Common and rare von Willebrand factor (VWF) coding variants, VWF levels, and factor VIII levels in African Americans: the NHLBI Exome Sequencing Project

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Key Points

• Several common and rare VWF missense variants contribute to phenotypic differences in VWF and FVIII among African Americans.
• Next-generation sequencing technology and improved genotype imputation can contribute to molecular genetics of VWD-related phenotypes.

Introduction

Von Willebrand factor (VWF) is a multimeric plasma glycoprotein that plays an important role in hemostasis and thrombosis.1 VWF adheres to sites of vascular injury, binds platelets, and stabilizes factor VIII (FVIII) in the circulation. High VWF levels are associated with increased risk of venous thrombosis, myocardial infarction, and ischemic stroke.2,3 Conversely, deficiency of VWF is associated with bleeding and a diagnosis of von Willebrand disease (VWD), the most common inherited bleeding disorder in humans.4

The human VWF gene is located on chromosome 12p13.3, spans 178 kb, and is composed of 52 exons ranging in size from 40 bases to 1.4 kb (exon 28).5 The translated pre–pro-VWF molecule contains 2813 amino acids, comprising a 22-residue signal peptide, a 741–amino acid propeptide, and a highly glycosylated 2050–amino acid mature VWF subunit, also known as VWF antigen, composed of repeated VWF domains commonly annotated A to D arranged in the order D1-D2-D’-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK (Figure 1 top). The architecture of these VWF domains is the result of complex duplications, and a recent revised VWF domain annotation based upon sequence homology and structure studies has been published6 (Figure 1 bottom). VWF mutations can be detected in many type 1 VWD cases and in nearly all type 2 and 3 patients.5,7 Type 2 VWD variants, characterized by defects in multimerization, proteolysis, or interaction with VWF ligands (platelets, collagen, or FVIII), tend to be localized to particular functional domains encoded by VWF exons 18 to 25 (D’-D3 domains, type 2N) exons 28 (A1-A2 domains, types 2A, 2B, and 2M), and exons 11 to 16, 24 to 26, and 51 to 52 (type 2A). On the other hand, type 1 and type 3 VWD mutations occur throughout the VWF gene.

Two-thirds of phenotypic variation in VWF level is attributable to heritable factors, with one-third of the heritable variance due to the influence of ABO blood group.8,9 Common VWF polymorphisms, such as the missense variant rs1063857 (encoding p.Thr789Ala), also...
influence VWF levels.\textsuperscript{10,11} VWF antigen (VWF:Ag) and FVIII coagulation activity levels (FVIII:C) are higher in AAs than in European Americans (EAs), and this difference is not explained by known differences in ABO allele frequencies.\textsuperscript{12-15} The ratio of VWF activity (ristocetin cofactor activity) to VWF:Ag is lower among AAs than EAs.\textsuperscript{15,16} Consequently, AAs are diagnosed more frequently with a variety of cardiovascular and pulmonary phenotypes (coronary heart disease, stroke, diabetes, asthma, COPD, acute lung injury, pulmonary artery hypertension, cystic fibrosis, and extremes of blood pressure, body mass, or low-density lipoprotein cholesterol). The sample for exome sequencing included a total of 1455 AAs from the ARIC, CARDIA, MESA, and WHI cohorts. Of the 1455 AA ESP participants with exome sequence data, 595 with VWF:Ag and FVIII:C phenotype measurements are included in the current analysis.

Until recently, molecular genetic analyses of VWD and population genomic studies of VWF were largely confined to populations of European descent. Two recent analyses of population-based sequencing data demonstrated considerable ethnic diversity in coding sequence variation at the VWF locus.\textsuperscript{17,18} Several VWF missense variants, previously reported in EAs as rare VWD-causing mutations (including p.Met740Ile, p.His817Gln, and p.Arg2185Gln), were considerably more common (minor allele frequencies [MAF] \(\geq 10\%\)) in AA.\textsuperscript{17,18} This observation, together with the lack of association between these variants and VWF-related phenotypes in 66 known differences in ABO allele frequencies.\textsuperscript{12-15} The ratio of VWF activity (ristocetin cofactor activity) to VWF:Ag is lower among AAs than EAs.\textsuperscript{15,16} Consequently, AAs are diagnosed more frequently with

