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Candidate Genetic Variants in the Fibrinogen, Methylenetetrahydrofolate Reductase and Intercellular Adhesion Molecule-1 Genes and Plasma levels of Fibrinogen, Homocysteine, and Intercellular Adhesion Molecule-1 Among Various Race/ethnic Groups: Data from the Women's Genome Health Study

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Abstract

Objectives—Examine the relationship between specific polymorphisms in the fibrinogen, homocysteine and intercellular adhesion molecule-1 genes and their respective inflammatory biomarker concentrations at baseline in women from different race/ethnic groups.

Background—Although inflammation is a core element of atherogenesis and plasma levels of fibrinogen, homocysteine and intercellular adhesion molecule-1 (ICAM-1) differ by race/ethnicity, little is known about the role of genetic polymorphisms in the fibrinogen (FGB), methylenetetrahydrofolate reductase (MTHFR), and intercellular adhesion molecule-1 (ICAM-1) genes in determining plasma levels of these biomarkers.

Methods—We genotyped specific polymorphisms in *FGB* (-455G>A/rs1800790), *MTHFR* (677C>T/rs1801133) and *ICAM-1* (Lys56Met/rs5491 and Gly241Arg/rs1799969) at baseline, and evaluated their relationship with respective inflammatory biomarker levels in 25, 565 white, 476 African-American (black), 277 Hispanic and 370 Asian women participating in the Women's Genome Health Study.

Results—Overall, the minor allele frequencies for –455G>A were similar among white, Hispanic and Asian women (17.2 to 21.9%) but significantly lower in black women (6.6%, p< 0.001). The minor allele was associated with elevated fibrinogen levels only in whites and Asians. After adjustment for age, body mass index, smoking, postmenopausal status, diabetes, hormone replacement therapy use, hypertension and education, black women had the highest fibrinogen levels compared to other race/ethnic groups. The minor allele frequency of the MTHFR 677C>T polymorphism was lowest in blacks (blacks 12.1%, whites 33.1%, Hispanics 39.0%, Asians 24.0%) and the T allele was only significantly associated with homocysteine levels in white women. Among whites, Hispanics and Asians, the Lys56Met polymorphism was rare compared to the frequency in

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blacks ($p < 0.001$). Neither the Lys56Met nor Gly241Arg polymorphisms were common in Asians. Nonetheless, both polymorphisms were generally associated with lower ICAM-1 levels; the lowest levels were observed in black women.

Conclusion—We found significant associations between certain candidate genetic polymorphisms and baseline plasma levels of fibrinogen, homocysteine and intercellular adhesion molecule-1 in women from various race/ethnic groups. The present investigation is hypothesis generating and suggests genetic determination of differential concentrations of these atherosclerosis-related inflammatory biomarkers differ among various race/ethnic groups.

Keywords

fibrinogen; ICAM-1; homocysteine; genetic polymorphisms; race/ethnicity

Introduction

In the United States, cardiovascular morbidity and mortality varies significantly by self-described race/ethnicity. African-Americans (blacks) die from cardiovascular disease (CVD) including myocardial infarction, stroke and sudden cardiac death at substantially higher rates than other Americans (1). In particular, black women have vascular mortality rates that are 30% higher than white women and a coronary heart disease prevalence rate that is 50% higher than that of Hispanic women (1). The underlying causes of these statistics are complex and multi-factorial, and likely include differences in risk factor burden, treatment, bias, social and environmental factors.

From a pathophysiologic standpoint, blacks who die from cardiac causes paradoxically tend to have less coronary atherosclerotic disease burden and more stable plaques at autopsy than whites (2–5). Also, research from our group and others indicate that black women have higher levels of baseline inflammation than women from other race/ethnic groups, a factor that might contribute to their observed higher vascular event rates (6–8). For example, baseline concentrations of C-reactive protein and fibrinogen levels are higher and intercellular adhesion molecule-1 levels are lower in black women compared to white, Hispanic and Asian women (7,8). However, little is known about the contribution of genetic factors to observed differences in inflammatory biomarker levels of CVD risk by race/ethnicity. An examination of polymorphisms in the promoter region of the β-fibrinogen gene (G-455>A, C-148>T) among whites, blacks (Caribbean and West African born) and South Asians residing in London revealed that the minor A allele was less prevalent in blacks and was not associated with elevated fibrinogen levels (9). Meanwhile, although the T allele was also less common in blacks, it was associated with higher fibrinogen levels in this race/ethnic (r/e) group. Limited research remains available about any association between polymorphisms in the homocysteine gene and plasma concentrations of this biomarker by race/ethnicity. Still, small case-control and cross-sectional investigations of the MTHFR C677T variant and atherosclerosis in whites and blacks have generally found no relationship between this variant and coronary heart disease (CHD) or sub-clinical atherosclerosis (10,11). Consequently, we examined the relationship between candidate polymorphisms in the fibrinogen, homocysteine and ICAM-1 genes and their associated plasma levels among self-describ ed white, black, Hispanic and Asian women living in the United States. Polymorphisms were chosen based on prior evidence of potential functionality, validated allele frequency and heterozygosity, and sequence proven allelic variation.

