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Biomarkers of inflammation and MRI-defined small vessel disease of the brain: the Cardiovascular Health Study

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Abstract

Background—To clarify the role of inflammation in the pathogenesis of small vessel disease of the brain, we investigated the association between common variation in the CRP and IL6 genes, plasma CRP and IL6 levels, and presence of MRI-defined white matter lesions (WML) and brain infarcts (BI) in elderly participants of the Cardiovascular Health Study.

Methods and Results—Tag single nucleotide polymorphisms (SNPs) in the CRP and IL6 genes were selected from the SeattleSNPs database. In cross-sectional analyses, logistic regression models adjusting for known CVD risk factors were constructed to assess the associations of plasma CRP and IL6 levels and common CRP and IL6 gene haplotypes with presence of WML or BI in Blacks (N=532) and Whites (N=2,905). Plasma IL6 and CRP levels were associated with presence of WML and BI in both races. In Whites, common haplotypes of the IL6 gene were significantly associated with WML and BI. The common haplotype tagged by the −174G/C promoter polymorphism was associated with an increased risk of WML (OR=1.14; 95% CI: (1.02; 1.28)). The common haplotype tagged by the −572G/C promoter polymorphism was associated with an increased risk of BI (OR=1.57; 95% CI: (1.15; 2.14)). Significant associations were lacking for WML or BI with IL6 gene variation in Blacks, or with CRP gene variation in either race.

Conclusions—This study provides evidence of a genetic basis underlying the relationship between plasma biomarkers of inflammation and small vessel disease of the brain. Further studies to elucidate the specific role of IL6 in disease pathogenesis are warranted.

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INTRODUCTION

Vascular disease of the brain is the third leading cause of death and the leading cause of severe long-term disability in the United States.[1] The burden of brain vascular disease is far greater than that of the clinically-recognizable acute neurological event, stroke.[2] Brain imaging techniques, such as magnetic resonance imaging (MRI), have revealed that white matter lesions (WML) and brain infarcts (BI) are remarkably common in the elderly.[3–6] While the majority of these MRI-detectable abnormalities do not produce acute clinical symptoms, they cannot be considered benign as they are often associated with an increased risk for cognitive deficits,[7, 8] motor function impairment,[9, 10] and future stroke.[11–13] The pathogenesis of these abnormalities remains poorly understood but may reflect vascular damage to the deep penetrating vessels of the brain resulting from arteriolosclerosis and leading to localized ischemic areas of necrosis and cavitation (i.e., BI) or diffuse rarefaction in the white matter (i.e., WML).[14]

Evidence is mounting suggesting that chronic inflammation contributes to the development and consequences of cardiovascular disease.[15] Numerous prospective studies have shown associations of serum levels of biomarkers of inflammation such as C-reactive protein (CRP), interleukin 6 (IL6), and fibrinogen with myocardial infarction, stroke, cardiovascular death, and peripheral arterial disease.[16–19] Little is known, however, about the relationship between inflammation and small vessel disease of the brain.[20] Previous studies have shown evidence of inflammatory activation and endothelial dysfunction in individuals with lacunar infarction and WML.[21, 22] More recently, higher CRP levels have been associated with presence and progression of WML in a population-based sample of non-demented elderly Europeans.[23] A significant association between higher CRP and IL6 levels and presence of silent BI was also reported in Japanese individuals.[24] While these studies provide support for a link between inflammatory markers and small vessel disease pathogenesis, it is yet unclear whether small vessel disease of the brain is directly and causally influenced by increased levels of inflammatory markers, or itself induces increased synthesis of inflammatory molecules, or both.

To further investigate the relationship between molecular markers of inflammation and small vessel disease of the brain, we conducted an association study of the sequence variation in 2 inflammation genes, CRP and IL6, with presence of WML and BI among elderly participants in the Cardiovascular Health Study (CHS).

