

Severe von Willebrand disease due to a defect at the level of von Willebrand factor mRNA expression: Detection by exonic PCR–restriction fragment length polymorphism analysis

(human/gene amplification/DNA/blood coagulation factors/DNA polymerase)

WILLIAM C. NICHOLS*[†], SUSAN E. LYONS*[†], JONATHON S. HARRISON[‡]§, ROBERT L. CODY[‡],
AND DAVID GINSBURG*[†]¶

*Howard Hughes Medical Institute and Departments of [†]Human Genetics and [‡]Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109

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ABSTRACT von Willebrand disease (vWD), the most common inherited bleeding disorder in humans, results from abnormalities in the plasma clotting protein von Willebrand factor (vWF). Severe (type III) vWD is autosomal recessive in inheritance and is associated with extremely low or undetectable vWF levels. We report a method designed to distinguish mRNA expression from the two vWF alleles by PCR analysis of peripheral blood platelet RNA using DNA sequence polymorphisms located within exons of the vWF gene. This approach was applied to a severe-vWD pedigree in which three of eight siblings are affected and the parents and additional siblings are clinically normal. Each parent was shown to carry a vWF allele that is silent at the mRNA level. Family members inheriting both abnormal alleles are affected with severe vWD, whereas individuals with only one abnormal allele are asymptomatic. The maternal and paternal silent alleles are identical at two coding sequence polymorphisms as well as an intron 40 variable number tandem repeat, suggesting a possible common origin. Given the frequencies of the two exon polymorphisms reported here, this analysis should be applicable to ≈70% of type I and type III vWD patients. This comparative DNA and RNA PCR–restriction fragment length polymorphism approach may also prove useful in identifying defects at the level of gene expression associated with other genetic disorders.

von Willebrand factor (vWF) is a central component of hemostasis, serving as the carrier for factor VIII and as the adhesive link between platelets and blood vessel wall. Synthesis of vWF is limited to endothelial cells and megakaryocytes, where it is assembled from a 220-kDa monomer subunit into multimers ranging up to 20 MDa (1). The human vWF gene spans 178 kilobases (kb) and contains 52 exons corresponding to an 8.7-kb mRNA (2–7). The vWF gene has been localized to the short arm of chromosome 12 (2), and an unprocessed pseudogene comprising the middle third of the gene has been mapped to chromosome 22 (8).

Abnormalities of vWF result in von Willebrand disease (vWD), the most common inherited bleeding disorder in humans; estimated prevalence is as high as 1% (9). Type I vWD, accounting for 70–80% of cases, is characterized by a quantitative defect in vWF and is autosomal dominant in inheritance. The reduced penetrance and limited sensitivity of currently available diagnostic tests may leave many cases undiagnosed (10). Type III vWD denotes a severe form of the disease associated with major hemorrhagic symptoms and characterized by extremely low or undetectable levels of vWF (11). The incidence of type III vWD has been estimated at ≈1 per million (12–14). In some cases one or both parents

have type I vWD, and the severe vWD appears a manifestation of homozygous (or compound heterozygous) type I. In other cases both parents are asymptomatic, and the apparent mode of inheritance is autosomal recessive (13–15). Whether these patterns represent two distinct genetic disorders or rather manifestations of the broad spectrum and variable expressivity of type I vWD is currently unclear.

Until recently, little was known about the molecular defects responsible for vWD. Given the complex multistep biosynthesis and processing of vWF, defects at a number of loci outside of the vWF gene could conceivably result in similar vWD phenotypes. All genetic linkage studies in vWD to date, however, have been consistent with defects within the vWF gene (16–20). In addition, missense mutations within the vWF gene have recently been reported in a qualitative variant of vWD, type IIA (21). Although large gene deletions have been reported in several type III vWD families (8, 22, 23), Southern blot analyses in the majority of vWD patients show no evidence for gross gene deletion or rearrangement at the vWF locus. Further analysis of the molecular basis for vWD in this large group of patients has been limited due to the large size of the vWF gene and the lack of a ready source of vWF mRNA.

We report a general method for detecting genetic defects at the level of vWF mRNA expression. Using DNA sequence polymorphisms located within exons of the vWF gene, expression from the two vWF alleles can be distinguished by RNA PCR analysis from peripheral blood platelets. By this approach we have identified the defective vWF allele in a pedigree with type III vWD. Family members inheriting two abnormal alleles are affected with severe vWD, whereas individuals with only one abnormal allele are asymptomatic. These methods should be applicable to the identification of defects at the level of mRNA in numerous genetic disorders.

