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ORIGINAL RESEARCH

Large-Scale Targeted Sequencing Study of Ischemic Stroke in the Han Chinese Population

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BACKGROUND: Ischemic stroke is likely caused by interactions of multiple genes and environmental determinants. However, large-scale sequencing studies to discern functional genetic variants and their interactions with clinical and lifestyle risk factors on ischemic stroke are limited.

METHODS AND RESULTS: We sequenced functional regions of 740 previously identified genes associated with atherosclerotic disease among 999 ischemic stroke cases and 1001 controls of Chinese ancestry. Multiple logistic regression models were used to examine the associations between variants and ischemic stroke and test interactions between variants and clinical and lifestyle risk factors. Functional variants achieving suggestive significance were replicated in an independent sample of 4724 ischemic stroke cases and 5029 controls. Driven by variant main effects, each minor allele of the correlated rs174535, rs174545, and rs3834458 variants at *MYRF-FADS1-FADS2* conferred an average 0.83-fold (95% CI, 0.78–0.88) decreased odds of stroke. Significant main effects of *MTHFR* rs1801133 missense variant were also observed, with each copy of the A allele associated with a 1.20-fold (95% CI, 1.13–1.27) higher odds of ischemic stroke. The functional *ALDH2* rs671 variant was identified in interaction analyses with alcohol drinking (*Meta-P*=3.39×10^{-17}). Each minor allele conferred a 0.54-fold (95% CI, 0.45–0.64) decreased odds of stroke among drinkers and a 0.89-fold (95% CI, 0.83–0.97) decreased odds among nondrinkers.

CONCLUSIONS: Significant associations at *MYRF-FADS1-FADS2* indicate that genetically elevated polyunsaturated fatty acids may decrease ischemic stroke risk in East Asians. Significant associations at *MTHFR* and *ALDH2* robustly confirm deleterious effects of genetically elevated homocysteine and alcohol intake, respectively, on ischemic stroke.

Key Words: gene–environment interaction ■ genetic analysis ■ ischemic stroke ■ targeted sequencing study

Stroke is the third-leading cause of death and disability-adjusted life-years globally.[1,2] China has the highest stroke morbidity and mortality in the world, with >2.4 million incident stroke cases and 1.1 million stroke deaths annually.[3–5] Ischemic stroke represents the most common stroke subtype, comprising 87% of total stroke cases.[6] As a complex phenotype, ischemic stroke is likely caused by both genetic and environmental determinants, as well as their interactions.[7] Candidate genes studies and genome-wide association studies (GWASs) have made important strides in identifying independent loci associated with ischemic stroke (Table S1).[8,9] Despite such

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CLINICAL PERSPECTIVE

What Is New?

- We identified 3 correlated variants (rs174535, rs174545, and rs3834458) at *MYRF-FADS1-FADS2* that inversely associated with ischemic stroke, and we provide additional evidence for associations between *MTHFR* rs1801133 and ischemic stroke, as well as interactions of *ALDH2* rs671 with alcohol consumption on risk of this complex phenotype.

What Are the Clinical Implications?

- Our findings at the *MYRF-FADS1-FADS2* locus provide the first causal evidence for a protective role of genetically elevated polyunsaturated fatty acids in ischemic stroke among individuals of East Asian ancestry.
- Observed associations of *MTHFR* rs1801133 and *ALDH2* rs671 with ischemic stroke provide support for homocysteine and alcohol, respectively, as important mechanisms in ischemic stroke and these findings support guidance to reduce homocysteine levels and limit alcohol consumption for the prevention of stroke.

Nonstandard Abbreviations and Acronyms

CATIS	China Antihypertensive Trial in Acute Ischemic Stroke
df	degree-of-freedom
GWAS	genome-wide association study
IIPAIS	Infectious Factors, Inflammatory Markers and Prognosis of Acute Ischemic Stroke
indel	insertion/deletion

advancements, the causal genes and variants at these loci remain largely unknown. In addition, there is a paucity of research examining interactions of these loci with known clinical and lifestyle risk factors for stroke.[10–13] Large-scale sequencing studies could help to refine GWAS signals, providing novel targets for the development of molecular-based therapies for stroke prevention and treatment. Furthermore, discerning environmental modifiers of gene–stroke associations could identify high-risk subgroups who might be specially benefited through known therapies or lifestyle modifications.

To identify genes and variants for stroke and related atherosclerosis phenotypes, we conducted a systematic literature search for published GWASs and candidate gene studies. In total, 740 genes from 407 previously identified loci were reported, and we conducted a targeted sequencing study among 999 ischemic stroke cases and 1001 healthy controls of Han Chinese ancestry. Our discovery-stage analyses assessed the main effects of sequenced variants, as well as their interactions with clinical and lifestyle risk factors, on stroke. Findings were replicated among an independent sample of 9753 Han Chinese participants, including 4724 ischemic stroke cases and 5029 controls. For the current study, we aimed to identify functional genetic variants for stroke as well as functional genetic variants that may interact with clinical and lifestyle risk factors to influence ischemic stroke risk. Such findings could provide novel insights into the underlying biological mechanisms of stroke and also inform the development of preventive strategies for stroke in genetically high-risk subgroups.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Participants

A total of 5723 ischemic stroke cases and 6030 geography-matched controls from China were included in the current study. Because environmental and clinical risk factors vary substantially by geography (Table S2), the controls were frequency matched to cases based on regions of China. For our study, regions included Middle China (including Anhui, Jiangsu, Henan, and Shandong provinces) and North China (including Hebei, Ningxia, Liaoning, Jilin, Inner Mongolia, and Heilongjiang provinces). Cases were recruited from the CATIS (China Antihypertensive Trial in Acute Ischemic Stroke) study (n=2652),[14] the IIPAIS (Infectious Factors, Inflammatory Markers and Prognosis of Acute Ischemic Stroke) study (n=890),[15] and community-based epidemiology studies in Jiangsu (n=2074) and Harbin (n=107).[16] Geography-matched controls were recruited from community-based health surveys including the China National Health Survey (n=318),[17] Northeast China Rural Cardiovascular Health Study (n=1312),[18] and other community-based epidemiology studies in Jiangsu (n=3665), and Harbin (n=735).[16,19] The discovery cohort was composed of 999 patients with ischemic stroke from the CATIS study with the earliest ages of stroke onset. This strategy was used to enrich the discovery sample for disease-causing variants. A total of 1001 healthy controls who were older and had fewer stroke risk factors (eg, lower body mass index, lower blood pressure, lower blood lipid levels, and lower frequency of hypertension, diabetes, and hyperlipidemia) were also selected. The remaining samples were used in the replication study. The

study was approved by the Institutional Review Boards at Tulane University in the United States and the Ethical Committees at Soochow University and other participating institutes in China. Written informed consent was obtained from all study participants.

Ischemic stroke was defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction,[20] which was confirmed in all patients by computed tomography or magnetic resonance imaging. Controls were judged to be free of atherosclerotic diseases (eg, ischemic stroke, transient ischemic attack, myocardial infarction, and peripheral arterial disease) on the basis of medical history and clinical examination.

Targeted Gene Sequencing and Variant Genotyping

Atherosclerosis is an established risk factor for ischemic stroke.[21] To maximize the number of genes sequenced, we included genes and variants for both stroke and related atherosclerosis phenotypes (coronary heart disease, myocardial infarction, carotid intima-media thickness, and arterial aneurysm). We conducted a systematic search of MEDLINE, the GWAS catalog,[22] and the HUGE Navigator phenopedia[23] for GWAS and candidate gene studies published between January 1996 and November 2014. Nominal significance was employed for candidate gene studies, while a genome-wide significance level (5×10^{-8}) was employed for GWAS. At identified GWAS loci, nearby genes for each variant were selected. In addition, expression quantitative trait loci databases were queried to select genes with differential expression according to the reported variant or a variant in linkage disequilibrium with the reported variant. The systematic search and database query identified a total of 740 genes from 407 one-megabase loci for targeted gene-sequencing study.

Among the 2000 discovery-stage participants, functional regions of the 740 identified genes, including promoters, 5′-untranslated region, exons, splice sites/junctions, and 3'-untranslated region, were sequenced on the Illumina HiSeq 4000 system (Illumina Inc., San Diego, CA) with custom capture using the NimbleGen SeqCap EZ Choice probes (Roche, Basel, Switzerland). The average depth and breadth of coverage was 140× and 99.4%, respectively. A total of 82 358 single-nucleotide polymorphisms (SNPs) and 8922 insertion/deletions (indels) were identified through sequencing. Some 81 365 SNPs and 8464 indels passed our stringent quality control protocol, which filtered 548 SNPs with missing rate >10%, 70 SNPs with differential missingness by case and control status ($P<1.0\times10^{-5}$), and 375 SNPs and 458 indels with Hardy–Weinberg equilibrium $P<1.0\times10^{-5}$ among control. Variants with minor allele count <10, including 72 524 SNPs and 7160 indels, were additionally excluded from the single variant analyses, leaving 8841 SNPs and 1304 indels for this analysis.

Among the 9753 replication stage participants, we used the SNPscan system (Genesky Biotechnologies Inc., Shanghai, China) to carry out genotyping of 48 functional variants (45 SNPs and 3 indels) identified in the discovery stage. A total of 34 variants (31 SNPs and 3 indels) were successfully genotyped. After excluding variants with missing rate >10%, minor allele count <10, or Hardy–Weinberg equilibrium $P<1.0\times10^{-2}$ in the controls, 31 SNPs and 3 indels remained for the analysis in the replication stage.

Measurement of Demographic, Lifestyle, and Clinical Variables

Data on demographic, lifestyle, medical history, and clinical variables were collected in cases and controls across studies using similar protocols and harmonized for our analysis. Demographic characteristics (age and sex), lifestyle risk factors (current drinking and current smoking), medical history (history of hypertension, hyperlipidemia, diabetes, and atherosclerotic disease), and medication use (antihypertensive medication, lipid-lowing medication, and hypoglycemic medication) were collected by trained staff using standard questionnaires. Blood pressure was estimated as the average of 3 blood pressure measurements obtained by trained nurses according to a common protocol adapted from procedures recommended by the American Heart Association.[24] Blood pressure was measured with a standard mercury sphygmomanometer and appropriate cuff size based on participant's arm circumference. Body weight and height were measured using a regularly calibrated stadiometer and balance-beam scale with patients wearing light clothing and no shoes. Body mass index was calculated as weight in kilograms divided by height in meters squared. Serum lipids and plasma glucose were measured after at least 8 hours of fasting at local clinical laboratories that participated in a national standardization program.

Statistical Analysis

Characteristics of study participants were presented as means and SDs for continuous variables and as numbers and percentages for categorical variables. Before association analyses, triglyceride values were log-transformed. In the discovery stage, multiple logistic regression models were used to examine the main effect of each variant on ischemic stroke. Interactions of each variant with demographic (sex), lifestyle (alcohol drinking and cigarette smoking) and clinical risk factors (body mass index, blood pressure, lipids,

Table 1. Characteristics of Ischemic Stroke Cases and Controls at Discovery and Replication Stages

Characteristics*	Discovery study			Replication study		
	Cases (n=999)	Controls (n=1001)	*P* value	Cases (n=4724)	Controls (n=5029)	*P* value
Age, y	62.4±10.4	65.4±6.4	<0.001	64.3±10.9	63.4±9.1	<0.001
Male, n (%)	622 (62.3)	520 (52.0)	<0.001	2974 (63.0)	2439 (48.5)	<0.001
Current cigarette smoking, n (%)	368 (36.8)	356 (35.6)	0.554	1528 (32.4)	1842 (36.8)	<0.001
Alcohol drinking, n (%)	287 (28.7)	476 (47.6)	<0.001	1175 (25.2)	1575 (31.8)	<0.001
Systolic blood pressure, mm Hg	167.2±16.7	127.5±12.4	<0.001	155.7±21.9	132.6±15.0	<0.001
Diastolic blood pressure, mm Hg	96.4±10.4	75.4±8.0	<0.001	91.2±12.8	80.9±9.2	<0.001
Body mass index, kg/m^2	25.1±3.3	23.6±3.4	<0.001	24.7±3.2	24.1±3.4	<0.001
Total cholesterol, mg/dL	197.2±45.2	206.1±40.6	<0.001	189.1±47.2	194.1±41.4	<0.001
Triglycerides, mg/dL	155.0±107.2	131.1±70.9	<0.001	160.3±135.5	152.3±131.1	0.002
Low-density lipoprotein cholesterol, mg/dL	114.5±39.1	114.8±36.0	0.934	112.5±38.7	113.7±45.6	0.275
High-density lipoprotein cholesterol, mg/dL	50.7±17.0	55.7±13.9	<0.001	47.6±17.4	58.0±37.5	<0.001
Fasting plasma glucose, mg/dL	123.4±52.8	100.5±19.1	<0.001	119.6±51.5	101.3±27.4	<0.001
Obesity,† n (%)	153 (17.5)	95 (9.6)	<0.001	341 (13.2)	595 (11.9)	0.093
History of hypertension, n (%)	793 (79.4)	113 (11.3)	<0.001	3316 (71.8)	1302 (26.0)	<0.001
History of hyperlipidemia, n (%)	60 (6.0)	24 (2.4)	<0.001	397 (8.9)	114 (3.5)	<0.001
History of diabetes, n (%)	187 (18.7)	0 (0.0)	<0.001	1025 (22.0)	436 (8.7)	<0.001
Use of antihypertensive medications, n (%)	479 (48.0)	0 (0.0)	<0.001	1661 (56.2)	1029 (36.5)	<0.001
Use of lipid-lowering medications, n (%)	28 (2.8)	8 (0.8)	0.001	141 (3.0)	25 (0.5)	<0.001
Use of hypoglycemic medications, n (%)	76 (7.6)	0 (0.0)	<0.001	503 (10.7)	66 (1.3)	<0.001

*Continuous variables are expressed as mean±SD. Categorical variables are expressed as number (percentage).
†Obesity was defined as body mass index ≥28 kg/m^2.

glucose, obesity, history of hypertension, and history of dyslipidemia) were assessed. The variant by risk factor interaction term as well as variant and risk factor were included in the logistic regression model. One degree-of-freedom (df) interaction and 2-df joint main effects and interaction tests were performed,[25,26] with the 2-df joint test used to maximize power to detect variants with both moderate main effects and moderate interaction effects.[25] All models were adjusted for the fixed effects of age, sex, province and the first 4 ancestry principal components.[27] Functional SNPs and indels achieving $P<1.0\times10^{-4}$ in any of the analyses were genotyped and tested in main effects, and 1-df and 2-df interaction analyses among replication study participants. Aggregate rare variant analyses were also performed. Rare variants were grouped by gene and tested for association with stroke using the sequence kernel association test, employing the same covariable adjustments as in the single marker analyses.[28] Gene-based signals achieving $P<1.0\times10^{-4}$ in the aggregate rare variant analyses were also moved forward for replication study. Statistical analyses were performed with PLINK version 1.9 and SAS version 9.4 (SAS Institute, Cary, NC).

Replication stage analyses employed the same multiple logistic regression models that were used in the discovery stage. Standard inverse variance weighted meta-analysis was used to combine results from each of the main effects and 1-df interaction analyses across the discovery and replication stages. For the 2-df joint tests, meta-analysis was conducted using methods developed by Manning et al.[29] All meta-analyses were conducted using METAL software (version 2010-02-08).[30] Variants achieving nominal significance in the replication stage and a Bonferroni-corrected meta-analysis *P* value $<5.0\times10^{-6}$ were considered statistically significant.

All of the analyses above were conducted under the assumption of an additive genetic model. For identified variants, sensitivity analyses assuming dominant and recessive genetic models were conducted. In addition, for variants identified in 1-df interaction or 2-df joint tests, sensitivity analyses using robust standard errors were conducted to control for possible inflation. Furthermore, given large differences in patterns of alcohol drinking between Chinese men and women,[17] significant variant-alcohol drinking interactions were further explored in sex-stratified analyses.

RESULTS

Characteristics of the discovery and replication study participants are presented according to case and control status in Table 1. In the discovery and replication stages, ischemic stroke cases were more likely to be men and have a history of hypertension, hyperlipidemia, diabetes, and medication use. On average, patients with ischemic stroke had higher systolic and diastolic blood pressure, body mass index, serum triglycerides, and fasting plasma glucose. Because of selection, ischemic stroke cases were younger than healthy controls in discovery samples. Ischemic stroke cases were less likely to drink alcohol in discovery and replication samples and smoke cigarettes in replication samples.

Discovery-stage analyses revealed 112 variants suggestively associated with ischemic stroke ($P<1.0\times10^{-4}$), including 85 SNPs and 27 indels (Figures 1 and 2, Figures S1 and S2, and Table S3 through S14). Seventeen variants were suggestively associated with stroke in the main-effects analyses (Table S3). In the 1-df interaction tests, 59 variants were suggestively identified for ischemic stroke, including 10 variants that interacted with alcohol drinking, 5 that interacted with smoking, 1 that interacted with low-density lipoprotein cholesterol, 31 that interacted with high-density lipoprotein (HDL) cholesterol, 13 that interacted with fasting plasma glucose, and 2 that interacted with history of hypertension (Tables S3 through S14). For the 2-df joint tests, a total of 85 variants were suggestively identified for ischemic stroke. These included 13 variants associated jointly with sex, 17 variants associated jointly with alcohol drinking, 16 associated jointly with smoking, 13 associated jointly with low-density lipoprotein cholesterol, 59 associated jointly with HDL-cholesterol, 16 associated jointly with fasting plasma glucose, 5 associated jointly with history of hyperlipidemia, nine variants associated jointly with history of hypertension, seven variants associated jointly with body mass index, 15 associated jointly with triglycerides, and 10 associated jointly with obesity (Tables S3 through S14). Aggregate rare variant analyses did not identify any genes suggestively associated with stroke (all $P>1.0\times10^{-4}$; data not shown).

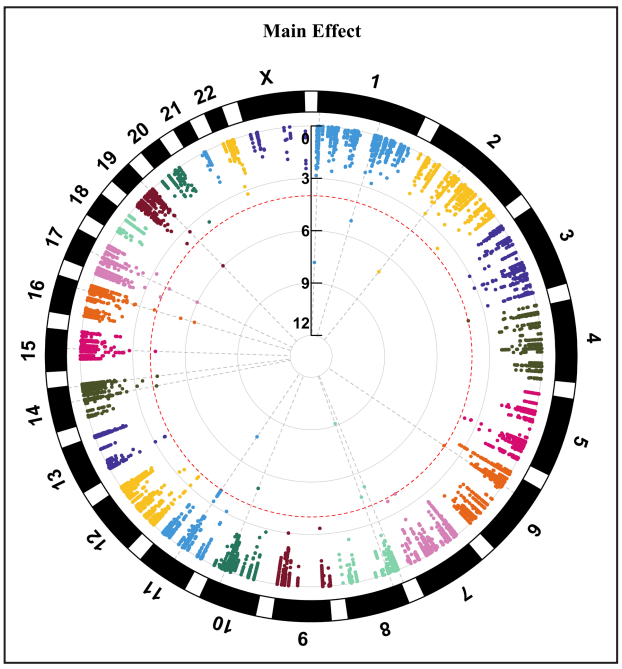

Figure 1. Circular Manhattan plot displaying variants achieving suggestive significance in the discovery-stage main-effects analyses.
Red dashed lines indicate suggestive significance ($P<1.0\times10^{-4}$) in the discovery stage.

Among the 112 variants identified in the discovery stage, 48 exonic variants (45 SNPs and 3 indels) were selected for replication study. Among them, 31 SNPs and 3 indels were successfully genotyped and further tested in the replication and meta-analyses (Tables S15 and S16). One suggestive signal from the discovery stage main effects analysis, *MTHFR* rs1801133, was replicated and achieved $P=7.5\times10^{-12}$ in meta-analyses of results from the discovery and replication studies (Table 2; Figure 3A). Each copy of the A allele conferred 1.20-fold (95% CI, 1.13–1.27) higher odds of ischemic stroke in meta-analyses.

