Variation in inflammation-related genes and risk of incident nonfatal myocardial infarction or ischemic stroke

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Abstract

Background—From initiation to plaque rupture, immune system components contribute to atherosclerosis. We investigated variation in inflammation-related genes – Interleukin (IL)-1β, IL-6, C-reactive protein (CRP), IL-10, IL-18, and the Tumor Necrosis Factor (TNF) superfamily [Lymphotoxin(LT)-α, TNF-α, LT-β] – with respect to nonfatal incident myocardial infarction (MI) or ischemic stroke risk.

Methods & Results—A population-based case-control study recruited postmenopausal and/or hypertensive Group Health members aged 30 to 79 years. We chose a subset of single nucleotide polymorphisms (SNPs) to describe common gene-wide variation on the basis of linkage disequilibrium. **36** SNPs, describing **38** common haplotypes for 5 genes and a 3-gene cluster, were genotyped among 856 MI cases, 368 stroke cases, and 2,688 controls. Associations of SNPs or PHASE-inferred haplotypes and risk were estimated using logistic regression; significance of gene-level associations was assessed with global Wald tests and permutation tests. Gene-wide IL-18 variation was associated with higher MI risk and an IL-1B haplotype was associated with lower stroke risk. In secondary analyses of SNPs, we observed associations of several IL-1B polymorphisms with risk of MI or stroke. IL-6, CRP, IL-10, and TNF superfamily gene variation was not associated with MI or stroke risk.

Conclusions—Our results support prior reports associating an IL-18 gene variant and MI risk, contribute additional evidence to reports of IL-1B and cardiovascular risk, and fail to confirm risk differences previously observed for CRP, IL-6, and TNF-α promoter variants.

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From initiation to plaque rupture, the immune system contributes to the atherosclerotic process. [1, 2] Levels of both C-reactive protein (CRP), an acute phase reactant, and interleukin (IL)-6, a pleiotrophic cytokine critical to its production, have been repeatedly found to be reliable predictors of cardiovascular disease. [3, 4] Other cytokines, including anti-inflammatory IL-10 and pro-inflammatory TNF-α, have been associated with cardiovascular outcomes among older adults. [5]

Based on prior associations between inflammation markers and cardiovascular disease and heritability estimates suggesting strong to moderate genetic component for levels of key inflammation markers, [6, 7] we speculated that variation within inflammation genes might result in lifelong exposures to higher or lower levels of inflammation system components. Such differences might then influence nonfatal incident myocardial infarction (MI) or ischemic stroke risk. A similar premise motivated a recent investigation of polymorphisms related to low-density lipoprotein levels, which found that the variants were also associated with lower coronary heart disease risk. [8] Using a population-based case-control study, we investigated sequence variations in IL-1B, IL-6, CRP, IL-10, IL-18, and the tumor necrosis factor (TNF) superfamily [lymphotoxin (LT)- α (LTA), TNF-α, LT-β (LTB)] genes. Because multi-locus haplotypes are expected to provide more information about relationship between genetic variation and phenotypes than single variants, [9] our approach considered gene-wide variation in our primary analyses.

METHODS

Setting

This study was conducted in the setting of Group Health, a large health maintenance organization commonly offered as an insurance option by many employers in the state of Washington. These analyses were conducted as part of ongoing population-based case-control studies of MI, stroke, and other cardiovascular outcomes. The methods, which have been described elsewhere, [10, 11] are summarized below. The Group Health Human Subjects Review Committee approved the study and all participants provided written informed consent.

Study Population

Study participants were members of Group Health aged 30 to 79 years who were either adults with pharmaceutically-treated hypertension or postmenopausal women who were alive at the time of study recruitment. We used computerized discharge abstracts and billing records to identify participants who suffered an incident MI or ischemic stroke during the study period (January 1, 1995 through December 31, 2002).

Controls, randomly sampled from the Group Health enrollment files on the basis of person-time, [12] were frequency-matched to the MI cases based on age (within decade), sex, calendar year of presentation, and medically treated hypertension. We excluded MI cases if they had a history of ischemic stroke, stroke cases if they had a history of MI, and control subjects if they had a history of either MI or stroke. Cases whose event was a complication of a procedure were not eligible for the study.

All subjects were assigned an index date. For the cases, the index date was the date of admission for the first MI or stroke; for the controls it was a computer-generated random date within the same calendar year for which they were chosen as controls.

