Molecular Basis of von Willebrand Disease Type IIB

Candidate Mutations Cluster in One Disulfide Loop between Proposed Platelet Glycoprotein Ib Binding Sequences

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Abstract

Many variants of von Willebrand disease (vWD) with qualitatively abnormal von Willebrand factor (vWF) are recognized. In vWD type IIB, the abnormal protein displays enhanced affinity for a platelet vWF receptor, the glycoprotein Ib-IX complex. 14 patients from 7 unrelated families with vWD type IIB were studied to determine the molecular basis for this phenotype. Specific oligonucleotide primers were used to amplify portions of vWF exon 28 encoding a domain that interacts with the platelet glycoprotein Ib-IX complex. Candidate missense mutations were identified for all 14 patients by DNA sequencing, allele specific oligonucleotide hybridization, and restriction endonuclease digestion. These sequence changes occur in an 11 amino acid segment within a single disulfide loop bounded by Cys(509) and Cys(695). All of these sequence changes are C → T transitions within CG dinucleotides. Six patients from two unrelated families were heterozygous for the encoded sequence Arg(543) → Trp. Seven patients from four unrelated families were heterozygous for the encoded sequence Arg(545) → Cys; this sequence change appears to have occurred independently three times, once as a new spontaneous mutation. One patient with apparently sporadic vWD type IIB was heterozygous for the encoded sequence Val(553) → Met, and this appears to be a new mutation. None of these sequence changes was found in 100 normal alleles. These findings suggest that vWD type IIB may be caused by relatively few distinct mutations, that these mutations may cluster within a specific region of one disulfide loop in vWF domain A1, and that this region can modulate the affinity of vWF for the platelet glycoprotein Ib-IX complex. (*J. Clin. Invest.* 1991. 87:1220–1226.) Key words: von Willebrand factor • polymerase chain reaction • structure-function relationships • nucleotide transitions • platelet GPIb-IX complex

Introduction

Von Willebrand disease (vWD)[1] is the most common inherited human bleeding disorder. Most patients have simple quantitative deficiency of von Willebrand factor (vWF) and are referred to as vWD type I, but ~ 20% produce a qualitatively abnormal protein and are referred to as vWD type II. vWD type II is usually inherited as an autosomal dominant trait, and most type II variants show a relative decrease in large vWF multimers (reviewed in reference 1). Analysis of vWD type II has the potential to reveal interesting and unsuspected structure-function relationships for vWF.

vWD type IIB is a rare subtype of vWD that is characterized by enhanced affinity of vWF for a specific platelet receptor, the glycoprotein Ib-IX complex (GPIb-IX) (2, 3). It is generally inherited as a dominant disorder, but apparently recessive inheritance has been reported (4, 5). The characteristic functional abnormality in this variant is demonstrated by the ristocetin-induced platelet aggregation (RIPA) assay. The antibiotic ristocetin causes dose-dependent platelet aggregation when added to platelet-rich plasma, and this aggregation requires the interaction of plasma vWF with platelet GPIb-IX. Most variants of vWD exhibit decreased or absent RIPA, manifested by a reduced extent of aggregation or a requirement for increased concentrations of ristocetin, because of decreased vWF concentration or function. In vWD type IIB, however, very low concentrations of ristocetin, which have no effect on normal platelet-rich plasma, result in full platelet aggregation (2).

The enhanced RIPA in vWD type IIB is not simply a useful laboratory artifact, but reflects the pathophysiology of the disorder. In this subtype, the absence of large multimers in plasma appears to be due to spontaneous binding of vWF to platelets and subsequent clearance from the circulation (6). Patients with vWD type IIB can synthesize a full range of vWF multimers as shown by the normal multimeric pattern of platelet vWF (7), and by the transient normalization of the plasma multimer pattern upon administration of 1-deamino-[8-D-arginine]-vasopressin (DDAVP) (6, 8). In addition, cultured endothelial cells from a patient with vWD type IIB synthesized a full range of vWF multimers, and these multimers displayed increased affinity for platelets (9). Conditions that increase the release or synthesis of vWF are associated with thrombocytopenia in vWD type IIB. These include pregnancy (10, 11), surgical stress (12), and treatment with DDAVP (13). Genetic linkage analysis in one family has shown that the mutation causing vWD type IIB is linked to and probably within the vWF gene (14). Characterization of the mutations that cause this phenotype should identify regions of vWF that affect the interaction of vWF with platelet GPIb-IX.