MATERIALS AND METHODS

Patients. Twelve members of a three-generation type III vWD family were investigated (Fig. 1). The proband (II-1) was initially referred to the University of Michigan for management of severe, recurrent epistaxis at the age of 39 years. She has had a severe bleeding diathesis throughout life, requiring frequent blood product support. During childhood, bleeding was manifest primarily by hemarthroses; however, in adult life repeated gastrointestinal bleeding and epistaxis have required infusion of cryoprecipitate several

Abbreviations: vWF, von Willebrand factor; vWD, von Willebrand disease; RFLP, restriction fragment length polymorphism; VNTR, variable number tandem repeat.

§Present address: Department of Medical Oncology, City of Hope National Medical Center, Duarte, CA 91010.

¶To whom reprint requests should be addressed.

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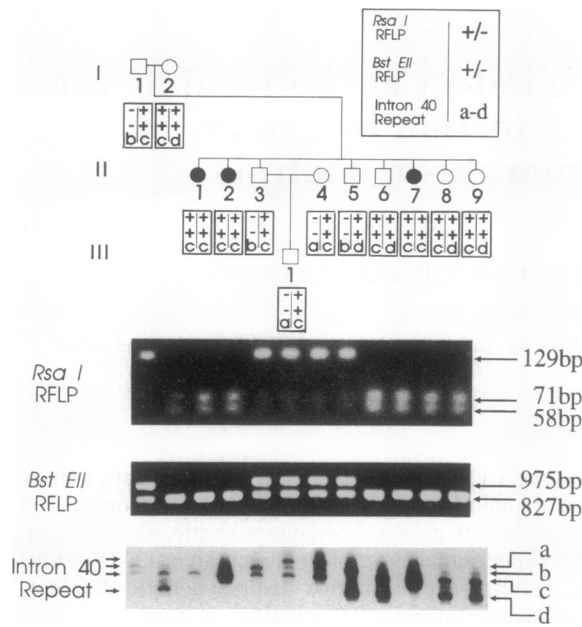


FIG. 1. Pedigree and haplotype analysis of a type III vWD family. Shown at top is the pedigree with generations indicated by Roman numerals at left and individuals identified by arabic numerals. The three affected members of the family (II-1, II-2, II-7) are shaded. Each individual's haplotypes are boxed; the polymorphisms represented by the haplotypes are indicated in the box at upper right. Shown below each individual are the PCR-restriction fragment length polymorphism (RFLP) analyses at all three loci; sizes of observed fragments are indicated in base pairs (bp) at right. The *Rsa* I PCR product is 129 bp in length; presence of the polymorphic *Rsa* I site at 2365 (+) results in cleavage of the 129-bp band to 71-bp and 58-bp bands. The *Bst*EII PCR product is 1166 bp; presence of the polymorphic *Bst*EII site at 4641 (+) results in cleavage of the 975-bp fragment to 827 bp (see Fig. 3) (smaller bands at 156 bp, 148 bp, and 35 bp are not shown). For the intron 40 repeat (shown at bottom), the four different alleles seen in the family (a-d) are indicated to the right and left. RFLP gels are approximately aligned below the corresponding family members.

times per week. Treatment with 1-deamino-8-D-arginine-vasopressin (dDAVP), estrogen, and progestational hormones has been unsuccessful. Agarose-gel vWF multimer analysis, done after 1 week without cryoprecipitate infusion, showed all multimers present but in extremely reduced amount. Two other siblings (II-2 and II-7) are also severely affected. Both parents (I-1 and I-2) are asymptomatic and of German ancestry, but neither is aware of any common relatives. Both a sister of I-2 and individual III-1 may have a history of abnormal bleeding, but no additional clinical information is available. No history of excessive or abnormal bleeding was obtained from the remainder of the family. Laboratory tests (Table 1) were performed by the Clinical Hematology Laboratory at The University of Michigan Medical Center. Agarose-gel multimer analysis was performed at Scripps Clinic Reference Laboratory.