Data collection

Data collection included a review of the medical record, a telephone interview, and a venous blood sample from subjects who agreed to participate. Information about eligibility and risk

factors for MI and stroke for the period before the index date was obtained by reviewing the outpatient medical record. Additional information regarding social and behavioral factors related to cardiovascular disease risk was obtained by telephone interview. Subjects were required to have had at least four health care visits prior to the index date to increase the likelihood that information would be available on health conditions. Incident events of myocardial infarction and stroke were verified by review of inpatient and outpatient medical records. For MI, confirmation was based on symptoms, electrocardiogram findings, enzyme levels, and physician diagnosis and treatment. For ischemic stroke, confirmation required the rapid onset of a neurologic deficit that persisted at least 24 hours or evidence of infarction on brain imaging studies.

Blood collection and laboratory analysis—A research nurse or certified phlebotomist visited the participants, obtained informed consent, and collected a venous blood sample. Blood was processed using standard methods and DNA was extracted from a single white-cell aliquot.

Genetic Assays—Genes related to inflammation were selected *a priori* and single nucleotide polymorphisms (SNPs) were identified by genomic resequencing by Seattle SNPs [13] (CRP, IL-1B, IL-6, IL-10, TNF, LTA, LTB) or Innate Immunity [14] (IL-18) among 23 European-American and 24 African-American DNA samples from the Coriell Repository. Sequencing included at least 94% of the gene and its immediate flanking region for all genes. For each gene, we chose a subset of highly-informative SNPs to describe common gene-wide variation on the basis of linkage disequilibrium using *ldSelect*. [15] With this method, all polymorphisms above our specified frequency threshold (5%) in the discovery populations were assayed directly or exceeded a specified level of correlation ($r^2 = 0.64$) with an assayed polymorphism.

Genotyping—Genotyping was performed by Illumina® with a GoldenGate custom panel using BeadArray® technology. Illumina® personnel were blinded to case-control status. From the final list of 71 SNPs, 60 SNPs in the 8 genes were successfully genotyped on the Illumina® platform. Among the 60 successful SNPs, 99.95% of nucleotide pairs were successfully called.

Data Analysis

We used PHASE [16] to infer haplotypes from the unphased SNPs genotyped for each gene and coded the resulting haplotype variables according to the number of copies present for each participant. For the TNF superfamily, primary analyses considered haplotypes constructed for the three-gene cluster. To accommodate uncertainty in haplotype estimation, participants were assigned multiple haplotype combinations along with a probability, which was used as a weight in analyses clustered on participant. [17] Haplotypes present in fewer than 2.5% of the control participants were grouped into a single "rare" bin to improve the power of global score tests. [18]

Statistical analyses were conducted using *Stata, version 8.2* (College Station, TX). Hardy-Weinberg Equilibrium was assessed using a Pearson's χ^2 test of expected proportions. Associations of haplotype and risk were calculated using logistic regression, which allowed estimation of odds ratios for each haplotype adjusted for race and the matching variables: age, sex, index year, and treated hypertension status. If a haplotype containing the common allele for all sites was not common, the most prevalent haplotype was used as the reference group. Gene-wide significance was assessed by fitting a model that included all common haplotypes and the group of rare haplotypes. For each gene, a Wald test of the hypothesis that none of the common haplotype's odds ratio, relative to the reference haplotype, differed from 1.0 served as a global test of association. Primary analyses considered an additive model for haplotype

associations; odds ratios represent the odds of an event associated with an additional copy of the haplotype relative to an additional copy of the reference haplotype.

In addition to the global test, we calculated a permutation test [a $max(z^2)$ statistic], which is expected to have greater power to detect associations in instances in which a single haplotype has a much larger association than all others. [18] For each gene and outcome (MI and stroke), we determined the haplotype with the largest test statistic and calculated an empirical p-value by shuffling a person's set of genotypes with respect to the phenotypes (case-control status, participant characteristics) to create 1,000 permuted datasets. The analyses were then repeated on each of the shuffled datasets to see how often a haplotype was observed to have a test statistic value as extreme as the maximum observed in the unshuffled dataset. The empiric p-value is the number of shuffled datasets with an equal or more extreme test statistic divided by the number of permutations.

