Ser968Thr Mutation within the A3 Domain of Von Willebrand Factor (VWF) in Two Related Patients Leads to a Defective Binding of VWF to Collagen

Anne-Sophie Ribba*, Irmine Loisel*, Jean-Maurice Lavergne, Irène Juhun-Vague1, Bernadette Obert, Ghislaine Cherel, Dominique Meyer, Jean-Pierre Girma

INSERM U 143, Hôpital de Bicêtre, Paris, 2CHU La Timone, Marseille, France

Keywords
- Recombinant von Willebrand factor, collagen binding, mutation, COS-7 cells transfection

Introduction

Von Willebrand factor (VWF) is a large multimeric glycoprotein composed of identical subunits associated through disulfide bonds. The mature subunits contain five types of structural domains present in one to three copies and designated A through D and CK (1). In plasma, VWF is essential for protecting factor VIII (FVIII) from proteolytic degradations. Under high shear stress conditions VWF also mediates platelet adhesion to the subendothelium at sites of vascular injury and promotes subsequent platelet aggregation (1). To promote its platelet functions, VWF acts as a molecular bridge between its platelet receptors (GPIb and GPIIb-IIIa) and components of the subendothelium like collagen. In these interactions the high molecular weight (HMW) forms of VWF bind to its various receptors more than the low sized multimers. Several studies have shown that VWF interacts with purified fibrillar type I and type III collagen (2) and with subendothelial microfibrillar type VI collagen (3–5).

Using proteolytic fragments of VWF and functional monoclonal antibodies, binding sites to fibrillar collagen have been identified within the A1 and A3 domains of VWF (6–8). Two regions extending between amino acids (aa) 542 to 622 and 948 to 998 have been involved in the binding (9). The binding site for microfibrillar type VI collagen has been located within the A1 domain (4, 10). Other studies have suggested that the A1 domain acts in concert with the A3 domain to bind circulating VWF to type VI collagen and to promote the subsequent high shear rate-induced platelet adhesion to subendothelium and aggregation (11). Using deleted recombinant VWF or recombinant VWF fragments, other groups have confirmed the presence of the main binding site for fibrillar type I and type III collagens within the A3 domain of VWF (12–14) and localized it between aa 1018 and 1114, appear to regulate the VWF binding to collagen. The crystal structure of the A3 domain (15, 16) has also been established and should allow to further investigate the mechanism of the VWF-collagen interaction.

Von Willebrand disease (VWD) is the most common congenital bleeding disorder. It results from quantitative (types 1 and 3) or qualitative (type 2) defects of VWF (17). In type 2, numerous molecular abnormalities have been identified which are responsible for modifying the structure of the binding site for FVIII (type 2N), or for platelet GPIb (types 2B and 2M) (database from Internet: http://mmg2.im.med.umich.edu/vwf) with, as a consequence, a defect of binding to either ligand of VWF. Other molecular abnormalities have been identified which result in VWF lacking HMW species (type 2A). Such molecular

*ASR and IL contributed equally to this work.

Correspondence to: Dr. A. S. Ribba, INSERM U 143, Hôpital de Bicêtre, 84 rue du Général Leclerc, 94276 le Kremlin-Bicêtre Cedex, France – Tel.: (33-1) 49-59-56-15, Fax: (33-1) 46-71-94-72, e-mail: ribba@infobiogen.fr
Abnormalities can alter the subunit dimerization (subtype IID), the multimerization of the dimers (subtype IIC) or the maturation and secretion of HMW multimers of VWF from endothelial cells (type 2A, group 1) (18). Molecular abnormalities can also modify the sensitivity to a plasmatic VWF-cleaving protease which mainly acts at the peptide bond 842Tyr-843Met of the subunit (type 2A, group 2) (19, 20). In all cases, the lack of HMW forms of VWF in plasma leads to an apparent decrease of its interaction with the platelet receptors GPIb and GPIIb-IIIa (21) and with collagen (22). However, so far, no molecular abnormalities have been associated with a defect of the VWF binding site for platelet GPIIb-IIIa (23) or for collagen.

We report here the identification, in two patients from the same family, of a new mutation Ser968Thr within the A3 domain of the mature VWF subunit. The mutation is associated with a decreased binding of VWF to collagen even though the full range of the VWF multimeric forms is present. Using site-directed mutagenesis and transient expression in COS-7 cells, we established that the Ser968Thr mutation induces an abnormal binding of recombinant VWF to collagen but does not alter the multimeric pattern, confirming the phenotype of the patients.

