA, USA
[181]Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, USA
[182]Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[183]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany
[184]German Center for Neurodegenerative Diseases (DZNE), Munich, Germany
[185]Boston University School of Medicine, Boston, MA, USA
[186]University of Kentucky College of Public Health, Lexington, KY, USA
[187]University of Newcastle and Hunter Medical Research Institute, New Lambton, Australia
[188]Univ. Montpellier, Inserm, U1061, Montpellier, France
[189]Centre for Research in Environmental Epidemiology, Barcelona, Spain

[190]Department of Neurology, Università degli Studi di Perugia, Umbria, Italy
[191]Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA
[192]Broad Institute, Cambridge, MA, USA
[193]Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France
[194]Bordeaux University Hospital, Department of Neurology, Memory Clinic, Bordeaux, France
[195]Neurovascular Research Laboratory. Vall d'Hebron Institut of Research, Neurology and Medicine Departments-Universitat Autònoma de Barcelona. Vall d'Hebrón Hospital, Barcelona, Spain
[196]University Medicine Greifswald, Department of Internal Medicine B, Greifswald, Germany
[197]DZHK, Greifswald, Germany
[198]Robertson Center for Biostatistics, University of Glasgow, Glasgow, UK
[199]Hero DMC Heart Institute, Dayanand Medical College & Hospital, Ludhiana, India
[200]Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
[201]Karolinska Institutet, Stockholm, Sweden
[202]Division of Emergency Medicine, and Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA
[203]Tohoku Medical Megabank Organization, Sendai, Japan
[204]Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA
[205]Department of Public Health and Caring Sciences / Geriatrics, Uppsala University, Uppsala, Sweden
[206]Epidemiology and Prevention Group, Center for Public Health Sciences, National Cancer Center, Tokyo, Japan
[207]Department of Internal Medicine and the Center for Clinical and Translational Science, The Ohio State University, Columbus, OH, USA
[208]Institute of Neuroscience and Physiology, the Sahlgrenska Academy at University of Gothenburg, Goteborg, Sweden
[209]Department of Basic and Clinical Neurosciences, King's College London, London, UK
[210]Department of Health Care Administration and Management, Graduate School of Medical Sciences, Kyushu University, Japan
[211]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Japan
[212]Landspitali National University Hospital, Departments of Neurology & Radiology, Reykjavik, Iceland
[213]Department of Neurology, Heidelberg University Hospital, Germany
[214]Department of Neurology, Erasmus University Medical Center
[215]Hospital Universitari Mutua Terrassa, Terrassa (Barcelona), Spain
[216]Albert Einstein College of Medicine, Montefiore Medical Center, New York, NY, USA
[217]John Hunter Hospital, Hunter Medical Research Institute and University of Newcastle, Newcastle, NSW, Australia
[218]Centre for Prevention of Stroke and Dementia, Nuffield Department of Clinical Neurosciences, University of Oxford, UK

[219]Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[220]Genetic and Genomic Epidemiology Unit, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
[221]The Wellcome Trust Centre for Human Genetics, Oxford, UK
[222]Beth Israel Deaconess Medical Center, Boston, MA, USA
[223]Wake Forest School of Medicine, Wake Forest, NC, USA
[224]Department of Neurology, University of Pittsburgh, Pittsburgh, PA, USA
[225]BioBank Japan, Laboratory of Clinical Sequencing, Department of Computational biology and medical Sciences, Graduate school of Frontier Sciences, The University of Tokyo, Tokyo, Japan
[226]Neurovascular Research Laboratory, Vall d'Hebron Institut of Research, Neurology and Medicine Departments-Universitat Autònoma de Barcelona. Vall d'Hebrón Hospital, Barcelona, Spain
[227]Department of Biostatistics, University of Liverpool, Liverpool, UK
[228]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
[229]Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany
[230]Department of Medicine I, Ludwig-Maximilians-Universität, Munich, Germany
[231]DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany
[232]Department of Cerebrovascular Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milano, Italy
[233]Karolinska Institutet, MEB, Stockholm, Sweden
[234]University of Tartu, Estonian Genome Center, Tartu, Estonia, Tartu, Estonia
[235]Department of Clinical and Experimental Sciences, Neurology Clinic, University of Brescia, Italy
[236]Translational Genomics Unit, Department of Oncology, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy
[237]Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Spain
[238]Psychiatric Genetics Unit, Group of Psychiatry, Mental Health and Addictions, Vall d'Hebron Research Institute (VHIR), Universitat Autònoma de Barcelona, Biomedical Network Research Centre on Mental Health (CIBERSAM), Barcelona, Spain
[239]Department of Neurology, IMIM-Hospital del Mar, and Universitat Autònoma de Barcelona, Spain
[240]IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
[241]National Institute for Health Research Comprehensive Biomedical Research Centre, Guy's & St. Thomas' NHS Foundation Trust and King's College London, London, UK
[242]Division of Health and Social Care Research, King's College London, London, UK
[243]FIMM-Institute for Molecular Medicine Finland, Helsinki, Finland
[244]THL-National Institute for Health and Welfare, Helsinki, Finland
[245]Iwate Tohoku Medical Megabank Organization, Iwate Medical University, Iwate, Japan
[246]BHF Glasgow Cardiovascular Research Centre, Faculty of Medicine, Glasgow, UK
[247]deCODE Genetics/Amgen, Inc., Reykjavik, Iceland
[248]Icelandic Heart Association, Reykjavik, Iceland

[249]Institute of Biomedicine, the Sahlgrenska Academy at University of Gothenburg, Goteborg, Sweden
[250]Department of Epidemiology, University of Maryland School of Medicine, Baltimore, MD, USA
[251]Institute of Cardiovascular and Medical Sciences, Faculty of Medicine, University of Glasgow, Glasgow, UK
[252]Chair of Genetic Epidemiology, IBE, Faculty of Medicine, LMU Munich, Germany
[253]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan
[254]Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[255]University Medicine Greifswald, Institute for Community Medicine, SHIP-KEF, Greifswald, Germany
[256]Department of Neurology, Caen University Hospital, Caen, France
[257]University of Caen Normandy, Caen, France
[258]Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, Netherlands
[259]Landspitali University Hospital, Reykjavik, Iceland
[260]Survey Research Center, University of Michigan, Ann Arbor, MI, USA
[261]University of Virginia Department of Neurology, Charlottesville, VA, USA