

## A Practical Approach to Genetic Testing for von Willebrand Disease

RAJIV K. PRUTHI, MBBS

von Willebrand disease (vWD) is the most commonly diagnosed congenital bleeding disorder. The laboratory diagnosis of type 2 variants and type 3 vWD is reasonably well defined, and characterization of the von Willebrand factor (vWF) gene has facilitated definition of their molecular basis. However, for type 1 vWD, the laboratory diagnosis poses a diagnostic dilemma, and knowledge of its molecular basis is evolving. Characterization of the *vWF* gene and refinement of genetic techniques have led to an evolving repertoire of genetic tests. Genetic testing is costly, and thus judicious use will be increasingly important for appropriate genetic counseling of patients with vWD and their family members. This article provides a practical approach to utilization of genetic testing in vWD.

*Mayo Clin Proc.* 2006;81(5):679-691

DDAVP = 1-desamino-8-D-arginine vasopressin (desmopressin); FVIII = factor VIII; FVIII:C = factor VIII coagulant activity; GP = glycoprotein; vWD = von Willebrand disease; vWF = von Willebrand factor; vWF:Ag = von Willebrand factor antigen; vWF:FVIIIb = von Willebrand factor VIII binding activity; vWF:RCo = von Willebrand factor ristocetin cofactor activity

The cornerstones of *primary hemostasis* are von Willebrand factor (vWF) and platelets. Platelet adhesion, to exposed vascular subendothelial collagen, is mediated by vWF (Figure 1), which results in platelet activation and aggregation. The simultaneous exposure of tissue factor leads to initiation of the process of *secondary hemostasis*, ie, activation of the procoagulant cascade and eventual formation of a hemostatic fibrin clot.<sup>2</sup>

Bleeding disorders are broadly classified into primary hemostatic defects (congenital and acquired vascular abnormalities, quantitative and qualitative platelet defects, and von Willebrand disease [vWD]) and secondary hemostatic defects (congenital and acquired coagulation factor deficiencies). The most common congenital bleeding disorder is vWD, which is due to an abnormality of vWF.

Although the laboratory diagnosis of type 2 variants and type 3 vWD is reasonably well defined, the laboratory diagnosis of type 1 vWD still poses a challenge. Characterization of the human genome in general<sup>3</sup> and the *vWF* gene

in particular and refinements in molecular techniques have facilitated the definition of the molecular basis of types 2 and 3 vWD, whereas the molecular basis of type 1 vWD is being defined. Such advances have also facilitated the development of a repertoire of genetic tests that, given their cost, warrant judicious utilization. Herein, a practical approach to the diagnosis of vWD is provided, and the repertoire, testing indications, and interpretation of genetic testing for vWD are reviewed.

### TESTING OF PATIENTS SUSPECTED OF HAVING BLEEDING DISORDERS

A detailed discussion on the approach to testing patients suspected of having bleeding disorders is beyond the scope of this article; however, a practical approach is outlined herein. A patient who presents with either spontaneous or postoperative hemorrhage or a family history of the same should undergo a detailed evaluation of personal and family history, physical examination, and laboratory testing for a bleeding disorder.

A thorough personal and family history and physical examination are important screening tools to identify a potential bleeding disorder,<sup>4</sup> although not all such patients will have an identifiable defect.<sup>5</sup> Issues to address in the patient history include age at onset of bleeding (which helps differentiate between congenital and acquired bleeding disorders) and pattern of bleeding, such as mucocutaneous (epistaxis, oral cavity, bruising, and menorrhagia), which is typically a manifestation of a primary hemostatic defect (eg, platelet defect or vWD), or soft tissue and articular hemorrhage, which are typically manifestations of a secondary hemostatic defect (clotting factor deficiency, eg, type 3 vWD or hemophilia). Details of surgery-related bleeding and a detailed medication review (antiplatelet agents such as aspirin or nonsteroidal anti-inflammatory agents) are important as well.

Two situations typically lead one to consider laboratory testing to diagnose or exclude patients with vWD who present with bleeding symptoms and patients who are referred for evaluation because of a family history of vWD. Patients who present with bleeding symptoms may not have previously undergone hemostatic testing. In such a situation, comprehensive evaluation should consist of a complete blood cell count (to determine the number of platelets), screening tests such as prothrombin time and activated partial thromboplastin time (which are reasonably sen-

From the Department of Internal Medicine and Division of Hematology, Special Coagulation DNA Diagnostic Laboratory, and Comprehensive Hemophilia Center, Mayo Clinic College of Medicine, Rochester, Minn.

Address correspondence to Rajiv K. Pruthi, MBBS, Division of Hematology, Mayo Clinic College of Medicine, 200 First St SW, Rochester, MN 55905 (e-mail: pruthi.rajiv@mayo.edu). Individual reprints of this article and the entire series on Genetics in Clinical Practice will be available for purchase from our Web site [www.mayoclinicproceedings.com](http://www.mayoclinicproceedings.com).

© 2006 Mayo Foundation for Medical Education and Research

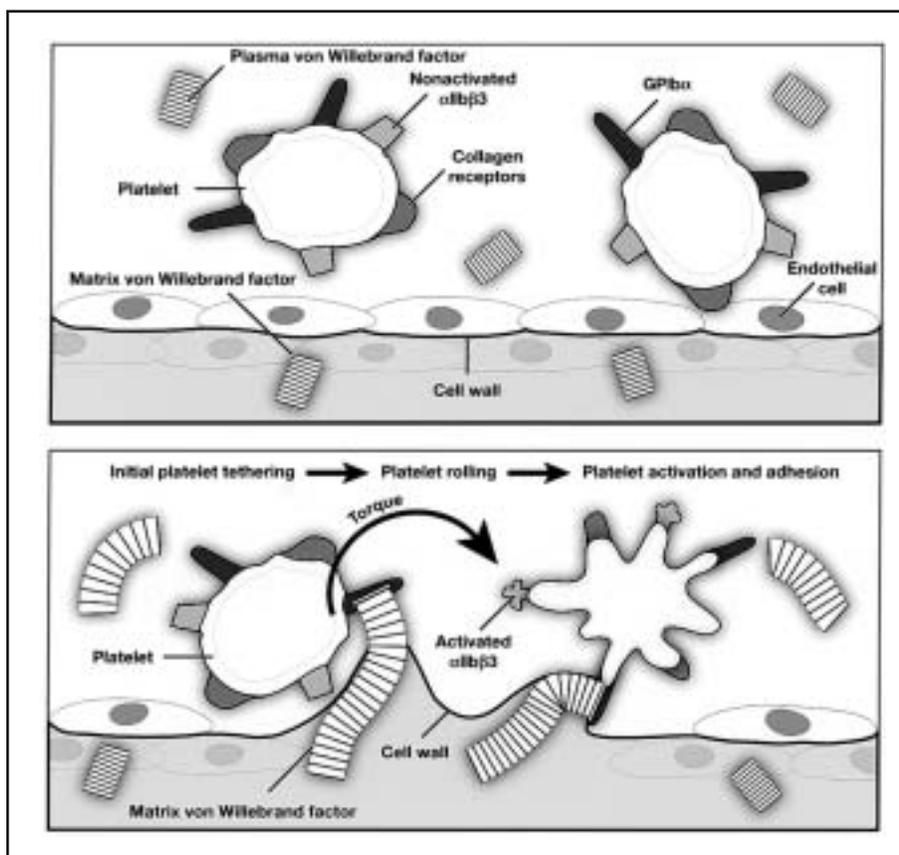


FIGURE 1. Role of von Willebrand factor in platelet adhesion and aggregation. Top, Inactive platelet with intact endothelium. Bottom, Disrupted endothelium with activated platelet leading to platelet adhesion. GP = glycoprotein. Adapted from *N Engl J Med*.<sup>1</sup>

sitive to clotting factor deficiency states), and performance of factor VIII (fVIII) and vWF assays (outlined subsequently). Depending on the findings, additional testing may be pursued (eg, assays of other clotting factors, factor XIII, platelet aggregation, and evaluation of the fibrinolytic pathway [antiplasmin and plasminogen activator inhibitor 1]).

In patients with a family history of vWD, it is reasonable to perform testing for vWD based on a vWD profile. Thus, the remainder of this article will focus on the biochemistry, laboratory testing, and an approach to molecular testing in vWD.

### BIOSYNTHESIS OF vWF

von Willebrand synthesis occurs in vascular endothelial cells and megakaryocytes. The primary translational product, pre- or pro-vWF, consists of a 22-amino acid signal peptide, a 741-amino acid propeptide, and a 2050 mature protein. After cleavage of the signal peptide, vWF precursor (pro-vWF) undergoes posttranslational modification in the

endoplasmic reticulum (N-linked glycosylation and dimerization by disulfide bond formation between the cysteine knot domains at the C-terminal ends), after which the pro-vWF dimers are transported to the Golgi apparatus. In the Golgi and post-Golgi compartments, the pro-vWF dimers undergo sulfation, O-linked glycosylation, and multimerization by forming disulfide bonds between the N-terminal ends of subunits, resulting in multimers ranging in size from 500 kDa to more than 10 million Da and proteolytic cleavage. After its synthesis, vWF is then either constitutively secreted directly into plasma or stored in the Weibel-Palade body of endothelial cells and in platelet granules to be released after appropriate stimulation<sup>6</sup> (eg, in response to stress, vascular injury, or 1-desamino-8-D-arginine vasopressin [DDAVP]). On secretion from endothelial cells, the ultralarge-molecular-weight multimers of vWF, which are the most hemostatically active, are cleaved into smaller multimers by a specific protease ADAMTS 13 by cleavage at the Tyr 1605-Met1606 bond within the A2 domain.<sup>7</sup>

## STRUCTURE AND FUNCTION OF vWF

Structural and functional relationships of vWF were recently reviewed.<sup>8</sup> The mature vWF peptide consists of 2050 amino acids organized into 4 domains (A-D) (Figure 2). Each domain contains functional residues that mediate vWF biological functions of platelet adhesion and aggregation as well as protection of fVIII from premature inactivation. The A1 (amino acid residues 497-716) domain binds to type VI collagen, whereas the A3 (amino acid residues 910-111) domain binds to type I and III collagen. In addition, the A1 domain contains the binding region for glycoprotein (GP) Ib $\alpha$ , a component of the platelet GPIb $\alpha$ -IX-V receptor complex and the heparin binding site. The fVIII binding domain of vWF is localized to the D' and D3 domains. The C domain contains the platelet GPIIb-IIIa complex binding site.