Preparation of Total Cellular RNA and Genomic DNA. Platelet-rich plasma prepared from 40 ml of peripheral blood by centrifugation at $100 \times g$ for 10 min was centrifuged at $2500 \times g$ for 10 min to form a platelet pellet. Total RNA was prepared from this pellet, and high-molecular weight lymphocyte DNA was prepared from peripheral blood, both as described (21).

PCR. DNA and RNA PCR reactions were done as described (21). For RNA PCR, $\approx 1 \mu\text{g}$ of total platelet RNA was used as template for the reverse transcriptase reaction by using either oligonucleotide 1 or 4 as reverse transcriptase primer (Table 2) followed by PCR amplification. One microgram of genomic DNA was used as template for DNA PCR.

Table 1. Clinical data

Subject	VIII:C	vWF:Ag	vWF activity	ABO
I-1	152	110	117	AB
I-2	141	69	66	O
II-1	<3	<12.5	<12.5	A
II-2	<3	<12.5	<12.5	A
II-3	165	81	116	B
II-5	315	151	155	A
II-6	80	70	41	B
II-7	<3	<12.5	<12.5	A
II-8	104	56	41	B
II-9	184	92	86	B

VIII:C, factor VIII procoagulant activity; vWF:Ag, vWF antigen; vWF activity, vWF ristocetin cofactor activity; ABO, ABO blood group. The normal range for VIII:C, vWF:Ag, and vWF activity is 50–150 units.

The technique of allele-specific PCR was used to selectively amplify the authentic vWF gene (24, 25). Primer 3 was positioned such that the 3'-terminal nucleotide matches the sequence of the authentic vWF gene but differs from that of the vWF chromosome 22 pseudogene (M. Bruck and D.G., unpublished data).

Cloning and DNA Sequence Analysis. For analysis of the polymorphism at 4641, PCR products generated with primers 2 and 3 were digested with *Eco*RI and *Bam*HI and directionally subcloned into M13mp18 or M13mp19. Independent M13 clones were sequenced with Sequenase, as described (21).

Analysis of Coding Sequence Polymorphisms. Amplification products (5 μl) were analyzed either by restriction enzyme digestion or by allele-specific oligonucleotide hybridization. Restriction analysis was done for the polymorphisms at nucleotides 2365 and 4641 by using restriction endonucleases *Rsa* I and *Bst*EII, respectively, under conditions recommended by the manufacturer (Boehringer Mannheim). Restriction products were electrophoresed through agarose and visualized by ethidium bromide staining.

The *Bst*EII digestion products were transferred to Hybond-N nylon (Amersham) and probed with full-length vWF cDNA radiolabeled by hexamer priming (26). After overnight hybridization in $6 \times$ standard saline phosphate EDTA (SSPE) ($1 \times$ SSPE = 0.15 M NaCl/0.01 M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ /0.001 M EDTA, pH 7.4)/1% SDS and sheared salmon sperm DNA at 100 ng/ml at 68°C , the filter was washed to $0.05 \times$ SSPE/0.1%

Table 2. Oligonucleotide primers

No.	Sequence	Location
1	ACAGAGACGT	5414-
2	<u>CACGAAT</u> TCTACTGCAGCAGGCTACTGGAC-CTGGTCT	3808+
3	<u>CACGGAT</u> CCGGGAGCGTCTCAAAGTCCTGG-ATGAGGATA	4973-
4	GCCAATCTTC	2535-
5	TATCTGTGTCGGCCCCCATGGTCAAGCTGG	2294+
6	AGCCAGAGACACAGCCCCATGCTCATGCACT	2422-
7	CGAGTGTACCAAAAAC	2358+
8	CGAGTGTGCCAAAAC	2358+
9	ACAGGTCTAGAGGATCCAAGTTGACCTGGC	31/1497+
10	GGGCAGGTCACAGCTCACTGGGTACACA	31/2521-
11	AGTACTCCTACATGGTGACCGTGGAGTACC	4622+
12	CTTCAGCGAGGCACAGTCCAAAGGGGACAT-CCTGC	4653+

Sequences are shown from 5' (left) to 3' (right). Underlining indicates the non-vWF sequence added to the 5' end to create synthetic restriction sites (*Eco*RI for primer 2 and *Bam*HI for primer 3). Location for the 5' nucleotide of each oligonucleotide within vWF cDNA is based on the sequence of Bonthron *et al.* (7), except oligonucleotides 9 and 10, which are based on the genomic sequencing data and numbering system of Mancuso *et al.* (5).