Among the functional variants that achieved suggestive significance in the discovery-stage 1-df tests, the interactions of alcohol with rs3782886 at *BRAP*, rs671 at *ALDH2*, and rs78069066 at *ADAM1A* (3 correlated variants with all pairwise $r^2\geq0.98$) were replicated and achieved $P=1.3\times10^{-15}$, $P=3.4\times10^{-17}$, and $P=1.6\times10^{-15}$, respectively, in the meta-analysis of discovery and replication stage findings (Table 3; Figure 3B). In meta-analysis, each copy of the rs671 A allele was associated with a 0.54-fold (95% CI, 0.45–0.64) decreased risk of ischemic stroke among drinkers and an attenuated 0.89-fold (95% CI, 0.83–0.97) decreased risk of ischemic stroke among nondrinkers. When further stratified by sex, protective effects were observed among male but not female participants (Tables S17).

Among those variants identified in the discovery-stage 2–df joint test, 7 variants from 3 loci—*MTHFR*, *BRAP-ALDH2-ADAM1A*, and *MYRF-FADS1-FADS2*—were replicated and achieved Bonferroni significance in meta-analyses (Table S16). *MTHFR* variant rs1801133 demonstrated significant 2-df joint effects with each of sex, body mass index, fasting plasma glucose, HDL cholesterol, low-density lipoprotein cholesterol, triglycerides, drinking, smoking, obesity, and history of hyperlipidemia on stroke (*Meta-P*=2.7×10^{-11}, 3.9×10^{-17}, 1.1×10^{-15}, 1.2×10^{-11}, 7.5×10^{-12}, 2.3×10^{-12}, 2.5×10^{-12}, 7.0×10^{-12}, 8.4×10^{-17}, and 7.2×10^{-9}, respectively). The results of the 2-df test were driven by the strong main effects of the *MTHFR* variant

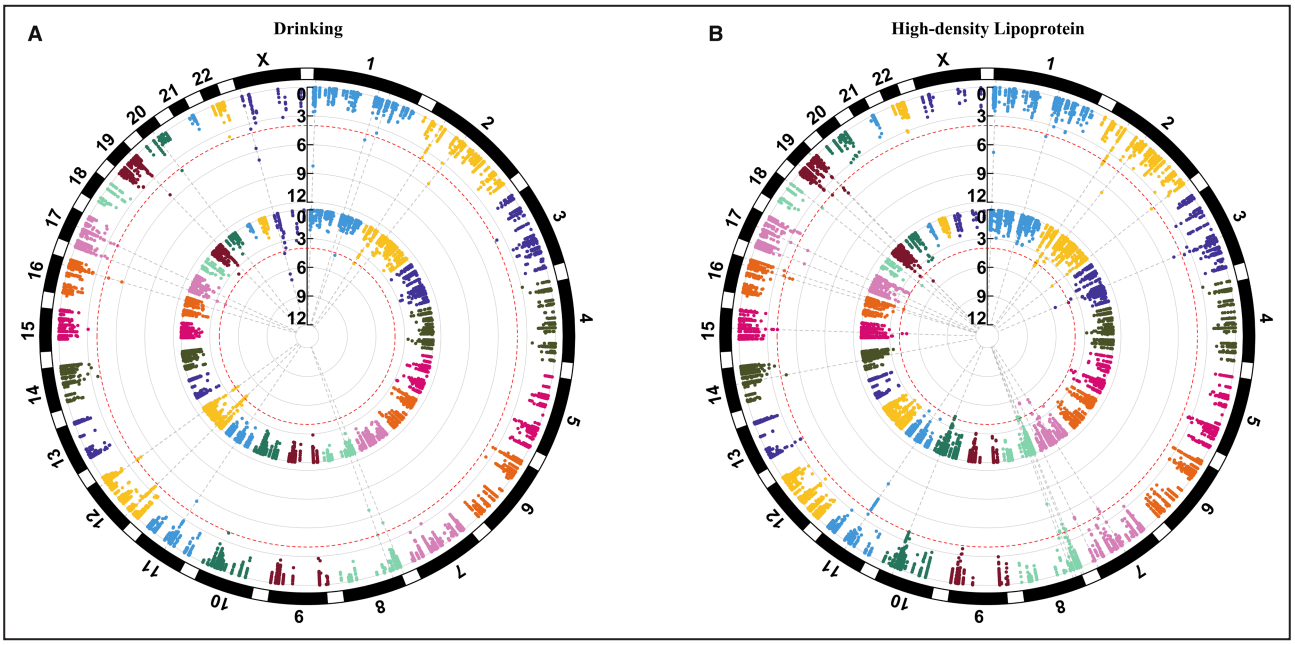

Figure 2. Circular Manhattan plots displaying variants achieving suggestive significance in the 1-df interaction (inner circle) and 2-df joint tests (outer circle).
A, Interaction with alcohol drinking; (**B**) interaction with high-density lipoprotein cholesterol. Red dashed lines indicate suggestive significance ($P<1.0\times10^{-4}$) in the discovery stage.

(Table 2), with findings from the 1-df tests generally nonsignificant across all risk factors (Table S16). In addition, the *BRAP-ALDH2-ADAM1A* variants rs3782886, rs671, and rs78069066 achieved meta-analysis $P=2.5\times10^{-23}$, 2.6×10^{-25}, and 2.7×10^{-23}, respectively, in the 2-df joint tests with alcohol drinking. These variants displayed significant main effects (Table 2), as well as significant 1-df interactions with alcohol drinking (Table 3) in meta-analyses. At the *MYRF-FADS1-FADS2* locus, correlated variants rs174535, rs174545, and rs3834458 (all pairwise $r^2\geq0.99$) were identified for stroke in 2-df joint tests with HDL cholesterol (*Meta-P*=1.2×10^{-12}, 1.7×10^{-13}, and 1.7×10^{-12}, respectively; Figure 3C). Significant associations of rs174535, rs174545, and rs3834458 were also observed in meta-analyses of main effects (*Meta-P*=4.8×10^{-11}, 2.8×10^{-11}, and 2.3×10^{-11}, respectively; Table 2).

Sensitivity analyses employing dominant and recessive genetic models demonstrated results consistent with the primary analyses, which assumed an additive model (Tables S18 through S19). Similarly, results of sensitivity analyses using robust standard errors for variants identified using the 1-df interaction or 2-df joint tests were also consistent with those of the primary analysis (Tables S20 and S21).

DISCUSSION

Our large-scale targeted sequencing study identified 3 independent loci associated with ischemic stroke in a combined sample of 11 753 Han Chinese participants. Three correlated ischemic stroke variants (rs174535, rs174545, and rs3834458) at *MYRF-FADS1-FADS2* were identified for the first time in East Asian participants. Discovered in 2-df joint tests with HDL-cholesterol, each minor allele conferred an average 0.8-fold decreased odds of stroke. Conversely, the well-known *MTHFR* rs1801133 missense variant was associated with a significant 1.2-fold increased odds of stroke in our main effects meta-analysis. At *BRAP-ALDH2-ADAM1A*, 3 correlated variants (rs3782886, rs671, and rs78069066) were identified in the 1-df interaction tests with alcohol drinking. These variants, likely reflecting the association of the functional *ALDH2* rs671 missense variant, conferred 0.54-fold decreased odds of ischemic stroke among drinkers in meta-analyses, with substantially attenuated associations among nondrinkers. In total, our findings provide novel functional insights into biological mechanisms underlying ischemic stroke.

The 3 correlated variants (rs174535, rs174545, and rs3834458) identified at *MYRF-FADS1-FADS2* appeared driven predominantly by variant main effects. Based on the most recent genome build, rs174545 lies in the 3′-untranslated region of *FADS1*, and rs3834458 is in an upstream region of *FADS2*. In contrast, *MYRF* variant rs174535 is exonic, with missense functionality in some isoforms. Despite the functional potential of the *MYRF* variant on the protein it encodes, previous studies have identified associations of these variants (or

Table 2. Variants Achieving Significance in Main Effects Meta-Analyses ($P<5\times10^{-6}$)

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	Odds ratios	95% CI	*P* value
rs1801133	1	11 856 378	*MTHFR*	A/G	0.55	Discovery	1.45	(1.26, 1.66)	1.54E-08
					0.48	Replication	1.16	(1.10, 1.23)	6.56E-07
						Meta-analysis	1.20	(1.13, 1.27)	7.54E-12
rs174535*	11	61 551 356	*MYRF*	C/T	0.34	Discovery	0.76	(0.67, 0.88)	1.35E-04
					0.37	Replication	0.84	(0.80, 0.89)	3.61E-08
						Meta-analysis	0.84	(0.79, 0.89)	4.81E-11
rs174545*	11	61 569 306	*FADS1*	G/C	0.33	Discovery	0.76	(0.67, 0.88)	1.48E-04
					0.37	Replication	0.84	(0.80, 0.89)	1.98E-08
						Meta-analysis	0.83	(0.78, 0.88)	2.78E-11
rs3834458*	11	61 594 920	*FADS2*	C/CT	0.33	Discovery	0.76	(0.67, 0.88)	1.85E-04
					0.36	Replication	0.84	(0.80, 0.89)	1.45E-08
						Meta-analysis	0.83	(0.78, 0.88)	2.30E-11
rs3782886†	12	112 110 489	*BRAP*	C/T	0.16	Discovery	0.91	(0.77, 1.09)	2.88E-01
					0.20	Replication	0.82	(0.76, 0.89)	1.15E-07
						Meta-analysis	0.84	(0.79, 0.89)	1.22E-07
rs671†	12	112 241 766	*ALDH2*	A/G	0.16	Discovery	0.90	(0.76, 1.08)	2.48E-01
					0.19	Replication	0.81	(0.75, 0.88)	2.44E-08
						Meta-analysis	0.83	(0.78, 0.88)	2.36E-08
rs78069066†	12	112 337 924	*ADAM1A*	A/G	0.16	Discovery	0.90	(0.76, 1.08)	2.38E-01
					0.20	Replication	0.83	(0.76, 0.89)	1.60E-07
						Meta-analysis	0.84	(0.79, 0.89)	1.28E-07

Based on an additive genetic model, adjusting for age, sex, province, and the first 4 genetic principal components. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; OA, other allele; and OR, odds ratio.

*rs174535, rs174545, and rs3834458 were highly correlated, smallest R^2=0.99.

†rs3782886, rs671, and rs78069066 were highly correlated, smallest R^2=0.98.

their linkage disequilibrium proxies) with concentrations of omega-6 polyunsaturated fatty acids (PUFAs).[31–35] Omega-6 PUFAs are regulated by the *FADS1* and *FADS2* genes,[36–38] suggesting that functional variants in these genes may underlie the observed associations. Similar to our findings, the minor allele of *FADS1* rs174547, a SNP in high linkage disequilibrium with the 3 identified variants, was associated with lower odds of stroke in a previous Mendelian randomization study in a predominantly European population.[39] Previous observational studies have provided evidence of the protective roles of PUFAs on stroke, and a clinical trial further showed that diets with higher PUFAs significantly lowered the incidence of stroke.[38,40,41] The anti-inflammatory and anticoagulant characteristics of PUFAs have been suggested as potential mechanisms of action for their beneficial effects. Our findings provide the first causal evidence for a protective role of genetically elevated PUFAs in ischemic stroke among individuals of East Asian ancestry.

The *MTHFR* rs1801133 variant strongly associated with ischemic stroke in our main-effects analysis and drove the significant findings of several 2-df joint tests with clinical and lifestyle risk factors. *MTHFR* encodes the methylenetetrahydrofolate reductase enzyme, which plays a critical role in converting homocysteine to methionine. Substitution of an alanine amino acid with valine at the 222nd residue, which is encoded by rs1801133, results in a more thermolabile protein and causes reduced enzymatic function and increased levels of homocysteine.[42,43] Elevated plasma homocysteine has been associated with stroke previously by observational studies.[43–46] Homocysteine may exert its effect by decreasing nitric oxide generation, inducing oxidative injury and increased platelet adhesion.[47,48] Homocysteine can be lowered by folic acid supplementation, and several clinical trials have demonstrated that homocysteine lowering via folic acid supplementation moderately reduces stroke risk.[49] Associations of homocysteine-regulating genetic variants, which are not confounded by traditional risk factors, could support its potential causal relationship with ischemic stroke. Indeed, rs1801133 has been associated with stroke in previous studies.[42,50–52] However, these studies have had relatively small sample sizes and used permissive statistical significance thresholds. We are the first to report this SNP in a large-scale study at a level surpassing genome-wide significance. The successful replication of this finding further implicates the potential causal role of homocysteine in stroke.

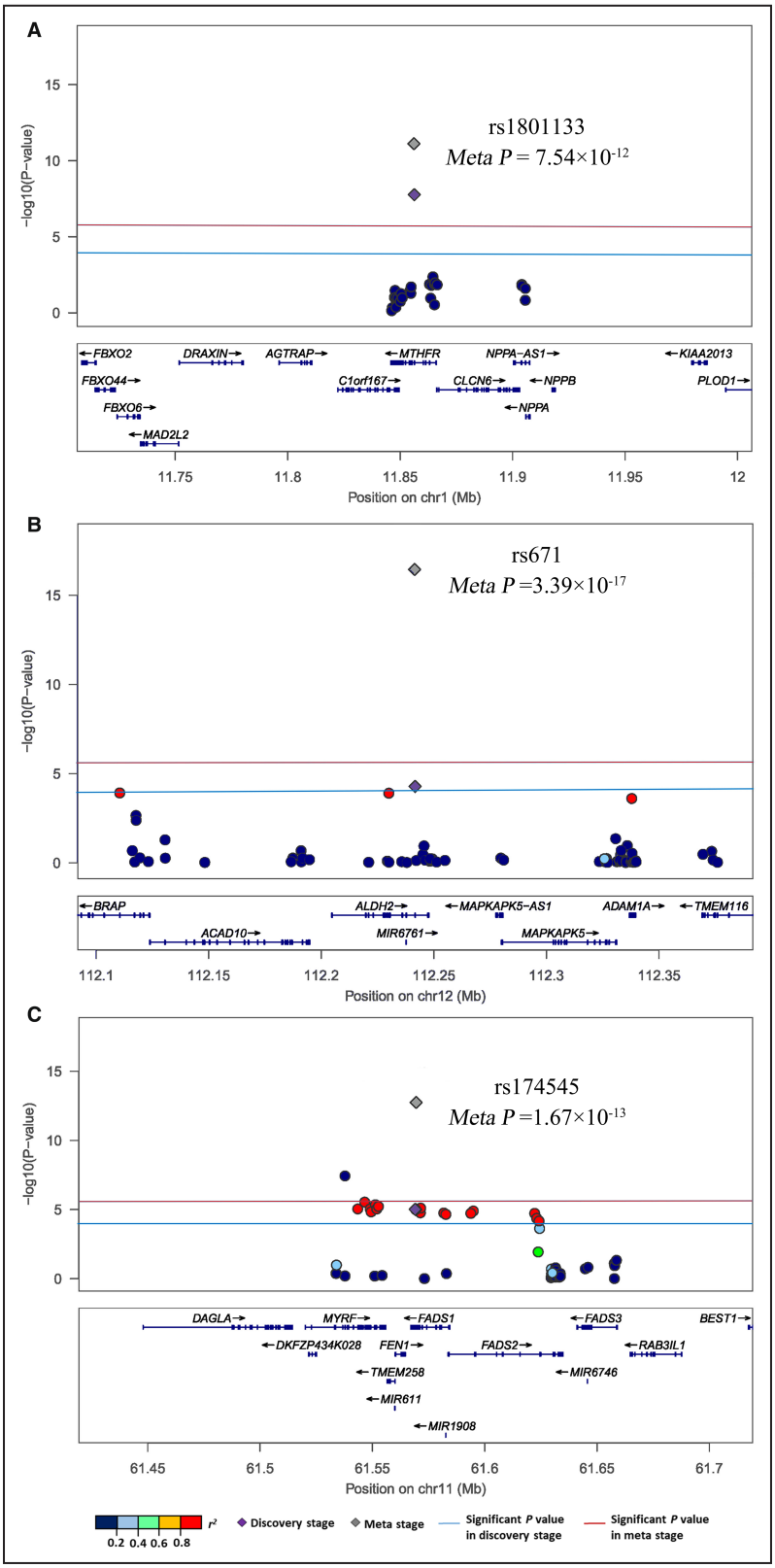

Figure 3. Regional association plots for significant stroke signals.
A, Main effects analysis of rs1801133; **B**, 1-df analysis of rs671 with alcohol drinking; C: 2-df analysis of rs174545 with high-density lipoprotein cholesterol.

Table 3. Variants Achieving Significance in 1-df Interaction Test Meta-Analyses ($P<5\times10^{-6}$)

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	Drinkers			Nondrinkers			1-df interaction *P* value
							OR	95% CI	*P* value	OR	95% CI	*P* value	
rs3782886*	12	112 110 489	*BRAP*	C/T	0.16	Discovery	0.37	(0.24, 0.59)	2.06E-05	1.01	(0.82, 1.23)	9.60E-01	1.19E-04
					0.20	Replication	0.61	(0.51, 0.74)	1.93E-07	0.88	(0.81, 0.96)	2.68E-03	1.72E-12
						Meta	0.57	(0.48, 0.68)	1.25E-10	0.90	(0.83, 0.97)	5.96E-03	1.33E-15
rs671*	12	112 241 766	*ALDH2*	A/G	0.16	Discovery	0.34	(0.21, 0.54)	6.47E-06	1.01	(0.83, 1.24)	9.02E-01	5.47E-05
					0.19	Replication	0.58	(0.48, 0.70)	1.39E-08	0.88	(0.80, 0.95)	2.27E-03	9.20E-14
						Meta	0.54	(0.45, 0.64)	3.55E-12	0.89	(0.83, 0.97)	5.60E-03	3.39E-17
rs78069066*	12	112 337 924	*ADAM1A*	A/G	0.16	Discovery	0.39	(0.25, 0.62)	4.36E-05	0.99	(0.81, 1.22)	9.42E-01	2.45E-04
					0.20	Replication	0.61	(0.50, 0.73)	1.14E-07	0.88	(0.81, 0.96)	3.19E-03	1.20E-12
						Meta	0.57	(0.48, 0.67)	1.01E-10	0.90	(0.83, 0.97)	5.99E-03	1.55E-15

Based on an additive genetic model, adjusting for age, sex, province, and the first 4 genetic principal components. CA indicates coded allele; CAF, coded allele frequency; Chr, chromosome; OA, other allele; and OR, odds ratio.

*rs3782886, rs671, and rs78069066 were highly correlated, smallest R^2=0.98.

Three variants at *BRAP-ALDH2-ADAM1A* (rs3782886, rs671, and rs78069066), which interacted with alcohol drinking, are specific to populations of Asian ancestry. Among drinkers, each copy of the minor alleles of these variants associated with a 0.5-fold decreased odds of ischemic stroke, a finding that was substantially attenuated among nondrinkers. The strong effect observed in drinkers resulted in a more attenuated but still genome-wide significant signal of this variant in the entire sample. Since the nondrinker group included former drinkers, we observed nominal associations within this subgroup. At the identified locus, *ALDH2* encodes an enzyme essential to alcohol metabolism. The well-known rs671 missense SNP results in a glutamic acid to lysine amino acid substitution at the 504th residue, decreasing activity of the encoded aldehyde dehydrogenase 2 enzyme. Decreased enzymatic activity allows acetaldehyde to accumulate,[53,54] resulting in reduced tolerance to alcohol and, likewise, reduced alcohol intake.[53,54] Given the important role of rs671 in alcohol metabolism, this SNP likely represents the causal variant underlying the signal observed at this locus and a genomic proxy for alcohol intake. Since drinkers carrying the rs671 variant are likely to consume less alcohol compared with noncarriers, these data suggest that decreased alcohol intake reduces ischemic stroke risk. Similar to our findings, previous studies have identified an association of rs671 with ischemic stroke,[55–57] including a recent study of 121 698 Chinese participants that demonstrated a positive log-linear relationship of genetically predicted alcohol intake with ischemic stroke.[58] Interestingly, when further stratified the population by sex, significant effects were only observed among male participants. These findings are consistent with those of Millwood and his colleagues[58] and likely relates to more modest alcohol drinking in women,[59] even within the "drinker" subgroup. Previous observational studies have supported an inverse association between low to moderate alcohol consumption and stroke risk.[60] Our findings, together with results from Millwood et al, indicate that these protective effects might be attributable to reverse causality or the influence of confounders. Overall, our findings provide further support of alcohol as a critical mechanism in stroke. Furthermore, our findings support guidance to limit alcohol consumption for the prevention of stroke.