MATERIAL AND METHODS

Subjects

Details of the study design and characteristics of the CHS cohort participants have been previously published.[25, 26] Briefly, between June 1989 and May 1990, 5,201 adults 65 years or older were recruited from a random sample of people on Medicare eligibility lists in 4 US communities: Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh (Allegheny County), PA. They were non-institutionalized, able to give informed consent, and able to respond to questions without the help of a surrogate respondent. To enhance minority representation in the cohort, 687 African-American participants were recruited to the study in 1992. Between enrollment and 1998–99, participants were seen in the clinic annually, and contacted by phone at 6-month intervals to collect information about hospitalizations and potential cardiovascular events. At clinic examinations, measures of cardiovascular risk were assessed. Between 1992 and 1994, 3,660 (62%) participants (3,073 Whites, 571 Blacks, and 16 belonging to other ethnic groups) underwent a MRI of the brain. Those who underwent MRI were younger and healthier than those who did not.[27]

Data Collection

Participants underwent an extensive baseline evaluation, including standard questionnaires, clinical examination, cognitive function assessment, and clinical laboratory testing. Parts of the baseline evaluation have been repeated annually. Variables considered in these analyses as potential risk factors or confounders were those from examination closest in time and before the MRI scan. IL6 was measured in serum by an ultra-sensitive ELISA method (R&D Systems, Minneapolis, MN) at the baseline examination and CRP was measured at the Year 5 examination by ELISA assay developed at the CHS central blood laboratory.[28]

Detailed descriptions of the MRI techniques and methods of analyses have been published. [29] Briefly, scanning protocols included sagittal T1-weighted localizer images and axial T1-weighted, spin density-, and T2-weighted images. All axial images had 5-mm thickness without interslice gaps. Blinded to any information about participants, neuroradiologists at the reading center identified brain infarcts [30] and estimated white matter grade using a 10-point system from 0 to 9 (most abnormal), using a library of templates.[27] In these analyses, presence of WML was defined as a white matter grade greater or equal to 2 and absence of WML was defined as a white matter grade of 0 or 1 (No or barely detectable WML, respectively). A brain infarct was defined as presence on MRI of an area of abnormal signal intensity 3 mm in size or greater in a vascular distribution and without mass effect.

Polymorphisms selection and genotyping

Single Nucleotide Polymorphisms (SNPs) were chosen based on pairwise linkage disequilibrium (LD) relationships [31] among common SNPs identified in African-Americans and European-Americans as part of the Seattle SNPs Variation Discovery resource.[32] A total of 5 SNPs in the CRP gene, and 9 SNPs in the IL6 gene were genotyped in 3,437 CHS MRI participants (532 Blacks, 2,905 Whites) who had a DNA sample and had given informed consent for use of their genetic material. The gene location and identity of these polymorphisms are shown in Figure 1. Genotyping was performed using TaqMan Assays by Design (Applied Biosystems) under conditions recommended by the manufacturer. Probe and primer sequences for each assay are available upon request.

Statistical Analysis

For each polymorphism, agreement of genotype frequencies with Hardy Weinberg equilibrium expectations was tested using a χ^2 goodness-of-fit test. All analyses were carried out stratified on race to minimize potential confounding due to population stratification. Non-normally distributed variables were log-transformed.

In each racial group, logistic regression models were used to evaluate the associations between MRI-findings and individual tagSNPs. For each SNP, genotypes were coded as number of copies (0, 1, or 2) of the variant allele. Significance of genotype effects were assessed by the Armitage's test, which is equivalent to the score test in the logistic regression model.[33] Models were adjusted for age, gender, current smoking, hypertension status, body mass index, diastolic blood pressure, education, diabetes status and HDL-cholesterol, and further for plasma levels of IL6 or CRP, as appropriate. Correction for multiple testing was performed using the direct simulation approach, a fast approximation to permutation.[34] Global tests of significance for association of the combined set of polymorphisms in each gene with MRI traits were evaluated by Fisher's product method.[35]

Significance of haplotype effects on presence of MRI findings was evaluated using a regression method for unphased haplotypes based on score statistics and implemented in the Haplo. Stats software.[36] Score statistics are constructed to test the null hypotheses of no haplotype effects on the probability of having MRI-defined brain vascular disease, adjusting for covariates.

Statistical significance was evaluated by permutation tests.[36] Only haplotypes with a frequency greater or equal to 3% were considered in the analyses to reduce risk of false positive findings related to low frequency haplotypes.[37] To estimate the magnitude of effects of haplotypes on traits, each individual was assigned the most probable pair of haplotypes, as estimated by EM algorithm implemented in the score method above, and haplotype effect was estimated using logistic regression models contrasting odds of having MRI findings between individuals carrying 0, 1, or 2 copies of the given haplotype.