Methods

Study Design

We examined the cross-sectional relationship between certain single gene nucleotide polymorphisms in the fibrinogen, homocysteine and ICAM-1 genes and serum levels of inflammatory biomarkers in different race/ethnic groups. Participant data came from the Women's Genome Health Study (WGHS), a genetic sub-study in the Women's Heath Study (WHS), a recently completed randomized, double-blinded, placebo-controlled trial of vitamin E and low-dose aspirin for the primary prevention of cardiovascular events and cancer among women (12–14). The study population consisted of apparently healthy women 45 years and older, who were enrolled between November 1992 and July 1995 and had no prior history of cardiovascular disease or cancer at study entry. EDTA plasma specimens were collected from 28,345 women and stored in liquid nitrogen until the time of analysis. Of these women, baseline data on age, smoking status, weight, height, diabetic status, hormone replacement therapy (HRT) use, hypertension, family history of myocardial infarction (MI), alcohol use, physical activity were available for 26,688 participants who form the basis of this analysis. An ELISA assay from R & D Systems, Minneapolis MN was used to measure ICAM-1 levels, whereas homocysteine concentrations were determined by enzymatic assay with reagents and calibrators from Catch Inc. (Seattle, WA). Fibrinogen was assayed using a Roche Diagnostics (Indianapolis, IN) immunoturbidimetric system with reagents and caibrators from Kamiya Biomedical Company (Seattle, WA). Information on race/ethnicity was obtained by participant self-report in one of six categories (white, Hispanic, black, Asian/Pacific Islander, American Indian/Alaskan native, other). Native Americans and "other" were excluded from this analysis due to small sample size. The study was approved by the institutional review board of Brigham and Women's Hospital (Boston, Massachusetts).

Genotype Determination

As previously described, genotype analysis was performed using an immobilized probe approach (Roche Molecular Systems, Alameda, California) (15). In brief, each DNA sample was amplified in a multiplex polymerase chain reaction (PCR) using biotinylated primers. Each PCR product pool was then hybridized to a panel of sequence-specific oligonucleotide probes immobilized on a linear array. The colorimetric detection method was based upon the use of streptavidin-horseradish peroxidase conjugates with hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine as substrates. Genotype assignment was performed using the proprietary Roche Molecular Systems StripScan image processing software (Roche Molecular Systems, Inc., CA). To confirm genotype assignment, scoring was carried out by two independent observers. Discordant results (<1% of all scoring results) were resolved by a joint reading, and where necessary by repeat genotyping.

Statistical Analysis

Allele and genotype frequencies in the whole sample population were compared with values predicted by Hardy-Weinberg equilibrium using the Chi-squared test. Within each race/ethnic group, we calculated residual biomarker levels after adjusting for age, smoking, body mass index (BMI), menopausal status, diabetes, HRT use, hypertension (≥140/90 mmHg or on anti-hypertensive medications), education and income. Linear regression analysis was performed to evaluate the relationship between genotypes and residual biomarker concentrations according to race/ethnic status assuming an additive contribution of each minor allele. Based on the present sample sizes, at the 0.05 significance levels, we had 80% power to detect effects explaining 0.04%, 1.65%, 2.15% and 2.8% of the variance among whites, blacks, Asians and Hispanics respectively. For example, for a minor allele with frequency 0.10, these effects correspond to a shift in the level of sICAM-1 per copy of minor allele of 0.033, 1.357, 1.709 and 3.039ng/ml respectively. The relationship between each polymorphism and corresponding

