REVIEW ARTICLE

Molecular Genetics of von Willebrand Disease

By David Ginsburg and E.J. Walter Bowie

VON WILLERBRAND disease (vWD) was first described by Erik von Willebrand in 1926 in several members of a family from the Åland archipelago in Finland. The proband, a 7-year-old girl, and 9 of her 11 siblings all had significant bleeding symptoms, four dying of hemorrhage between the ages of 2 and 4 and the proband herself dying of hemorrhage at the age of 13 at the time of her fourth menstrual period. von Willebrand coined the term “hereditary pseudohaemophilia” for the disease that subsequently bore his name. In retrospect, the first case of vWD may actually have been described by Minot and Lee in 1920, and a similar clinical disorder was reported independently by four American groups in 1928.3,4 In 1953, an association between decreased factor VIII (FVIII) procoagulant activity and vWD was first described, leading to some confusion concerning the protein defects responsible for hemophilia A and vWD.3,4 An explosion in the understanding of von Willebrand factor (vWF) and FVIII began with the immunologic characterization of the proteins in the early 1970s, culminating in the cDNA cloning of FVIII in 1984 and vWF in 1985.7,10 Molecular defects responsible for hemophilia and vWD were first detected by Southern blot analysis in 1983 and 1987, respectively. The subsequent identification of point mutations in DNAs from large numbers of hemophiliacs and vWD patients was made possible by the discovery of the polymerase chain reaction in 1985 and the application of Taq polymerase in 1987.14,15 This review will focus on the molecular genetics of vWD. The biosynthesis, structure, and function of the vWF protein will only be briefly introduced. For a more detailed discussion of these latter topics, the interested reader is referred to several excellent recent reviews.16-20

vWF BIOSYNTHESIS

The vWF monomeric subunit of approximately 250 KD in molecular weight is assembled into multimers containing up to 100 subunits with molecular weights in excess of 20×10^6 daltons. vWF is synthesized exclusively in endothelial cells and megakaryocytes and has become a standard marker of endothelial cell origin for histochemical studies. vWF is first synthesized as a large precursor form that initially dimerizes and subsequently multimerizes with coincident processing to the mature vWF subunit. Within the endothelial cell, vWF is secreted via both constitutive and regulated pathways. Dimerization of vWF and early carbohydrate processing begin in the endoplasmic reticulum with final carbohydrate processing and multimerization restricted to golgi and post-golgi compartments. A specific storage compartment for vWF has been identified within the endothelial cell termed the Weibel-Palade body. This structure contains densely packed vWF with a characteristic appearance under the electron microscope. The only other protein known to be contained within the Weibel-Palade body is the recently described selectin, GMP140 (P-selectin).21,22 Platelet vWF is stored within the α-granule.16

STRUCTURE AND FUNCTION OF vWF

In plasma, vWF serves two major functions. Via specific binding to the platelet surface as well as to one or more discrete ligands within the subendothelium, vWF provides a major adhesive link between the platelet and the vessel wall at sites of vascular injury. In addition, vWF serves as the carrier for FVIII in plasma, conferring increased stability and localizing FVIII to sites of platelet plug and subsequent fibrin clot formation.

The various binding functions of vWF appear to be localized to discrete domains within the molecule as shown by studies of proteolytic vWF fragments (summarized in Fig 1 and reviewed in Ruggeri and Zimmerman.17 Girma et al.18 Ruggeri,19 and Sadler20). The FVIII binding domain of vWF has been localized to a 272 amino acid tryptic fragment at the N-terminus of vWF.23,24 In addition, two monoclonal antibodies mapping to an epitope spanning amino acids 78 through 96 were shown to block FVIII binding to vWF, suggesting a further localization for this function. Recent evidence suggests that the mature vWF N-terminus is required for FVIII binding,27 although the role of the vWF propeptide is controversial.27,28 Regions involved in binding to platelet glycoprotein Ib (GPIb), heparin, and collagen have been localized to a 48/52-Kd tryptic fragment spanning Val449 through Lys728.29,30 An additional potential collagen binding domain has been mapped to residues 911-1114.32,33 Finally, an RGDS sequence thought to function as a ligand for the GPIIb/IIIa platelet surface integrin is located at amino acids 1744-1747.