## DISEASE SYNOPSIS

### EPIDEMIOLOGY

Congenital vWD is an autosomal disorder that reflects abnormalities of vWF. Defining its exact prevalence poses a challenge given the variables that affect clinical and laboratory diagnosis of vWD; nevertheless, it is the most commonly recognized congenital bleeding disorder. Estimates of prevalence vary with study and range from 1 per 10,000 persons<sup>10</sup> to 0.82%<sup>11</sup> to 1.3%,<sup>12</sup> with type 1 vWD being the most commonly represented subtype. Prevalence of type 3 vWD is better defined as 1.38 to 1.51 cases per million in the United States and Europe, respectively,<sup>13</sup> and 0.1 to 3 per million persons in a second report.<sup>14</sup>

### DIAGNOSIS AND CLASSIFICATION OF vWD

Since the initial description of vWD,<sup>15</sup> improvements in laboratory and molecular techniques, discovery of the vWF gene, and elucidation of the molecular pathological findings have led to the recognition and classification of a variety of different vWD subtypes,<sup>16,17</sup> and further clarification of the molecular pathological findings of vWD continues to refine the classification.<sup>18,19</sup>

### SELECTION OF vWF TESTS

Comprehensive reviews on the laboratory diagnosis of vWD have recently been published<sup>20,21</sup> and will be briefly reviewed herein. The diagnosis of vWD is established based on a personal and/or a family history of abnormal clinical bleeding and results of diagnostic tests. Since screening tests for vWD, including bleeding time and Platelet Function Analyzer (PFA-100, Dade Behring, Deerfield, Ill) lack sufficient sensitivity,<sup>20,22</sup> a vWF panel is required. The diagnosis is based on a reduced vWF antigen

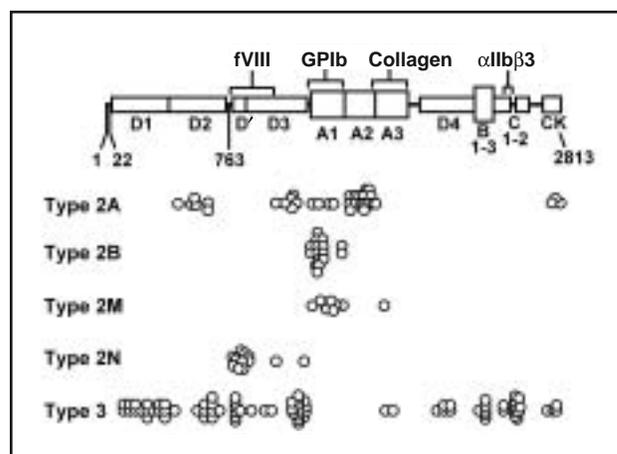


FIGURE 2. The von Willebrand factor peptide showing ligand-binding domains and location of mutations. fVIII = factor VIII; GP = glycoprotein. Adapted from the *Annual Review of Medicine*, volume 56 ©2005,<sup>9</sup> with permission by Annual Reviews at www.annualreviews.org.

(vWF:Ag), vWF activity assay (ristocetin cofactor activity [vWF:RCo]), and fVIII coagulant activity (fVIII:C).<sup>23</sup> Results should be analyzed within the context of blood group.

Analysis of vWF multimer distribution is important for typing and subtyping of vWD. The assay is labor intensive, involving protein electrophoresis followed by radioactive or immunofluorescent detection of vWF multimers on the gel. The vWF multimeric analysis should not be ordered with the initial screen, unless it is available reflexively (ie, for further evaluation of abnormally low vWF test results).<sup>20</sup> Additional tests may include performance of the collagen binding assay, ristocetin-induced platelet aggregation, and vWF fVIII binding assay (vWF:fVIII:B) (Table 1).

The fVIII:C provides information on the ability of vWF to serve as a carrier protein for fVIII and is typically normal

TABLE 1. Approach to the Assessment of vWD\*

Bleeding history
Complete blood cell count
vWD profile testing
vWF:Ag
vWF:RCo
fVIII:C
ABO blood group
Optional tests if initial data suggestive of vWD
vWF
vWF:CBA
vWF:fVIII:B
RIPA
Genetic tests if indicated

\*Ag = antigen; CBA = collagen binding assay; fVIII:B = factor VIII binding assay; fVIII:C = factor VIII coagulant activity; RCo = ristocetin cofactor; RIPA = ristocetin-induced platelet aggregation; vWD = von Willebrand disease; vWF = von Willebrand factor.

TABLE 2. Laboratory Values in vWD\*

vWD subtype	vWF:RCo	vWF:Ag	fVIII:C	vWF:CBA	vWF:fVIIIIB	Platelet count	Low-dose RIPA	Multimers
1	Decreased	Decreased	Decreased/normal	Decreased/normal	Normal	Normal	Absent	Decreased production of all multimeric forms
2A	Decreased	Decreased	Decreased/normal	Decreased	Normal	Normal	Absent	Decreased high-molecular-weight multimers
2B	Decreased	Decreased	Decreased/normal	Decreased	Normal	Decreased	Present	Decreased high-molecular-weight multimers
2M	Decreased	Normal/decreased	Normal	Decreased	Normal	Normal	Absent	Normal
2N	Normal	Normal	Decreased	Decreased	Decreased	Normal	Absent	Normal
3	Marked decrease/absent	Marked decrease/absent	Marked decrease	Marked decrease		Normal	Absent	Absent

\*Ag = antigen; CBA = collagen binding assay; fVIIIIB = factor VIII binding assay; fVIII:C = factor VIII coagulant activity; RCo = ristocetin cofactor; RIPA = ristocetin-induced platelet aggregation (0.5 mg/dL); vWD = von Willebrand disease; vWF = von Willebrand factor.

or decreased. (It is always decreased in patients with type 2N [Normandy] and type 3 vWD). Of note, fVIII:C levels do not always parallel the vWF:Ag levels. Current vWF:Ag assays are typically based on the enzyme-linked immunosorbent assay or automated latex immunoassay, and vWF functional assays (vWF:RCo) are based on the ability of ristocetin to bind (fresh washed or fixed) platelets and the high-molecular-weight multimers of vWF. Of the vWD assays, the vWF:RCo assay has a significant variability, depending on the method.<sup>24</sup>

The vWF collagen binding assay is being increasingly used in the diagnosis and management of vWD, is based on the enzyme-linked immunosorbent assay, and provides information on the presence or absence of the higher-molecular-weight multimers and vWF collagen binding function. Thus, this assay complements rather than replaces the vWF:RCo assay.<sup>25,26</sup> Additional assays that are not widely available include ristocetin-induced platelet aggregation, which provides a measure of hyperreactivity of patient vWF to low concentrations of ristocetin that induce platelet aggregation characteristic of type 2B variants of vWD. The vWF:fVIIIIB detects the fVIII binding defect on vWF.<sup>27</sup>

Based on levels of vWF:RCo, vWF:Ag, fVIII activity, and distribution of vWF multimers, vWD can be broadly classified into quantitative (types 1 and 3) or qualitative (type 2) abnormalities of plasma vWF<sup>18</sup> (Table 2). Quantitative abnormalities include a mild to moderate reduction of qualitatively normal vWF (type 1 vWD) or absent vWF (type 3 vWD), whereas in qualitative (type 2 vWD) defects, the plasma vWF exhibits defective structure and function, resulting in a reduction of the high-molecular-weight multimers and a discordant decrease in vWF:RCo (compared with vWF:Ag) in types 2A and 2B (Figure 3). Several clinical and laboratory variables affect the diagnosis of vWD, including variable phenotypic expression of vWD, even within fami-

lies with clinical bleeding, rendering the diagnosis of vWD, especially type 1, complex and difficult.<sup>28,29</sup>

#### KEY CONSIDERATIONS IN THE DIAGNOSIS OF vWD

Multiple variables that affect vWF levels can make a firm diagnosis of vWD difficult. In aggregate, mildly reduced vWF:Ag and vWF:RCo levels do not always establish a diagnosis of vWD<sup>29</sup>; conversely, low normal vWF:Ag and vWF:RCo activity does not always exclude the diagnosis. Of the several genetic and environmental modifiers known to affect vWF levels, the best known genetic modifier is the patient's ABO blood group.<sup>30</sup> Persons with blood group type O have on average 25% lower vWF levels than patients with non-O blood groups.<sup>31</sup> In addition, inflammation, stress, pregnancy, or estrogen therapy may increase vWF levels above baseline and potentially mask diagnosis of mild vWD.<sup>32</sup> Thus, repeated follow-up testing is advised to confirm or exclude the diagnosis of vWD.<sup>33</sup> In addition, although vWF:Ag assays have good precision and reproducibility, the vWF:RCo assay has greater variability,<sup>34,35</sup> resulting in potential for misdiagnosis and/or misclassification.<sup>21</sup> Furthermore, the low sensitivity of the available tests is highlighted in one report, in which approximately 40% of obligate heterozygotes with type 1 vWD had normal test results,<sup>36</sup> and in another report, which demonstrated variability in test results over time.<sup>37</sup> Thus, clinical correlation with patient symptoms and follow-up laboratory testing may be indicated.<sup>38</sup>

Lack of clear delineation of the normal state vs the disease state has led to a proposal to consider mild reduction in vWF as a risk factor for bleeding and to reserve the diagnosis of vWD for patients with clearly dominant disease (ie, moderate to marked reduction in vWF levels and bleeding histories).<sup>9</sup> Currently, clinical molecular genetic testing for confirmation of diagnosis of type 1 vWD is not

recommended, although evolving information suggests it may be useful in selected cases in the near future.

