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Are Myocardial Infarction–Associated Single-Nucleotide Polymorphisms Associated With Ischemic Stroke?

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Background and Purpose—Ischemic stroke (IS) shares many common risk factors with coronary artery disease (CAD). We hypothesized that genetic variants associated with myocardial infarction (MI) or CAD may be similarly involved in the etiology of IS. To test this hypothesis, we evaluated whether single-nucleotide polymorphisms (SNPs) at 11 different loci recently associated with MI or CAD through genome-wide association studies were associated with IS.

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Methods—Meta-analyses of the associations between the 11 MI-associated SNPs and IS were performed using 6865 cases and 11 395 control subjects recruited from 9 studies. SNPs were either genotyped directly or imputed; in a few cases a surrogate SNP in high linkage disequilibrium was chosen. Logistic regression was performed within each study to obtain study-specific βs and standard errors. Meta-analysis was conducted using an inverse variance weighted approach assuming a random effect model.

Results—Despite having power to detect odds ratio of 1.09–1.14 for overall IS and 1.20–1.32 for major stroke subtypes, none of the SNPs were significantly associated with overall IS and/or stroke subtypes after adjusting for multiple comparisons.

Conclusions—Our results suggest that the major common loci associated with MI risk do not have effects of similar magnitude on overall IS but do not preclude moderate associations restricted to specific IS subtypes. Disparate mechanisms may be critical in the development of acute ischemic coronary and cerebrovascular events. **(*Stroke*. 2012; 43:980-986.)**

Key Words: cerebral infarct ■ genetics ■ ischemia

Ischemic strokes (IS) comprise 87% of all strokes.[1] Nearly 800 000 individuals in the United States have a stroke each year, making stroke one of the leading causes of serious, long-term disability in developed countries.[1,2] The tendency of stroke to aggregate within families implies a genetic etiology, although few genetic variants have as yet been unequivocally linked to the common forms of IS.

Some of the difficulty in identifying stroke susceptibility genes may arise from heterogeneity within the stroke phenotype, making it imperative to carry out genetic studies within specific subtypes of IS. IS syndromes can, in fact, be recognized by experienced clinicians with a high degree of inter-rater agreement.[3] The major categories, which are based on the presumed stroke mechanism, include atherosclerotic (ie, large-artery) stroke, cardioembolic stroke, and small-vessel (lacunar) stroke. Given disparate mechanisms, it is likely that different risk alleles would contribute to these different stroke subtypes. Previous studies showing that a family history of stroke is more common in younger compared with older age of onset strokes[4–6] raise the additional possibility that the genetic contribution to stroke may differ by age of onset.

IS shares many risk factors with myocardial infarction (MI) and coronary artery disease (CAD), including smoking and high blood pressure. The possibility that common susceptibility genes might predispose to both IS and MI/CAD prompted us to examine whether genetic variants previously associated with MI/CAD might also be associated with IS. To address this hypothesis, we evaluated whether DNA sequence variants at 11 different loci recently associated with MI or CAD through genome-wide association (GWA) studies were also associated with IS. Our analysis included 6865 IS cases from 9 different studies, allowing us to test whether the genetic variants were associated not only with overall IS, but also with the major IS subtypes and with age of stroke onset.

Methods

Study Populations

This analysis includes 6865 IS cases and 11 395 control subjects from 9 different studies: Australian Stroke Genetics Collaborative (ASGC), Bio-Repository of DNA in Stroke (BRAINS), Besta Cerebrovascular Diseases Registry (CEDIR), Genes Affecting Stroke Risk and Outcomes Study (GASROS), Genetics of Early Onset Stroke (GEOS), Graz Stroke Study (GRAZ), Ischemic Stroke Genetics Study and Siblings with Ischemic Stroke Study (ISGS/SWISS), Risk Assessment of Cerebrovascular Events (RACE), and the Vitamin Intervention for Stroke Prevention (VISP) study. All stroke cases, except those from VISP study, were adjudicated for ischemic stroke subtype using the original TOAST system,[3] which assigns each case to 1 of the following categories: cardioembolic, large-artery, small-vessel, other known causes, and undetermined causes. Control subjects free of stroke were selected based on study-specific criteria. For this report, cases and control subjects with a known history of MI were excluded from the analyses. Details of the study designs for each participating study are included in the online-only Data Supplement Methods (http://stroke.ahajournals.org).

All participating studies were conducted with the consent of study subjects and were approved by the institutional review board in each institution.

SNP Selection

SNPs shown previously to be associated with MI or CAD in whites were identified through a review of genome-wide association studies published before May 2010. MI/CAD-associated SNPs reported in these previously published GWA studies were selected for inclusion in this study only if their associations with MI/CAD reached genome-wide significance ($P<5\times10^{-8}$) and were replicated in at least 1 large, independent, white cohort. Based on these criteria, we identified 11 loci from nine chromosomes that were consistently associated with MI/CAD (see Table 1).[7–13]

Genotyping and Imputation

The 11 MI-associated single-nucleotide polymorphisms (SNPs) selected for inclusion in this study were genotyped by existing commercial genome-wide SNP platforms, KASPar SNP array (KBioscience, Herts, United Kingdom) and/or by Taqman assays (Applied Biosystems, Foster City, CA). For SNPs that were not directly genotyped, genotypes were obtained either through imputation or the targeted SNP was replaced by a surrogate SNP that was in high linkage disequilibrium (LD $r^2>0.8$) with the target SNP. A detailed description on the genotyping and imputation methods for each participating site is provided in online-only Data Supplement Table S1.

Statistical Analysis

Site-specific logistic regression analysis was performed to test for associations of each individual SNP with IS under an additive model, and then site-specific β coefficients and standard errors (SE) were pooled in meta-analyses. A genotype risk score was also computed for each individual by summing the number of MI/CAD-risk alleles across each of the 11 loci (score 1). Each SNP was given equal weights rather than weights based on their effect sizes (β) for MI/CAD in the risk score calculation given that we do not know the

Table 1. Previous Published SNPs Associated With MI or CAD

SNP	Nonrisk/Risk Allele*	Frequency of Risk Allele	Region	Position	Reported Gene	Previous Reported Odds Ratio on MI	Reference
rs11206510	C/T	0.81	1p32	55,268,627	PCSK9	1.15	8
rs646776	C/T	0.81	1p13	109,620,053	CELSR2-PSRC1-SORT1	1.17	10
rs17465637	A/C	0.72	1q41	220,890,152	MIA3	1.13	8,12
rs6725887	T/C	0.14	2q33	203,454,130	WDR12	1.17	8
rs9818870	C/T	0.16	3q22	139,604,812	MRAS	1.15	7
rs12526453	G/C	0.65	6p24	13,035,530	PHACTR1	1.12	8
rs4977574	A/G	0.56	9p21	22,088,574	ANRIL/CDKN2B-AS1	1.28	8,9,11
rs1746048	T/C	0.84	10q11	44,095,830	CXCL12	1.14	8,12
rs3184504	C/T	0.39	12q24	110,368,991	SH2B3	1.13	13
rs1122608	T/G	0.75	19p13	11,024,601	SMARCA4-LDLR	1.15	8
rs9982601	C/T	0.13	21q22	34,520,998	SLC5A3-MRPS6-KCNE2	1.20	8

SNP indicates single-nucleotide polymorphism; MI, myocardial infarction; CAD, coronary artery disease.
*Risk allele is the allele associated with increased risk of MI or CAD.

effects of these SNPs on stroke and the assumption that they would have similar effects on stroke as they do on MI seems unjustified. Additionally, we computed a genotype risk score that summed the number of MI/CAD-risk alleles across 10 loci, excluding SNP rs4977574 on chromosome 9, because SNPs near this locus have previously been reported to be associated with large-artery stroke (score 2).[14] Each genotype risk score was used as a continuous independent variable in the logistic regression model to obtain the joint additive effects of all MI/CAD-associated SNPs on the risk of IS. The β of the genotype risk score represents the increase in log odds of stroke associated with having one additional MI/CAD-risk allele. The GRAZ study was not included in the score analyses because three of the 11 SNPs were not genotyped directly and could not be imputed with acceptable quality scores (rs6725887, rs9818870, and rs9982601). Overall, about 35% of the individuals in whom 1 or more SNPs were not genotyped or imputed were removed from the genotype risk score analysis. For ease of result interpretation, all odds ratios were designated with the previously published nonrisk allele as the reference so that the odds ratio reflects the effect of the MI-associated risk allele. Association analyses were adjusted for study-specific covariates (eg, age, sex, and population structure; online-only Data Supplement Table S2).

