Shared Genetic Susceptibility to Ischemic Stroke and Coronary Artery Disease

A Genome-Wide Analysis of Common Variants

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Background and Purpose—Ischemic stroke (IS) and coronary artery disease (CAD) share several risk factors and each has a substantial heritability. We conducted a genome-wide analysis to evaluate the extent of shared genetic determination of the two diseases.

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Methods—Genome-wide association data were obtained from the METASTROKE, Coronary Artery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM), and Coronary Artery Disease (C4D) Genetics consortia. We first analyzed common variants reaching a nominal threshold of significance ($P<0.01$) for CAD for their association with IS and vice versa. We then examined specific overlap across phenotypes for variants that reached a high threshold of significance. Finally, we conducted a joint meta-analysis on the combined phenotype of IS or CAD. Corresponding analyses were performed restricted to the 2167 individuals with the ischemic large artery stroke (LAS) subtype.

Results—Common variants associated with CAD at $P<0.01$ were associated with a significant excess risk for IS and for LAS and vice versa. Among the 42 known genome-wide significant loci for CAD, 3 and 5 loci were significantly associated with IS and LAS, respectively. In the joint meta-analyses, 15 loci passed genome-wide significance ($P<5\times10^{-8}$) for the combined phenotype of IS or CAD and 17 loci passed genome-wide significance for LAS or CAD. Because these loci had prior evidence for genome-wide significance for CAD, we specifically analyzed the respective signals for IS and LAS and found evidence for association at chr12q24/SH2B3 ($P_{IS}=1.62\times10^{-7}$) and ABO ($P_{IS}=2.6\times10^{-4}$), as well as at HDAC9 ($P_{LAS}=2.32\times10^{-12}$), 9p21 ($P_{LAS}=3.70\times10^{-6}$), RAI1-PEMT-RASD1 ($P_{LAS}=2.69\times10^{-5}$), EDNRA ($P_{LAS}=7.29\times10^{-4}$), and CYP17A1-CNNM2-NT5C2 ($P_{LAS}=4.9\times10^{-4}$).

Conclusions—Our results demonstrate substantial overlap in the genetic risk of IS and particularly the LAS subtype with CAD. **(*Stroke*. 2014;45:24-36.)**

Key Words: coronary artery disease ■ genetics ■ meta-analysis ■ polymorphism, single nucleotide ■ stroke

Stroke and coronary artery disease (CAD) are among the most common causes of premature death and loss of disability-adjusted life years worldwide.[1,2] Both conditions are risk factors for one another[3,4] and in combination they are used for the assessment of risk or as a therapeutic target in clinical trials. Stroke and CAD share several risk factors and many aspects of their underlying pathophysiology. This shared biology applies to ischemic stroke (IS) and particularly to the subtype of atherosclerotic stroke (large artery stroke [LAS]).[4,5] Twin and family studies have demonstrated that both IS and CAD are highly heritable[6,7] with some evidence of a shared heritability for both diseases.[8]

Recent genome-wide association studies (GWASs) have identified some common genetic variants that are associated with IS[9–11] and multiple loci that are associated with CAD.[12,13] Interestingly, some of the variants that were originally found to affect CAD risk also associate with LAS,[14,15] suggesting a shared genetic architecture. However, there has been no systematic study assessing shared genetic susceptibility to both IS and CAD or to LAS and CAD on a genome-wide level in large datasets.

Combining genome-wide data from the METASTROKE, CARDIoGRAM, and C4D consortia, we examined whether IS and its subtype LAS share genetic risk with CAD with respect to common genetic variation. We further explored the most robustly associated variants for CAD for their association with both IS and LAS and vice versa. Finally, we conducted a joint meta-analysis of IS and CAD to search for variants that are associated with the combined and thus broader vascular phenotype.

Methods

Participating Studies and Study Design

The study sample consisted of GWAS case–control samples from the METASTROKE,[9] CARDIoGRAM,[12] and C4D[16] consortia (Table I in the online-only Data Supplement). All participating studies used a case–control or nested case–control design. Most participating studies were cross-sectional, whereas some were prospective, population-based studies.

The METASTROKE consortium included 15 GWASs involving 12 389 IS cases and 62 004 controls. Among them were 2167 LAS cases and 49 159 LAS controls, and 2365 cardioembolic stroke (CES) cases and 56 140 CES controls. Genotyping in individual cohorts was performed using Affymetrix or Illumina platforms, and ≈2.5 million imputed genotypes were generated. Individual METASTROKE results of the association analyses from every center were analyzed using a fixed-effects inverse-variance weighted model with Meta Analysis Helper.[9,17] All data were quality controlled as previously described.[9]

The CARDIoGRAM consortium included 14 GWASs involving 22 233 CAD cases and 64 762 controls. The genotyping platforms used and imputation approach was similar to METASTROKE. The C4D consortium included 3 studies involving a total of 11 165 CAD cases and 10 964 controls. Genotyping was performed using Illumina arrays containing a common set of ≈575 000 genotyped single nucleotide polymorphisms (SNPs).[16] The meta-analysis of all CAD studies was performed using a fixed-effects or random-effects model depending on the extent of heterogeneity as described previously.[18] All data were quality controlled as previously described.[16,18]

Phenotype definitions of stroke and CAD are described in the original reports.[9,12,16] In brief, stroke was defined as a typical clinical syndrome with radiological confirmation. Stroke subtyping was done using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system. Definitions for CAD slightly varied between cohorts but usually included myocardial infarction, symptoms of angina pectoris, and >50% coronary artery stenosis. Participating studies were approved by relevant institutional review boards, and all participants provided written or oral consent for genetic research using protocols approved by the relevant institutional body.

