Brief Report:

The Variable Number of Tandem Repeat Polymorphism of Platelet Glycoprotein Ibα and Risk of Coronary Heart Disease

Vahid Afshar-Kharghan¹, Nevenka Matijevic-Aleksic², Chul Ahn², Eric Boerwinkle², Kenneth K. Wu², and José A. López¹

¹ Department of Medicine, Thrombosis Research Section, Baylor College of Medicine, Houston, TX 77030
² The University of Texas at Houston Health Science Center, Houston TX, 77030

This work was supported by grant R01 HL65205 from the National Institute of Health and NHLBI contracts N01 HC-55015, -55016, -55018, -55019, -55020, -55021 and -55022. V. A-K is a recipient of the LLS & MPD Foundation’s Translational Research Grant and AHA, Texas Affiliate’s BGIA.

*Address correspondence to this author at: Thrombosis Research Section, Department of Medicine, BCM 286, N1319, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Telephone: 713-798-3470; FAX: 713-798-3415; e-mail: josel@bcm.tmc.edu.
ABSTRACT

Glycoprotein (GP) Ib-IX-V complex plays an important role in formation of platelet-fibrin clot at the area of damaged vessel wall. One polymorphism of GP Ibα, the main component of GP Ib-IX-V complex, is due to variable numbers of tandem repeats in the macroglycopeptide region of this molecule. We studied the association between the presence of different VNTR alleles of GP Ibα and the frequency of coronary heart disease among individuals recruited to a large community-based case-cohort study (Atherosclerosis Risk in Communities or ARIC study). We found that the distribution of VNTR alleles of GP Ibα is different among Caucasians and African-Americans. The B allele (with 3 VNTR repeats) of GP Ibα is relatively more common among African-Americans compared to Caucasians. In African-Americans, the CC genotype (homozygous with 2 VNTR repeats) is associated with a lower risk of CHD events than all other genotypes.
INTRODUCTION

The glycoprotein (GP) Ib-IX-V complex contains four polypeptides, GP Ibα, GP Ibβ, GP IX and GP V.1 The largest is GP Ibα, which contains the binding site for VWF and for several other proteins important in the genesis of vascular disease: thrombin, Factor XI, P-selectin, and leukocyte Mac-1.2-5

Glycoprotein Ibα is highly polymorphic, one polymorphism being due to variable numbers of tandem repeats (VNTR) in a region encoding the macroglycopeptide, a mucin-like stalk separating the ligand-binding region from the plasma membrane. This region may have from one to four 39-base tandem repeats, each encoding a 13–amino acid sequence. The four variants are named A, B, C, and D, with four, three, two and one repeat, respectively. It has been hypothesized that because added repeats increase the length of the macroglycopeptide region of GP Ibα, they might also increase the sensitivity of the longer molecule to shear stress, a known modulator of the GP Ibα–VWF interaction.1

Here, we analyzed the association of the GP Ibα VNTR alleles with risk of incident coronary heart disease (CHD) in the ARIC (Atherosclerosis Risk in Communities) case-cohort study.
METHODS

Study Population

We analyzed the Atherosclerosis Risk in Communities (ARIC) Study population, consisting of a population-based cohort totaling 15,792 men and women between 45 and 64 years of age from four US communities.\textsuperscript{6} We defined CHD incidence as (1) a definite or probable myocardial infarction (MI), (2) a silent MI detected by new ECG changes between examinations, (3) a definite CHD death, or (4) a coronary revascularization procedures.

This was a case-cohort study, with frequencies of GP Ib\(\alpha\) alleles determined for CHD cases and a stratified random sample of the ARIC cohort. We identified 349 individuals as cases with CHD. For the reference cohort, we oversampled participants with low average carotid intima–media thickness measurements at baseline (< 30 percentile) and also stratified the sampling by age and sex. We included 383 individuals as a reference cohort (of whom 22 were also CHD cases). Before selecting the case–cohort sample, we excluded participants identified as having prevalent CHD, stroke, or transient ischemic neurologic attack (TIA). The study included participants from 2 ethnic groups, Caucasians and African-Americans.