Meta-analysis was performed using a variance-weighted approach assuming a random effect model (DerSimonian and Laird method),[15] which takes heterogeneity of the genetic effects across studies into account. Between-study heterogeneity was estimated using Cochran Q statistic, which is the summation of the squared, weighted difference between the study-specific effect and the overall effect size (estimated by the fixed effect), as well as I^2 statistics, which represent the percent of total variation across studies due to heterogeneity after accounting for variability due to chance. All meta-analyses were performed using the *metan* module implemented in STATA 10 (StataCorp, College Station, TX). Analyses were carried out for total IS, by stroke subtype, and by age of stroke onset ($\leq$50 years, 51–69 years, and $\geq$70 years).

With our total sample size (6865 cases), we had 80% power to detect odds ratios of 1.09$\approx$1.14 for overall IS and odds ratios of 1.20–1.32 for major stroke subtypes ($\approx$1000 cases) for variants with allele frequency 10–50% (Bonferroni-corrected α=0.0045=0.05/11 SNPs). In this report, nominal probability values were provided for all the analyses.

Results

Study Population Characteristics

This study included 6865 cases and 11 395 control subjects recruited from 9 studies across North America, Europe, Australia, and South Asia. Stroke cases ranged in age between 15–101 years. Among cases with subtype information available, the 3 major stroke subtypes, cardioembolic, large-artery (atherosclerotic), and small-vessel (lacunar), accounted for $\approx$20.9% (n=1216), 19.6% (n=1137), and 18.0% (n=1043) of the cases, respectively; the remaining cases were attributed to either "other known causes" (5.6%; n=324) or "undetermined causes" (35.9%; n=2084). The majority of the study participants were of European ancestry with the exception of those enrolled in the RACE study (1890 cases and 4625 control subjects), which has enrolled participants of South Asian origin. Detailed characteristics of the participating studies are provided in online-only Data Supplement Table S3.

Associations of MI/CAD SNPs and Overall IS Risk

In the total sample, the meta-analysis odds ratios for all 11 SNPs ranged from 0.92–1.11 (Table 2). Only 2 SNPs were nominally associated with IS: rs11206510 at *PCSK9* (odds ratio [OR], 0.92; 95% confidence interval [CI], 0.86–0.99; P=0.03; allele T) and rs3184504 at *SH2B3* (OR, 1.11; 95% CI, 1.01–1.21; P=0.03; allele T). This association would not have withstood a conservative Bonferroni correction (α=0.05/11, or 0.0045). Although there was no significant evidence for heterogeneity of effect size across studies for rs11206510, the direction of effect was not consistent across studies with ORs ranging from 0.80 (RACE study) to 1.12 (CEDIR study) (Cochran Q statistic=8.1, P=0.4) (online-only Data Supplement Figure S1). rs3184504, on the other hand, showed significance evidence for heterogeneity (Cochran Q statistic=20.9, P=0.01) with 1 study (BRAINS) showing opposite effect as compared with other studies. We also examined the joint effect of these MI/CAD-associated SNPs by testing for association with the genotype scores and found no significant effect for either genotype score 1 or score 2 (OR, 1.02; P=0.2 for both scores). Removing the RACE study, the only study that had participants of South Asian origin, did not change results significantly although the association with rs11206510, and rs3184504 became less significant (OR, 0.94; P=0.10 and 1.10, P=0.06, respectively; online-only Data Supplement Table S4).

Table 2. Association Results of the 11 MI/CAD Risk Loci and Risk Scores With Ischemic Stroke

SNP	MI/CAD Risk Allele	Heterogeneity Between Studies		Association With Overall Ischemic Stroke		Minimal OR Detectable in Current Study†
		Q (*P*)	I^2, %	OR (95% CI)*	*P*	
rs11206510	T	8.12 (0.42)	1.53	0.92 (0.86, 0.99)	0.03	1.13
rs646776	T	9.02 (0.34)	11.34	0.95 (0.89, 1.01)	0.12	1.13
rs17465637	C	3.09 (0.93)	0	1.03 (0.97, 1.09)	0.37	1.11
rs6725887	C	13.38 (0.06)	47.67	1.03 (0.90, 1.16)	0.69	1.13
rs9818870	T	5.45 (0.61)	0	1.03 (0.95, 1.11)	0.47	1.12
rs12526453	C	8.81 (0.36)	9.2	1.00 (0.95, 1.06)	0.92	1.10
rs4977574	G	4.17 (0.84)	0	1.02 (0.97, 1.07)	0.54	1.09
rs1746048	C	5.37 (0.72)	0	1.03 (0.96, 1.10)	0.38	1.14
rs3184504	T	20.87 (0.01)	61.67	1.11 (1.01, 1.21)	0.03	1.09
rs1122608	G	6.31 (0.61)	0	1.00 (0.95, 1.06)	0.92	1.11
rs9982601	T	6.20 (0.52)	0	1.00 (0.93, 1.09)	0.95	1.13
Score 1	NA	12.31 (0.09)	43.15	1.02 (0.99, 1.05)	0.23	n/a
Score 2	NA	14.44 (0.04)	51.52	1.02 (0.99, 1.06)	0.23	n/a

MI indicates myocardial infarction; CAD, coronary artery disease; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NA, not applicable; *P*, probability value.

*ORs reflect the effect of the MI-associated risk allele with the previously published MI nonrisk allele as the reference allele defined in Table 1.

†Minimal ORs that current sample size (6865 overall ischemic stroke cases) can detect with at least 80% power (α=0.0045).

We further stratified the associations between MI/CAD-associated SNPs and overall IS according to age of stroke onset: early (≤50 years), intermediate (51–69 years), and late onset (≥70 years). None of the associations were statistically significant in the age-stratified analyses, either (online-only Data Supplement Table S5).

Associations With Major IS Subtypes

We next examined the effects of MI/CAD-associated SNPs on TOAST-defined subtypes (Table 3). Nominally significant associations ($0.03<P<0.05$) were observed for 4 of the different TOAST subtypes. The MI/CAD-associated risk allele at rs9818870 in *MRAS* was associated with the cardioembolic stroke subtype (OR, 1.17; 95% CI, 1.02–1.35; $P=0.03$); the MI-associated risk allele at rs17465637 in *MIA3* was associated with the small-vessel stroke subtype (OR, 1.12; 95% CI, 1.00–1.26; $P=0.05$); and the MI-associated risk allele at rs3184504 in *SH2B3* was associated with strokes due to other known causes (OR, 1.33; 95% CI, 1.02–1.72; $P=0.04$). In addition, rs11206510 was associated with

Table 3. Association Results of the 11 MI/CAD Risk Loci and Risk Scores With Ischemic Stroke, by TOAST Subtypes