Statistical Analysis

For the analysis of variants showing a nominal threshold of significance ($P<0.01$) for IS, LAS, or CAD, data were taken from the METASTROKE (for IS and LAS) and the combined CARDIoGRAM and C4D (for CAD) sample. Variants with a P value of <0.01 for a given phenotype were then tested for association with the alternate phenotype(s) to determine whether the observed distribution of P values significantly deviated from the expected distribution. To ensure that only independent loci are incorporated in the analysis, we performed LD-based pruning with an r^2 cut off of 0.3 retaining the SNP with the lowest P value in the original study for each locus. QQ plots were drawn to plot –log (P values) where SNPs with effects in opposite directions were plotted separately from SNPs with effects in the same direction. To determine the deviation of the P value distribution shown in the QQ plots, P values were z transformed. Under the null hypothesis, the z transformed effects follow a standard normal

distribution. One-tailed significance was determined by comparing the absolute values of the z scores to a random normal 1-tailed distribution using a standard t test. 2×2 contingency tables were constructed for different P values and r^2 cut offs and Fisher exact test were used to evaluate the significance of the contingency tables.

Directionality of effects (odds ratio associated with the minor allele) of top variants for the 3 phenotypes (IS, LAS, and CAD) in other phenotypes (CAD for IS and LAS variants; IS and LAS for CAD variants) were examined by calculating the proportion of effects going in the same direction and comparing this proportion to that expected by chance (50%). For this, an exact binomial test was performed. The analysis was repeated on the LD-pruned data to ensure independence of tested SNPs. Bonferroni correction was applied to determine study-wide significance.

To rule out that the agreement in P values at individual risk loci is limited to single variants, we calculated the correlation of P values using Spearman rank correlation for defined genomic regions (consistent drop of P values <0.05) for each potentially shared risk locus. This allows to quantify the agreement between the P value distributions of the different phenotypes using Spearman ρ as a read out, where ρ=1 is defined as a perfect positive correlation and ρ=−1 as a perfect inverse correlation.

Meta-Analysis Methods

We performed meta-analyses of the combined data from CARDIoGRAM (for CAD) and METASTROKE (for IS and LAS) using 2 methods. First, we performed subtype-specific meta-analyses using the protocol published by Mägi et al[19] and Mägi and Morris.[20] This method was originally developed for sex-specific GWASs but can also be applied to other dichotomous covariates. The algorithm is implemented in the GWAMA software[19,20] and accounts for possible heterogeneity between study subgroups by formally allowing for interaction between genotypes and subgroups under an additive model. Here a subgroup-differentiated P value below individual P values for individual subgroups is indicative of an association with both subphenotypes. To evaluate whether the resulting meta-analysis P values are significant after correcting for multiple testing, we evaluated the false discovery rate of these P values. The R package fdrtool was used to estimate q values, a direct measure of the proportion of false-positive results in the presence of a statistically significant result.

Second, we used the method of Zaykin and Kozbur,[21] which is similar to the method by Lin and Sullivan[22] to account for overlap of an estimated ≈38 000 controls between the CARDIoGRAM and METASTROKE samples from the Kooperative Gesundheitsforschung in der Region Augsburg (KORA), Wellcome Trust Case-Control Consortium 2 (WTCCC2), Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), and deCODE studies (the exact number of overlapping controls could not be determined in the absence of individualized data), which may lead to the inflation of meta-analysis P values. This program compensates for this lack of independence in test statistics created by the use of the same controls by computing the correlation between studies and using this measure for correction of P values obtained from a standard meta-analysis. In the absence of exact numbers for overlapping controls, we simulated different scenarios of overlapping controls.

Results

Analysis of Variants Meeting a Low Threshold of Significance of Association With CAD, IS, and LAS

We first tested whether SNPs with some evidence for association with CAD also associate with IS, and vice versa. Specifically, we constructed a QQ plot in the IS GWAS meta-analysis using variants that displayed a P value of <0.01 for CAD in CARDIoGRAM/C4D.[12,16] We next constructed a QQ plot in the CAD GWAS meta-analysis using variants that displayed a P value of <0.01 for IS in METASTROKE.[9] For both analyses, deviation of the observed from the expected distribution was highly significant with $P<10^{-82}$ (Figure 1A).

Next, we generated corresponding QQ plots for LAS and CAD. Again, deviation of the observed from the expected distribution was highly significant in both analyses ($P<10^{-27}$; Figure 1B). Corresponding QQ plots for CES and CAD also showed some deviation of the observed from the expected distribution (Figure 1C). However, the deviation was less pronounced than for LAS and CAD. Focusing on variants with a P value of <0.0001 revealed a significant excess of shared signals between IS and CAD (3.75×10^{-8}) and between LAS and CAD ($P=3.4\times10^{-3}$) but not between CES and CAD (P=1.0; Figure I in the online-only Data Supplement).

Cross-Analysis of Robustly Associated Variants for CAD and IS

We next analyzed directionality of effects (odds ratio associated with the minor allele) for all variants that have previously shown genome-wide significance for association with CAD in CARDIoGRAMplusC4D[13] in the METASTROKE GWASs for IS and LAS. Among 46 CAD variants from 42 loci, 33 variants (72%) from 31 loci showed point estimates for IS that were directionally consistent for CAD (P=0.0045; exact binomial test, 2-sided; Table II in the online-only Data Supplement). Three variants from 3 loci were significantly associated with IS (Table 1) at the 95% confidence level after Bonferroni correction (P<0.00108 for testing of 46 variants). The effects for IS were in the same direction as for CAD for all 3 of the variants. Corresponding results for LAS were 34 variants from 31 loci (74%; P=0.0016) and 5 variants from 5 loci with study-wide significance (Table 1; Table II in the online-only Data Supplement). When considering LD-pruned SNPs (r^2<0.3) the results were similar with only 2 SNPs (rs11203042 and rs3217992) at 2 loci being removed from analysis. Among 44 CAD variants from 42 loci, 31 variants from 31 loci (70%) showed point estimates for IS that were directionally consistent (P=0.0096). Corresponding results for LAS were 32 variants from 31 loci (73%; P=0.0037).