#### CLINICAL FEATURES

Substantial deficiency of vWF (eg, levels lower than 20% to 30%) results in a primary hemostatic defect, and most patients typically experience mild mucocutaneous bleeding symptoms, including lifelong ease of bruising, epistaxis and menorrhagia in women, and posttraumatic and postoperative surgical bleeding. The absence of spontaneous bleeding in mild to moderate phenotypes can lead to a false sense of security on the part of both physicians and patients, such that seemingly minor procedures (eg, colon polypectomy or dental extraction) can result in pronounced hemorrhage without appropriate perioperative hemostatic management. Consistent with its fVIII carrier function, progressively decreasing vWF will result in a secondary decrease in fVIII and, if severe enough, will result in an additional hemostatic defect. Thus, patients with type 3 vWD, with very low fVIII levels, can experience soft tissue and joint hemorrhage as seen in patients with moderately severe hemophilia.

#### MANAGEMENT

After the diagnosis of vWD has been established, a DDAVP treatment trial should be considered for patients with types 1, 2A, and 2M vWD.<sup>39</sup> Intravenous administration of 0.3 µg/kg of this synthetic analogue of the antidiuretic hormone vasopressin results in release of vWF from its endothelial storage sites, with maximal plasma levels occurring at 30 to 90 minutes after DDAVP administration.

Patients with type 1 vWD typically experience a 2- to 5-fold increase of vWF and fVIII over baseline, which generally lasts 8 to 10 hours<sup>40</sup>; however, this response may be short-lived in selected patients with type 1 vWD, and in patients with type 2 variants of vWD, response and duration of response may be unsatisfactory. Generally, DDAVP is not helpful for patients with type 2B vWD given that release of endogenous vWF results in worsening of thrombocytopenia<sup>41</sup>; however, the clinical importance of this finding is being debated.<sup>42</sup> Patients with type 3 vWD will not respond and should not undergo a DDAVP trial.<sup>43</sup>

For patients who respond, DDAVP is a reasonable alternative for prevention or treatment of minor bleeding or for minor procedures such as dental extraction and in the management of menorrhagia in women with vWD. An intranasal formulation of DDAVP (Stimate, ZLB Behring, King of Prussia, Pa) is licensed for use in the United States. A single spray typically delivers 150 µg, which is sufficient for children or adults with body weights less than 50 kg; however, adults typically require 300 µg.<sup>44</sup> A more concentrated preparation for subcutaneous use is not licensed for

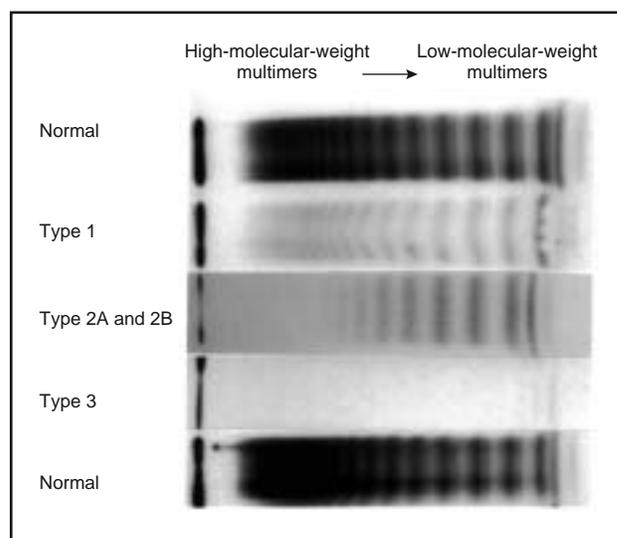


FIGURE 3. von Willebrand factor multimers.

use in the United States. For patients who do not respond to DDAVP and for those in whom it is contraindicated, purified plasma-derived vWF concentrates are the therapy of choice.<sup>45</sup> The mainstay of management consists of prophylaxis and treatment of bleeding episodes, which was recently reviewed.<sup>1</sup>

#### GENETIC BACKGROUND OF vWF

The *vWF* gene is located near the tip of the short arm of human chromosome 12, consists of 52 exons, and spans approximately 180 kb.<sup>46</sup> The intron-exon boundaries roughly correlate with the vWF functional domains, and there is an unprocessed partial pseudogene located on chromosome 22q11.2 that is approximately 97% identical to exons 23 through 34 of the *vWF* gene. Although this high degree of homology warrants careful interpretation of the results of molecular testing, the use of gene-specific polymerase chain reaction primers has facilitated such interpretation.<sup>47</sup> In general, quantitative abnormalities of the *vWF* gene are due to promoter, frameshift nonsense mutations, and large deletions, whereas missense mutations result in qualitative defects. The presence of multiple exonic polymorphisms makes interpretation of the results of molecular testing difficult; however, *in vitro* expression of such mutations has clarified their deleterious nature. The presence of a highly pleomorphic variable number tandem repeat within intron 40 has been informative for linkage analysis.<sup>48,49</sup> Mutations and polymorphisms in the *vWF* gene are currently being cataloged in an international database, which can be accessed via the Internet. References for the amino acid number system used herein can be accessed at [www.shef.ac.uk/vwf/](http://www.shef.ac.uk/vwf/).

**TYPE 1 vWD**

Type 1 vWD, due to partial quantitative deficiency of vWF,<sup>18</sup> accounts for approximately 70% of cases of vWD and is characterized by mild to moderate proportionate reductions in plasma vWF:Ag, vWF:RCo with normal or decreased fVIII:C, and a normal distribution of vWF multimers.<sup>33</sup> Included in this category are several subtypes of vWD from the original classification<sup>17</sup> (I platelet normal, I platelet low, IA, I-1, I-2, and I-3). Although the subclassification based on platelet vWF content<sup>50,51</sup> has been demonstrated to be of clinical importance (ie, poor response to DDAVP in the platelet low subgroup<sup>51</sup>), providing such complex testing on a routine clinical basis is not practical.<sup>21</sup>

Knowledge of the molecular basis of type 1 vWD is only beginning to evolve.<sup>52,53</sup> Even though the *vWF* gene had been identified, early linkage studies of families with type 1 vWD suggested weak<sup>54</sup> or inconsistent linkage to the *vWF* locus; in addition, earlier studies demonstrated linkage to the *vWF* gene in only 41% of families,<sup>56</sup> even though comprehensive sequencing of the *vWF* gene yielded 112 candidate mutations for which structure function relationships were being defined. Moreover, selected patients with type 1 vWD were found to be heterozygous carriers of type 3 defect,<sup>57</sup> but not all such individuals have symptomatic bleeding.<sup>58</sup>

Studies of animal models have suggested locus heterogeneity as an explanation for the mild quantitative deficiency of vWF associated with type 1 vWD (eg, defects in glycosylation of the vWF protein<sup>59,60</sup>), resulting in a shortened vWF survival.

A recent report found segregation of a specific mutation Tyr1584Cys in up to 15% of families with type 1 vWD. In addition, expression studies demonstrated increased intracellular accumulation of the variant carrying the mutation,<sup>61</sup> thus, potentially providing, for the first time, a genetic test that may assist in clarification of the diagnosis of vWD in patients with borderline vWF assay test results. Moreover, more recent studies have demonstrated stronger linkage to the *vWF* gene.<sup>62</sup> Therefore, evolution of knowledge of the molecular basis of type 1 vWD will clarify the role of genetic testing in type 1 vWD.

**TYPE 3 vWD**

Type 3 vWD is autosomal recessive and characterized by a severe reduction in vWF:Ag and vWF:RCo and a concordant reduction in fVIII:C activity, resulting in a more severe phenotype. Its prevalence ranges from 0.5 to 5.3 per million.<sup>14,63</sup> The most common mutation reported in patients with type 3 vWD is a frameshift mutation in exon 18, which was found in approximately 50% of a cohort of patients<sup>64</sup> and is a common mutation reported in German patients.<sup>65</sup> Although uncommon, gene deletions, detected by Southern blot analysis, are predictive of development of alloantibodies

directed against transfused vWF.<sup>66</sup> Additional characterized deleterious mutations include missense and nonsense mutations that result in loss of vWF messenger RNA expression<sup>58</sup> and frameshift and splice site mutations. A complete listing of various mutations can be found at [www.shef.ac.uk/vwf/](http://www.shef.ac.uk/vwf/).