SNP	Cardioembolic (n=1216)		Large-Artery (n=1137)		Small-Vessel (n=1043)		Other Known Causes (n=324)		Undetermined Causes (n=2087)	
	OR (95% CI)*	*P*	OR (95% CI)*	*P*	OR (95% CI)*	*P*	OR (95% CI)*	*P*	OR (95% CI)*	*P*
rs11206510	1.02 (0.89, 1.18)	0.78	0.91 (0.80, 1.04)	0.17	1.05 (0.91, 1.21)	0.50	0.89 (0.72, 1.11)	0.32	0.86 (0.76, 0.99)	0.03
rs646776	0.93 (0.83, 1.05)	0.25	0.91 (0.81, 1.03)	0.12	0.95 (0.84, 1.07)	0.38	1.01 (0.78, 1.31)	0.94	0.93 (0.82, 1.05)	0.24
rs17465637	1.01 (0.89, 1.16)	0.86	0.98 (0.87, 1.09)	0.69	1.12 (1.00, 1.26)	0.05	0.96 (0.79, 1.17)	0.69	1.01 (0.92, 1.10)	0.90
rs6725887	0.98 (0.83, 1.16)	0.84	1.01 (0.85, 1.19)	0.93	0.91 (0.76, 1.09)	0.30	1.45 (0.93, 2.26)	0.10	0.96 (0.83, 1.11)	0.58
rs9818870	1.17 (1.02, 1.35)	0.03	0.97 (0.83, 1.13)	0.66	0.91 (0.77, 1.07)	0.24	1.32 (0.92, 1.89)	0.13	0.99 (0.87, 1.12)	0.90
rs12526453	1.06 (0.95, 1.18)	0.31	0.93 (0.83, 1.03)	0.15	0.90 (0.80, 1.02)	0.09	1.03 (0.85, 1.25)	0.74	1.00 (0.91, 1.09)	0.99
rs4977574	1.02 (0.89, 1.17)	0.82	1.09 (0.99, 1.20)	0.09	1.03 (0.94, 1.14)	0.52	0.91 (0.69, 1.18)	0.47	1.01 (0.94, 1.09)	0.72
rs1746048	0.99 (0.88, 1.12)	0.93	1.05 (0.92, 1.2)	0.44	1.01 (0.86, 1.18)	0.94	0.97 (0.70, 1.33)	0.85	1.00 (0.91, 1.1)	0.96
rs3184504	1.05 (0.91, 1.22)	0.51	1.16 (0.96, 1.39)	0.11	1.03 (0.89, 1.20)	0.68	1.33 (1.02, 1.72)	0.04	1.12 (0.98, 1.27)	0.11
rs1122608	0.98 (0.88, 1.09)	0.67	1.06 (0.95, 1.18)	0.33	1.03 (0.92, 1.15)	0.66	1.03 (0.85, 1.26)	0.75	0.99 (0.91, 1.07)	0.74
rs9982601	1.05 (0.89, 1.25)	0.55	1.04 (0.89, 1.22)	0.63	1.06 (0.88, 1.27)	0.55	1.15 (0.83, 1.60)	0.39	1.02 (0.85, 1.22)	0.85
score1	1.02 (0.98, 1.07)	0.29	1.02 (0.97, 1.06)	0.47	1.01 (0.95, 1.07)	0.85	1.06 (0.98, 1.14)	0.16	1.00 (0.96, 1.04)	0.97
score2	1.04 (0.99, 1.08)	0.11	1.01 (0.96, 1.08)	0.63	1.00 (0.94, 1.07)	0.91	1.08 (1.01, 1.17)	0.03	1.00 (0.96, 1.05)	0.92

MI indicates myocardial infarction; CAD, coronary artery disease; TOAST, Trial of Org 10172 in Acute Stroke Treatment; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; *P*, probability value.

*ORs reflect the effect of the MI-associated risk allele with the previously published MI nonrisk allele as the reference allele defined in Table 1.

strokes due to other undetermined causes, although the allele conferring protection against MI was associated with stroke risk (OR, 0.86; 95% CI, 0.76–0.99; $P=0.03$). The ASGC study had a much higher OR and a wide CI for rs11206510 association, but this study contributed only 11 cases of undetermined causes in the analysis. Excluding the ASGC study from the meta-analysis resulted in an OR of 0.86 (95% CI, 0.76–0.97; $P=0.01$) for the association between rs11206510 and undetermined causes of stroke. The *ANRIL* rs4977574 SNP was very modestly (and not significantly) associated with the large-artery stroke subtype (OR, 1.09; 95% CI, 0.99–1.20; $P=0.09$), nor was it associated with any other stroke subtype. None of these results withstand correction for multiple testing. Forest plots showing the most strongly associated SNP with each subtype are in online-only Data Supplement Figure S2.

Discussion

It is of interest to consider whether common loci influence susceptibility to MI/CAD and stroke to the extent that such loci could elucidate common genetic and/or biological pathways. Given that the 11SNPs tested in this study were associated with MI/CAD in previous GWA studies, they probably are among the common SNPs having the largest effect sizes. The risk alleles at these loci were associated with increases of 12–20% in MI risk, with the exception of rs4977574 in *ANRIL*, for which the risk allele was associated with a 28% increase in Europeans.[7,8,13] In contrast, none of the MI/CAD risk alleles at these loci were significantly associated with risk of overall IS, with the estimated odds ratios ranging from 0.92–1.11 (ie, 8% reduction to 11% increase in odds of stroke per allele).

It is possible that failure to detect associations of these SNPs with the combined IS end point might be partially attributable to the heterogeneity of the IS phenotype. The large size of our sample provided us with the opportunity to evaluate the association of these SNPs to subtypes of ischemic stroke. Similar to the combined IS end point, we did not find any significant associations of MI/CAD SNPs (or the joint genotyping risk scores) with any of the stroke subtypes. Given that this study included more than 6800 total cases and more than 1000 cases for the major stroke subtypes, we have sufficient power to detect even small effects on overall stroke although more modest power to detect associations with stroke subtype. Therefore, our findings suggest that these MI/CAD-associated SNPs do not have effects of similar magnitude on overall stroke as they do on MI/CAD. If an association does exist, it is likely to be very modest for overall IS or restricted to a single stroke subtype.

Although the SNP associations with stroke subtypes did not reach statistical significance, it is interesting that the strongest associations we observed in the subtype analysis were genes involved in cell regulation or signaling. For example, the strongest association with cardioembolic stroke was the MI-risk allele at rs9818870 in *MRAS* (OR, 1.17; $P=0.03$). *MRAS*, an intracellular signal transducer, is highly expressed in the cardiovascular system and may be involved in cell adhesion.[7,8,16] The strongest association with small-vessel stroke was the MI-risk allele at rs17465637 in *MIA3* (OR, 1.12; $P=0.05$), which encodes melanoma inhibitory activity 3 and is associated with cell adhesion and migration in inflammatory pathways[17] It should also be noted that rs4977574 near *ANRIL* was very modestly associated with the large-artery subtype (OR, 1.09; $P=0.09$). Although the association did not achieve statistical significance in our study, other SNPs in the *ANRIL* region on chromosome 9p21 have previously been associated with large-artery stroke, with the strongest signal found with SNP rs1537378 (OR, 1.21; 95% CI, 1.09–1.35; $P=0.0005$).[14] Interestingly, rs1537378 is not in high LD with our target SNP rs4977574 ($r^2=0.5$), suggesting these 2 SNPs may represent different association signals or each of them may be tagging the causal variant imperfectly (assuming the association is indeed true). *ANRIL* encodes an antisense noncoding RNA that may disrupt other genes in the region, including *CDKN2A and CDKN2B*, 2 genes that play a key role in regulating cell proliferation, senescence, and apoptosis. We also observed the MI-associated risk allele at rs3184504 in *SH2B3* to be nominally significantly associated with strokes due to other known causes (OR, 1.33; 95% CI, 1.02–1.72; $P=0.04$). It has been hypothesized that this SNP could reduce the anti-inflammatory activity of *SH2B3*, which is expressed in the vasculature, thereby leading to the development of plaque development and/or progression in coronary arteries.[13]

In contrast, SNPs whose effects on MI are likely to be mediated though lipid metabolism, for example, rs1122608 at *SMARCA4/LDLR* (19p13), rs646776 at the *CELSR2/PSRC1/SORT1* locus (1p13), and rs11206510 at PCSK9 (1p32), have minimal effects on IS or any of its subtypes, and in some cases, even showed opposite effects on stroke. For example, the MI/CAD-associated risk allele of rs11206510 at *PCSK9*, a gene involved in cholesterol homeostasis, was modestly associated with decreased risk of overall stroke (OR, 0.92; $P=0.03$) or strokes due to undetermined causes (OR, 0.86; $P=0.03$). Our analyses suggest that genes involved in lipid metabolism do not predispose individuals to increased stroke risk as they do for MI, although replications will be needed to confirm these associations.

Taken together, our results suggest that the major common loci associated with MI/CAD risk do not have large effects on stroke. However, although our analyses did not reveal any significant associations between MI/CAD SNPs and stroke, the chromosomal regions harboring these loci might still contain stroke-related SNPs that are not in high LD with the MI-associated SNPs, as the example of chromosome 9p region.[14] A limitation of our meta-analysis is that all included studies are case-control–based, and some have included recurrent stroke, thus allowing for the possibility of selection toward milder strokes (due to survival bias). It is possible that these genetic MI/CAD variants could be associated with more severe forms of stroke, but longitudinal studies that follow healthy cohorts for stroke occurrence prospectively would be needed to address this issue. Finally, our analyses were based on 11 SNPs unequivocally associated with MI and/or CAD identified from the first wave of meta-analyses for these traits. A second wave of even larger meta-analyses of these traits is in progress that will probably generate additional associations. We can expect most of these newly associated

SNPs to have even smaller effect sizes on MI and CAD. In fact, in September 2011, 17 additional CAD-associated SNPs were identified by even larger meta-analyses, and the reported effect sizes for these newly identified SNPs, as expected, were smaller (ranging from 1.06–1.17) than the original 11 SNPs used in the present analysis.[18–21] However, this does not necessarily mean that these SNPs will have smaller effect sizes on stroke. Indeed, it is very possible that such SNPs may be in gene pathways that are more relevant to stroke than are the pathways associated with the currently known MI SNPs. Thus, it will be important to monitor new SNPs identified, even those with small effect sizes, as they may generate new biological insights into the etiology of stroke.