The current study has several strengths. As one of the largest targeted sequencing studies of ischemic stroke to date, power was enhanced to detect not only variant main effects but their interactions with important clinical and lifestyle risk factors. To our knowledge, this study also represents the only large-scale sequencing study conducted in an East Asian ancestry population, allowing us to carefully assess ancestry specific variants. By examining homogeneous Han Chinese samples in

the discovery and replication stages, population stratification was minimized. Furthermore, stringent quality control measures were employed for sequencing, genotyping, and covariable and outcome measurements. Still, certain limitations should also be mentioned. The sample size in the discovery stage was relatively small, limiting power to identify rare variants. Furthermore, the "extreme-trait" design and more general "winner's curse" may have resulted in somewhat overestimated effect sizes in the discovery-stage analysis.[61] Because discovery-stage participants had more severe disease compared with replication-stage participants, it is possible that heterogeneity in disease etiology was present across analytic stages, which could have led to a failure to replicate true signals. Furthermore, although frequency matching was successful in the discovery stage, regional imbalances were identified in the replication stage. However, these differences should have been accounted for when adjusting for province (within regions) in the statistical analysis. In addition, to maximize study efficiency, we only sequenced genes or genes in genomic regions previously related to atherosclerosis phenotypes. By design, this limited our ability to identify any novel stroke loci. Also, only exonic variants, according to the latest genome build, were selected for replication study. Among them, several were not successfully genotyped in the replication stage. Noncoding variants with important regulatory effects and coding variants that could not be successfully genotyped by the current study could functionally influence stroke susceptibility and warrant future follow-up.[62] Because environmental and clinical risk factors vary substantially by geography and associate with stroke, we employed geography matching and further adjusted for province in our analysis. This should minimize heterogeneity in environmental and clinical factors, particularly in the replication stage, where cases were selected from multiple studies conducted across various regions of China. However, similar to other observational studies, we cannot rule out residual confounding in our analyses.

In conclusion, this large-scale targeted sequencing study implicated 3 loci—*FADS1-FADS2*, *MTHFR*, and *ALDH2*—in ischemic stroke. By identifying variants at the *FADS1-FADS2* locus, we provide the first genetic evidence for a causal, protective effect of PUFAs on ischemic stroke in an East Asian population. Furthermore, the observed deleterious effect of *MTHFR* variant rs180113 on stroke provides some of the first robust genomic evidence for a causal role of elevated homocysteine in this condition. We are also among the first to describe an interaction of the *ALDH2* rs671 variant and alcohol intake on ischemic stroke, bolstering support for the potentially harmful etiologic effects of excessive alcohol consumption on this condition. In total, our findings provide valuable mechanistic information on ischemic stroke susceptibility and highlight the synergistic effects of genetic and environmental factors in determining risk of ischemic stroke.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S21
Figure S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Candidate genes studies or genome-wide association studies of ischemic stroke

Fist author	Journal	Publication year	Study method	Study design	Sample type	Study population	Sample Size	Main Results
Rubattu S	Circulation	1999	case-control	candidate gene	population based	European	796	rs5063
Endler G	Br J Haematol	2000	case-control	candidate gene	population based	European	176	rs1799768
Imai Y	Atherosclerosis	2000	case-control	candidate gene	population based	Asian	666	rs662
Kokubo Y	Stroke	2000	case-control	candidate gene	population based	Asian	1448	*ApoE* epsilon2
Reiner AP	Stroke	2000	case-control	candidate gene	population based	European	382	rs1126643
Baker RI	Blood	2001	case-control	candidate gene	population based	Austrilia	424	rs2243093
Bang Co	Cerebrovasc Dis	2001	case-control	candidate gene	population based	Asian	160	rs1799889
Dai K	Thromb Res	2001	case-control	candidate gene	population based	Asian	220	*vWF* Sma I polymorphism
Herrmann SM	Arterioscler Thromb Vasc Biol	2001	case-control	candidate gene	population based	European	560	rs45567233
Morrison AC	Stroke	2001	cohort	candidate gene	population based	European	1215	rs5443
Shen N	Zhonghua Yi Xue Za Zhi	2001	case-control	candidate gene	population based	Asian	219	HLA-DQA1*0301
Wu Y	Kobe J Med Sci	2001	case-control	candidate gene	population based	Asian	137	rs1801133
Morrison AC	Genet Epidemiol	2002	case-control	candidate gene	population based	European	1182	rs328
Revilla M	Neurosci Lett	2002	case-control	candidate gene	population based	European	164	rs1800795
Voetsch B	Stroke	2002	case-control	canddiate gene	population based	European	236	rs662
Chi LQ	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2003	case-control	candidate gene	population based	Asian	163	*GR* G1666T
Hoekstra T	Stroke	2003	cohort	candidate gene	population based	European	637	rs1799768
Kolovou GD	Angiology	2003	case-control	candidate gene	population based	European	405	ApoE epsilon2
Li Z	Stroke	2003	case-control	candidate gene	population based	Asian	3664	rs1801133
Martiskainen M	Stroke	2003	case-control	candidate gene	population based	European	132	rs1800790

Souza DR	Arg Neuropsiquiatr	2003	case-control	candidate gene	population based	European	414	rs429358 and rs7412
Wang XY	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2003	case-control	candidate gene	population based	Asian	162	rs7493
Xia J	Zhonghua Yi Xue Za Zhi(Taipai)	2003	case-control	candidate gene	population based	Asian	230	rs1801692
Zhao SP	Clin Chim Acta	2003	case-control	candidate gene	population based	Asian	213	rs328
Aznar J	Thromb Haemost	2004	case-control	candidate gene	population based	European	343	rs1799963
Baum L	Clin Chem Lab Med	2004	case-control	candidate gene	population based	Asian	545	rs1801133
Cipollone F	JAMA	2004	case-control	candidate gene	population based	European	1728	rs20417
Dong QL	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2004	case-control	candidate gene	population based	Asian	176	rs1800790
Frikke-Schmidt R	Eur Heart J	2004	cohort	candidate gene	population based	European	9238	rs11669576
Jannes J	Stroke	2004	case-control	candidate gene	population based	European	483	rs2020918
KöIsch H	Neurology	2004	case-control	candidate gene	population based	European	442	rs4925
Lanca V	Rev Port Cardiol	2004	case-control	candidate gene	population based	European	184	rs4646903
Lee BC	Neurosci Lett	2004	case-control	candidate gene	population based	Asian	317	rs18004629
Muñoz X	Hum Mutat	2004	case-control	candidate gene	population based	European	173	*GAS6* c.834+7G>A
Rubattu S	J Hypertens	2004	case-control	candidate gene	population based	European	451	rs1799752
Rubattu S	Stroke	2004	case-control	candidate gene	population based	European	442	rs5065
Santamaria A	Stroke	2004	case-control	candidate gene	population based	European	436	rs1801020
Slowik A	Stroke	2004	case-control	candidate gene	population based	European	276	rs5918
Suzuki Y	Neurology	2004	cross-sectional	candidate gene	population based	Asian	2195	ADH2*1 allele
Voetsch B	Arch Neurol	2004	case-control	canddiate gene	population based	European	236	rs705379
Wallerstedt SM	J Hypertens	2004	case-control	candidate gene	population based	European	1032	rs16139
Alluri RV	Eur J Neurol	2005	case-control	candidate gene	population based	India	118	rs1801133

Bevan S	Stroke	2005	case-control	candidate gene	population based	European	1013	rs40512, rs26950, rs702531, and rs829259
Champrro A	Cerebrovasc Dis	2005	case-control	candidate gene	population based	European	194	rs1800795
Chen J	J Mol Med(Berl)	2005	case-control	candidate gene	population based	Asian	1234	rs2794521
Dziedzic T	Cerebrovasc Dis	2005	case-control	candidate gene	population based	European	349	rs16944
Howard TD	Stroke	2005	case-control	candidate gene	population based	African	124	rs1800779 and rs2070744
Iacoviello L	Arterioscler Thromb Vasc Biol	2005	case-control	candidate gene	population based	Asian	268	rs16944
Lavergne E	Arterioscler Thromb Vasc Biol	2005	case-control	candidate gene	population based	European	929	rs3732378
Lin YC	Atherosclerosis	2005	case-control	candidate gene	population based	Asian	457	*TLR4* C119A
Lõhmussaar E	Stroke	2005	case-control	candidate gene	population based	European	1375	rs10507391
Ranade K	Stroke	2005	cohort	candidate gene	population based	European	2634	rs662
Rubattu S	Thromb Haemost	2005	case-control	candidate gene	population based	European	580	*F7* C122T
Saleheen D	Stroke	2005	case-control	candidate gene	population based	Asian	450	rs966221
Shearman AM	Stroke	2005	cohort	candidate gene	population based	European	2709	rs2234693
Slowik A	Cerebrovasc Dis	2005	case-control	candidate gene	population based	European	201	rs5985
Staton J	Stroke	2005	case-control	candidate gene	population based	European	315	rs3024718
van Rijn MJ	Neurology	2005	cross-sectional	candidate gene	family based	European	464	rs12188950 and rs3887175
Wiklund PG	Stroke	2005	case-control	candidate gene	population based	European	542	rs1799889
Zhang X	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2005	case-control	candidate gene	population based	Asian	102	*PAFAH1B1* 994C/T
Andrikovics H	Cerebrovasc Dis	2006	case-control	candidate gene	population based	European	394	rs2066718 and rs2230806
Baum L	Clin Chem Lab Med	2006	case-control	candidate gene	population based	Asian	816	rs328
Brophy VH	Stroke	2006	case-control	candidate gene	population based	European	485	5 SNPs of *PDE4D* gene
Funk M	Endler G	2006	case-control	candidate gene	population based	European	481	rs510317

Hegener HH	Clin Chem	2006	case-control	candidate gene	population based	European	518	rs266729 and rs182052
Hermans MP	Diabet Med	2006	cross-sectional	candidate gene	population based	European	165	rs1801133
Kaneko Y	Hypertens Res	2006	case-control	candidate gene	population based	Asian	559	rs768963
Kim Y	Neurosci Lett	2006	case-control	candidate gene	population based	Asian	227	rs2249358
Kim Y	Stroke	2006	case-control	candidate gene	population based	Asian	478	rs1800470
Lai J	Neurol India	2006	case-control	candidate gene	population based	Asian	112	rs16944
Lee BC	Neurosci Lett	2006	case-control	candidate gene	population based	Asian	272	rs1801282
Sie MP	Stroke	2006	cohort	candidate gene	population based	European	6996	rs1800470 and rs1800469
Staton JM	J Neurol Neurosurg Psychiatry	2006	case-control	candidate gene	population based	European	315	rs1396476, rs2910829 and rs966221
Szolnoki Z	J Mol Neurosci	2006	case-control	candidate gene	population based	European	580	rs5186
van Rijn MJ	J Neurol Neurosurg Psychiatry	2006	cohort	candidate gene	population based	European	6808	*IGF1* 192bp/-
van Rijn MJ	Stroke	2006	cohort	candidate gene	population based	European	6471	rs4961
Woo D	Stroke	2006	case-control	candidate gene	population based	Mixed	839	rs2910829 and rs152312
Yamada Y	Arterioscler Thromb Vasc Biol	2006	case-control	candidate gene	population based	Asian	2927	rs1800796
Yamaguchi S	Int J Mol Med	2006	case-control	candidate gene	population based	Asian	2705	rs235326, rs2107538 and rs4680
Zee RY	Stroke	2006	case-control	candidate gene	population based	European	518	rs702553
Zhang SY	Zhongguo Wei Zhong Bing Ji Jiu Yi Xue	2006	case-control	candidate gene	population based	Asian	61	*HLA-DRB1**0301
Zhang WL	Yi Chuan Xue Bao	2006	case-control	candidate gene	population based	Asian	1478	*ALOX5AP* SG13S114T/A
Zhu XY	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2006	case-control	candidate gene	population based	Asian	272	*LCAT* 608C/T
Abboud S	Plos one	2007	case-control	candidate gene	population based	European	563	rs505151
Alanne M	Hum Genet	2007	cohort	candidate gene	population based	European	14140	rs7178239
Benn M	J Clin Endocrinol Metab	2007	cohort	candidate gene	population based	European	9157	rs1042031

Berger K	Hum Genet	2007	case-control	candidate gene	population based	European	3648	rs1799864, rs1295686, rs1062535, rs1799983, and rs6131
Cole JW	BMC Neurol	2007	case-control	candidate gene	population based	European	194	rs6797312
Djoussé L	Am Heart J	2007	case-control	candidate gene	population based	European	1451	rs28362459
Fu Y	Zhonghua Yi Xue Za Zhi	2007	case-control	candidate gene	population based	Asian	245	rs1800588
Grewal RP	BMC Med Genet	2007	case-control	candidate gene	population based	African	879	*NOS3* repeat of a 27-bp
Hata J	Hum Mol Genet	2007	cohort	candidate gene	population based	Asian	2224	rs9943582
Kaushal R	Hum Genet	2007	case-control	candidate gene	population based	European	839	rs9579646 and rs4769874
Kuroda J	Eur J Neurol	2007	case-control	candidate gene	population based	Asian	1189	rs4673
Lee C	Stroke	2007	case-control	candidate gene	population based	Asian	782	rs16135
Liu J	Clin Chim Acta	2007	case-control	candidate gene	population based	Asian	232	rs1043618
Matarin M	Lancet Neurol	2007	case-control	GWAS	population based	European	517	27 loci with $P<1\times10-5$
Moon KS	J Mol Neurosci	2007	case-control	candidate gene	population based	Asian	729	T6235C *CYP1A1* polymorphism
Parfenov MG	J Neurol Sci	2007	case-control	candidate gene	population based	European	208	rs769446, rs42938, and rs7412
Quarta G	J Investig Med	2007	case-control	candidate gene	population based	European	451	rs708272
Saidi S	J Stroke Cerebrovasc Dis	2007	case-control	candidate gene	population based	African	253	rs1799768
Slowik A	Cerebrovasc Dis	2007	case-control	candidate gene	population based	European	276	rs7493
Stanzione R	Am J Hypertens	2007	case-control	candidate gene	population based	European	580	rs1042714
Tseng CH	Eur J Clin Invest	2007	case-control	candidate gene	population based	Asian	450	rs4646994
Voetsch B	Stroke	2007	case-control	canddiate gene	population based	European	246	*GPx-3* gene promoter haplotype
Volcik KA	Atherosclerosis	2007	cohort	canddiate gene	population based	African	3330	rs2228315
Worrall BB	Stroke	2007	case-control	candidate gene	population based	European	886	rs419598
Zhang Y	Clin Chim Acta	2007	case-control	candidate gene	population based	Asian	285	*GP* Iba VNTR polymorphism

Abboud S	Eur J Hum Genet	2008	case-control	candidate gene	population based	European	563	rs405509 and rs440446
Banerjee	Brain Res Bull	2008	case-control	candidate gene	population based	Asian	388	rs1800587 and rs966221
Can Demirdöğen B	Clin Biochem	2008	case-control	candidate gene	population based	European	186	rs662
Cheng J	Acta Neurol Scand	2008	case-control	candidate gene	population based	Asian	618	rs2229765
Cheng J	Clin Chim Acta	2008	case-control	candidate gene	population based	Asian	618	rs1800779 and rs2070744
Fatar M	Cerebrovasc Dis	2008	case-control	candidate gene	population based	European	340	rs1030868, rs2241145, rs2287074, rs2287076, and rs7201
Fava C	Hypertension	2008	cohort	candidate gene	population based	European	5753	rs2108622
Genius J	Cerebrovasc Dis	2008	case-control	candidate gene	population based	European	297	rs4673
Gretarsdottir S	Ann Neeurol	2008	case-control	GWAS	population based	European	36370	rs2200733
Gschwendtner A	Stroke	2008	case-control	candidate gene	population based	European	1337	rs751141, rs7357432, and rs2291635
Hagiwara N	Eur J Neurol	2008	case-control	candidate gene	population based	Asian	1492	rs2281939
Han SH	Blood Coagul Fibrinolysis	2008	case-control	candidate gene	population based	Asian	284	rs7950273
Jood K	J Thromb Haemost	2008	case-control	candidate gene	population based	European	1200	rs6050, rs2070011, rs2066864, rs1049636 and rs1800792
Kohsaka S	Atherosclerosis	2008	cohort	candidate gene	population based	African	3462	rs20417
Lazaros L	Acta Neurol Scand	2008	cross-sectional	candidate gene	population based	European	370	rs2234693 and rs9340799
Lin Y	BMC Med Genet	2008	case-control	candidate gene	population based	Asian	513	rs4903565
Maasz A	Circ J	2008	case-control	candidate gene	population based	Asian	509	rs662799
Maász A	J Neurol	2008	case-control	candidate gene	population based	European	295	rs3135506
Mararin M	Stroke	2008	case-control	candidate gene	population based	European	517	rs10116277 and rs1333042
Matarin M	J Cereb Blood Flow Metab	2008	case-control	candidate gene	population based	European	747	rs3756541 and rs2303124
Matarin M	Stroke	2008	case-control	candidate gene	population based	European	517	rs1333040 and rs2383207
Moe KT	Eur J Neurol	2008	case-control	candidate gene	population based	Asian	327	rs1801133

Möllsten A	J Hypertens	2008	case-control	candidate gene	population based	European	824	rs4646994 and rs5186
Morrison AC	Cerebrovasc Dis	2008	cohort	candidate gene	population based	African	3814	rs3213646, rs1042164, rs7439293, and rs11628722
Munshi A	J Neurol Sci	2008	case-control	candidate gene	population based	Asian	624	rs4646994
Naganuma T	Hereditas	2008	case-control	candidate gene	population based	Asian	227	rs699473
Pruissen DM	Blood	2008	case-control	candidate gene	population based	European	957	rs6003
Saidi S	Cerebrovasc Dis	2008	case-control	candidate gene	population based	Asian	634	rs1801106 and rs5918
Shi C	Clin Chim Acta	2008	case-control	candidate gene	population based	Asian	196	*eNOS* 4ab variant
Smith NL	J Thromb Haemost	2008	case-control	candidate gene	population based	European	3057	rs1800291 and rs1936645
Trompet S	Exp Gerontol	2008	cohort	candidate gene	population based	European	5389	rs1041981
Yamada Y	Int J Mol Med	2008	case-control	candidate gene	population based	Asian	1284	17 loci from 8 genes
Yamada Y	Stroke	2008	case-control	candidate gene	population based	Asian	2892	rs1800977, rs3027898, rs1059703, and
Zafarmand MH	Hypertension	2008	cohort	candidate gene	population based	European	15236	rs4961
Zhang K	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2008	case-control	candidate gene	population based	Asian	588	rs662799
Zhang L	Pharmacogenet Genomics	2008	case-control	candidate gene	population based	Asian	550	rs751141
Al-Allawi NA	Neurol India	2009	case-control	candidate gene	population based	Asian	120	rs1801133
Almawi WY	J Stroke Cerebrovasc Disease	2009	case-control	candidate gene	population based	Asian	238	rs1801133
Celiker G	Clin Appl Thromb Hemost	2009	case-control	candidate gene	population based	European	269	rs1799752
Chen J	Clin Sci(Lond)	2009	case-control	candidate gene	population based	Asian	2001	rs3739390
DE Gaetano M	J Thromb Haemost	2009	case-control	candidate gene	population based	European	815	rs1324214
Demirdöğen BC	Cell Biochem Funct	2009	case-control	candidate gene	population based	European	277	rs705379
Goracy I	J Appl Genet	2009	case-control	candidate gene	population based	European	285	rs1801133
Greisenegger S	Clin Chem	2009	case-control	candidate gene	population based	European	918	rs17611