THE vWF GENE AND cDNA

vWF cDNAs were independently isolated by four groups in 1985.7,10 The complete primary amino acid sequence of the mature vWF subunit was also independently determined by Titani et al.34 using direct amino acid sequence analysis. In addition to the expected agreement with the...
The human vWF gene has been localized to chromosome 12p12 → pter. Localization studies using a cDNA probe from the midportion of vWF identified not only the authentic gene on chromosome 12 but a second sequence on chromosome 22. The latter has recently been localized to 22q11.22-q11.23. The complete exon/intron structure of the vWF gene has been established by Mancuso et al. The 52 exons span 178 kb, approximately 0.1% of human chromosome 12. Exons range from 40 bp to 1.4 kb for the largest exon (exon 28). The latter encodes the entire A1 and A2 repeats, a critical region of the molecule spanning several important functional domains (Fig 1) and also containing most of the mutations responsible for type IIA and type IIB vWD (see below). The vWF intron/exon structure shows little correlation with the homologous repeat structure described above. Although there is some limited correspondence of introns in the D domains, the A1 and A2 domains are contained in a single exon, whereas the A3 domain extends across 5 exons. Nearly the entire vWF pseudogene on chromosome 22 has now been sequenced and shows 97% homology to the authentic chromosome 12 gene, indicating a very recent evolutionary origin. The pseudogene represents a nonprocessed duplication spanning exons 23-34. The presence of multiple stop codons in the coding sequence provides conclusive evidence that this is not a functional gene in humans. Southern blot studies confirm the absence of this pseudogene in a number of other mammalian species. The location and high degree of sequence identity between the pseudogene and authentic gene present particularly difficult problems for the identification of mutations in vWD (see below).

Information on potential regulatory elements within the vWF gene includes the analysis of 2.2 kb of upstream sequence. Although there is a typical "TATA" box at −30, no "CCAAT" or "GC" box elements are present. Transfection studies using vWF promoter sequences have been difficult and little information is currently available to explain the extremely high level of expression of vWF in endothelial cells and megakaryocytes and its remarkable tissue specificity.

vWD

vWD is an extremely heterogeneous disorder, with over 20 distinct clinical subtypes described (Table 1). The numerous vWD variants generally all fit into one of two classes, characterized by either quantitative (types I and III) or qualitative (type II and other variants) defects in vWF. For the former class, vWF of relatively normal appearing structure and function may be present, but in significantly decreased amounts. In type I, vWF levels are generally between 20% and 50% of normal, and in type III, levels are extremely low or undetectable. The type I variant of vWD is by far the most common, accounting for at least 70% of clinical cases. Type III vWD is much less common with frequency estimated at approximately 1 per million. Overall prevalence figures for all cases of vWD have ranged as high as 1% to 3% of the population.
The treatment of choice for mild type I vWD is DDAVP. Intravenous or intranasal administration of this vasopressin analogue generally results in marked elevation of vWF and FVIII levels, providing adequate hemostasis in the majority of patients.\(^{57,66}\) This treatment is much less effective in the type III and type II variants and may be contraindicated in type IIB vWD.\(^{67,68}\) In this latter group of patients, as well as type I vWD patients who have otherwise failed DDAVP, the treatment of choice is replacement of vWF either in the form of pooled plasma fractions (cryoprecipitate) or, more recently, partially purified FVIII concentrates that also contain intact vWF multimers.\(^{57}\) These latter products are thought to be free of viral transfusion risks on the basis of heat or detergent treatment. Whether cryoprecipitate or one of the purified products should be the first line treatment for severe (type III) vWD and type II variants remains controversial.

**ANIMAL MODELS FOR vWD**

In addition to its high prevalence in human populations, vWD has also been frequently encountered in other animal species, including dogs, rabbits, cats, mice, pigs, and horses.\(^{52,69-74}\) Heterozygotes for porcine vWD are generally thought to be free of viral transfusion risks on the basis of heat or detergent treatment. Whether cryoprecipitate or one of the purified products should be the first line treatment for severe (type III) vWD and type II variants remains controversial.