Although molecular genetic techniques have conclusively linked type 3 vWD to the *vWF* gene locus and mutations have been defined, observations remain that require satisfactory explanation. Selected patients may be homozygotes or compound heterozygous for 2 null alleles and are classified as type 3 vWD yet have asymptomatic parents. In addition, complicating the widespread clinical application of molecular genetic analysis of type 3 vWD is the fact that mutations are distributed throughout the approximately 180 kb of coding and noncoding regions of the *vWF* gene (Figure 2). The presence of a processed pseudogene further complicates testing, even though primers for selective amplification of the *vWF* gene have been carefully designed. Laboratories that offer direct and indirect genetic testing<sup>48</sup> for vWD are listed with GeneTests ([www.genetests.org](http://www.genetests.org)). In summary, for the quantitative variants, despite the limitations discussed herein, genetic testing for type 3 vWD is rational; however, further clarification of the molecular basis of type 1 vWD is needed before implementation of clinical genetic testing.

**TYPE 2 vWD**

Type 2 variants of vWD, which account for approximately 20% to 30% of patients with vWD, are characterized by qualitatively abnormal vWF with decreased platelet-dependent function associated with decreased or absent hemostatically active high-molecular multimers. Qualitative variants include types 2A, B, N, and M, and included within this broad category are variants defined under the original classification scheme. The molecular basis of type 2 variants has been intensely studied, providing clear information on the deleterious nature of mutations.

**TYPE 2A vWD**

Type 2A vWD is typically autosomal dominant and accounts for approximately 75% of all type 2 vWD. There is a variable reduction in vWF:Ag, with a discordant reduction in vWF:RCo activity, indicative of a qualitative vWF abnormality. The higher and intermediate plasma vWF multimers are reduced or absent, and the lower-molecular-weight multimers are relatively increased or have an abnormal infrastructure. Plasma and platelet multimer abnormalities may be concordant or discordant, depending on the underlying molecular defect.

Included in this category of vWD are several subtypes previously classified as IB, IIA, IIA-1, IIA-2, IIA-3, IIC, IIE, IIF, IIG, IIH, and II-1.<sup>17</sup> Missense mutations that result in

type IIA vWD occur predominantly in the A2 domain (Gly 742 to Glu 875) (Figure 2) and result in abnormal vWF patterns by 2 distinct mechanisms. The first group includes mutations that impair the assembly and secretion of normal vWF multimers, resulting in decreased higher-molecular-weight vWF multimers in both plasma and platelets.<sup>67</sup> The second group includes mutations that result in normal assembly and secretion of vWF. However, the mutant vWF has an increased sensitivity to proteolytic degradation in plasma, resulting in decreased plasma high-molecular-weight multimers but a normal platelet vWF multimer pattern.<sup>68</sup> The cleavage site in a subset of patients with type 2A vWD was shown to be the Tyr842-Met843 bond, in which mutations may result in a conformational change, resulting in increased sensitivity to proteolysis.

The molecular defect in subtype IID results in defects of dimerization at the C terminus of vWF and defects of polymerization to multimers at their N termini.<sup>69</sup> Mutations have been described in the D1 and D2 domains in subtype IIC and in the D3 domain in subtypes IIE, IIF, and IIC Miami.<sup>19</sup> Additional mutations are being described and cataloged in the vWF mutation database.

#### TYPE 2B vWD

Type 2B vWD, which accounts for approximately 20% of all type 2 vWD, is autosomal dominant and characterized by a variable reduction in vWF:Ag and a discordant reduction in vWF:RCo activity, with a loss of the higher and intermediate plasma vWF multimers but normal distribution of platelet vWF multimers. This variant is distinguished from type 2A vWD by the presence of mild to moderate thrombocytopenia, which occurs as a result of an increased affinity of vWF for the platelet GPIb-IX complex, resulting in spontaneous binding of plasma vWF to platelets and platelet agglutination *in vivo* and clearance of the platelet-bound larger multimers from plasma. In addition, platelet aggregation in response to low concentrations of ristocetin demonstrates an exaggerated response. Loss of higher-molecular-weight multimers is not a characteristic of types I New York<sup>70</sup> and Malmo<sup>71</sup> in which hyperresponsiveness to ristocetin is a feature. Most deleterious mutations are missense mutations that occur in the A1 domain, which contains the GPIb-IX complex binding domain, and *in vitro* studies demonstrate that these mutations result in gain of function. Type 2B vWD must be distinguished from a pseudo-vWD or platelet-type vWD, which is similar in presentation. Patients in the latter group have a primary platelet defect that results from mutations in the platelet vWF receptor (GPIb-IX).<sup>72</sup>

#### TYPE 2M vWD

In type 2M vWD, although the vWF:Ag and the distribution of vWF multimers are normal, the vWF:RCo activity

is reduced, reflecting a functional defect of the vWF multimers. This subtype includes patients with larger than normal vWF multimers (vWD Vicenza), which result from uncleaved pro-vWF or ultrahigh-molecular-weight multimers. Selected variants have mutations in the A1 domain, resulting in decreased binding affinity for platelet GPIb; however, mutations in the D3 domain have been found in families with the Vicenza subtype.<sup>73</sup> Most described mutations are missense changes.

#### TYPE 2N (NORMANDY) vWD

Mutations in the fVIII binding domain of vWF result in suboptimal binding of fVIII to vWF, which can be detected by performing fVIII binding assays or predicted by DNA-based testing<sup>27</sup> (Figure 2). This binding defect results in a shorter half-life of plasma fVIII; thus, plasma fVIII activity is reduced typically to 5% to 20% of mean normal. Levels of vWF:Ag and vWF:RCo activity are normal as is the vWF multimer distribution. This subtype mimics mild hemophilia A; however, it is distinguished by its autosomal recessive pattern of inheritance rather than the X-linked recessive pattern of hemophilia A.

In a recent international survey, type 2N vWD was detected in 58 (4.8%) of 1198 patients previously diagnosed as having mild hemophilia A.<sup>74</sup> Three vWF gene mutations in the D' domain, Thr791Met, Arg816Trp, and Arg854Gln, accounted for 96% of patients with type 2N vWD.<sup>74</sup> Type 2N vWD should be considered in patients with a diagnosis of "mild hemophilia A" with a non-X-linked inheritance. Typically, heterozygotes have normal fVIII levels, and homozygotes have reduced fVIII activity. However, apparent heterozygotes with low fVIII levels typically have inherited a second allele, resulting in a vWD phenotype (compound heterozygotes for type 1 and type 2N vWD).<sup>75</sup> In summary, the more detailed genotype-phenotype correlations and relatively well-localized mutations provide rationale for genetic testing in the type 2 qualitative variants of vWD.

#### INHERITANCE OF vWD

Of the quantitative defects, type 1 vWD is an autosomal dominant bleeding disorder with incomplete penetrance, whereas type 3 vWD is autosomal recessive. Of the quantitative variants, types 2A, 2B, and 2M vWD are considered autosomal dominant, although selected patients classified under type 2A (eg, type IIC) have autosomal recessive inheritance. Type 2N (Normandy) vWD is considered autosomal recessive.

#### LABORATORY TESTING IN vWD

Results of laboratory testing should be interpreted with certain caveats in mind.

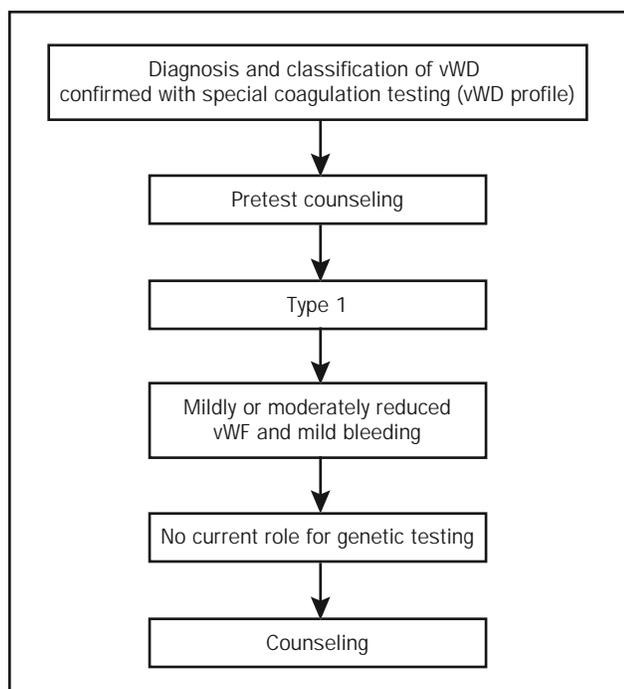


FIGURE 4. Rational approach to genetic testing in von Willebrand disease (vWD) type 1. vWF = von Willebrand factor.

#### GENETIC MODIFIERS OF vWF

People with blood group O have an average vWF level that is 25% to 30% lower than the non-O blood groups.<sup>31</sup> Therefore, ABO blood group typing should be part of initial testing. In addition, secretor-null persons have lower vWF levels compared with heterozygous or homozygous individuals.<sup>76</sup>

#### ENVIRONMENTAL MODIFIERS OF vWF

The development of acquired defects of vWF, as can be seen in patients with aortic stenosis,<sup>77</sup> mimics congenital type 2 vWD. In addition, presence of myeloproliferative disorders and monoclonal protein disorders<sup>78,79</sup> may also lead to acquired abnormalities of vWF. Combined oral contraceptive pills have been demonstrated to increase vWF levels.<sup>80</sup> However, although such data for women with vWD are not available, repeat vWF testing should be undertaken 4 to 6 weeks after discontinuation of oral contraceptives.

