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Possible Race and Gender Divergence in Association of Genetic Variations with Plasma von Willebrand Factor: A Study of ARIC and 1000 Genome Cohorts

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Abstract

The synthesis, secretion and clearance of von Willebrand factor (VWF) are regulated by genetic variations in coding and promoter regions of the VWF gene. We have previously identified 19 single nucleotide polymorphisms (SNPs), primarily in introns that are associated with VWF antigen levels in subjects of European descent. In this study, we conducted race by gender analyses to compare the association of VWF SNPs with VWF antigen among 10,434 healthy Americans of European (EA) or African (AA) descent from the Atherosclerosis Risk in Communities (ARIC) study. Among 75 SNPs analyzed, 13 and 10 SNPs were associated with VWF antigen levels in EA male and EA female subjects, respectively. However, only one SNP (RS1063857) was significantly associated with VWF antigen in AA females and none was in AA males. Haplotype analysis of the ARIC samples and studying racial diversities in the VWF gene from the 1000 genomes database suggest a greater degree of variations in the VWF gene in AA subjects as compared to EA subjects. Together, these data suggest potential race and gender divergence in regulating VWF expression by genetic variations.

Introduction

von Willebrand factor (VWF) is a multimeric glycoprotein ligand essential in initiating hemostasis at sites of vessel injury [1]. It can also promote thrombosis by mediating platelet adhesion to activated endothelium and/or subendothelium at sites of ruptured atherosclerotic plaques, or aggregating platelets under pathological high shear stress found at sites of arterial stenosis [2,3]. An elevated plasma level of VWF is an independent risk factor for coronary heart disease (CHD), ischemic stroke, and peripheral artery disease [4,5], whereas low VWF antigen and activity could result in bleeding associated with von Willebrand disease (VWD) [6]. Plasma VWF antigen levels vary significantly among healthy individuals [7,8]. Both environmental and genetic factors contribute to this variation. The former includes conditions known to stimulate endothelial cells to secret VWF [9,10], whereas the latter plays a dominant role in determining a baseline level of circulating VWF [11,12]. ABO blood types greatly influence the baseline level of plasma VWF. Individuals with blood type O have a lower level of circulating VWF, resulting from an accelerated clearance of VWF from the circulation [13]. Genetic variations in the promoter and coding regions of the VWF gene are known to affect VWF levels [14,15]. We have previously identified intronic single nucleotide polymorphisms (SNPs) and their haplotypes that are associated with plasma VWF levels in 7,856 subjects of European descent (EA) from the Atherosclerosis Risk in Communities (ARIC) cohort [16]. We have also detected a significant ethnic diversity of variations in the VWF gene among subjects included in the 1000 genomes database [17]. In this study, we further examined potential race and gender differences in this association between VWF antigen and VWF gene variations. This study is important because AA subjects often have a higher level of circulating VWF antigen [7,8,15]. Recent studies by Bellissimo D. B., et al [19] and by us [17] show that some “mutations” that were originally associated...
SNPs Associated with Plasma VWF Levels Analyzed for Race-by-Gender.

Table 2. SNPs Associated with Plasma VWF Levels Analyzed for Race-by-Gender.

