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, PHARM.D*

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.

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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 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...
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).

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>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer Sequence</th>
<th>Annealing Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJA-4 C1019T</td>
<td>Forward 5’-biotin-AACCTGACCACAGAGGAGGAGGAC-3’</td>
<td>62°</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’-CATCCCAAGCAGCCAGAC-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Internal 5’-GGGACGACTTGGGGG3’</td>
<td></td>
</tr>
<tr>
<td>MMP-3 –1171delA</td>
<td>Forward 5’-biotin-CACGCCACCTTGCTTAAG-3’</td>
<td>55°</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’-CTGCCAACCTGTTCTCT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Internal 5’-AAAGACATGTTTTTT-3’</td>
<td></td>
</tr>
<tr>
<td>PAI-1 –668delG</td>
<td>Forward 5’-GGGCAACAGAGAGTCTTGGG-3’</td>
<td>60°</td>
</tr>
<tr>
<td></td>
<td>Reverse 5’-TCCCATCCTCTGGCATT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Internal 5’-TGGACAGCTGGGGG-3’</td>
<td></td>
</tr>
</tbody>
</table>

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

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.

Table 1. Polymerase Chain Reaction and Pyrosequencing Primers and Conditions

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

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

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>GJA-4 C10119T</th>
<th>MMP-3 -1171delA</th>
<th>PAI-1 -668delG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>C/C</td>
<td>−/−</td>
<td>−/G</td>
</tr>
<tr>
<td>African American</td>
<td>25.3</td>
<td>19.8</td>
<td>37.0</td>
</tr>
<tr>
<td>European American</td>
<td>48.4</td>
<td>32.3</td>
<td>15.1</td>
</tr>
<tr>
<td>Japanese‡</td>
<td>71.7</td>
<td>6.7</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* † * ‡

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.

### REFERENCES


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.