The region of vWF that binds to GPIb-IX has been localized to a peptide including amino acid residues 480/481–718 of the mature subunit (15, 16) that is encoded by exon 28 of the vWF gene (17). Mutations in this region of the protein can be detected by PCR amplification of cDNA sequences, starting with vWF mRNA isolated from platelets (18). Alternatively,

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Received for publication 24 September 1990 and in revised form 19 November 1990.

1. *Abbreviations used in this paper:* GP, glycoprotein; RIPA, ristocetin-induced platelet aggregation; vWD, von Willebrand disease; vWF, von Willebrand factor.

J. Clin. Invest.

0021-9738/91/04/1220/07 $2.00
Volume 87, April 1991, 1220–1226

PCR methods could be used to characterize exon 28 sequences starting with genomic DNA isolated from leukocytes. The latter approach is complicated by the presence of a vWF pseudogene that differs only ~ 3% in sequence from the authentic gene and corresponds to exons 23–24 (19, 20).

Differences between the sequences of the vWF pseudogene and corresponding segment of the gene can be exploited to design oligonucleotide primers that allow amplification of vWF gene fragments without interference from the pseudogene (20). With this approach, three different candidate mutations were identified in seven unrelated families with vWD type IIB. These findings suggest that vWD type IIB is caused by a relatively small number of distinct mutations that cluster in an 11 amino acid segment of repeated domain A1, within a disulfide loop bounded by Cys(509) and Cys(695), and that this segment can modulate the affinity of vWF for platelet GPIb-IX.

Methods

Materials. Deaza T7-sequencing mixes, T7 DNA polymerase, T4 DNA polymerase, and T4 polynucleotide kinase were obtained from Pharmacia Inc. (Piscataway, NJ). Restriction endonucleases were obtained from Bethesda Research Laboratories (Gaithersburg, MD), Boehringer Mannheim (Indianapolis, IN), or New England Biolabs (Beverly, MA). The Klenow fragment of *Escherichia coli* DNA polymerase I, T4 DNA ligase, and calf intestine alkaline phosphatase were from Boehringer Mannheim. Taq DNA polymerase (Amplitaq) was purchased from Perkin Elmer-Cetus (Norwalk, CT). Deoxycytidine 5′-[α-^{32}P]triphosphate ([α^{32}P]dCTP), deoxyadenosine 5′-[α-^{35}S]-thiotriphosphate ([^{35}S]dATPαS), and [γ-^{32}P]ATP were purchased from Amersham Corp. (Arlington Heights, IL). Nitrocellulose membranes were from Schleicher & Schuell Inc., (Keene, NH). Oligonucleotides were synthesized on an Applied Biosystems synthesizer model 380A or 380B.

Patients. The 14 patients studied were from 7 unrelated Italian families affected with vWD type IIB. 4 families were reported previously in detail (2, 4, 21). The remaining 3 index patients and their families are typical for vWD type IIB with prolonged bleeding time, dominant transmission, increased RIPA, and type IIB multimer pattern in plasma. Platelet-type or pseudo-vWD was excluded by mixing experiments to confirm that the defect was in patient plasma and not in patient platelets (22, 23).

Polymerase chain reaction. The polymerase chain reaction was performed (24) with a Perkin Elmer-Cetus DNA Thermal Cycler. Typically, 100 ng of human peripheral blood leukocyte DNA were amplified with 2.5 U of Taq polymerase and ~ 70 pmol of each oligonucleotide primer in a total volume of 100 μl. Reactions without enzyme were preheated at 99°C for 5 min and then at 96°C for an additional 5 min after addition of Taq polymerase. DNA was amplified for 30 cycles of: 1 min at 96°C (denaturing), 1 min at 53–55°C (annealing), and 2 min at 72°C (extension) with a final extension time of 5 min at 72°C.

The primers employed for amplification of exon 28 were: No. 226, TGG GAA TAT GGA AGT CAT TG; No. 227a, CCG ATC CTT CCA GGA CGA AC; No. 375a, TCT TGG CAG ATG CAT GTA GC (20).

The amplified products were phosphorylated with T4 polynucleotide kinase, electrophoresed on 1% agarose gels (SeaPlaque; FMC BioProducts, Rockland, ME), and visualized by staining with ethidium bromide. Some fragments were polished with T4 DNA polymerase before phosphorylation. The desired fragment was excised and purified on Geneclean glass beads (Bio 101, La Jolla, CA), cloned into the Sma I site of plasmid pBluescript II KS+ (Stratagene Inc., La Jolla, CA), and propogated in *E. coli* strain XL1-Blue (Stratagene Inc.).