**Methods**

**Subjects**

Participants included 4468 self-identified AAs with VWF and/or FVIII measurements from 4 population-based cohorts: the Atherosclerosis Risk in Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA), Multi-Ethnic Study of Atherosclerosis (MESA), and Women’s Health Initiative (WHI). Clinical information was collected by self-report and in-person examination. Detailed descriptions and VWF and FVIII laboratory methods for each cohort are provided in supplemental Methods. The 4468 AAs included 595 participants with exome sequence data and 3873 with imputed genotypes using preexisting GWAS data, as described below. In accordance with the Declaration of Helsinki, all participants provided written informed consent as approved by local institutional review boards.

**Exome sequencing and VWF coding variant identification**

We used VWF exonic sequence data from the NHLBI Exome Sequencing Project to assess the association of coding variants with VWF:Ag and FVIII:C levels in a large population-based sample of AAs.

**Imputation of VWF coding variant genotypes into a larger AA sample**

Using an imputation procedure, we probabilistically determined the genotypes for the 147 VWF missense variants with MAF > 0.1% that were identified through exome sequencing in a larger sample of 3873 AAs with VWF:Ag and/or FVIII:C levels and Affymetrix 6.0 genome-wide genotyping data from the parent ARIC, CARDIA, MESA, and WHI cohorts. We used as our imputation reference panel a subset of 2163 ESP participants (1692 AAs, 471 EAs) with exome sequence data who had been genotyped on Affymetrix 6.0 GWAS platform. Variant- and sample-level processing, quality control, and filtering of the Affymetrix 6.0 genotype data were performed as described previously.\textsuperscript{20-21} We then used minimac\textsuperscript{20} to probabilistically impute genotypes, as described previously.\textsuperscript{20}

Sequence variants were excluded at the calling stage for a read depth <10\%. Prior to data analysis, single-nucleotide variants (SNVs) were additionally excluded on the basis of imputation quality. We used the imputation quality metric Rsq, which ranges from a value of 1 (no genotype uncertainty) to 0 (complete uncertainty). Using previously defined MAF-based thresholds, SNVs passing the quality-control threshold had an average Rsq of \(\approx 0.8\). Of the total of 147 SNV from the VWF gene with MAF > 0.1% identified by
Table 1. Characteristics of 4468 AA participants by cohort

<table>
<thead>
<tr>
<th>Study</th>
<th>ARIC</th>
<th>CARDIA</th>
<th>MESA</th>
<th>WHI</th>
<th>Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>2354</td>
<td>353</td>
<td>1607</td>
<td>154</td>
<td>4468</td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>53.3 (5.8)</td>
<td>24.9 (4.4)</td>
<td>62.2 (10.1)</td>
<td>65.2 (6.5)</td>
<td>54.8 (12.4)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>63</td>
<td>61</td>
<td>54</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>29.7 (6.1)</td>
<td>25.4 (5.7)</td>
<td>29.9 (5.5)</td>
<td>31.3 (5.1)</td>
<td>29.3 (6.2)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>28.9%</td>
<td>33.9%</td>
<td>24.7%</td>
<td>29.4%</td>
<td>27.8%</td>
</tr>
<tr>
<td>BMI, body mass index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VWF antigen, IU/dL (SD)</td>
<td>148 (48)</td>
<td>102 (38)</td>
<td>115 (47)</td>
<td>123 (63)</td>
<td>128 (15)</td>
</tr>
<tr>
<td>FVIII activity, IU/dL (SD)</td>
<td>184 (48)</td>
<td>102 (38)</td>
<td>115 (47)</td>
<td>123 (63)</td>
<td>128 (15)</td>
</tr>
<tr>
<td>FVIII/VWF ratio</td>
<td>1.18 (0.33)</td>
<td>1.12 (0.53)</td>
<td>1.20 (0.40)</td>
<td>1.15 (0.62)</td>
<td>1.18 (0.37)</td>
</tr>
<tr>
<td>Ristocetin cofactor activity</td>
<td>NA</td>
<td>92 (40)</td>
<td>NA</td>
<td>92 (40)</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean (SD) values are shown for age, body mass index, VWF antigen, FVIII activity, FVIII/VWF ratio, and ristocetin cofactor activity.

BMI, body mass index; NA, not available; SD, standard deviation.