**Table 1. Summary of vWD Phenotypes**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Frequency</th>
<th>Clinical Features</th>
<th>Diagnosis</th>
<th>Molecular Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type I</strong></td>
<td>1-30,000; most common vWD variant ( &gt; 70% of vWD)</td>
<td>Mild to moderate bleeding; autosomal dominant; incomplete penetrance (approximately 60%).</td>
<td>vWF:Ag, vWF:RCo, and FVIII all proportionately decreased (20-50%). Normal multimer distribution.</td>
<td>Unknown.</td>
</tr>
<tr>
<td><strong>Type II</strong></td>
<td>1-5 x 10^6</td>
<td>Severe bleeding disorder; ? autosomal recessive inheritance.</td>
<td>Markedly decreased or undetectable vWF:Ag, vWF:RCo, and FVIIIIC.</td>
<td>vWF gene deletions; nonsense mutation; other cis-defects in mRNA expression.</td>
</tr>
<tr>
<td><strong>Type II A</strong></td>
<td>Approximately 10-15% of clinical vWD cases</td>
<td>Mild to moderate bleeding disorder; autosomal dominant, more complete penetrance than type I; generally poor response to DDAVP.</td>
<td>Variable decreased vWF:Ag, vWF:RCo, and FVIIIIC; absent high and intermediate size vWF multimers with prominent satellite bands;</td>
<td>Misense mutations clustered within vWF A2 domain. Two subgroups: group 1-Defect in intracellular transport; group 2-? proteolysis in plasma after sequestration.</td>
</tr>
<tr>
<td><strong>Type II B</strong></td>
<td>Uncommon variant (&lt; 5% of clinical vWD)</td>
<td>Mild to moderate bleeding disorder; autosomal dominant, more complete penetrance than type I; DDAVP contraindicated.</td>
<td>Variable decreased vWF antigen vWF:RCo and FVIIIIC; loss of large multimers; enhanced RIPA; thrombocytopenia.</td>
<td>Misense mutation clustered in vWF A1 domain result in increased or spontaneous binding to platelet GPlb.</td>
</tr>
<tr>
<td>Types IIC-H, others</td>
<td>Rare (case reports)</td>
<td>Variable bleeding disorder; generally autosomal dominant though some autosomal recessive (type IIC).</td>
<td>Variable decreased vWF:Ag, vWF:RCo, and FVIIIIC. Diagnosis generally based on unique abnormalities in multimer pattern.</td>
<td>Unknown, some may represent compound heterozygotes for other variants.</td>
</tr>
<tr>
<td><strong>FVIII binding defects</strong></td>
<td>Rare (case reports)</td>
<td>Variable bleeding disorder, homozygotes (or compound heterozygotes) may present as autosomal hemophilia A.</td>
<td>Variable vWF:Ag and vWF: RCo. Disproportionately low FVIIIIC. Generally normal multimers. Decreased or absent vWF binding to FVIII.</td>
<td>Misense mutations within the N-terminus of mature vWF which interfere with FVIII binding.</td>
</tr>
<tr>
<td><strong>Platelet-type vWD</strong></td>
<td>Rare (case reports)</td>
<td>Similar to type II B vWD.</td>
<td>Can be distinguished from type II B by mixing studies with normal platelets and plasma.</td>
<td>Misense mutation within GPlb α-chain probably resulting in increased or spontaneous binding to vWF.</td>
</tr>
</tbody>
</table>

Although others suggest a considerably lower range of approximately 1:10,000,\(^{56,59}\) The sensitivity and specificity of the current standard diagnostic tests (vWF:Ag, vWF ristocetin cofactor activity [vWF:RCo], FVIII procoagulant activity [FVIIIIC], and bleeding time) may be as low as 60%.\(^{63}\) Additional variability is contributed by a number of other factors, including blood group and estrogen level.\(^{56,64,65}\) Mean vWF:Ag levels can vary from 75% for blood type O individuals to 123% for type AB, when compared with a standard normal donor plasma pool. As a result, the diagnosis of vWD may be more readily established in patients who are blood type O. Clinical symptoms are more common in women, most likely due to the hemostatic stresses of pregnancy and menstruation. The bewildering complexity of vWD classification and the low accuracy of the current diagnostic tests present major problems for the practicing clinical hematologist. These issues lend additional importance to the identification of vWF mutations, to eventually facilitate the precise diagnosis and classification of vWD at the molecular level.
asymptomatic, while homozygotes have less than 1% of normal vWF levels and a severe bleeding diathesis closely resembling type III human vWD. vWD has been recognized in over 30 breeds of dogs, including examples of type III, type I, and type II vWD. The prevalence of vWD is extremely high in several inbred dog strains (up to 65% of Doberman Pinchers and Airedale Terriers). Murine vWD has been recently described in the RI11S/J mouse, identified by a screen of 25 inbred mouse strains, again suggesting a high spontaneous frequency for this disorder among mammalian species. vWD in the RI11S/J mouse appears to most closely resemble human type I vWD. It is autosomal dominant in inheritance associated with a partial defect in plasma vWF, prolonged bleeding time, and decreased platelet aggregation. Type I vWD in this animal model appears to be a “true dominant” disorder with homozygotes and heterozygotes having similar phenotype. These data also suggest that type III vWD is not simply the homozygous form of type I vWD (see below).