| SNP                  | Race by Sex | Mean ± S.E. (Genotype frequency %) | P valuea |
|---------------------|-------------|------------------------------------|----------|
|                     | AA          | AB                                 | BB       |          |
| RS1063857 (Exon 18) | EA-F        | 104.8±0.9 (41%)                    | 109.8±0.8 (46%) | 115.3±1.6 (13%) | <.0001 |
|                     | EA-M        | 106.5±1.0 (40%)                    | 113.0±0.9 (47%) | 119.7±1.7 (13%) | <.0001 |
|                     | AA-F        | 117.7±3.2 (16%)                    | 126.6±1.8 (50%) | 136.0±2.2 (33%) | <.0001 |
|                     | AA-M        | 113.5±4.2 (16%)                    | 121.2±2.3 (50%) | 128.3±2.7 (35%) | 0.0076 |
| RS216315 (Intron 22) | EA-F       | 91.4±5.7 (1%)                      | 100.7±1.4 (16%) | 110.1±0.6 (83%) | <.0001 |
|                     | EA-M        | 97.9±6.4 (1%)                      | 105.9±1.6 (15%) | 112.374±0.7 (84%) | 0.0001 |
|                     | AA-F        | 123.6±5.1 (7%) *                   | 128.7±1.3 (93%) | 0.3382 |
|                     | AA-M        | 117.9±5.9 (7%) *                   | 122.9±1.7 (93%) | 0.4186 |
| RS111610629 (Intron 22) | EA-F     | 106.5±0.7 (56%)                    | 110.3±0.9 (38%) | 113.7±2.4 (6%) | 0.0004 |
|                     | EA-M        | 108.9±0.9 (55%)                    | 113.3±1.0 (39%) | 118.2±2.6 (6%) | <.0001 |
|                     | AA-F        | 124.9±1.8 (34%)                    | 131.1±2.1 (39%) | 134.7±5.2 (7%) | 0.0362 |
|                     | AA-M        | 119.5±2.3 (49%)                    | 127.5±2.5 (43%) | 114.8±6.1 (8%) | 0.0277 |
| RS216318 (Intron 21) | EA-F       | 92.1±5.5 (1%)                      | 100.7±1.4 (16%) | 110.1±0.6 (83%) | <.0001 |
|                     | EA-M        | 97.9±6.4 (1%)                      | 105.8±1.6 (15%) | 112.4±0.7 (84%) | <.0001 |
|                     | AA-F        | 120.5±4.6 (8%) *                   | 129.0±1.4 (92%) | 0.0788 |
|                     | AA-M        | 117.9±5.8 (8%) *                   | 123.0±1.7 (92%) | 0.4004 |
| RS216295 (Intron 17) | EA-F       | 110.1±0.6 (82%)                    | 101.6±1.4 (17%) | 92.9±5.5 (1%) | <.0001 |
|                     | EA-M        | 112.3±0.7 (83%)                    | 106.4±1.6 (16%) | 91.7±6.8 (1%) | <.0001 |
|                     | AA-F        | 130.5±1.6 (66%)                    | 125.3±2.4 (31%) | 110.8±7.8 (3%) | 0.0153 |
|                     | AA-M        | 123.5±1.9 (68%)                    | 120.2±3.1 (29%) | 124.9±8.9 (3%) | 0.6384 |
| RS216298 (Intron 16) | EA-F       | 94.8±5.4 (1%)                      | 101.2±1.4 (17%) | 110.1±0.6 (82%) | <.0001 |
|                     | EA-M        | 95.1±6.2 (1%)                      | 106.0±1.6 (16%) | 112.5±0.7 (83%) | <.0001 |
|                     | AA-F        | 113.0±8.1 (3%)                    | 124.9±2.5 (26%) | 130.1±1.5 (71%) | 0.0351 |
|                     | AA-M        | 120.2±9.2 (3%)                    | 120.7±3.3 (25%) | 123.3±1.9 (72%) | 0.7643 |
| RS216299 (Intron 16) | EA-F       | 96.3±5.0 (1%)                      | 101.5±1.4 (17%) | 110.0±0.6 (82%) | <.0001 |
|                     | EA-M        | 95.5±5.9 (1%)                      | 106.2±1.6 (16%) | 112.4±0.7 (83%) | <.0001 |
|                     | AA-F        | 112.0±7.9 (3%)                    | 125.0±2.5 (26%) | 130.2±1.5 (71%) | 0.0242 |

Methods

The Atherosclerosis Risk in Communities (ARIC, http://www.cscu.unc.edu/aric/) is a prospective cohort study designed to assess subclinical atherosclerosis and clinical atherosclerotic events and cardiovascular risk factors [21]. Among 15,792 ARIC participants, 10,434 were included in this study after exclusion of subjects lacking data on 1) VWF antigen (n = 280), 2) VWF SNPs (n = 3,032), or 3) ABO genotypes (n = 1,898). The study also excluded non-EA and non-AA individuals (n = 1,756) with VWD in Caucasians are found in 10–20% of African subjects without evidence of clinical bleeding, even though some of these “VWD mutants” are dose-dependently associated with VWF levels in apparently healthy AA subjects [20]. Together, these data suggest that 1) the association between variations in the VWF gene and plasma levels of VWF antigen may vary by race and 2) this racial diversity may impact on how genetic variations influence VWF synthesis, secretion and clearance. In this study, we further examined the racial diversity in this association between VWF antigen and VWF gene variations between the EA and AA subjects from the ARIC cohort.