Secondary analyses considered the association of individual SNPs and MI or stroke risk. These analyses used logistic regression and assumed an additive model for SNPs. We evaluated the adequacy of this assumption with a likelihood ratio test comparing the additive model to general association (2df) model. We explored the possibility that results differed by sampling strata or clinical characteristics by fitting logistic regression models that included product terms. To further explore potential interactions we subsequently used Logic Regression, an adaptive regression method that constructs predictors as Boolean combinations of binary covariates, [19] to search for potential high-level interactions between SNPs. From the 60 SNPs successfully genotyped in the study population, 15 were found to be homozygous in all participants or extremely rare (<2% minor allele frequency), 8 were tightly linked ($r^2 > 0.8$) with another genotyped SNP, and 1 showed extreme differences from the genotype frequencies predicted by Hardy-Weinberg Equilibrium. Many of these SNPs were initially selected to describe variation among African-Americans or were intentionally chosen to improve the probability of successfully-genotyping a tag SNP for a given bin. These sites were not included in analyses of individual SNPs, leaving **36** variants. No correction was made for multiple testing and our conclusions of significance are based on a Type I error rate of 0.05 per test. For our primary hypotheses, we used the global and permutation tests to assess significance using a significance level of 0.05 for each gene and outcome (MI and stroke).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Some characteristics of the study participants are shown in Table 1. Our analyses included 2,688 controls, 856 MI cases, and 368 ischemic stroke cases. We observed expected differences between cases and controls, with diabetes, higher blood pressure, and higher cholesterol more common among the MI and stroke cases.

Gene results

A summary of the genes investigated in this study is shown in Table 2. The **36** bi-allelic tag SNPs considered for analyses provided good coverage (>90%) of the common SNPs found in the European-descent variation discovery panels for all of the genes except for IL-18 and the TNF superfamily. Among these 36 SNPs, the allele frequency for *rs30224498* (IL-10) significantly differed from the expectation of Hardy-Weinberg Equilibrium among self-described White participants; *rs360722* (IL-18) and *rs2229094* (LTA) differed from the expected frequencies among self-described African-Americans.

Common haplotypes accounted for 87 to 98% of all observed haplotypes for the genes in this analysis. Global gene-wide tests indicated an association of gene variation and MI risk for the IL-18 gene (p=0.009). The permutation tests also suggested an association of an IL-1B haplotype and ischemic stroke risk (p=0.022).

IL-18—The analyses for IL-18 included five common haplotypes defined by six common SNPs, plus a group of rare haplotypes. Table 3 summarizes the association of IL-18 haplotypes and SNPs with MI and ischemic stroke risk. In rows, a matrix of nucleotides shows the alleles present for each common haplotype, the haplotype prevalence for cases and controls, and odds ratios and 95% confidence intervals for MI and stroke risk. In columns, each SNP's minor allele frequency among controls is shown along with the odds ratios and 95% confidence intervals for MI and stroke risk. Among these, we observed a gene-wide association of IL-18 haplotypes and MI risk. Haplotype 5, present in 32% of control participants and 28% of MI cases, contains the minor allele for *rs2043055*, a SNP in the gene's first intron, and appeared to be the haplotype responsible for the gene-wide association with MI risk. Compared to the reference haplotype, which contained the common allele for all genotyped sites, each additional copy of haplotype 5 was associated with a lower MI risk (odds ratio, 0.74; 95% confidence interval, 0.64 to 0.87). IL-18 haplotypes were not associated with stroke risk.

In analyses of individual IL-18 SNPs, each copy of *rs2043055*'s minor allele was also associated with a lower risk of MI (0.81; 0.72 to 0.91). No IL-18 SNP was associated with ischemic stroke risk.

IL-1B—Our analyses of the IL-1B gene included six common haplotypes defined by six common SNPs plus a group of rare haplotypes (Table 4). While IL-1B haplotypes were not associated with MI risk, we observed an association of one IL-1B haplotype and stroke risk. Haplotype 4, prevalent in 22% of controls and 26% of stroke cases, contains the minor allele for *rs1143629*, a SNP in the gene's second intron. Compared to the reference haplotype, which contains minor alleles for *rs3917356* and *rs1143633*, each additional copy of haplotype 4 was associated with higher stroke risk (1.38; 1.12 to 1.71).

When we considered individual IL-1B SNPs in additive models, additional copies of the *rs1143629* minor allele were associated with higher MI risk (1.13; 1.01 to 1.27) and additional copies of the *rs3136558* were associated with lower MI risk (0.86; 0.75 to 0.98). For stroke, the minor alleles for each of the two modestly correlated ($r^2 = 0.54$) minor alleles present on the reference haplotype was associated with lower stroke risk.

CRP, IL-6, IL-10, TNF Superfamily—For the CRP, IL-6, IL-10, and the TNF superfamily, we did not observe a significant global test or max statistic for either MI or stroke. The results were similar when SNPs were analyzed separately; additional copies of the minor allele were not associated with MI or stroke risk for any SNP in these genes. Tables for these genes are available in an online appendix.

