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Pseudohemophilia of Erik von Willebrand caused by homozygous one nucleotide deletion in exon 18 of the VW-factor gene

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Author contributions: Michiels JJ, Berneman Z and Schroyens W analysed the clinical features of congenital severe type 1 and 3 VWD and obligate heterozygous carriers; Gadisseur A and Michiels JJ analysed the molecular characteristics of severe type 1 and 3 VWD patients and wrote the manuscript.

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Abstract

The original description of a novel severe bleeding disorder as "Hereditary Pseudohemophilia" by Erik von Willebrand can currently be labelled as von Willebrand disease (VWD) type 3. VWD type 3 is autosomal recessive caused by homozygous or double heterozygous null mutations in the von Willebrand factor (VWF) gene and typically characterized by prolonged bleeding time and APTT, FⅧ: C levels below 2%, undetectable VWF: Ag, VWF: RC0 and VWF: CB and absence of ristocetin induced platelet aggregation (RIPA). Autosomal recessive von Willebrand disease type 3 VWD with virtual complete VWF deficiency are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA). Reports on severe recessive VWD compound heterozygous for a null allele and a missense mutation and homozygous or double heterozygous for missense mutations are associated with very low but measurable FⅧ and VWF: Ag and should be reclassified as severe recessive type 1 VWD. Homozygous missense or compound missense/null mutations related to recessive severe type 1 VWD have been indentified in the VWF prosequence D1 and D2 domains, the D4, B1-3, C1-2 domains, and only a very few in the dimmerization site (D3 domain). The detection of even tiny amounts of VWF: Ag after desmopressin acetate (DDAVP) or in hidden sites like platelets allows the differentiation between patients with VWD type 3 and homozygous or double heterozygous recessive severe type 1. Carriers of a null allele related to VWD type 3 or a missense mutation related with severe recessive type 1 VWD may present with mild VWD with low penetrance of bleeding in particular when associated with blood group O. Heterozygous obligatory carriers (OC) of a null mutation or a missense mutation related to recessive VWD type 3 or severe type 1 both present with asymptomatic or mild VWD type 1 in particular when associated with blood group O. The response to DDAVP of OC of either a nonsense or a missense mutation appears to be abnormal and diagnostic with a 3-times higher response of FⅧ: C as compared to VWF: Ag. In contrast, the responses to DDAVP of FⅧ: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

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Key words: Autosomal recessive von Willebrand disease type 3 and 1; Molecular etiology; Carrier of von
Willebrand disease null or missense allele; Desmopressin acetate responses

Core tip: The novel lethal bleeding disorder described as “Hereditary Pseudohemophilia by von Willebrand (VW)” in 1926 is caused by a homozygous nonsense mutation (one nucleotide deletion in exon 18) of the VW-factor gene consistent with autosomal recessive VW disease (VWD) type 3. Heterozygous carriers presented with VWD type 1 with variable penetrance of mild mucocutaneous bleeding manifestations. The present editorial reviews the clinical, laboratory and molecular features of severe recessive type 1 and 3 VWD and obligate heterozygous carriers of VWF nonsense and missense mutations.


INTRODUCTION

In 1926 Erik von Willebrand first described a novel severe bleeding disorder which he named “Hereditary Pseudohemophilia” in at least 4 affected members of the original large family S, living on the Föglo Aland Island[5]. In this report we review the available clinical, laboratory and molecular features of the original family S, which can now be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nucleotide deletion of exon 18) and of mild VWD type 1 with variable penetrance of bleeding manifestations in heterozygous carriers.

CLINICAL FEATURES

The pedigree of family S, originally described by Erik von Willebrand in 1926, has been updated and numbered by Blombäck in 1999 (Figure 1)[6].

The proband Hjördis S, case 16, aged 5 years was admitted on April 29 1924 to the hospital Diakoniantalten in Helsinki, Finland. At the age of 1 year, her bleeding tendency was observed after falling and hurting her nose and bled unusually long. At 3 years of age, she fell and had a deep cut in the upper lip. She bled heavily for 3 d and became bloodless and almost unconscious. She had to be confined to bed for 10 wk before recovering. After this she had frequent bruising, and regular episodes of epistaxis and gingival bleeding. An ankle distortion was followed by a severe articular bleeding with intense pain for some weeks (hemarthrosis). When a bleeding time according to the Duke method was performed she continued to bleed for 2 h and this had to be stopped by compression. Erik von Willebrand never visited the Aland Islands himself. Eight years later at the age of 13, Hjördis bled to death during her fourth menstrual period.