*include current and former smokers.
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Table 1. Demographic and Plasma VWF for 10,434 ARIC Participants by Race.

| Characteristics | Race | N (%) | Mean ± (s.d) | P value |
|-----------------|------|-------|--------------|---------|
| Subject included | N (%) | 2,378 (22.8) | 8,056 (77.2) |         |
| Gender          | F    | 1,505 | 4,286        | < 0.001 |
|                 | M    | 873   | 3770         |         |
| BMI             | Mean (s.d) | 30.12 (6.37) | 27.01 (4.67) | < 0.001 |
| Diabetes        | N (%) | 452 (19.3) | 726 (9.1)    | < 0.001 |
| Hypertension    | N (%) | 1,308 (55.0) | 2,256 (27.8) | < 0.001 |
| History of smoking | N (%) | 1,260 (53.1) | 4,818 (59.8) | < 0.001 |
| VWF [mean ± (s.d)] | F | 128.15 (54.42) | 108.44 (41.10) | < 0.001 |
|                 | M    | 122.74 (51.16) | 111.34 (42.36) | < 0.001 |

A Biracial Comparison of VWF Gene Polymorphisms
and those who did not consent to DNA use (n = 100). Covariates included ABO genotypes (O vs. non-O), age, gender, body mass index (BMI), hypertension, diabetes, and smoking status, all of which are known to affect VWF antigen levels. VWF measurements, SNP genotyping, haplotype construction, and data analyses were previously described [16]. A polyclonal VWF antibody was used to detect VWF antigen in plasma samples, but if this antibody binds VWF multimers from EA and AA subjects with an equal affinity has not been experimentally determined. The difference between EA and AA subjects in allele frequencies of these VWF variants were further validated with

48) and those who did not consent to DNA use (n = 100). Covariates included ABO genotypes (O vs. non-O), age, gender, body mass index (BMI), hypertension, diabetes, and smoking status, all of which are known to affect VWF antigen levels. VWF measurements, SNP genotyping, haplotype construction, and data analyses were previously described [16]. A polyclonal VWF antibody was used to detect VWF antigen in plasma samples, but if this antibody binds VWF multimers from EA and AA subjects with an equal affinity has not been experimentally determined.

The difference between EA and AA subjects in allele frequencies of these VWF variants were further validated with information from the April 2012 Integrated Variant Set release of the 1000 Genomes Project [ftp://ftp.1000genomes.ebi.ac.uk/vol1ftp/release/20110521/ALL.wgs.phase1_release_v3.20101123.sns_indels_sv.sites.vcf.gz]. This database compiles information on genomic variations among 1,092 subjects from 14 ethnicities [25] and has been used to demonstrate a significant ethnic allelic diversity of VWF SNPs among Africans, Americans of European decent, Europeans and Asians [17].
Results and Discussion

Characteristics of the 10,434 subjects are summarized in Table 1 and found to be consistent with our previous reports [16;22]. AA subjects (N = 2,378) had significantly higher BMI and prevalences of diabetes and hypertension, but a lower prevalence of smoking compared to EA subjects (N = 8,056). The mean level of plasma VWF varied significantly in race-by-gender groups. VWF levels found in female subjects of this ARIC cohort are comparable to a VWF varied significantly in race-by-gender groups. VWF levels of diabetes and hypertension, but a lower prevalence of smoking subjects (N = 2,378) had significantly higher BMI and prevalences 1 and found to be consistent with our previous reports [16;22]. AA subjects (N = 8,056) because the difference in VWF antigen accounted for. These data need to be validated in larger cohorts between EA and AA subjects. Among these 15 positive SNPs, 13 and 10 reached statistical significance in EA males and EA females, respectively, with 5 (38.5%) and 2 (20%) of them being significant only in males and females, respectively. In contrast, only one of these 15 positive SNPs (RS1063857) was associated with VWF levels in AA females and none in AA males (Table 2). RS1063857, which was associated with EA males and females, is a synonymous SNP located in exon 18 of the VWF gene. Nucleotide sequence in exon 18 encodes for the D' domain that is involved in VWF multimerization [24] and binding FVIII [25]. This SNP also generated the strongest association in our previous study of EA subjects [16] and was associated with VWF antigen levels in a GWA study by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium [26]. Together, these data suggest potential race and gender diversities in allele frequencies for these SNPs. These race and gender diversities were further validated using information provided in the 1000 Genomes Project. Among the 15 positive SNPs, 7 (53.3%) had allele frequencies of 1.9 – 9.6 fold higher in Africans than in EA subjects and 1 (6.7%) was 2.7 fold more common in EA subjects (Table 3). As expected, Americans of European decent and European subjects had a highly comparable level of allele frequencies for all 15 VWF SNPs. However, 6 SNPs that differ most between Africans and EA were very rare in Asians (> 100 fold less frequent).