Gschwendtner A	Ann Neurol	2009	case-control	candidate gene	population based	European	5226	rs7044859, rs496892, rs564398, rs7865618, rs1537378, rs2383207, and rs10757278
Gudbjartsson DF	Nat Genet	2009	case-control	candidate gene	population based	European	46133	rs7193343
Haidari M	Cerebrovasc Dis	2009	case-control	candidate gene	population based	European	712	*E-selectin* S128R and L554F polymorphisms
Hsieh FI	Diabetes Care	2009	case-control	candiate gene	population based	Asian	1074	*PPARgamma* C2821T
Hu WL	Brain Res Bull	2009	case-control	candiate gene	population based	Asian	536	rs3731245 and rs2383206
Ikram MA	N Engl J Med	2009	cohort	GWAS	population based	European	19602	rs11833579 and rs12425791
Karvanen J	Genet Epidemiol	2009	case-control	candidate loci	population based	European	33282	rs1333049 and rs11670734
Kim NS	Clin Biochem	2009	case-control	candidate gene	population based	Asian	504	*PON1* 1266G/A
Li XX	Mol Biol Rep	2009	case-control	candidate gene	population based	Asian	618	rs5498
Luke MM	Cerebrovasc Dis	2009	case-control	candidate gene	population based	European	1377	rs10757274, rs20455, rs3900940, and rs1010
Luke MM	Stroke	2009	case-control	candidate gene	population based	Mixed	5244	7 SNPs among White, 5 SNPs among Black
MacClellan LR	Stroke	2009	case-control	candidate gene	population based	European	327	rs10478723, rs1800542, rs10507875 and rs4885493
Munshi A	J Neurol Sci	2009	case-control	candidate gene	population based	Asian	500	rs966221
Naqanuma T	Clin Biochem	2009	case-control	candidate gene	population based	Asian	486	rs1760944, rs3136814, and rs1130409
Park SA	BMB Rep	2009	case-control	candidate gene	population based	Asian	713	rs12267682
Rezaii AA	Immunol Invest	2009	case-control	candidate gene	population based	Asian	309	rs380092
Saidi S	Acta Neurol Scand	2009	case-control	candidate gene	population based	African	773	rs699 and rs5051
Shimada T	Diabetes Res Clin Pract	2009	cross-sectional	candidate gene	population based	Asian	874	rs237025
Sieqerink B	J Thromb Haemost	2009	case-control	candidate gene	population based	European	957	rs6050
Smith JG	Circ Cardiovasc Genet	2009	case-control	candidate loci	population based	European	4565	rs10757274, rs2383207, and rs1333049
Sun JZ	Neurol India	2009	case-control	candidate gene	population based	Asian	215	rs1801133

Sun Y	Clin Sci(Lond)	2009	case-control	candidate gene	population based	Asian	1410	rs966221
Szolnoki Z	Clin Neurol Neurosurg	2009	case-control	candidate gene	population based	European	688	rs7291467 and rs909253
Um JY	J Mol Neurosci	2009	case-control	candidate gene	population based	Asian	801	rs2107538
Wahlstrand B	J Hypertens	2009	cohort	candidate gene	population based	European	5262	rs10757278
Wang B	J Neurol Sci	2009	case-control	candidate gene	population based	Asian	792	rs429358/rs7412
Wang Q	Acta Pharmacol Sin	2009	case-control	candidate gene	population based	Asian	1128	rs2794521
Wang XB	Zhongguo Shi Yan Xue Ye Xue Za Zhi	2009	case-control	candidate gene	population based	Asian	760	*PROCR* A6936G
Wang Z	Hypertens Res	2009	case-control	candidate gene	population based	Asian	2492	rs4944832
Wei YS	DNA Cell Biol	2009	case-control	candidate gene	population based	Asian	545	rs281865545
Wu L	Stroke	2009	case-control	candidate gene	population based	Asian	2383	rs2230500
Xu H	Clin Sci(Lond)	2009	case-control	candidate gene	population based	Asian	1140	rs966221
Yamada Y	Atherosclerosis	2009	case-control	GWAS	population based	Asian	6341	rs6007897, rs4044210, rs1671021, and rs1062708
Yamaguchi M	Med Sci Monit	2009	case-control	candidate gene	population based	Asian	466	rs670950
Zhang W	Stroke	2009	case-control	candidate gene	population based	Asian	3638	rs2305948 and rs2071559
Bai CH	J Biomed Sci	2010	case-control	candidate gene	family based	Asian	347	rs3074372
Buraczynska K	Clin Biochem	2010	case-control	candidate gene	population based	European	484	rs1024611
Chen J	Hum Mol Genet	2010	case-control	candidate gene	population based	Asian	1328	rs2507800
Chen K	Beijing Da Xue Xue Bao	2010	case-control	candidate gene	population based	Asian	1490	rs11833579
Deng S	Prog Neuropsychopharmacol Biol Psychiatry	2010	case-control	candidate gene	population based	Asian	652	rs2108622
Ding H	Circ Res	2010	case-control	candidate gene	population based	Asian	6885	*DDAH1* 4 bp -/+
Ding H	Pharmacogenet Genomics	2010	case-control	candidate gene	population based	Asian	2360	*CYP4A11* C296T and *CYP4F2* V433M

Ding H	Stroke	2010	case-control	candidate loci	population based	Asian	1680	rs10204475, rs10486776, and rs11052413
Domingues-Montanari S	Atherosclerosis	2010	case-control	candidate gene	population based	European	799	rs10947803
Domingues-Montanari S	Cerebrovasc Dis	2010	case-control	candidate gene	population based	European	1873	*ALOX5AP* SG13S114
Fava C	Pharmacogenet Genomics	2010	cohort	candidate gene	population based	European	5875	rs41507953
Freitas RN	Eur J Cardiovasc Prev Rehabil	2010	cohort	candidate gene	population based	European	20835	rs17238540
Giusti B	Thromb Haemost	2010	case-control	candidate gene	population based	European	1712	rs10037045, rs682985, rs1051319, rs202680, rs2274976, rs1979277, and rs20721958
Isordia-Salas I	Cerebrovasc Dis	2010	case-control	candidate gene	population based	Mexican	361	rs1801133
Kim NS	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	1350	rs16147
Kuhlenbaeumer G	Cerebrovasc Dis	2010	case-control	candidate gene	population based	European	3338	rs3093075, rs1130864, and rs1800947
Lai CQ	Am J Clin Nutr	2010	case-control	candidate gene	population based	European	1147	rs3851059 and rs7087728
Lee BC	Neurol Res	2010	case-control	candidate gene	population based	Asian	297	rs7535475 and rs7512140
Lee JD	Neurol Res	2010	case-control	candidate gene	population based	Asian	292	rs2738446 and rs2738450
Li N	Brain Res Bull	2010	case-control	candidate gene	population based	Asian	742	rs1800587 and rs966221
Li Y	Pharmacogenet Genomics	2010	case-control	candidate gene	population based	Asian	468	rs4646994
Lopaciuk S	Blood Coagul Fibrinolysis	2010	case-control	candidate gene	population based	European	300	rs6046
Majumdar V	Biochem Biophys Res Commun	2010	case-control	candidate gene	population based	Asian	1034	rs9536314
Majumdar V	J Atheroscler Thromb	2010	case-control	candidate gene	population based	Asian	126	*eNOS* intron 4a/b polymorphism
Matsushita T	Hum Mol Genet	2010	cohort	candidate gene	population based	Asian	2637	rs4376531 and rs2280887
Munshi A	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	780	rs2234693
Munshi A	Cytokine	2010	case-control	candidate gene	population based	Asian	950	rs1800896
Munshi A	J Neurol Sci	2010	case-control	candidate gene	population based	Asian	797	rs1799998

Nakazato T	J Hum Hypertens	2010	case-control	candidate gene	population based	Asian	405	rs3754701, rs3769048, and rs7590387
Saidi S	Acta Neurol Scand	2010	case-control	candidate gene	population based	African	773	rs1799983
Saidi S	J Renin Angiotensin Aldosterone Syst	2010	case-control	candidate gene	population based	Asian	773	rs1799998
Shyu HY	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	232	rs1800566
Shyu HY	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	232	*NQO1* C609T
Szilvási A	Genet Test Mol Biomarkers	2010	case-control	candidate gene	population based	European	154	rs4148211
Tong Y	Biochem Biophys Res Commun	2010	case-control	candidate gene	population based	Asian	1296	rs1800796
Tong Y	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	1496	rs1800629
Volcik KA	Stroke	2010	cohort	canddiate gene	population based	Mixed	12284	rs1799969
Xu C	Stroke	2010	case-control	candidate loci	population based	Asian	3984	rs11206510
Yamaguchi M	Hereditas	2010	case-control	candidate gene	population based	Asian	479	rs10780199
Yoshida T	Int J Mol Med	2010	case-control	candidate gene	population based	Asian	1884	rs9925481 and rs4923918
Yu JT	Clin Chim Acta	2010	case-control	candidate gene	population based	Asian	873	rs16147
Zhang N	Brain Res Bull	2010	case-control	candidate gene	population based	Asian	427	rs1946518
Bondarenko EA	Genetika	2011	case-control	candidate gene	population based	European	847	rs152312
Cheng YC	G3(Bethesda)	2011	case-control	GWAS	population based	European	1816	rs1986743 and rs2304556
Cheong MY	Yonsei Med J	2011	case-control	candidate gene	population based	Asian	1260	rs822391 and rs822396
Dahlberg J	J Hypertens	2011	case-control	candidate gene	population based	European	4505	rs1057293 and rs1743966
Fu Y	Eur Neurol	2011	case-control	candidate gene	population based	Asian	278	rs699947, rs1570360, and rs3025039
Gouveia LO	Atherosclerosis	2011	case-control	candidate gene	population based	European	1091	rs6007897 and rs4044210
Hata J	Nihon Eiseigaku Zasshi	2011	case-control	GWAS	population based	Asian	2224	rs9943582, rs4376531, and rs2230500
Hirose T	Hypertens Res	2011	cohort	candidate gene	population based	Asian	529	rs6609080

Ji R	Cerebrovasc Dis	2011	case-control	candidate gene	population based	Asian	1005	*ALOX5AP* -581_582 Ins A polymorphism
Kalita J	Clin Chim Acta	2011	case-control	candidate gene	population based	Asian	386	rs4646994
Kim JO	Stroke	2011	case-control	candidate gene	population based	Asian	1109	rs699947
Lee JD	J Clin Neurosci	2011	case-control	candidate gene	population based	Asian	569	rs4293222 and rs4360791
Leu HB	Atherosclerosis	2011	cohort	candidate gene	population based	Asian	3330	rs1764391
Li X	Eur J Intern Med	2011	case-control	candidate gene	population based	Asian	652	rs662799
Li XM	Nan Fang Yi Ke Da Xue Xue Bao	2011	case-control	candidate gene	population based	Asian	500	rs189897
Liu F	Mol Biol Rep	2011	case-control	candidate gene	population based	Asian	640	rs266729
Liu SY	Neurol India	2011	case-control	candidate gene	population based	Asian	293	rs660895
Luk AO	Atherosclerosis	2011	cohort	candidate gene	population based	Aisan	1327	rs2290608, rs1062535, rs328, and rs1799983
Meschia JF	Stroke	2011	case-control	GWAS	family based	European	332	No SNP achieved genome-wide significance, there was clustering of the most associated SNPs on chromosomes 3p (NOS1) and 6p.
Milton AG	Int J Stroke	2011	case-control	candidate gene	population based	European	481	rs152312
Munshi A	Eur J Neurol	2011	case-control	candidate gene	population based	Asian	1025	rs1800629
Oh SH	J Neurol Sci	2011	case-control	candidate gene	population based	Asian	979	rs1870377
Olsson S	Eur J Neurol	2011	case-control	candidate gene	population based	European	1512	rs2277984, rs3745565, and rs7857345
Peng Z	Lipids Health Dis	2011	case-control	candidate gene	population based	Asian	331	rs1800470 and rs1800469
Qin X	Clin Chim Acta	2011	case-control	candidate gene	population based	Asian	703	rs2107538
Sun H	Mol Biol Rep	2011	case-control	candidate gene	population based	Asian	1017	rs9579646
They-They TP	Acta Neurol Scand	2011	case-control	candidate gene	population based	European	224	rs1801133
Tong Y	Pharmacogenet Genomics	2011	case-control	canddiate loci	population based	Asian	1296	rs11833579
Tu Y	Pharmacogenet Genomics	2011	case-control	candidate gene	population based	Asian	1115	rs1799998

Wan XH	J Neurol Sci	2011	case-control	candidate gene	population based	Asian	1673	rs10849373
Wnuk M	Neurol Neurochir Pol	2011	case-control	candidate loci	population based	European	729	rs2200733
Yan JT	Acta Pharmacol Sin	2011	case-control	candidate gene	population based	Asian	115	rs841
Yin YY	Neurol India	2011	case-control	candidate gene	population based	Asian	597	rs4376531
Zakai NA	J Thromb Haemost	2011	cohort	candidate gene	population based	European	5255	rs3093261, rs6046, rs3781387, rs4918851, rs4648004, and rs3138055
Zhao D	J Mol Neurosci	2011	case-control	candidate gene	population based	Asian	703	rs805297 and rs805296
Arrequi M	Gene	2012	cohort	candidate loci	population based	European	1891	rs2943634
Babu MS	Gene	2012	case-control	candidate gene	population based	Asian	1029	rs4646972
Carty CL	Circ Cardiovasc Genet	2012	cohort	candidate loci	population based	Mixed	38816	5 SNPs were significant in AA; 2 SNPs were significant in EA; 1 SNP was significant in AI.
Chai Y	CAN Neurosci Ther	2012	case-control	candidate gene	population based	Asian	844	rs659366
Chen X	J Thromb Haemost	2012	case-control	candidate gene	population based	Asian	2528	rs4731702
Esen FI	Neurol Res	2012	case-control	candidate gene	population based	Turkish	451	rs2243093
Gui G	J Neuroinflammation	2012	case-control	candidate gene	population based	Asian	2415	rs1799964 and rs1800629
Harbuzova Viu	Fiziol Zh	2012	case-control	candidate gene	population based	European	294	rs1800801
He Y	Chin Med J(Engl)	2012	case-control	candidate gene	population based	Asian	800	rs918592
Holliday EG	Nat Genet	2012	case-control	GWAS	population based	European	56816	rs556621
ISGC Consortium	Nat Genet	2012	case-control	GWAS	population based	European	14033	rs11984041, rs2200733, and rs19065993
Jiang J	Vasc Med	2012	case-control	candidate gene	population based	Asian	427	rs10304
Jin X	J Int Med Res	2012	case-control	candidate gene	population based	Asian	801	rs2228314
Kim DE	BMC Res Notes	2012	case-control	candidate gene	population based	Asian	164	rs11833579
Kim JH	DNA Cell Biol	2012	case-control	candidate gene	population based	Asian	941	rs5067, rs5065, rs198372, and rs198373

Kim SK	J Interferon Cytokine Res	2012	case-control	candidate gene	population based	Asian	784	rs520540, rs602128, and rs679620
Liu Y	Clin Exp Hypertens	2012	case-control	candidate gene	population based	Asian	658	rs696831
Manso H	Atherosclerosis	2012	case-control	candidate gene	population based	European	1081	rs2293050, rs2139733, rs7308402, and rs1483757
Markoula S	J Neurol Sci	2012	case-control	candidate gene	population based	European	854	rs4986938
Munshi A	Gene	2012	case-control	candidate gene	population based	Asian	1029	rs12153798 and rs702553
Munshi A	J Neurol Sci	2012	case-control	candidate gene	population based	Asian	1025	rs320
Munshi A	Mol Biol Rep	2012	case-control	candidate gene	population based	Asian	994	rs2108622
Olsson S	Stroke	2012	case-control	candidate gene	population based	European	6450	rs380092
Pedersen A	Thromb Res	2012	case-control	candidate gene	population based	European	1512	rs8176592 and rs8176541
Polonikov A	Hypertens Res	2012	case-control	candidate gene	population based	European	667	rs713041
Polonikov A	J Mol Neurosci	2012	case-control	candidate gene	population based	European	667	*GSTT1* deletion
Shetova IM	Zh Nevrol Psikhiatr Im S S Korsakova	2012	case-control	GWAS	population based	European	950	rs1842993
Shyu HY	J Atheroscler Thromb	2012	case-control	candidate gene	population based	Asian	431	rs13181
Traylor M	Lancet Neurol	2012	case-control	GWAS	population based	European	74393	verified 2 previous accosiations at $P<5\times10-8$, and identified 12 potentially novel loci at$P<5\times10-6$
Yan G	Neurol India	2012	case-control	candidate gene	population based	Asian	582	rs1799998
Yuan M	J Clin Neurosci	2012	case-control	candidate gene	population based	Asian	504	rs2299939
Zhang HF	Mol Biol Rep	2012	case-control	candidate gene	population based	Asian	1435	rs1800449
Zhang W	Stroke	2012	case-control	candidate loci	population based	Asian	3321	rs10757278
Zhao DX	J Neurol Sci	2012	case-control	candidate gene	population based	Asian	703	rs5361
Zhao J	J Mol Neurosci	2012	case-control	candidate gene	population based	Asian	450	rs2619112 and rs7217186

Zhao N	J Neuroinflammation	2012	case-control	candidate gene	population based	Asian	2287	rs4795895 in overall; rs1799864 and rs1799987 in hypertensive group; rs3744508, rs730012, rs569108, rs1800469, and rs909253 in non-hypertensive group.
Ataman OV	Tsitol Genet	2013	case-control	candidate gene	population based	European	394	rs1800801
Biscetti F	Hum Genet	2013	case-control	candidate gene	population based	European	856	rs2073618, rs2073617, and rs3134069
Chehaibi K	J Mol Neurosci	2013	case-control	candidate gene	population based	Tunisian	388	rs2016520
Chen CC	Clin Biochem	2013	case-control	candidate gene	population based	Asian	1003	rs689466
Cui X	Cell Physiol Biochem	2013	case-control	candidate gene	population based	Asian	809	rs2070600
Dai Y	ScientificWorldJournal	2013	case-control	candidate gene	population based	Asian	890	rs7308402
Demirdöğen BC	Hum Exp Toxicol	2013	case-control	candidate gene	population based	Turkish	339	rs4646903
Feng J	J Thromb Thrombolysis	2013	case-control	candidate gene	population based	Asian	770	rs3861950
Guo L	BMC Med Genet	2013	case-control	candidate gene	population based	Asian	360	rs4742170, rs1929992, and rs10975519
Han Y	Exp Biol Med (Maywood)	2013	case-control	candidate gene	population based	Asian	1263	rs2389995 and rs2240419
Hanson E	Plos one	2013	case-control	candidate gene	population based	European	1512	rs3733403, rs925451, and rs1593
Heckman MG	Eur J Neurol	2013	case-control	candidate gene	population based	Mixed	1704	5 SNPs among Whites and 4 SNPs among Black
Jeon YJ	Arterioscler Thromb Vasc Biol	2013	case-control	candidate gene	population based	Asian	1121	rs29101642 and rs2292832
Jing M	Cell Physiol Biochem	2013	case-control	candidate gene	population based	Asian	928	rs11730582
Kawai T	J Atheroscler Thromb	2013	cohort	candidate gene	population based	Asian	353	rs1501299
Li R	J Mol Neurosci	2013	case-control	candidate gene	population based	Asian	718	*ECE-1b* C-338A
Li Y	CNS Neurosci Ther	2013	case-control	candidate gene	population based	Asian	646	rs653765
Liu H	J Clin Neurol	2013	case-control	candidate gene	population based	Asian	380	rs40593
Liu ME	Atherosclerosis	2013	case-control	candidate gene	population based	Asian	1344	rs3735590