Allele specific oligonucleotide hybridization. Aliquots (~ 100 ng) of PCR amplified DNA fragments were denatured with 0.3 M NaOH for 15 min at 70°C, neutralized with 2.0 M ammonium acetate, pH 5.0, adsorbed onto a nitrocellulose membrane using a slot-blotting apparatus (Schleicher & Schuell Inc.), and fixed by baking for 2 h at 80°C in vacuo. Synthetic oligonucleotides that matched either the wild-type sequence or the proposed mutant sequence were labeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase. Hybridization conditions and wash buffer containing tetramethylammonium chloride were as described previously (25). Oligonucleotides employed for the detection of the C(4166) → T substitution were: wild-type, C(4166), ATG ATG GAG CGG CTG CGCAT; mutant T(4166), ATG ATG GAG TGG CTG CGC AT. Final washing conditions were three washes for 20 min each at 65°C in 3 M tetramethylammonium chloride, 50 mM Tris-Cl, pH 8.0, 2 mM EDTA-Na, 1 mg/ml sodium dodecyl sulfate. Oligonucleotides employed for the detection of the G(4196) → A substitution were: wild-type, G(4196), GGG TCC GCG TGG CCG TG; mutant, A(4196), GGG TCC GCA TGG CCG TG. Final washing conditions were as above except that the temperature was 58°C.

Miscellaneous methods. Restriction mapping and Southern blotting, DNA subcloning and sequencing, and preparation of peripheral blood leukocyte DNA were performed as described previously (17, 26).

Results

Amplification and characterization of vWF exon 28 sequences. Oligonucleotide primers for PCR amplification of exon 28 were chosen such that the 3′ ends of the primers could not anneal to the homologous segment of the pseudogene. Conditions were established that permitted the specific amplification of gene sequences without coamplification of the pseudogene sequences. This specificity was demonstrated by digestion with restriction endonucleases that generate different patterns for the gene and pseudogene sequences (20).

Fragments of exon 28 were amplified from each of 7 index patients with vWD type IIB and from selected relatives, and also from 50 unrelated controls. The patient population was from Italy, and most (40 of 50) of the controls chosen were also from Italy to minimize the likelihood that a sequence polymorphism prevalent among Italians but uncommon in other populations would be misidentified as a candidate mutation.

Amplification with primers 226 (5′) and 227a (3′) yielded a fragment of 936 bp that extends from 92 bp 5′ of exon 28 to nucleotide 4769 of the cDNA sequence, corresponding to the 5′ 844 bp of exon 28 and encoding amino acid residues 463–743 of mature vWF. Numbering of cDNA nucleotides assigns nucleotide 1 to the site of transcription initiation, which is 250 nt before the initiation codon ATG. Similarly, amplification with primers 226 (5′) and 375a (3′) yielded a fragment of 1,549 bp that contains all 1,379 bp of exon 28 and encodes amino acid residues 463–921 of mature vWF. Amplified DNA fragments were either cloned for DNA sequencing, or analyzed by restriction mapping or oligonucleotide hybridization.

Missense sequence changes were identified within exon 28 for all 14 patients from 7 unrelated families. In each case, the variant sequence was identified directly for the index patient by DNA sequencing of subclones isolated from PCR reactions. The variant sequence was also confirmed to be present in the genome of the patient and affected relatives, and absent in that of controls by either allele-specific oligonucleotide hybridization, characterization of restriction fragment length differences, or both. In no case was a candidate vWD type IIB mutation found in any of 100 unrelated control vWF alleles.

Candidate mutation Arg(543) → Trp. Two unrelated patients (patients 1 and 2) were identified with the same cDNA sequence change of C(4166) → T. This changes the encoded

amino acid sequence from Arg → Trp at residue 543 of the mature vWF subunit sequence (Fig. 1 *A*). Both patients were heterozygous for this sequence, consistent with the inheritance of vWD type IIB as a dominant trait in their families. This sequence variation does not alter a convenient restriction endonuclease site, and therefore allele-specific oligonucleotide hybridization was employed to confirm the genotype of the patients and family members, and to exclude the presence of this sequence in controls (Fig. 1 *B*).