We further analyzed all variants that showed P values $<10^{-5}$ with IS in METASTROKE (n=6 variants) for directionality of effects in the CARDIoGRAM meta-analyses for CAD. The choice of a more liberal P value ($P<10^{-5}$) was based on the paucity of variants reaching genome-wide significance for IS. In all cases point estimates for CAD were directionally consistent for IS (P=0.0313; for directionality; exact binomial test, 2-sided; Table II in the online-only Data Supplement). One variant was significantly associated with CAD at the 95% confidence level after Bonferroni correction for testing of multiple variants (Table 1). Finally, we analyzed variants that showed P values $<10^{-5}$ with the LAS subtype in METASTROKE (n=11 variants) for directionality in the CAD data set. Again, the majority (82%) showed effects going in the same direction (P=0.065; Table II in the online-only Data Supplement). One variant was significantly associated with CAD (Table 1). Considering LD-pruned SNPs did not change the results. None of the 3 loci that showed P values $<1\times10^{-5}$ with CES were associated with CAD (all P_{CAD}>0.2).

Meta-Analyses of Combined Data From CARDIoGRAM and METASTROKE

As a further step we performed meta-analyses for the combined data from CARDIoGRAM (for CAD) and from

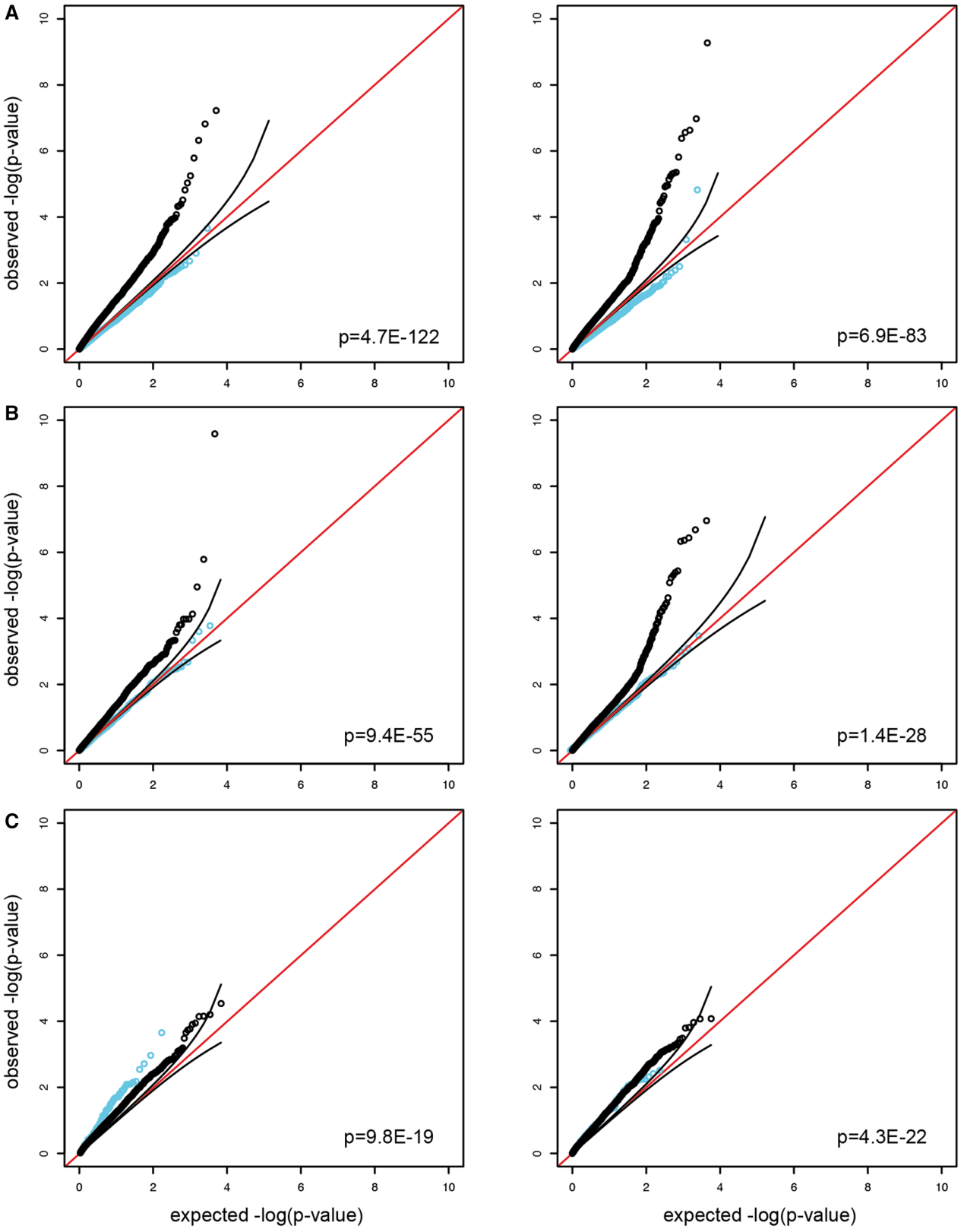

Figure 1. QQ plots for individual vascular phenotypes considering variants reaching a low threshold of significance ($P<0.01$) in alternate vascular phenotypes: coronary artery disease (CAD) variants in all ischemic stroke (IS; **left**; **A**) and all IS variants in CAD (**right**; **A**), CAD variants in large artery stroke (LAS; **left**; **B**) and LAS variants in CAD (**right**; **B**); CAD variants in cardioembolic stroke (CES; **left**; **C**) and CES variants in CAD (**right**; **C**). Single nucleotide polymorphisms (SNPs) with effects going into the same direction in the respective samples are shown in black. SNPs with effects going into opposite directions in the respective samples are shown in light blue. Data were drawn from METASTROKE, CARDIoGRAM, and C4D. Red line, expected line corresponding to a normal distribution; black lines, 95% confidence intervals of the expected distribution. For display purposes variants from the 9p21 locus are omitted from the figure. *P* values correspond to the analysis of directionally consistent SNPs (black line).