Subgroups & interactions—Results were similar when the sampling strata (postmenopausal women, participants with treated hypertension) were considered separately. Findings did not differ in analyses restricted to participants who self-described their race as White. We evaluated several interactions between genes and clinical characteristics (including age, sex, obesity, diabetes) and drug-gene interactions for common classes of hypertension medications and statins and found no more significant differences than expected by chance alone. In Logic Regression models used to explore potential interactions among SNPs permutation tests indicated that for both MI and stroke the data did not contain significant signal for SNP-SNP interaction.

DISCUSSION

In this study of common genetic variation among eight inflammation-related genes, one gene (IL-18) was associated with MI risk and another (IL-1B) with ischemic stroke risk. A common IL-18 haplotype was associated with lower MI risk (OR, 0.74; 95% CI, 0.64 to 0.87]) and an IL-1B haplotype was associated with an increased risk of stroke (1.38; 1.12 to 1.71). Of **36** SNPs, three were associated with incident MI and two were associated with ischemic stroke.

Our results for IL-18, a pro-inflammatory cytokine related to production of both T and B cells, are consistent with previous studies of the IL-18 gene's relationship to cardiovascular outcomes. Although there are no previous reports on the SNP associated with MI in this study, an IL-18 haplotype was associated with both lower levels of circulating IL-18 and lower risk of cardiovascular death (HR, 0.57; 95% CI, 0.36 to 0.92) among a cohort of coronary artery disease patients. [20] The presumed SNP marking the haplotype of interest is located in the gene's 3′ untranslated region (+183A>G [*rs5744292*]). While we did not genotype this variant directly, *rs2043055*, the SNP marking haplotype 5 in our analyses was in modest, but significant linkage disequilibrium ($r^2 = 0.34$) with it among the European-descent variation discovery samples. Both this SNP and haplotype 5 were also associated with lower MI risk among our participants.

Our finding of increased stroke risk associated with an IL-1B haplotype marked by *rs1143629* supports some findings from previous research. In another study of the IL-1B gene, which codes for a proinflammatory cytokine, a polymorphism in the gene's 3′ flanking region (−511C>T [*rs16944*]) was associated with increased risk for small vessel disease stroke. [21] Although we did not genotype this polymorphism directly, *rs1143629* was in high ($r^2 = 0.81$) linkage disequilibrium with it among the European-descent variation discovery panel. In our analyses of *rs1143629*, we observed a modest, but significantly higher risk of MI risk for additional copies of the minor allele. For ischemic stroke, the risk estimate was similar, but the confidence interval included 1.0. These findings are in contrast to an observation by Iacovello *et al* that homozygotes for the minor allele of the *rs16944* polymorphism were at lower risk of MI and ischemic stroke. [22] Our findings of lower MI risk for *rs3136558* and lower stroke risk for *rs3917356* and *rs1143633* appear to be novel. Although other gene clusters coding for IL-1 proteins, such as IL-1RA, have been widely investigated in relation to stroke, we did not genotype any polymorphisms in these genes.

Our results for IL-10, an anti-inflammatory cytokine that inhibits a range of pro-inflammatory cytokines are consistent with an absence of an association with MI risk. [23, 24] No IL-10 SNP or haplotype was associated with MI or ischemic stroke risk among our study population.

Our analysis of polymorphisms of the TNF superfamily, a series of three genes (LTA, LTB, TNF) in close proximity within the major histocompatibility locus whose products play an important role in immune system regulation, did not support previous findings relating gene variation to cardiovascular outcomes. Among coronary artery disease patients, two TNF promoter region polymorphisms (including −308G>A [*rs1800629*]) were not associated with MI risk, [23] while other studies observed a lower risk of stroke for carriers of the *rs1800629* minor allele. [25, 26] In our data, *rs1800629* was not associated with stroke risk, but in a post-hoc analysis, minor allele homozygotes were observed to have lower MI than carriers of two copies of the common allele [AA vs. GG, OR, 0.47; 95% CI, 0.25 to 0.87].

Although a case-control genome-wide scan identified LTA SNPs (*rs909253* and others in high LD with it) associated with MI risk among Japanese participants,[27] confirmation of these results have been mixed. A recent large-scale case-control study and meta-analysis of subsequent studies concluded that common LTA polymorphisms are not strongly associated with coronary disease susceptibility. [28] Our results contribute additional evidence for a lack

of association; among our study population *rs909253* was not associated with MI or stroke risk.

