Published by Elsevier Inc.

ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2004.03.053

Genotypes Associated With Myocardial Infarction Risk Are More Common in African Americans Than in European Americans

David E. Lanfear, MD,* Sharon Marsh, PHD,* Sharon Cresci, MD,* William D. Shannon, PHD,* John A. Spertus, MD, FACC,† Howard L. McLeod, PHARMD*

St. Louis and Kansas City, Missouri

OBJECTIVES This study was designed to describe the frequencies of multiple myocardial infarction (MI) risk-associated genotypes among different racial groups.

BACKGROUND Racial disparities in the prevalence of cardiovascular disease (CVD) are well known. Recent large Japanese case-control studies identified connexin-37 (GJA-4), plasminogen activator inhibitor-1 (PAI-1), and stromelysin-1 (MMP-3) polymorphisms as risk factors for MI, but the prevalence of these genotypes among different racial groups in the U.S. needs to be determined.

METHODS Genomic deoxyribonucleic acid from 95 healthy African Americans (AA) and 95 healthy European Americans (EA) was used for genotyping. Deoxyribonucleic acid containing the region of interest was amplified using the polymerase chain reaction, followed by genotyping using pyrosequencing.

RESULTS All three MI-risk genotypes were observed in both populations and were in Hardy-Weinberg equilibrium. The frequencies of two of the three "risk-associated" genotypes were significantly higher in the AA population: GJA4 C1019T T/T: AA, 20%, EA, 7% ($p = 0.053$); MMP3 −1171delA A/A: AA, 78%, EA, 24% ($p < 0.001$); PAI-1 −668delG G/G: AA, 55%, EA, 16% ($p < 0.001$). The likelihood of two or more high-risk genotypes was 3.3% among EA subjects and 51% in the AA group ($p < 0.001$). We found that 9.1% of AA had all three high-risk genotypes, compared with 0% among the EA group ($p = 0.0097$).

CONCLUSIONS We found higher frequencies of disease-associated genotypes in AA than in EA. Our results also show that more AA than EA carry multiple risk-associated genotypes. Future studies need to assess whether such genetic profiles predict adverse outcomes in U.S. populations and contribute to racial disparities in CVD burden. (J Am Coll Cardiol 2004;44:165–7) © 2004 by the American College of Cardiology Foundation

African Americans (AA) appear to be disproportionately affected by cardiovascular disease (CVD) compared with European Americans (EA). The prevalence of CVD among AA men and women is nearly 40% compared to 23% to 30% for EA (1). Further, the rates of incident myocardial infarction (MI), death due to coronary heart disease (CHD), and heart failure are all higher in AA than EA (1,2). The understanding and eradication of such disparities has become a national priority (3) and is a primary goal of the Department of Health and Human Services' *Healthy People 2010* agenda (4).

Although the presence of such differences is clear, the underlying reasons are not completely understood. Certainly differences in access to health care and socioeconomic status, as well as differences in the processes of medical care, may explain potential disparities in outcome (5). However, the extent to which genetic variation may contribute to the excess CVD burden on AA remains relatively unexplored. Recent advances in cardiovascular genetics have highlighted interactions between common genetic variants and CVD (6), making possible the exploration of the role of these genetic variations in racial health disparities.

A recent landmark article by Yamada et al. (7) explored 112 polymorphisms in 71 candidate genes and their relationship to the risk of having an MI. After screening 909 Japanese subjects, researchers validated the results in an additional 4,152 subjects. Three single-nucleotide polymorphisms (SNP) in three candidate genes were identified that showed significant correlations to incident MI: connexin-37 (HUGO name GJA-4), C1019T (T allele) in men, and stromelysin-1 (MMP-3) −1171delA (insertion allele) and plasminogen activator inhibitor-1 (PAI-1) −668delG (insertion allele) in women. An even more recent study from the same group included 1,661 Japanese subjects and confirmed a significant correlation of the MMP-3 and GJA-4 polymorphisms to the presence of CHD (8).

The functional significance of these variants has been explored. Both the MMP-3 and PAI-1 polymorphisms are in the promoter regions of the respective genes, and previous investigators have shown allele-specific interactions with nuclear proteins, and altered transcription levels in

From the *Washington University School of Medicine, Departments of Medicine, Genetics, Molecular Biology, and Pharmacology, and Division of Biostatistics, St. Louis, Missouri; and the †Mid America Heart Institute-University of Missouri Kansas City, Kansas City, Missouri. This work was supported in part by the NIH Pharmacogenetics Research Network (U01 GM63340) and the data have been submitted to PharmGKB.org.

Manuscript received December 15, 2003; revised manuscript received March 5, 2004, accepted March 23, 2004.