Pregnancy is known to increase vWF levels, especially in type 1 vWD<sup>81,82</sup>; thus, testing should be repeated when the patient is not pregnant. Generally, patients with type 2 vWD do not have a significant increase in vWF:RCo.<sup>83</sup> Short-term physical exertion leads to a rapid significant increase in vWF levels.<sup>84</sup> The presence of inflammation, malignancy, or hyperthyroidism increases vWF levels, whereas hypothyroidism is associated with reduced vWF

levels. After the diagnosis of vWD has been established, family counseling and genetic testing are typical issues that arise.

#### RATIONALE FOR GENETIC TESTING IN vWD

Challenges that face clinical molecular genetic testing for vWD include the fact that the gene for vWF is large and highly polymorphic<sup>85</sup> and has a pseudogene homologous to exons 23 to 34.<sup>47</sup> Furthermore, the molecular basis for the most common type 1 vWD is being defined, and mutations in the rare but most severe type 3 vWD are not localized to any specific region of the vWF peptide. Thus, currently, clinical genetic testing for types 1 and 3 is not generally available. The clustering of mutations in type 2 vWD variants makes clinical molecular testing more feasible.

As with any genetic disorder, the expense and lack of easy access to genetic testing should prompt a critical evaluation of indications and utility of genetic testing in a patient or family with vWD. From the diagnostic standpoint, the most important aspects of genetic testing in vWD, with the potential to alter patient management, include (1) genetic testing to differentiate mild hemophilia A from type 2N vWD, in which both genetic counseling and appropriate selection of clotting factor concentrates are key to patient management; (2) delineation of the causative mutation in families with type 3 vWD, in which management of future pregnancies may be needed (eg, consideration of termination of affected fetuses or management of delivery); and (3) the need to distinguish between types 2A and 2B vWD. This last factor is predicated on the relative contraindication of DDAVP in patients with type 2B vWD, which is being debated.<sup>86</sup>

Practically, however, the diagnosis of type 2N vWD is made based on the vWF:FVIIIb assay, and the diagnosis of type 2B vWD can be made with the standard profile of tests, including the ristocetin-induced platelet aggregation; thus, genetic testing may be used for confirmation of the diagnosis but is not essential. In contrast, delineation of causative mutation in families with type 3 vWD is essential if the information is to be used for prenatal testing. Although linkage analysis has been successful, recombination events, de novo mutations, and presence of the pseudogene increase the potential for misdiagnosis; furthermore, heterozygous carriers of mutations can have a normal vWD profile of tests and may be asymptomatic, further complicating the decision-making process, especially when termination of pregnancy is considered. Finally, careful phenotypic description of vWD using currently available protein-based testing may predict the genetic defect except in patients with types 1 and 3 vWD.

## RATIONAL APPROACH TO GENETIC TESTING IN vWD

The rationale for genetic testing of affected individuals can be summarized as follows.

### TYPE 1 vWD

**Genetic Testing of Affected Individuals.** Genetic testing should not be performed as the initial screening test (Figure 4). Given its typically mild phenotype, the evolving state of knowledge of the molecular basis of type 1 vWD and the implication of locus heterogeneity, genetic testing of the proband for establishing the diagnosis of vWD is not currently recommended. Currently, genetic testing to differentiate patients with borderline reduced or borderline normal vWF levels from a normal state is not recommended. The role of testing for the presence or absence of Tyr1584Cys mutations remains to be defined.<sup>61</sup> Further characterization of mutations found in pedigrees with vWD may aid in confirmation of the diagnosis but will not affect routine clinical management.<sup>21</sup>

**Genetic Testing of At-Risk Family Members.** Laboratory testing of potentially affected family members should consist of special coagulation testing (vWD profile). Given the inconsistent genotype-phenotype correlation, genetic testing has no role as the initial screening test, even though the affected individuals in the family may have had a mutation identified.<sup>55</sup>

**Prenatal Testing.** In type 1 vWD, given the typically mild to moderate phenotype and complex issues related to genotype and phenotype correlation and finally the risks associated with the procedure, prenatal testing has no role.

### TYPE 3 vWD

Genetic testing of families with type 3 vWD provides information that can significantly affect patient care; however, a critical appraisal of its utility is needed in each family (Figure 5). To address this, the following issues need to be considered: (1) do parents of the proband desire additional children; if they do (2) will they undergo prenatal testing; if they will (3) how will that information be used (ie, will the parents desire termination of pregnancy if the fetus is found to be affected or is the information simply to know if the fetus is affected, which may assist in management of labor and delivery). For both possibilities, in experienced hands, fetal cord blood sampling may provide the information needed for both termination and management of the pregnancy, although chorionic villus sampling will permit an earlier diagnosis.<sup>48</sup> For management of labor and delivery, unlike the case with severe hemophilia,<sup>87</sup> little is known about the risk of peripartum intracranial hemorrhage, but it seems to be low.

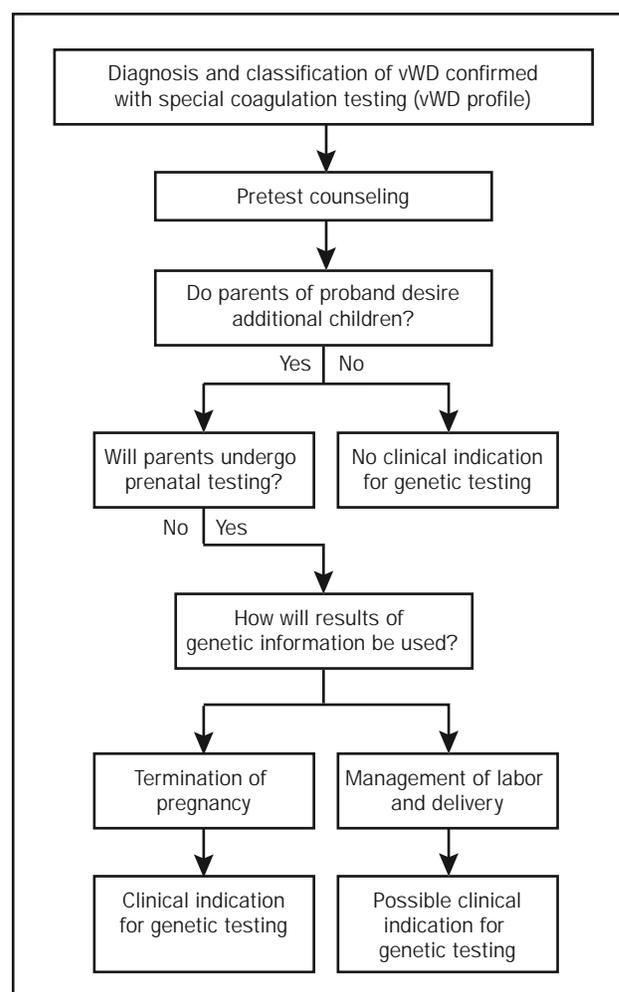


FIGURE 5. Critical pretest considerations for genetic testing in type 3 von Willebrand disease (vWD). Genetic testing may predict the risk of development of alloantibodies against von Willebrand factor. Fetal cord blood sampling, in experienced hands, is an alternative.

**Genetic Testing of Affected Individuals.** The disease has a severe phenotype, so the mutations are clearly deleterious when present in both alleles of an individual's vWF genome (Figure 6). Nevertheless, although genotyping the index patient may provide an estimate of the risk of developing inhibitors, it will not significantly influence routine clinical management for the affected individual. Direct DNA testing to delineate the mutation or assessment of haplotype for potential linkage analysis would be useful for genetic counseling and prenatal diagnosis of the parents in anticipation of management of future pregnancies (eg, consideration of termination of pregnancy or management of delivery or postpartum care of the neonate).

**Genetic Testing of At-Risk Family Members.** Initial testing of potentially affected family members consists of

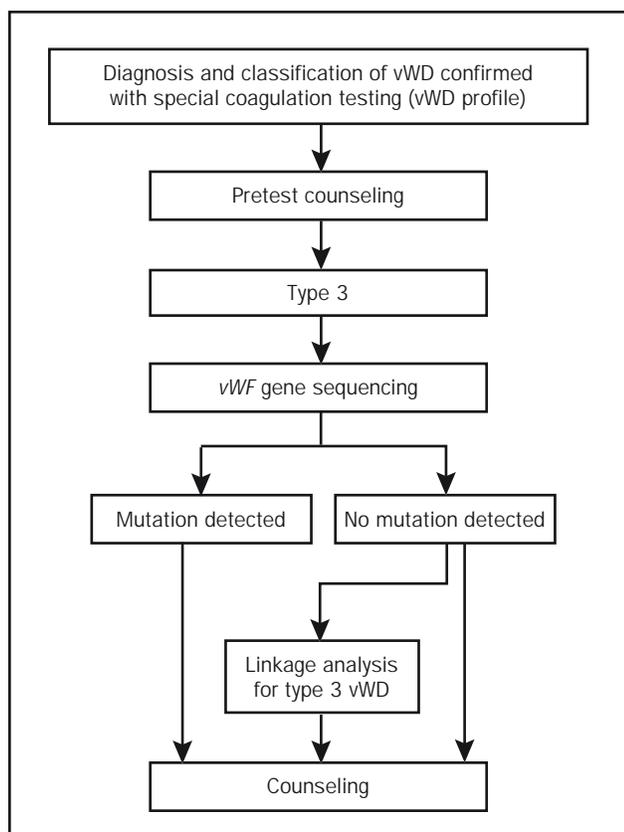


FIGURE 6. Rational approach to genetic testing in von Willebrand disease (vWD) type 3. von Willebrand factor (vWF) gene sequencing is performed if genetic testing for the family is thought to be indicated.

the vWD profile discussed herein; however, heterozygous carriers of type 3 mutations can have normal coagulation test results. Thus, in this group of patients, direct genetic testing is the only way to identify carriers. Although this information is critical for appropriate genetic counseling for the risk of having affected children, genetic testing as the only modality of testing is not advised.