The reason for the high frequency of vWD in humans and other mammalian species remains unclear. vWD may have a protective effect against atherosclerosis in the porcine model, although no such effect has yet been shown in humans. However, given the generally late age of onset for clinically significant heart disease, a protective effect from vWD probably would not exert a strong evolutionary pressure and should be easily outweighed by even mild changes in hemorrhagic risk. Interestingly, vWD also appears to afford some resistance to bacterial endocarditis in the porcine model. In addition, a sequence similarity has recently been noted between the YopM outer membrane protein of the Yersinia pestis organism (the pathogenic bacteria of plague) with the α-chain of GPIb, the platelet surface receptor for vWF. YopM– mutants of Yersinia pestis show decreased virulence in mice. If an interaction between YopM and vWF is important for virulence, a potential relative protection from this or other pathogenic bacteria may have provided a strong selective pressure for vWD. The large size of the vWF gene may also afford an ample target for mutation, a proposed explanation for the high mutation rates observed in several other disorders associated with unusually large proteins and genes, such as hemophilia A and muscular dystrophy.

For any autosomal dominant disease, the proportion of cases representing new mutations should be directly related to the decrease in reproductive fitness associated with the disorder. Because vWD is in general a mild disorder, the decrease in reproductive fitness would be expected to be small, and thus the relative proportion of new mutations in von Willebrand disease should be low. Consistent with this hypothesis only a few patients have been identified in whom vWD appears to have arisen as a new genetic event (see below).

GENETIC LINKAGE ANALYSIS IN vWD

Given the complexity of vWF biosynthesis, secretion, and function, defects at a variety of genetic loci could potentially result in a vWD phenotype. As an example, platelet-type vWD, clinically very similar to type IIB vWD, is now known to be due to a molecular defect in the GPIbα chain gene (see below). Indeed, genetic locus heterogeneity could be partially responsible for the extensive phenotypic heterogeneity observed in vWD. Such a mechanism could also explain the apparent discordance between the relative frequencies of type I and type III vWD; ie, if the true incidence of type I vWD is 1% to 3%, this would imply a gene frequency of 0.01 to 0.005, which should translate into a homozygote frequency (expected to have severe vWD) of 1/4,000 to 1/40,000, much greater than the observed frequency of approximately 1/1,000,000. Similarly, the “true autosomal” pattern of murine vWD might also suggest a regulatory locus outside of the vWF gene.

In studies of porcine vWD, a restriction fragment length polymorphism (RFLP) identified within the porcine vWF gene was shown to be tightly linked to the inheritance of vWD with a LOD score of 5.3 (at θ = 0). Southern blot analysis showed no evidence of gene deletion or rearrangement. Taken together, these observations suggest a point mutation or small insertion or deletion within the vWF gene as the molecular basis for porcine vWD. A large number of RFLPs have been identified within the human vWF gene, including a highly polymorphic tetranucleotide repeat in intron 40. For polymorphisms located within the midportion of the vWF gene, care must be taken to distinguish RFLPs within the authentic gene from pseudogene polymorphisms.

Linkage analysis of type I vWD is complicated by problems inherent in the incomplete penetrance of this disorder and low sensitivity and specificity of conventional diagnostic tests. Tight linkage has been demonstrated for two human type IIA vWD pedigrees (LOD scores of 3.68 and 5.78). Although RFLP analyses in a number of other vWD pedigrees have been consistent with linkage, none have individually obtained statistical significance (LOD score > 3.0). Taken together with the recent identification of specific vWF gene mutations in several groups of patients, these data indicate that vWD is generally due to defects within the vWF gene itself. With the exception of platelet-type vWD, defects at other loci have not yet been identified.

GENE DELETIONS IN vWD

Gene deletion or rearrangement is a common mechanism for human genetic disease. Once DNA probes for vWF became available, large numbers of patients were screened by Southern blot analysis for the presence of such abnormalities. However, gene deletions have only been reported in six families, associated with type III vWD in five (Table 2). The one exception is a patient with an apparent de novo deletion in the midportion of the vWF gene resulting in a type II vWD phenotype (Table 3). Although the numbers are small, there appears to be a correlation between the presence of vWF inhibitors (anti-vWF allo-antibody) and the presence of gene deletion. Similar correlations have been noted in the hemophilias, particularly hemophilia B.

