Possible Effect of Secretor Locus on Plasma Concentration of Factor VIII and von Willebrand Factor

By Karen Helene Ørstavik, Leif Kornstad, Howard Reinsner, and Kåre Berg

A significant fraction (30%) of the genetically determined variance in plasma concentration of the von Willebrand factor antigen (vWF:Ag) has been shown to be related to ABH determinants. Individuals with blood group O, who have the highest amounts of blood group H substance, have the lowest concentration of vWF:Ag. The Lewis substances, Le^a and Le^b, are biochemically closely related to the ABH substances as both can be produced from the same precursor substance. We studied the effect of the presence of the Lewis antigens on the plasma concentration of vWF:Ag and factor VIII antigen (VIII:Ag) in 323 individuals of different ABO groups from a series of twins and in 58 blood donors of blood group O. Among persons belonging to blood group O, those with the Le^a antigen had a higher concentration of both vWF:Ag and VIII:Ag than individuals lacking Le^a. Le(a^+b^-) people are nonsecretors and Le(a^-b^+) people are secretors of ABH substance. Thus, the lowest concentration of vWF:Ag and VIII:Ag was found in group O secretors. The effect is most likely due to an effect of the secretor locus. This finding may be of importance for the detection of carriers of hemophilia A and for the diagnosis of type I von Willebrand disease.

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THE PLASMA CONCENTRATION of factor VIII (F VIII) and von Willebrand factor (vWF) show a very wide range in individuals without a bleeding disorder. Family studies have demonstrated a significant genetic effect on both components of the VIII/vWF complex. An effect of the ABH blood group determinants was first described by Preston and Barr, and has been confirmed in several later studies. In an extensive twin study we found that 60% of the total variance in the plasma concentration of vWF antigen (vWF:Ag) was due to genetic factors. Thirty percent of the genetic variance was due to an effect of the ABO blood group. The ABO locus is therefore important for the determination of the plasma concentration of vWF.

The Lewis substances Le^a and Le^b show great structural similarity to the ABH blood group substances, and are found in secretions in high concentration as glycoproteins and in plasma in much lower concentration as glycolipids. The Lewis antigens present on the RBCs are, in contrast to the ABH antigens, not synthesized in the erythrocyte precursor cells, but adsorbed from plasma onto the red cell surface. Le^a substance is produced by individuals with the genotype Le/Le or Le/le. Most of the Le^a substance is converted into Le^b in people with the Se gene present (genotypes Se/Se and Se/se). Thus, individuals with the red cell Lewis phenotype Le(a^-b^-) are secretors and individuals with the phenotype Le(a^+b^-) are nonsecretors of ABH substance. Persons who are Le/le have the Lewis phenotype Le(a^-b^-); among these approximately 80% are secretors and 20% are nonsecretors (in the Norwegian population).

The H substance is the immediate precursor of the A and B antigens. As the O allele of the ABO locus is silent, people belonging to the blood group O have the highest amount of H substance. The amount is intermediate in A1 individuals and low in people with the blood groups A1, B, A1B, and A1B. The Factor VIII antigen (VIII:Ag) and vWF:Ag concentrations seem to be inversely related to the amount of H substance, since the concentrations are lowest in group O, intermediate in group A1, and highest in group A1 individuals. The highest concentration of H substance in plasma has been found in group O individuals who are secretors. It might therefore be expected that the concentration of VIII:Ag and vWF:Ag would be lower in secretors than in nonsecretors. We looked for an association between the presence of the Le^a antigen on the red cells and the concentration of vWF:Ag and VIII:Ag in the previously published twin series and in a series of blood donors. The results are published in this report.