Lu JX	Braz J Med Biol Res	2013	case-control	candidate gene	population based	Asian	750	rs1946518
Lu X	Hum Genet	2013	case-control	candidate gene	population based	Asian	2538	rs199469469
Ma AJ	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2013	case-control	candidate gene	population based	Asian	360	rs679620, rs522616, and rs3025058
Ma S	Biochem Genet	2013	case-control	candidate gene	population based	Asian	730	rs4918
Ma Y	Gene	2013	case-control	candidate gene	population based	Asian	622	*CD40* -1C/T
Oh SH	Clin Neurol Neurosurg	2013	case-control	candidate gene	population based	Asian	1128	rs28365031
Olsson S	Eur J Neurol	2013	case-control	candidate gene	population based	European	833	rs1035798
Park HJ	Int J Immunogenet	2013	case-control	candidate gene	population based	Asian	387	rs3117604
Ross OA	Plos one	2013	case-control	candidate gene	population based	European	1144	rs78501403, rs61749020, and rs3815188
Rubattu S	Eur J Intern Med	2013	case-control	candidate gene	population based	European	703	*NPR3* 55C>A
Shimizu M	J Clin Neurosci	2013	case-control	candidate gene	population based	Asian	264	rs4987262
Stepanyan A	Neurosci Lett	2013	case-control	candidate gene	population based	European	255	rs628117
Sun H	Chin Med J(Engl)	2013	case-control	candidate gene	population based	Asian	1423	rs2029253 and rs6538697
Tong YQ	Genet Mol Res	2013	case-control	canddiate gene	population based	Asian	200	*IL-4* VNTR polymorphism
Türkanoğlu Özçelik A	Gene	2013	case-control	candidate gene	population based	Turkish	390	rs2266782 and rs2266780
Williams FM	Ann Neurol	2013	case-control	candidate loci	population based	European	13167	rs505922
Xie G	Plos one	2013	case-control	candidate gene	population based	Asian	1475	rs1554286, rs3021094, and rs1800872
Zhang J	Genet Test Mol Biomarkers	2013	case-control	candidate gene	population based	Asian	1402	rs6050 and rs1800790
Zhang LJ	Lipids Health Dis	2013	case-control	candidate gene	population based	Asian	663	rs12218
Zhang Z	Biomed Res Int	2013	case-control	candidate gene	population based	Asian	926	rs1800587
Zhao J	Neurol Sci	2013	case-control	candidate gene	population based	Asian	677	rs768963
Zhong G	BMC Med Genet	2013	case-control	candidate gene	population based	Asian	836	rs705381 and rs854571

Zhu R	J Mol Neurosci	2013	case-control	candidate loci	population based	Asian	749	rs1746048 and rs501120
Bai Y	stroke	2014	case-control	candidate gene	population based	Asian	3471	rs2043211
Cai J	Zhejiang Da Xue Xue Bao Yi Xue Ban	2014	case-control	candidate gene	population based	Asian	608	rs11611246
Cao Y	Int J Neurosci	2014	case-control	candidate loci	population based	Asian	2492	*PAI-1* 4G/5G polymorphism
Chi LF	Neuroreport	2014	case-control	candidate gene	population based	Asian	551	rs776746
Choi HJ	Diabetes Res Clin Pract	2014	case-control	candidate gene	population based	Asian	810	rs7903146
Cotlarciuc I	Stroke	2014	case-control	candidate gene	population based	European	74393	rs9379800, rs17271121, rs12664474, rs2287921, rs1801131, and rs566295
Dichgans M	Stroke	2014	case-control	GWAS	population based	European	161388	21 SNPs with genome-wide significance
Gu S	BMC Med Genet	2014	case-control	candidate gene	population based	Asian	594	rs4986893 and rs4244285
Jiang B	Stroke	2014	case-control	candidate gene	population based	European	823	rs1799963
Li C	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2014	case-control	candidate gene	population based	Asian	1037	rs2236307
Li Y	Lipids Health Dis	2014	case-control	candidate gene	population based	Asian	409	rs35814191
Luo M	CNS Neurosci Ther	2014	case-control	candidate loci	population based	Aisan	1486	rs2208454
Nakamura K	Eur J Neurol	2014	case-control	candidate gene	population based	Asian	475	*GLA* 196G>C
Papapostolou A	Gene	2014	case-control	candidate gene	population based	European	423	rs4769055, rs3803277, and rs202068154
Wen D	Mol Neurobiol	2014	case-control	candidate loci	population based	Mixed	3548	*MMP-1* -1607 1G/2G and *MMP-3* -1612 5A/6A
Wu G	Int J Neurosci	2014	case-control	candidate loci	population based	Asian	4650	*ITGA2* C807T
Xiong X	Hum Genet	2014	case-control	candidate gene	population based	Asian	5792	rs1122608
Xu X	Zhonghua Yi Xue Yi Chuan Xue Za Zhi	2014	case-control	candidate gene	population based	Asian	586	rs1927911 and rs2149356
Yin C	J Clin Neurosci	2014	case-control	candidate gene	population based	Asian	567	rs351855
Zee RY	Clin Sci(Lond)	2014	cohort	candidate gene	population based	European	23294	10 SNPs from *ARHGEF10* and *ROCK1*

Zhao J	J Neurol Sci	2014	case-control	candidate loci	population based	Mixed	47026	*ACE* I/D polymorphism
Zhu R	Neurol Res	2014	case-control	candidate gene	population based	Asian	742	rs13290387
Zhu Y	J Thromb Thrombolysis	2014	case-control	candidate loci	family based	Asian	458	rs11833579
Au A	Sci Rep	2015	case-control	candidate loci	population based	Mixed	4016	rs11053646 and rs505151
Auer PL	JAMA Neurol	2015	case-control	exome sequencing	population based	Mixed	6000	*PDE4DIP* and ACOT4 genes
Bazina A	Gene	2015	case-control	candidate loci	population based	European	301	*IL-6* -174G>C polymorphisms
Bi J	J Stroke Cerebrovasc Dis	2015	case-control	candidate loci	population based	Asian	234	rs10757278, rs1537378, and rs1333047
Buraczynska K	Neuromolecular Med	2015	case-control	candidate loci	population based	Asian	732	*MMP-9* C(-1562)T
Carty CL	Stroke	2015	case-control	GWAS	population based	African	14746	rs4471613
Dang M	Neuromolecular Med	2015	case-control	candidate loci	population based	Asian	996	rs6438833
Fan Y	PLoS One	2015	case-control	candidate loci	population based	Asian	1835	rs17222919
Gao T	J Renin Angiotensin Aldosterone Syst	2015	case-control	candidate loci	population based	Asian	4290	*AGT* M235T and T174M
Hanscombe KB	Stroke	2015	case-control	GWAS	population based	Mixed	111913	*FXIIIB*
He P	Genet Mol Res	2015	case-control	candidate loci	population based	Asian	240	*APM-1* +276G/T
Li Q	J Stroke Cerebrovasc Dis	2015	case-control	candidate loci	population based	Asian	769	rs1378577 and rs57137919
Lv Q	Genet Mol Res	2015	case-control	candidate loci	population based	Asian	440	*MTHFR* A1298C
Oliveira-Filho J	J Stroke Cerebrovasc Dis	2015	case-control	candidate loci	population based	European	140	rs20417
Pereira NL	Circ Cardiovasc Genet	2015	case-control	candidate gene	population based	European	1784	rs5063
Shao J	Curr Neurovasc Res	2015	case-control	candidate loci	population based	Asian	373	rs768963
Su L	J Mol Neurosci	2015	case-control	candidate loci	population based	Asian	1632	rs2200733, and rs6843082
Tang H	J Stroke Cerebrovasc Dis	2015	case-control	candidate loci	population based	Asian	206	*LEPR* Lys109Arg and Gln223Arg
Yang Q	Int J Clin Exp Pathol	2015	case-control	candidate loci	population based	Asian	1669	rs174546 and rs174601

Yang Q	Lipids Health Dis	2015	case-control	candidate loci	population based	Asian	1065	rs2902940
Yi X	Gene	2015	case-control	candidate loci	population based	Asian	396	rs17110453 and rs751141
Yi X	J Stroke Cerebrovasc Dis	2015	case-control	candidate loci	population based	Asian	774	rs10507391 and rs776746
Yu Y	J Renin Angiotensin Aldosterone Syst	2015	case-control	candidate loci	population based	Mixed	5883	*CYP11B2* -344C/T
Zhang B	Genet Test Mol Biomarkers	2015	case-control	candidate loci	population based	Asian	774	rs9333025
Zhang Z	J Neurol Sci	2015	case-control	candidate loci	population based	Asian	846	rs12425791 and rs11833579
Zhang Z	Mol Neurobiol	2015	case-control	candidate loci	population based	Asian	919	*PRKCH* 1425G/A
Zhu XY	Int J Neurosci	2015	case-control	candidate loci	population based	Asian	4440	rs1801133
Cheng YC	Stroke	2016	case-control	GWAS	population based	Mixed	35221	rs11196288
He XW	J Neurol Sci	2016	case-control	candidate loci	population based	Asian	1540	rs4076317
Hinds DA	Hum Mol Genet	2016	case-control	GWAS	population based	European	74393	rs9797861
Kumar P	J Stroke Cerebrovasc Dis	2016	case-control	candidate loci	population based	Asian	500	*IL-10* -1082G/A
Lee TH	J Am Heart Assoc	2016	case-control	GWAS	population based	Asian	4292	rs2415317, rs934075, rs944289, rs2787417, and rs1952706
Malik R	Neurology	2016	case-control	GWAS	population based	European	29633	rs532436, rs2107595, rs2723334, and rs12932445
CHARGE Consortium, SiGN, and ISGC Consortium	Lancet Neurol	2016	case-control	GWAS	population based	Mixed	155765	rs12204590
SiGN; ISGC Consortium	Lancet Neurol	2016	case-control	GWAS	population based	Mixed	435001	rs12122341
Qiu S	PLoS One	2016	case-control	candidate loci	population based	Asian	5728	*VEGFR* +1192C>T and +1719A>T
Song Y	J Stroke Cerebrovasc Dis	2016	case-control	candidate loci	population based	Mixed	11270	*MTHFR* C677T
Sung YF	Stroke	2016	case-control	candidate loci	population based	Asian	1660	ALDH2*2
Traylor M	Neurology	2016	case-control	GWAS	population based	European	1976	genes from OXPHOS pathway
Williams SR	Neurology	2016	case-control	GWAS	population based	European	74393	rs3093068, rs16842599, and rs11265260

Zhang Z	Int J Neurosci	2016	case-control	candidate loci	population based	Asian	16672	rs12425791
Au A	Atherosclerosis	2017	case-control	candidate loci	population based	Mixed	8149	rs662799, rs3135506, rs1801701, rs1042031, and rs2230806
He T	J Stroke Cerebrovasc Dis	2017	case-control	candidate loci	population based	Mixed	6356	rs3918242
He T	J Stroke Cerebrovasc Dis	2017	case-control	candidate loci	population based	Mixed	4357	*LPL HindIII* variant
Lee TH	Sci Rep	2017	case-control	candidate loci	population based	Asian	2073	rs2594966, rs2594973, and rs4684776
Liu X	Genet Test Mol Biomarkers	2017	case-control	candidate loci	population based	Asian	772	*IL-10*-1082A/G
Malik R	Proc Natl Acad Sci U S A	2017	case-control	exome sequencing	population based	Mixed	12905	rs6647 and rs2023938
Rannikmäe K	Neurology	2017	case-control	candidate loci	population based	Mixed	57422	rs9515201 and rs79043147
Rodríguez-Esparragón F	Int J Neurosci	2017	case-control	candidate loci	population based	European	305	*PON2* S311C
Williams SR	Stroke	2017	case-control	GWAS	population based	European	2100	rs505922
Zhang G	J Stroke Cerebrovasc Dis	2017	case-control	candidate loci	population based	Mixed	6312	*MMP-1* -1607 1G/2G and *MMP-12* -82 A/G
Zhang L	Thromb Haemost	2017	case-control	candidate loci	population based	Asian	715	rs3024735 and 2273971
Zhang Z	Mol Neurobiol	2017	case-control	candidate loci	population based	Asian	914	rs2682818
Alhazzani AA	J Stroke Cerebrovasc Dis	2018	case-control	candidate loci	population based	Mixed	24885	*Factor V* G1691A
Bao MH	J Stroke Cerebrovasc Dis	2018	case-control	candidate loci	population based	Mixed	2319	rs6265
Cole JW	PLoS One	2018	case-control	candidate loci	population based	Mixed	26473	rs9574 and rs2069951
Li S	Curr Neurovasc Res	2018	case-control	candidate loci	population based	Mixed	2245	rs10435816, rs7025417, rs11792633, and rs7044343
Luo L	Biosci Rep	2018	case-control	candidate loci	population based	Asian	535	rs774320676 and rs928508030
Malik R	Ann Neurol	2018	case-control	GWAS	population based	Mixed	896016	rs1799983, rs9521634, and rs720470
Malik R	Nat Genet	2018	case-control	GWAS	population based	Mixed	521612	22 novel loci were identified
Misra S	Gene	2018	case-control	candidate loci	population based	Mixed	7389	*MMP-9* (-1562C/T) and *MMP-12* (-1082 A/G)
Mortensen JK	Cerebrovasc Dis	2018	case-control	candidate loci	population based	European	1405	rs25531

Nie F	Curr Neurovasc Res	2018	case-control	candidate loci	population based	Mixed	27203	rs12425791
Rao AS	Circ Genom Precis Med	2018	case-control	candidate loci	population based	European	337536	rs11591147
Khounphinith E	Int J Med Sci	2019	case-control	candidate loci	population based	Asian	1765	rs6882076
Luo H	J Stroke Cerebrovasc Dis	2019	case-control	candidate loci	population based	Mixed	11785	*FGβ* -148 C/T and -455 G/A
Ren Z	Aging (Albany NY)	2019	case-control	candidate loci	population based	Asian	4802	rs7703688
Wei YS	J Cell Mol Med	2019	case-control	candidate loci	population based	Asian	1127	rs2240183
Wu Y	BMC Med Genet	2019	case-control	candidate loci	population based	Asian	972	rs3093193, rs3093144 and rs12459936
Xiao T	Crit Rev Eukaryot Gene Expr	2019	case-control	candidate loci	population based	Asian	1080	rs12425791 and rs11833579
Zheng PF	BMC Cardiovasc Disord	2019	case-control	candidate loci	population based	Asian	1783	rs7819412
Zheng Z	Sci Rep	2019	case-control	candidate loci	population based	Asian	1157	rs12415607
Zuo S	Medicine (Baltimore)	2019	case-control	candidate loci	population based	Mixed	5127	*IL-10*-1082A/G
Han X	Cytokine	2020	case-control	candidate loci	population based	Asian	1157	rs10757278 and rs9333358
Jaworek T	Stroke	2020	case-control	exome sequencing	population based	Mixed	1449	*NAT10* gene
Keene KL	Stroke	2021	case-control	GWAS	population based	African	22000	24 loci were identified
Ken-Dror G	Ann Neurol	2021	case-control	GWAS	population based	European	2087	37 SNPs within the 9q34.2 region
Kumar A	Neurology	2021	case-control	GWAS	population based	Asian	5697	1p21, 16q24, 3p26 and 16p13
Liu C	BMC Cardiovasc Disord	2021	case-control	candidate loci	population based	Asian	547	rs4646188
Liu X	J Gene Med	2021	case-control	candidate loci	population based	Asian	610	rs966221
Traylor M	Lancet Neurol	2021	case-control	GWAS	population based	Mixed	262136	rs72934535, rs4621303, rs2293576, rs12445022, and rs9958650
Wang Q	Medicine (Baltimore)	2021	case-control	candidate loci	population based	Asian	1119	rs4977574
Yuan H	J Cardiovasc Pharmacol	2021	case-control	candidate loci	population based	Asian	977	rs12037987 and rs10776752
Hu Y	Stroke	2022	case-control	WGS	population based	Mixed	33949	7q22, *AUTS2*, 13q33, *RAP1GAP2*, and *TEX13C*

Table S2. Characteristics of participants according to region at discovery and replication stages.

Characteristics*	Discovery study			Replication study		
	Middle (n=589)	North (n=1411)	*P* value	Middle (n=5883)	North (n=4340)	*P* value
Age, years	65.9 ± 19.6	63.0 ± 8.2	<0.001	65.3 ± 9.9	61.5 ± 9.8	<0.0001
Male, n (%)	297 (50.4)	845 (49.9)	0.0001	3276 (55.7)	2137 (55.2)	0.6619
Smoking, n (%)	116 (19.7)	608 (43.1)	<0.001	2032 (34.6)	1338 (34.7)	0.9306
Drinking, n (%)	125 (21.2)	638 (45.2)	<0.001	1723 (29.7)	1027 (26.7)	0.0027
SBP, mm Hg	145.8 ± 24.5	148.0 ± 24.7	0.0726	140.7 ± 21.2	148.2 ± 22.3	<0.0001
DBP, mm Hg	85.0 ± 14.5	86.3 ± 13.8	0.0590	84.8 ± 11.3	87.3 ± 13.4	<0.0001
BMI, kg/m^2	24.0 ± 3.2	24.4 ± 3.5	0.0138	23.8 ± 3.4	24.8 ± 3.2	<0.0001
TC, mg/dL	195.5 ± 41.0	204.4 ± 43.6	<0.0001	184.8 ± 42.6	202.3 ± 44.4	<0.0001
TG, mg/dL	131.6 ± 75.6	147.7 ± 96.3	<0.0001	147.7 ± 124.5	168.5 ± 144.7	<0.0001
LDL, mg/dL	100.5 ± 33.3	120.2 ± 37.6	<0.0001	106.5 ± 32.4	123.1 ± 52.7	<0.0001
HDL, mg/dL	55.1 ± 14.9	52.6 ± 16.1	0.0009	50.3 ± 13.9	57.5 ± 44.4	<0.0001
FPG, mg/dL	108.3 ± 38.8	113.2 ± 42.3	0.0123	104.3 ± 38.7	113.8 ± 40.8	<0.0001
Obesity†, n (%)	51 (10.1)	197 (14.5)	0.0139	407 (10.2)	529 (14.6)	<0.0001
History of hypertension, n (%)	246 (41.8)	660 (46.8)	0.0403	2706 (46.2)	1912 (50.8)	<0.0001
History of hyperlipidemia, n (%)	27 (4.6)	57 (4.0)	0.6248	269 (4.9)	242 (11.1)	<0.0001
History of diabetes, n (%)	42 (7.1)	145 (10.3)	0.0284	1075 (18.3)	386 (10.1)	<000001

*Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as number (percentage).
† Obesity was defined as BMI ≥ 28 kg/m^2

Table S3. Suggestive loci identified in the discovery stage main effects analyses (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	Beta	SE	*P* value
rs1801133	1	11856378	*MTHFR*	A/G	0.55	0.37	0.07	1.54E-08
chr1_156647165	1	156647165	*NES*	CG/C	0.06	0.64	0.14	7.88E-06
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.68	0.13	1.01E-07
rs4512220	6	44923138	*SUPT3H*	C/G	0.28	0.28	0.07	8.90E-05
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	0.67	0.15	7.26E-06
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	1.20	0.20	7.71E-10
chr8_27403038	8	27403038	*EPHX2*	GC/G	0.06	0.60	0.14	2.31E-05
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	0.85	0.19	8.80E-06
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	1.28	0.23	2.24E-08
rs60585534	14	65222652	*SPTB*	C/T	0.14	0.37	0.09	6.32E-05
chr14_100135024	14	100135024	*HHIPL1*	T/TG	0.02	1.08	0.27	4.72E-05
rs71116579	15	58885348	*LIPC; ADAM10*	A/ATTTTTTTTTTTTTTTTTTTTTT	0.11	0.46	0.11	5.11E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	0.68	0.15	3.70E-06
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	1.17	0.23	5.64E-07
chr17_46986377	17	46986377	*UBE2Z*	AC/A	0.03	0.85	0.21	6.07E-05
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	1.54	0.32	1.04E-06
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.71	0.15	1.09E-06

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S4. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with sex (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			2df joint tests P-value
						Beta	SE	P-value	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	0.12	0.13	3.69E-01	7.91E-08
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.09	0.29	7.60E-01	4.60E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	-0.50	0.26	5.71E-02	1.92E-07
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	-0.18	0.30	5.63E-01	3.96E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.05	0.40	8.98E-01	5.86E-09
chr8_27403038	8	27403038	*EPHX2*	GC/G	0.06	0.33	0.29	2.57E-01	6.69E-05
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	-0.23	0.40	5.68E-01	4.93E-05
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	0.24	0.46	6.00E-01	1.30E-07
chr14_100135024	14	100135024	*HHIPL1*	T/TG	0.02	0.94	0.55	9.01E-02	9.75E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	-0.14	0.30	6.33E-01	2.17E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	0.01	0.49	9.85E-01	3.65E-06
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	-0.15	0.64	8.09E-01	7.08E-06
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	-0.51	0.29	8.37E-02	2.53E-06