Genotyping

We genotyped each individual for the GP Ib\(\alpha\) VNTR polymorphism by polymerase chain reaction as described previously.\textsuperscript{7}

Data Analysis

We used analysis of covariance to compute age-, race-, and sex-adjusted frequencies of different genotypes, and means or percentage values of study variables.
(including lipid profile, plasma VWF and fibrinogen concentrations, history of hypertension, diabetes mellitus, tobacco or alcohol abuse) for CHD cases versus non-cases after appropriate weighting for the stratified case-cohort sampling design. We computed the risk ratios and 95% confidence intervals for the time to development of CHD using a weighted proportional hazard regression, accounting for the stratified random sampling and the case-cohort design by Barlow’s method. Because the prevalence of different GP Ibα genotypes was significantly different between Caucasians and African-Americans, we performed separate weighted proportional hazard regression analyses for Caucasians and African-Americans. In the weighted proportional hazard regression model, we adjusted for sex, age and the other factors related to CHD. The number of subjects with BB (5 cases and 5 non-cases) and DD (1 case and 3 non-cases) genotypes were very small and were combined with BC and CD, respectively, in analysis. Only 4 individuals with the A allele were identified, and were omitted from the analysis.
RESULTS AND DISCUSSION

Ethnicity and GP Ibα genotype

The association of three genotype groups (BC+BB, CC, and CD+DD) with age, sex, ethnicity and conventional risk factors is shown in Table 1. The CC and CD+DD groups were smaller by percentage in African-Americans than Caucasians, with a corresponding increase in the BC+BB group. Aramaki et al. has previously shown the higher frequency of the B allele in African-Americans compared to Caucasians.9

CHD incidence and GP Ibα genotype

In the entire population, we found no significant differences in genotypes between incident CHD cases and non-cases (Table 2). However, when we examined the two ethnic groups separately, we found an association in African-Americans between the non-CC genotypes and incident CHD (Table 2). Put another way, African-Americans with the CC genotype had a significantly lower incidence of CHD than those with either the BC+BB or CD+DD genotypes. The weighted proportional hazard regression analysis showed that GP Ibα genotypes correlate with the development of CHD in African-Americans (Table 2). In contrast, Caucasians in either the B/C+B/B or C/D+D/D groups did not have a significantly higher risk of CHD (risk ratio 1.1, and 1.4, respectively).

We also analyzed the association according to the presence of the C allele (supplementary data). The C allele was associated with lower incident CHD in African Americans (p=0.003), whereas no such association was noted in the whole group or in Caucasians (p=0.091 and 0.521, respectively). African Americans homozygous for the C allele had significantly lower incident CHD compared to those with one C allele.
We have found a protective effect of the CC genotype in African-Americans participating in the ARIC study, but not in the group overall or in Caucasians. This may indicate a interaction between GP Ibα genotype and another environmental or genetic variable that is more prevalent in African-Americans than Caucasians. Alternatively, it is possible that the increased prevalence of the B allele in African-Americans unmasks an increased risk for cardiovascular disease associated with this variant.

Of interest, two GP Ibα haplotypes for GP Ibα have been reported with much higher frequency in African-Americans than in Caucasians, the C/Met145 variant and the B/Thr145 variant, present in this population with frequencies of 2.2% and 6.5%, respectively. Because Met145 has been associated with an increased prevalence and severity of coronary artery disease the protective effect of the C/C genotype in the African-American population is even more striking.

The hypothesis that the length of GP Ibα affects its function, and as a result the propensity to develop CHD, is not a new one. Other groups have also studied the role of the GP Ibα VNTR polymorphism in case–control studies, with contradictory results. All of these studies were cross-sectional case-control studies. The design of these studies makes it less likely that an ideal control group will be chosen, with the case and control subjects being fully matched for all other risk factors. In the current study, 15,792 individuals were followed prospectively over a period of 10 years.

Another mechanism by which the VNTR polymorphism may influence CHD susceptibility is suggested by the results of our study. Most of the individuals in the non-CC group were heterozygous for two length variants, either carrying the BC or the CD
genotypes. Thus, it is possible that it is the disparity in length between the two allele products in a heterozygous individual that influences the interaction of GP Ibα with the receptor (it has been postulated that GP Ibα may be able to associate into dimers or tetramers to form the functional VWF-binding unit\(^1\)). Nevertheless, because of the relative shortage of BB homozyotes, a larger study is needed to determine whether it is the length of the macroglycopeptide stalk or a length disparity in heterozygotes that determines the increased risk in the non-CC African-American individuals.