MATERIALS AND METHODS

Material. Plasma samples were examined from 323 twins and 58 blood donors. The twins had previously been examined in a study of the effect of genetic factors on the plasma concentrations of vWF:Ag and VIII:Ag, and included 74 identical pairs, 84 like-sexed fraternal pairs, and seven twins where the cotwin was lacking. The twins were bled in their homes or in the laboratory after an overnight fast. Blood was collected into Vacutainer tubes containing 1/10 vol 3.13% sodium citrate. The blood donors all belonged to ABO blood group O, and the samples were selected to give about the same number of individuals of each of the three Lewis phenotypes. The blood donors were bled at the end of ordinary blood donations and were not fasting. Blood was collected into Vacutainer tubes containing 1/100 vol 15% EDTA. All donors were retested in the Lewis system on this occasion and the phenotypes were in all cases confirmed. The twins were either 33 to 39 or 57 to 62 years old, with approximately the same number of individuals in each age group. The blood donors were between 22 and 61 years old with a mean of 40 years in all three Lewis phenotypes.

vWF:Ag and VIII:Ag. vWF:Ag was determined by an electroimmunoassay using a rabbit antibody, and VIII:Ag was determined by a radioimmunoassay using a human antibody, as previously described. For some individuals plasma was not available for the VIII:Ag assay. The concentration was given relative to a standard plasma pool defined to contain 100 U/dL.

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Blood grouping. ABO and Lewis grouping were performed using standard laboratory procedures. The twins were Lewis typed at the Institute of Medical Genetics using antisera from Behringwerke or Dade Laboratories. The donors were typed at the National Blood Group Reference Laboratory using antisera prepared at the laboratory.

Statistical methods. vWf:Ag and VIII:Ag concentrations were not normally distributed, but normality was obtained after logarithmic transformation. Natural logarithms were therefore used for all statistical analyses. t Tests and z scoring were performed as described in standard textbooks.

**RESULTS**

Twin series. The relationships between the Lewis blood groups and the vWf:Ag and VIII:Ag concentrations are shown in Tables 1 and 2. Many anti-Le<sup>e</sup> sera give weak and unreliable results with red cells of ABO groups other than O and A<sub>1</sub>, while there are no problems of this kind when testing for Le<sup>a</sup>. Therefore, the material was divided into one group having and one group lacking the Le<sup>a</sup> antigen on their erythrocytes. A significantly higher concentration of vWf:Ag and VIII:Ag was found in Le(a<sup>-</sup>) compared with Le(a<sup>-</sup>) persons belonging to blood group O. There was no difference in the vWf:Ag and VIII:Ag concentrations of group A, Le(a<sup>-</sup>) and Le(a<sup>-</sup>) persons. The number of persons with ABO blood group other than O and A<sub>1</sub> was too small for statistical analysis. A difference in vWf:Ag and VIII:Ag concentration between Le(a<sup>-</sup>) and Le(a<sup>-</sup>) persons was not found when all ABO blood groups were examined.

A positive correlation between age and concentration of vWF and F VIII has been shown in many studies, and was confirmed in this material. The relationship between Lewis blood group and vWf:Ag and VIII:Ag concentration was therefore also examined on values for vWf:Ag and VIII:Ag that had been corrected for age effect by z transformation as described previously. The same results were obtained as for the uncorrected values (results not shown).

Blood donor series. In order to study the effect of the relatively infrequent le<sup>e</sup>/le<sup>e</sup> genotype and to evaluate the relative importance of the Lewis and the ABH secretor loci for the vWf:Ag concentration, blood samples were collected from nearly the same number of blood donors of all three Lewis phenotypes. The donors were all of blood group O. This selection was made to rule out the possibility of any erroneous results of the Le<sup>a</sup> typing. No difference in vWf:Ag concentration was found between Le(a<sup>-</sup>b<sup>-</sup>) and Le(a<sup>-</sup>b<sup>-</sup>) individuals (Table 3). The higher concentration in persons with the Le<sup>a</sup> antigen found in the twin series was also observed in the blood donors (P < .034).