Her mother, Mrs Augusta, case 6, aged 44 years, had a history of frequent and persistent nose bleedings during her entire youth, but not lately. Menstruation (menarche) began at age 16 years and had a duration of 6 d or more, always copious, especially lately. She had experienced normal deliveries without severe bleeding. The bleeding time was normal. Augusta, the mother of Hjördis, (case 4, Figure 1), and four of 10 siblings in the family had an increased bleeding tendency.

Her father Mr Oskar S, case 5, aged 48 years, had rather sturdy nose bleedings, when he was young, and did not bruise more easily than other people. Neither of his parent (cases 1 and 2) had been bleeders. One of his sisters and several of her children (family E) had a moderate bleeding tendency.

Her oldest sister, Dagny S, case 7, had her first severe nose bleeding after a slight trauma at the age of 1 year. After this she had several nose bleedings and died from intestinal bleeding at the age of 2 years.

Her sister Anna S, case 8, began to have frequent nose bleedings from the age of 1 year. When she was 4 years old, she fell and two teeth penetrated her tongue. She had a bleeding that could not be stopped and died.

Her sister Dagny S, case 11, had thrush when she was a few weeks old. When her mother tried to loosen the membranes, there was a bleeding that almost would not stop. She bled much after insect bites and died at the age of 2 years from intestinal bleeding.

Her younger sister, case 17, aged 3 years, bruised easily since the age of 1 year and experienced heavy nose bleeds for the first time at age 1.5 years. Thereafter nose bleeds fairly often recurred. The bleeding often started spontaneously, and once went on for a whole week. The bleeding time was considerable prolonged. She had prolonged bleedings after insect bites. At the age of 5 she developed influenza, started to vomit blood and died within 20 h.

Her younger brother, case 19 (proven heterozygous for del 18 in 1990s), suffered in his youth from a slightly increased bleeding tendency, although he did not report

**LABORATORY FEATURES**

The inheritance of the disease in Family S could be followed through four generations\(^1-5\). In the female bleeders, the bleeding diathesis is manifested in a mild form or in a severe form, whereas the males show only the mild form of bleeding diathesis (Figure 1). Among female affected members with severe bleeding five deaths in one generation had occurred, four in childhood and one shortly after menarche (Figure 1). The women are of two types, those with a single and those with double trait. The former (heterozygotes) may had a milder form of bleeding, the latter (homozygotes) a severe lethal form. There was no opportunity to study the so-called homozygote women because they died from fatal bleeding before the reproductive age and long before FⅧ and the von Willebrand factor were identified as causes of hemophilia and VWD more than 3 decades later in the late 1950s. This means that data on the level of FⅧ:C and VWF parameters are lacking in those deceased women with the double trait (Table 1). This may explain why Jurgens \(et \ al\)^6 have falsely interpreted the bleeding disorder as a constitutional thrombopathy mainly based on the very prolonged bleeding times. Jurgens \(et \ al\)^7 mentioned several years later a deficiency of an antihemophilic factor (FⅧ) in heterozygous affected family members with mild VWD, but the cases with severe bleeding could not studied anymore at that time.

**Molecular Studies**

The DNA samples were screened for mutations with PCR, followed by direct sequencing in the “hot spot” regions in exons 18, 28, 32, 43 and 45 found in the Swedish-Finnish patients\(^8,9\). In the original family S one nucleotide deletion in exon 18 was identified in heterozygous carriers, which had been found to be a “hot spot” in the majority of VWD type 3 patients in Sweden\(^10\). This mutation interrupts the reading frame leading to an early translational stop (null allele). Five individuals (numbers 13, 14, 19, 20 and 25 in Figure 2) all having VWD type 1 were found to be heterozygous for the deletion. The deceased family member 12 must also have carried the same deletion, as his daughter is a carrier. In the third generation, at least four individuals (numbers 12, 13, 14, 19, Figure 2) carry the deletion. These results indicated that the deletion originated from the parents of family S (number 5 and 6, Figures 1 and 2) who are thought to be heterozygous. All five daughters, who died from uncontrolled bleedings, very likely would have been homozygous for the deletion in exon 18 consistent with pseudohemophilia, now called VWD type 3.
The three siblings, Harald, Sylvia and Runar, (cases 12, 13 and 14, proven heterozygous for del 18) had more less severe nose bleeds, especially in their youth but never experienced a pronounced bleeding tendency during life-long follow-up. These data demonstrate that the original family S, described by Erik von Willebrand as pseudohemophilia A, has to be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nucleotide deletion of exon 18). In the family S in addition to the deletion in exon 18, two mutations at S1263 and P1266 in exon 28 were identified in two siblings (numbers 24 and 25, Table 1, Figure 2) with an unrelated and clinically normal father, who married into the family S. The transition G→A at S1263 is neutral, and the other C→T at P1266 results in an amino acid substitution of proline to leucin (P1266L). P1266L is a frequent mutation in Sweden and has been described as VWD type 1 Malmö with increased ristocetin induced platelet aggregation (RIPA) (mild 2B).

**DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE TYPE 3 VWD**

The inheritance of a pronounced bleeding tendency in subsequent reports of families with VWD type 3 is autosomal recessive\(^{11-18}\). Patients with VWD type 3 are typically characterized by prolonged bleeding time (BT) and APTT, FVIII: C levels below 2%, undetectable VWF: Ag, VWF: RCo and VWF: CB levels before and after desmopressin acetate (DDAVP) and absence of RIPA\(^{13}\). VWD type 3 patients with FVIII: C levels above 2% and detectable levels of VWF: Ag and response of FVIII: C to DDAVP should be reclassified as severe type 1 VWD caused by double heterozygous for a nonsense/missense mutation or homozygous or double heterozygous missense mutation causing a severe secretion defect\(^{11,12,13}\).

In 31 cases diagnosed as VWD type 3, (age 2 to 80, median 15 years) described by Schnepenheim et al\(^{13}\) bleeding manifestations were recorded as easy bruising and prolonged epistaxis in 31 (100%), spontaneous joint bleeding in 23 (76%), muscle bleeding in 7 (22%) and gastrointestinal bleedings in 3 (10%). The bleeding manifestations and complications of childbirth have been nicely evaluated in 385 Iranian patients diagnosed as autosomal recessive type 3 VWD (SSC-ISTH classification) and compared to age matched severe hemophilia A\(^{18}\). Among patients with type 3 VWD the incidences of spontaneous hemarthrosis (37%) and muscle bleedings (52%) are lower most likely because FVIII: C levels are higher (1%-9%) as compared to severe hemophilia A (≤ 1%).

Type 3 VWD with virtual complete VWF deficiency (severe VWD) and absence of FVIII: C (pseudohemophilia) are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA) in the majority and rarely compound heterozygous for a null allele and a missense mutation or homozygous for a missense mutation\(^{11-18}\). The null alleles are located all over the VWF gene in nearly all exons 3-52\(^{18}\). The data base of the SSC of the ISTH reports 58 null alleles and 14 missense alleles involved in the etiology of type 3 VWD\(^{18}\). Missense mutations related to severe recessive VWD type 1 are mainly located in the D1-D2 domains (D47H, S85P, Y87S, D141Y, D141N, C275S, W377C, H427N, and in the D4, B1-3, C1-2, CK domains (P2063S, C2174G, C2362F, N2546Y, C2671Y, C2754W, and C2804Y), but not in the D3, A1 and A2 domains except one (C1071F/null)\(^{18}\). Consequently, some so-called type 3 VWD patients, who are compound heterozygous for a null allele and a missense mutation and may have detectable but very low VWF levels, are incorrectly diagnosed as VWD type 3 and should be reclassified as severe type 1 VWD\(^{19,27}\).

**DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE SEVERE TYPE 1 VWD**

A considerable number of missense mutations related to autosomal recessive severe type 1 VWD have been identified in the VWF prosequence (D1 and D2 domains)\(^{3}\) and the D4, B1-3, C1-2 and CK (dimerization) domains, but only a very few in the dimerization site (D3 domain (Tables 2 and 3))\(^{19,20}\). There are two reports on double heterozygous missense/null mutation D141Y/
null and C275S/null associated with VWD severe type 1 and not type 3 with documented hemarthros in one of them (Table 2)\[19\. Expression studies the missense mutation D141Y and C275S showed a severe secretion defect of mainly dimers while higher molecular weight bands like tetramers and hexamers were barely detectable\[20\. Homozygotes for the missense mutations W377C\[21\] and for R273W\[20\] in the propeptide D1 domain have been described to be associated with autosomal recessive severe type 1 (not type 3) VWD phenotype (Table 2). Homozygous missense mutation C570S in the D2 domain has been described as the cause of recessive severe type 1 VWD laboratory phenotype mimicking a type 2C (II C) VWF multimers\[21\].