*Of the total of 4468 AA participants, VWF levels were measured in 2989 and FVIII levels were measured in 4430. Both measurements were available in 2951 participants. Ristocetin cofactor activity was measured in 165 CARDIA participants.

Results

The characteristics of the AA participants by cohort are summarized in Table 1. The overall correlation between VWF and FVIII levels was 0.70. Significant differences between cohorts were observed for age, sex, body mass index, and smoking status. Differences in mean VWF:Ag and FVIII:C levels between cohorts most likely reflect differences in demographic characteristics as well as interassay variability between laboratories (see supplemental Methods).

Of the 30 VWF missense variants, the consensus prediction for 7 were predicted to be deleterious and 23 were predicted to be neutral, though there was considerable heterogeneity between each of the individual prediction methods (supplemental Table 2). We determined pairwise LD using the full set of Exome Sequencing Project AA (supplemental Figure 1). Only 2 pairs of variants showed moderate allelic correlation or LD (defined as $r^2 > 0.5$): rs11063987 (p Ser1435Asn) with rs11063989 (p.Ile1380Val) ($r^2 = 0.89$) and rs75645183 (p.Asp837Ala) with rs78302129 (p.Ser1725Pro) ($r^2 = 0.73$), p.Arg2185Gln, p.As1472His, and p.Ser1435Asn/Ile1380Val were in weak LD with one another (pairwise $r^2$ in the range of 0.1-0.2).

Association results for VWF missense variants

A total of 9 of the 30 VWF missense variants tested (30%) were significantly ($P < 0.0017$) associated with VWF:Ag and/or FVIII:C (Table 2). A complete list of association results for all 30 missense variants is provided in supplemental Table 3. There was little evidence of heterogeneity of results (supplemental Table 3), and effect sizes were relatively consistent across cohorts (supplemental Figure 2). A total of 6 of the 9 variants were common (MAF > 5%) and 3 were infrequent or rare (MAF < 1%) in AA. The rare variant encoding p.Ser1486Leu disrupts a predicted O-linked glycosylation site adjacent to the A1 domain but has not been reported previously.

Each additional copy of the rs1063856 (p Thr789Ala) A allele (A/a789) was associated with 9 IU/dL higher VWF:Ag and 7 IU/dL higher FVIII:C levels. Each additional copy of the rs2229446 (p.Arg2185Gln) minor allele was associated with 13 IU/dL lower VWF:Ag and FVIII:C levels. rs57950734 (p.His817Gln) minor allele was associated with a significant 18 IU/dL per allele decrease in FVIII:C level but had no significant association with VWF:Ag level. Three common exon 28 coding variants (p.Ile1380Val, p.Asn1435Ser, and p.Asp1472His) were associated with 5 to 6 IU/dL higher FVIII:C levels. Each additional copy of the p.Ala879Gln (A/a879) was associated with 9 IU/dL higher VWF:Ag and 7 IU/dL higher FVIII:C levels. Each additional copy of the p.Ala879Gln (A/a879) was associated with 9 IU/dL higher VWF:Ag and 7 IU/dL higher FVIII:C levels.
Table 2. VWF missense variants associated with VWF antigen and/or FVIII coagulant activity in AAs