RESULTS

Selected characteristics of the participants are shown in Table 1 by race. In the group of elderly individuals, prevalence of WML (65%) and that of BI (28%) at the initial examination were similar in black and white participants.

There was a significant and graded association between plasma IL6 levels and presence of WML in both races. Individuals with higher plasma IL6 levels had a significantly greater risk of having WML (odds ratio (OR) per standard deviation of Log(IL6) (95% confidence interval): 1.28 (1.11; 1.47) for Whites; 1.55 (1.12; 2.15) for Blacks) (Table 2). Adjustment for covariates did not significantly attenuate this relationship (Table 2). Similar results were obtained for plasma CRP (OR: 1.13 (1.06; 1.22), Whites; 1.09 (0.93; 1.28), Blacks), although statistical significance was reached only in Whites. Consistently, there was a graded association between plasma IL6 levels and presence of BI (OR: 1.41 (1.21; 1.63), Whites; 1.32 (0.94; 1.85), Blacks) (Table 2), but statistical significance was reached only in Whites. Adjustment for covariates did not modify this association (Table 2). Plasma CRP levels were also associated, although marginally, with presence of BI in the two racial groups (OR: 1.09 (1.01; 1.19), Whites; 1.21 (1.00; 1.47), Blacks).

Analyses were also carried out excluding individuals with prevalent stroke, coronary heart disease, heart failure, and transient ischemic attacks (N=988). Associations of the 2 inflammatory biomarkers with WML and BI were essentially unchanged (Table 2).

We also determined whether sequence variation in the 2 genes may be associated with presence of WML and BI. Within each racial group, all polymorphisms were in Hardy Weinberg equilibrium, except for SNP 2892 (rs2069837) in Whites. This polymorphism was excluded from further analysis in this group.

IL6 gene polymorphisms were associated with both WML and BI in Whites but not in Blacks. In Whites, a common IL6 haplotype (frequency=40%) was associated with a significantly greater risk of WML (OR: 1.14 (1.02; 1.28)) (Table 3). This haplotype was tagged by the promoter SNP 1510 (−174 G/C) and SNP 3572 in intron 3, both of which were in significant linkage disequilibrium (r^2=0.86). Consistently, these 2 SNPs showed similar associations with WML, although only that of SNP 1510 with WML reached statistical significance after correction for multiple tests (Table 3). Magnitude of associations of IL6 haplotypes and genotypes with WML was not modified after adjusting for plasma IL6 or CRP levels or both, although statistical significance was attenuated (not shown).

Another IL6 haplotype (frequency=3%) was significantly associated with greater risk of BI (OR: 1.57 (1.15; 2.14)) (Table 4) and this association persisted after adjusting for plasma IL6 or CRP levels, or both. This haplotype was tagged by SNP 1111 (−572 G/C), located in the gene promoter. While this SNP showed a trend toward a significant association with BI in single-SNP analysis (OR: 1.23 (0.94; 1.62)), statistical significance was not reached after correction for multiple testing (P=0.13) (Table 4). Moreover, the association of the IL6 haplotype with BI was not modified after adjusting for the effects of SNP 1111 (OR: 2.6 (1.3; 5.3), P=0.005).

Despite their significant association with plasma CRP levels in this cohort [38], we did not find any evidence that CRP haplotypes and/or genotypes were associated with WML or BI in either Blacks or Whites (not shown).

DISCUSSION

In the CHS cohort of elderly individuals, we showed a significant association of circulating IL6 and CRP with WML and BI. In addition, common variation in the IL6 gene was significantly associated with increased risk of WML and BI in Whites but not Blacks. A common IL6 haplotype and the −174 G/C (rs1800795) promoter polymorphism that tags it were significantly associated with a modest increase in risk of WML. Another common IL6 haplotype, tagged by the −572 G/C (rs1800796) promoter polymorphism, was significantly associated with a modest increase in risk of BI. No significant associations of CRP sequence variation with MRI traits were observed in either race.