We did not observe evidence to support a role for IL-6 gene variation in MI or ischemic stroke risk. In population studies, (−174G>C [*rs1800795*]) has been associated with cardiovascular outcomes including MI risk [29] and increased CHD and mortality risk, [30] but we did not observe any associations of this, or any other IL-6 SNP or haplotype, with risk of MI or stroke.

Our results do not support existing evidence suggesting that variations in the CRP gene sequence are associated with cardiovascular disease. For instance, among the European-American cohort in the Cardiovascular Health Study, the minor allele of one CRP polymorphism (1919T [*rs1417938*]) was associated with increased stroke risk and CVD mortality while the minor alleles of two other CRP SNPs (2667 [*rs1800947*] and 3872 [*rs1205*]) were associated with decreased CVD mortality risk. [31] We genotyped these SNPs and did not observe associations with MI or stroke, but our study was limited to survivors of incident events and the strongest associations in CHS were found among the fatal cases.

Summary

We investigated gene variation among eight inflammation-related genes by analyzing **38** common haplotypes described by **36** SNPs in a large population-based case-control study of incident nonfatal MI and ischemic stroke. Our strategy for genotyping provided a thorough description of most common variation within genes, and several sources of high-quality clinical information were available. Our sample size provided ample power to detect modest associations: for a variant with a prevalence of 10%, we had 90% statistical power to detect an odds ratio of 1.5 for MI and 1.7 for ischemic stroke.

Participants in our study were those who survived incident events and were able to provide a blood sample. For this reason, variants related to case-fatality or post-event survival would be under-represented in our study population. We also chose to investigate common genetic variation; so any associations of rare polymorphisms with cardiovascular events would not be captured in this study. The associations observed in this study might reflect associations of SNPs linked to those that we genotyped. While we relied on global tests for our primary analyses to reduce the number of statistical tests performed and limit false-positives, it is still possible that some of our results are chance findings. In particular, the secondary analyses of SNPs involved **36** tests for MI and ischemic stroke. At a 0.05 significance level, we would have expected approximately 2 significant results by chance alone for each outcome. Finally, although we attempted to characterize all common genetic variation in these genes, our coverage for two genes was modest (>60%) among participants of European descent and among African-Americans. Thus, it is possible that untyped variants in these genes may be associated with risk.

The results from our study support prior reports associating an IL-18 variant and MI risk, contribute additional evidence to conflicting reports of IL-1B variation and cardiovascular risk, and fail to confirm associations previously observed for CRP, IL-6, IL-10, and TNF-α promoter variants.

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Table I

Characteristics of cases and contros

n=	Controls 2688	Myocardial infarction cases 856	Ischemic stroke cases 368
Sex, % male	42	43	31
Caucasian, %	91	91	91
Age, years	65 + 10	66 + 10	69 + 9
Body Mass Index, kg/m2	30 + 6	30 + 6	30 + 7
Current Smoking, %	10	18	13
Group Health membership, years	22 + 12	19 + 12	20 + 13
Diabetes,%	11	24	25
Any CVD % [*]	11	23	14
Current statin use, %	10	12	8
Blood pressure before index	138 + 19	142 + 20	146 + 22
Systolic blood pressure, mmHg	81 + 11	81 + 11	82 + 12
Diastolic blood pressure, mmHg			
Cholesterol, mg/dl	221 + 54	232 + 48	229 + 44
High-density cholesterol, mg/dl	53 + 17	49 + 15	52 + 17

Unless otherwise indicated, numbers in table are means +/− standard deviation

[*] Any CVD includes a history of congestive heart failure, angina, PTCA, or CABG surgery

Table 2

Summary of gene results

gene	size (base pairs)	percent sequenced	tag SNPs, n	common SNPs captured, %	haplotypes, n	percent common	global p-values[1]		max (z2) p-value[2]	
							MI	Stroke	MI	Stroke
C-reactive protein (CRP)	6,836	98%	5	100%	5	94%	0.996	0.402	0.989	0.207
Interleukin 1 beta (IL-1B)	17,447	100%	6	95%	6	87%	0.334	0.071	0.345	0.022
Interleukin 6 (IL-6)	8,019	94%	7	94%	8	98%	0.436	0.284	0.374	0.223
Interleukin 10(IL-10)	7,879	100%	6	100%	7	98%	0.566	0.313	0.431	0.606
Interleukin 18(IL-18)	22,816	100%	6	63%	5	94%	0.009	0.308	0.0008	0.349
Tumor necrosis factor superfamily				68%	7	91%	0.943	0.885	0.668	0.669
Lymphotoxin alpha (LTA)	5,033	100%	4							
Tumor necrosis factor, alpha (TNF)	4,412	100%	2							
Lymphotoxin beta (LTB)	4,830	100%	1							

[1] Global p-values were calculated using a Wald test for the hypothesis that no common haplotype's odds ratio differed from 1.0

[2] max(z2) p-values were calculated by comparing the maximum score test from the actual dataset to the maximum score test from datasets in which a person's set of genotypes were permuted withr espect to phenotype data.