Table 1. Association Signals and Directional Consistency of Effects of Top Variants for Coronary Artery Disease, Ischemic Stroke, and Large Artery Stroke

				Coronary Artery Disease			Ischemic Stroke			Large Artery Stroke		
Lead SNPs	Band	Gene in Region	Minor Allele	*P* Value	Odds Ratio (95% CI)	Effects Go in Same Direction*	*P* Value	Odds Ratio (95% CI)	Effects Go in Same Direction†	*P* Value	Odds Ratio (95% CI)	Effects Go in Same Direction†
Top variants (known loci) for CAD/MI in the CARDIoGRAMplusC4D sample[13]												
rs12413409	10q24.32	CYP17A1/ CNNM2/ NT5C2	A	1.24E-06‡	0.89 (0.84–0.93)	. . .	0.0603	0.95 (0.89–1.00)	Yes	0.00049‡	0.80 (0.70–0.90)	Yes
rs12936587	17p11.2	RAI1-PEMT-RASD1	A	1.98E-07‡	0.93 (0.90–0.96)	. . .	0.0051	0.95 (0.92–0.98)	Yes	2.69E-05‡	0.86 (0.80–0.92)	Yes
rs3184504‖	12q24.12	chr12q24/ SH2B3	T	9.33E-07‡	1.07 (1.04–1.11)	. . .	1.01E-06‡	1.08 (1.05–1.12)	Yes	0.00015‡	1.14 (1.06–1.22)	Yes
rs2023938	7p21.1	HDAC9	C	2.10E-03‡	1.08 (1.03–1.13)	. . .	1.65E-06‡	1.14 (1.08–1.20)	Yes	2.33E-09‡	1.38 (1.24–1.53)	Yes
rs579459	9q34.2	ABO	C	2.14E-07‡	1.10 (1.06–1.14)	. . .	0.00026‡	1.08 (1.04–1.12)	Yes	0.0054	1.13 (1.04–1.22)	Yes
rs1333049	9p21.3	CDKN2BAS	C	2.96E-56‡	1.24 (1.21–1.28)	. . .	0.0053	1.05 (1.01–1.09)	Yes	3.70E-06‡	1.19 (1.11–1.28)	Yes
Top variants ($P<10^{-5}$) for all ischemic stroke in the METASTROKE sample												
rs17696736‖	12q24.13	chr12q24/ SH2B3	G	6.56E-08‡	1.07 (1.04–1.10)	Yes	5.96E-08‡	1.10 (1.06–1.14)	. . .	0.0024	1.11 (1.04–1.20)	Yes
Top variants ($P<10^{-5}$) for large artery stroke in the METASTROKE sample												
rs1333047	9p21.3	CDKN2BAS	T	1.44E-53‡	1.24 (1.20–1.27)	Yes	0.0063	1.05 (1.01–1.08)	Yes	1.64E-06‡	1.20 (1.11–1.19)	. . .

Shown are variants that were significantly associated with both coronary artery disease (CAD) and ischemic stroke (IS), or both CAD and large artery stroke (LAS), or all 3 phenotypes (study-wide level of significance: $P<0.00108$ for CAD, $P<0.008$ for IS, and $P<0.0045$ for LAS). Results are shown for the CARDIoGRAM[12]§ and METASTROKE[9] samples. CI indicates confidence interval; and SNP, single nucleotide polymorphism.

*Compared with IS or LAS.

†Compared with CAD/MI, associations reaching study-wide significance ($P<0.00108$, $P<0.008$, and $P<0.0045$) are shown with a (‡).

§Note that the CARDIoGRAM sample represents a subsample of the CARDIoGRAMplusC4D sample.

‖rs3184504 and rs17696736 are in high linkage disequilibrium ($r^2=0.72$, $D'=0.91$).

METASTROKE (for IS and LAS) to identify variants that are associated with the broader vascular end point. This meta-analysis revealed 15 loci that exceeded the threshold for genome-wide significance for the combined CAD/IS phenotype (Table 2; Figure 2A) and 17 loci that exceeded the threshold for genome-wide significance for the combined CAD/LAS phenotype (Table 2; Figure 2B). All of these loci have been published previously for genome-wide significant association with CAD.[13] Of note however in the combined datasets, several loci showed *P* values that were >1 order of magnitude lower than those in individual meta-analyses on the individual diseases. This applied to 3 loci of the CAD/IS meta-analysis and 5 loci of the CAD/LAS meta-analysis (Table 2). All loci were still significant after false discovery rate correction (Table 2).

We next focused on loci that showed the strongest independent association with IS or LAS in addition to their genome-wide significance in the combined data set. For IS these were chr12q24/SH2B3 ($P_{IS}=1.62\times10^{-7}$) and ABO ($P_{IS}=2.65\times10^{-4}$; Table 2). For LAS, these were HDAC9 ($P_{LAS}=2.39\times10^{-12}$), 9p21 ($P_{LAS}=3.85\times10^{-6}$), RAI1-PEMT-RASD1 ($P_{LAS}=2.69\times10^{-5}$), CYP17A1-CNNM2-NT5C2 ($P_{LAS}=4.92\times10^{-4}$), and EDNRA ($P_{LAS}=7.29\times10^{-4}$; Table 2). In all cases *P* values for individual variants within the respective genetic regions (defined as a consistent drop of *P* values <0.05) significantly correlated between CAD and stroke phenotypes suggesting that the association signals originate from the same genetic variants (chr12q24/SH2B3: Spearman $\rho_{IS/CAD}=0.68$, $P=3.8\times10^{-87}$; ABO: $\rho_{IS/CAD}=0.82$, $P=2.2\times10^{-08}$; HDAC9: $\rho_{LAS/CAD}=0.83$, $P=4.8\times10^{-12}$; 9p21: $\rho_{LAS/CAD}=0.85$, $P=2.9E^{-35}$; RAI1-PEMT-RASD1: $\rho_{LAS/CAD}=0.78$, $P=5.6\times10^{-17}$; CYP17A1/CNNM2/NT5C2: $\rho_{LAS/CAD}=0.46$, $P=7.5\times10^{-15}$; EDNRA: $\rho_{LAS/CAD}=0.85$, $P=6.5\times10^{-13}$; Figure II in the online-only Data Supplement).