residual plasma biomarker concentrations was tested using linear regression. A two-tailed p-value of 0.05 was considered a statistically significant result. All analyses were carried out using SAS/Genetics 9.1 package (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 shows the baseline characteristics of the study population according to race/ethnicity. Black women were more likely to have a history of hypertension, diabetes, smoking and had a higher BMI than white, Hispanic or Asian women. Asian women had the lowest BMI, while Hispanic women were more likely than other women to have a history of hypercholesterolemia. Table 2 shows the relationship between the specific biomarker levels after adjustment for age, BMI, smoking, menopausal status, HRT use, diabetes, hypertension, income and education in Hispanics, blacks and Asians compared to whites. After adjustment for the aforementioned variables, homocysteine levels were not significantly different in Hispanic and black women compared to white women, whereas fibrinogen levels remained higher for all three race/ethnic subgroups compared to white women. Black women had the highest fibrinogen levels followed by Hispanic women. ICAM-1 levels were lower in black and Asian women than in other women.

Allele and genotype frequencies for –455G>A SNP in the fibrinogen gene were generally similar for white, Hispanic and Asian women, but were not in Hardy-Weinberg equilibrium (HWE) in whites ($p < 0.001$) and Asians ($p = 0.04$) [Table 3]. The overall genotyping completion rate was ≥ 95% per gene variant. Two percent of the samples were randomly selected for re-genotyping and 100% concordance was found. Thus, we did not find genotyping errors that might be responsible for the observed Hardy-Weinberg disequilibrium. Because of our stringent genotyping criteria, we believe that the latter results are due to chance and not due to genotyping error. Moreover, the observed allele frequencies in our sample population correspond to previously published estimates (9). The minor allele frequency (MAF) of the –455G>A SNP was lowest in black women at 6.6%. The minor allele of the 677C>T SNP in the homocysteine gene was relatively prevalent in whites, Hispanics and Asians and was in HWE in all race/ethnic groups. Two polymorphisms in the ICAM-1 gene (Lys56Met and Gly241Arg) by race/ethnic status were evaluated. The minor allele frequency associated with the Lys56Met polymorphism is relatively rare (<3.0%), except in blacks where the frequency of the Met allele was 16.3 %; allele and genotype frequencies were in HWE in all groups studied. The Gly241Arg SNP was more common in whites and Hispanics than in blacks and Asians. The linkage disequilibrium (D′) for white, Hispanic, black and Asian women are 0.61, 1.00, 0.77, and 1.00 respectively.

Table 4 shows the association of residual biomarker levels with the examined polymorphisms by race/ethnic designation. Among whites all examined SNPS were related to corresponding inflammatory biomarker levels *[677C>T, adjusted $R^2 = 0.0061$; -455G>A, adjusted $R^2 = 0.0074$; Lys56Met, adjusted $R^2 = 0.0085$; Gly241Arg, adjusted $R^2 = 0.0183$]*. Both polymorphisms in the ICAM-1 gene were associated with lower ICAM-1 levels in whites. In Hispanics, the Gly241Arg SNP in the ICAM-1 gene *(adjusted $R^2 = 0.0283$)* and the Lys56Met SNP (adjusted $R^2 = 0.0127$; p=0.045) were associated with ICAM-1 levels. Similar to the observation in Hispanic women, there was no association between the 677C>T and –455G>A SNPs and homocysteine or fibrinogen levels respectively in black women. However, both ICAM-1 SNPs were significantly associated with lower ICAM-1 levels among black women *(Lys56Met, adjusted $R^2 = 0.4247$; Gly241Arg, adjusted $R^2 = 0.0088$)*. Finally, in Asian women both the –455G>A *(adjusted $R^2 = 0.0497$)*, and Lys56Met *(adjusted $R^2 = 0.2168$)* SNPs were significantly related to higher and lower levels of fibrinogen and ICAM-1 levels respectively.

Discussion

We examined the cross-sectional relationship between polymorphisms in the fibrinogen, homocysteine and ICAM-1 genes, associated inflammatory biomarker levels and race/ethnicity among participants in the WGHS. Although black women had the highest fibrinogen levels, the minor allele of the –455G>A polymorphism was only associated with elevated fibrinogen levels in whites and Asians. Homocysteine levels were only significantly related to the MTHFR 677C>T polymorphism in white women. The Lys56Met ICAM-1 gene polymorphism was generally rare in whites, Hispanics and Asians and was associated with lower ICAM-1 levels in these women. The Gly241Arg polymorphism was also associated with lower ICAM-1 levels. Our findings indicate that after taking into account measured environmental factors, certain polymorphisms in the fibrinogen, homocysteine and ICAM-1 genes previously associated with atherosclerosis relate to serum concentrations of these inflammatory markers in a differential manner based on race/ethnicity.