Conclusions

Common variants previously associated with MI/CAD risk do not have effects of similar magnitude on overall IS but do not preclude moderate associations restricted to specific IS subtypes.

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Disclosures

None.

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SUPPLEMENTAL MAERIAL

Title: Are myocardial infarction-associated single nucleotide polymorphisms associated with ischemic stroke?
Cheng et al.

Participating Consortia

From the GARNET Consortium, support was provided from researchers at the Data Coordinating Center at the University of Washington (Dr. Cathy C. Laurie, Dr. Sarah Nelson, Dr. Bruce Weir, Ms. Kate Wehr and Ms. Jenna I. Udren), the Genotyping Center at the Center for Inherited Disease Research (CIDR) (Dr. Corinne Boehm, Ms. Marcia Adams and Ms. Michelle Zilka), and the NIH (Dr. Teri A. Manolio and Ms. Corina Din-Lovinescu).

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Supplemental Methods

ASGC is a collaboration of 4 stroke centers (Newcastle, Gosford, Adelaide, and Perth) across Australia bringing together the major collections of stroke cases with biobanked samples nationally. *Case participants* were hospitalized with a first cerebral infarction identified through hospital admission from each of the study centers. The abstracted hospital records of cases were reviewed and adjudicated for ischemic stroke subtype by a pair of neurologists according to the widely used TOAST system that assigns each case to a single category. All cases were confirmed with imaging, predominantly CT scanning and/or MRI in a smaller number of cases. *Control participants* were selected by age and gender matching with cases from the Hunter Community Study. This longitudinal cohort consists of 3000 community dwelling participants aged 55 to 85 years old randomly selected through the state electoral roll and recruited using a modified Dillman procedure (mailed invitation followed by telephone call). Controls were excluded if they reported a history of stroke. Full details of this cohort have been published previously[1]. For this analysis, 12.9% of cases and 9.5% of controls that had a history of MI (based on self-report) were removed from analysis.

BRAINS is an ongoing, multicentre, in-hospital study which recruits consenting acute stroke patients into a highly characterized biobank. All adult (>18 years of age) stroke patients are recruited with either ischaemic and haemorrhagic pathology MRI confirmed lesions. Ischaemic stroke subtypes are further sub-classified according to TOAST criteria[2]. Known monogenic causes of stroke are excluded. BRAINS has two principal arms. The first arm recruits UK European stroke patients while the second arm recruits South Asian stroke patients from multiple sites in the UK and also from sites in India. Control data for the European arm is provided by the Wellcome Trust Case Control Consortium while control subjects for the South Asian arm are recruited simultaneously as the affected stroke patient and usually is the proband's spouse. For the purposes of this study, only ischemic strokes and subjects from the European arm were included, and individuals with prior history of MI (7.6%) were removed as well.

CEDIR includes consecutive Italian patients referred to Besta Institute from 2000 for stroke, TIA, vascular cognitive impairment and other cerebrovascular diseases (venous thrombosis, aneurisms, arterio-venous malformations, etc.). Clinical, neuroradiological, neurophysiological and biochemical data are recorded prospectively in the CEDIR database. Genomic DNA was extracted from whole blood with commercial kit. Samples were genotyped in 2009 at Istituto Auxologico Italiano using the Illumina Human610-Quadv1_B or Human660W-Quad_v1_A BeadChip (Illumina, San Diego, CA, USA). Ischemic stroke cases, defined as focal neurological deficit persisting for more than 24 h with evidence of cerebral infarction on neuroimaging, first ever or recurrent, were selected for this study. Controls are Italian blood donors enrolled at Mario Negri Institute within the PROCARDIS Study. Controls with a history of MI or CAD were ineligible to participate in the study. Cases with a history of MI or CAD (12.5% of all cases) were excluded from analysis. Diagnosis of CAD was based on medical records or self-reports. Details of the study have been described previously[3-5].

GEOS is a population-based case-control study designed to identify genes associated with early-onset ischemic stroke and to characterize interactions of identified stroke genes and/or SNPs with environmental risk factors[6, 7]. Participants were recruited from the greater Baltimore-Washington area in 4 different time periods: Stroke Prevention in Young Women-1 (SPYW-1) conducted from 1992-1996, Stroke Prevention in Young Women-2 (SPYW-2) conducted from 2001-2003, Stroke Prevention in Young Men (SPYM) conducted from 2003-2007, and Stroke Prevention in Young Adults (SPYA) conducted in 2008. *Case participants* were hospitalized with a first cerebral infarction identified by discharge surveillance from one of the 59 hospitals in the greater Baltimore-Washington area and direct referral from regional neurologists. The abstracted hospital records of cases were reviewed and adjudicated for ischemic stroke subtype by a pair of neurologists according to previously published procedures with disagreements resolved by a third neurologist. The ischemic stroke subtype classification system retains information on all probable and possible causes, and is reducible to the more widely used TOAST system that assigns each case to a single category. *Control participants* without a history of stroke were identified by random-digit dialing and were balanced to cases by age and region of residence in each recruitment periods. Genomic DNA was isolated from a variety of sample types, including cell line, whole blood, mouth wash and buccal swab. Samples were genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR) using the Illumina HumanOmni1-Quad_v1-0_B BeadChip (Illumina, San Diego, CA, USA). Individuals were excluded if they were unexpected duplicates, gender discrepancy and unexpected relatedness. For this analysis, 5.1% of cases and 0.6% of controls that had a history of MI (based on self-report) were removed from analysis.

Graz Study included white patients with ischemic strokes admitted to the stroke unit of the Medical University Graz Department of Neurology between 2002 and 2007[8]. All patients underwent either CT or MRI of the brain and a standardized protocol including a laboratory examination and carotid ultrasound or magnetic resonance angiography and ECG. More extensive cardiac examination, including transesophageal echocardiography or transthoracic echocardiography and Holter, was done in subjects with suspected

cardiac embolism. A total of 670 stroke patients were included in the study with 509 (76%) having a first ever stroke. The Stroke subtype was assessed according to modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria and were done by trained stroke neurologists. Controls were participants of the Austrian Stroke Prevention Study[1] and came from the same catchment area. All control subjects were recruited between 1991 and 2000 and were free of stroke and dementia. They underwent full risk factor assessment, brain MRI, Duplex scanning of the carotid arteries, ECG, and transthoracic echocardiography. A total of 28.9% of stroke patients and 8.2% of controls with historical evidence for MI and angina pectoris (based on ROSE questionnaire[9]) were excluded from analyses.

ISGS/SWISS The Ischemic Stroke Genetic Study (ISGS) is a multicenter inception cohort study[10]. Cases were recruited from inpatient stroke services at five United States academic medical centers. Cases are adult men and women over the age of 18 years diagnosed with first-ever ischemic stroke confirmed by a study neurologist on the basis of history, physical examination and CT or MR imaging of the brain. Cases had to be enrolled within 30 days of onset of stroke symptoms. Cases were excluded if they had: a mechanical aortic or mitral valve at the time of the index ischemic stroke, central nervous system vasculitis, or bacterial endocarditis. They were also excluded if they were known to have: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Fabry disease, homocystinuria, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), or sickle cell anemia. Diagnostic evaluation included: head CT (95%) or MRI (83%), electrocardiography (92%), cervical arterial imaging (86%), and echocardiography (74%). Medical records from all cases were centrally reviewed by a vascular neurology committee and assigned ischemic stroke subtype diagnoses according to criteria from the Trial of ORG10172 (TOAST)[2], the Oxfordshire Community Stroke Project [11], and the Baltimore-Washington Young Stroke Study[12]. DNA was donated to the NINDS DNA Repository (Coriell Institute, Camden, NJ) for eligible samples with appropriate written informed consent. The Siblings with Ischemic Stroke Study (SWISS) is a multicenter affected sibling pair study[13]. Probands with ischemic stroke were enrolled at 66 US medical centers and 4 Canadian medical centers. Probands are adult men and women over the age of 18 years diagnosed with ischemic stroke confirmed by a study neurologist on the basis of history, physical examination and CT or MR imaging of the brain. Probands were required to have a history of at least one living sibling with a history of stroke. Probands were excluded if they had: a mechanical aortic or mitral valve at the time of the index ischemic stroke, central nervous system vasculitis, or bacterial endocarditis. Probands were also excluded if they were known to have: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Fabry disease, homocystinuria, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), or sickle cell anemia. Siblings were enrolled using proband-initiated contact[14] or direct contact when permitted by Institutional Review Boards. Concordant siblings had their diagnosis of ischemic stroke confirmed by review of medical records by a central vascular neurology committee. Concordant siblings had the same eligibility criteria as probands. Subtype diagnoses were assigned to the index strokes of probands and concordant siblings according to TOAST criteria. Discordant

siblings of the proband were confirmed to be stroke-free using the Questionnaire for Verifying Stroke-free Status[15]. A repository of lymphoblastoid cell lines was created and is curated by the Coriell Institute, Camden, NJ.
Readily available US controls were utilized, including stroke-free participants from the Baltimore Longitudinal Study of Aging and the National Institute of Neurological Diseases and Stroke neurologically normal control series taken from the Coriell Cell Repositories. All controls had been previously genotyped and described in detail elsewhere[16]. About 15% of study participants that had a history of MI were removed from the analyses, and the MI cases were identified by self-report.