Give the low incidence of type III vWD (see above) and the presence of deletions in only a small subset of these patients, this would appear to be a
Table 2. Type III vWD

<table>
<thead>
<tr>
<th>Amino Acid Substitution</th>
<th>Molecular Defect</th>
<th>No. of Independent Families</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ig gene deletion (17 kb entire gene)</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Ig gene deletion (17 kb entire gene)</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.3 kb deletion (exon 42)</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>cis-defect in vWF mRNA expression</td>
<td>1</td>
<td>84</td>
</tr>
<tr>
<td>Rpro365*</td>
<td>C1993T</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>R1772*</td>
<td>C7603T</td>
<td>3†</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>cis-defect in vWF mRNA expression</td>
<td>3†</td>
<td>97</td>
</tr>
</tbody>
</table>

*Termination codon.
†All 3 alleles have identical RFLP haplotypes.

Table 3. Miscellaneous Defects

<table>
<thead>
<tr>
<th>Amino Acid Substitution</th>
<th>Nucleotide Substitution</th>
<th>No. of Independent Families</th>
<th>Functional References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII binding mutants</td>
<td></td>
<td>1</td>
<td>133</td>
</tr>
<tr>
<td>R19W</td>
<td>C2344T</td>
<td>1</td>
<td>133</td>
</tr>
<tr>
<td>T28M</td>
<td>C2372T</td>
<td>1</td>
<td>127,132</td>
</tr>
<tr>
<td>R53W</td>
<td>C2448T</td>
<td>2</td>
<td>130,134</td>
</tr>
<tr>
<td>R91Q</td>
<td>G2961A</td>
<td>4</td>
<td>128-130</td>
</tr>
<tr>
<td>Other variants</td>
<td></td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>G561S</td>
<td>G3970A</td>
<td>1</td>
<td>140</td>
</tr>
<tr>
<td>F606I</td>
<td>T4105A</td>
<td>1</td>
<td>139</td>
</tr>
</tbody>
</table>

Platelet type
("pseudo-vWD")

<table>
<thead>
<tr>
<th>Amino Acid Substitution</th>
<th>Functional References</th>
</tr>
</thead>
<tbody>
<tr>
<td>G239V</td>
<td>GP Ib α-chain</td>
</tr>
<tr>
<td>M239V</td>
<td>GP Ib α-chain</td>
</tr>
</tbody>
</table>
heterozygotes and presumed homozygotes having identical vWD phenotypes.\textsuperscript{70} Taken together, these observations suggest that type III and type I vWD may arise via different molecular mechanisms.

Despite considerable progress in characterizing vWD mutations in a variety of vWD subtypes, the molecular defects responsible for type I vWD remain unknown. Gene deletions, and presumably other defects resulting in complete loss of vWF expression from the vWF gene, appear to result in recessive type III vWD. If loss of vWF expression from one allele results in a silent carrier for type III vWD, an alternative mechanism must be proposed for the autosomal pattern of type I vWD inheritance. Type I vWD could be due to defects within the vWF gene giving rise to an abnormal protein whose function interferes with the normal allele in a "dominant-negative" manner. The multimeric nature of vWF provides a plausible mechanism for such an interaction because an abnormal protein product from one allele could affect not only its own secretion, assembly, or function, but also the product of the normal allele with which it forms heteromultimers. Similar interactions between mutant and normal α1 subunits in the trimeric type I collagen molecule account for the autosomal dominance of osteogenesis imperfecta type II.\textsuperscript{99} Alternatively, type I vWD could be due to a defect(s) at another genetic locus, perhaps in a gene involved in vWF biosynthesis, processing, or secretion.

**TYPE IIA vWD**

Identifying single point mutations within the vWF gene is a difficult problem. As noted above, because of its large size, direct sequence analysis of the entire vWF gene is not currently a practical approach. Concentrating on qualitative vWF variants, in which a mutation within the coding sequence might be expected, narrows the search to the coding exons (or mRNA). DNA sequence analysis is also complicated by the autosomal dominant nature of most vWD variants, requiring distinction between the single mutant allele and the other normal allele. In addition, mutations located within the mid portion of the vWF gene must be distinguished from the two pseudogene alleles. The pseudogene problem can be circumvented by directly analyzing vWF mRNA\textsuperscript{100} or, alternatively, using allele-specific PCR strategies to amplify only the authentic gene.\textsuperscript{51,84,86,100-102} The latter approach is facilitated by knowledge of the authentic and pseudogene sequences determined by Mancuso et al.\textsuperscript{90,91}