Our findings of significant associations between circulating IL6 and CRP with MRI-defined brain small vessel disease are consistent with previous reports. In the population-based Rotterdam Scan Study of 1,033 individuals of similar age as the CHS participants, higher plasma CRP levels were significantly associated with presence and progression of WML, and marginally associated with greater prevalence and incidence of lacunar infarcts.[23] Higher plasma levels of CRP and IL6 were associated with increased risk for silent BI in two independent cohorts of elderly Japanese.[24, 39]

Sequence variation in the IL6 and CRP genes has previously been shown to influence circulating levels of the two cytokines in several studies, including CHS.[38, 40] In particular, the −174G/C polymorphism has been associated with increased plasma IL6 and CRP [40–42], raising the possibility that the observed association between IL6 gene variation and WML may be mediated via effects of this gene on levels of circulating inflammatory proteins. IL6 sequence variation and IL6 plasma levels remained significantly and independently associated with presence of WML in statistical models estimating their simultaneous effects, suggesting that the mechanism of the association between −174 G/C and WML may not be solely through the polymorphism influence on IL6 levels. Indeed, the −174 C/G SNP or its related haplotype accounted for only about 1% of the inter-individual variation in IL6 levels in Whites (not shown). This polymorphism may have additional functional properties relevant to cardiovascular disease pathogenesis. Indeed, a recent proteomic screen of the human serum from 151 healthy men identified apolipoprotein C1 and HSP60 as circulating proteins whose serum levels were significantly associated with IL6 −174 genotypes.[43] These data raise the possibility that sequence variation in the IL6 gene may have pleiotropic effects on multiple, yet-unrecognized protein components or pathways, which may be of importance to pathogenesis of brain small vessel disease.

Several additional arguments must also be considered to explain the independent associations of IL6 polymorphism and plasma levels in our analyses. The single CRP and IL6 plasma level measurements in this study may not accurately represent the cumulative inflammatory burden experienced over the period of disease development and, thus, may not comprehensively reflect the true relationship between genotype, intermediate biomarker levels, and disease. Moreover, plasma IL6 and CRP measurement variability, including that due to intra-individual variation and to assay experimental variability [28, 44, 45] may have also occasioned incomplete adjustment for potential mediation among gene, gene products, and phenotypes.

The strong epidemiological associations that exist among risk factors, WML and BI suggest that they may share a common pathophysiology.[46] Our data showing an association of variation in the IL6 gene with both WML and BI are consistent with such a hypothesis, although the

underlying specific genetic variants that may influence pathophysiology may or may not be shared between the two diseases. A previous case-control study in a small sample of Spanish individuals found a significant association of the −174G/C polymorphism with increased risk of lacunar stroke.[47] In a subset of the CHS cohort, this polymorphism was significantly associated with MRI-defined BI.[48] In our study, the haplotype associated with increased risk of BI was tagged by the −572 G/C promoter polymorphism. Nonetheless, effects of this polymorphism, alone, are unlikely to fully account for the observed association, suggesting that a yet-unrecognized variant or variants on this haplotype background may influence risk of BI in this population.

There was no association of sequence variation in the IL6 gene with WML or BI in Blacks, despite evidence of a relationship between plasma IL6 and the two MRI traits. Possible explanations include a reduced statistical power in the smaller sample of Blacks, and/or differences in allele frequencies between the 2 racial groups, or both. For example, the power to detect a 1.2 fold increased risk of WML associated with the −174G/C polymorphism in our sample of Blacks was only 15%. Analyses in larger cohorts are warranted to further investigate the role of the IL6 gene in small-vessel disease in African-Americans.

There was no association of common variation in the CRP gene with presence of WML or BI in either racial group. These results are in agreement with recent findings from the Rotterdam Scan study and the MEMO study.[49] Significant associations of CRP haplotypes with cardiovascular events, including stroke, have been recently reported in the CHS cohort.[38] We cannot exclude the possibility that common CRP gene variation may have an influence on large-vessel disease pathogenesis or on the transition from covert disease to clinical disease.[38]