<table>
<thead>
<tr>
<th>Variant</th>
<th>Chromosome/BP</th>
<th>Rs Number</th>
<th>Annotation</th>
<th>EAF AA</th>
<th>EAF EA</th>
<th>Functional Prediction</th>
<th>Beta (SE)</th>
<th>Beta (SE)</th>
<th>P Value</th>
<th>Ln(VWF)</th>
<th>Ln(FVIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr12:6153534</td>
<td>1063856</td>
<td>Thr789Ala</td>
<td>0.58</td>
<td>0.3600</td>
<td>1.00</td>
<td>Benign</td>
<td>8.62 (1.56)</td>
<td>0.0664 (0.0109)</td>
<td>1.05E-09</td>
<td>4432</td>
<td>7.22 (1.36)</td>
</tr>
<tr>
<td>chr12:6145649</td>
<td>5790734</td>
<td>His817Gln</td>
<td>0.11</td>
<td>0.0003</td>
<td>0.97</td>
<td>Deleterious</td>
<td>7.47 (2.57)</td>
<td>-0.0317 (0.018)</td>
<td>0.078</td>
<td>4432</td>
<td>7.18 (2.23)</td>
</tr>
<tr>
<td>chr12:6145649</td>
<td>1106988</td>
<td>Ile1380Val</td>
<td>0.11</td>
<td>0.0035</td>
<td>0.82</td>
<td>Benign</td>
<td>6.33 (2.71)</td>
<td>0.059 (0.0188)</td>
<td>0.017</td>
<td>4360</td>
<td>9.39 (2.42)</td>
</tr>
<tr>
<td>chr12:6128260</td>
<td>1106987</td>
<td>Asn1435Ser</td>
<td>0.11</td>
<td>0.0003</td>
<td>0.85</td>
<td>Benign</td>
<td>6.20 (2.50)</td>
<td>0.0555 (0.0186)</td>
<td>0.028</td>
<td>4392</td>
<td>9.72 (2.41)</td>
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<tr>
<td>chr12:6131277</td>
<td>14244724</td>
<td>Ser1486Leu</td>
<td>0.09</td>
<td>0.0001</td>
<td>0.75</td>
<td>Benign</td>
<td>4.74 (2.57)</td>
<td>0.0317 (0.018)</td>
<td>0.078</td>
<td>4432</td>
<td>7.18 (2.23)</td>
</tr>
<tr>
<td>chr12:6130072</td>
<td>7634212</td>
<td>Arg2185Gln</td>
<td>0.19</td>
<td>0.0015</td>
<td>0.97</td>
<td>Benign</td>
<td>7.13 (2.60)</td>
<td>-0.1013 (0.0141)</td>
<td>0.046</td>
<td>4382</td>
<td>7.16 (2.23)</td>
</tr>
</tbody>
</table>

Linear regression models were initially run with natural-log–transformed VWF and FVIII level as the dependent variable, with adjustment for age, sex, and genetic ancestry (as inferred from principal component analysis), as described under “Methods.” The β coefficient corresponds to the mean difference in natural-log–transformed VWF or FVIII between an individual carrying 1 copy of the “effect allele” compared with individuals carrying 0 copies of the effect allele. The effect allele refers to the second amino acid under “Annotation.” Effect allele frequency indicates the frequency of the effect allele in AAs and EAs. To provide more clinically relevant mean VWF or FVIII effect sizes, standard errors per each additional copy of the minor allele were also reported for corresponding regression models using untransformed VWF or FVIII.

*Allele frequency data in EAs from NHLBI Exome Sequencing Project (ESP) Exome Variant Server (evs.gs.washington.edu/EVS).
† Imputation quality Rsq is an estimate of the correlation between the imputed genotype and the actual genotype and serves as a measure of imputation accuracy.

Discussion

Recent DNA sequencing studies of multicentric populations found that many VWF missense variants, including some previously identified in European ancestry VWD probands, are common in healthy AA individuals. Since AA have a lower VWF ristocetin cofactor activity to VWF antigen (supplemental Table 4), although power to detect an association was limited by the small sample size. The ratio of VWF activity to VWF antigen (supplemental Table 4) provided evidence of association between the variant p.Arg2185Gln and VWF:Ag levels (all P < 0.010). In contrast, p.Arg2185Gln became more strongly associated with higher VWF:Ag and FVIII:C levels; p.His817Gln remained independently associated with lower VWF:Ag and FVIII:C levels; and p.Asn1435Ser remained independently associated with lower VWF:Ag and FVIII:C levels.

We further assessed the exon 28 region by performing haplotype analysis of the remaining variants within exon 28. Among the remaining variants, p.Thr789Ala variant remained independently associated with lower VWF:Ag and FVIII:C levels, and p.Arg2185Gln remained independently associated with lower VWF:Ag and FVIII:C levels. When accounting for the joint effects of the remaining 8 SNVs on overall VWF:Ag variance, the 8 SNVs in aggregate explained 3.3% of the overall VWF:Ag variance. When accounting for the joint effects of the remaining 8 SNVs on overall FVIII:C variance, the 8 SNVs in aggregate explained 1.0% of the overall FVIII:C variance.