Table S5. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with body-mass index (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			2df joint tests P-value
						Beta	SE	P-value	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.004	0.02	8.43E-01	3.51E-07
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.04	0.05	3.82E-01	3.95E-05
rs4512220	6	44923138	*SUPT3H*	C/G	0.28	-0.02	0.02	5.04E-01	3.15E-05
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	0.08	0.06	1.24E-01	2.45E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	-0.04	0.06	5.70E-01	2.51E-06
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	-0.08	0.08	2.91E-01	1.67E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-0.10	0.07	1.77E-01	2.87E-05

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S6. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with fasting plasma glucose ($P < 1 \times 10^{-4}$)

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.05	0.05	3.23E-01	2.79E-07
rs386600283	1	94998750	*F3*	A/G	0.02	-0.42	0.10	5.96E-05	1.09E-05
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.15	0.09	9.96E-02	2.84E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.04	0.10	6.96E-01	2.96E-06
rs146404695	5	58571684	*PDE4D*	C/T	0.02	-0.37	0.09	1.61E-05	1.89E-05
rs2071303	6	26091336	*HFE*	T/C	0.35	-0.15	0.05	1.69E-03	5.52E-05
rs144146728	6	49403301	*MUT*	T/C	0.03	-0.37	0.09	5.00E-05	1.99E-04
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.00	0.16	9.99E-01	4.21E-08
rs7075480	10	100176627	*HPS1*	A/G	0.06	-0.31	0.08	6.89E-05	3.40E-04
rs12571249	10	100186959	*HPS1*	G/A	0.06	-0.31	0.08	9.07E-05	4.04E-04
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	0.11	0.20	5.95E-01	4.15E-07
rs11174397	12	62645916	*FAM19A2; USP15*	G/C	0.14	-0.28	0.06	1.04E-05	1.02E-05
rs11174398	12	62646348	*FAM19A2; USP15*	C/T	0.14	-0.28	0.06	9.92E-06	1.03E-05
rs11174399	12	62646556	*FAM19A2; USP15*	A/G	0.14	-0.28	0.06	1.04E-05	1.02E-05
rs117545816	12	112148254	*ACAD10*	A/G	0.05	-0.33	0.09	9.98E-05	3.80E-04
rs4646779	12	112237981	*ALDH2*	C/T	0.03	-0.37	0.09	1.78E-05	9.98E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	0.09	0.22	6.95E-01	4.37E-06
rs2250526	17	57951973	*TUBD1*	A/G	0.42	-0.20	0.05	4.29E-05	4.83E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	-0.14	0.09	1.44E-01	7.50E-06
rs41345851	20	23063779	*CD93*	T/C	0.15	-0.23	0.05	1.38E-05	7.82E-05
rs41418351	20	23064912	*CD93*	C/T	0.15	-0.21	0.05	8.44E-05	4.39E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S7. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with HDL-cholesterol (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	SE	*P-value*	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.24	0.19	1.90E-01	1.64E-07
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.06	0.36	8.78E-01	5.46E-05
rs3213768	2	53943696	*ASB3; GPR75-ASB3*	T/C	0.25	0.70	0.18	7.55E-05	1.07E-04
rs2287339	2	53992593	*ASB3; GPR75-ASB3*	T/A	0.25	0.71	0.18	5.19E-05	7.50E-05
rs76943041	2	53993382	*ASB3; GPR75-ASB3*	G/A	0.25	0.71	0.18	5.78E-05	6.52E-05
rs3755116	2	53993503	*ASB3; GPR75-ASB3*	A/G	0.25	0.70	0.18	7.28E-05	8.24E-05
rs3755115	2	53994160	*ASB3; GPR75-ASB3*	A/C	0.25	0.72	0.18	4.80E-05	6.03E-05
rs3755114	2	53994493	*ASB3; GPR75-ASB3*	G/A	0.25	0.72	0.18	4.22E-05	8.17E-05
rs76328173	2	54087470	*GPR75; GPR75-ASB3*	A/G	0.25	0.73	0.18	4.40E-05	3.86E-05
rs3755113	2	54088024	*GPR75*	T/C	0.25	0.69	0.18	1.03E-04	9.18E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.67	0.28	1.81E-02	1.56E-07
rs76224543	2	240061403	*HDAC4*	T/C	0.04	1.43	0.34	2.39E-05	5.30E-05
rs4687994	3	119013558	*ARHGAP31*	A/G	0.33	0.84	0.18	3.33E-06	1.62E-05
rs1194182	7	80231504	*CD36*	G/C	0.37	-0.86	0.19	4.66E-06	2.70E-05
rs2366855	7	80253455	*CD36*	A/T	0.38	-0.84	0.19	9.96E-06	5.74E-05
rs73167652	7	150700637	*NOS3*	G/A	0.08	1.24	0.27	3.45E-06	9.39E-06
rs3730305	7	150704400	*NOS3*	A/C	0.08	1.22	0.27	4.24E-06	1.38E-05
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	-0.61	0.42	1.44E-01	5.30E-05
rs2916715	8	6357307	*ANGPT2*	C/T	0.10	0.99	0.25	7.67E-05	1.26E-05
rs1961222	8	6377433	*ANGPT2*	T/C	0.08	1.00	0.26	1.01E-04	3.76E-05
rs328	8	19819724	*LPL*	G/C	0.08	1.12	0.28	5.98E-05	4.68E-05

rs11570891	8	19822810	*LPL*	T/C	0.08	1.11	0.28	6.17E-05	6.51E-05
rs1803924	8	19823674	*LPL*	T/C	0.08	1.09	0.28	8.30E-05	6.88E-05
rs3735964	8	19824045	*LPL*	A/C	0.08	1.09	0.28	8.06E-05	6.52E-05
rs1059611	8	19824563	*LPL*	C/T	0.08	1.10	0.28	7.13E-05	6.11E-05
rs10645926	8	19824626	*LPL*	CTT/C	0.08	1.10	0.28	7.05E-05	5.59E-05
rs15285	8	19824667	*LPL*	T/C	0.08	1.07	0.28	1.50E-04	5.36E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	-0.07	0.51	8.85E-01	3.84E-08
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	0.18	0.43	6.71E-01	9.91E-05
rs78414630	10	104842172	*CNNM2; NT5C2*	T/C	0.08	1.07	0.27	6.43E-05	3.33E-04
rs12573199	10	104848844	*NT5C2*	T/A	0.08	1.04	0.27	9.53E-05	4.93E-04
rs12573200	10	104848855	*NT5C2*	T/A	0.08	1.04	0.27	9.54E-05	4.89E-04
rs12573221	10	104849144	*NT5C2*	C/A	0.08	1.07	0.27	6.41E-05	3.39E-04
rs11191554	10	104855278	*NT5C2*	T/C	0.17	0.90	0.23	6.85E-05	1.21E-04
rs78436955	10	104900841	*NT5C2*	T/C	0.07	1.05	0.27	8.99E-05	4.65E-04
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	0.78	0.53	1.41E-01	3.65E-08
rs174528	11	61543499	*MYRF*	C/T	0.35	-0.53	0.20	9.85E-03	8.88E-06
rs174530	11	61546592	*MYRF*	G/A	0.34	-0.54	0.20	7.72E-03	2.86E-06
rs174533	11	61549025	*MYRF*	A/G	0.34	-0.53	0.21	1.01E-02	8.96E-06
rs174534	11	61549458	*MYRF*	G/A	0.34	-0.48	0.20	1.73E-02	1.44E-05
rs174535	11	61551356	*MYRF*	C/T	0.34	-0.53	0.20	1.00E-02	4.37E-06
rs174536	11	61551927	*MYRF*	C/A	0.34	-0.50	0.21	1.50E-02	8.94E-06
rs174537	11	61552680	*MYRF*	T/G	0.34	-0.51	0.21	1.28E-02	5.85E-06
rs174545	11	61569306	*FADS1*	G/C	0.33	-0.51	0.21	1.44E-02	9.95E-06
rs174546	11	61569830	*FADS1*	T/C	0.33	-0.53	0.21	9.99E-03	1.02E-05

rs174548	11	61571348	*FADS1*	G/C	0.33	-0.49	0.21	1.84E-02	1.01E-05
rs174549	11	61571382	*FADS1*	A/G	0.33	-0.47	0.21	2.33E-02	1.69E-05
rs174550	11	61571478	*FADS1*	C/T	0.33	-0.50	0.21	1.52E-02	7.72E-06
rs174560	11	61581764	*FADS1*	C/T	0.33	-0.47	0.21	2.46E-02	1.75E-05
rs174561	11	61582708	*MIR1908*	C/T	0.33	-0.46	0.21	2.59E-02	2.20E-05
rs174568	11	61593816	*FADS2*	T/C	0.33	-0.50	0.21	1.48E-02	1.85E-05
rs3834458	11	61594920	*FADS2*	C/CT	0.33	-0.51	0.21	1.38E-02	1.25E-05
rs174600	11	61622227	*FADS2*	C/T	0.35	-0.49	0.20	1.54E-02	1.86E-05
rs174601	11	61623140	*FADS2*	T/C	0.35	-0.43	0.20	3.56E-02	4.25E-05
rs97384	11	61624181	*FADS2*	T/C	0.35	-0.48	0.20	1.58E-02	6.67E-05
rs60585534	14	65222652	*SPTB*	C/T	0.14	0.48	0.20	1.45E-02	2.61E-05
rs3751542	15	58856033	*LIPC*	T/C	0.45	0.68	0.17	5.05E-05	2.43E-04
rs3829460	15	58857945	*LIPC*	T/A	0.45	0.72	0.17	1.73E-05	9.49E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	-0.78	0.47	9.66E-02	3.48E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-0.20	0.58	7.33E-01	1.68E-05
rs1061228	16	88782079	*PIEZO1*	A/G	0.15	0.76	0.20	9.51E-05	2.02E-04
chr17_7577678	17	7577678	*TP53*	CT/C	0.05	0.88	0.28	1.37E-03	3.80E-06
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	0.66	0.70	3.44E-01	6.57E-06
rs6105	18	61565062	*SERPINB2*	G/C	0.07	1.11	0.27	2.77E-05	6.94E-05
chr19_15997151	19	15997151	*CYP4F2*	C/CA	0.02	0.95	0.48	4.65E-02	8.31E-05
rs838136	19	49256388	*FUT1*	C/T	0.37	-0.84	0.19	7.69E-06	2.17E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.08	0.38	8.37E-01	7.29E-06
chr19_51323764	19	51323764	*KLK1*	G/GCAT	0.04	0.94	0.33	5.07E-03	3.93E-05

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S8. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with LDL-cholesterol (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	0.01	0.07	8.85E-01	5.17E-07
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.06	0.15	6.66E-01	5.35E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.21	0.13	1.04E-01	3.00E-06
rs9861471	3	79816891	*ROBO1*	C/G	0.37	-0.25	0.07	6.10E-04	6.99E-05
rs4512220	6	44923138	*SUPT3H*	C/G	0.28	0.16	0.08	4.21E-02	7.92E-05
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	0.20	0.16	2.33E-01	1.20E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.33	0.21	1.18E-01	2.92E-08
chr8_27403038	8	27403038	*EPHX2*	GC/G	0.06	0.35	0.16	2.79E-02	3.80E-05
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	0.18	0.24	4.67E-01	1.75E-07
chr14_86098827	14	86098827	*FLRT2; LINC02328*	A/AT	0.04	0.78	0.22	4.52E-04	6.49E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	0.04	0.27	8.93E-01	5.63E-06
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	-0.28	0.36	4.44E-01	1.44E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.05	0.16	7.51E-01	3.13E-05
chr22_19954915	22	19954915	*COMT*	C/CA	0.02	1.72	0.40	2.19E-05	1.22E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S9. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with triglycerides (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	0.11	0.13	4.28E-01	1.98E-07
chr1_156647165	1	156647165	*NES*	CG/C	0.06	0.21	0.28	4.62E-01	6.88E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	-0.03	0.26	9.10E-01	3.77E-06
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	0.11	0.31	7.13E-01	9.88E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	-0.07	0.43	8.63E-01	1.24E-08
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	0.76	0.44	8.61E-02	5.98E-05
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	0.58	0.50	2.47E-01	1.41E-07
rs174530	11	61546592	*MYRF*	G/A	0.34	-0.04	0.14	7.52E-01	7.63E-05
rs174535	11	61551356	*MYRF*	C/T	0.34	-0.05	0.14	7.25E-01	8.15E-05
rs174537	11	61552680	*MYRF*	T/G	0.34	-0.04	0.14	7.60E-01	9.30E-05
rs174550	11	61571478	*FADS1*	C/T	0.33	-0.06	0.14	6.66E-01	9.35E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	0.96	0.32	2.91E-03	3.14E-06
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-0.14	0.47	7.65E-01	5.24E-06
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	-0.28	0.66	6.71E-01	5.71E-06
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	-0.31	0.30	3.02E-01	1.57E-06

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S10. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with alcohol drinking ($P < 1\times10^{-4}$)

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.33	0.14	1.69E-02	6.22E-09
chr1_156647165	1	156647165	*NES*	CG/C	0.06	0.01	0.30	9.84E-01	2.34E-05
chr1_182077613	1	182077613	*ZNF648; LINC01344*	CT/C	0.07	-1.27	0.31	5.52E-05	2.67E-04
rs4148217	2	44099433	*ABCG8*	A/C	0.12	0.85	0.21	6.67E-05	1.61E-04
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.60	0.28	3.00E-02	2.36E-06
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	-0.38	0.32	2.34E-01	1.03E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.24	0.42	5.60E-01	1.82E-07
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	-0.16	0.50	7.56E-01	5.52E-06
rs3736211	12	24985637	*BCAT1*	C/T	0.12	0.83	0.21	8.36E-05	4.00E-04
rs60104197	12	24986679	*BCAT1*	A/G	0.12	0.85	0.21	4.84E-05	2.03E-04
rs12228257	12	24987129	*BCAT1*	A/G	0.12	0.83	0.21	7.37E-05	3.22E-04
rs3782886	12	112110489	*BRAP*	C/T	0.16	-0.88	0.23	1.19E-04	6.66E-05
rs4646776	12	112230019	*ALDH2*	C/G	0.16	-0.89	0.23	1.23E-04	8.51E-05
rs671	12	112241766	*ALDH2*	A/G	0.16	-0.95	0.23	5.47E-05	3.06E-05
rs78069066	12	112337924	*ADAM1A*	A/G	0.16	-0.83	0.23	2.45E-04	9.97E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	0.12	0.31	7.05E-01	4.41E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-0.71	0.51	1.61E-01	1.63E-06
rs2664593	17	4545132	*ALOX15*	G/C	0.19	0.69	0.18	9.30E-05	2.86E-04
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	-0.36	0.68	5.96E-01	9.09E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.14	0.30	6.41E-01	3.94E-06
chr20_33762783	20	33762783	*PROCR*	G/GTCT	0.11	-0.52	0.24	2.99E-02	4.98E-05

rs8094	23	15415583	*PIR*	T/C	0.49	-0.92	0.17	8.12E-08	1.18E-07
rs6632666	23	15496733	*PIR-FIGF*	T/C	0.48	-0.87	0.17	3.49E-07	2.17E-06
rs2285666	23	15610348	*ACE2*	C/T	0.46	-0.68	0.17	5.75E-05	2.62E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S11. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with cigarette smoking (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.22	0.14	1.12E-01	3.18E-08
chr1_156647165	1	156647165	*NES*	CG/C	0.06	-0.12	0.29	6.95E-01	3.86E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.23	0.28	3.94E-01	5.82E-07
chr4_2930427	4	2930427	*ADD1*	GC/G	0.08	1.01	0.30	8.73E-04	6.25E-06
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	-0.12	0.30	7.00E-01	3.41E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	1.03	0.48	3.03E-02	6.74E-09
chr8_27403038	8	27403038	*EPHX2*	GC/G	0.06	0.28	0.30	3.39E-01	9.38E-05
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	-0.06	0.38	8.76E-01	4.41E-05
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	-0.24	0.49	6.30E-01	1.65E-07
rs60585534	14	65222652	*SPTB*	C/T	0.14	-0.33	0.19	8.14E-02	8.08E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	0.21	0.31	5.01E-01	1.84E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-0.18	0.47	7.03E-01	3.39E-06
chr17_46986546	17	46986546	*UBE2Z*	TC/T	0.02	0.70	0.83	4.02E-01	9.81E-06
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.47	0.31	1.25E-01	2.74E-06
rs8094	23	15415583	*PIR*	T/C	0.49	-0.77	0.16	1.28E-06	1.96E-06
rs6632666	23	15496733	*PIR-FIGF*	T/C	0.48	-0.70	0.16	1.18E-05	6.42E-05
rs2285666	23	15610348	*ACE2*	C/T	0.46	-0.63	0.16	9.01E-05	3.73E-04
rs5194	23	115304830	*AGTR2*	A/G	0.38	-0.67	0.17	7.55E-05	1.92E-04
rs11091046	23	115305126	*AGTR2*	A/C	0.38	-0.68	0.17	5.39E-05	1.32E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S12. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with obesity (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.06	0.21	7.81E-01	5.66E-07
chr1_156647165	1	156647165	*NES*	CG/C	0.06	0.02	0.45	9.68E-01	4.30E-05
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	-0.49	0.41	2.34E-01	6.00E-05
rs4512220	6	44923138	*SUPT3H*	C/G	0.28	-0.30	0.22	1.76E-01	1.31E-05
chr8_1818398	8	1818398	*ARHGEF10*	G/GA	0.06	0.75	0.60	2.08E-01	1.84E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.19	0.63	7.63E-01	2.12E-06
chr11_61537791	11	61537791	*MYRF*	GC/G	0.03	-0.76	0.73	3.01E-01	3.47E-05
chr14_90864456	14	90864456	*CALM1*	C/CG	0.38	-0.61	0.23	8.03E-03	2.29E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	-1.29	0.65	4.60E-02	2.45E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	-0.45	0.44	3.01E-01	6.86E-05

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S13. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with history of hypertension ($P < 1 \times 10^{-4}$)

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs225125	1	8087006	*ERRFI1*	G/A	0.02	-0.90	0.72	2.11E-01	2.05E-06
chr2_102508323	2	102508323	*MAP4K4*	TG/T	0.08	0.69	0.48	1.46E-01	1.06E-06
chr5_131718063	5	131718063	*SLC22A5*	CA/C	0.04	-0.90	0.45	4.65E-02	1.53E-05
rs72456916	6	44795920	*SUPT3H*	A/AAAGTT	0.37	-0.74	0.18	6.15E-05	6.89E-05
rs9369514	6	44797271	*SUPT3H*	C/A	0.37	-0.72	0.18	8.02E-05	9.42E-05
rs4512220	6	44923138	*SUPT3H*	C/G	0.28	-0.59	0.19	2.22E-03	2.29E-05
chr8_27403032	8	27403032	*EPHX2*	GC/G	0.04	0.49	0.66	4.58E-01	1.61E-05
chr16_73095268	16	73095268	*ZFHX3; HCCAT5*	G/GC	0.03	1.03	1.06	3.33E-01	6.78E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	0.43	0.49	3.79E-01	3.32E-05

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S14. Suggestive loci identified in the discovery stage 1 df interaction and 2df joint tests with history of hyperlipidemia (P <1×10^{-4})

Variant	Chr	Position	Gene	CA/OA	CAF	1 df interaction test			*2 df joint test P-value*
						Beta	**SE**	***P-value***	
rs1801133	1	11856378	*MTHFR*	A/G	0.55	-0.40	0.35	2.64E-01	5.75E-08
chr1_156647165	1	156647165	*NES*	CG/C	0.06	1.28	1.09	2.41E-01	4.90E-05
chr10_104718215	10	104718215	*CNNM2*	T/TG	0.04	0.49	1.11	6.61E-01	7.50E-05
chr16_73095267	16	73095267	*ZFHX3; HCCAT5*	T/TGCC	0.06	0.37	0.84	6.63E-01	2.82E-05
chr19_51323750	19	51323750	*KLK1*	G/GAT	0.06	-0.92	0.80	2.49E-01	3.21E-06