SDS for 30 min at 68°C. Exposure to Kodak X-Omat film was for 15 min at room temperature.

The *Rsa* I polymorphism was analyzed by allele-specific oligonucleotide hybridization with oligonucleotide probes 7 and 8 (Table 2) using minor modifications of the procedure of Roth *et al.* (27). A final wash in 5× SSPE/0.1% SDS was done for 10 min at temperatures ≈0–5°C above the calculated probe melting temperature. Filters were exposed to film with an intensifying screen for 1–48 hr at –80°C.

Analysis of Intron 40 Variable Number Tandem Repeat (VNTR). Amplification using primer pair 9 and 10 (Table 2) was performed after end-labeling primer 10 with [γ -³²P]ATP and polynucleotide kinase. After PCR, the reactions were extracted once with phenol/chloroform and precipitated with 1/3 vol of 10 M ammonium acetate and 2.5 vol of 95% (vol/vol) ethanol. Precipitation products were collected by centrifugation and resuspended in 50 μ l of distilled H₂O. Ten microliters of each PCR was digested with 10 units of *Rsa* I in a 15- μ l reaction under conditions suggested by the manufacturer. Five microliters each digestion was fractionated on an 8% polyacrylamide/8.3 M urea sequencing gel. The dried gel was exposed to Kodak X-Omat film with an intensifying screen overnight at –80°C.

Southern Analysis of Genomic DNA. Five to 10 μ g of high-molecular weight DNA was digested with *Eco*RI, *Hind*-III, or *Bam*HI (Bethesda Research Laboratories); electrophoresed through 1% agarose; transferred to Hybond-N nylon; and probed with radiolabeled full-length vWF cDNA as described (2).

RESULTS

Identification of vWF Coding Sequence Polymorphisms. The strategy shown in Fig. 2 was used to identify defects at the level of vWF mRNA expression as a potential mechanism for vWD. For this approach, vWF sequence polymorphisms are characterized that are located within exons and, thus, can be scored by PCR at both DNA and mRNA levels. Once an exonic sequence polymorphism for which a given patient is

heterozygous at the DNA level is identified, RNA PCR can be used to detect loss of expression from either vWF allele at the mRNA level.

The first such PCR polymorphism, an A → G substitution at nucleotide 2365 [based on the sequence of Bonthron *et al.* (7)] located within exon 18 (5), was identified by comparison of published vWF cDNA and genomic sequence data (4, 5, 7, 28). This change results in the presence or absence of an *Rsa* I restriction site, facilitating detection by PCR–RFLP digest analysis (Fig. 1). From the screening of 100 normal vWF alleles, this polymorphism was found to have a frequency of 0.35, consistent with the recent report of Kunkel *et al.* (29).

A second high-frequency exonic RFLP was inadvertently identified in our studies of type IIA vWD (21). In one patient, only the mutant type IIA vWF allele was detected in peripheral blood platelet mRNA by RNA PCR analysis of exon 28, whereas both alleles were seen on PCR analysis of genomic DNA. The patient was hypothesized to be a compound heterozygote with a defect at the level of vWF mRNA expression on the non-type IIA vWF allele (21). However, subsequent RNA analysis of the *Rsa* I PCR–RFLP in exon 18, for which the patient was heterozygous, demonstrated equivalent mRNA expression from both alleles in this region of the gene. Further analysis in this patient by using different exon 28 primers showed normal expression from both vWF alleles at the mRNA level for this portion of the gene as well. DNA sequencing of exon 28 from this patient identified a single base substitution (C-4641 → T) on the normal vWF allele. This substitution had not previously been observed in any published sequence (4, 5, 7, 28) and is silent with respect to the amino acid sequence. This change is at position 20 (numbered 5' → 3') of the 30-base 5' oligonucleotide used in the RNA PCR studies (primer 11), and the resulting single-base mismatch leads to selective amplification of only the abnormal allele. This C-4641 → T substitution, which abolishes a *Bst*EII restriction site (Fig. 1), was evaluated by PCR–RFLP analysis with a panel of 103 normal DNAs and found to be a common polymorphism with an allele frequency of 0.36.