GASROS All ischemic stroke subjects were enrolled as part of an ongoing single-center prospective cohort study of consecutive patients with ischemic stroke aged ≥ 18 years admitted to the Massachusetts General Hospital Stroke Unit after presenting to the emergency department within 24 hours of symptom onset between 2003 and 2009. Ischemic stroke was defined as a clinical syndrome of any duration associated with a radiographically proven acute infarct consistent with a vascular pattern of involvement and without radiographic evidence of a demyelinating or neoplastic disease or other structural disease, including vasculitis, subacute bacterial endocarditis, vasospasm due to subarachnoid hemorrhage or cocaine abuse, or primary intracerebral hemorrhage. Diagnosis of acute cerebral ischemia was confirmed for all AIS subjects in the present study by admission diffusion-weighted MRI completed within 48 hours after symptom onset. Controls are recruited from outpatient clinics serving the same community, and are similar to cases for age, sex and race. Controls are 55 years or older and are excluded if they have a history of ischemic stroke. The Institutional Review Board approved all aspects of this study, and informed consent for collection of data was obtained for all subjects or their legal guardians. Details of the study have been described previously[16]. Controls with a history of MI were ineligible to participate in the study. Cases with a history of MI (3% of all cases) were excluded from analysis. Diagnosis of MI was based on self report or medical records.

RACE Risk Assessment of Cerebrovascular Events (RACE) is an existing case-control study of stroke now involving over 5000 imaging confirmed cases of stroke and 5000 controls, recruited from six centers in Pakistan. DNA, plasma, serum, whole blood and information from the baseline assessment of demographic details, habits, lifestyles and other characteristics have been collected from the participants. Cases are eligible for inclusion in the study if they: (ii) are aged at least 18 years; (ii) present with a sudden onset of neurological deficit respecting a vascular territory with sustained deficit at 24 hours verified by medical attention within 72 hours after onset (onset is defined by when the patient was last seen normal and not when found with deficit); and (iii) the diagnosis is supported by CT/MRI; and (iv) present with a Modified Rankin Score < 2 prior to the stroke. The TOAST classification method is used to classify ischemic stroke based on aetiology whereas the Oxfordshire classification is used to classify stroke neuro-anatomically. Controls are selected from the same participating hospital centres as the stroke cases so that they derive from the same catchment areas. In order to minimize any potential selection biases, controls are recruited in the following order of priority: (i) non-blood related or blood related visitors of patients of the out-patient department; (ii) non-

blood related visitors of stroke patients; (iii) patients of the out-patient department presenting with minor complaints (eg. back pain, minor gastric complaints). Controls with any of the following are excluded from RACE: (i) a prior history of stroke or TIA; (ii) a prior history of CAD (MI/CABG); or (iii) who are unable to provide informed consent. A locally-piloted and validated epidemiological questionnaire has been administered to participants by medically qualified research officers that seeks >400 items of information in relation to: ethnicity (eg, personal and paternal ethnicity, spoken language, place of birth and any known consanguinity); demographic characteristics; lifestyle factors (eg, tobacco and alcohol consumption, dietary intake and physical activity); personal and family history of cardiovascular disease; and medication usage. Controls with a history of CAD were ineligible to participate in the study. Cases with a history of CAD (11% of all cases) were excluded from analysis. Diagnosis of CAD was based on clinical history and electrocardiogram.

VISP was a multi-center, double-blind, randomized, controlled clinical trial that enrolled patients aged 35 or older with Homocysteine levels above the 25th percentile at screening and a non-disabling cerebral infarction (NDCI) within 120 days of randomization (P.I. James Toole, MD, Wake Forest University School of Medicine (WFU); R01 NS34447)[17, 18]. NDCI was defined as an ischemic brain infarction not due to embolism from a cardiac source, characterized by the sudden onset of a neurological deficit. The deficit must have persisted for at least 24 hours, or if not, an infarction in the part of the brain corresponding to the symptoms must have been demonstrated by CT or MRI imaging. The trial was designed to determine if daily intake of a multivitamin tablet with high dose folic acid, vitamin B6 and vitamin B12 reduced recurrent cerebral infarction (1° endpoint), and nonfatal myocardial infarction (MI) or mortality (2° endpoints). Subjects were randomly assigned to receive daily doses of the high-dose formulation (n=1,827), containing 25mg pyridoxine (B6), 0.4mg cobalamin (B12), and 2.5mg folic acid; or the low-dose formulation (n=1,853), containing 200µg pyridoxine, 6µg cobalamin and 20µg folic acid. Enrollment in VISP began in August 1997, and was completed in December 2001, with 3,680 participants enrolled, from 55 clinic sites across the US and Canada and one site in Scotland. A subset of VISP participants gave consent and were included in the GWAS component of VISP, supported by the National Human Genome Research Institute (NHGRI), Grant U01 HG005160, as part of the Genomics and Randomized Trials Network (GARNET). Samples were genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR), and genotyping was performed using the Illumina HumanOmni1-Quad_v1-0_B BeadChip (Illumina, San Diego, CA, USA). Individuals were excluded if they were unexpected duplicates or had gender discrepancies. All VISP participants are stroke cases, therefore we obtained GWAS data (dbGAP) for ~1000 external controls from the High Density SNP Association Analysis of Melanoma: Case-Control and Outcomes Investigation (Study Accession: phs000187.v1.p1). These samples were also genotyped on the Illumina HumanOmni1-Quad. 17.3% of the cases were removed from analyses because of a prior history of MI based on medical questionnaire.

Supplemental Tables

Table S1. Genotyping and Imputation

	ASGC	BRAINS	CEDIR	GEOS	Graz	ISGS/SWISS	GASROS	RACE	VISP
Genotyping platform	Illumina 610 Quad bead chip	Illumina 610 or 660	Illumina Human610-Quadv1_B or Human660W-Quad_v1_A BeadChip	Illumina HumanOmni1-Quad_v1-0_B BeadChip	Illumina Human610-Quad BeadChip (ontrols); Tagman (cases)	Illumina 610 or 660 (cases) and Illumina HumanHap 550Kv1 or 550Kv3 (Controls)	Affymetrix 6.0	KASPAR, KBIOSCIENCE	Illumina HumanOmni1-Quad_v1-0_B BeadChip
Genotyping calling algorithm	Illumina BeadStudio Genotyping Module	Illumina BeadStudio Genotyping Module	Illumina BeadStudio Genotyping Module version 3.3.7	Illumina BeadStudio Genotyping Module version 3.3.7	Controls:llumina BeadStudio Cases:SDS 2.3 (ABI)	Illumina BeadStudio Genotyping Module	Birdsuite	-	Illumina BeadStudio Genotyping Module
Call rate threshold	> 95%	> 95%	> 95%	> 95%	> 95% (control)	> 95%	> 95%	> 95%	> 95%
Imputation software	MACH v 1.0.16	MACH v 1.0.16	n/a	n/a	MACH version 1.0.15 (control)	MACH v 1.0.16	n/a	n/a	n/a
LD threshold (r^2) for surrogate markers	n/a	n/a	0.8	0.8	n/a	n/a	0.8	0.8	0.8
Imputed quality (Dosage r2) score threshold for imputed SNP	0.3	0.3	n/a	n/a	n/a	0.3	n/a	n/a	n/a
Genotyping information for individual MI/CAD SNP (surrogate marker ID provided if applicable)									
rs11206510	---	---	---	---	Genotyped in case/imputed in control	---	---	---	---