The multimeric pattern of homzygous R273W and C570S clearly showed the absence of high molecular weight multimers and a pronounced monomer band mimicking type 2C (II C) subtype VWD\[21\]. Expression studies of recombinant R273W, W377C and C570S showed a severe secretion defect mainly consisting of dimers and failed to form intermediate and high molecular weight multimers\[19-21\]. These findings demonstrate that mutations in the D1 and D2 VWFpp domain completely abolishes multimerization of VWF. Homozygous asympomatic carriers of such missense mutation are asymptomatic or diagnosed as mild type 1 VWD with borderline values of VWF parameters around 0.50 U/dL. Heterozygotes for a missense mutation in the D1 or D2 domain typically show a pronounced VWF dimer band.

Homzygous missense mutation C2364F in the B1-3 domain and double heterozygous C2364F/null has been reported to be associated with severe type 1 VWD featured by FVIII C levels of 12 to 32 U/L, very low but detectable VWF: Ag and undetectable VWF: RCo\[22-26\]. C2364F heterozygous carriers were asymptomatic, had normal or slightly prolonged BT; subnormal values for VWF: Ag and VWF: RCo with a normal VWF: RCo/Ag ratio, and a normal VWF multimeric pattern in a low 0.8% or 0.9% agarose resolution gel (asymptomatic “dominant” VWD type 1)\[22\]. However, analysis of VWF in plasma from cases with severe autosomal recessive VWD homozygous for a missense mutation C2362F or compound C2362F/null (exon 42 of the B1-3 domain) as well as heterozygous carrier of C2364F all showed a heightened proteolytic pattern with marked increase of 176 and 140 kDa degradation products mimicking type 2A (II A) VWD\[25\]. Other causes of severe autosomal recessive type 1 VWD include homozygous C2364Y (B1-3 domain) or double heterozygous C2364Y/intron 13 splice site\[23\], homozygous C2671Y (exon 49) or double heterozygous missense mutation C2671Y/del (exon 49) of the VWF gene\[27\]. DDAVP in recessive VWD severe type 1 induces a poor for VWF: Ag and VWF: RCo but significant increase of FVIII C levels. In some cases of autosomal recessive severe type 1 VWD patients FVIII C, VWF: Ag and VWF: RCo reached values of > 0.50, 0.11 and 0.09 U/L respectively after DDAVP\[22\].

**RECESSIVE SEVERE TYPE 1 VWD DUE TO MUTATIONS IN THE CK DOMAIN**

The replacement of cysteine residues in the CK dimerization domain of the VWF gene causes two completely different laboratory phenotypes of VWD either severe VWD type 3 or VWD type 2D (II D)\[28-36\]. Homozygous or double heterozygous loss of cysteine mutations C2739Y, C2754W, C2804 and C2806 results in severe autosomal recessive type 1 VWD with nearly complete absence of VWF\[29-36\]. Homozygous C2754W mutation is associated with VWD severe type 1 and a mild type 1 VWD in heterozygous carriers (Table 3). Expression studies of C2754W show intracellular production of mainly monomers and dimmers (indicating a dimerization defect) with no secretion of mutant VWF indicating that homozygous C2754W mutation indeed will lead to severe type 1 or 3 VWD\[36\].

Experimental and clinical data are in line with the concept that loss of a single disulfide band in the CK domain of VWF leads to a recessive quantitative VWF deficiency with very low VWF: Ag (VWD type 3) if an intrachain disulfide band is involved (C2739Y or C2754W), and to a dominant-negative qualitative defect of VWF with abnormal multimers if an interchain-disulfide bond is involved, which leads to the characteristic type dominant type VWD type 2D (II D) multimeric pattern (Figure 3)\[35,36\].