Haplotype Analysis of the Kindred. Fig. 1 shows that all members of the family were genotyped, and haplotypes were assigned for the *Rsa* I and *Bst*EII coding-sequence polymorphisms, as well as a 665-bp intron 40 VNTR reported to contain three different TCTA repeat units (5). Four alleles were detected for the 3' end of the VNTR and were arbitrarily (from largest to smallest) designated a–d; these four alleles were inherited in a Mendelian fashion by the family members. The total number of alleles and their frequencies for this portion of the intron 40 VNTR must await further analysis. A recent report by Peake *et al.* (30) detected eight different alleles for the middle portion of the VNTR in 106 normal vWF genes. The three severely affected individuals (II-1, II-2, and II-7) were homozygous for all three polymorphisms.

RNA PCR Studies. Although the three affected sisters (II-1, II-2, and II-7) were homozygous for the two exonic polymorphisms, their father (I-1) and two brothers (II-3, II-5) were heterozygous at both positions. PCR analysis of platelet mRNA revealed that while individual II-5 expressed both the *Bst*EII⁺ and the *Bst*EII[–] alleles, individuals I-1 and II-3 expressed only the *Bst*EII[–] allele (Fig. 3). DNA and mRNA PCRs were all done with the same primer set. Allele-specific oligonucleotide analysis for the *Rsa* I polymorphism demonstrated mRNA expression from only the *Rsa* I allele in individuals I-1 and II-3 and from both alleles in individual II-5 (data not shown). Individuals II-3 and II-5 inherited the same normal paternal allele (–b) but opposite maternal alleles with the maternal (+d) allele normally expressed (II-5) and the (+c) allele not expressed (II-3), as determined by RNA PCR. Thus, both the maternal and paternal (+c) alleles are silent at the mRNA level. As predicted by the haplotype

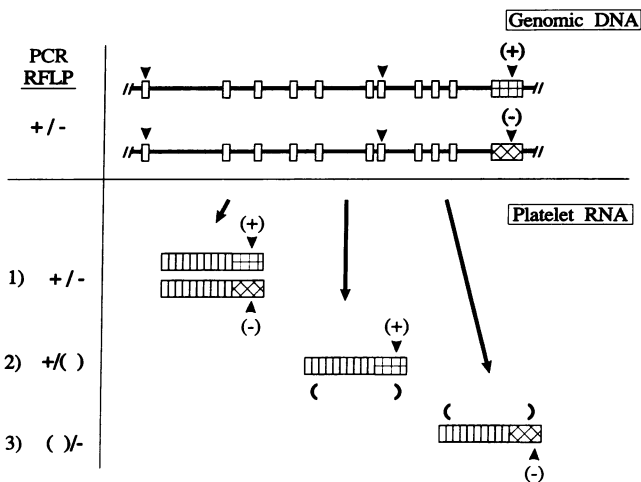


Fig. 2. Schematic of method for detecting defects in vWF mRNA expression. A group of exonic PCR sequence polymorphisms is assembled (represented by ▼). Genomic DNA is screened to identify one or more PCR–RFLP for which the individual is heterozygous (shown by +/-). PCR of the corresponding region of mRNA is subsequently applied to detect potential reduction to hemizygosity for either allele. As indicated, RNA PCR may demonstrate expression of both alleles (+/-) or only one of the two alleles [+/() or ()/-]. Reduction to hemizygosity at the RNA level could result from a number of different abnormalities in mRNA expression, including defects in transcription, mRNA splicing, and mRNA stability.