rs646776	---	---	---	---	Genotyped in case/imputed in control	---	rs629301	---	---
rs17465637	Imputed	Imputed	rs2291834	---	Genotyped in case/imputed in control	rs17532708	---	---	---
rs6725887	---	---	---	rs6722332	Not available	---	---	---	rs6722332
rs9818870	Imputed	Imputed	rs2306374	---	Not available	Imputed	---	---	---
rs12526453	Imputed	Imputed	rs4714990	rs7739181	Genotyped in case/imputed in control	Imputed	rs4714990	---	rs7739181
rs4977574	---	---	---	---	Genotyped in case/imputed in control	---	---	rs10757278	---
rs1746048	---	---	---	rs607363	Genotyped in case/imputed in control	---	---	---	rs1632484
rs3184504	---	---	---	---	Genotyped in case/imputed in control	---	rs653178	---	---
rs1122608	Imputed	Imputed	rs8102273	rs7275	Genotyped in case/imputed in control	Imputed	---	---	rs7275
rs9982601	Imputed	Imputed	rs973754	rs9305545	Not available	Imputed	rs9978407	---	rs9305545

n/a: not applicable; --- Direct genotype

Table S2. Site-specific statistical analysis

	ASGC	BRAINS	CEDIR	GEOS	Graz	ISGS/SWISS	GASROS	RACE	VISP
Model	Logistic Regression	Logistic Regression	Logistic Regression	Logistic regression	Logistic Regression	Logistic Regression	Logistic Regression	Logistic Regression	Logistic Regression
Adjustment covariates*	Age, Sex and first two principal components	Sex and first two principal components	Age	Age, recruitment periods and the first two MDS components (population substructure)	Age and sex	Age, sex and first two principal components	Age, Sex and first two principal components	Age, gender and Subject Ethnicity	Age, sex and the first two MDS components (population substructure)
Statistical Package	PLINK v1.07 & STATA 10	PLINK v1.07 & R package	PLINK v1.07 & R package	PLINK v1.07 & STATA 10	PASW statistics 18	PLINK v1.07 & R package	PLINK v1.07 & STATA 10	STATA 10	PLINK v1.07

* Association models: ASGC, ISGS/SWISS, GASROS, RACE and VISP were adjusted for age, sex and population structure (RACE using ethnic background instead of principal components for population structure). BRAINS was adjusted for sex and population structure only because age was unavailable for controls. CEDIR was adjusted for age only because very few controls are women (12.5%) and the population was from the more genetic homogeneous northern Italy region. GEOS was adjusted for age, recruitment periods and population structure since different genders were recruited during different study periods. GRAZ was adjusted for age and sex only because there was minimal population heterogeneity in the region.

Table S3. Population characteristics

	ASGC		BRAINS		CEDIR		GASROS		GEOS	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
N	669	872	364	444	314	409	516	1202	425	495
Age (yrs) mean ± S.D.	70.7 ±13.4	65.6±7.5	68.2±14.1	>65	55 ± 15.3	51 ± 8.2	66.7±14.6	47.5±8.5	41 ± 7.0	39.5 ± 6.7
% female	43.1	54.7	41.3	64.2	38.2	12.5	39.7	40.9	39.1	43.6
% recurrent stroke	36%	---	0%	---	8%	---	25%	---	0%	---
Subtype, n (%)										
Cardioembolic	156 (23.3)	---	33 (9.1)	---	49 (15.6)	---	192 (37.2)	---	84 (19.8)	---
Large Artery	179 (26.8)	---	107 (29.4)	---	56 (17.8)	---	109 (21.1)	---	34 (8.0)	---
Small Vessel	189 (28.3)	---	110 (30.2)	---	22 (7.0)	---	45 (8.7)	---	53 (12.5)	---
Other Known	124 (18.5)	---	0	---	50 (15.9)	---	43 (8.3)	---	28 (6.6)	---
Undetermined	11 (1.6)	---	114 (31.3)	---	137 (43.6)	---	127 (24.6)	---	226 (53.2)	---
Cardiovascular risk factors at baseline										
HBP (%)	66.2	52.4	66.82	n.a.	51.0	n.a.	62.3	55.2	29.2	15.4
DM (%)	19.4	11.2	13.5	n.a.	12.1	n.a.	21.2	17.8	10.6	2.0
Hyperlipidemia (%)	44.1	49.9	n.a.	n.a.	58.6	n.a.	40.2	33.6	26.4	23.0
Current smoking (%)	19.1	6.2	15.62	n.a.	40.1	n.a.	20.4	18.3	40.7	23.2
MI risk score 1 mean ± S.D.	11.9±2.0	11.9±2.1	12.0±2.0	12.1±2.0	12.0±2.0	11.4±1.9	12.0 ± 2.0	11.8±1.9	12.1 ± 1.9	12.0 ± 2.0
MI risk score 2 mean ± S.D.	10.9±1.8	11.0±1.9	11.0±1.9	11.1±1.8	10.8 ± 1.9	10.3±1.8	10.9±1.9	10.8±1.8	11.0 ± 1.8	10.9 ± 1.8

n.a. information not available; --- not applicable (controls).

Table S3. Population characteristics (continue)

	GRAZ		ISGS/SWISS		RACE		VISP	
	Case	Control	Case	Control	Case	Control	Case	Control
N	670	813	991	1488	1890	4625	1026	1047
Age (yrs), mean ± S.D.	66.3±14.8	65.1±8.1	66.6±13.6	64.1±17.3	60.3±13.9	52.4 ±10.6	67.7± 10.8	51.2± 12.6
% female	58.5	58.1	43.3	51.9	23.7	46.5	34.6	40.6
% recurrent stroke	24%	---	9.3%	---	0%	---	0%	---
Subtype, n (%)								
Cardioembolic	112 (16.7)	---	216 (21.8)	---	374 (19.8)	---	n.a.	---
Large Artery	121 (18.1)	---	220 (22.2)	---	311 (16.5)	---	n.a.	---
Small Vessel	160 (23.9)	---	190 (19.2)	---	274 (14.5)	---	n.a.	---
Other Known	25 (3.7)	---	0	---	54 (2.9)	---	n.a.	---
Undetermined	227 (33.9)	---	365 (36.8)	---	877 (46.4)	---	n.a.	---
Cardiovascular risk factors at baseline								
HBP (%)	58.8	49.7	64.5	34.8	56.31	29.38	68.6	n.a.
DM (%)	27.7	8.6	20.6	10.9	32.89	15.57	22.5	n.a.
Hyperlipidemia (%)	53.9	27.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Current smoking (%)	20.1	11.3	18.3	5.8	27.18	31.37	14.7	n.a.
MI risk score 1 mean ± S.D. range	n.a.	n.a.	12.1±2.0	12.1±2.0	11.9±2.0	11.9±1.9	12.0 ±2.0	11.9±2.07
MI risk score 2 mean ± S.D. range	n.a.	n.a.	11.1±1.9	11.1±1.9	10.9±1.7	11.0±1.5	11.0 ± 1.9	10.9 ± 1.9

n.a. information not available; --- not applicable (controls)

Table S4. Association results of the 11 MI/CAD-risk loci and risk scores with ischemic stroke, excluding RACE study

		Heterogeneity between studies		Association with overall IS	
SNP	MI/CAD-risk allele	Q (P-value)	I^2, %	OR (95% CI) *	*P*
rs11206510	T	6.23 (0.51)	0	0.94 (0.87, 1.01)	0.10
rs646776	T	8.65 (0.28)	19.03	0.96 (0.89, 1.04)	0.31
rs17465637	C	3.08 (0.88)	0	1.03 (0.96, 1.10)	0.41
rs6725887	C	9.11 (0.17)	34.12	0.99 (0.88, 1.11)	0.85
rs9818870	T	4.97 (0.55)	0	1.04 (0.96, 1.13)	0.34
rs12526453	C	8.77 (0.27)	20.14	1.00 (0.94, 1.07)	0.95
rs4977574	G	3.85 (0.80)	0	1.02 (0.97, 1.08)	0.42
rs1746048	C	5.10 (0.65)	0	1.02 (0.94, 1.10)	0.68
rs3184504	T	20.86 (0)	66.45	1.10 (1.00, 1.22)	0.06
rs1122608	G	5.89 (0.55)	0	0.99 (0.93, 1.06)	0.82
rs9982601	T	5.64 (0.47)	0	1.02 (0.93, 1.11)	0.72
score1	n/a	12.08 (0.06)	50.31	1.02 (0.98, 1.05)	0.30
score2	n/a	13.48 (0.04)	55.48	1.02 (0.98, 1.06)	0.35

OR: odds ratio; CI: confidence interval; n/a: not applicable; *P*: p-value

* OR: odds ratios reflects the effect of the MI-associated risk allele with the previously published MI non-risk allele as the reference allele defined in Table 1.