While elevated fibrinogen levels are associated with increased CVD risk (16), studies evaluating functional polymorphisms in the fibrinogen gene and any relationship with CVD events are sparse and generally show a weak or no relationship (17,18). Moreover, the role of race/ethnicity on the latter is largely unexplored with a few studies evaluating blacks and whites. To date, the –455G>A polymorphism in the promoter region of the fibrinogen gene has been most commonly examined. Some reports suggest that the A/A genotype is associated with elevated fibrinogen levels in white populations (19,20). Additionally, the frequency of the A allele differs between whites and blacks with most reports indicating 15–22% and < 7% allele frequencies in these race/ethnic groups respectively (9,20). The allele frequencies associated with the –455G>A SNP based on race/ethnicity in our study are similar to those observed in these previous studies. Our data extend previous reports by as well demonstrating that the frequency of the minor A allele is similar between Hispanic, Asian and white Americans; moreover, as is the case for blacks, among Hispanics this allele was not associated with fibrinogen levels. In general, U.S blacks and women tend to have higher fibrinogen concentrations (8,21) than other groups, a finding that is probably largely dictated by environmental factors and of course possibly the impact of other polymorphisms. This assertion is supported by 1) the observation of lower fibrinogen levels among blacks living in London compared to whites and South Asians (9); 2) evidence that another polymorphism –148C>T associated with elevated fibrinogen levels that is in complete allelic association with –455G>A in whites but not in blacks (9); 3) previous work from our group in this cohort and others demonstrating that fibrinogen levels were highly correlated with smoking, body mass index and race/ethnicity (16); 4) research suggesting that single site fibrinogen gene SNPs only account for < 2% of variation in serum fibrinogen levels (20).

Because elevated homocysteine levels result in heightened vascular risk (22–24), investigations about factors that result in raised serum concentrations including deficiencies in vitamins B6, B12 and folate have naturally led to examination of genetic causes. The mutation 677C>T in the MTHFR gene is relatively common in white populations (25,26) and is implicated in hyperhomocystenemia. Although homozygotes with the TT genotype tend to have the highest levels of homocysteine, consistent evidence about any relationship of this polymorphism with vascular risk is lacking (27,28). In this study, homocysteine levels were only associated with the 677C>T polymorphism in white women, a finding that is in part related to the lower observed minor allele frequencies in the other r/e groups, but also likely due to the influence of environmental factors on homocysteine levels. In other race/ethnic groups, little information is available regarding the effect of MTHFR on homocysteine levels, or its role in cardiovascular events (10,29). In general, homocysteine levels do not appear to vary significantly based on race/ethnic designation, (8,30,31) although certain cultural customs such as vegetarianism might predispose to higher homocysteine levels in some ethnic groups.

Although the lys56met and gly241arg variants in the ICAM-1 gene have been implicated in atherogenesis, these polymorphisms are linked with lower ICAM-1 levels and research from our group demonstrated no association between ICAM-1 levels, these variants and CVD (32, 33). Research from a multi-ethnic sample from Bielinski et al. also show no relationship between these ICAM-1 SNPs and the coronary artery calcium (35). Both polymorphisms were relatively rare among Asian women (MAF < 3%), but the frequency of the lys56met was higher in black women (16.3%) compared to other women, a finding that corroborates other data showing allele frequencies of 20–30% in black women living in Kifili, Kenya and the U.S (34,35). Experimental evidence suggests that the 56met allele probably exerts a protective influence against cerebral malaria, a lethal illness for which millions are at risk in Africa (36). Thus, environmental forces might have selected for a higher frequency of the 56met allele in populations from the African Diaspora. Intriguingly, the lys56met polymorphism is also associated with relatively lower levels of ICAM-1 among black women compared to other women in our study. However, this finding might relate to the inability of the common commercial monoclonal ICAM-1 antibody assay used in this study and others to detect the plasma ICAM-1 in persons homozygous for the 56met allele (37,35). Recent work about ICAM-1 SNPs and biomarker levels demonstrate that after exclusion of T-allele carriers (lys56met), rs5496 and rs1799969 were significantly associated with ICAM-1 levels in blacks and Hispanics respectively (35); notably HapMap data obtained from Yorubans show that the LD between rs5496 and rs5491 is $D' = 0.064$.