Significant at P < 0.05.
As illustrated in Figure 1, the VWF-related phenotype associated missense variants tend to be localized to functional domains of VWF, such as the D', D3, and D4 (VIII-binding; multimerization) or A1 (platelet-binding). Notably, all VWF missense variants associated with VWF:Ag and/or FVIII:C levels in this study occur more frequently among AAs than EAs.19 Thus, our data extend recent findings on the importance of ethnic diversity of VWF sequence variants and demonstrate several of these variants may contribute to phenotypic differences in VWF:Ag and FVIII:C levels among AA.

The G allele of the common VWF rs1063856 polymorphism, which encodes the Ala form of the exon 18 p.Thr789Ala missense variant, was associated with higher VWF:Ag and FVIII:C levels in our AA sample. This finding confirms prior reports in healthy subjects and patients with diabetes of European ancestry10,11,30-33 and extends the p.Thr789Ala association to African-descent populations. rs1063856 G alleles are perfectly correlated (LD r2 = 1.0) and extends the p.Thr789Ala association to African-descent populations. rs1063856 G alleles are perfectly correlated (LD r^2 = 1.0). The rs1063856G/rs1063856G alleles (corresponding to p.Ala789) represent the VWF ancestral haplotype. These 2 exon 18 sites are polymorphic in all human populations studied to date; the frequency of the p.Ala789 variant varies widely from ~70% in African to ~40% in European to ~5% in East Asian populations.34,35 Therefore, the worldwide pattern of allele frequency differences recapitulates the known racial/ethnic variation in VWF and FVIII levels.12-15 suggesting the p.Thr789Ala variant of exon 18, p.His817Gln (MAF = 0.01), was strongly associated with lower FVIII:C levels, disproportionately to its effect on VWF:Ag levels. p.His817Gln has been reported in 2 European patients with type 2N VWD who were compound heterozygotes for p.His817Gln and p.Arg822Trp,38 and in 1 patient homozygous for p.His817Gln. In the report of Bellissimo et al.,39 the mean FVIII:C level in 10 AA carriers (including 1 homozygote) was normal. Our larger sample included up to 809 heterozygotes and 52 homozygotes for the minor p.His817 allele. Therefore, the most likely reason for the discrepant results is our larger sample size and greater statistical power to detect an association for sequence variants with modest phenotypic effects. p.His817Gln is a plausible type 2N variant as it lies within the FVIII binding region and is adjacent to another type 2N variant, p.Arg816Trp.7 An in vitro study of p.His817Gln demonstrated that this amino acid change resulted in a significant decrease in FVIII binding capacity, further supporting the functional role of p.His817Gln in VWF-FVIII interaction.38

Our data confirm that the D4 domain variant p.Arg2185Gln is common in healthy AAs,17 p.Arg2185Gln was associated with lower VWF:Ag and FVIII:C levels in our large, population-based AA

<table>
<thead>
<tr>
<th>Variant</th>
<th>Rs number</th>
<th>Annotation</th>
<th>VWF β (SE)</th>
<th>VWF P value</th>
<th>FVIII β (SE)</th>
<th>FVIII P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr12:6153534</td>
<td>1063856</td>
<td>Thr789Ala</td>
<td>5.92 (1.48)</td>
<td>6.4E-05</td>
<td>7.68 (1.30)</td>
<td>4.4E-09</td>
</tr>
<tr>
<td>chr12:6145649</td>
<td>57950734</td>
<td>Hiss17Gln</td>
<td>-1.48 (2.63)</td>
<td>.57</td>
<td>-16.72 (2.36)</td>
<td>1.6E-12</td>
</tr>
<tr>
<td>chr12:6128280</td>
<td>11069387</td>
<td>Asn1435Ser</td>
<td>.087 (2.70)</td>
<td>.97</td>
<td>6.60 (2.45)</td>
<td>.007</td>
</tr>
<tr>
<td>chr12:6128269</td>
<td>150077670</td>
<td>Met4139Val</td>
<td>-20.75 (13.09)</td>
<td>.11</td>
<td>-3.06 (11.09)</td>
<td>.78</td>
</tr>
<tr>
<td>chr12:6128446</td>
<td>11069398</td>
<td>Asp1472His</td>
<td>7.63 (1.87)</td>
<td>4.8E-05</td>
<td>4.55 (1.70)</td>
<td>.007</td>
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<tr>
<td>chr12:6128117</td>
<td>149424724</td>
<td>Ser1486Leu</td>
<td>-32.1 (9.07)</td>
<td>4.1E-04</td>
<td>-1.92 (8.17)</td>
<td>.81</td>
</tr>
<tr>
<td>chr12:6103072</td>
<td>76342212</td>
<td>Arg2185Gln</td>
<td>-12.7 (2.16)</td>
<td>4.54E-09</td>
<td>-7.02 (1.94)</td>
<td>3.1E-04</td>
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<tr>
<td>chr12:6094771</td>
<td>61750625</td>
<td>Arg2287Trp</td>
<td>-41.5 (8.57)</td>
<td>1.3E-06</td>
<td>-21.2 (7.01)</td>
<td>.007</td>
</tr>
</tbody>
</table>