Table S5. Association results of the 11 MI/CAD-risk loci and risk scores with overall ischemic stroke, by age of onset

SNP	Age ≤ 50 years (n=1,535)		50 < Age <70 (n=2,110)		Age ≥ 70 years (n=2,613)	
	OR (95% CI)*	P	OR (95% CI)*	P	OR (95% CI)*	P
rs11206510	0.97 (0.81, 1.15)	0.71	0.94 (0.84, 1.05)	0.28	0.90 (0.79, 1.02)	0.11
rs646776	0.95 (0.84, 1.06)	0.34	1.00 (0.92, 1.10)	0.95	0.90 (0.80, 1.03)	0.12
rs17465637	1.09 (0.98, 1.21)	0.13	0.97 (0.89, 1.05)	0.45	1.01 (0.91, 1.13)	0.84
rs6725887	1.01 (0.86, 1.18)	0.90	1.00 (0.85, 1.17)	1	1.00 (0.76, 1.32)	0.99
rs9818870	1.13 (0.99, 1.30)	0.08	0.93 (0.83, 1.05)	0.23	1.05 (0.91, 1.21)	0.48
rs12526453	0.96 (0.85, 1.08)	0.47	1.05 (0.95, 1.15)	0.34	0.98 (0.87, 1.11)	0.76
rs4977574	1.01 (0.87, 1.17)	0.91	1.05 (0.96, 1.15)	0.28	1.02 (0.90, 1.14)	0.80
rs1746048	0.99 (0.88, 1.12)	0.92	1.04 (0.93, 1.16)	0.49	0.99 (0.87, 1.13)	0.92
rs3184504	1.12 (1.02, 1.24)	0.02	1.11 (0.94, 1.31)	0.23	1.07 (0.96, 1.19)	0.20
rs1122608	1.08 (0.97, 1.19)	0.15	1.00 (0.92, 1.09)	0.98	0.97 (0.87, 1.08)	0.58
rs9982601	1.02 (0.84, 1.24)	0.85	1.04 (0.93, 1.17)	0.50	0.91 (0.78, 1.05)	0.20
score1	1.03 (0.99, 1.07)	0.18	1.03 (0.98, 1.08)	0.28	0.98 (0.95, 1.02)	0.35
score2	1.03 (0.99, 1.07)	0.17	1.02 (0.97, 1.08)	0.37	0.98 (0.94, 1.02)	0.33

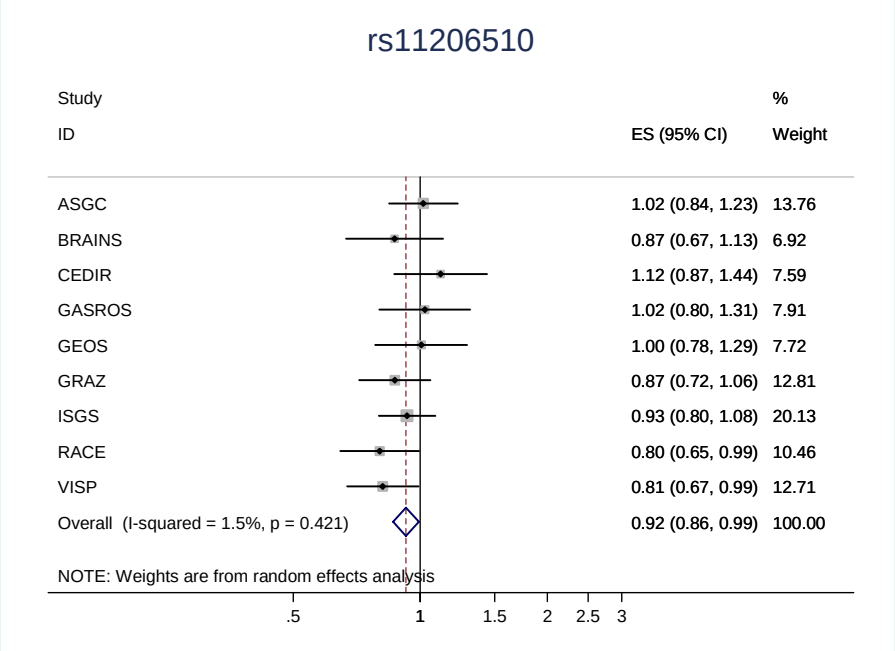
n: number of cases; OR: odds ratio; P: p-value

* OR: odds ratios reflects the effect of the MI-associated risk allele with the previously published MI non-risk allele as the reference allele defined in Table 1.

Supplemental Figures

Figure S1.

A.



B.

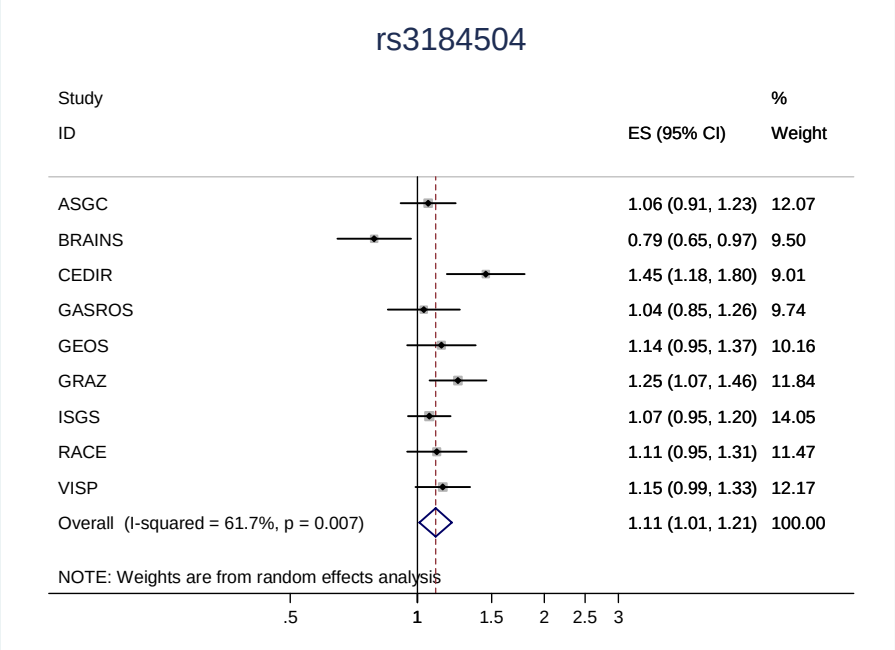
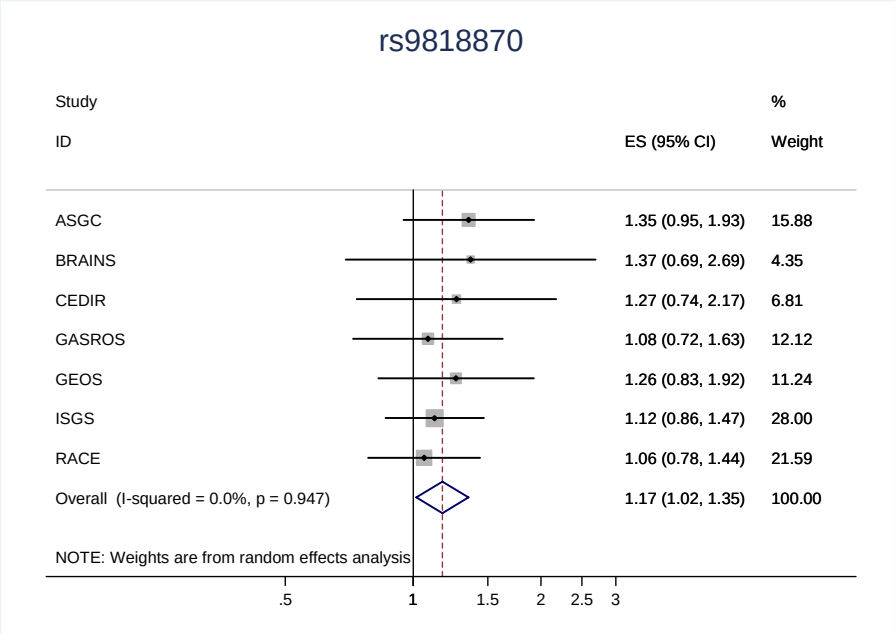