**RECESSIVE TYPE 1 VWD DUE TO A HOMOZYGOUS MISSENSE MUTATION IN THE D2 DOMAIN**

The 1534C > A mutation in the consensus splicing site of intron 13 (D2 domain) induces exon 14 skipping with the introduction of a premature termination after codon 586, resulting in a truncated VWF\[37\]. Moreover, the 1534C > A mutation induces the activation of a cryptic
splicing site, 62 nucleotides upstream from the normal site\(^3\). The spliceosome produces a normal transcript of normal VWF. Gallinaro described a family with autosomal recessive type 1 VWD caused by homozygous intron 13 splicing site mutation 1534-3C > A (Figure 3)\(^\text{[17]}\).

The proband, a 34-year old man had a history of severe epistaxis requiring blood transfusion and recurrent mild epistaxis and gingivorrhagia not requiring medical attention. The proband had VWF values between 10 to 15 IU/dL with normal ratios of VWF: RCo/VWF: Ag (0.86), decreased VWF: CB/VWF: Ag ratio (0.6), and increased ratio of FⅧ/VWF: Ag (3.5) indicating a secretion defect. (dot) The plasma VWF multimeric pattern showed a homogenous decrease in all oligomers with a subtly loss of large VWF multimers and a more pronounced loss of platelet VWF multimers (VWD type 1 plasma low/platelet low). The response to DDAVP was restricted for VWF parameters but good for FⅧ: C thereby confirming a severe secretion defect. After DDAVP VWF: Ag increased from 15 to 28 IU/dL (1.9x) and FⅧ from 51 to 146 IU/dL (2.9x) followed by normal half life times for VWF and FⅧ (Figure 3). The father (Ⅰ-1) and sister (Ⅱ-2) heterozygous for the 1534-3C > A mutation never bled whereas the heterozygous mother suffered from mild menorrhagia, hematomas and bleeding after delivery. The plasma VWF values of affected family members were in the low normal range and relatively decreased as compared to FⅧ (Figure 3). Interestingly the response of FⅧ: C to DDAVP was rather good but severely restricted for all VWF parameters as compared the completely normal increase of FⅧ to values above 3.0 IU/dL consistent with carrier state of a missense mutation related to a secretion defect (Figure 3)\(^\text{[37]}\).

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\(^1\)BT (bleeding time) was performed using Ivy method; \(^2\)RIPA was performed with 1.2 mg/mL ristocetin; RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; aPTT: activated partial thromboplastin time.

---

### Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Blood group</th>
<th>(^1)BT min</th>
<th>(^2)RIPA s</th>
<th>FⅧ (U/dL)</th>
<th>VWF: Ag (U/dL)</th>
<th>VWF: RCo (U/dL)</th>
<th>VWF: CB (U/dL)</th>
<th>Plat. VWF: Ag (U/dL)</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ⅰ-1 (father)</td>
<td>O</td>
<td>7</td>
<td>34.3</td>
<td>99%</td>
<td>120</td>
<td>101.2</td>
<td>70</td>
<td>82.9</td>
<td>48.1</td>
</tr>
<tr>
<td>Ⅰ-2 (mother)</td>
<td>O</td>
<td>-</td>
<td>31.2</td>
<td>87.5%</td>
<td>123</td>
<td>64.6</td>
<td>44</td>
<td>56.4</td>
<td>56.4</td>
</tr>
<tr>
<td>Ⅰ-1 (proband)</td>
<td>O</td>
<td>20</td>
<td>37.8</td>
<td>5.4%</td>
<td>51</td>
<td>14.5</td>
<td>12.5</td>
<td>9.8</td>
<td>9</td>
</tr>
<tr>
<td>Ⅱ-1 (sister)</td>
<td>O</td>
<td>6</td>
<td>34.2</td>
<td>85.6%</td>
<td>146</td>
<td>76.5</td>
<td>77</td>
<td>86.7</td>
<td>56.8</td>
</tr>
</tbody>
</table>

Normal range: 2-9 \(^1\)BT (bleeding time) was performed using lvy method; 30-40 \(^2\)RIPA was performed with 1.2 mg/mL ristocetin; RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; aPPT: activated partial thromboplastin time.