1,000 permutations were performed for CRP, IL-1B, IL-6, IL-10, and the TNF superfamily; 10,000 were performed for IL-18 (after 1,000 permutations, the empirical p-value was 0.000)

Gene size and percent sequenced refer to variation discovery performed by SeattleSNPs and InnateImmunity

Common SNPs captured refers to the percentage of a gene's common SNPs captured by the tag SNPs successfully genotyped in this study.

Percent common refers to the percentage of haplotypes common among control participants; haplotypes less common than 2.5% were grouped into a single "rare" bin for analyses

Table 4

Association of IL-1B gene variation with risk of MI or Stroke

	rs143629	rs3917356	rs3136558	rs1143633	rs3917365	rs3917366	Control, % (n=2688)	MI, % (n=856)	MI (OR, 95% CI)	Stroke, % (n=368)	Stroke (OR, 95% CI)
MAF, % (among controls)	34	46	23	35	8	23					
haplotype 1	A	A	A	A	G	C	31	30	Ref.	27	Ref.
haplotype 2	A	A	A	G	G	C	8	8	1.01 (0.81 to 1.24)	8	1.17 (0.87 to 1.58)
haplotype 3	A	G	G	G	G	A	18	16	0.92 (0.78 to 1.09)	19	1.21 (0.96 to 1.52)
haplotype 4	G	G	A	G	G	C	22	24	1.15 (0.99 to 1.34)	26	1.38 (1.12 to 1.71)
haplotype 5	G	G	A	G	G	A	3	2	0.96 (0.68 to 1.35)	2	0.97 (0.59 to 1.59)
haplotype 6	G	G	A	G	A	C	6	6	1.05 (0.83 to 1.33)	5	0.91 (0.62 to 1.33)
other							13	13	0.99 (0.83 to 1.19)	13	1.09 (0.84 to 1.42)
global wald test									p = 0.334		p = 0.071
MI (OR, 95% CI)	1.13 (1.01 to 1.27)	0.93 (0.83 to 1.04)	0.86 (0.75 to 0.98)	0.98 (0.88 to 1.10)	0.96 (0.79 to 1.17)	0.89 (0.78 to 1.02)					
Stroke (OR, 95% CI)	1.13 (0.96 to 1.33)	0.84 (0.72 to 0.98)	1.03 (0.86 to 1.24)	0.83 (0.70 to 0.99)	0.77 (0.57 to 1.04)	1.03 (0.86 to 1.24)					

The grid, above, shows the alleles composing each haplotype. The minor allele is indicated in bold type. Rows (horizontal), show haplotype prevalence for cases and controls with odds ratios representing the risk for an additional copy of the haplotype compared to the reference haplotype. Vertical (columns) show SNP minor allele frequencies among controls and odds ratios representing the risk of MI or stroke associated with an additional copy of the minor allele. SNPs are labeled according to their NCBI reference sequence number. Odds ratios are from logistic regression models adjusted for age, sex, race, treated hypertension status, & event year.