**DISCUSSION**

We have previously shown that the ABO locus is a major locus for the determination of plasma vWf:Ag concentration. In the present study two independent series also showed an effect of the Lewis groups. The effect was related to the presence or absence of the Le<sup>a</sup> antigen, with a higher concentration in Le(a<sup>-</sup>b<sup>-</sup>) people belonging to blood group O. A lower vWf:Ag concentration was found in the donors compared with the O group twins. However, blood was obtained differently in the two series and therefore they cannot be compared directly. Samples from the blood donors were obtained after ordinary blood donation. This may explain the low mean vWf:Ag concentration in the blood donor series.

The difference between individuals with and without the Le<sup>a</sup> antigen could be due to an effect of the Le<sup>a</sup> antigen itself or an effect of the secretor locus. One reason why a direct effect of Le<sup>a</sup> substance seems unlikely is that people with the Le(a<sup>-</sup>b<sup>-</sup>) red cell phenotype nevertheless have the Le<sup>a</sup>

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**Table 1. Plasma Concentration of vWf:Ag(U/dL) in Lewis(a<sup>-</sup>) and Lewis(a<sup>-</sup>) Individuals Within Different ABO Blood Groups**

<table>
<thead>
<tr>
<th>ABO Blood Group</th>
<th>vWf:Ag Median</th>
<th>In vWf:Ag Mean ± SD (No.)</th>
<th>VIII:Ag Median</th>
<th>In VIII:Ag Mean ± SD (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>75</td>
<td>4.36 ± 0.47 (26)</td>
<td>57</td>
<td>4.15 ± 0.43 (97)</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>96</td>
<td>4.55 ± 0.24 (8)</td>
<td>83</td>
<td>4.43 ± 0.46 (28)</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>96</td>
<td>4.57 ± 0.42 (29)</td>
<td>98</td>
<td>4.62 ± 0.43 (99)</td>
</tr>
<tr>
<td>B</td>
<td>111</td>
<td>4.80 ± 0.38 (11)</td>
<td>124</td>
<td>4.62 ± 0.55 (18)</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;B</td>
<td>45</td>
<td>3.80 ± 0.52 (1)</td>
<td>114</td>
<td>4.74 ± 0.15 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>4.52 ± 0.44 (75)</td>
<td>81</td>
<td>4.42 ± 0.49 (248)</td>
</tr>
</tbody>
</table>

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**Table 2. Plasma Concentration of VIII:Ag(U/dL) in Lewis(a<sup>-</sup>) and Lewis(a<sup>-</sup>) Individuals Within Different ABO Blood Groups**

<table>
<thead>
<tr>
<th>ABO Blood Group</th>
<th>VIII:Ag Median</th>
<th>In VIII:Ag Mean ± SD (No.)</th>
<th>VIII:Ag Median</th>
<th>In VIII:Ag Mean ± SD (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>125</td>
<td>4.89 ± 0.76 (21)</td>
<td>98</td>
<td>4.60 ± 0.47 (90)</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>152</td>
<td>5.05 ± 0.69 (6)</td>
<td>111</td>
<td>4.84 ± 0.63 (28)</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>130</td>
<td>4.91 ± 0.60 (26)</td>
<td>145</td>
<td>5.00 ± 0.57 (82)</td>
</tr>
<tr>
<td>B</td>
<td>155</td>
<td>4.80 ± 0.38 (11)</td>
<td>145</td>
<td>4.96 ± 0.42 (16)</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;B</td>
<td>—</td>
<td>—</td>
<td>145</td>
<td>5.01 ± 0.62 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
<td>4.93 ± 0.65 (64)</td>
<td>118</td>
<td>4.82 ± 0.56 (220)</td>
</tr>
</tbody>
</table>

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*Test within O blood group: P < .028.*

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antigen in secretions, although in smaller amounts than Le(a+b-) individuals. The phenotypes Le(a+b-) and Le(a-b+) both require the presence of the Le gene, while the absence of this gene gives the phenotype Le(a-b-). As Le(a-b+) and Le(a-b-) people have similar vWF:Ag concentrations, lower than the average concentration in Le(a-b+) persons, the vWF:Ag concentration is not likely to be determined by the Le gene. A main characteristic of the Le(a-b+) people is that they are all nonsecretors of ABH substance, while all Le(a-b-) and about 80% of the Le(a-b+) persons carry the Se gene.