Table 3. Multivariate regression model of VWF missense variants and VWF antigen and FVIII coagulation activity

Table 4. Association of VWF exon 28 haplotypes with VWF antigen and FVIII levels in AAs (N = 612)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Ile1380Val</th>
<th>Asn1435Ser</th>
<th>Asp1472His</th>
<th>Arg2185Gln</th>
<th>Freq</th>
<th>VWF level</th>
<th>P value</th>
<th>FVIII level</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.500</td>
<td>Referent</td>
<td>—</td>
<td>Referent</td>
<td>—</td>
</tr>
<tr>
<td>H2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.210</td>
<td>13.7 (5.7)</td>
<td>.017</td>
<td>11.3 (4.4)</td>
<td>.010</td>
</tr>
<tr>
<td>H3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.123</td>
<td>0.68 (7.3)</td>
<td>.93</td>
<td>0.13 (5.4)</td>
<td>.98</td>
</tr>
<tr>
<td>H4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.095</td>
<td>0.47 (7.7)</td>
<td>.95</td>
<td>1.11 (5.7)</td>
<td>.85</td>
</tr>
<tr>
<td>H5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.070</td>
<td>-6.9 (9.6)</td>
<td>.47</td>
<td>-4.7 (7.1)</td>
<td>.50</td>
</tr>
</tbody>
</table>

β and standard error (SE) refer to the mean difference in VWF or FVIII levels for individuals carrying a single copy of the indicated haplotype compared with individuals carrying 2 copies of the referent haplotype (H1).

Freq, haplotype frequency; SE, standard error.
sample; overall, the mean VWF:Ag level in AA carriers of p.Arg2185Gln variant was 124 IU/dL compared with a mean level of 136 IU/dL in noncarriers. p.Arg2185Gln was identified in 1 subject in a series of Canadian type 1 VWD patients. Together, these data suggest that p.Arg2185Gln is a common functional variant associated with lower VWF:Ag and FVIII:C levels in AAs.

The missense variant p.Arg2287Trp located within the D4 domain had an allele frequency of <1% in our AA sample. This amino acid substitution was associated with a large magnitude of effect on lowering VWF:Ag levels and is predicted to be functionally deleterious (supplemental Table 2). The p.Arg2287Trp variant was reported in combination with a p.Pro2063Ser polymorphism in a European ancestry type 1 VWD patient with reduced VWF and normal FVIII and normal VWF clearance. Based on in vitro analysis and expression of recombinant p.Arg2287Trp missense variant, it was suggested to be a causative mutation due to a mild reduction in the amount of secreted VWF.