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**Figure 3** State of the Art characterization of a family with autosomal recessive von Willebrand disease type 1 by Casonato and co-workers using a complete set of laboratory assays related to von Willebrand disease diagnosis, von Willebrand factor multimeric analysis in plasma and platelets and the response of FⅧ, VWF: Ag and VWF: RCo to desmopressin acetate before and at several time points after desmopressin acetate according to standardized recommendations anno 2006. RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.
Table 4 Laboratory phenotype and clinical symptoms in 69 patients with true von Willebrand factor deficiency type 1 heterozygous for the von Willebrand factor null allele (parents of type III von Willebrand disease)

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of patients</th>
<th>Blood Group</th>
<th>FVII:C (%)</th>
<th>VWF: Ag</th>
<th>VWF: RCo</th>
<th>Mild bleedings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Zhang et al[3]</td>
<td>25</td>
<td>A</td>
<td>0.81</td>
<td>81-121</td>
<td>45</td>
<td>37-94</td>
</tr>
<tr>
<td>Eikenboom et al[4]</td>
<td>17</td>
<td>O</td>
<td>0.74</td>
<td>74-93</td>
<td>32</td>
<td>12-70</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>A</td>
<td>0.94</td>
<td>93-128</td>
<td>61</td>
<td>11-128</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>O</td>
<td>0.39</td>
<td>93-128</td>
<td>52</td>
<td>37-98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38-93</td>
<td>53</td>
<td>40-66</td>
</tr>
</tbody>
</table>

Mild bleeding was defined by one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations without abnormal bleeding after both extraction or surgery, hemarthrosis or muscle bleeding. VWF: von Willebrand factor.

Table 5 Von Willebrand factor antigen (VWF: Ag) levels in heterozygous carriers for a null allele related to pseudohemophilia A von Willebrand disease type 3 and for the mutation C2364F related to severe recessive type 1 von Willebrand disease

<table>
<thead>
<tr>
<th>Carriers</th>
<th>Number of patients</th>
<th>VWF: Ag mean ± SD (IU/dL)</th>
<th>VWF: Ag range (IU/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null allele:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>15</td>
<td>43.2 ± 10.8</td>
<td>30-66</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>15</td>
<td>61.3 ± 23.6</td>
<td>25-98</td>
</tr>
<tr>
<td>C2364F:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood group O</td>
<td>8</td>
<td>35.2 ± 16.2</td>
<td>25-55</td>
</tr>
<tr>
<td>Blood group non-O</td>
<td>15</td>
<td>61.5 ± 26.6</td>
<td>30-140</td>
</tr>
</tbody>
</table>


DETECTION OF SYMPTOMATIC AND ASYMPTOMATIC OBBLIGATORY CARRIERS OF RECESSIVE VWD TYPE 3 AND SEVERE TYPE 1

The only objective and correct way to characterise true type 1 VWF deficiency heterozygous for the VWF null allele or missense mutation is to analyse the bleeding manifestations and FVII:C/VWF parameters in obligate heterozygous parents of recessive type 3 or severe type 1 VWF patients. In the study of 27 patients with congenital type 1 VWF deficiency associated with one null allele analysed by Schneppenheim et al[13], 20 were asymptomatic and only 7 presented very mild bleeding, mainly bruising and epistaxis. All except one, had a normal BT. The mean values for FVII:C, VWF: Ag, and VWF: RCo were 0.76, 0.39 and 0.39 U/mL respectively with an increased FVII:C/VWF: Ag ratio of 1.9 and a normal VWF: RCo/Ag ratio of 1 consistent with true type 1 VWF deficiency. In the study of Zhang et al[3] including 25 patients heterozygous for the VWF null allele and blood group non-O, 12 had no history of bleeding and 13 presented with very mild bleeding (one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations with no abnormal bleeding after tooth extraction). The mean values for FVII:C and VWF: Ag were 0.81 and 0.45 respectively with an increased ratio for FVII:C/VWF: Ag of 1.8 (Table 4). In the same study of Zhang et al[3] out of 17 patients heterozygous for the VWF null allele but having blood group O, 8 had no bleeding history and 11 presented minor bleedings (65%, Table 4). The mean values for FVII:C and VWF: Ag were 0.74 and 0.32 respectively with an increased ratio for FVII:C/VWF: Ag of 2.3 (Table 2). In the study of Eikenboom the values VWF: Ag ranging from normal to increased above 2 indicating the difficulty to distinguish true congenital type 1 VWF deficiency from VWF deficiency related to blood group O. Using the recently developed sensitive bleeding score assessment, Castaman et al[38] compared the severity of bleeding symptoms in 70 OC of recessive type 3 VWD, 42 OC of recessive type 1 VWD and in 215 normal controls. OC of VWD type 3 with a null mutation had clearly less severe bleeding than patients diagnosed as type 1 VWD. OC of type 1 VWD with a missense mutation were distinct from normal controls, presenting more epistaxis, cutaneous bleeding and usually did not significantly bleed after surgery, further pointing to the wide heterogeneity of VWD as a heterozygous congenital disorder of the VWF gene mutations (Table 5).