The mechanism for the effect of the ABO locus on the plasma concentration of the VIII/vWF complex is not known, but could relate to an effect on secretion from the site of synthesis or to an effect on the catabolism of the complex. Blood group A, B, and H oligosaccharide structures have been found in purified VIII/vWF complex preparations. Vascular endothelial cells are the site of synthesis of vWF, and we have previously shown that the effect of the ABO locus on the plasma concentration is mainly on this part of the VIII/vWF complex. ABH substances are also produced by the vascular endothelium. However, the ABH substance present here, as well as in other mesodermal tissues, seems to be independent of the Se and Le gene product but controlled by the H locus. The Se locus has been thought of as a regulatory locus controlling the expression of the H gene in secretory tissues. A more recent view is that Se is a structural gene, expressed in epithelial tissues, that encodes an α-2-fucosyltransferase with different acceptor specificity from the H-gene-encoded α-2-fucosyltransferase expressed in hematopoietic and other mesodermal tissues. The higher plasma concentration of ABH substances in secretors compared with nonsecretors is therefore hardly caused by a different production in the endothelial cells. Thus, it is difficult at the present time to explain the effect of the ABO locus and the possible effect of the Se locus on the plasma concentration of the VIII/vWF complex.

A similar association to ABO and Lewis groups has been found for the serum concentration of cholesterol and for peripheral arterial disease. The lowest concentration of serum cholesterol was also found here in blood group O secretors, and these individuals had the lowest frequency of peripheral arterial disease. However, F VIII and not vWF has been found to be a risk factor for coronary heart disease.

The effect of the ABO blood groups on the concentration of F VIII and vWF may have practical implications for the detection of carriers of hemophilia A. Green et al. recently showed that inclusion of effect of ABO blood group improved the efficacy of a linear discriminant for carrier detection. In the present study we found a possible effect of the secretor locus on the vWF:Ag and VIII:Ag concentration. It remains to be seen if inclusion of Lewis blood group information will similarly improve such a discriminant. The frequency of secretors varies widely in different populations. It ranges from nearly 100% in some Amerindian and Eskimo populations to hardly 60% in some black populations. It is therefore important to compare ethnically homogenous populations in carrier detection.

In a recent paper Gill et al. found an increased number of group O individuals in patients with type I von Willebrand disease. They discussed the possibility that there is a subset of patients with decreased concentration of vWF on the basis of blood group rather than on specific inherited abnormal vWF production. It seems probable that in these patients there also will be an increased number of group O secretors since these are the individuals with the lowest plasma concentration of vWF:Ag.

REFERENCES


Table 3. Plasma Concentration of vWF:Ag(U/dL) in ABO Group O Blood Donors of Different Lewis Blood Groups

<table>
<thead>
<tr>
<th>Lewis Blood Group</th>
<th>vWF:Ag Median (U/dL)</th>
<th>Mean ± SD (U/dL)</th>
<th>(No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a+b-)</td>
<td>55</td>
<td>4.02 ± 0.32</td>
<td>(20)</td>
</tr>
<tr>
<td>Le(a-b+)</td>
<td>44</td>
<td>4.38 ± 0.44</td>
<td>(23)</td>
</tr>
<tr>
<td>Le(a-b-)</td>
<td>44</td>
<td>3.81 ± 0.45</td>
<td>(15)</td>
</tr>
</tbody>
</table>

t Test between Le(a+b-) and Le(a-b+) + Le(a-b+): P = .034.
Possible effect of secretor locus on plasma concentration of factor VIII and von Willebrand factor

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