Brief report

Bombay phenotype is associated with reduced plasma-VWF levels and an increased susceptibility to ADAMTS13 proteolysis

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ABO blood group is an important determinant of plasma von Willebrand factor antigen (VWF:Ag) levels, with lower levels in group O. Previous reports have suggested that ABO(H) sugars affect the susceptibility of VWF to ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 repeats-13) cleavage. To further test this hypothesis, we collected plasma from individuals with the rare Bombay blood group. VWF:Ag levels were significantly lower in Bombay patients (median, 0.69 IU/mL) than in groups AB, A, or B (P < .05) and lower than in group O individuals (median, 0.82 IU/mL). Susceptibility of purified VWF fractions to recombinant ADAMTS13 cleavage, assessed using VWF collagen-binding assay (VWF:CB), was increased in Bomays compared with either group O or AB. Increasing urea concentration (0.5 to 2 M) increased the cleavage rate for each blood group but eliminated the differences between groups. We conclude that reduction in the number of terminal sugars on N-linked glycan increases susceptibility of globular VWF to ADAMTS13 proteolysis and is associated with reduced plasma VWF:Ag and VWF:CB levels. (Blood. 2005;106:1988-1991)

Introduction

The antigens of the ABO system (A, B, and H) consist of complex carbohydrate molecules. H (Fucα1→2 Galβ1→4 GlcNAc β1→) antigen is an essential carbohydrate acceptor for either α-1,3-N-acetylgalactosaminyltransferase (A transferase) or α-1,3-galactosyltransferase (B transferase), which are both encoded by the ABO locus (9q34).1 In group A, B, or AB individuals, the A and B transferases convert precursor H antigen into either A (GalNAc α1→3 [Fuc α1→2] Galβ 1→4 GlcNAc β1→) or B (Gal α1→3 [Fuc α1→2] Galβ 1→4 GlcNAc β1→) determinants, respectively. In group O individuals, the O allele does not encode any functional transferase enzyme so that they continue to express terminal H structures only.2 In human tissues, H antigen can be synthesized by 2 distinct α-1,2-fucosyltransferases. One is the H gene (FUT1)–encoded H enzyme that regulates expression of ABH antigens in red blood cells.3 The other is the Secretor gene (FUT2)–encoded Se enzyme that regulates expression of ABH antigens in the gastrointestinal tract and secretions.4 Individuals with the very rare Bombay phenotype are non-Secretors and also fail to express H antigens in their secretions.6 Para-Bombay individuals also fail to express H transferase (FUT 1).5 Such people cannot synthesize A or B antigenic structures regardless of their ABO blood group genotype, and ABH antigens are absent from both their erythrocytes and secretions.5 Para-Bombay individuals also fail to express H transferase, but do express the FUT2 (Secretor)–encoded α-1,2-fucosyltransferase, so that ABH antigens are present in their secretions but not on erythrocytes.5,7

It is well established that ABO blood group exerts a major quantitative effect on plasma von Willebrand factor (VWF) levels, with significantly lower levels in group O individuals.8,9 Moreover, ABH antigenic determinants have been identified on the N-linked glycans of circulating VWF according to the blood group of the individual.10 However, the mechanism through which these glycans influence plasma-VWF antigen (VWF:Ag) levels remains unclear. Animal studies have shown that VWF glycans may influence rate of hepatic clearance,11 and previous data suggested it may be mediated by the H antigen.12 On the other hand, Bowen recently reported that VWF of different ABO blood groups exhibited different susceptibility to specific cleavage by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type-1 repeats-13) (O ≥ B > A ≥ AB).13 To further investigate how glycan expression on VWF influences plasma VWF:Ag levels, we have collected plasma samples from a series of Bombay and para-Bombay individuals. As these individuals lack the H antigen, they provide a critical test of current hypotheses. We report the novel observation that Bombay phenotype is associated with plasma-VWF levels similar or lower than group O. In addition, we demonstrate that Bombay VWF demonstrates significant increased susceptibility to cleavage by ADAMTS13, via a conformation-dependent mechanism.

Study design

VWF glycans, VWF antigen, and VWF multimer distribution

Plasma samples from 47 anonymized individuals with Bombay blood groups were collected from blood transfusion centers. No clinical details on these individuals were available. The Bombay (n = 30) and para-Bombay (n = 17) phenotype of each case was established by serologic testing. Plasma samples previously collected from a series of healthy volunteer
Purification of VWF and ADAMTS13 expression

VWF was purified from human group AB, group O, and Bombay plasmas, as previously described. In brief, group-specific plasma was cryoprecipitated. The pellet was then resuspended in TC buffer (20 mM Tris [tris(hydroxymethyl)aminomethane]–HCl, 10 mM sodium citrate, pH 7.4) and passed through a Sephacore CL-2B HiPrep 26/60 gel filtration column (AmershamPharmacia, Buckinghamshire, United Kingdom). Eluate fractions were assessed for VWF content, multimer distribution, and purity as previously described.