Appendix A

Association of IL-6 gene variation with risk of MI or Stroke

	rs2069824	rs2069827	rs1800795	rs2066992	rs2069840	rs2069843	rs2069861	Control, % (n= 2688)	MI, % (n= 856)	MI (OR, 95% CI)	Stroke, % (n= 368)	Stroke (OR, 95% CI)
MAF, % (among controls)	7	9	41	7	33	3	9					
haplotype 1	A	C	C	C	G	G	G	9	8	Ref.	9	Ref.
haplotype 2	A	C	C	A	G	G	G	7	6	.97 (0.74 to 1.29)	7	0.96 (0.65 to 1.43)
haplotype 3	A	C	C	C	**C**	G	G	32	34	.21 (0.98 to 1.50)	30	0.96 (0.71 to 1.31)
haplotype 4	A	C	C	C	G	A	G	3	3	.11 (0.75 to 1.66)	3	1.38 (0.81 to 2.34)
haplotype 5	A	C	**G**	C	G	G	G	23	23	.16 (0.93 to 1.46)	23	1.04 (0.75 to 1.46)
haplotype 6	A	C	**G**	C	G	G	A	9	8	.07 (0.82 to 1.40)	9	1.04 (0.71 to 1.52)
haplotype 7	A	**A**	**G**	C	G	G	G	9	8	.02 (0.78 to 1.33)	9	0.98 (0.68 to 1.43)
haplotype 8	**G**	C	C	C	G	G	G	7	7	.19 (0.90 to 1.58)	8	1.28 (0.86 to 1.90)
other								2	2	.29 (0.84 to 2.00)	3	1.73 (1.01 to 2.97)
global wald test										p = 0.436		p = 0.284
MI (OR, 95% CI)	1.06 (0.86 to 1.31)	0.88 (0.73 to 1.07)	0.97 (0.86 to 1.08)	0.85 (0.69 to 1.06)	1.09 (0.97 to 1.23)	1.01 (0.71 to 1.42)	0.94 (0.78 to 1.15)					
Stroke (OR, 95% CI)	1.23 (0.92 to 1.66)	0.95 (0.72 to 1.25)	0.99 (0.84 to 1.17)	0.89 (0.65 to 1.21)	0.91 (0.77 to 1.08)	1.35 (0.86 to 2. II)	1.02 (0.78 to 1.33)					

The grid, above, shows the alleles composing each haplotype. The minor allele is indicated in bold type.

Rows (horizontal), show haplotype prevalence for cases and controls with odds ratios representing the risk for an additional copy of the haplotype compared to the reference haplotype.

Vertical (columns) show SNP minor allele frequencies among controls and odds ratios representing the risk of MI or stroke associated with an additional copy of the minor allele.

SNPs are labeled according to their NCBI reference sequence number

Odds ratios are from logistic regression models adjusted for age, sex, race, treated hypertension status, & event year.

Appendix B

Association of CRP gene variation with risk of MI or Stroke

	rs1417938	rs1800947	rs143629	rs2808630	rs3093077	Control, % (n= 2688)	MI, % (n= 856)	MI (OR, 95% CI)	Stroke, % (n= 368)	Stroke (OR, 95% CI)
MAF, % (among controls)	30	6	33	29	7					
haplotype 1	T	C	G	G	A	29	29	Ref.	27	Ref.
haplotype 2	T	C	A	A	A	27	26	0.97 (0.83 to 1.12)	28	1.11 (0.89 to 1.39)
haplotype 3	T	G	A	A	A	6	6	1.00 (0.78 to 1.28)	6	1.07 (0.76 to 1.51)
haplotype 4	A	C	G	A	A	29	29	1.01 (0.87 to 1.16)	29	1.05 (0.84 to 1.30)
haplotype 5	T	C	G	A	C	4	4	1.01 (0.75 to 1.36)	3	0.87 (0.55 to 1.38)
other						6	6	0.97 (0.76 to 1.24)	8	1.38 (1.01 to 1.90)
global wald test								p = 0.996		p = 0.402
MI (OR, 95% CI)	1.01 (0.90 to 1.14)	1.02 (0.81 to 1.29)	0.97 (0.87 to 1.09)	1.02 (0.90 to 1.15)	0.89 (0.72 to 1.11)					
Stroke (OR, 95% CI)	1.01 (0.85 to 1.20)	0.99 (0.71 to 1.38)	1.06 (0.90 to 1.25)	0.90 (0.76 to 1.08)	0.98 (0.72 to 1.33)					

The grid, above, shows the alleles composing each haplotype. The minor allele is indicated in bold type.

Rows (horizontal), show haplotype prevalence for cases and controls with odds ratios representing the risk for an additional copy of the haplotype compared to the reference haplotype.

Vertical (columns) show SNP minor allele frequencies among controls and odds ratios representing the risk of MI or stroke associated with an additional copy of the minor allele.

SNPs are labeled according to their NCBI reference sequence number

Odds ratios are from logistic regression models adjusted for age, sex, race, treated hypertension status, & event year.