Despite the clear strengths of our study, including a well-characterized sample of older adults and a comprehensive tagSNP strategy in genes encoding two major markers of inflammation, some limitations must be acknowledged. First, although *in vitro* experiments showed that sequence variants in the IL6 promoter, including −174G/C, are functional [50, 51], it is important to recognize that association studies cannot distinguish the causal SNP (or combination of SNPs), among those in linkage disequilibrium (LD). Analysis of pairwise LD among polymorphic markers located in the IL6 gene region of chromosome 7 and genotyped as part of the HapMap project, shows that the −174G/C polymorphism is in significant LD with multiple SNPs within the IL6 gene but not beyond (not shown). Second, the cross-sectional nature of our analyses opens the possibility that the results obtained here were confounded by an unrecognized association of IL6 polymorphisms with associated prevalent cardiovascular conditions. However, although statistical significance of the associations between polymorphisms and traits was attenuated, the magnitude of these associations remained unchanged after exclusion of individuals with prevalent clinical disease (not shown). Finally, we have not examined possible interactions between the 2 inflammation genes. Hence, genetic variation, whose individual contribution is minimal – and perhaps undetectable – but when considered in the context of the other gene is non-negligible, may have been missed.

In summary, the present findings add to the growing body of evidence of a link between inflammation and small vessel disease of the brain and provide support for a genetic basis underlying this relationship. The molecular mechanisms by which the IL6 gene influences risk for WML and BI remain to be established. Small vessel atherosclerosis has been causally implicated in a proportion of BI.[52] Atherosclerosis lesion formation in different vascular beds may share common etiologic mechanisms. Increased levels of IL6 have been recently associated with intra-cranial large vessel atherosclerosis [53], raising the possibility that IL6's role in atherogenesis may be similar in small and large vessels. IL6 is the principal regulator of acute-phase proteins and, therefore, plays a major role in the activation of the coagulation-

fibrinolysis system.[54] WML have been associated with hypercoagulable conditions.[55] Elevated circulating endothelial-derived adhesion molecules have been reported in patients with WML or lacunar infarcts.[21, 22, 56] Leukocyte-mediated injury to the small vessels and associated upregulation of endothelial adhesion molecules can lead to blood brain barrier disruption, which has been implicated in the pathogenesis of white matter lesions.[57, 58] Whether IL6 plays a role in these pathogenetic mechanisms remains to be explored.

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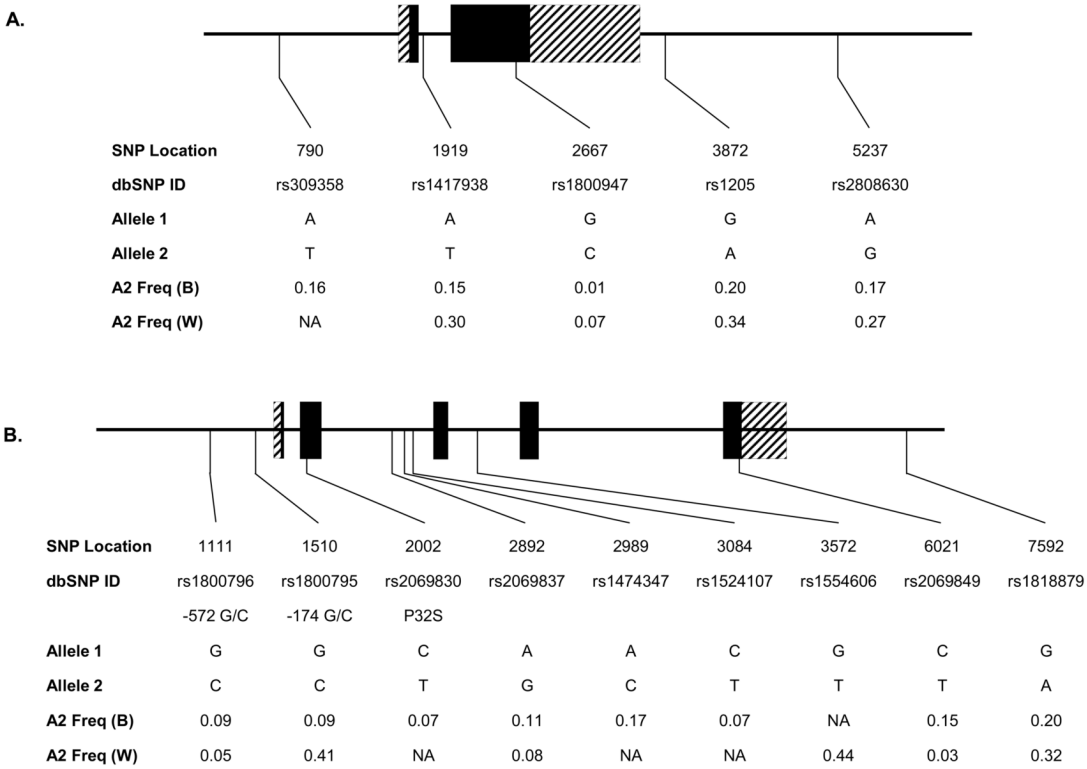