**Materials and Methods**

**Routine Coagulation Tests**

Measurement of Ivy bleeding time (BT) and assays of VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo) and factor VIII (VIII:C) were performed as previously reported (24). One hundred units of VWF:Ag, VWF:RCo and VIII:C are defined as the amount present in 1 dL of a pool of plasmas from 20 normal individuals. Platelet aggregation was performed on platelet-rich plasma (PRP) by measuring the extent of aggregation using arachidonic acid (1mg/mL) or various concentrations of ristocetin (1.25, 1 and 0.5 mg/mL), collagen (0.72 and 0.36 mg/mL) and ADP (2.5 and 1 μM). The range of VWF:Ag, VWF:RCo and VIII:C levels for normal individuals was between 50 and 150 U/dL, the bleeding time was between 4 and 8 minutes and the platelet count, determined on blood samples, was between 150 and 400 10^9/L.

**Plasma**

The study was performed on nine members from three generations of a French family with their informed consent. Control plasmas were from 16 normal volunteers and 13 patients with type 1 VWD as defined by international classification (17). All control plasmas exhibited a normal multimeric pattern of VWF. Results were compared to those obtained using a pool of plasma from 20 normal subjects.

**Mutation Identification**

Genomic DNA was extracted from peripheral blood leukocytes. Exon 28 was studied by denaturing gradient gel electrophoresis (DGGE) as previously described (25). Exons 29 to 32 of the VWF gene coding for the A3 domain were amplified by PCR in two steps using an automated thermal cycler (Perkin Elmer, Foster City, USA) and then analyzed by DGGE.

The first amplification of exons 29 to 32 of VWF gene was performed with primers allowing the specific amplification of the VWF gene and avoiding that of the pseudogene (26, 27). 200 to 500 ng of genomic DNA were incubated in PCR buffer (10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin) with 10 pmoles of specific primers, 0.2 mM dNTP, 1 U of Taq polymerase in final volume of 50 μL. After 5 min of denaturation at 95°C, samples were subjected to 20 amplification cycles. Each cycle comprised 2 min denaturation at 95°C, 2 min hybridization at 55°C or 63°C according to the primers couple, and 2 min elongation at 72°C. The PCR was terminated by a final elongation for 7 min at 72°C. A fragment of 911 base pairs (bp) corresponding to exons 29

<table>
<thead>
<tr>
<th>Primer’s name</th>
<th>Sequence</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2931A (st)</td>
<td>5’ACCTGTGTGCTACCTCCTTGTTG3'</td>
<td>nt 10454</td>
</tr>
<tr>
<td>2931B (ast)</td>
<td>5’CACAACATCCAAAGTAACCCAGC3’</td>
<td>nt 11365</td>
</tr>
<tr>
<td>32A (st)</td>
<td>5’AGTCCATTTTGGCACTTGTG3’</td>
<td>nt 13656</td>
</tr>
<tr>
<td>32B (ast)</td>
<td>5’CTCCATGAAGAACTTTAAAG3’</td>
<td>nt 14047</td>
</tr>
<tr>
<td>29A (st)</td>
<td>5’CCCCGCGCGCGCGGCGGCGGCGGGCTGTTG3’</td>
<td>nt 10454</td>
</tr>
<tr>
<td>29B(ast)</td>
<td>5’CTCTGGCGAAACTCTTATT3’</td>
<td>nt 10581</td>
</tr>
<tr>
<td>29C (st)</td>
<td>5’TCCAGCTTCTTTATTTGTAG3’</td>
<td>nt 10539</td>
</tr>
<tr>
<td>29D (ast)</td>
<td>5’CCCCGCGCGCGCGGCGGCGGCGGGCTCTCGACCTGTG3’</td>
<td>nt 10644</td>
</tr>
<tr>
<td>30A (st)</td>
<td>5’CCCCGCGCGCGCGGCGGCGGCGGCGGCTCTCGACCTGTG3’</td>
<td>nt 10678</td>
</tr>
<tr>
<td>30B (ast)</td>
<td>5’ATGCCAAATATAGACAGAAGG3’</td>
<td>nt 10902</td>
</tr>
<tr>
<td>31A (st)</td>
<td>5’CCCCGCGCGCGCGGCGGCGGCGGCGGATGCTG3’</td>
<td>nt 11053</td>
</tr>
<tr>
<td>31B (ast)</td>
<td>5’TGAATTCGATCGTACAGAAAG3’</td>
<td>nt 11301</td>
</tr>
<tr>
<td>32C (st)</td>
<td>5’CCCCGCGCGCGCGGCGGCGGCGGCGGTGCTG3’</td>
<td>nt 13685</td>
</tr>
<tr>
<td>32D (ast)</td>
<td>5’TGAGGGAAGTAAAGAAAGGATT3’</td>
<td>nt 13911</td>
</tr>
</tbody>
</table>
to 31 was amplified with primers 2931A and 2931B (Table 1). A fragment of 400 bp corresponding to exon 32 was amplified with primers 32A and 32B (Table 1).