Patient 1 was described previously as patient 90, family XLIV (21); patient 10, family 2 (2); and patient III_3 (4). Patient 2 was described previously as a member of a large pedigree affected with vWD type IIB (family 3) (2), and is the mother of the affected child whose cultured umbilical vein endothelial cells were employed to study the synthesis and properties of vWF type IIB (9). Inheritance of the altered allele was demonstrated in four affected relatives of patient 2 over three generations (data not shown).

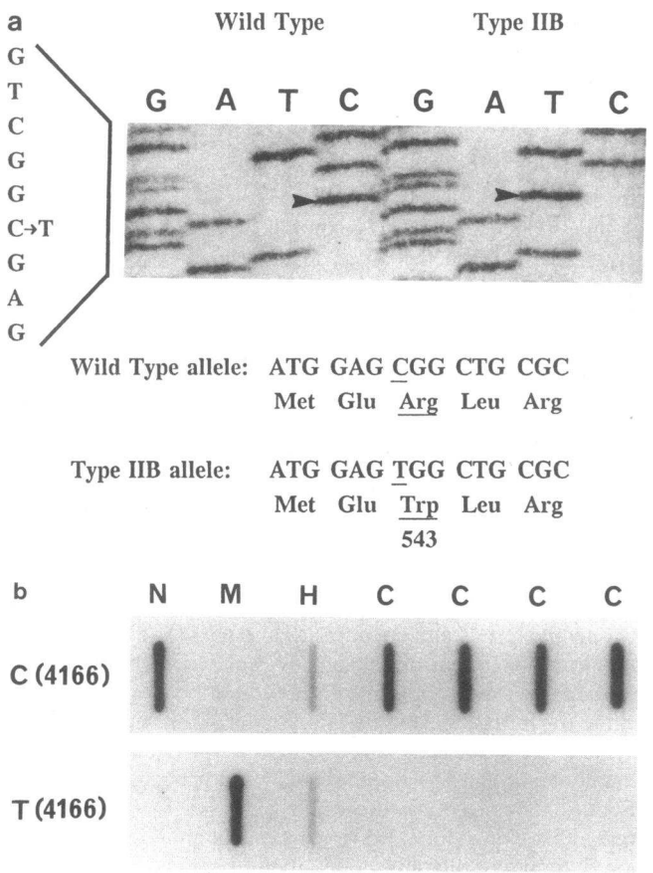

Figure 1. The Arg(543) → Trp substitution in vWF. (*a*) DNA sequence of normal and candidate mutant vWF alleles from patient 1. Representative sequences for the wild-type and mutant alleles are shown. The indicated C(4166) → T transition alters the encoded sequence from Arg(543) to Trp. (*b*) Allele specific oligonucleotide hybridization to PCR products. PCR products with primers 226 and 227a were analyzed by slot blotting and hybridization with radiolabeled oligonucleotide probes. The upper panel shows hybridization with a probe specific for the wild-type C(4166), and the lower panel shows hybridization with a probe oligonucleotide specific for the substitution T(4166). The DNA templates used for amplification were: *N*, cloned normal allele from patient 1; *M*, cloned mutant allele from patient 1; *H*, genomic DNA from heterozygous patient 1; *C*, genomic DNA from four unrelated controls.