SNP Location	790	1919	2667	3872	5237
dbSNP ID	rs309358	rs1417938	rs1800947	rs1205	rs2808630
Allele 1	A	A	G	G	A
Allele 2	T	T	C	A	G
A2 Freq (B)	0.16	0.15	0.01	0.20	0.17
A2 Freq (W)	NA	0.30	0.07	0.34	0.27

SNP Location	1111	1510	2002	2892	2989	3084	3572	6021	7592
dbSNP ID	rs1800796	rs1800795	rs2069830	rs2069837	rs1474347	rs1524107	rs1554606	rs2069849	rs1818879
	-572 G/C	-174 G/C	P32S						
Allele 1	G	G	C	A	A	C	G	C	G
Allele 2	C	C	T	G	C	T	T	T	A
A2 Freq (B)	0.09	0.09	0.07	0.11	0.17	0.07	NA	0.15	0.20
A2 Freq (W)	0.05	0.41	NA	0.08	NA	NA	0.44	0.03	0.32

Figure 1.

Gene location and allele frequency of the assayed polymorphisms in the CRP (A.) and IL6 (B.) genes by race. Exons are indicated by boxes and are numbered. Coding sequence is shown in black. Polymorphism position is given according to the SeattleSNPs numbering. The corresponding dbSNP ID and common polymorphism designation are also shown. A2 Freq: Frequency of allele 2; B: Blacks; W: Whites.

Table 1

Mean (standard deviation) or proportion for selected characteristics of participants present at the initial examination

	Whites (N=3,073)	Blacks (N=571)	P
Age (years)	75.4 (5.0)	73.9 (5.7)	<0.0001
Men (%)	42.5	37.3	0.02
Body mass index (kg/m^2)	26.3 (4.4)	28.2 (4.9)	<0.0001
Current smokers (%)	8.5	15.0	<0.0001
Education (years)	14.3 (4.6)	12.9 (5.1)	<0.0001
Hypertension (%)	55.0	75.1	<0.0001
Diabetes (%)	12.5	23.4	<0.0001
LDL cholesterol (mg/dL)	127.2 (33.3)	129.2 (36.4)	0.19
HDL cholesterol (mg/dL)	53.0 (14.6)	57.1 (14.6)	<0.0001
Systolic blood pressure (mm Hg)	133.9 (20.4)	140.4 (21.4)	<0.0001
Diastolic blood pressure (mm Hg)	69.8 (10.5)	75.3 (10.9)	<0.0001
WML prevalence (%)	65.6	63.9	0.44
Brain infarcts prevalence (%)	27.7	28.3	0.78
Interleukin 6 (pg/mL)	2.0 (1.7)	2.3 (2.1)	<0.0001*
C-Reactive Protein (mg/L)	4.7 (8.4)	6.3 (8.5)	<0.0001*

* P value for comparison of geometric means

Table 2

Association of plasma IL6 and CRP levels with presence of white matter lesions and brain infarcts at the 1st MRI examination