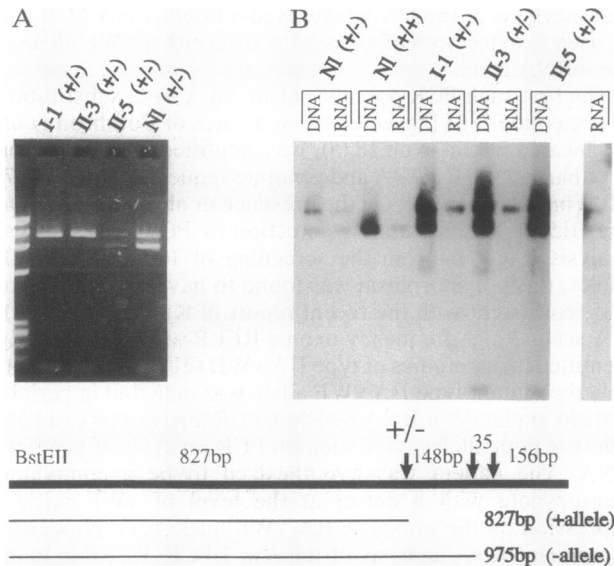


FIG. 3. RNA PCR-RFLP analysis of heterozygous family members. The C-4641 \rightarrow T substitution results in the loss of a *BstEII* site, as shown schematically at bottom. The locations of the three *BstEII* sites in exon 28 are marked by arrows with the polymorphic site marked (+/-). The diagnostic fragments of 827 bp (+allele) and 975 bp (-allele) are shown and are evident in A and B. The smaller fragments of 156 bp, 148 bp, and 35 bp have been run off the gel. (A) Ethidium bromide-stained agarose gel of RNA PCR products digested with *BstEII*. Platelet RNA was reverse-transcribed, amplified, digested, and fractionated on 2% agarose. Marker (ϕ X174 DNA digested with *HaeIII*) is shown in the leftmost lane. While individual II-5 is heterozygous for polymorphism at the RNA level, individuals I-1 and II-3 are hemizygous for the *BstEII*⁻ allele. A normal heterozygote control (+/-) is shown in lane N1 (+/-). (B) Southern blot analysis of *BstEII*-digested PCR products of genomic DNA and platelet RNA. Genomic DNA and platelet RNA were amplified, restricted with *BstEII*, fractionated on 2% agarose, transferred to nylon, and probed with radiolabeled full-length vWF cDNA. Although individuals I-1 and II-3 are heterozygous at the DNA level, only the *BstEII*⁻ allele is detected in both platelet RNA samples. Individual II-5 is heterozygous at both the DNA and RNA levels. For controls, a normal heterozygote [N1 (+/-)] and a normal homozygote [N1 (+/+)] are shown.

analysis, individual III-1 also carries the maternal (+/+c) silent allele, and this fact was confirmed by platelet RNA PCR-RFLP analysis for the *BstEII* marker (data not shown). Southern blot analysis of genomic DNA from all family members detected no evidence for gross vWF gene deletion or rearrangement (data not shown).

DISCUSSION

Although all published linkage studies of vWD to date are consistent with a defect(s) within the vWF gene, large gene deletions or rearrangements are rarely identified, suggesting that most cases of vWD are due to small insertions/deletions or point mutations within the vWF gene (2, 8, 16-19, 22, 23). Further characterization of the molecular defect(s) responsible for vWD has been difficult, given the large size and complexity of the vWF gene, the presence of a partial unprocessed pseudogene, and the lack of a readily available source of patient vWF mRNA. The advent of the PCR enabled detection of missense mutations at the vWF locus in association with type IIA vWD, aided by the localization of these mutations within a discrete region of vWF (21).

Since the quantitative decrease in vWF seen in type I and type III vWD could result from a variety of defects located anywhere within the 178-kb vWF gene, direct sequence analysis for these variants is currently impractical. Many of

these defects could, however, be due to disruption of vWF mRNA transcription or processing resulting in loss of mRNA expression from the affected allele. To screen for this class of mutations, we have developed an approach based on the analysis of vWF exonic sequence polymorphisms (Fig. 2). Affected individuals are first screened at the DNA level to identify one or more heterozygous exon polymorphism that is subsequently applied to RNA PCR analysis of peripheral blood platelet vWF mRNA. Apparent reduction to hemizygosity at the mRNA level indicates loss of RNA expression, which we term a "silent" allele. Given the allele frequencies of the two PCR-RFLPs reported here and our preliminary observation that they are in linkage equilibrium (W.C.N. and D.G., unpublished data), $\approx 70\%$ of individuals (including type I and type III vWD patients) should be heterozygous for at least one of these two polymorphisms. Using a larger panel of such PCR-RFLPs, this approach should be applicable to nearly all type I/III vWD patients.

Comparative DNA and mRNA PCR-RFLP analysis, as described here, should also be applicable to a number of other genetic disorders. Indeed, the use of RNA PCR has resulted in the fortuitous detection of silent alleles at both the insulin receptor locus and the branched chain α -ketoacid dehydrogenase locus by comparison with missense mutations on the opposite allele in compound heterozygotes (31, 32). Using a panel of PCR-RFLPs, our approach should be useful for a large number of patients with diverse genetic diseases.