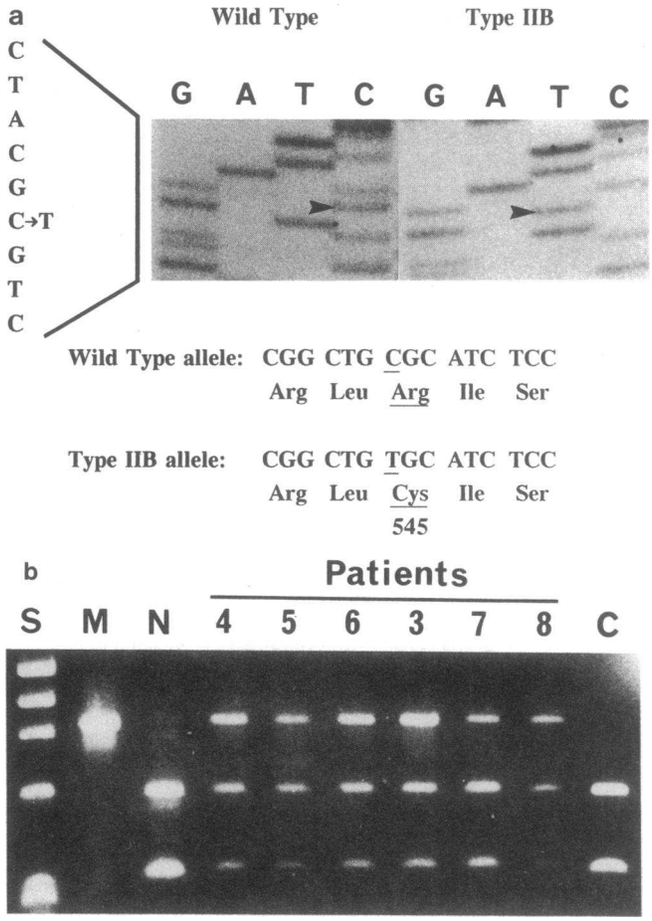

Figure 2. The Arg(545) → Cys substitution in vWF. (*a*) DNA sequence of normal and candidate mutant vWF alleles from patient 4. Representative sequences for the wild type and mutant alleles are shown. The indicated C(4172) → T transition alters the encoded sequence from Arg(545) to Cys. (*b*) Detection of the C(4172) → T transition by FspI digestion of PCR products. PCR was performed with primers 226 and 227a. The wild-type pattern consists of two fragments of 599 and 337 bp, and the C(4172) → T transition is not digested. Lane *S* is ΦX174/HaeIII standards of 1353, 1078, 872, 603, 310, and 281/271 bp. The DNA templates used for amplification were: lane *M*, cloned mutant allele from patient 4; lane *N*, cloned normal allele from patient 4; *patients*, genomic DNA from the indicated patients 3–8; lane *C*, genomic DNA from a control.

Candidate mutation Arg(545) → Cys. Four unrelated patients and three affected relatives (patients 3–9) were identified with the cDNA sequence change of C(4172) → T, changing the encoded amino acid sequence from Arg → Cys at residue 545 in the mature vWF subunit (Fig. 2 *A*). This sequence change destroys an FspI site. Control alleles were screened for this sequence change by restriction mapping of PCR fragments (Fig. 2 *B*). All of the patients were shown to be heterozygous, and none of 100 control chromosomes contained this sequence variation. Four of the patients (patients 4, 6, 7, and 8) are known to be related. Patient 7 is the mother of patient 6, patient 8 is the aunt of patient 4, and patients 6 and 8 have common grandparents. Thus the Arg(545) → Cys change and vWD type IIB show apparent coinheritance across four generations in this family.