While our study has uniquely examined the relationship between race/ethnicity, inflammatory biomarkers associated with cardiovascular risk and putative genetic polymorphisms, limitations of our study must be addressed. First, our data are cross-sectional and therefore causality and any association with CVD outcomes cannot be determined. Second, race/ethnic background was self-reported and thus does not take into account heterogeneity within race/ethnic groups or genetic admixture. However, it is critical to note that self-described race/ethnicity is a powerful predictor of vascular outcomes in the U.S, an observation that is influenced by gene-environment interactions. Moreover, we have previously reported that 99.7% of self-reported whites were accurately categorized as white utilizing principal component analysis to identify ancestry (38). Third, since CVD is a complex, polygenic condition, multiple polymorphisms undoubtedly interact to influence plasma inflammatory biomarker levels. Moreover, it is possible that other polymorphisms in the examined genes that are not in linkage disequilibrium with the ones that we studied could result in different results. Fourth, although the number of non-white compared to white subjects is small, we had at least 80% power to detect up to 2.8 % variance in biomarker levels in non-whites. Also, as previously noted, the polymorphisms tested in our study were solely selected based on prior evidence of potential functionality, validated allele frequency and heterozygosity, and sequence proven allelic variation. Thus, linkage disequilibrium/haplotype structure was not considered. Hence, further studies using linkage disequilibrium/haplotype-SNP tagging information from public genome databases such as HapMap are warranted. We did not correct for multiple comparisons but these polymorphisms have been widely characterized and are functionally relevant; additional studies are needed to examine the associations of the genetic polymorphisms with respective biomarker levels in non-whites.

Future work is also needed to prospectively examine the association between these and other candidate genetic polymorphisms with CVD risk in non-white populations. Attention must be given to comprehensive, standardized measurement of various environmental factors that goes beyond the typical measured factors such as smoking and physical activity to include assessment of other often unmeasured critical environmental influences such as neighborhood environment and other stressors that also heavily influence CVD health outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Loyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberg J, Thom T, Wilson M, Hong Y. for the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics 2008 Update. A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2007Epub ahead of print
2. Burke A, et al. Traditional Risk Factors and the Incidence of Sudden Coronary Death with and without Coronary Thrombosis in Blacks. Circulation 2002;105(4):419–424. [PubMed: 11815422]
3. Strong JP, Oalmann MC, Newman WP 3rd, Tracy RE, Malcom GT, Johnson WD, McMahan LH, Rock WA Jr, Guzman MA. Coronary Heart Disease in Young Black and White Males in New Orleans: Community Pathology Study. Am Heart J 1984;108(3 Pt 2):747–759. [PubMed: 6475744]
4. Tang W, Detrano RC, Brezden OS, Georgiou D, French WJ, Wong ND, Doherty TM, Brundage BH. Racial Differences in Coronary Calcium Prevalence Among High-risk Adults. Am J Cardiol 1995;75 (16):1088–1091. [PubMed: 7762490]
5. Jain T, Peshock R, McGuire DK, Willett D, Yu Z, Vega GL, Guerra R, Hobbs HH, Grundy SM. Dallas Heart Study Investigators. African Americans and Caucasians Have a Similar Prevalence of Coronary Calcium in the Dallas Heart Study. J Am Coll Cardiol 2004;44(5):1011–1017. [PubMed: 15337212]
6. Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH Jr, Grundy SM, de Lemos JA. Race and Gender Differences in C-Reactive Protein Levels. J Am Coll Cardiol 2005;46(3):464–469. [PubMed: 16053959]
7. Albert MA, Glynn RJ, Buring J, Ridker PM. C-Reactive Protein Levels Among Women of Various Ethnic Groups Living in the United States (from the Women's Health Study). Am J Cardiol 2004;93 (10):1238–1242. [PubMed: 15135696]
8. Albert MA, Glynn RJ, Buring JE, Ridker PM. Relation Between Soluble Intercellular Adhesion Molecule-1, Homocysteine, and Fibrinogen Levels and Race/Ethnicity in Women without Cardiovascular Disease. Am J Cardiol 2007;99(9):1246–1251. [PubMed: 17478152]
9. Cook DG, Cappuccio FP, Atkinson RW, Wicks PD, Chitolie A, Nakandakare ER, Sangnella GA, Humphries SE. Ethnic Differences in Fibrinogen Levels: The Role of Environmental Factors and the Beta-fibrinogen Gene. Am J Epidemiol 2001;153(8):799–806. [PubMed: 11296154]
10. Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE. Prospective Study of Coronary Heart Disease Incidence in Relation to Fasting Total Homocysteine, Related Genetic Polymorphisms, and B Vitamins: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 1998;98(3):204–210. [PubMed: 9697819]
11. Markus H, Kapozsta Z, Ditrich R, Wolfe C, Ali N, Powell J, Mendell M, Cullinane M. Increased Common Carotid Intima-Media Thickness in UK African Caribbeans and its Relation to Chronic Inflammation and Vascular Candidate Gene Polymorphisms. Stroke 2001;32(11):2465–2471. [PubMed: 11692002]
12. Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med 2005;352:1293–304. [PubMed: 15753114]

13. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. Jama 2005;294:56–65. [PubMed: 15998891]

14. Ridker PM, Chasman D, Zee R, et al. Rationale, Design, and Methodology of the Women's Genome Health Study: A Genome-Wide Association Study of More than 25,000 Initially Healthy American Women. Clin Chem 54(2):249–255. [PubMed: 18070814]

15. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, Chen JJ, Pullinger CR, Malloy MJ, Siest G, Kane JP. A multilocus genotyping assay for candidate markers of cardiovascular disease risk. Genome Res 1999;9:936–949. [PubMed: 10523522]

16. Fibrinogen Studies Collaboration. Association of Plasma Fibrinogen Levels with Established Cardiovascular Disease Risk Factors, Inflammatory Markers, and Other Characteristics: Individual Participant Meta-Analysis of 154,211 Adults in 31 Prospective Studies: The Fibrinogen Studies Collaboration. Am J Epidemiol 2007;166(8):867–879. [PubMed: 17785713]

17. Morgan TM, Krumholtz HM, Lifton RP, Spertus JA. Nonvalidation of Reported Genetic Risk Factors for Acute coronary Syndrome in a Large-Scale Replication Study. JAMA 2007;297(14):1551–1561. [PubMed: 17426274]

18. de Matt MP. Effects of Diet, Drugs, and Genes on Plasma Fibrinogen Levels. Ann N Y Acad Sci 2001;936:509–521. [PubMed: 11460508]

19. Humphries SE, Ye S, Talmud P, Bara L, Wilhelmsen L, Tiret L. European Atherosclerosis Research Study: Genotype at the Fibrinogen Locus (G-455-A Beta-Gene) is Associated with Differences in Plasma Fibrinogen Levels in Young Men and Women from Different Regions in Europe. Evidence for Gender-Genotype-Environment Interaction. Arterioscler Thromb Vasc Biol 1995;15(1):96–104. [PubMed: 7749821]

20. Reiner AP, Carty CL, Carlson CS, Wan JY, Rieder MJ, Smith JD, Rice K, Fornage M, Jaquish CE, Williams OD, Tracy RP, Lewis CE, Siscovick DS, Boerwinkle E, Nickerson DA. Association Between Patterns of Nucleotide Variation Across the Three Fibrinogen Genes and Plasma Fibrinogen Levels: The Coronary Artery Risk in young Adults (CARDIA) Study. J Thromb Haemost 2006;4(6): 1279–1287. [PubMed: 16706972]

21. Folsom AR, Qamheih HT, Flack JM, Hilner JE, Liu K, Howard BV, Tracy RP. Plasma Fibrinogen: Levels and Correlates in Young Adults. The Coronary Artery Risk in young Adults (CARDIA) Study. Am J Epidemiol 1993;138(12):1023–1036. [PubMed: 8266905]

22. Gangi V, Kafai MR. Demographic, Health Lifestyle, and Blood Vitamin Determinants of Serum Total Homocysteine Concentrations in the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 2003;77:826–833. [PubMed: 12663279]

23. Sacco RL, Anand K, Lee HS, Boden-Albala B, Stabler S, Allen R, Paik MC. Homocysteine and the Risk of Ischemic Stroke in a Triethnic Cohort the Northern Manhattan Study. Stroke 2004;35:2263–2269. [PubMed: 15345803]

24. Homocysteine Studies Collaboration. Homocysteine and Risk of Ischemic Heart Disease and Stroke: A Meta-Analysis. JAMA 2002;288(16):2015–2022. [PubMed: 12387654]

25. Stevenson RE, Schwartz CE, Du YZ, Adams MJ Jr. Differences in Methylenetetrahydrofolate Reductase Genotype Frequencies, Between Whites and Blacks. Am J Hum Genet 1997;60(1):229–230. [PubMed: 8981967]