The second amplification of 30 cycles allowed to amplify exons 29, 30, 31, and 32, independently, from PCR fragments of 911 bp and 400 bp using five couples of primers (29A-29B, 29C-29D, 30A-30B, 31A-31B and 32C-32D) (Table 1). PCR conditions were approximately the same as those described before except for hybridization temperature which was 55°C or 60°C according to the primers couple used. PCR products were then analyzed by DGGE. Fragments with altered mobility were excised from the gel, reamplified and sequenced both strands using dRhodamine Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, USA).

Plasmid Construct

Plasmid pSVVWFA containing the normal human full-length cDNA of VWF (28) was digested by restriction enzymes Nhel (nucleotide (nt) 5358 = aa 941) and EcoRV (nt 8483 = aa 1982). Nhel-EcoRV VWF cDNA fragment (3.2 kb) was then purified after electrophoresis on 0.8% low melting agarose gel and subcloned into the plSN 1100 Superlinker Phagemid (Pharmacia Biotech, Saclay, France), at the Nhel and EcoRV cloning sites. The new plasmid was called pSL-3'NE.

Site-directed mutagenesis was performed using the Quick Change™ Kit (Stratagene, La Jolla, CA) onto the plasmid pSL-3'NE using two phosphorylated and complementary mutagenesis primers (Oligo Express, Paris, France): S968T (strand): 5'CGTCTCACTCAGGTG and AS968T (antistrand): 5'GCTTCCATACTGCAGCACTG (Stratagene, La Jolla, CA) onto the plasmid pSL-3'NE using two phosphorylated and complementary mutagenesis primers (Oligo Express, Paris, France): S968T (strand): 5'CGTCTCACTCAGGTG and AS968T (antistrand): 5'GCTTCCATACTGCAGCACTG.

By sequencing that no undesired nt substitution was present, the mutated NheI-EcoRV fragment was subcloned into pSVVWFA digested in parallel with the same enzymes to obtain the mutated full-length cDNA plasmid, called pSVVVWS968T. The new plasmid was amplified with Maxi Plasmid Purification™ kit (Qiagen, Chatsworth, USA) and stored at –20°C before used.

Recombinant VWF

Three rVWF, wild type VWF (WTrVWF), T968rVWF and hybrid S/T968rVWF, were produced for this study. COS-7 cells were transfected by electroporation using expression vectors and cultured as previously described (29).

Hybrid S/T968rVWF results from cotransfection experiments using a mixture of 1/1) of plasmids pSVVWFA and pSVVWFS968T to mimic the heterozygous VWF from patients’ plasma.

Recombinant VFDF3 was a gift from Professor J. J. Sixma (Department of Haematology, University Hospital Utrecht, Utrecht, The Netherlands). It was expressed in BHK cell line overexpressing furin. Its characterization was performed by Lankhof et al. (13).

Monoclonal and Polyclonal Anti-VWF Antibodies

A series of murine monoclonal antibodies (MoAbs) (30) and two polyclonal antibodies directed against VWF were prepared. MoAb 487 and MoAb 9 are directed against the C-terminal part of the VWF subunit. MoAb 9 blocks the binding of VWF to GPIb-IIIa (23). MoAbs 505 and B20 are directed against the A3 domain of VWF and block the interaction of VWF with collagen. MoAbs 724 and 701 were raised by immunization with reduced VWF. All the antibodies used in this study were purified IgG fraction.

Labeling of the Antibodies

IgG were labeled with 125Iodine (Amersham, Les Ulis, France) using the Iodo-gen method. Specific radioactivity was between 1.0 and 3.0 μCi/μg. Labeled IgG were stored at 4°C for 1 month without apparent loss of reactivity with VWF.

PolyAb was also coupled to horseradish peroxidase to be used in ELISA. Coupled IgG were aliquoted and stored at –20°C.