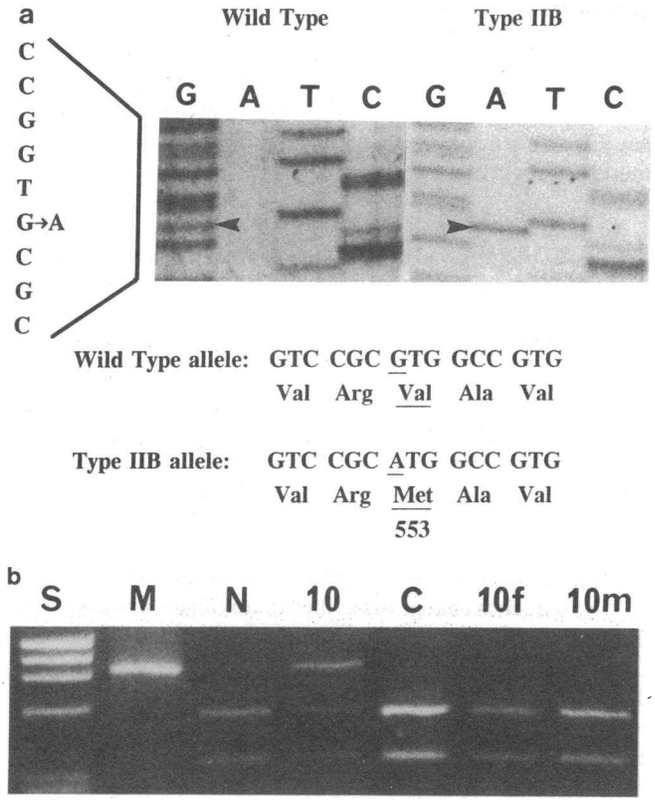

Figure 3. The Val(553) → Met substitution in vWF. (*a*) DNA sequence of a normal and candidate mutant vWF allele from patient 10. The indicated G(4196) → A transition alters the encoded sequence from Val(553) to Met. (*b*) Detection of the Val(553) → Met substitution by MvnI digestion of PCR products. Fragments were enzymatically amplified with primers 226 and 227a. The wild-type restriction pattern consists of two fragments of 575 and 361 bp, and the G(4196) → A transition is not digested. Lane *S* contains standards as described in the legend for Fig. 2. The DNA templates used for amplification were: lane *M*, cloned mutant allele from patient 10; lane *N*, cloned normal allele; lane *10*, genomic DNA from patient 10; lane *C*, genomic DNA from a control; lane *10f*, genomic DNA from father of patient 10; lane *10m*, genomic DNA from mother of patient 10.

The DNA sequences of the proposed mutant alleles were examined over the segment that corresponds to nucleotides 3925–4749 of the cDNA sequence. This segment includes the sites for several previously reported DNA sequence polymorphisms (17), and this provides a basis for distinguishing the genetic backgrounds in which the C(4172) → T sequence change occurs. Two polymorphic sites were informative: G/A(4391) and G/C(4664). Three different alleles bearing the candidate mutation T(4172) were observed. Patient 3 had the pattern T(4172)/A(4391)/G(4664), patients 4 and 5 (both from Vicenza) had the pattern T(4172)/G(4391)/G(4664), and patient 9 had the pattern T(4172)/G(4391)/C(4664). These sequence data suggest that the candidate mutation Arg(545) → Cys has occurred independently at least three times among the four unrelated families in this study.

One patient (patient 9) with this sequence change, from the Naples area, was previously reported to have sporadic and possibly recessive vWD type IIB (patient I_3; reference 4). The patient was heterozygous for this sequence. The mother (I_2) and father (I_1) of this patient did not carry the C(4172) → T change as determined by FspI digestion of amplified exon 28 (data not shown). Illegitimate paternity was excluded on the basis of analysis of blood groups and HLA markers (4). Thus, patient 9 appears to have acquired both this sequence change and the vWD type IIB phenotype as the result of a spontaneous mutation in a single generation, suggesting that the Arg(545) → Cys substitution is a new mutation causing vWD type IIB.

Candidate mutation Val(553) → Met. One patient (patient 10) previously reported to have possible recessive vWD type IIB (patient II_3) (4) was identified with the cDNA sequence change of G(4196) → A, changing the encoded amino acid sequence from Val → Met at residue 553 in the mature vWF subunit (Fig. 3 *A*). This nucleotide sequence change destroys a restriction site for the enzyme MvnI (Fig. 3 *B*). This substitution was not identified in any of 100 normal controls. Analysis of the parents, however, showed that both were homozygous for the normal sequence at this position. As reported previously (4), illegitimate paternity was excluded with a very high likelihood by analysis of blood groups and HLA polymorphic markers. These data suggest that the Val(553) → Met substitution in patient 10 is a new mutation causing vWD type IIB.

Discussion

The segment of vWF that binds platelet glycoprotein Ib-IX has been studied extensively by protein chemistry and immunochemistry methods. This binding activity resides in a ~ 50 kD tryptic peptide that contains amino acids 449–728 of the mature vWF subunit (15). This fragment inhibits platelet aggregation induced by vWF type IIB (27), suggesting that both normal and type IIB vWF bind similarly to GPIb-IX. A smaller 39/34-kD dispase fragment extending from Leu(480) or Val(481) to Gly(718) also possesses GPIb-IX binding activity (16). This smaller active peptide is encoded entirely by vWF exon 28 (Fig. 4) (17), and it corresponds roughly to one copy of the triplicated A domains in the middle of the subunit, domain A1 (28, 29).

This domain contains a large disulfide loop defined by the intrachain Cys(509)-Cys(695) disulfide bond (16, 30). Binding to GPIb-IX is decreased in affinity but not abolished by reduction and alkylation of this disulfide bond (16, 31). Most of this disulfide loop can be destroyed by digestion with trypsin, which cleaves after Arg(511) and Lys(673) (Fig. 4), and the resultant fragment binds to GPIb-IX with reduced affinity (30). Studies employing monoclonal antibodies and synthetic peptides to inhibit the binding of vWF to GPIb-IX have suggested that the noncontiguous segments Cys(474)-Pro(488) and Cys(695)-Pro(708) mediate this binding (32), while the intervening 205 amino acids including the Cys(509)-Cys(695) loop may be required to maintain the appropriate conformation of these discontinuous binding segments (30). At this time there is no reported evidence that alterations in the structure of this loop can enhance the binding of vWF to platelet GPIb-IX.