Appendix C

Association of IL-10 gene variation with risk of MI or Stroke

	rs1800894	rs2222202	rs3021094	rs1554286	rs3024498	rs3024505	Control, % (n= 2688)	MI, % (n= 856)	MI (OR, 95% CI)	Stroke, % (n= 368)	Stroke (OR, 95% CI)
MAF, % (among controls)	4	47	10	21	25	16					
haplotype 1	G	G	A	G	A	G	30	32	Ref.	32	Ref.
haplotype 2	G	G	A	A	A	G	13	14	0.98 (0.82 to 1.17)	16	1.16 (0.91 to 1.48)
haplotype 3	G	G	**C**	**A**	A	G	7	7	0.93 (0.75 to 1.17)	6	0.83 (0.59 to 1.17)
haplotype 4	G	**A**	A	G	A	G	3	2	0.73 (0.52 to 1.04)	3	0.94 (0.59 to 1.51)
haplotype 5	G	**A**	A	G	A	**A**	16	15	0.89 (0.75 to 1.05)	13	0.82 (0.63 to 1.06)
haplotype 6	G	**A**	A	G	**G**	G	25	24	0.95 (0.82 to 1.10)	23	0.88 (0.71 to 1.09)
haplotype 7	**A**	**A**	A	G	A	G	4	4	1.03 (0.76 to 1.40)	4	1.01 (0.65 to 1.57)
other							2	3	1.11 (0.80 to 1.54)	2	0.85 (0.52 to 1.40)
global wald test									p = 0.566		p = 0.313
MI (OR, 95% CI)	1.08 (0.81 to 1.45)	0.93 (0.83 to 1.03)	1.02 (0.85 to 1.23)	1.01 (0.88 to 1.16)	0.98 (0.87 to 1.12)	0.91 (0.78 to 1.07)					
Stroke (OR, 95% CI)	1.09 (0.72 to 1.65)	0.87 (0.74 to 1.01)	0.83 (0.62 to 1.09)	1.14 (0.94 to 1.38)	0.92 (0.77 to 1.10)	0.85 (0.68 to 1.07)					

The grid, above, shows the alleles composing each haplotype. The minor allele is indicated in bold type.

Rows (horizontal), show haplotype prevalence for cases and controls with odds ratios representing the risk for an additional cop/ of the haplotype compared to the reference haplotype.

Vertical (columns) show SNP minor allele frequencies among controls and odds ratios representing the risk of MI or stroke associated with an additional copy of the minor allele.

SNPs are labeled according to their NCBI reference sequence number

Odds ratios are from logistic regression models adjusted for age, sex, race, treated hypertension status, & event year.

Appendix D

Association of LTA-TNF-LTB gene variation with risk of MI or Stroke

	LTA rs143629	LTA rs143629	LTA rs143629	TNF rs143629	TNF rs143629	LTb rs143629	Control, % (n= 2688)	MI, % (n=856)	MI (OR, 95% CI)	Stroke, % (n= 368)	Stroke (OR, 95% CI)
MAF, % (among controls)	17	27	6	16	8	9					
haplotype 1	C	A	A	G	A	C	46	47	Ref.	48	Ref.
haplotype 2	C	A	A	A	A	C	16	16	1.00 (0.85 to 1.16)	14	0.85 (0.67 to 1.07)
haplotype 3	C	A	A	G	A	A	8	8	0.97 (0.79 to 1.19)	8	0.92 (0.67 to 1.25)
haplotype 4	C	G	A	G	A	C	4	4	0.97 (0.74 to 1.28)	4	0.93 (0.64 to 1.36)
haplotype 5	C	G	A	G	G	C	6	6	1.00 (0.80 to 1.27)	6	0.93 (0.66 to 1.30)
haplotype 6	G	G	A	G	A	C	10	11	1.01 (0.84 to 1.23)	11	0.97 (0.75 to 1.27)
haplotype 7	G	G	C	G	A	C	6	5	0.83 (0.65 to 1.07)	5	0.81 (0.56 to 1.17)
other							3	3	1.04 (0.74 to 1.45)	4	1.30 (0.85 to 1.99)
global wald test									p = 0.937		p = 0.650
MI (OR, 95% CI)	0.95 (0.82 to 1.10)	0.97 (0.86 to 1.10)	0.83 (0.65 to 1.06)	1.01 (0.87 to 1.18)	1.03 (0.84 to 1.25)	0.98 (0.81 to 1.20)					
Stroke (OR, 95% CI)	0.95 (0.76 to 1.17)	0.95 (0.80 to 1.13)	0.84 (0.59 to 1.19)	0.87 (0.70 to 1.09)	1.07 (0.81 to 1.40)	0.95 (0.71 to 1.27)					

The grid, above, shows the alleles composing each haplotype. The minor allele is indicated in bold type.

Rows (horizontal), show haplotype prevalence for cases and controls with odds ratios representing the risk for an additional copy of the haplotype compared to the reference haplotype.

Vertical (columns) show SNP minor allele frequencies among controls and odds ratios representing the risk of MI or stroke associated with an additional copy of the minor allele.

SNPs are labeled according to their NCBI reference sequence number

Odds ratios are from logistic regression models adjusted for age, sex, race, treated hypertension status, & event year.