Type 2M VWD is characterized by a decreased VWF activity to antigen ratio and defective binding of VWF to platelets due to mutations in the VWF A1 domain. A lower VWF activity to antigen ratio and increased prevalence of the type 2M phenotype has been reported in AAs. In AAs, the minor alleles of 3 common exon 28 A1 domain polymorphisms (p.Ile1380Val, p.Asn1435Ser, and p.Asp1472His) are present together on 1 common haplotype (frequency = 0.095), while another common allele contains p.Asp1472His with or without p.Arg2185Gln (frequency = 0.333). Flood et al demonstrated that p.Asp1472His alone may have the strongest association with lower VWF activity to antigen ratio. In our multivariable-adjusted regression analysis, the p.Asp1472His variant was independently associated with higher VWF:Ag levels. The haplotype containing p.Asp1472His alone was associated with higher VWF:Ag, while the Val1380/Ser1435/His1472 haplotype was not significantly associated with VWF:Ag. p.Asp1472His is located within the p.Asp1459-p.His1472 ristocetin binding site and was shown to decrease VWF ristocetin cofactor levels by affecting direct binding to ristocetin. Collectively, these results suggest that p.Asp1472His may lower the VWF activity to antigen ratio by lowering VWF ristocetin activity and increasing VWF:Ag levels. p.Ser1486Leu is a novel rare variant in at a critical residue in a highly conserved cluster of 8 predicted O-glycosylation sites flanking the A1 domain that appear to modulate platelet glycoprotein Ib binding under shear stress. A p.Val1485Leu substitution at the adjacent amino acid (in combination with other exon 28 variants) was reported to be common among Turkish patients with VWD. Notably, site-directed mutagenesis of Ser1486 to Ala resulted in normal VWF synthesis and multimerization but decreased VWF hemostatic function. On the other hand, the substitution of Ser with Leu at 1486 is predicted to be “benign” at the protein sequence conservation level, while the substitution of Ser1486 with Ala is predicted to be “possibly damaging” by Polyphen2. Therefore, the putative mechanism of effect of the Ser1486Leu variant on lowering VWF:Ag remains to be determined.

Our data provide strong evidence that at least 6 distinct non-synonymous VWF variants independently contribute to VWF and FVIII phenotypic differences among healthy AA individuals. In aggregate the VWF missense variants identified in our study explained 3.3% of the overall VWF phenotypic variance and 2.7% of the overall FVIII phenotypic variance. These changes are somewhat larger than the proportion of VWF phenotypic variance explained by common VWF variants in whites from the ARIC study (0.9%-1.5%). By comparison, ABO blood group (defined as O type vs non-O type) contributes to ~15% of VWF:Ag phenotypic variation. In addition to ABO and VWF, several other genomic loci contribute to VWF:Ag and FVIII:C levels. Still, the total variance explained by all common variants (12.8% for VWF:Ag and 10% for FVIII:C) is notably less than the heritability estimates of 32% to 75% and 40% to 61% previously reported for VWF and FVIII, respectively. A recent linkage analysis using sibling data identified a new locus on chromosome 2 explaining 19% of additional plasma VWF variance. Therefore, family-based study designs and studies that include larger samples of minority participants and analysis of rare variants may help to identify “missing heritability.” The ethnic diversity of VWF coding sequence variants underscores the importance of studying ethnically diverse populations to expand our knowledge of normal genetic variation and to distinguish pathogenic changes from benign variants. Our results demonstrate that, when applied to well-characterized population-based samples, these approaches can greatly contribute to our understanding of the role of both common and rare variants to the molecular genetics of VWF-related phenotypes.

Several limitations of the current study should be noted. First, heterogeneity between laboratory assays and/or sample processing between cohorts may have introduced between-group variability. Even though the absolute values for VWF:Ag and FVIII:C differ between studies, the rank order of the values should be consistent across studies and therefore not affect the associations of high or low values with specific genetic variants. Nonetheless, interassay variability, along with variation in genotype imputation quality, may have decreased our statistical power to find associations of smaller magnitudes. Second, though none of the participants carrying common or rare VWF variants reported bleeding symptoms, a detailed clinical bleeding history was not ascertained in our cohort studies. Third, the predicted functional consequences derived from in silico sequence-conservation-based algorithms should be interpreted cautiously, since the performance of these tools can vary markedly and optimum predictions are achieved by different tools in different genes. Finally, it is important to point out that the bleeding tendency of VWD is a complex, incompletely penetrant phenotype. VWD is estimated to be present in up to 1% of the population, yet there is considerable overlap between individuals with low-normal VWF levels and patients diagnosed with mild type 1 VWD. Environmental factors and extra-allelic genetic factors that influence VWF levels, such as ABO blood group, ethnicity/genetic background, age, body mass index, pregnancy, and medications, additionally contribute to the phenotypic complexity. Application of next-generation sequence analysis in a genome-wide manner among individuals with low VWF and bleeding symptoms may help to identify cosegregating genetic variants that contribute to bleeding risk.

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References


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Common and rare von Willebrand factor (VWF) coding variants, VWF levels, and factor VIII levels in African Americans: the NHLBI Exome Sequencing Project

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