The most characteristic laboratory abnormality of vWF type IIB is its increased affinity for platelet GPIb-IX, and the mutations that cause this phenotype should identify structures of vWF that can modulate the affinity of the protein for this receptor. We have characterized three candidate missense mutations among 14 patients from 7 unrelated families with vWD type IIB. 2 of the patients (patients 1 and 2) were part of the

population used to define the phenotype of vWD type IIB in the original description of this variant (2). The sequences identified encode the amino acid substitutions Arg(543) → Trp, Arg(545) → Cys, and Val(553) → Met. Coinheritance of the Arg(545) → Cys substitution across four generations, and the Arg(543) → Cys substitution through three generations, confirms the previously reported linkage of vWD type IIB phenotype to the vWF gene (14).

The Arg(543) → Trp and Arg(545) → Cys substitutions are nonconservative substitutions that remove a positively charged residue. The Val(553) → Met substitution appears conservative because both valine and methionine are hydrophobic. Methionine contains a sulfoether bond, however, and substitution of methionine for valine is the least common mutation among large aliphatic amino acids in natural proteins (33). All three substitutions lie within the disulfide loop separating the discontinuous segments that may be important for the binding of vWF to GPIb-IX (Fig. 4). These findings suggest that the affinity of vWF for platelet GPIb-IX is increased by mutations in a small region of the vWF subunit, not previously associated with this function.

In principle, these sequence changes could be polymorphisms that are linked to the cause of vWD type IIB, and not mutations responsible for the disease. The indirect genetic data suggesting that they are mutations is, however, compelling. All patients studied had sequence changes within a 31-nucleotide segment of exon 28 and none of these sequence changes was found among 100 control vWF alleles, 80 of which were from the same country as the patients. The Arg(545) → Cys substitution was found in three distinct vWF gene backgrounds, suggesting that it has arisen independently at least three times. For these sequence changes to be polymorphisms would require that each of three rare polymorphisms be in extreme linkage disequilibrium with lesions that cause vWD type IIB, but not with other common sequence polymorphisms. The likelihood that this would occur is very small and the most parsimonious interpretation is that the observed sequence changes are mutations.

In addition, two patients (patient 9, Arg(545) → Cys; and patient 10, Val(553) → Met) appear to have acquired de novo both the vWD type IIB phenotype and a candidate missense mutation in a single generation. This pattern is very unlikely to occur by chance and suggests that each of these substitutions causes vWD type IIB. These studies provide strong genetic evidence that vWD type IIB is heterogeneous at the level of protein structure, that most such mutations cluster within a short segment of the protein, and that remarkably few mutations may account for a large percentage of vWD type IIB. Formal proof that these sequence changes cause vWD type IIB will require expression and phenotypic characterization of the corresponding mutant recombinant vWF proteins.

The sequence changes described in this report are all C → T transitions within CG dinucleotides. The cytidine residue in CG dinucleotides is frequently methylated (34) and may undergo deamination to yield thymidine (35), thus contributing to an unusually high rate of mutation at CG dinucleotides. Such CG dinucleotides are mutational hotspots in many genes; for example, frequent independent mutations have been documented at several CG sites in both the factor VIII and factor IX genes (36, 37). Among the patients of this study, the C → T transition associated with the Arg(545) → Cys substitution in vWF may have occurred independently at least three times,