**ACKNOWLEDGEMENT**

The authors thank the staff and participants in the ARIC study for their important contributions.

**SUPPLEMENTAL MATERIAL IS AVAILABLE ONLINE AT THE TIME OF FINAL PUBLICATION ONLY.**
REFERENCE LIST


<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotypes</th>
<th></th>
<th></th>
<th></th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B/C+B/B</td>
<td>C/C</td>
<td>C/D+D/D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>24%</td>
<td>27%</td>
<td>19%</td>
<td>0.655</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus, %</td>
<td>3%</td>
<td>6%</td>
<td>9%</td>
<td>0.675</td>
<td></td>
</tr>
<tr>
<td>Systolic Blood-Pressure, mmHg</td>
<td>117</td>
<td>119</td>
<td>118</td>
<td>0.835</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol, mg/dL</td>
<td>204</td>
<td>217</td>
<td>209</td>
<td>0.204</td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>123</td>
<td>123</td>
<td>124</td>
<td>0.987</td>
<td></td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>56</td>
<td>54</td>
<td>51</td>
<td>0.453</td>
<td></td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>124</td>
<td>139</td>
<td>133</td>
<td>0.498</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>288</td>
<td>304</td>
<td>294</td>
<td>0.475</td>
<td></td>
</tr>
<tr>
<td>VWF, %</td>
<td>133</td>
<td>118</td>
<td>102</td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>Cigarette years</td>
<td>272</td>
<td>234</td>
<td>210</td>
<td>0.693</td>
<td></td>
</tr>
<tr>
<td>Ethanol intake, g/W</td>
<td>32</td>
<td>30</td>
<td>31</td>
<td>0.987</td>
<td></td>
</tr>
<tr>
<td>Waist/Hip ratio</td>
<td>0.89</td>
<td>0.92</td>
<td>0.91</td>
<td>0.260</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>55</td>
<td>54</td>
<td>54</td>
<td>0.482</td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>40%</td>
<td>43%</td>
<td>47%</td>
<td>0.906</td>
<td></td>
</tr>
<tr>
<td>African-Americans, %</td>
<td>39%</td>
<td>22%</td>
<td>5%</td>
<td>0.003*</td>
<td></td>
</tr>
</tbody>
</table>

*P values for B/C+B/B vs C/C, B/C+B/B vs C/D+D/D, and C/C vs C/D+D/D are 0.074, 0.0001, and 0.009 respectively.
### Table 2: GP Ibα genotypes and CHD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All</th>
<th>Caucasians</th>
<th>African-Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHD</td>
<td>Non-CHD</td>
<td>RH (95% CI)</td>
</tr>
<tr>
<td>BC+BB</td>
<td>61</td>
<td>50</td>
<td>1.6 (0.8-3.2)</td>
</tr>
<tr>
<td>CC</td>
<td>239</td>
<td>279</td>
<td>&amp;</td>
</tr>
<tr>
<td>CD+DD</td>
<td>45</td>
<td>30</td>
<td>1.8 (0.87-3.6)</td>
</tr>
</tbody>
</table>

*p-value*: for the comparison of GPIbα genotypes against incident CHD status. *p*-value is computed by adjusting the effects of age, gender, and race for all subjects, and by adjusting the effects of age and gender for Caucasians and African-Americans.

In African-Americans, *p*=0.014 for C/C vs. B/C+B/B and *p*=0.0001 for C/C vs. C/D+D/D

**RH (95%CI):** Relative Hazard (95% confidence interval), adjusted for age, gender, race, hypertension, diabetes mellitus, total cholesterol, HDL-cholesterol, smoking and alcohol intake.

&: reference value in calculation of adjusted relative hazard

---

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
The variable number of tandem repeat polymorphism of platelet glycoprotein Ib α and risk of coronary heart disease

Vahid Afshar-Kharghan, Nevenka Matijevic-Aleksic, Chul Ahn, Eric Boerwinkle, Kenneth K Wu and Jose A Lopez