**Prenatal Testing.** Direct DNA testing or linkage analysis is useful for genetic counseling and prenatal testing for potential termination of an affected pregnancy or management of delivery.<sup>48</sup>

#### TYPE 2 vWD

**Genetic Testing of Affected Individuals and At-Risk Family Members.** Differentiation of mild hemophilia A and vWD type 2N (Normandy) has the most significant impact on clinical management (use of vWF vs fVIII concentrates) and genetic counseling (autosomal inheritance pattern). The fVIII binding assays to confirm reduced vWF:fVIII should be performed, and genetic testing for the common mutations that are localized to the fVIII bind-

ing region on vWF can be used to verify the diagnosis (Figure 7).

Differentiation of types 2A and 2B vWD provides useful information that alters clinical management. As discussed, the standard vWD profile is typically sufficient to diagnose type 2B vWD, and genetic testing can be used to confirm the diagnosis. Although patients with type 2B are characterized by the presence of variable degrees of thrombocytopenia, such a clear distinction is not always possible; hence, genetic testing can be used for diagnosis. Although debated, the importance of such a distinction is believed to affect management, since the therapeutic use of vasopressin (DDAVP) is believed to be contraindicated in patients with type 2B vWD because of the potential for worsening the thrombocytopenia.

**Prenatal Testing.** Considering the relatively mild phenotype of type 2 vWD and risks of testing procedures, prenatal testing is not indicated.

#### METHODS OVERVIEW OF SPECIFIC GENETIC TESTS IN vWD

Recent reviews on commonly used methods in genetic testing are available<sup>88</sup> and will not be reiterated. In general, for direct DNA testing, initial screening methods can be used to detect mutations that typically should be confirmed with direct sequencing of the relevant regions. In addition, restriction length fragment polymorphisms can be used for focused testing. Indirect testing is available for vWD; however, it is rarely indicated except for severe type 3 vWD. The laboratories that offer such testing and the respective methods used are listed on the GeneTests Web site ([www.genetests.org](http://www.genetests.org)).

#### GENETIC COUNSELING

Although complex, both pretest and posttest counseling are critical components of genetic testing. A thorough explanation of the inheritance of vWD and limitations of information obtained with genetic testing is important. The training, time, and effort needed for optimal communication of the impact of genetic testing on multiple aspects of the patient and family make this a daunting task for the untrained individual.<sup>89,90</sup> In addition to the medical impact, issues related to psychosocial, economic, patient confidentiality, and health and life insurability, although beyond the scope of this article, are important and add another layer of complexity.<sup>91</sup> Thus, although physicians (eg, hematologists and obstetricians or other primary care physicians) may be knowledgeable about key issues, the time needed for such counseling in a busy clinical practice is not always available. Genetic counselors are likely best suited to provide such nonbiased, comprehensive counseling.

Since the absence of bleeding symptoms does not exclude the possibility of an individual being affected with

vWD, especially for milder phenotypes, family members of patients with vWD should undergo specialized coagulation testing (vWD profile). Except for families with type 1 vWD, the vWD profile test results, supplemented sometimes by genotyping information, reliably identify affected family members. Although counseling will be the same as for any autosomal dominant or recessive disorder, variable penetrance renders such counseling complex. Given the generally mild phenotype, prenatal testing is generally not performed. For the most severe phenotype, type 3 vWD, all potentially affected family members should undergo specialized coagulation testing, and for couples considering conception, the option of linkage analysis and prenatal testing should be discussed.

#### COUNSELING FOR SPECIFIC SUBTYPES OF vWD

**Type 1 vWD.** Type 1 vWD is autosomal dominant, but understanding and communicating the concept of variable penetrance of clinically important bleeding pose a challenge. Family members who inherit heterozygous mutations but have no clinically important bleeding may have normal results of laboratory testing even within the same family, yet other families with apparently heterozygous mutations may have clinically important bleeding. The latter case may be due to unsuspected compound heterozygosity for a second deleterious mutation. Thus, although genotyping an individual may demonstrate an apparently deleterious mutation, clinical management of both the patient and family members depends on results of coagulation testing (vWD profile) and clinical phenotype.

**Type 3 vWD.** Counseling for type 3 vWD is the same as for any autosomal recessive disorder. Patients with large deletions and selected homozygous nonsense mutations are at risk of development of alloantibodies against vWF.<sup>92</sup> However, not all patients with such mutations will develop antibodies. Practically speaking, knowledge of the risk of development of inhibitors may prompt closer surveillance; however, vWF factor concentrates are still the product of choice, although use of fVIII bypassing agents may be useful for management of hemorrhage.<sup>93</sup> Counseling a family with type 3 vWD typically involves explaining why parents of an affected child may be asymptomatic. Generally, affected patients are either homozygous or compound heterozygous for deleterious mutations, and either parent may have inherited a heterozygous mutation, which generally does not result in a bleeding phenotype, or selected families may have consanguineous parents. Management of the patient, as mentioned, will depend on clinical phenotype and results of vWD profile testing; however, results of molecular genetic analysis are useful for a discussion on the risk of having affected children and for prenatal testing.

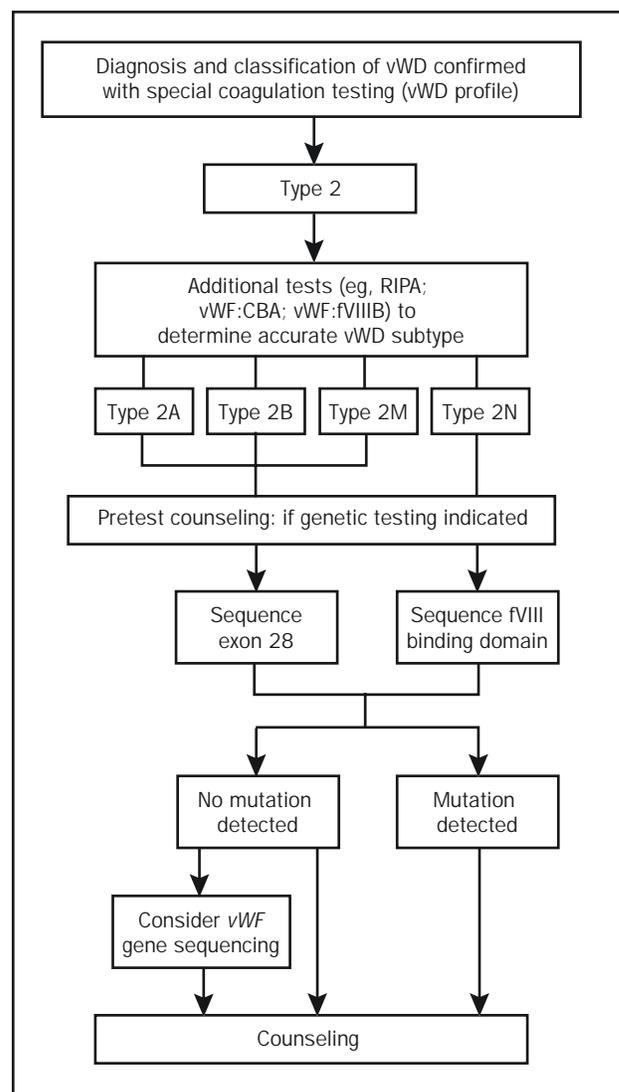


FIGURE 7. Rational approach to genetic testing in von Willebrand disease (vWD) type 2. von Willebrand factor (vWF) gene sequencing was considered if testing for mutations not found within exon 28. RIPA = ristocetin-induced platelet aggregation; vWF:CBA = von Willebrand factor collagen binding activity; vWF:fVIII = von Willebrand factor VIII binding activity.

**Type 2 vWD.** Types 2A, 2B, and 2M vWD are inherited in an autosomal dominant fashion; type 2N and certain subtypes (eg, type IIC) are autosomal recessive. The diagnosis of type 2N vWD is critical for differentiation from mild hemophilia A, since the latter is inherited as an X-linked recessive disorder; thus, counseling will be significantly different. In addition, management of prophylaxis and treatment of bleeding consist of the use of vWF-containing concentrates rather than fVIII concentrates. Accurate diagnosis of the type 2 variants is critical since the vWF protein is dysfunctional, and thus exogenous vWF concen-

trates are typically administered rather than DDAVP, which would release endogenous dysfunctional vWF.