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S15. Loci achieving $P<1\times10^{-4}$ in the discovery stage main effects analyses and selected for replication study

Variant	Chr	Position	Gene	CA/OA	CAF	Stage	Beta	SE	*P* value
rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	0.37	0.07	1.54E-08
					0.48	Replication	0.15	0.03	6.56E-07
						Meta	0.18	0.03	7.54E-12
rs174535	11	61551356	*MYRF*	C/T	0.34	Discovery	-0.27	0.07	1.35E-04
					0.37	Replication	-0.17	0.03	3.61E-08
						Meta	-0.18	0.03	4.81E-11
rs174545	11	61569306	*FADS1*	G/C	0.33	Discovery	-0.27	0.07	1.48E-04
					0.37	Replication	-0.17	0.03	1.98E-08
						Meta	-0.19	0.03	2.78E-11
rs3834458	11	61594920	*FADS2*	C/CT	0.33	Discovery	-0.27	0.07	1.85E-04
					0.36	Replication	-0.17	0.03	1.45E-08
						Meta	-0.19	0.03	2.30E-11
rs3782886	12	112110489	*BRAP*	C/T	0.16	Discovery	-0.09	0.09	2.88E-01
					0.20	Replication	-0.20	0.04	1.15E-07
						Meta	-0.18	0.03	1.22E-07
rs671	12	112241766	*ALDH2*	A/G	0.16	Discovery	-0.10	0.09	2.48E-01
					0.19	Replication	-0.21	0.04	2.44E-08
						Meta	-0.19	0.03	2.36E-08
rs78069066	12	112337924	*ADAM1A*	A/G	0.16	Discovery	-0.10	0.09	2.38E-01
					0.20	Replication	-0.19	0.04	1.60E-07
						Meta	-0.18	0.03	1.28E-07

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S16. Loci achieving P<1×10^{-4} in the discovery stage 1 df interaction or 2 df joint tests and selected for replication study

Environmental Variable	Variant	Chr	Position	Gene	CA/OA	CAF	Stage	Variant-environment interaction			2df joint test *P*-value
								Beta	SE	*P*-value	
Sex	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	0.12	0.13	3.69E-01	7.91E-08
						0.48	Replication	-0.11	0.06	6.36E-02	7.84E-07
							Meta-analysis	-0.07	0.05	1.81E-01	2.72E-11
Body-mass index	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.004	0.02	8.43E-01	3.51E-07
						0.48	Replication	-0.01	0.01	3.79E-01	4.12E-11
							Meta-analysis	-0.009	0.01	3.83E-01	3.92E-17
Fasting plasma glucose	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.05	0.05	3.23E-01	2.79E-07
						0.48	Replication	0.002	0.018	9.05E-01	8.55E-10
							Meta-analysis	-0.004	0.017	8.37E-01	1.08E-15
	rs146404695	5	58571684	*PDE4D*	C/T	0.02	Discovery	-0.37	0.09	1.61E-05	1.89E-05
						0.03	Replication	-0.05	0.05	2.53E-01	7.08E-02
							Meta-analysis	-0.13	0.04	2.05E-03	5.98E-03
	rs144146728	6	49403301	*MUT*	T/C	0.03	Discovery	-0.37	0.09	5.00E-05	1.99E-04
						0.02	Replication	-0.02	0.07	7.76E-01	9.01E-01
							Meta-analysis	-0.14	0.05	9.34E-03	2.56E-02
	rs7075480	10	100176627	*HPS1*	A/G	0.06	Discovery	-0.31	0.08	6.89E-05	3.40E-04
						0.07	Replication	0.03	0.04	4.36E-01	6.82E-01
							Meta-analysis	-0.04	0.03	2.93E-01	5.66E-01
	rs2250526	17	57951973	*TUBD1*	A/G	0.42	Discovery	-0.20	0.05	4.29E-05	4.83E-05
						0.41	Replication	0.03	0.02	1.52E-01	2.39E-01

							Meta-analysis	-0.002	0.02	9.01E-01	9.77E-01
	rs41345851	20	23063779	*CD93*	T/C	0.15	Discovery	-0.23	0.05	1.38E-05	7.82E-05
						0.15	Replication	0.01	0.03	7.49E-01	9.50E-01
							Meta-analysis	-0.04	0.02	1.13E-01	2.84E-01
	rs41418351	20	23064912	*CD93*	C/T	0.15	Discovery	-0.21	0.05	8.44E-05	4.39E-04
						0.15	Replication	0.004	0.025	8.74E-01	9.26E-01
							Meta-analysis	-0.03	0.02	1.27E-01	2.93E-01
HDL-cholesterol	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.24	0.19	1.90E-01	1.64E-07
						0.48	Replication	0.10	0.09	2.20E-01	4.68E-07
							Meta-analysis	0.04	0.08	5.69E-01	1.24E-11
	rs2287339	2	53992593	*ASB3; GPR75-ASB3*	T/A	0.25	Discovery	0.71	0.18	5.19E-05	7.50E-05
						0.26	Replication	0.12	0.10	2.27E-01	1.44E-01
							Meta-analysis	0.26	0.09	2.38E-03	1.50E-02
	rs76328173	2	54087470	*GPR75; GPR75-ASB3*	A/G	0.25	Discovery	0.73	0.18	4.40E-05	3.86E-05
						0.27	Replication	0.16	0.10	1.10E-01	6.88E-02
							Meta-analysis	0.29	0.09	7.89E-04	5.94E-03
	rs1194182	7	80231504	*CD36*	G/C	0.37	Discovery	-0.86	0.19	4.66E-06	2.70E-05
						0.36	Replication	-0.09	0.09	3.15E-01	4.81E-01
							Meta-analysis	-0.23	0.08	4.00E-03	1.25E-02
	rs73167652	7	150700637	*NOS3*	G/A	0.08	Discovery	1.24	0.27	3.45E-06	9.39E-06
						0.08	Replication	0.26	0.16	9.76E-02	2.54E-01
							Meta-analysis	0.52	0.14	1.41E-04	4.34E-04
	rs2916715	8	6357307	*ANGPT2*	C/T	0.10	Discovery	0.99	0.25	7.67E-05	1.26E-05
						0.09	Replication	-0.29	0.16	7.15E-02	1.96E-01

							Meta-analysis	0.09	0.14	5.30E-01	3.39E-01
	rs1961222	8	6377433	*ANGPT2*	T/C	0.08	Discovery	1.00	0.26	1.01E-04	3.76E-05
						0.07	Replication	-0.25	0.18	1.68E-01	3.55E-01
							Meta-analysis	0.16	0.15	2.68E-01	1.46E-01
	rs328	8	19819724	*LPL*	G/C	0.08	Discovery	1.12	0.28	5.98E-05	4.68E-05
						0.08	Replication	-0.02	0.15	8.73E-01	9.79E-01
							Meta-analysis	0.24	0.13	7.28E-02	5.56E-02
	rs1803924	8	19823674	*LPL*	T/C	0.08	Discovery	1.09	0.28	8.30E-05	6.88E-05
						0.08	Replication	-0.04	0.15	7.85E-01	9.63E-01
							Meta-analysis	0.22	0.13	9.42E-02	7.81E-02
	rs3735964	8	19824045	*LPL*	A/C	0.08	Discovery	1.09	0.28	8.06E-05	6.52E-05
						0.08	Replication	-0.04	0.15	7.77E-01	9.60E-01
							Meta-analysis	0.22	0.13	9.54E-02	7.78E-02
	rs1059611	8	19824563	*LPL*	C/T	0.08	Discovery	1.10	0.28	7.13E-05	6.11E-05
						0.08	Replication	-0.03	0.15	8.38E-01	9.20E-01
							Meta-analysis	0.23	0.13	8.15E-02	5.16E-02
	rs10645926	8	19824626	*LPL*	CTT/C	0.08	Discovery	1.10	0.28	7.05E-05	5.59E-05
						0.08	Replication	-0.07	0.16	6.36E-01	6.59E-01
							Meta-analysis	0.21	0.14	1.26E-01	1.88E-01
	rs15285	8	19824667	*LPL*	T/C	0.08	Discovery	1.07	0.28	1.50E-04	5.36E-05
						0.19	Replication	0.11	0.11	3.09E-01	5.72E-01
							Meta-analysis	0.24	0.10	1.96E-02	2.25E-02
	rs12573199	10	104848844	*NT5C2*	T/A	0.08	Discovery	1.04	0.27	9.53E-05	4.93E-04
						0.07	Replication	-0.19	0.17	2.73E-01	5.25E-01

							Meta-analysis	0.17	0.14	2.33E-01	4.79E-01
	rs12573200	10	104848855	*NT5C2*	T/A	0.08	Discovery	1.04	0.27	9.54E-05	4.89E-04
						0.07	Replication	-0.15	0.17	3.65E-01	6.32E-01
							Meta-analysis	0.19	0.14	1.79E-01	3.87E-01
	rs12573221	10	104849144	*NT5C2*	C/A	0.08	Discovery	1.07	0.27	6.41E-05	3.39E-04
						0.07	Replication	-0.19	0.17	2.70E-01	5.28E-01
							Meta-analysis	0.18	0.14	2.18E-01	4.62E-01
	rs174535	11	61551356	*MYRF*	C/T	0.34	Discovery	-0.53	0.20	1.00E-02	4.37E-06
						0.37	Replication	-0.02	0.09	7.97E-01	9.18E-09
							Meta-analysis	-0.11	0.08	1.97E-01	1.24E-12
	rs174545	11	61569306	*FADS1*	G/C	0.33	Discovery	-0.51	0.21	1.44E-02	9.95E-06
						0.37	Replication	-0.11	0.09	2.17E-01	2.07E-09
							Meta-analysis	-0.18	0.08	3.34E-02	1.67E-13
	rs3834458	11	61594920	*FADS2*	C/CT	0.33	Discovery	-0.51	0.21	1.38E-02	1.25E-05
						0.36	Replication	-0.13	0.09	1.68E-01	1.13E-09
							Meta-analysis	-0.19	0.08	2.36E-02	1.70E-12
	rs1061228	16	88782079	*PIEZO1*	A/G	0.15	Discovery	0.76	0.20	9.51E-05	2.02E-04
						0.14	Replication	0.17	0.12	1.61E-01	8.82E-02
							Meta-analysis	0.34	0.10	1.10E-03	4.83E-04
	rs6105	18	61565062	*SERPINB2*	G/C	0.07	Discovery	1.11	0.27	2.77E-05	6.94E-05
						0.06	Replication	-0.02	0.18	9.31E-01	4.58E-01
							Meta-analysis	0.33	0.15	2.51E-02	1.77E-02
LDL-cholesterol	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	0.01	0.07	8.85E-01	5.17E-07
						0.48	Replication	-0.04	0.03	9.47E-02	2.24E-07

							Meta-analysis	-0.04	0.03	1.32E-01	7.50E-12
Triglycerides	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	0.11	0.13	4.28E-01	1.98E-07
						0.48	Replication	-0.09	0.05	5.62E-02	9.04E-08
							Meta-analysis	-0.07	0.05	1.30E-01	2.31E-12
	rs174535	11	61551356	*MYRF*	C/T	0.34	Discovery	-0.05	0.14	7.25E-01	8.15E-05
						0.37	Replication	-0.04	0.05	4.78E-01	8.87E-08
							Meta-analysis	-0.04	0.05	4.30E-01	3.07E-11
Alcohol drinking	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.33	0.14	1.69E-02	6.22E-09
						0.48	Replication	0.24	0.07	2.01E-04	4.46E-09
							Meta-analysis	0.14	0.06	1.87E-02	2.50E-12
	rs4148217	2	44099433	*ABCG8*	A/C	0.12	Discovery	0.85	0.21	6.67E-05	1.61E-04
						0.11	Replication	0.08	0.10	4.40E-01	4.15E-01
							Meta-analysis	0.23	0.09	1.50E-02	6.54E-02
	rs3782886	12	112110489	*BRAP*	C/T	0.16	Discovery	-0.88	0.23	1.19E-04	6.66E-05
						0.20	Replication	-0.68	0.10	1.72E-12	2.68E-19
							Meta-analysis	-0.71	0.09	1.33E-15	2.51E-23
	rs671	12	112241766	*ALDH2*	A/G	0.16	Discovery	-0.95	0.23	5.47E-05	3.06E-05
						0.19	Replication	-0.73	0.10	9.20E-14	4.23E-21
							Meta-analysis	-0.76	0.09	3.39E-17	2.63E-25
	rs78069066	12	112337924	*ADAM1A*	A/G	0.16	Discovery	-0.83	0.23	2.45E-04	9.97E-05
						0.20	Replication	-0.68	0.10	1.20E-12	2.40E-19
							Meta-analysis	-0.71	0.09	1.55E-15	2.66E-23
	rs2664593	17	4545132	*ALOX15*	G/C	0.19	Discovery	0.69	0.18	9.30E-05	2.86E-04
						0.19	Replication	0.07	0.08	3.92E-01	4.62E-01

							Meta-analysis	0.19	0.08	1.39E-02	1.99E-02
Cigarette smoking	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.22	0.14	1.12E-01	3.18E-08
						0.48	Replication	0.20	0.06	9.81E-04	2.62E-08
							Meta-analysis	0.13	0.06	1.80E-02	7.04E-12
Obesity	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.06	0.21	7.81E-01	5.66E-07
						0.48	Replication	-0.10	0.11	3.91E-01	4.59E-11
							Meta-analysis	-0.09	0.10	3.76E-01	8.39E-17
History of hypertension	rs72456916	6	44795920	*SUPT3H*	A/AAAGTT	0.37	Discovery	-0.74	0.18	6.15E-05	6.89E-05
						0.37	Replication	0.06	0.07	4.08E-01	4.28E-01
							Meta-analysis	-0.04	0.07	5.14E-01	2.24E-01
	rs9369514	6	44797271	*SUPT3H*	C/A	0.37	Discovery	-0.72	0.18	8.02E-05	9.42E-05
						0.35	Replication	0.06	0.07	4.07E-01	4.04E-01
							Meta-analysis	-0.04	0.07	5.28E-01	2.07E-01
History of hyperlipidemia	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	-0.40	0.35	2.64E-01	5.75E-08
						0.48	Replication	-0.34	0.16	3.66E-02	6.13E-04
							Meta-analysis	-0.35	0.15	1.80E-02	7.17E-09

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S17. Association between identified variants and stroke according to drinking status and sex.

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	Drinkers					Non-drinkers			
							Male (n=2784)		Female (n=727)			Male (n=3719)		Female (n=4379)	
							OR (95% CI)	*P* value	OR (95% CI)	*P* value		OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs3782886*	12	112110489	*BRAP*	C/T	0.16	Discovery	0.34 (0.21, 0.55)	1.08E-05	0.70 (0.19, 2.60)	5.93E-01		0.84 (0.64, 1.11)	2.15E-01	1.26 (0.94, 1.71)	1.27E-01
					0.20	Replication	0.55 (0.45, 0.67)	4.76E-09	1.39 (0.85, 2.29)	1.91E-01		0.81 (0.72, 0.92)	8.39E-04	0.93 (0.82, 1.04)	1.92E-01
						Meta	0.51 (0.42, 0.61)	1.20E-12	1.28 (0.80, 2.03)	3.01E-01		0.82 (0.73, 0.91)	3.78E-04	0.96 (0.86, 1.07)	5.03E-01
rs671*	12	112241766	*ALDH2*	A/G	0.16	Discovery	0.29 (0.17, 0.47)	1.17E-06	0.97 (0.31, 3.01)	9.59E-01		0.86 (0.65, 1.13)	2.78E-01	1.26 (0.93, 1.72)	1.39E-01
					0.19	Replication	0.52 (0.42, 0.63)	2.77E-10	1.38 (0.84, 2.26)	2.09E-01		0.81 (0.72, 0.92)	9.04E-04	0.92 (0.82, 1.03)	1.61E-01
						Meta	0.47 (0.39, 0.57)	1.58E-14	1.30 (0.82, 2.05)	2.59E-01		0.82 (0.73, 0.92)	5.16E-04	0.96 (0.86, 1.07)	4.32E-01
rs78069066*	12	112337924	*ADAM1A*	A/G	0.16	Discovery	0.34 (0.21, 0.55)	1.08E-05	0.95 (0.31, 2.96)	9.32E-01		0.83 (0.63, 1.09)	1.78E-01	1.26 (0.93, 1.71)	1.37E-01
					0.20	Replication	0.54 (0.44, 0.66)	2.31E-09	1.40 (0.85, 2.30)	1.85E-01		0.82 (0.72, 0.93)	1.31E-03	0.92 (0.82, 1.04)	1.81E-01
						Meta	0.50 (0.42, 0.61)	5.35E-13	1.31 (0.83, 2.07)	2.38E-01		0.82 (0.73, 0.92)	4.95E-04	0.96 (0.86, 1.07)	4.72E-01

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S18. Comparison of variants main effects under additive, dominant and recessive models.

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	Additive model		Dominant model		Recessive model	
							OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	1.45 (1.26, 1.66)	1.54E-08	1.57 (1.29, 1.91)	6.46E-06	1.75 (1.39, 2.20)	1.63E-06
					0.48	Replication	1.16 (1.10, 1.23)	6.56E-07	1.19 (1.08, 1.30)	2.26E-04	1.25 (1.13, 1.37)	8.47E-06
						Meta-analysis	1.20 (1.13, 1.27)	7.54E-12	1.25 (1.15, 1.36)	1.62E-07	1.31 (1.20, 1.43)	2.38E-09
rs174535*	11	61551356	*MYRF*	C/T	0.34	Discovery	0.76 (0.67, 0.88)	1.35E-04	0.76 (0.63, 0.92)	3.71E-03	0.59 (0.44, 0.80)	5.09E-04
					0.37	Replication	0.84 (0.80, 0.89)	3.61E-08	0.80 (0.74, 0.87)	3.35E-07	0.80 (0.71, 0.90)	2.24E-04
						Meta-analysis	0.84 (0.79, 0.89)	4.81E-11	0.80 (0.74, 0.86)	5.00E-09	0.77 (0.69, 0.86)	2.31E-06
rs174545*	11	61569306	*FADS1*	G/C	0.33	Discovery	0.76 (0.67, 0.88)	1.48E-04	0.77 (0.64, 0.92)	4.74E-03	0.57 (0.42, 0.78)	3.70E-04
					0.37	Replication	0.84 (0.80, 0.89)	1.98E-08	0.80 (0.73, 0.87)	1.14E-07	0.80 (0.71, 0.90)	3.11E-04
						Meta-analysis	0.83 (0.78, 0.88)	2.78E-11	0.79 (0.74, 0.86)	1.07E-08	0.77 (0.69, 0.86)	3.14E-06
rs3834458*	11	61594920	*FADS2*	C/CT	0.33	Discovery	0.76 (0.67, 0.88)	1.85E-04	0.77 (0.65, 0.94)	8.00E-03	0.56 (0.41, 0.76)	2.05E-04
					0.36	Replication	0.84 (0.80, 0.89)	1.45E-08	0.80 (0.73, 0.87)	9.95E-08	0.80 (0.71, 0.90)	2.29E-04
						Meta-analysis	0.83 (0.78, 0.88)	2.30E-11	0.79 (0.74, 0.86)	2.69E-09	0.76 (0.68, 0.85)	1.70E-06
rs3782886†	12	112110489	*BRAP*	C/T	0.16	Discovery	0.91 (0.77, 1.09)	2.88E-01	0.90 (0.74, 1.10)	3.10E-01	0.86 (0.51, 1.43)	5.59E-01
					0.20	Replication	0.82 (0.76, 0.89)	1.15E-07	0.81 (0.74, 0.88)	1.40E-06	0.69 (0.56, 0.85)	4.79E-04

						Meta-analysis	0.84 (0.79, 0.89)	1.22E-07		0.82 (0.76, 0.89)	1.36E-06		0.71 (0.58, 0.86)	5.56E-04
rs671[†]	12	112241766	*ALDH2*	A/G	0.16	Discovery	0.90 (0.76, 1.08)	2.48E-01		0.89 (0.73, 1.09)	2.66E-01		0.85 (0.50, 1.43)	5.35E-01
					0.19	Replication	0.81 (0.75, 0.88)	2.44E-08		0.80 (0.73, 0.87)	2.40E-07		0.68 (0.55, 0.85)	5.48E-04
						Meta-analysis	0.83 (0.78, 0.88)	2.36E-08		0.81 (0.75, 0.88)	2.21E-07		0.71 (0.58, 0.86)	5.94E-04
rs78069066[†]	12	112337924	*ADAM1A*	A/G	0.16	Discovery	0.90 (0.76, 1.08)	2.38E-01		0.88 (0.72, 1.08)	2.21E-01		0.90 (0.57, 1.50)	6.89E-01
					0.20	Replication	0.83 (0.76, 0.89)	1.60E-07		0.81 (0.75, 0.89)	2.33E-06		0.68 (0.55, 0.84)	3.63E-04
						Meta-analysis	0.84 (0.79, 0.89)	1.28E-07		0.82 (0.76, 0.89)	1.44E-06		0.71 (0.59, 0.86)	5.55E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele; OR=odds ratio

[*] rs174535, rs174545, and rs3834458 were highly correlated, smallest R^2=0.99

[†] rs3782886, rs671, and rs78069066 were highly correlated, smallest R^2=0.98

Table S19. Comparison of variants interaction effects under additive, dominant and recessive models.