A closer look at loci that were significant in the combined meta-analyses revealed that some loci showed a strong association with both phenotypes reaching a similar level of significance for the IS and CAD phenotypes (Figure 3; Figure II in the online-only Data Supplement), whereas for other loci the association was largely confined to a single phenotype (Figure 4; Figure II in the online-only Data Supplement).

To account for the overlap in controls between the stroke and CAD samples, we further performed conventional sample-size dependent meta-analyses[21] for 2 different scenarios covering the estimated number of controls that overlapped between the 2 samples (Table III in the online-only Data Supplement). The results compared well with the primary subtype-specific meta-analysis except for HDAC9 (for all IS/CAD) and SORT1 (for LAS/CAD), which reached genome-wide significance in the

Table 2. Association Signals for Risk Loci Significantly Associated With the Combined Coronary Artery Disease/Stroke Phenotypes in Meta-Analyses

rs_number	Band	Gene in Region	CAD *P* Value	IS *P* Value	Effects Go in the Same Direction	CAD and IS Combined *P* Value	FDR q Value
Ischemic stroke							
rs1333049	9p21.3	CDKN2BAS	2.96E-56	0.005	Yes	1.09E-56	5.00E-51
rs11065987	12q24.12	chr12q24/SH2B3	5.13E-09	1.62E-07	Yes	4.05E-14*	9.60E-10
rs10455872	6q25.3	SLC22A3/LPAL2/ LPA	3.15E-13	0.322	Yes	1.72E-12	3.75E-08
rs1122608	19p13.2	LDLR/SMARCA4	3.32E-11	0.002	Yes	2.59E-12*	5.52E-08
rs4714955	6p24.1	PHACTR1	6.30E-12	0.498	No	4.24E-11	8.30E-07
rs11556924	7q32.2	ZC3HC1	2.55E-10	0.217	Yes	9.37E-10	1.59E-05
rs964184	11q23.3	ZNF259	1.50E-10	0.871	Yes	1.17E-09	1.95E-05
rs579459	9q34.2	ABO	2.14E-07	0.0003	Yes	1.81E-09*	2.95E-05
rs2219939	15q25.1	ADAMTS7	2.65E-09	0.042	No	2.49E-09	3.97E-05
rs7582720	2q33.1	WDR12	3.76E-09	0.052	No	4.18E-09	6.30E-05
rs599839	1p13.3	SORT1	1.41E-09	0.938	Yes	1.07E-08	0.00014
rs12190287	6q23.2	TCF21	2.32E-09	0.823	No	1.69E-08	0.00022
rs12449964	17p11.2	RAI1-PEMT-RASD1	1.64E-07	0.005	Yes	2.23E-08	0.00028
rs17114036	1p32.2	PPAP2B	9.78E-09	0.162	Yes	2.66E-08	0.00032
rs9351814	6q13	C6orf155	1.45E-07	0.015	Yes	4.93E-08	0.00055
Large artery stroke							
rs1333049	9p21.3	CDKN2BAS	2.96E-56	3.70E-06	Yes	1.20E-59*	5.92E-54
rs10455872	6q25.3	SLC22A3/LPAL2/ LPA	3.15E-13	0.009	Yes	9.25E-14	2.18E-09
rs2107595	7p21.1	HDAC9	0.042	2.32E-12	Yes	2.60E-12	5.72E-08
rs1122608	19p13.2	LDLR/SMARCA4	3.32E-11	0.017	Yes	1.56E-11	3.31E-07
rs4714955	6p24.1	PHACTR1	6.30E-12	0.173	No	2.11E-11	4.42E-07
rs12936587	17p11.2	RAI1-PEMT-RASD1	1.98E-07	2.69E-05	Yes	1.93E-10*	3.56E-06
rs11065987	12q24.12	chr12q24/SH2B3	5.13E-09	0.002	Yes	3.20E-10*	5.77E-06
rs11556924	7q32.2	ZC3HC1	2.55E-10	0.167	Yes	7.74E-10	1.33E-05
rs599839	1p13.3	SORT1	1.41E-09	0.023	Yes	8.17E-10	1.40E-05
rs964184	11q23.3	ZNF259	1.50E-10	0.468	No	9.14E-10	1.55E-05
rs12190287	6q23.2	TCF21	2.32E-09	0.814	Yes	1.69E-08	0.00023
rs12413409	10q24.32	CYP17A1-CNNM2-NT5C2	1.24E-06	0.0005	Yes	1.77E-08*	0.00023
rs6841581	4q32.21	EDNRA	8.45E-07	0.0007	Yes	1.78E-08*	0.00024
rs17114036	1p32.2	PPAP2B	9.78E-09	0.133	Yes	2.29E-08	0.00029
rs899997	15q25.1	ADAMTS7	4.75E-09	0.391	No	2.41E-08	0.00030
rs7582720	2q33.1	WDR12	3.76E-09	0.682	No	2.55E-08	0.00032
rs579459	9q34.2	ABO	2.14E-07	0.005	Yes	2.96E-08	0.00036

Shown are loci with $P<5e-8$. Single nucleotide polymorphisms (SNPs) showing the lowest meta-*P* values in the respective region are reported. Data were drawn from METASTROKE and CARDIoGRAM. Results are shown for both ischemic stroke (IS) and large artery stroke (LAS). CAD indicates coronary artery disease; and FDR, false discovery rate.