## CONCLUSION

Genetic testing in the management of families with hereditary bleeding disorders in general and vWD in particular is evolving toward becoming part of routine care. Since there may be substantial costs of testing, as well as multifactorial impacts of testing results, the onus of optimal utilization rests with health care professionals. Comprehensive hemophilia centers and qualified genetic counselors can play important roles in counseling patients and their family members. A role for genetic testing for vWD can be expected to evolve considerably during the next few years as data from the European studies, coordinated by Peake (in the United Kingdom) and Rodeghiero (in Italy), become available, as well as its impact on the management of patients with vWD and their families.

## REFERENCES

- Mannucci PM. Treatment of von Willebrand's disease. *N Engl J Med*. 2004;351:683-694.
- Mann KG. Biochemistry and physiology of blood coagulation. *Thromb Haemost*. 1999;82:165-174.
- Lorentz CP, Wieben ED, Tefferi A, Whiteman DAH, Dewald GW. Primer on medical genomics, part I: history of genetics and sequencing of the human genome. *Mayo Clin Proc*. 2002;77:773-782.
- Rodeghiero F, Castaman G, Tosetto A, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. *J Thromb Haemost*. 2005;3:2619-2626.
- Sramek A, Eikenboom JC, Briet E, Vandenbroucke JP, Rosendaal FR. Usefulness of patient interview in bleeding disorders. *Arch Intern Med*. 1995;155:1409-1415.
- Wagner DD. Cell biology of von Willebrand factor. *Annu Rev Cell Biol*. 1990;6:217-246.
- Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001;276:41059-41063.
- Ruggeri ZM. Structure of von Willebrand factor and its function in platelet adhesion and thrombus formation. *Best Pract Res Clin Haematol*. 2001;14:257-279.
- Sadler JE. New concepts in von Willebrand disease. *Annu Rev Med*. 2005;56:173-191.
- Nilsson IM. Von Willebrand's disease—fifty years old. *Acta Med Scand*. 1977;201:497-508.
- Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. *Blood*. 1987;69:454-459.
- Werner EJ, Broxson EH, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: a multiethnic study. *J Pediatr*. 1993;123:893-898.
- Weiss HJ, Ball AP, Mannucci PM. Incidence of severe von Willebrand's disease [letter]. *N Engl J Med*. 1982;307:127.
- Mannucci PM, Bloom AL, Larrieu MJ, Nilsson IM, West RR. Atherosclerosis and von Willebrand factor, I: prevalence of severe von Willebrand's disease in western Europe and Israel. *Br J Haematol*. 1984;57:163-169.
- von Willebrand E. Hereditär pseudohefemofili. *Finska Lakaresallsk Handl*. 1926;5:7-112.
- Holmberg L, Nilsson IM. Genetic variants of von Willebrand's disease. *Br Med J*. 1972;3:317-320.
- Ruggeri ZM. Classification of von Willebrand disease. In: Verstraete M, Vermynen J, Lijnen R, Arnout J, eds. *Thrombosis and Haemostasis*. Leuven, Belgium: Leuven University Press; 1987:419-445.
- Sadler JE. Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. A revised classification of von Willebrand disease. *Thromb Haemost*. 1994;71:520-525.
- Schneppenheim R, Budde U, Ruggeri ZM. A molecular approach to the classification of von Willebrand disease. *Best Pract Res Clin Haematol*. 2001;14:281-298.
- Favaloro EJ. Appropriate laboratory assessment as a critical facet in the proper diagnosis and classification of von Willebrand disorder. *Best Pract Res Clin Haematol*. 2001;14:299-319.
- Laffan M, Brown SA, Collins PW, et al. The diagnosis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia*. 2004;10:199-217.
- Quiroga T, Goycoolea M, Munoz B, et al. Template bleeding time and PFA-100 have low sensitivity to screen patients with hereditary mucocutaneous hemorrhages: comparative study in 148 patients [published correction appears in *J Thromb Haemost*. 2005;3:622]. *J Thromb Haemost*. 2004;2:892-898.
- Sadler JE, Rodeghiero F, ISTH SSC Subcommittee on von Willebrand Factor. Provisional criteria for the diagnosis of VWD type 1. *J Thromb Haemost*. 2005;3:775-777.
- Preston FE. Assays for von Willebrand factor functional activity: a UK NEQAS survey [letter] [published correction appears in *Thromb Haemost*. 1999;81:478]. *Thromb Haemost*. 1998;80:863.
- Riddell AF, Jenkins PV, Nitu-Whalley IC, McCraw AH, Lee CA, Brown SA. Use of the collagen-binding assay for von Willebrand factor in the analysis of type 2M von Willebrand disease: a comparison with the ristocetin cofactor assay. *Br J Haematol*. 2002;116:187-192.
- Federici AB, Canciani M, Goodeve A, et al. Collagen binding (VWF : CB) is less sensitive than ristocetin cofactor activity (VWF : RCo) in suggesting von Willebrand factor (VWF) molecular defects in patients with von Willebrand disease type 1: results from the European Multicenter MCMMDM-1VWD study [abstract]. *J Thromb Haemost*. 2005;3(suppl 1). Abstract P0269.
- Nishino M, Girma JP, Rothschild C, Fressinaud E, Meyer D. New variant of von Willebrand disease with defective binding to factor VIII. *Blood*. 1989;74:1591-1599.
- Levy G, Ginsburg D. Getting at the variable expressivity of von Willebrand disease. *Thromb Haemost*. 2001;86:144-148.
- Sadler JE. Von Willebrand disease type 1: a diagnosis in search of a disease. *Blood*. 2003;101:2089-2093.
- Orstavik KH, Magnus P, Reiser H, Berg K, Graham JB, Nance W. Factor VIII and factor IX in a twin population: evidence for a major effect of ABO locus on factor VIII level. *Am J Hum Genet*. 1985;37:89-101.
- Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*. 1987;69:1691-1695.
- Rickles FR, Hoyer LW, Rick ME, Ahr DJ. The effects of epinephrine infusion in patients with von Willebrand's disease. *J Clin Invest*. 1976;57:1618-1625.
- Triplett DA. Laboratory diagnosis of von Willebrand's disease. *Mayo Clin Proc*. 1991;66:832-840.
- Favaloro EJ, Facey D, Grispoli L. Laboratory assessment of von Willebrand factor: use of different assays can influence the diagnosis of von Willebrand's disease, dependent on differing sensitivity to sample preparation and differential recognition of high molecular weight VWF forms. *Am J Clin Pathol*. 1995;104:264-271.
- Favaloro EJ, Aboud M, Arthur C. Possibility of potential VWD misdiagnosis or misclassification using LIA technology and due to presence of rheumatoid factor. *Am J Hematol*. 2001;66:53-56.
- Castaman G, Eikenboom JC, Missiaglia E, Rodeghiero F. Autosomal dominant type 1 von Willebrand disease due to G3639T mutation (C1130F) in exon 26 of von Willebrand factor gene: description of five Italian families and evidence for a founder effect. *Br J Haematol*. 2000;108:876-879.
- Abildgaard CF, Suzuki Z, Harrison J, Jefcoat K, Zimmerman TS. Serial studies in von Willebrand's disease: variability versus "variants." *Blood*. 1980;56:712-716.
- Hambleton J. Diagnosis and incidence of inherited von Willebrand disease. *Curr Opin Hematol*. 2001;8:306-311.
- Rodeghiero F, Castaman G, Di Bona E, Ruggeri M. Consistency of responses to repeated DDAVP infusions in patients with von Willebrand's disease and hemophilia A. *Blood*. 1989;74:1997-2000.
- Mannucci PM, Canciani MT, Rota L, Donovan BS. Response of factor VIII/von Willebrand factor to DDAVP in healthy subjects and patients with hemophilia A and von Willebrand's disease. *Br J Haematol*. 1981;47:283-293.
- Casonato A, Sartori MT, de Marco L, Girolami A. 1-Desamino-8-D-arginine vasopressin (DDAVP) infusion in type IIB von Willebrand's disease: shortening of bleeding time and induction of a variable pseudothrombocytopenia. *Thromb Haemost*. 1990;64:117-120.
- McKeown LP, Connaghan G, Wilson O, Hansmann K, Merryman P, Gralnick HR. 1-Desamino-8-arginine-vasopressin corrects the hemostatic defects in type 2B von Willebrand's disease. *Am J Hematol*. 1996;51:158-163.
- Pasi KJ, Collins PW, Keeling DM, et al. Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia*. 2004;10:218-231.