	Odds Ratio (95% Confidence Interval) for association with presence of WML				Odds Ratio (95% Confidence Interval) for association with presence of BI			
	Whites (N=3,073)		Blacks (N=571)		Whites (N=3,073)		Blacks (N=571)	
	IL6	CRP	IL6	CRP	IL6	CRP	IL6	CRP
Model 1								
1st Quartile	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
2nd Quartile	1.24 (0.99; 1.55)	1.28 (1.03; 1.59)	1.87 (1.09; 3.19)	0.82 (0.49; 1.38)	1.38 (1.07; 1.78)	0.99 (0.78; 1.25)	1.05 (0.57; 1.92)	1.05 (0.58; 1.89)
3rd Quartile	1.32 (1.05; 1.65)	1.31 (1.05; 1.62)	1.26 (0.75; 2.13)	0.89 (0.53; 1.51)	1.43 (1.10; 1.85)	1.16 (0.92; 1.47)	1.17 (0.63; 2.17)	1.07 (0.60; 1.93)
4th Quartile	1.48 (1.18; 1.86)	1.59 (1.27; 1.98) **	2.16 (1.26; 3.73) *	1.28 (0.74; 2.19)	1.81 (1.40; 2.33)**	1.18 (0.93; 1.49)	1.37 (0.74; 2.51)	1.45 (0.81; 2.58)
P trend	0.007	0.0006	0.02	0.37	0.0001	0.29	0.74	0.56
Per SD (log)	1.28 (1.11; 1.47)	1.13 (1.06; 1.22)	1.55 (1.12; 2.15)	1.09 (0.93; 1.28)	1.41 (1.21; 1.63)	1.11 (1.03; 1.20)	1.32 (0.94; 1.85)	1.16 (0.98; 1.38)
Model 2								
1st Quartile	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
2nd Quartile	1.22 (0.97; 1.53)	1.21 (0.97; 1.51)	1.83 (1.05; 3.18)	0.80 (0.47; 1.37)	1.35 (1.04; 1.75)	0.95 (0.74; 1.21)	1.09 (0.58; 2.04)	1.04 (0.56; 1.93)
3rd Quartile	1.27 (1.01; 1.60)	1.20 (0.97; 1.50)	1.25 (0.71; 2.17)	0.86 (0.50; 1.48)	1.40 (1.07; 1.83)	1.10 (0.86; 1.41)	1.12 (0.58; 2.18)	1.11 (0.60; 2.04)
4th Quartile	1.43 (1.12; 1.82) *	1.48 (1.17; 1.84) **	2.20 (1.23; 3.90) *	1.16 (0.67; 2.04)	1.75 (1.34; 2.28)**	1.11 (0.86; 1.42)	1.37 (0.71; 2.65)	1.62 (0.85; 3.11)
P trend	0.03	0.01	0.03	0.55	0.0008	0.54	0.80	0.43
Per SD (log)	1.24 (1.07; 1.44)	1.11 (1.03; 1.19)	1.55 (1.10; 2.19)	1.06 (0.90; 1.25)	1.37 (1.17; 1.60)	1.09 (1.01; 1.19)	1.34 (0.93; 1.93)	1.21 (1.00; 1.47)
Model 3								
1st Quartile	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)	1 (referent)
2nd Quartile	1.09 (0.84; 1.41)	1.15 (0.89; 1.50)	1.95 (1.02; 3.71)	0.84 (0.44; 1.61)	1.33 (0.99; 1.80)	0.96 (0.72; 1.29)	1.28 (0.62; 2.67)	0.81 (0.39; 1.66)
3rd Quartile	1.28 (0.97; 1.67)	1.13 (0.86; 1.47)	1.22 (0.63; 2.34)	0.83 (0.43; 1.58)	1.38 (1.01; 1.87)	1.07 (0.80; 1.44)	1.19 (0.56; 2.56)	1.03 (0.51; 2.08)
4th Quartile	1.42 (1.08; 1.87)*	1.33 (1.01; 1.75)*	2.13 (1.08; 4.20)*	0.90 (0.44; 1.86)	1.87 (1.38; 2.54)**	1.14 (0.84; 1.54)	1.68 (0.81; 3.52)	1.47 (0.70; 3.08)
P trend	0.06	0.26	0.06	0.94	0.0009	0.69	0.55	0.47
Per SD (log)	1.23 (1.05; 1.46)	1.09 (0.99; 1.21)	1.54 (1.03; 2.30)	1.18 (0.94; 1.48)	1.46 (1.22; 1.74)	1.09 (0.99; 1.21)	1.42 (0.96; 2.11)	1.18 (0.94; 1.48)

* P<0.05;