In addition to individual SNPs, we also constructed haplotypes that included rare VWF SNPs in order to identify SNPs that are co-transmitted together. Using the fastPHASE 1.2 program, we identified 10 and 13 major haplotype blocks that are in linkage disequilibrium for EA and AA subjects, respectively (Figure 1). SNPs in each LD haplotype varied significantly between EA and AA subjects, indicating a highly diverse SNP co-aggregation between EA and AA subjects.

In conclusion, we have identified VWF SNPs that appear to be differentially associated with plasma levels of VWF antigen between EA and AA subjects as well as between male and female subjects. We did not detect a significant race-by-SNPs interaction for several reasons. First, the numbers of AA subjects were relatively small (N = 2,378) compared to EA subjects (N = 8,056) because the difference in VWF antigen associated with these intronic variations are expected to be small. However, this small sample size is partially compensated by higher allele frequencies in AA subjects for most of these SNPs (Table 2 & Table 3). Second, the VWF gene is more variable in AA subjects as suggested by their haplotypes (Figure 1). Finally, these VWF genetic variations may exert a smaller influence on AA subjects because of their relative higher baseline levels of VWF antigen. Covariates known to affect VWF antigen levels (ABO, age, gender, body mass index, hypertension, diabetes, and smoking status) were adjusted for the data analyses, but other covariates may exist and are not accounted for. These data need to be validated in larger cohorts of mix ethnicities, but they, nevertheless, suggest that the association between genetic and antigenic variations of VWF could be influenced by race and gender. This notion of racial divergence is consistent with recent reports that selective “mutations” previously associated with the bleeding disorder VWD were detected at 10–21% of AA subjects without documented bleeding [17;19]. The finding suggests that these “mutations” are unlikely to cause the disease. Our data, therefore, suggest caution in associating a specific SNP with a bleeding or thrombotic state without considering the impact of race and gender.

| Table 3. Allele Frequency of VWF SNP in Four Ethnic Groups (%) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| SNP             | Ref. allele     | Alt. allele     | African         | EA              | European        | Asian           |
| RS1063857       | A               | G               | 65              | 27              | 38              | 8               |
| RS216315        | A               | G               | 98              | 94              | 91              | 79              |
| RS1160629       | A               | C               | 27              | 22              | 25              | 0.17            |
| RS216318        | A               | C               | 98              | 93              | 91              | 79              |
| RS216295        | T               | C               | 85              | 9               | 91              | 77              |
| RS216298        | C               | T               | 87              | 91              | 91              | 77              |
| RS216299        | A               | G               | 87              | 91              | 91              | 77              |
| RS12304995      | C               | T               | 62              | 31              | 37              | 18              |
| RS11609815      | C               | G               | 41              | 22              | 25              | 0.17            |
| RS11063995      | T               | C               | 41              | 22              | 25              | 0.17            |
| RS11612401      | G               | C               | 11              | 2               | 25              | 0.17            |
| RS1800380       | C               | T               | 27              | 22              | 25              | 0.17            |
| RS1609728       | C               | A               | 7               | 19              | 25              | 0.17            |
| RS2239161       | G               | A               | 97              | 91              | 88              | 77              |
| RS2239160       | A               | G               | 86              | 9               | 88              | 77              |

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Supporting Information

Table S1  Genotype frequencies of VWF SNPs by race and gender. This table lists frequencies of all VWF SNPs that were studied and categorized based on race and gender.

Author Contributions

Conceived and designed the experiments: KKW ARF JFD. Performed the experiments: ZZ FY AF MC LEC. Analyzed the data: AB LEC JFD. Wrote the paper: ZZ ARF LEC JFD.

Figure 1. VWF haplotypes. The haplotypes were constructed using the default setting that (1) ignores pairwise comparisons of markers more than 500 kb apart, (2) excludes subjects with more than 50% of missing genotypes, (3) examines haplotypes found 1% or more in the population, (4) removes markers with Hardy-Weinberg disequilibrium, and (5) uses R² to define haplotype blocks.

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