26. McAndrew PE, Brandt JT, Pearl DK, Prior TW. The Incidence of the Gene for the Thermolabile Methylene Tetrahydrofolate Reductase in African-Americans. Thromb Res 1996;83(2):195–198. [PubMed: 8837319]

27. Zee RY, Mora S, Cheng S, Erlich HA, Lindpaintner K, Rifai N, Buring JE, Ridker PM. Homocysteine, 5,10-methylenetetrahydrofolate Reductase 677C>T Polymorphism, Nutrient Intake, and Incident Cardiovascular Disease in 24,968 Initially Healthy Women. Clin Chem 2007;53(5):845–851. [PubMed: 17332146]

28. Brugada R, Marian AJ. A Common Mutation in Methlenetetrahydrofolate Reductase Gene is not a Major Risk of Coronary Artery Disease or Myocardial Infarction. Atherosclerosis 1997;128(1):107–112. [PubMed: 9051203]

29. Dilley A, Hooper WC, El-Jamil M, Renshaw M, Wegner NK, Evarr BL. Mutations in the Genes Regulating Methylene Tertahydrofolate Reductase (MTHFR C→T677) and Cystathione Beta-

Synthase (CBS G→A919, DBS T→C833) are not Associated with Myocardial Infraction in African Americans. Thromb Res 2001;103(2):109–115. [PubMed: 11457468]

30. Sosin MD, Patel JV, Bhatia GS, Hughes EA, Davis RC, Lip GY. Effects of White European, African Caribbean and South Asian Ethnicity on Homocysteine Levels in Patients with Systolic Heart Failure. Int J Cardiol. 2007Epub ahead of print
31. Cappuccio FP, Bell R, Perry IJ, Gilg J, Ueland PM, Refsum H, Sagnella GA, Jeffery S, Cook DG. Homocysteine Levels in Men and Women of Different Ethnic and Cultural Background Living in England. Atherosclerosis 2002;164(1):95–102. [PubMed: 12119198]
32. Zee RY, Cheng S, Eelich HA, Lindpaintner K, Rifai N, Buring JE, Ridker PM. Intercellular Adhesion Molecule 1 (ICAM1) Lys56Met and Gly241Arg Gene Variants, Plasma-Soluble ICAM1 Concentrations, and Risk of Incident Cardiovascular Events in 23,014 Initially Healthy White Women. Stroke 2007;38(12):3152–3157. [PubMed: 17962597]
33. Albert MA, Glynn RJ, Buring JE, Ridker PM. Differential Effect of Soluble Intercellular Adhesion Molecule-1 on the Progression of Atherosclerosis as Compares to Arterial Thrombosis: A Prospective Analysis of the Women's Health Study. Atherosclerosis. 2007Epub ahead of print
34. Zimmerman PA, Wieseman M, Spalding T, Boatin BA, Nutman TB. A New Intercellular Adhesion Molecule-1 Allele Identified in West Africans is Prevalent in African-Americans in Cintrast to Other North-American Racial Groups. Tissue Antigens 1997;50(6):654–656. [PubMed: 9458121]
35. Bielinski SJ, pankow JS, Li N, Hsu FC, Adar SD, Jenny NS, Bowden DW, Wasserman BA, Arnett D. ICAM1 and VCAM1 polymorphisms, coronary artery calcium, and circulating levels of soluble ICAM:1: The multi-ethnic study of atherosclerosis (MESA). Atherosclerosis. 2008epub ahead of print
36. Fernandez-Reyes D, Craig AG, Kyes SA, Peshu N, Snow RW, Berendt AR, Marsh K, Newbold CI. A High Frequency African Coding Polymorphism in the N-terminal Domain of ICAM-1 Predisposing to Cerebral Malaria in Kenya. Hum Mol Genet 1997;6(8):1357–1360. [PubMed: 9259284]
37. Register TC, Burdon KP, Kenchik L, Bowden DW, Hawkins GA, Nicklas BJ, Lohman K, Hsu FC, Langefeld CD, Carr JJ. Variability of Serum Soluble Intercellular Adhesion Molecule-1 Measurements Attributable to a Common Polymorphism. Clin Chem 2004;50(11):2185–2187. [PubMed: 15502096]
38. Ridker PM, Pare G, Parker A, et al. Loci related to metabolic syndrome pathways including LEPR, HNF1A, IL6R and GCKR associate with plasma C-reactive protein: The Women's Genome Health Study. Am J Hum Genet 2008;82(5):1185–92. [PubMed: 18439548]