As noted above, the polymorphism at position 4641 resulted in inadvertent allele-specific PCR initially interpreted incorrectly as loss of mRNA expression (21). Although allele-specific PCR can be successfully achieved by using primers with single-base mismatches positioned at the critical 3' nucleotide (24, 25) (as used for specific amplification of the authentic vWF gene over the pseudogene in this study), low levels of mismatched product are still frequently observed. The remarkable allele specificity inadvertently achieved with primer 11 (Table 2) is thus particularly surprising, given the location of the mismatch at base 20 of the 30-mer primer. Use of identical primer pairs for RNA and DNA PCR and confirmation by analysis of more than one RFLP should minimize the occurrence of this type of false positive result.

The two silent alleles detected in this type III vWD family are identical at two coding sequence polymorphisms as well as the intron 40 repeat. In addition, no difference between the two alleles could be detected by analysis at six other vWF coding polymorphisms (W.C.N. and D.G., unpublished data). This raises the possibility that the two alleles may carry the same mutation and could represent a common vWD allele. This finding could also result from undetected recent common ancestry between the parents, or alternatively, two independent defects that arose on the same genetic background. The population frequency for this particular haplotype is currently unknown.

Southern analysis detected no evidence for a gross vWF gene deletion or rearrangement. In addition, the heterozygosity at the DNA level detected in exons 18 and 28 and intron 40 rules out a deletion of at least these portions of the vWF gene. Potential abnormalities in one or both of these silent alleles include defects in transcription, processing, and mRNA catabolism. Numerous RNA-processing mutations giving rise to silent alleles have been identified in other genetic disorders (33-36). Given the complexity of the vWF gene (51 introns), a defect interfering with normal RNA splicing might be particularly likely. Other possible defects include mutations affecting transcription or nonsense/missense mutations within the coding sequence. The latter have been shown to result in reduced mRNA stability in some instances (33).

All six individuals in this family who are heterozygous for a silent vWF allele are asymptomatic with normal clinical

values, except individual II-8, who could be classified as having type I vWD (Table 1). Interestingly, asymptomatic carriers of type III vWD are frequently noted to have abnormal or low normal laboratory values (8, 14, 15, 22, 23, 30, 37). Given the low penetrance of vWD and the contribution of other factors, such as blood type and estrogen levels to phenotypic expression (10, 38), it is not clear that type III vWD can truly be distinguished from homozygous type I vWD. Type III vWD may be a heterogeneous disorder resulting from homozygosity (or compound heterozygosity) for a wide range of abnormal vWF alleles, including very mild or asymptomatic type I alleles and potentially mild qualitatively abnormal (type II) alleles. Heterozygosity for silent alleles, as identified here, might account for many of these asymptomatic type I alleles, whereas mutations giving rise to abnormal vWF, which interfere with multimer expression from the normal allele [dominant negative mutations (39)], might be responsible for the majority of symptomatic type I vWD alleles.

If a feedback mechanism exists for regulation of total cellular vWF mRNA, this could account for a high frequency of asymptomatic carriers among patients with silent alleles. In the homozygous individual, lack of expression from both alleles would result in severe vWD, whereas in the heterozygous individual, loss of RNA from the defective allele would trigger a compensatory increase in expression from the remaining normal allele, resulting in an asymptomatic or carrier phenotype. No such compensation would occur in vWF defects associated with stable mRNAs and would result in intermediate symptoms in heterozygotes, manifest as type I vWD. Such a mechanism could help explain the broad range of clinical severity among type I and type III vWD patients. Consistent with this hypothesis, the reported vWF gene deletions associated with severe vWD, which would also result in loss of mRNA expression from the defective allele, have been associated with a silent phenotype in the obligate heterozygotes. In preliminary studies from our laboratory, loss of expression from one allele has been observed in a second type III family with expression from both alleles observed in three type I patients.

Over the past several years, considerable progress has been made in our understanding of the structure and function of vWF and the molecular basis of vWD. A clearer picture of the phenotypic heterogeneity is beginning to emerge as the molecular defects responsible for distinct vWD variants are defined. With continued progress, it may eventually become possible to provide rapid and accurate DNA-based diagnosis and classification for this common and complex bleeding disorder.

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