The first point mutations responsible for vWD were reported for the type IIA variant.\textsuperscript{109} A 176-Kd proteolytic fragment present in normal plasma and localized to the C-terminus of the mature vWF subunit has been observed to be markedly increased in the plasma of type IIA vWD patients.\textsuperscript{105} A similar proteolytic fragment and the associated vWF multimer satellite bands were also shown to result from proteolytic degradation in studies of cultured human umbilical vein endothelial cells derived from a patient with type IIA vWD.\textsuperscript{104} The hypothesis that increased sensitivity to proteolysis in the vicinity of a type IIA vWD mutation might account for the 176-Kd fragment focused attention on the corresponding segment of vWF in exon 28. PCR sequence analysis of vWF mRNA obtained from platelets or directly from exon 28 genomic DNA sequences has led to the identification of a number of mutations in type IIA vWD patients, generally all clustered within the A2 homologous repeat (Table 4 and Fig 1).

Expression of mutant vWF sequences by transfection in mammalian cells has provided important insights into the molecular basis of type IIA vWD. Transfection results allow subclassification of type IIA vWD patients into two distinct subgroups.\textsuperscript{106,105} In group 1, the associated point mutation leads to a defect in intracellular transport with vWF observed to be retained within the endoplasmic reticulum.\textsuperscript{105} In the heterozygous state, increased retention of the larger multimers (more likely to contain one or more variant subunits) could result in relatively more efficient secretion of the smaller multimers, accounting for the characteristic pattern observed in type IIA vWD plasma. By contrast, expression of recombinant vWF from group 2 results in normal appearing vWF multimers in tissue culture cells.\textsuperscript{105} One of these mutations (Arg834 → Trp) has been identified on at least two distinct genetic backgrounds in six unrelated type IIA vWD patients DNAs (Table 4).

The characteristic loss of large multimers in group 2 appears to occur via a second mechanism. Several studies have reported that large multimer loss in some type IIA vWD patients results from proteolysis occurring after synthesis and secretion\textsuperscript{108,106-109} mediated by a plasma or platelet specific protease, possibly a calpain.\textsuperscript{108,110} The site of proteolysis within vWF generating the characteristic 176-Kd fragment has recently been localized to Tyr842-Met843, in close proximity to many of the identified type IIA mutations (Table 4).\textsuperscript{110} A model for loss of large vWF multimers as a result of this single proteolytic cleavage has been proposed.\textsuperscript{111} Collection of blood from some type IIA patients into a cocktail of protease inhibitors results in relative preservation of multimer structure.\textsuperscript{106,107} This group of patients may well correspond to the group 2 mutations. In support of this hypothesis, a close correlation was observed between preservation of vWF multimer structure

<table>
<thead>
<tr>
<th>Table 4. Type IIA vWD Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid Substitution</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>V551F</td>
</tr>
<tr>
<td>G742R</td>
</tr>
<tr>
<td>G742E</td>
</tr>
<tr>
<td>S743L</td>
</tr>
<tr>
<td>L777P</td>
</tr>
<tr>
<td>V800L</td>
</tr>
<tr>
<td>R834W</td>
</tr>
<tr>
<td>V844D</td>
</tr>
<tr>
<td>S850P</td>
</tr>
<tr>
<td>I865T</td>
</tr>
<tr>
<td>E875K</td>
</tr>
</tbody>
</table>

*Greater than or equal to 2 independent alleles, based on haplotype analysis.\textsuperscript{109}

There are a total of 11 mutations in 17 families
in platelet vWF (protected from proteases within the platelet α-granule) and expression of intact multimers in in vitro transfection studies. Interestingly, in 1983 Weiss et al first suggested a subdivision of type IIA vWD based on platelet multimer pattern, a subclassification that probably will correspond to the subgroups now suggested on the basis of molecular pathogenesis. The list of currently reported potential type IIA mutations is shown in Table 4. In all, 11 mutations have been identified in 17 families. In one study, mutations were identified in 9 of 11 patients studies. Thus, it is likely that a panel of mutations accounting for the vast majority of type IIA vWD may eventually be established, permitting accurate diagnosis and classification at the DNA level for this common vWD subtype.