**P* value for the combined phenotype is >1 order of magnitude lower than in individual meta-analyses on the individual phenotypes. Note that variants at individual loci may differ from those reported in Table 1.

respective subtype-specific meta-analyses but not in the conventional sample-size–dependent meta-analyses.

Discussion

This study demonstrates that common variants at a substantial number of genetic loci influence risk of both IS and CAD. This conclusion is supported by the results of several approaches: First, selecting common variants that had reached a nominal threshold ($P<0.01$) of significance in previous studies and testing them for association with the respective other vascular phenotype; second, analyzing common variants that had reached a high threshold of significance in previous studies;

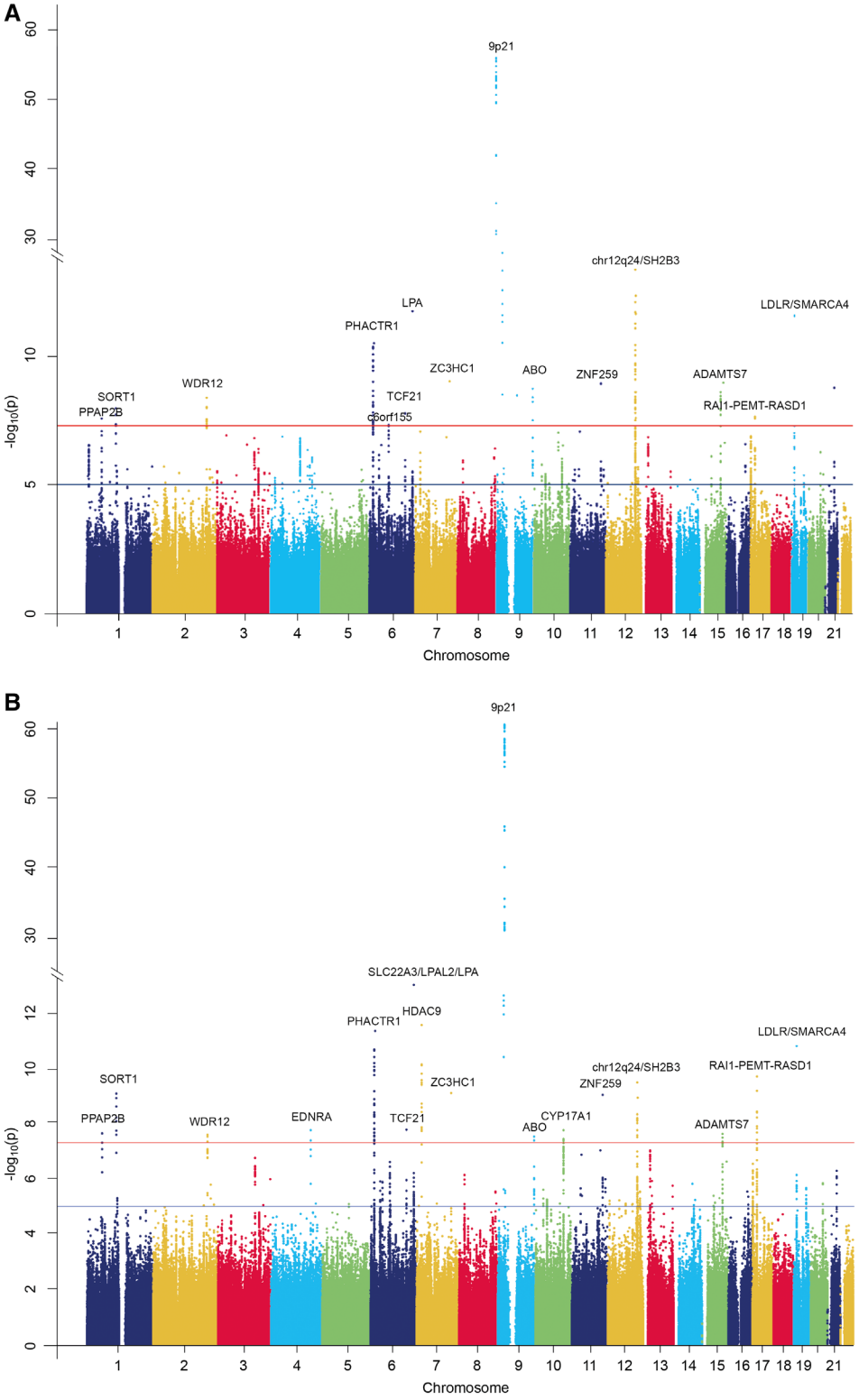

Figure 2. Manhattan plots of $-\log_{10}(P)$ against genomic position: Results are shown for (**A**) the combined end point of all ischemic stroke or coronary artery disease (CAD) and (**B**) the combined end point of large artery stroke or CAD. Genome-wide meta-analysis association results by genomic position at autosomal single nucleotide polymorphisms. Data were drawn from METASTROKE and CARDIoGRAM.

and third, meta-analysis of the combined vascular end point of CAD and IS, as well as CAD and LAS.

The QQ plots suggest that multiple variants at multiple loci including variants reaching a low threshold of significance for association with IS, CAD, or both, and thus not previously reported as risk loci for arterial disease, contribute to shared genetic susceptibility to IS and CAD. This agrees with the growing evidence that common traits are affected by a large number of causative alleles with very small effects.[23] As illustrated both by the QQ plots and the analysis of variants meeting a high threshold of significance, the excess of shared signals between CAD and LAS was more pronounced than the excess of signals between CAD and CES. This might indicate that some of the shared risk variants for CAD and LAS act