Reactivity of MoAbs towards rVWF

MoAbs (5 μg/mL in carbonate/bicarbonate buffer, pH 9.6) were coated onto microtiter plates at 4°C for 18 h. Following postcoating with 50 mTris-HCl buffer, pH 7.4, containing 150 mM NaCl and 3% bovine serum albumin (TBS-BSA), serial dilutions of rVWF (0 to 5 U/mL in TBS-BSA) were incubated at 37°C for 18 h. After washing, bound VWF was estimated using 121I-polyAb.

VWF Structure Analysis

The multimeric structure of VWF was analyzed by 0.1% sodium dodecyl sulfate (SDS), 1% agarose gel electrophoresis using IEF agarose (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as described (31). After electrophoresis, the gel was incubated with 121I-polyAb. The multimeric structure of VWF was revealed by autoradiography.

Subunit analysis of VWF was performed by SDS- 5% PAGE under reducing conditions. Following blotting and incubation with 125I-labeled polyclonal antibody directed against reduced VWF (32), the proteins were revealed by autoradiography.

VWF-GP Ib Binding Assays

Platelet binding assays of VWF were performed as described by Siguret et al. (33) using 10 U/dL of VWF:Ag, 108 fixed platelets/mL in the presence of various concentrations of ristocetin (0 to 1.5 mg/mL) or botrocetin (0 to 1 μg/mL) and a constant amount of 125I-MoAb 487 to VWF as marker. The binding was expressed as the percentage of bound radioactivity to platelets. Non-specific binding was < 2% when estimated with conditioned media from cells transfected containing the expression vector without the full-length cDNA of VWF. Each experiment was performed three times in duplicate.

VWF Binding to Collagen

Assay of VWF binding to collagen was performed according to Lankhof et al. (13). Human placenta type I collagen (Sigma, St. Quentin Fallavier, France) was solubilized in 100 mM acetic acid (500 μg/mL) overnight at 4°C and subsequently dialyzed against 50 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (PBS) for 48h at 4°C to obtain fibrillar collagen. Microtiter plates were immediately coated with 100 μL of fibrillar collagen (50 μg/mL) for 48 h at 4°C and subsequently washed with PBS. Following postcoating for 30 min at 20°C with PBS containing 1% bovine serum albumin (BSA) (Calbiochem, La Jolla, CA), 100 μL of serial dilutions of plasma VWF or recombinant VWF in PBS-1% BSA were incubated for 2 h at 20°C. The wells were then extensively washed with PBS. The binding of VWF to collagen was measured at 490 nm by ELISA using 100 μL of a 1/2000 dilution in PBS-1% BSA of peroxidase-conjugated polyAb to VWF.

In all experiments, plates coated with the pool of MoAbs were used to determine the amount of VWF by ELISA. rVFDF3el or FVIII/VWF-deficient plasma (Diagnostica Stago, Asnières, France) were used as negative controls. Nonspecific binding was obtained by testing conditioned media from non-transfected cells. VWF collagen binding activity (VWF:CBA) is expressed as a function of VWF:Ag level for each dilution of plasma or recombinant VWF. The binding of VWF to collagen was estimated by the VWF:CBA/VWF:Ag ratio. The binding of normal pooled plasma VWF or WTrVWF was taken as 100%. Each experiment was performed three to five times in duplicate.
Results

Patients

The propositus (MC) is a 17 y. female suffering from life long bleeding episodes with epistaxis, ecchymosis, menorrhagia. She exhibited a slightly prolonged bleeding time (8-12.5 min). Platelet count was between 383 and $506 \times 10^9$/L. Platelet aggregation was normal when induced by arachidonic acid, or in the presence of various concentrations tested of ristocetin, ADP or collagen. She had a blood group O+ phenotype. VWF assays were performed on two independent samples, the last plasma sample being analyzed twice. The VWF levels were at the lower limit of the normal with VWF:Ag between 53 and 61 U/dL, and VWF:RCo between 45 and 58 U/dL, contrasting with normal levels of VIII:C between 81 to 112 U/dL. The multimeric pattern of her plasma VWF was normal (Fig. 1). Her mother (MN) is a 43 y. woman who suffered from epistaxis, ecchymosis, menorrhagia and severe post-partum bleeding, treated by transfusion. Bleeding time was normal or slightly prolonged (8-10 min). Platelet count was between 247 and 393 $\times 10^9$/L. Platelet aggregation induced by ristocetin, arachidonic acid, ADP or collagen was normal. Her blood group phenotype was O-. VWF assays were performed on four independent samples, the last sample being analyzed twice. VWF:Ag and VWF:RCo levels varied between 30 and 60 U/dL and VIII:C levels between 38 and 70 U/dL. The multimeric pattern of her plasma VWF was normal (Fig. 1). Seven other members of the family had no hemorrhagic syndrome and exhibited a normal phenotype.