Abbreviations and Acronyms

AA = African Americans
CHD = coronary heart disease
CVD = cardiovascular disease
DNA = deoxyribonucleic acid
EA = European Americans
GJA-4 = connexin-37
MI = myocardial infarction
MMP-3 = stromelysin-1
PAI-1 = plasminogen activator inhibitor-1
PCR = polymerase chain reaction
SNP = single nucleotide polymorphism

vitro (9,10). Mechanistic studies of the GJA-4 polymorphism are still needed; at present this polymorphism has been correlated to the presence of carotid atherosclerosis (11).

Both of these large case-control studies were performed in Japanese subjects, and there is little information about the frequency of these polymorphisms in AA or EA subjects. As a first step toward a large disease-association study, the frequencies of these three variants were assessed as both individual SNPs and in aggregate in healthy population samples of AA and EA subjects.

METHODS

Subjects. After written informed consent and human studies committee approval, genomic deoxyribonucleic acid (DNA) was extracted from whole blood of 95 EA and 95 AA healthy volunteers. The DNA was resuspended in 10 mM Tris/1 mM EDTA pH 8 and used directly for polymerase chain reaction (PCR) analysis.

Genotyping. The DNA segments containing the region of interest were amplified with PCR. Polymerase chain reaction primers were designed using Primer Express version 1.5 (ABI, Foster City, California), and the pyrosequencing primers were designed using the Pyrosequencing SNP Primer Design Version 1.01 software (Pyrosequencing, Uppsala, Sweden). The PCR primers and conditions are listed in Table 1. Polymerase chain reaction was carried out using Amplitaq Gold PCR master mix (ABI, Foster City, California), 5 pmole of each primer (IDT, Coralville, Iowa), and 1 ng DNA. Pyrosequencing was carried out as previously described (12) using the Pyrosequencing PSQ_hs96A instrument and software.

Statistics. The frequency of homozygosity for the risk-associated allele or the composite genotype was compared between AA versus EA subjects using Fisher exact test. All p values were adjusted for multiple comparisons using permutation testing, as previously described (13).

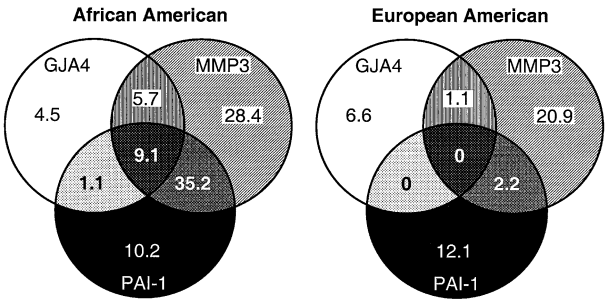

Figure 1. Frequency (%) of compound risk-associated genotypes in African-American (AA) and European-American (EA) subjects with complete genotype information (n = 91 for EA, n = 88 for AA). GJA4 = connexin-37; MMP3 = stromelysin-1; PAI-1 = plasminogen activator inhibitor-1.

RESULTS

Our results for allele frequency of PAI-1 C1019T and MMP-3 −1171delA in both populations and GJA4 −668delG in EA were consistent with those of other investigators (11,14,15). The risk-associated genotypes for MMP-3 and PAI-1 are more common in AA than EA subjects (Table 2) ($p < 0.001$ for each). Even more striking is the number of subjects with more than one high-risk genotype (Fig. 1). Among AA subjects 51.1% were homozygous for at least two high-risk alleles, compared with only 3.3% in the EA group ($p < 0.001$). No EA subjects were homozygous for all three high-risk alleles; however, eight AA subjects (9.1%) did fulfill this criterion ($p = 0.0097$).

DISCUSSION

African Americans are at higher risk of CVD, including CHD and MI (1). The extent to which this excess burden is accounted for by genetic determinants remains unknown.

Table 1. Polymerase Chain Reaction and Pyrosequencing Primers and Conditions

Polymorphism	Primer Sequence	Annealing Temp. (C°)
GJA-4 C1019T	Forward 5′-biotin-AACCTGACCACAGAGGAGAGGC-3′ Reverse 5′-CATCCCAGGCAGCCAGACT-3′ Internal 5′-GGGACGACTTGGGG-3′	62°
MMP-3 −1171delA	Forward 5′-biotin-CACGGCACCTGGCCTAAAG-3′ Reverse 5′-CTGCCACCACTCTGTTCTCCTT-3′ Internal 5′-ACAAGACATGGTTTTT-3′	55°
PAI-1 −668delG	Forward 5′-GGGGCACAGAGAGAGTCTGGA-3′ Reverse 5′-biotin-TCCCTCATCCCTGCCATGT-3′ Internal 5′-TGGACACGTGGGG-3′	60°

GJA-4 = connexin-37; MMP-3 = stromelysin-1; PAI-1 = plasminogen activator inhibitor-1.