Table 1

Baseline Characteristics (N=26, 688)

	White (N=25,565)	Black (N=476)	Asian (N=370)	Hispanic (N=277)
Age, yrs	54.7 ± 7.1	54.1 ± 6.3	53.4 ± 5.6	53.9 ± 6.2
BMI, kg/m^2	25.9 ± 5.0	28.6 ± 5.6	23.4 ±3.4	26.2 ± 4.9
HTN, %	24.6	45.0	25.1	24.2
Diabetes, %	2.6	9.0	3.0	6.1
Hypercholesterolemia, %	29.5	30.7	34.1	36.5
Current Smoking,%	11.5	16.0	3.0	9.8
HRT, %	43.7	38.3	38.7	40.4
Homocysteine, μmol/L	10.5 (8.7–12.9)	10.5 (8.7–12.8)	9.8(8.1–11.5)	10.4 (8.4–13.2)
Fibrinogen, mg/dL	350.0 (307.0–401.7)	395.6 (341.8–454.5)	344.1 (299.1–393.4)	365.0 (318.6–402.7)
sICAM-1, ng/mL	343.2 (301.9–394.9)	309.5 (218.1–375.4)	312.0 (266.4–358.4)	350.1 (307.6–401.6)
Menopause, %	54.4	51.4	57.4	44.4
≥4-yr College Degree, %	43.7	47.9	77.7	43.8
≥$50K Income, %	54.5	48.7	84.9	48.9

Biomarker levels presented as median (IQR)

All other measures are presented as mean ± SD

sICAM-1 = soluble ICAM-1

Table 2

Residual Biomarker Concentration* According to Race/Ethnicity

Biomarker	White•	Hispanic		Black		Asian	
		β coefficient	P value	β coefficient	P value	β coefficient	P value
Hcys	-	0.301	0.304	−0.092	0.219	−0.656	0.008
FGB	-	12.589	0.005	28.726	<0.0001	7.336	0.054
sICAM-1	-	5.102	0.269	−59.790	<0.0001	−23.605	<0.0001

*Biomarker variable represents residual levels after adjustment forage, body-mass index, smoking, postmenopausal status, hypertension, diabetes, HRT use, income and education

•White is the reference group

Table 3

Allele Distributions by Race/Ethnicity

Biomarker of interest	SNP	Locus	Major Allele	Minor Allele	White		Hispanic		Black		Asian	
					MAF	HW	MAF	HW	MAF	HW	MAF	HW
Hcys	677C>T/rs1801133	1p36.22	C	T	0.331	0.224	0.390	0.431	0.121	0.650	0.240	0.186
FGB	-455G>A/rs1800790	4q32.1	G	A	0.211	<0.001	0.172	0.384	0.066	0.430	0.219	0.043
sICAM-1	Lys56Met/rs5491	19p13.2	Lys	Met	0.003	1.000	0.012	1.000	0.163	<0.001	0.030	1.000
sICAM-1	Gly241Arg/rs1799969	19p13.2	Gly	Arg	0.116	0.755	0.164	0.817	0.039	1.000	0.023	<0.009

MAF = Minor Allele Frequency

HW = Deviation from Hardy-Weinberg equilibrium

Hcys = Homocysteine; FGB = Fibrinogen

Table 4

Association of Residual Biomarker Levels* with Examined SNPs** by Race/Ethnicity

Biomarker	SNP	White		Hispanic		Black		Asian	
		β coefficient	P value	β coefficient	P value	β coefficient	P value	β coefficient	P value
Hcys	677C>T/rs1801133	0.552	<0.0001	0.053	0.908	0.305	0.578	0.258	0.435
FGB	-455G>A/rs1800790	10.431	<0.0001	6.146	0.527	6.318	0.511	25.564	<0.0001
SICAM-1	Lys56Met/rs5491	−86.927	<0.0001	−60.992	0.045	−144.819	<0.0001	−148.704	<0.0001
	Gly241Arg/rs1799969	−21.942	<0.0001	−25.434	0.005	−40.514	0.027	−1.134	0.949

*Biomarker variable represents residual levels after adjustment for age, body-mass index, smoking, postmenopausal status, hypertension, diabetes, HRT use, income and education

**Additive mode