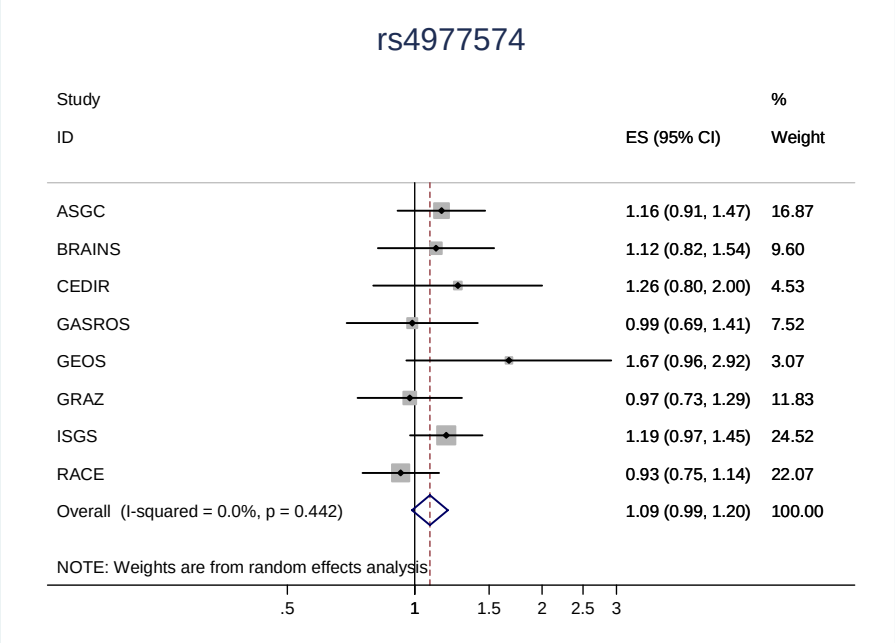
Figure S1. Forest plots showing association between (A) rs11206510 at *PCSK9* and (B) rs3184504 at *SH2B3* with overall stroke risk.

Figure S2

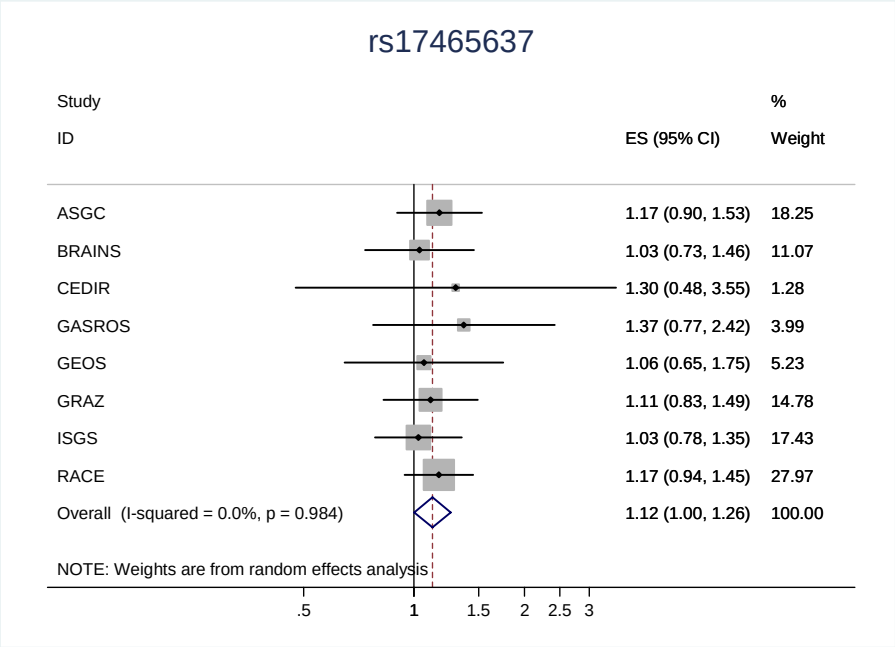
A.



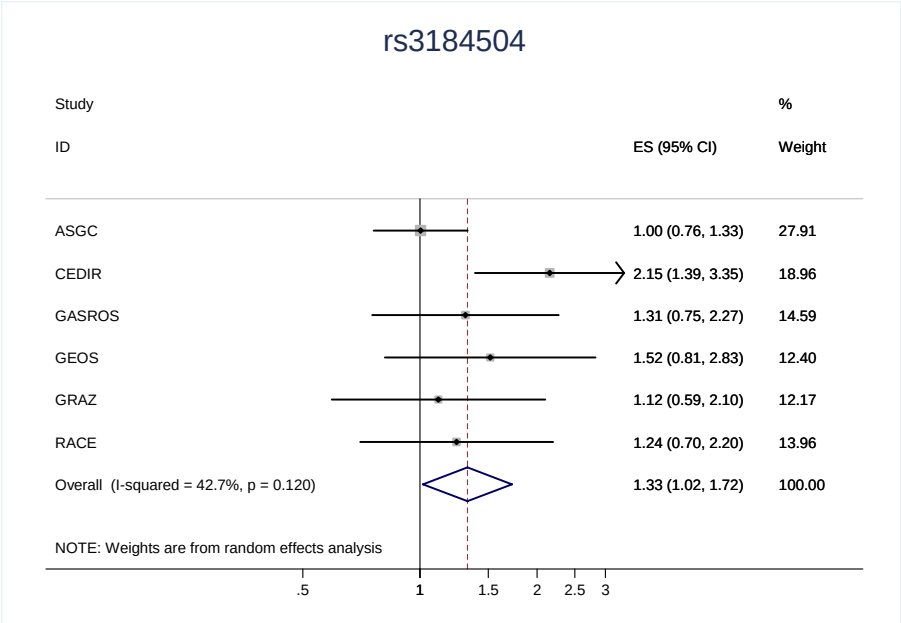
B.



C.



D.



E.

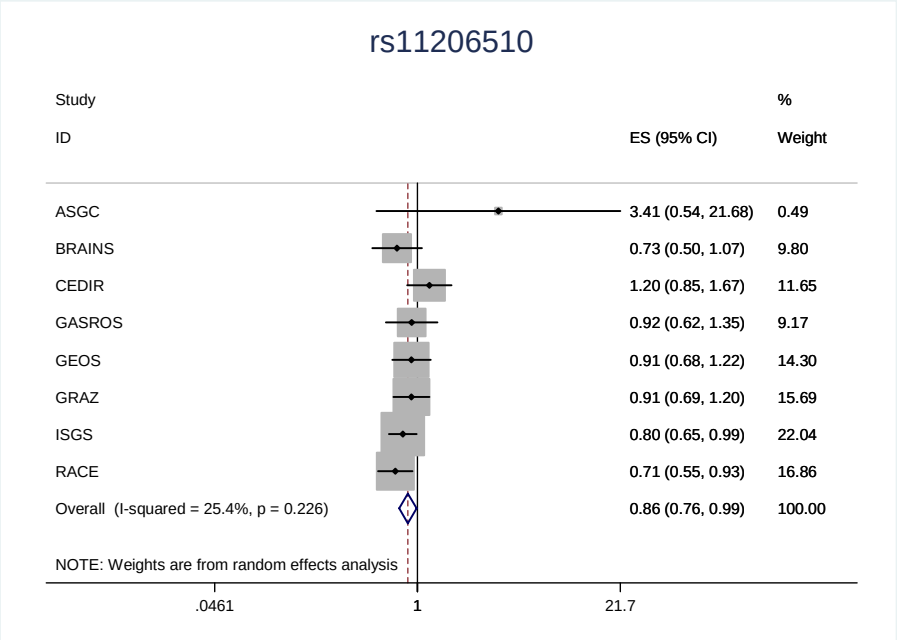


Figure S2. Forest plots showing the most significant SNP associations with each stroke subtype. A. rs9818870 at *MRAS* with cardioembolic subtype. B. rs4977574 at *ANRIL* with large artery subtype. C. rs17465637 at *MIA3* with small vessel subtype. D. rs3184504 at *SH2B3* with other known causes. E. rs11206510 at *PCSK9* with undetermined causes.

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