**TYPE IIB vWD**

Type IIB vWD is a relatively uncommon variant characterized by a unique “gain of function.” In this disorder, vWF demonstrates an increased reactivity with its platelet a-granule) and expression of intact multimers in in platelet vWF (protected from proteases within the platelet vWF demonstrates an increased reactivity with its platelet a-granule) and expression of intact multimers in in platelet vWF (protected from proteases within the platelet vWF GPIb-α receptor interaction and subsequent clearance from the circulation. The larger vWF multimers are more reactive with platelets and are thus selectively cleared, resulting in the characteristic low molecular weight multimer pattern observed in type IIB vWD plasma. Since the vWF GPIb binding domain had been localized to a peptide fragment within vWF exon 28, several groups have analyzed DNA sequence from type IIB vWD patients, concentrating on this region. By this approach, seven specific single amino acid substitutions (and one insertion) have been identified in type IIB vWD patients (see Table 5) all clustered within a small segment of the vWF A1 repeat (Fig 1). Expression of the Trp550→Cys mutation in a recombinant vWF fragment resulted in increased binding to platelets, consistent with the type IIB vWD phenotype. Full-length recombinant vWF containing the Arg543→Trp mutation also demonstrated increased platelet binding as a result of the Arg578→Gln and Pro574→Leu substitutions, confirming their identify as authentic type IIB vWD mutations.

Four mutations account for nearly 90% of the type IIB vWD patients studied to date (Table 5). Several of these mutations have been demonstrated to be recurrent independent genetic events on the basis of RFLP haplotype analysis and in at least three cases, the amino acid substitution appears to be a new mutation. In one of these cases, the mutation was shown to have originated on one allele during the development of the germ-line in the founder and was subsequently passed on to a subset of his offspring inheriting that allele (germ-line mosaicism). Five of the eight substitutions represent C→T transitions at CpG dinucleotides, proposed hot spots for mutation within the human genome. Thus, in the case of type IIB vWD, screening for a small panel of mutations should have a high sensitivity for the detection of type IIB vWD, and should facilitate precise diagnosis and classification of this disorder at the DNA level.

**vWF/FVIII interaction and autosomal hemophilia**

As discussed above, vWF and FVIII are closely associated in plasma as a noncovalent molecular complex. vWF is critical for FVIII transport and stability in plasma and the decreased vWF levels resulting from most vWD variants are also generally associated with a proportional decrease in FVIII antigen and procoagulant activity. In patients with severe or type III vWD, FVIII levels are markedly reduced and contribute significantly to the resulting bleeding diathesis. The close association of vWF and FVIII led to initial difficulties in distinguishing classic hemophilia A from “pseudohemophilia” (vWD). Hemophilia A is an X-linked disorder due to mutations within the F VIII gene on the X chromosome long arm. In the past few years, over 100 distinct mutations responsible for the hemophilia phenotype have been identified, most through the use of PCR techniques. Female patients affected with hemophilia are rarely encountered, often ascribed to unequal lyonization in a carrier. However, in several pedigrees this unusual form of hemophilia appears to be inherited in an autosomal fashion. In several cases, plasma vWF was demonstrated to have a markedly decreased binding capacity for FVIII. One of these original reports suggested the name vWD “Normandy” (the patient’s province of origin) for vWF variants defined by deficient FVIII binding without other qualitative or quantitative abnormalities. With the identification of additional patients with similar defects from a number of locations in the United States as well as Europe, a more general terminology should probably be adopted.

Recently, specific mutations within the vWF genes have been identified as the apparent explanation for the decreased vWF FVIII binding (Table 3). A single amino acid substitution, Thr28→Met, has been identified in one of the original vWD “Normandy” pedigrees. Recombinant vWF containing this substitution demonstrated markedly decreased binding capacity for vWF/FVIII interaction and autosomal hemophilia.
decreased FVIII binding. A study of three additional patients with low FVIII levels (6% to 22%) identified a homozygote for an Arg91 → Gln substitution, a homozygote for Arg53 → Trp, and a third patient who was a compound heterozygote for both of these latter mutations. The Arg91 → Gln mutation was also identified on 1 vWF allele in each of two unrelated mild type I vWD patients with disproportionately low FVIII levels. Plasma vWF from one of these patients had previously been shown to exhibit an abnormal interaction with FVIII.

Recombinant vWF containing the Arg91 → Gln substitution demonstrated markedly decreased capacity for FVIII binding. Interestingly, an amino acid polymorphism was identified on the other vWF allele from one of the type I patients, located just 2 amino acids upstream and resulting in the similar substitution, Arg89 → Gln. Expression of vWF containing this latter substitution resulted in FVIII binding indistinguishable from wild-type. The functional significance of the Arg53 → Trp mutation, along with a fourth substitution (Arg19 → Trp) have also recently been confirmed by recombinant expression studies.