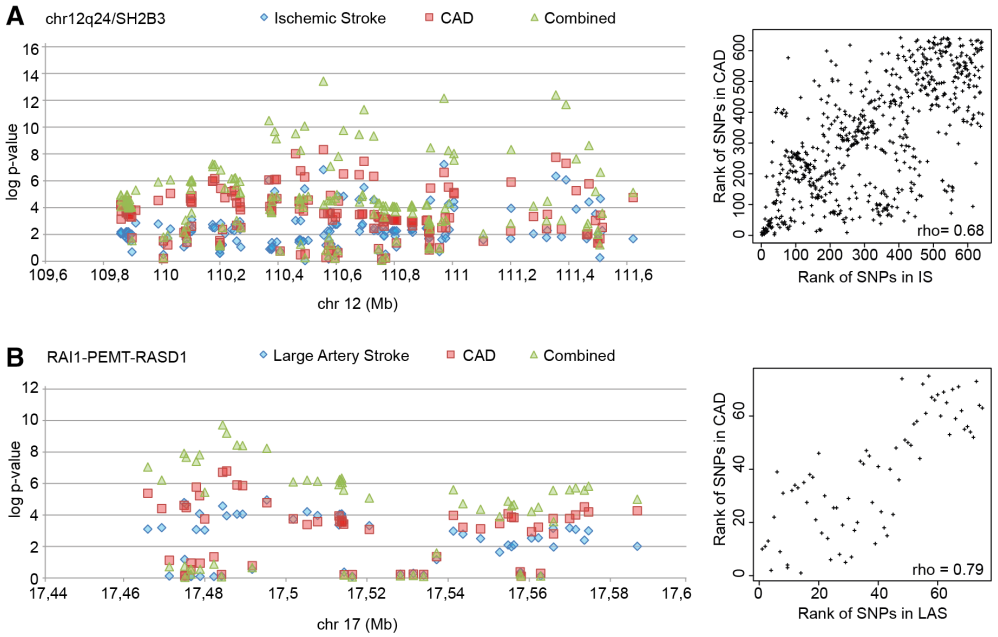

Figure 3. Regional association plots (**left**) and corresponding Spearman correlation plots (**right**) of *P* values for individual variants of (**A**) the chr12q24/SH2B3 locus for ischemic stroke (IS) and coronary artery disease (CAD) and (**B**) the RAI1-PEMT-RASD1 locus for large artery stroke (LAS) and CAD. For clarity, only a subset of variants is displayed (see Figure II in the online-only Data Supplement for all variants). Data were drawn from METASTROKE and CARDIoGRAM. SNP indicates single nucleotide polymorphism.

through mechanism that are relatively specific for atherosclerotic disease.

Several loci thus far not identified in isolated GWASs of IS or LAS showed a strong and consistent signal when considered jointly with CAD. Several lines of statistical evidence support a role for these loci in IS risk: (1) *P* values for individual variants were $<1\times10^{-3}$, (2) the combined *P* value in the joint meta-analysis with CAD was genome-wide significant and ≥1 order of magnitude below the *P* value found for CAD alone, and (3) *P* values for individual variants significantly correlated between CAD (where these loci reached genome-wide significance) and IS or LAS.

Loci reaching genome-wide significance in the joint meta-analyses can be broadly classified into 3 categories: loci that showed a clear signal for both IS and CAD (eg, chr12q24/SH2B3; Figure 3A), loci that showed a clear signal for both LAS and CAD (eg, RAI1-PEMT-RASD1; Figure 3B), and loci for which the association was confined to CAD (eg, SORT1 or TCF21; Figure 4).

The locus with the strongest association signal for IS was at chr12q24/SH2B3 and to date had not been reported for this phenotype. This locus also showed one of the strongest signals in the combined meta-analysis indicating that chr12q24/SH2B3 is a major susceptibility locus for cardiovascular disease. Variants in this region have been shown previously to be associated with various other traits including blood pressure,[24,25] blood lipids,[26] platelet count,[27] and type-1 diabetes mellitus.[28] Several of these traits are linked to IS, CAD, or both. Odds ratios for IS and CAD were similar and *P* values for individual variants for IS and CAD significantly correlated indicating that the association signals for the 2 phenotypes originate from the same genetic variants.

Variants at ABO, the locus with the second strongest signal for IS, have likewise been associated with a variety of traits including low-density lipoprotein,[26] von Willebrand factor,[15] and venous thromboembolism.[29] Again, *P* values for individual variants for IS and CAD significantly correlated and the odds ratios for IS, CAD, and LAS were all similar with no significant heterogeneity (Table II in the online-only Data Supplement). Several observations suggest that the effects of this locus on vascular risk are mediated by an influence on end-stage coagulation and thrombosis,[15,29,30] which would be consistent with shared mechanisms in CAD and the broader phenotype of IS.[15]

Loci significantly associated both with CAD and the more restricted phenotype of LAS included 9p21.3, the locus with the strongest signal in the combined meta-analysis, HDAC9, and several loci not previously reported to be associated with LAS. Among the most significant loci is RAI1-PEMT-RASD1 (17p11.2), which to date has not been reported as a risk locus for LAS. Once again, *P* values for individual variants for LAS and CAD significantly correlated at this locus. Variants at RAI1-PEMT-RASD1 also significantly associated with IS, but the odds ratio and level of significance were lower than for LAS, suggesting that the association with IS is driven by the association with LAS. Interestingly, the RAI1-PEMT-RASD1 locus to date has not been associated with other traits or diseases known to relate to the vascular system. Another locus significantly associated with both LAS and CAD and not previously reported as being associated with LAS is EDNRA. This locus has been associated with carotid artery atherosclerosis,[31] suggesting that this locus acts by promoting early atherogenesis.