Recombinant human ADAMTS13 was purified by ADAMTS13–VWF cleavage assays were performed using either recombinant human ADAMTS13 or plasma-derived ADAMTS13. In brief, 5 to 20 nM ADAMTS13 was preincubated with 10 μM BaCl2 for 10 minutes at 37°C. The activated ADAMTS13 was then incubated at 37°C with 8 nM of comparable blood group–specific (O or AB or Bombay) high-molecular-weight (HMW)–VWF in reaction mix containing urea (0.5–4 M), 10 mM ADAMTS13 was preincubated with 10 mM BaCl2 for 10 minutes at 37°C. In keeping with previous reports, we observed a circulating VWF distinct to that observed in normal plasma VWF are associated with an alteration in the glycan structure of VWF:Ag levels in Bombay patients (median VWF:Ag 0.82 IU/dL), compared with group O (median VWF:Ag 0.69 IU/dL) were significantly lower than in groups A, B, and AB (P < .05). Moreover, Bombay VWF:Ag levels were also lower than in group O individuals (median VWF:Ag = 0.82 IU/dL), although this difference failed to achieve statistical significance (P = .133; Mann-Whitney analysis) (Figure 1B). Bombay and para-Bombay phenotypes result from null mutations at the FUT1 and FUT2 loci, which are both located on chromosome 19, remote from the ABO locus on chromosome 9. Consequently, this effect of Bombay phenotype on plasma VWF levels is conclusive evidence that the effect of ABO group on plasma VWF:Ag levels is due to a direct functional effect of the ABH determinants on VWF, rather than linkage disequilibrium between the ABO locus and another unidentified VWF regulatory locus.

Through cleavage at the Tyr1605-Met1606 bond within the VWF A2 domain, ADAMTS13 regulates plasma-VWF multimer composition. In keeping with a previous report, we found group O VWF was cleaved significantly more quickly than group AB. If the ABO effect on plasma-VWF level is mediated by susceptibility to ADAMTS13 cleavage, then cleavage of Bombay VWF should be at least as rapid as that of group O. In fact, we demonstrated that Bombay HMW-VWF is cleaved significantly faster than either group O or group AB (P < .001) (Figure 2A–C). This marked difference was apparent over the full range of ADAMTS13 concentrations studied (5–20 nM) (data not shown). In a parallel set of experiments, cryodepleted plasma was used as the source of
Previous studies have demonstrated that N-linked glycan structures directly influence the folding of glycoproteins by reducing conformational freedom of the local peptide backbone. We hypothesized that glycan changes may alter the conformation of VWF and thus alter accessibility to the ADAMTS13 cleavage site. To investigate this hypothesis, we repeated ADAMTS13 cleavage assays over a range of urea concentrations (0.5-4 M) to mimic changes in shear forces responsible for unraveling VWF multimer. As urea concentration increased, we found that the rate of VWF proteolysis increased for each blood group, but Bombay VWF continued to be cleaved most quickly (Figure 2D). However, as the concentration of urea progressively increased, the differential effects observed between AB, O, and Bombay became less apparent. This observation suggests that oligosaccharide chain composition may influence the conformation of VWF, such that removal of terminal sugars allows the A2 domain to adopt a conformation more permissive for cleavage by ADAMTS13. Alternatively, the glycan structure of VWF may influence the ability of HMW-VWF multimers to unwind in vivo in response to shear stress.

Reduction in the number of sugars on the oligosaccharide chains of VWF is clearly associated with an increased susceptibility to cleavage by ADAMTS13. Whether this susceptibility to ADAMTS13 proteolysis is responsible for a quantitative effect on plasma VWF:Ag levels remains to be determined, as the magnitude of the Bombay effect on cleavage is not clearly matched by a comparable effect on plasma-VWF level. Furthermore, it is interesting that despite the increased rate of cleavage, plasma multimer analysis in Bombay and para-Bombay individuals appeared normal. In particular, we observed no loss of HMW multimers as seen in typical patients with type 2A von Willebrand disease (VWD), and the ratio of VWF:CB to VWF:Ag (CBA/Ag) was similar to that observed in other blood groups (Figure 1C-D). This apparent paradox of increased susceptibility to ADAMTS13, yet normal plasma multimer distribution, has been previously described in association with the Tyr1584Cys polymorphism of VWF. The paradox may reflect the fact that the increased ADAMTS13 susceptibility associated with both Tyr1584Cys and the Bombay phenotype is markedly less than that arising from classic type 2A mutations. Further studies are required to clarify how VWF proteolysis by ADAMTS13, plasma multimer distribution, and VWF clearance are integrated in vivo.

Reference:


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