44. Lethagen S, Harris AS, Sjorin E, Nilsson IM. Intranasal and intravenous administration of desmopressin: effect on F VIII/vWF, pharmacokinetics and reproducibility. *Thromb Haemost*. 1987;58:1033-1036.
45. United Kingdom Haemophilia Centre Doctors' Organisation. Guidelines on the selection and use of therapeutic products to treat haemophilia and other hereditary bleeding disorders. *Haemophilia*. 2003;9:1-23.
46. Keeney S, Cumming AM. The molecular biology of von Willebrand disease. *Clin Lab Haematol*. 2001;23:209-230.
47. Mancuso DJ, Tuley EA, Westfield LA, et al. Human von Willebrand factor gene and pseudogene: structural analysis and differentiation by polymerase chain reaction. *Biochemistry*. 1991;30:253-269.
48. Peake IR, Bowen D, Bignell P, et al. Family studies and prenatal diagnosis in severe von Willebrand disease by polymerase chain reaction amplification of a variable number tandem repeat region of the von Willebrand factor gene. *Blood*. 1990;76:555-561.
49. Mercier B, Gaucher C, Mazurier C. Characterisation of 98 alleles in 105 unrelated individuals in the F8VWF gene. *Nucleic Acids Res*. 1991;19:4800.
50. Weiss HJ, Hoyer LW, Rickles FR, Varma A, Rogers J. Quantitative assay of a plasma factor deficient in von Willebrand's disease that is necessary for platelet aggregation: relationship to factor VIII procoagulant activity and antigen content. *J Clin Invest*. 1973;52:2708-2716.
51. Mannucci PM, Lombardi R, Bader R, et al. Heterogeneity of type I von Willebrand disease: evidence for a subgroup with an abnormal von Willebrand factor. *Blood*. 1985;66:796-802.
52. Hilbert L, Ribba A, Mazurier C, et al. In vitro expression of ten missense mutations in the VWFC-terminal domain identified in patients diagnosed with type I vWD: results from the MCMDM-1VWD study [abstract]. *J Thromb Haemost*. 2005;3(suppl 1). Abstract P0865.
53. Ribba A, Stepanian A, Habard D, et al. Two novel type I von Willebrand disease causing mutations (G160W and N166I) in the von Willebrand factor propeptide, identified in the European study MCMDM-1VWD, are responsible for multimerization and secretion defects [abstract]. *J Thromb Haemost*. 2005;3(suppl 1). Abstract P0265.
54. Nichols WC, Ginsburg D. von Willebrand disease. *Medicine (Baltimore)*. 1997;76:1-20.
55. Castaman G, Eikenboom JC, Bertina RM, Rodeghiero F. Inconsistency of association between type I von Willebrand disease phenotype and genotype in families identified in an epidemiological investigation. *Thromb Haemost*. 1999;82:1065-1070.
56. Nicolle AL, Talks KL, Hanley J. The genetics of bleeding disorders: a report on the UK Haemophilia Centre Doctors' Organisation annual scientific symposium, 10th October 2003. *Haemophilia*. 2004;10:390-396.
57. Zhang ZP, Lindstedt M, Falk G, Blomback M, Egberg N, Anvret M. Nonsense mutations of the von Willebrand factor gene in patients with von Willebrand disease type III and type I. *Am J Hum Genet*. 1992;51:850-858.
58. Eikenboom JC, Ploos van Amstel HK, Reitsma PH, Briet E. Mutations in severe, type III von Willebrand's disease in the Dutch population: candidate missense and nonsense mutations associated with reduced levels of von Willebrand factor messenger RNA. *Thromb Haemost*. 1992;68:448-454.
59. Mohlke KL, Nichols WC, Westrick RJ, et al. A novel modifier gene for plasma von Willebrand factor level maps to distal mouse chromosome 11. *Proc Natl Acad Sci U S A*. 1996;93:15352-15357.
60. Mohlke KL, Purkayastha AA, Westrick RJ, et al. MVWF, a dominant modifier of murine von Willebrand factor, results from altered lineage-specific expression of a glycosyltransferase. *Cell*. 1999;96:111-120.
61. O'Brien LA, James PD, Othman M, et al. Founder von Willebrand factor haplotype associated with type I von Willebrand disease. *Blood*. 2003;102:549-557.
62. Eikenboom J, Van Marion V, Putter H, et al. Linkage analysis of von Willebrand disease type I phenotype, von Willebrand factor levels and bleeding symptoms with Von Willebrand factor gene locus in the European Multicenter MCMDM-1VWD study [abstract]. *J Thromb Haemost*. 2005;3(suppl 1). Abstract OR279.
63. Berliner SA, Seligsohn U, Zivelin A, Zwang E, Soffer G. A relatively high frequency of severe (type III) von Willebrand's disease in Israel. *Br J Haematol*. 1986;62:535-543.
64. Zhang ZP, Falk G, Blomback M, Egberg N, Anvret M. A single cytosine deletion in exon 18 of the von Willebrand factor gene is the most common mutation in Swedish vWD type III patients. *Hum Mol Genet*. 1992;1:767-768.
65. Schneppenheim R, Krey S, Bergmann F, et al. Genetic heterogeneity of severe von Willebrand disease type III in the German population. *Hum Genet*. 1994;94:640-652.
66. Shelton-Inloes BB, Chehab FF, Mannucci PM, Federici AB, Sadler JE. Gene deletions correlate with the development of alloantibodies in von Willebrand disease. *J Clin Invest*. 1987;79:1459-1465.
67. Lyons SE, Bruck ME, Bowie EJ, Ginsburg D. Impaired intracellular transport produced by a subset of type IIA von Willebrand disease mutations. *J Biol Chem*. 1992;267:4424-4430.
68. Battle J, Lopez Fernandez MF, Campos M, et al. The heterogeneity of type IIA von Willebrand's disease: studies with protease inhibitors. *Blood*. 1986;68:1207-1212.
69. Schneppenheim R, Budde U, Obser T, et al. Expression and characterization of von Willebrand factor dimerization defects in different types of von Willebrand disease. *Blood*. 2001;97:2059-2066.
70. Weiss HJ, Sussman II. A new von Willebrand variant (type I, New York): increased ristocetin-induced platelet aggregation and plasma von Willebrand factor containing the full range of multimers. *Blood*. 1986;68:149-156.
71. Holmberg L, Berntorp E, Donner M, Nilsson IM. von Willebrand's disease characterized by increased ristocetin sensitivity and the presence of all von Willebrand factor multimers in plasma. *Blood*. 1986;68:668-672.
72. Miller JL. Platelet-type von Willebrand disease. *Thromb Haemost*. 1996;75:865-869.
73. Schneppenheim R, Federici AB, Budde U, et al. Von Willebrand disease type 2M "Vicenza" in Italian and German patients: identification of the first candidate mutation (G3864A; R1205H) in 8 families. *Thromb Haemost*. 2000;83:136-140.
74. Mazurier C, Meyer D, Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the ISTH. Factor VIII binding assay of von Willebrand factor and the diagnosis of type 2N von Willebrand disease—results of an international survey. *Thromb Haemost*. 1996;76:270-274.
75. Siguret V, Lavergne JM, Cheral G, et al. A novel case of compound heterozygosity with "Normandy"/type I von Willebrand disease (vWD): direct demonstration of the segregation of one allele with a defective expression at the mRNA level causing type I vWD. *Hum Genet*. 1994;93:95-102.
76. O'Donnell J, Boulton FE, Manning RA, Laffan MA. Genotype at the secretor blood group locus is a determinant of plasma von Willebrand factor level. *Br J Haematol*. 2002;116:350-356.
77. Vincentelli A, Susen S, Le Tourneau T, et al. Acquired von Willebrand syndrome in aortic stenosis. *N Engl J Med*. 2003;349:343-349.
78. Kumar S, Pruthi RK, Nichols WL. Acquired von Willebrand's syndrome: a single institution experience. *Am J Hematol*. 2003;72:243-247.
79. Federici AB, Rand JH, Bucciarelli P, et al. Subcommittee on von Willebrand Factor. Acquired von Willebrand syndrome: data from an international registry [published correction appears in *Thromb Haemost*. 2000;84:739]. *Thromb Haemost*. 2000;84:345-349.
80. David JL, Gaspard UJ, Gillain D, Raskinet R, Lepot MR. Hemostasis profile in women taking low-dose oral contraceptives. *Am J Obstet Gynecol*. 1990;163(1, pt 2):420-423.
81. Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. *Thromb Haemost*. 1984;52:176-182.
82. Kadir RA, Lee CA, Sabin CA, Pollard D, Economides DL. Pregnancy in women with von Willebrand's disease or factor XI deficiency. *Br J Obstet Gynaecol*. 1998;105:314-321.
83. Greer IA, Lowe GD, Walker JJ, Forbes CD. Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. *Br J Obstet Gynaecol*. 1991;98:909-918.
84. Hansen JB, Olsen JO, Osterud B. Physical exercise enhances plasma levels of extrinsic pathway inhibitor (EPI). *Thromb Haemost*. 1990;64:124-126.
85. Mancuso DJ, Tuley EA, Westfield LA, et al. Structure of the gene for human von Willebrand factor. *J Biol Chem*. 1989;264:19514-19527.
86. Kouides PA, Kasper C, Lipton R, et al. Desmopressin use in type 2B von Willebrand disease in North America: a survey of members of the Hemophilia Research Society of North America [abstract]. *Blood*. 2002;100:2750a. Abstract 98a.
87. Kulkarni R, Lusher JM. Intracranial and extracranial hemorrhages in newborns with hemophilia: a review of the literature. *J Pediatr Hematol Oncol*. 1999;21:289-295.
88. Tefferi A, Wieben ED, Dewald GW, Whiteman DAH, Bernard ME, Spelsberg TC. Primer on medical genomics, part II: background principles and methods in molecular genetics. *Mayo Clin Proc*. 2002;77:785-808.
89. Genetic counseling. *Am J Hum Genet*. 1975;27:240-242.
90. Biesecker BB. Goals of genetic counseling. *Clin Genet*. 2001;60:323-330.
91. Hook CC, DiMugno EP, Tefferi A. Primer on medical genomics, part XIII: ethical and regulatory issues. *Mayo Clin Proc*. 2004;79:645-650.
92. Eikenboom JC. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Best Pract Res Clin Haematol*. 2001;14:365-379.
93. Maddox JM, Anderson JA, Plews D, Ludlam CA. Management of acquired von Willebrand's syndrome in a patient requiring major surgery. *Haemophilia*. 2005;11:633-637.

The Genetics in Clinical Practice series will continue in the July issue.