Finally, several loci displayed highly significant associations with CAD, whereas showing no association with LAS or

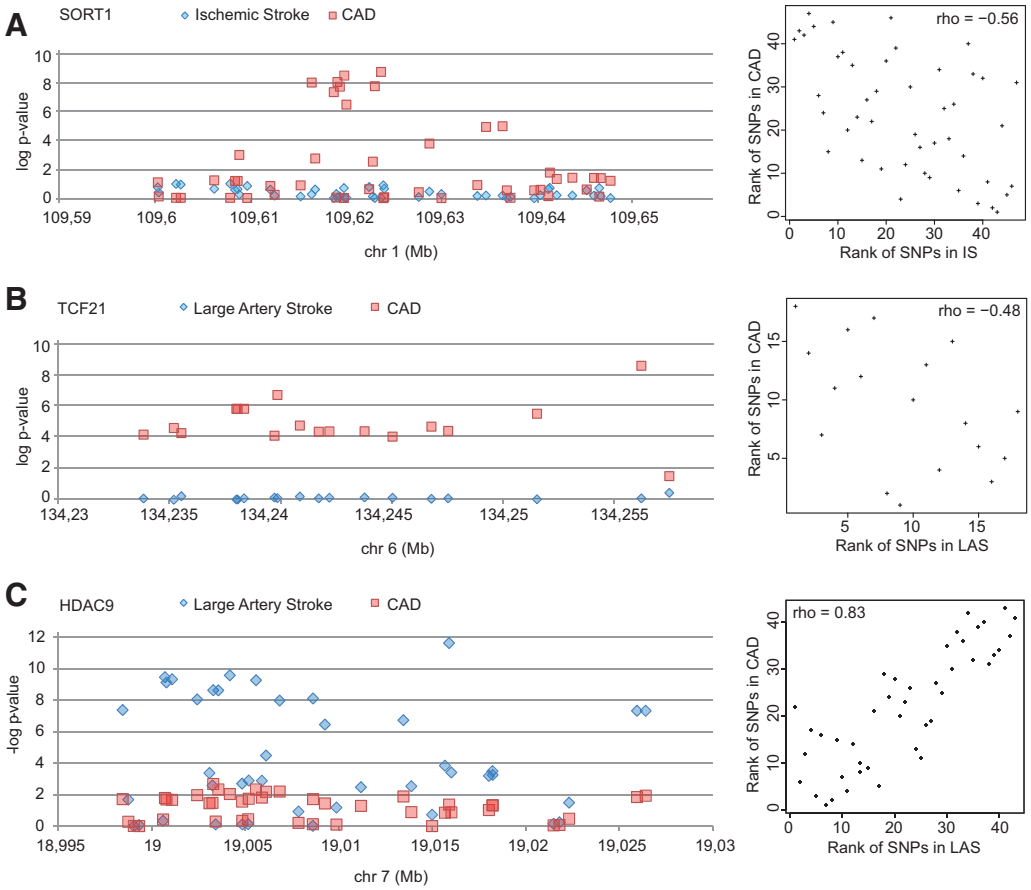

Figure 4. Regional association plots (**left**) and corresponding Spearman correlation plots (**right**) of (**A**) the SORT1 locus for ischemic stroke (IS) and coronary artery disease (CAD), (**B**) the TCF21 locus for large artery stroke (LAS) and CAD, and (**C**) the HDAC9 locus for LAS and CAD. For clarity, only a subset of variants is displayed (see Figure II in the online-only Data Supplement for all variants). Data were drawn from METASTROKE and CARDIoGRAM. SNP indicates single nucleotide polymorphism.

IS. This included TCF21 (6q23.2), PHACTR1 (6p24.1), and WDR12 (2q33.1), which are among the strongest signals for CAD.[12,13] The finding suggests partially distinct mechanisms by which common genetic variants contribute to the risk of CAD and LAS.

Our findings must be interpreted in light of the known comorbidity between IS and CAD. We did not control for comorbid vascular disease because the information was not available for most of the participants. However, the pattern of association between established CAD loci and IS differs from what would be expected based on comorbidity or referral bias in that the chr12q24/SH2B3 locus displayed a similar strength of association with IS and CAD, and RAI1-PEMT-RASD1 (17p11.2) showed a similarly strong association with LAS and CAD. Also, several of the top signals for CAD displayed no association with IS or LAS. We can largely exclude a referral bias favoring the selection of patients with stroke with a diagnosis of CAD because the majority of subjects included into METASTROKE were recruited through acute stroke services or through population-based studies. There may have been some enrichment for patients with a history of stroke among subjects recruited into CARDIoGRAM/C4D. However, with the exception of HDAC9, all top signals in the combined meta-analysis showed stronger associations with CAD than with IS, which cannot be explained by comorbidity or referral bias.

Our data add to the understanding of familial aggregation of IS and CAD. A parental history of CAD is a risk factor for stroke and a family history of stroke is a risk factor for CAD and acute coronary syndromes.[8] In fact, a family history of stroke was found to be as common in acute coronary syndromes as in patients with acute cerebrovascular events.[8] Our finding of shared genetic influences between IS and CAD provides some explanation for the aggregation of different arterial phenotypes within families.

Translating findings from genetic association studies into clinical practice remains a challenge. Recent GWASs have revealed a large number of loci that are associated with classical vascular risk factors,[24] and genetic risk scores based on multiple SNPs for blood pressure[24] or lipid levels are associated with vascular end points including stroke and CAD. Up to now, however, the clinical use of such scores in predicting vascular risk is rather limited. This may change as additional information from even more markers is added. More importantly, identification of the biological pathways and mechanisms by which shared genetic influences modulate vascular risk might eventually lead to novel therapeutic strategies with a broad impact on vascular disease.

Our study has limitations. First, sample sizes for IS and CAD differed substantially. Second, there was some overlap in controls between the IS and CAD studies. We attempted to account for these limitations through the use of appropriate analytic algorithms and found that our results were remarkably stable when performing meta-analyses assuming a wide range in the proportion of overlapping controls. The statistical strength of the subtype-specific meta-analysis[19] is illustrated by the results for HDAC9, which showed a strong association in the joint subtype-specific meta-analysis, despite a weak signal in CAD.

In conclusion, this is the first study examining shared genetic influences between IS and CAD by meta-analyzing GWAS data. Our data provide insights into shared mechanisms and may, in part, explain why vascular events in one organ predict vascular events in the other organ.

Appendix

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