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	Drinkers			Non-drinkers			1df Interaction *P* value
							OR	95% CI	*P* value	OR	95% CI	*P* value	
Additive model													
rs3782886*	12	112110489	*BRAP*	C/T	0.16	Discovery	0.37	(0.24, 0.59)	2.06E-05	1.01	(0.82, 1.23)	9.60E-01	1.19E-04
					0.20	Replication	0.61	(0.51, 0.74)	1.93E-07	0.88	(0.81, 0.96)	2.68E-03	1.72E-12
						Meta	0.57	(0.48, 0.68)	1.25E-10	0.90	(0.83, 0.97)	5.96E-03	1.33E-15
rs671*	12	112241766	*ALDH2*	A/G	0.16	Discovery	0.34	(0.21, 0.54)	6.47E-06	1.01	(0.83, 1.24)	9.02E-01	5.47E-05
					0.19	Replication	0.58	(0.48, 0.70)	1.39E-08	0.88	(0.80, 0.95)	2.27E-03	9.20E-14
						Meta	0.54	(0.45, 0.64)	3.55E-12	0.89	(0.83, 0.97)	5.60E-03	3.39E-17
rs78069066*	12	112337924	*ADAM1A*	A/G	0.16	Discovery	0.39	(0.25, 0.62)	4.36E-05	0.99	(0.81, 1.22)	9.42E-01	2.45E-04
					0.20	Replication	0.61	(0.50, 0.73)	1.14E-07	0.88	(0.81, 0.96)	3.19E-03	1.20E-12
						Meta	0.57	(0.48, 0.67)	1.01E-10	0.90	(0.83, 0.97)	5.99E-03	1.55E-15
Dominant model													
rs3782886*	12	112110489	*BRAP*	C/T	0.16	Discovery	0.37	(0.23, 0.60)	4.26E-05	1.02	(0.80, 1.30)	8.81E-01	2.27E-04
					0.20	Replication	0.58	(0.47, 0.71)	2.93E-07	0.88	(0.79, 0.97)	1.15E-02	6.42E-12
						Meta	0.54	(0.45, 0.65)	2.21E-10	0.90	(0.82, 0.99)	2.28E-02	7.55E-15
rs671*	12	112241766	*ALDH2*	A/G	0.16	Discovery	0.32	(0.19, 0.52)	4.47E-06	1.03	(0.81, 1.31)	8.30E-01	4.80E-05
					0.19	Replication	0.55	(0.44, 0.67)	1.85E-08	0.87	(0.78, 0.96)	7.64E-03	3.51E-13
						Meta	0.50	(0.41, 0.61)	2.96E-12	0.89	(0.81, 0.98)	1.72E-02	1.04E-16
rs78069066*	12	112337924	*ADAM1A*	A/G	0.16	Discovery	0.37	(0.23, 0.60)	4.1E-05	0.99	(0.78, 1.26)	9.50E-01	3.25E-04
					0.20	Replication	0.57	(0.47, 0.71)	1.60E-07	0.88	(0.80, 0.98)	1.56E-02	3.60E-12
						Meta	0.53	(0.44, 0.65)	1.11E-10	0.90	(0.82, 0.99)	2.39E-02	5.55E-15
Recessive model													
rs3782886*	12	112110489	*BRAP*	C/T	0.16	Discovery[a]	NA	NA	NA	0.94	(0.54, 1.65)	8.35E-01	NA
					0.20	Replication	0.46	(0.24, 0.85)	1.41E-02	0.75	(0.59, 0.95)	1.60E-02	3.57E-04

						Meta	NA	NA	NA	0.77	(0.62, 0.96)	2.14E-02	NA
rs671*	12	112241766	*ALDH2*	A/G	0.16	Discovery	0.22	(0.03, 1.85)	1.62E-01	0.95	(0.53, 1.71)	8.69E-01	1.80E-01
					0.19	Replication	0.42	(0.22, 0.81)	9.64E-03	0.76	(0.60, 0.97)	2.44E-02	2.35E-04
						Meta	0.40	(0.21, 0.75)	3.94E-03	0.78	(0.63, 0.98)	3.21E-02	9.12E-05
rs78069066*	12	112337924	*ADAM1A*	A/G	0.16	Discovery	0.22	(0.03, 1.85)	1.63E-01	0.98	(0.56, 1.74)	9.56E-01	1.61E-01
					0.20	Replication	0.45	(0.24, 0.85)	1.38E-02	0.74	(0.59, 0.94)	1.19E-02	4.14E-04
						Meta	0.43	(0.23, 0.78)	5.85E-03	0.77	(0.62, 0.96)	1.91E-02	1.52E-04

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele; OR=odds ratio

[a]: For rs3782886, when using recessive model, there were no stroke cases with two mutations.

* rs3782886, rs671, and rs78069066 were highly correlated, smallest R^2=0.98

Table S20. Comparison of identified variants 1 df interaction effects using standard error and robust standard error.

Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	1df Interaction *P* value using standard error	1df Interaction *P* value using robust standard error
rs3782886	12	112110489	*BRAP*	C/T	0.16	Discovery	1.19E-04	3.83E-05
					0.20	Replication	1.72E-12	3.53E-13
						Meta	1.33E-15	8.91E-17
rs671	12	112241766	*ALDH2*	A/G	0.16	Discovery	5.47E-05	1.55E-05
					0.19	Replication	9.20E-14	1.44E-14
						Meta	3.39E-17	1.54E-18
rs78069066	12	112337924	*ADAM1A*	A/G	0.16	Discovery	2.45E-04	1.04E-04
					0.20	Replication	1.20E-12	2.31E-13
						Meta	1.55E-15	1.30E-16

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Table S21. Comparison of identified variants 2 df joint effects using standard error and robust standard error.

Environmental Variable	Variant	Chr	Position (Build 37)	Gene	CA/OA	CAF	Stage	2df Joint *P* value using standard error	2df Joint *P* value using robust standard error
Body-mass index	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	3.51E-07	2.17E-07
						0.48	Replication	4.12E-11	6.78E-11
							Meta-analysis	3.92E-17	4.90E-17
Fasting plasma glucose	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	2.79E-07	2.68E-07
						0.48	Replication	8.55E-10	1.26E-09
							Meta-analysis	1.08E-15	2.94E-15
HDL-cholesterol	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	1.64E-07	1.10E-07
						0.48	Replication	4.68E-07	8.64E-07
							Meta-analysis	1.24E-11	1.49E-11
	rs174535	11	61551356	*MYRF*	C/T	0.34	Discovery	4.37E-06	1.65E-05
						0.37	Replication	9.18E-09	6.68E-09
							Meta-analysis	1.24E-12	1.08E-12
	rs174545	11	61569306	*FADS1*	G/C	0.33	Discovery	9.95E-06	3.36E-05
						0.37	Replication	2.07E-09	2.59E-09
							Meta-analysis	1.67E-13	3.81E-13
	rs3834458	11	61594920	*FADS2*	C/CT	0.33	Discovery	1.25E-05	4.58E-05
						0.36	Replication	1.13E-09	1.60E-09
							Meta-analysis	1.70E-12	2.88E-13
LDL-cholesterol	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	5.17E-07	3.83E-07
						0.48	Replication	2.24E-07	2.19E-07
							Meta-analysis	7.50E-12	4.78E-12
Triglycerides	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	1.98E-07	2.04E-07
						0.48	Replication	9.04E-08	1.31E-08

							Meta-analysis	2.31E-12	4.27E-13
	rs174535	11	61551356	*MYRF*	C/T	0.34	Discovery	8.15E-05	6.54E-05
						0.37	Replication	8.87E-08	1.13E-07
							Meta-analysis	3.07E-11	5.93E-11
Alcohol drinking	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	6.22E-09	1.33E-08
						0.48	Replication	4.46E-09	1.34E-09
							Meta-analysis	2.50E-12	2.06E-11
	rs3782886	12	112110489	*BRAP*	C/T	0.16	Discovery	6.66E-05	1.04E-06
						0.20	Replication	2.68E-19	5.91E-23
							Meta	2.51E-23	6.83E-26
	rs671	12	112241766	*ALDH2*	A/G	0.16	Discovery	3.06E-05	2.82E-07
						0.19	Replication	4.23E-21	1.89E-25
							Meta	2.63E-25	7.69E-32
	rs78069066	12	112337924	*ADAM1A*	A/G	0.16	Discovery	9.97E-05	2.40E-06
						0.20	Replication	2.40E-19	4.81E-23
							Meta	2.67E-23	1.43E-28
Cigarette smoking	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	3.18E-08	3.98E-08
						0.48	Replication	2.62E-08	1.01E-08
							Meta-analysis	7.04E-12	1.98E-12
Obesity	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	5.66E-07	4.08E-07
						0.48	Replication	4.59E-11	9.72E-11
							Meta-analysis	8.39E-17	1.12E-16
History of hyperlipidemia	rs1801133	1	11856378	*MTHFR*	A/G	0.55	Discovery	5.75E-08	3.99E-08
						0.48	Replication	6.13E-04	6.47E-04
							Meta-analysis	7.17E-09	8.17E-09

CA=Coded allele; CAF= Coded allele frequency; Chr=chromosome; OA=Other allele

Figure S1. Circular Manhattan plots displaying variants achieving suggestive significance in the 1df interaction (inner circle) and 2df joint tests (outer circle). Red dashed lines indicate suggestive significance ($P<1.0\times10^{-4}$) in the discovery stage

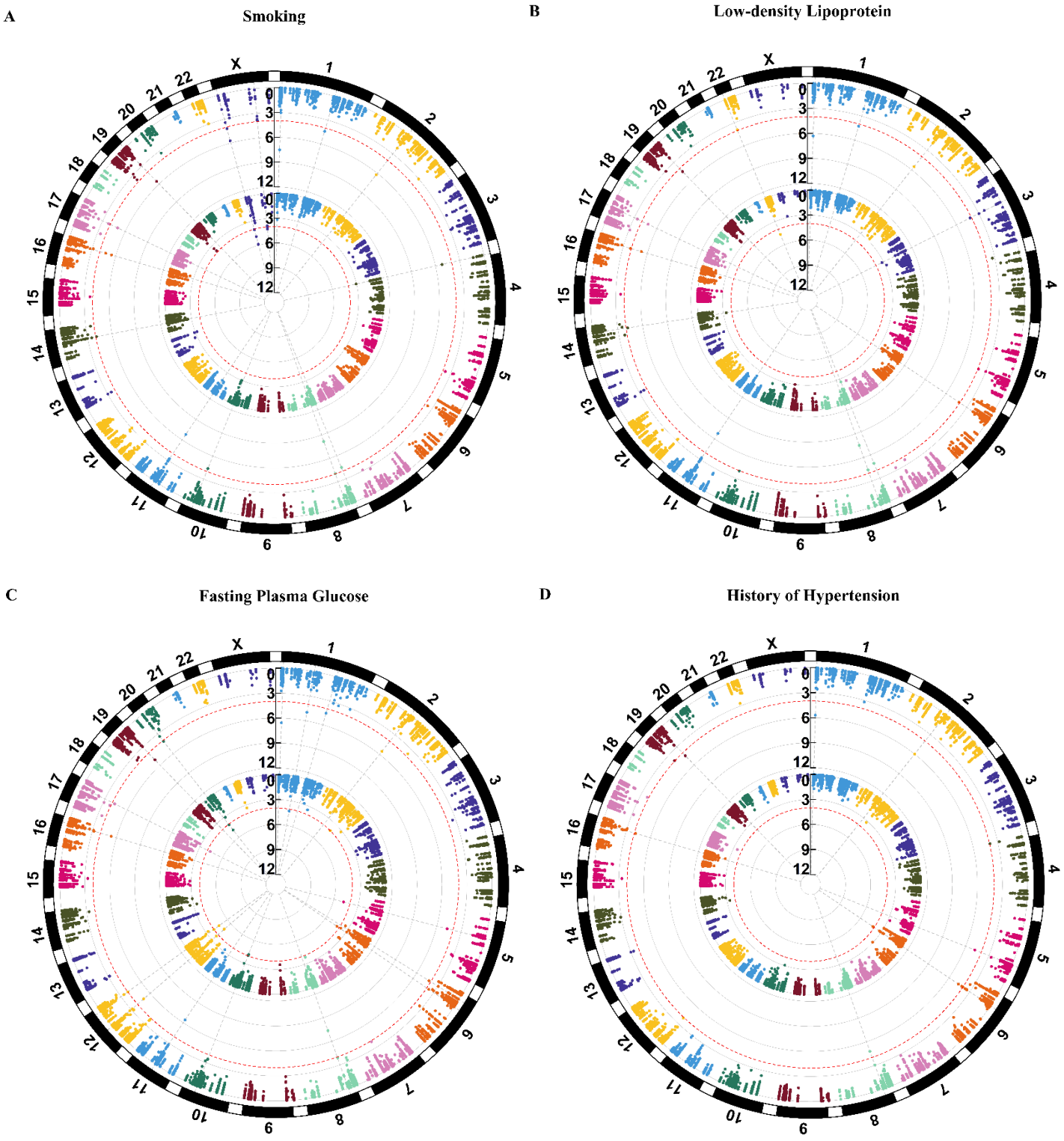
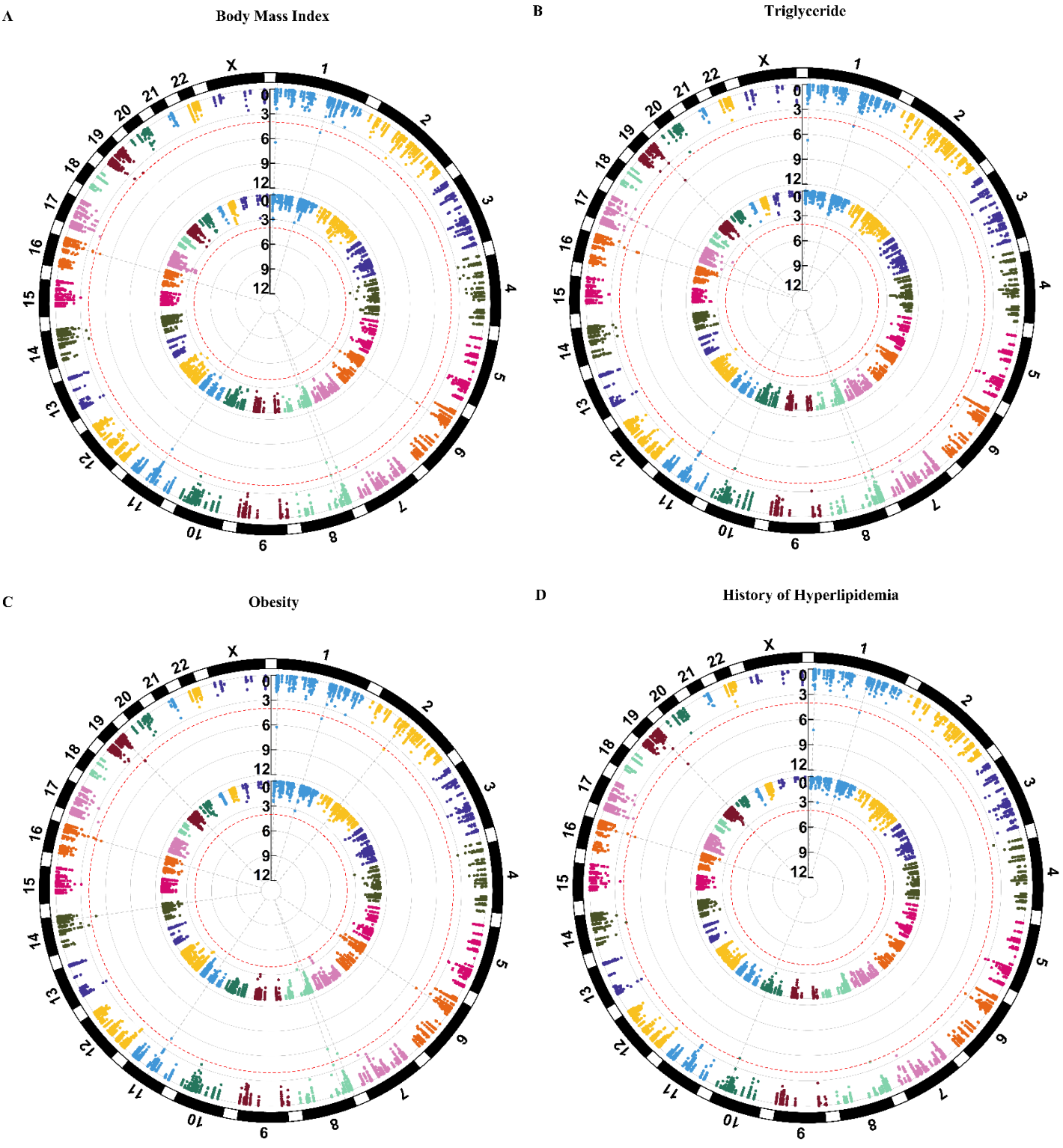


Figure S2. Circular Manhattan plots displaying variants achieving suggestive significance in only the discovery stage 2df joint test. Inner circle displays 1 df interaction tests and outer circle displays 2 df joint tests. Red dashed lines indicate suggestive significance (P<1.0×10^{-4}) in the discovery stage.



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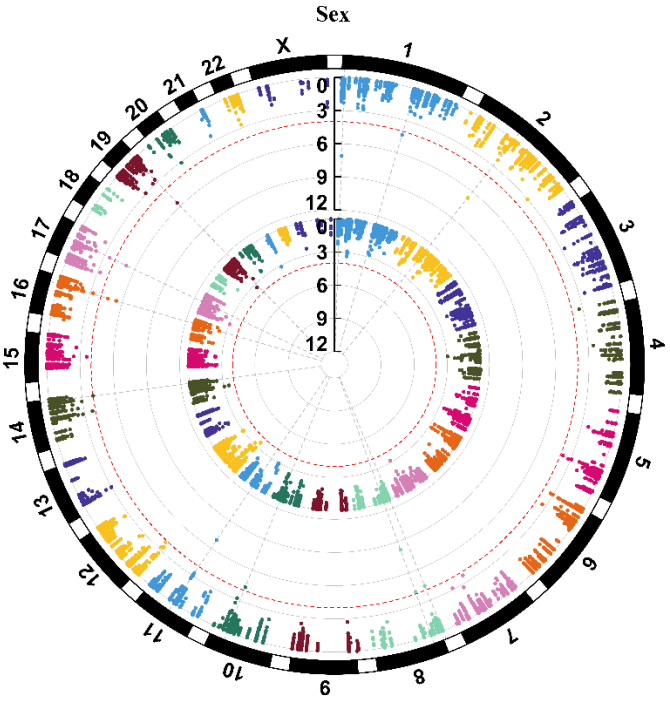
Sex
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