Mutation Detection

Analysis by DGGE of exons 28 to 32 of VWF gene showed in both patients only the presence of a nt substitution in exon 30 in addition to known polymorphisms. Sequencing of exon 30 of both patients demonstrated the presence at the heterozygous state of a thymidine to adenosine transversion at nt 5441, changing the serine 968 for threonine within the A3 domain of the mature subunit. The mutation was not found in the other members of the family nor on alleles of 300 normal individuals excluding the presence of a new polymorphism.

Collagen Binding of Plasma VWF from Patients

The Ser968Thr mutation being localized within the A3 domain involved in the binding of VWF to collagen, the binding capacity of both patients’ VWF was compared with that of normal and type 1 VWD plasmas using human fibrillar type I collagen. The effect of unrelated plasma proteins was analyzed using F.VIII/VWF-deficient plasma.

Fig. 1 shows a typical dose-response curve of binding of plasma VWF from control and patients MN and MC to type I collagen. The slope of the curve (VWF:CBA/VWF:Ag) for the patients is equal to 64 and 70% of the control, respectively.

Fig. 2 summarizes our results. The mean ± SD of VWF:CBA/VWF:Ag ratio was 109 ± 22% for healthy volunteers ($n = 46$) and 102 ± 22% for VWD type 1 patients ($n = 29$). Patients MN and MC exhibited a lower ratio upon repeated testings. It was 75 ± 12% for MN ($n = 6$) and 73 ± 9% for MC ($n = 6$), respectively. Plasma VWF from other members of the family exhibited normal binding (not shown).

As a control, we observed that ristocetin- and botrocetin-induced binding of patients’ plasma VWF to platelet GPIIb was normal.

Expression and Characterization of Recombinant Mutated T968rVWF and Hybrid S/T968rVWF

The recombinant proteins WTrVWF, T968rVWF and hybrid S/T968rVWF were transiently expressed in COS–7 cells. The VWF:Ag secreted in the conditioned medium was similar for the three rVWF (~ 12 U/dL).

After concentration to 40-100 U/dL, the three rVWF were analyzed for their structure and binding capacity to collagen and GPIIb.

SDS-agarose gel electrophoresis of rVWF showed the presence of the full range of multimers in each of three recombinant proteins (Fig. 3). In addition, analysis of the subunit composition of rVWF by SDS-PAGE under reducing conditions followed by Western blotting showed the presence of a single band with an apparent molecular weight of 250 kDa (not shown).

Figs. 2 and 3 show the decreased capacity of T968rVWF and hybrid S/T968rVWF to bind to type I collagen. The typical dose response curves (Fig. 3) have slopes of 53 and 71% of that of WTrVWF, respectively. The mean VWF:CBA/VWF:Ag ratio was 59 ± 4% for...
Thromb Haemost 2001; 86: 848–54

T968rVWF (n = 6) as compared with WTrVWF (Fig. 2). It was 76 ±
12% (n = 6) for hybrid S/T968rVWF, that is comparable to that
observed for mutated plasma VWF from patients. The negative control
rVWFdelA3 had no binding capacity for collagen (Fig. 3).

The reactivity of MoAbs 487, 505, B200, 9, 701, 724 and 418 for
T968rVWF and S/T968rVWF was assayed by IRMA in which the
MoAb was coated and the 125I-polyAb was used to estimate bound
rVWF. In all cases the binding curves were superimposable to that of
WTrVWF used as control. In particular MoAb 505 and MoAb B200
which block the interaction between VWF and collagen normally
recognized mutated rVWF.

Mutated rVWFs tested for their ability to bind to platelet GPIb in the
absence or the presence of either ristocetin or botrocetin were not
significantly distinct from WTrVWF.