Table 2. Genotype Frequency (%) Among 95 African Americans and 95 European Americans

Polymorphism	GJA-4 C10119T*			MMP-3 -1171delA†			PAI-1 -668delG†		
Genotype	C/C	C/T	T/T	−/−	−/A	A/A	−/−	−/G	G/G
African American	25.3	54.9	19.8	2.2	19.8	78	7.6	37	55.4
European American	48.4	44.1	7.5	32.3	44.1	23.6	38.7	46.2	15.1
Japanese‡	71.7	25.4	2.9	5.7	31.3	63.8	44.8	44.9	10.3

*$p = 0.053$; †$p < 0.001$. Comparisons for homozygous high-risk allele in African American vs. European American populations. ‡Frequencies taken from control population in reference 7.

Abbreviations as in Table 1.

With the identification and validation of common genetic variants that carry an elevated risk of MI, there is now the potential to explore this question. Our results, showing markedly higher frequencies of both single and compound risk-associated genotypes in AA than in EA subjects, suggest a potential genetic contribution to the observed racial differences in CHD risk. However, this should be interpreted with caution because the frequency of these genotypes in AA is greater than the observed incidence of CHD and MI in this population. This raises the question of whether these risk-associated genotypes carry the same significance in AA as has been shown in Japanese subjects. A differential impact of these polymorphisms, if present, could suggest either that they are in linkage with a causative variant or that their influence requires other genetic modifiers that may differ between races. This important question can now be asked in studies in AA and EA with CHD or MI.

Though this is a small study (190 total subjects), our findings are significant for several reasons. First, they describe the genetic landscape for patients likely to be encountered in U.S. populations and can be used to derive sample size for future studies. Second, we have extended previous observations to note the frequency of multiple genetic risk factors in the same individuals. This is pertinent because even though the risks associated with these genotypes are very significant by statistical standards, individually they were of somewhat modest clinical magnitude. On the other hand, if genotypes at several relevant loci were known, it might be possible to more accurately identify individuals at significantly higher CVD risk who could benefit from a more aggressive early strategy to prevent adverse outcomes. Thus, the fact that a substantial proportion of both AA and EA populations can be identified with multiple risk-associated alleles is intriguing for the alleles' potential utility in future health screening. Such data are needed to design and carry out larger, more definitive studies to assess the cumulative risk of MI in patients with these compound genotypes, as well as their prognostic importance for those with established disease. Finally, these data emphasize the potential opportunity to assess the relevance of common genetic variants to the differences in CVD among various racial groups, while giving an intriguing hypothesis from which to start this vital endeavor.

Reprint requests and correspondence: Dr. Howard L. McLeod, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8069, St. Louis, Missouri 63110-1093. E-mail: hmcleod@im.wustl.edu.

REFERENCES

1. American Heart Association. Heart Disease and Stroke Statistics—2003 Update. Dallas, TX: American Heart Association, 2003.
2. McCullough PA, Philbin EF, Spertus JA, Kaatz S, Sandberg KR, Weaver WD. Confirmation of a heart failure epidemic: findings from the Resource Utilization Among Congestive Heart Failure (REACH) study. J Am Coll Cardiol 2002;39:60–9.
3. Smedley BD, Stith AY, Nelson AR, Institute of Medicine (U.S.). Committee on Understanding and Eliminating Racial and Ethnic Disparities in Health Care. Unequal treatment: confronting racial and ethnic disparities in health care. Washington, DC: National Academy Press, 2003.
4. U.S. Department of Health and Human Services. Healthy People 2010: Understanding and Improving Health. Washington, DC: U.S. Government Printing Office, 2000.
5. Canto JG, Allison JJ, Kiefe CI, et al. Relation of race and sex to the use of reperfusion therapy in Medicare beneficiaries with acute myocardial infarction. N Engl J Med 2000;342:1094–100.
6. Nabel EG. Cardiovascular disease. N Engl J Med 2003;349:60–72.
7. Yamada Y, Izawa H, Ichihara S, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 2002;347:1916–23.
8. Hirashiki A, Yamada Y, Murase Y, et al. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol 2003;42:1429–37.
9. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996;271:13055–60.
10. Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A 1995;92:1851–5.
11. Boerma M, Forsberg L, Van Zeijl L, et al. A genetic polymorphism in connexin 37 as a prognostic marker for atherosclerotic plaque development. J Intern Med 1999;246:211–8.
12. Rose CM, Marsh S, Ameyaw MM, McLeod HL. Pharmacogenetic analysis of clinically relevant genetic polymorphisms. Methods Mol Med 2003;85:225–37.
13. Westfall PH, Young SS. Resampling-Based Multiple Testing: Examples and Methods for P-Value Adjustment. New York, NY: Wiley, 1993.
14. Festa A, D'Agostino R, Jr., Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Circulation 2003;107:2422–7.
15. Rundek T, Elkind MS, Pittman J, et al. Carotid intima-media thickness is associated with allelic variants of stromelysin-1, interleukin-6, and hepatic lipase genes: the Northern Manhattan Prospective Cohort Study. Stroke 2002;33:1420–3.