All of these vWF mutations are located within the N-terminal portion of vWF, the region implicated in binding to FVIII. As noted above, two independent monoclonal antibodies that block FVIII binding to vWF have been localized to a 19 amino acid peptide (Thr78-Thr96) with the N-terminal segment. Interestingly, the Arg91 → Gln substitution is localized in the middle of this small epitope. Together, these data provide strong evidence that this region plays a critical role in the FVIII/vWF interaction. The corresponding segment within FVIII that binds vWF has been localized to an acidic peptide, spanning amino acids 1677 to 1684. The Thr78-Thr96 epitope is markedly basic, and the Arg91 substitution results in a loss of one of these basic residues. However, the finding that Arg89 → Gln has no effect on FVIII binding indicates that this interaction is not simply based on charge.

Thus, these mutations at the vWF N-terminus (Table 3) all interfere with the ability of vWF to bind FVIII and define a new variant of vWD. In the homozygote (or compound heterozygote) this defect results in an autosomal form of hemophilia characterized by decreased FVIII levels and a clinical pattern indistinguishable from mild to moderate classic hemophilia A. In the heterozygote, this defect is generally silent and only detected by incidental screening, or when it occurs in conjunction with a coexistent type I or other vWD variant. Coinheritance of a FVIII binding defective vWF could account for some of the phenotypic heterogeneity occasionally observed among hemophilia A patients with identical FVIII gene mutations.

OTHER vWD VARIANTS

A large number of other vWD variants have been described, most as single case reports, generally identified by subtle abnormalities in vWF multimeric structure (Table 1). Diagnosis of these variants requires high quality multimer analysis, generally available at only a few reference laboratories. Thus, their frequency may be underestimated. Some of these variants may represent compound heterozygosity for other known subtypes. Preliminary reports of potential mutations responsible for several rare variants have recently appeared.

Several families have been reported with a unique disorder termed platelet-type or "pseudo" vWD. This condition is remarkably similar to type IIB vWD, with decreased large vWF multimers, thrombocytopenia, and increased vWF/platelet interaction. However, unlike type IIV vWD, in which the defect has been shown to lie within the GPIb binding domain of vWF (Table 5), the defect in platelet type vWD is in the platelet itself, specifically within the GPIb/IX complex. The two disorders can be distinguished by platelet and plasma mixing studies. Point mutations within the GPIb chain gene potentially responsible for platelet-type vWD in two families have recently been reported (Table 3).

PRENATAL DIAGNOSIS

With the dramatic advances in our understanding of vWD molecular genetics, powerful tools are now available for potential prenatal diagnosis. In those cases in which the precise molecular defect is known (Tables 2 through 5), reliable and accurate diagnosis can easily be achieved from amniotic fluid or chorionic villus biopsy by PCR techniques, as now regularly applied for a variety of genetic disorders. As noted above, a large number of polymorphisms within the VWF gene have now been identified including a highly informative variable number tandem repeat (VNTR) in intron 40. However, given the potential for locus heterogeneity in vWD (ie, vWD resulting from defects in genes other than vWF; see above), caution should be exercised in interpreting these results.

CONCLUSIONS

Since the cloning of vWF cDNA in 1985 and characterization of the complete genomic structure of the vWF gene in 1989, considerable progress has been made in characterizing the specific molecular defects responsible for the heterogeneous disorder known as vWD. A large number of specific molecular defects have now been identified and precise characterization may now be possible in the majority of type IIA, type IIB, and potentially also type IIV vWD cases. However, the most common variant, type I vWD, still remains a major challenge. Continued progress in this area will not only improve our understanding of the pathogenesis of vWD, but should also lead to more rapid and precise diagnosis and classification for this common disorder. The problems of incomplete vWD penetrance and poor diagnostic sensitivity and accuracy for the currently available clinical laboratory tests provide strong incentives for the development of DNA-based diagnostics. In addition, prenatal diagnosis is now possible either at the level of single point mutations or by RFLP analysis (assuming linkage to the vWF gene) and will probably be applied with increasing frequency. Understanding the molecular basis of vWD also has important implications for vWF structure and function and is helping to define critical binding domains.
within the vWF molecule. Insights gained from these studies may eventually lead to improved therapeutic approaches not only for VWD, but for a variety of other genetic and acquired hemorrhagic and thrombotic disorders.

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