Discussion

In the present study we report the identification of a new mutation of
VWF gene that changes Ser 968 to Thr (S1731T of the preproVWF) in
the A3 loop involved in its interaction with collagen (7, 8, 12-14). The
mutation was identified at the heterozygous state in two subjects from
the same family (mother and daughter). The daughter (propositus) ex-
hibited a moderate bleeding disorder associated with slightly prolonged
bleeding time, moderately decreased levels of VWF, contrasting with
higher levels of VIII:C, and normal VWF multimeric pattern. Platelet
aggregation was normal in the presence of various inducers thus ex-
cluding a platelet abnormality that could have explained the bleeding
symptoms. The mother exhibited a similar phenotype but associated
with a borderline bleeding time and FVIII levels comparable to those of
VWF. Both subjects had a blood group O phenotype that could be
responsible for their levels of VWF at the lower limit of normal. Thus
the bleeding disorder observed in both subjects fitted neither with a
well-characterized platelet abnormality nor with a typical VWD. How-
ever, subjects’ VWF repeatedly demonstrated decreased binding to
collagen whereas their interaction with platelet GPIb, induced by either
ristocetin or botrocetin, was normal. In addition seven other members
of the family, who did not carry the Ser968Thr mutation, demonstrated
no bleeding symptoms and a normal VWF binding to collagen. In the
present study the collagen-binding capacity of VWF was estimated by
an ELISA test already used by several groups (22, 34) to distinguish
various types of VWD with respect to the antigen level and/or the
multimeric pattern of VWF. Plasma VWF from type 2A and type 2B
lacking large multimers has been reported to exhibit a decreased colla-
gen binding capacity (22). As the correction of the multimeric pattern
normalizes this abnormality (34-36), the collagen-binding defect of
type 2A and 2B VWF appears to only depend upon the lack of HMW
multimers (37). It was shown in addition that point mutations in type
2M VWD leading to a loss of binding capacity of VWF to platelet GPIb
in the presence of the full range of VWF multimers keep a normal bind-
ing capacity for collagen (38, 39). Thus the mutated VWF at position
968 does not exhibit the qualitative defects observed in type 2A, 2B or

![Fig. 2](image2.png)

![Fig. 3](image3.png)
type 2M VWD. Our results testing plasma from patients with type 1 VWD confirmed that type 1 VWF demonstrates normal binding to collagen (40). Our observations thus clearly show that the two subjects with the Ser968Thr mutation exhibited a defective binding of VWF to collagen. The functional and structural studies of the mutated T968-rVWF and hybrid S/T968rVWF confirmed that this specific mutation is directly responsible for the decreased binding to collagen observed in the two subjects. However, we cannot exclude that a second mutation of VWF is responsible for the associated bleeding disorder. If such a mutation were present, it should be located on the same allele as the Ser968Thr mutation and outside the A1 to A3 domains as shown by the normal phenotype of the other members of the family. However the presence of a second mutation on the VWF gene appears unlikely because our data demonstrate that the tested biological functions of subjects’ VWF are normal except for collagen binding. Thus it is likely that the Ser968Thr mutation that decreases the affinity of VWF for collagen participates to the bleeding disorder by altering the first step of platelet adhesion to the vessel wall.

It is noteworthy that the Ser968Thr substitution is located within the 948-998 sequence of the A3 loop previously described by Roth et al. (9) as being directly involved in its interaction with type I and III collagen. It may thus be hypothesized that Ser968 belongs to the interactive binding site on VWF and that the γ methylation leading to Thr causes a partial loss of affinity for collagen. However several pieces of evidence suggest that Ser 968 is not directly implicated in VWF interaction with the ligand. This second hypothesis is strongly supported by the crystal structure of the A3 domain of VWF already elucidated (15, 16). The VWF A3 domain displays an αβ fold, consisting of a central β-sheet with six strands, surrounded by seven α-helices. Five α-strands are parallel and one, which is located at an edge of the sheet, is anti-parallel (15, 16). Ser 968 is located in the β2-strand at the amino edge of the β-sheet, i.e. hidden inside the tertiary structure of the molecule but not exposed at the surface. Because that surface is supposed to expose the interactive binding site to collagen it is unlikely that Ser 968 belongs to it. In addition the results of binding experiments by Cruz et al (14) using a VWF/A1/A3 chimeric fragment also strongly suggest that VWF interactive site with collagen is located within the 1018-1114 sequence thus excluding Ser 968. Finally our data showing that MoAb 505 totally blocks VWF binding to collagen but normally interacts with T968rVWF is also in favor of the second hypothesis. It is thus likely that the Ser968Thr mutation is responsible for conformational changes propagated to a distant surface region of the A3 domain rather than for a disruption of the interactive binding site of VWF to collagen.

Acknowledgements

We thank Dr. K. Pouyamayou from CHU La Timone, Marseille, France, for providing the patients’ plasma and Professor J. J. Sixma from the Department of Haematology, University Hospital Utrecht, Utrecht, The Netherlands, for the gift of recombinant VWFdelA3.

References


Received July 11, 2000 Accepted after resubmission May 7, 2001