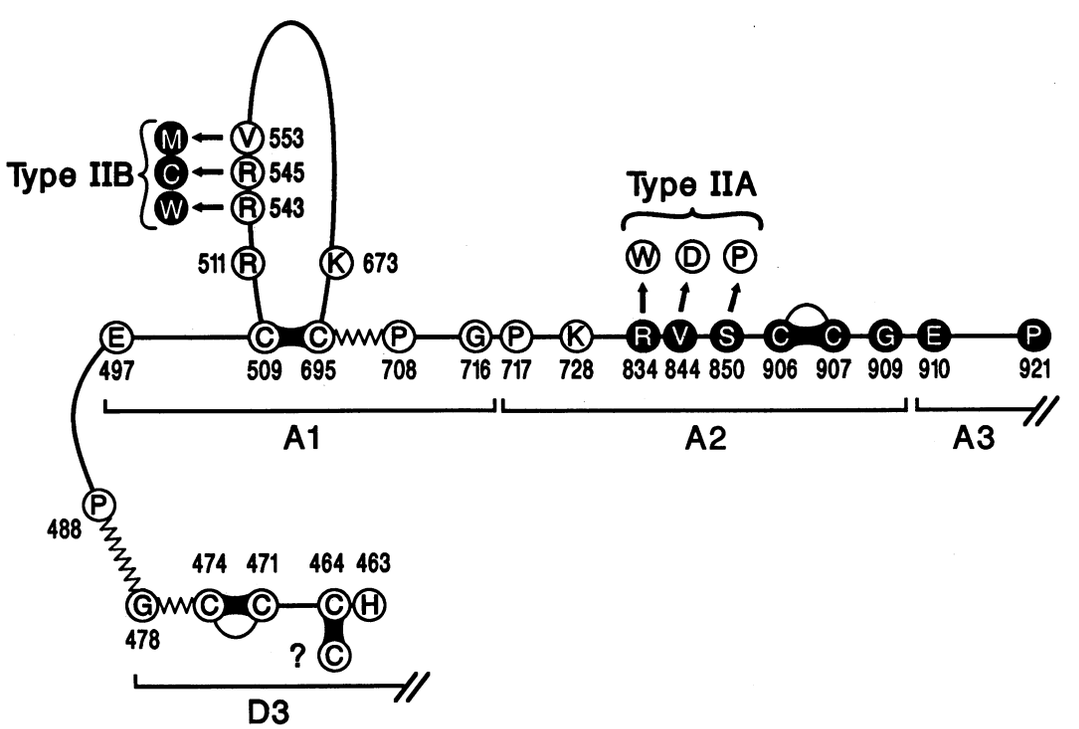

Figure 4. Candidate vWD mutations in exon 28. The segment of mature vWF encoded by exon 28, amino acids 463–921, is shown schematically. Disulfide bonds are indicated. The position of repeated domains *D3*, *A1*, *A2*, and *A3* are shown. The sequence changes identified in patients with vWD type IIB are shown, as are those reported previously in patients with vWD type IIA. The zigzag segments from Cys(474)-Pro(488) and Cys(695)-Pro(708) indicate regions reported to be important for interaction of vWF with platelet GPIb-IX. The transition from open to filled shading after Lys(728) indicates the site of trypsin cleavage that separates a GPIb-IX binding fragment from the remainder of the subunit. Other features are described in the text.

and the C → T transition associated with the Val(553) → Met substitution occurred once as a new mutation. Study of additional patients may confirm that the apparent high prevalence of a few C → T transitions in vWD type IIB is generally due to recurrent independent mutations in only a few CG dinucleotides.

The apparent clustering of mutations in vWD type IIB can be compared to the similar phenomenon in vWD type IIA (18, 38, 39). Candidate mutations in vWD type IIA cluster within a segment that is carboxyl-terminal to the region known to interact with platelet GPIb-IX. These mutations may cause the vWD type IIA phenotype either by preventing the synthesis of large vWF multimers or by enhancing the sensitivity of the mutant vWF to proteolytic degradation in the circulation (18, 38, 39) (Fig. 4). In both subtypes of vWD, potential mutations within different small segments of the vWF protein correlate with each distinct phenotype.

The proposed vWD type IIB mutations appear to modulate the binding of vWF to platelet GPIb-IX, perhaps by causing a conformational change in vWF that increases its binding affinity. A similar conformation change might normally accompany the binding of plasma vWF to subendothelial connective tissue, thereby promoting the adhesion of circulating platelets. Accordingly, vWF type IIB might adopt this active conformation in the absence of the inducing conditions that accompany vascular injury in vivo.

Acknowledgments

We wish to thank Dr. David Ginsburg (University of Michigan, Ann Arbor, MI) for sharing data on sequence changes in von Willebrand disease type IIB before publication, and Dr. Francesco Rodeghiero (San Bortolo Hospital, Vicenza, Italy) for providing samples from patients with von Willebrand disease.

This research was supported in part by National Heart, Lung, and Blood Institute grant HL-14147 (Specialized Center of Research in Thrombosis) and Training grant T32 HLBI 07088 from the National Institutes of Health, and by grants from Consiglio Nazionale della Ricerche, Progretto Finalizzato Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie, and Fondazione A. Bianchi Bonomi.

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