** P<0.01 for comparison with 1st quartile;

P trend over quartiles; SD: standard deviation; log: logarithm-transformed biomarker plasma levels; Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, current smoking, education, diastolic blood pressure, body mass index, and hypertension (MRI-defined WML); adjusted for age, sex, current smoking, systolic blood pressure, diastolic blood pressure, HDL cholesterol, body mass index, diabetes, and hypertension (MRI-defined BI). Model 3: adjusted for variables in Model 2 and excluding individuals with prevalent stroke, transient ischemic attack, coronary heart disease, and heart failure (N=2,283, Whites; N=426, Blacks). Cutoff for Quartiles of plasma IL6 in Whites and Blacks, respectively: 1st: 0.35–1.08; 0.36–1.18 pg/mL; 2nd: 1.09–1.55; 1.19–1.70 pg/mL; 3rd: 1.56–2.31; 1.71–2.58 pg/mL; 4th: 2.32–44.17; 2.59–23.37 pg/mL. Cutoff for Quartiles of plasma CRP in Whites and Blacks, respectively: 1st: 0.13–1.13; 0.16–1.47 mg/L; 2nd: 1.14–2.43; 1.49–3.45 mg/L; 3rd: 2.44–5.34; 3.46–7.86 mg/L; 4th: 5.35–143.0; 7.89–79.40 mg/L.

Table 3

Association of IL6 haplotypes and genotypes with presence of MRI-defined WML in Whites.

		SNP							
		1111	**1510**	**3572**	**6021**	**7592**			
Haplotype Index	**Haplo. Freq.**						**OR* (95% CI)**	**P†**	**Global P†**
1	40.5	G	C	T	C	A	1.14 (1.02; 1.28)	**0.03**	0.07
2	3.1	C	G	G	C	C	0.98 (0.72; 1.33)	0.91	
3	23.2	G	G	G	C	A	0.93 (0.81; 1.07)	0.32	
4	27.7	G	G	G	C	C	0.98 (0.86; 1.12)	0.68	
OR ‡ (95% CI)		0.89 (0.69; 1.15)	1.13 (1.01; 1.27)	1.10 (0.99; 1.23)	0.84 (0.60; 1.18)	0.97 (0.86; 1.10)			
P¶		0.13	**0.03**	0.08	0.46	0.58			
Global P	**0.03**								

*Odds ratio per copy of haplotype adjusted for age, sex, current smoking, education, diastolic blood pressure, body mass index, and hypertension.

†Permutation-based P value from score test. P values for individual haplotypes test the null hypothesis that the odds of having WML of a particular haplotype are the same as that of all other haplotypes combined. The global P value tests the null hypothesis that the odds of having WML are the same for all haplotypes.

‡Odds ratio per copy of variant allele adjusted for age, sex, current smoking, education, diastolic blood pressure, body mass index, and hypertension.

¶adjusted for multiple tests.

Haplo. Freq.: Haplotype frequency. SNP: Single nucleotide polymorphism

Table 4

Association of IL6 haplotypes and genotypes with presence of MRI-defined BI in Whites.

		SNP							
		1111	**1510**	**3572**	**6021**	**7592**			
Haplotype Index	**Haplo. Freq.**						**OR* (95% CI)**	**P†**	**Global P†**
1	40.5	G	C	T	C	A	1.02 (0.90; 1.16)	0.78	**0.01**
2	3.1	C	G	G	C	C	1.57 (1.15; 2.14)	**0.001**	
3	23.2	G	G	G	C	A	0.98 (0.85; 1.13)	0.76	
4	27.7	G	G	G	C	C	0.97 (0.84; 1.12)	0.70	
OR ‡ (95% CI)		1.23 (0.94; 1.62)	1.02 (0.90; 1.15)	1.01 (0.89; 1.14)	0.90 (0.61; 1.32)	0.98 (0.85; 1.13)			
P ¶		0.13	0.48	0.51	0.53	0.87			
Global P	0.61								

*Odds ratio per copy of haplotype adjusted for age, sex, current smoking, systolic blood pressure, diastolic blood pressure, HDL cholesterol, body mass index, diabetes, and hypertension.

†Permutation-based P value from score test.

‡Odds ratio per copy of variant allele adjusted for age, sex, current smoking, systolic blood pressure, diastolic blood pressure, HDL cholesterol, body mass index, diabetes, and hypertension.

¶adjusted for multiple tests.

Haplo. Freq.: Haplotype frequency. SNP: Single nucleotide polymorphism