Obligatory carriers (OC) of a nonsense mutation related to VWD type 3 and OC of missense mutation related to severe recessive VWD type 1 in the population are asymptomatic or manifest mild bleeding, and have VWF levels at 50% of normal (true type 1 VWD according to the law of Mendel). Such OC of a null allele or missense mutation may become more symptomatic when associated with blood group O or another modifier of the VWF level. Castaman and Eikenboom demonstrated that ABO blood group significantly influences the VWF: Ag levels in OC of a null allele related to VWD type 3 or the missense mutation C2364F related to severe recessive VWD type 1 (Table 5). From a genotypic point of view, OC of a null allele in type 3 VWD are very similar to asymptomatic or mild type 1 VWD patients with a single missense allele.

Based on careful analysis of reports on recessive VWD we proposed in 2006 the Antwerp Classification of recessive VWD type 3, recessive severe type 1 VWD, and true type 1 VWD heterozygous for a null allele or missense mutation with variable penetrance of bleeding manifestations but symptomatic when associated with blood group O (Table 6). In subsequent studies the variable penetrance of bleeding manifestations mild VWD type 1 is clearly related to blood group O[41,42].
Table 6 The 2006 Antwerp Classification of recessive von Willebrand disease type 3, recessive severe on Willebrand disease type 1 and obligatory carriers of a null or missense allele with asymptomatic or mild on Willebrand disease type 1 and variable penetrance of bleeding tendency

<table>
<thead>
<tr>
<th>Category VWD</th>
<th>BT</th>
<th>FⅧ: C (%)</th>
<th>VWF (%) Ag</th>
<th>RCo</th>
<th>RIPA</th>
<th>Bleeding type</th>
<th>VWF gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe type 3</td>
<td>N</td>
<td>1-9</td>
<td>zero</td>
<td>zero</td>
<td>zero</td>
<td>Severe</td>
<td>Double Nonsense</td>
</tr>
<tr>
<td>Recesive</td>
<td>N</td>
<td>9-40</td>
<td>1-10</td>
<td>0-6</td>
<td>zero</td>
<td>Moderate</td>
<td>Double Nonsense</td>
</tr>
<tr>
<td>Severe type 1</td>
<td>N</td>
<td>35-150</td>
<td>35-150</td>
<td>N</td>
<td>Asymp</td>
<td>Very mild</td>
<td>Single non-sense</td>
</tr>
<tr>
<td>Recesive VWD</td>
<td>N</td>
<td>30-140</td>
<td>15-90</td>
<td>15-90</td>
<td>N</td>
<td>Asymp</td>
<td>Single non-sense</td>
</tr>
<tr>
<td>Carrier type 3</td>
<td>N</td>
<td>20-80</td>
<td>10-40</td>
<td>0-30</td>
<td>N</td>
<td>Mild</td>
<td>Single non-sense</td>
</tr>
<tr>
<td>Minor influence (&lt;10%)</td>
<td>N</td>
<td>5-20</td>
<td>5-20</td>
<td>5-20</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
<tr>
<td>Carrier type 1 (polymorphism)</td>
<td>N</td>
<td>5-20</td>
<td>5-20</td>
<td>5-20</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
<tr>
<td>Mild type 1</td>
<td>N</td>
<td>5-20</td>
<td>5-20</td>
<td>5-20</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
<tr>
<td>Recessive or variable penetrance and multigenetic background</td>
<td>N</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
<tr>
<td>Dominant type 1</td>
<td>N</td>
<td>20-80</td>
<td>10-40</td>
<td>0-30</td>
<td>N</td>
<td>Mild</td>
<td>Single non-sense</td>
</tr>
<tr>
<td>Secretion defect</td>
<td>N</td>
<td>20-80</td>
<td>20-50</td>
<td>20-50</td>
<td>N</td>
<td>Mild</td>
<td>Single non-sense</td>
</tr>
<tr>
<td>Dominant type 1</td>
<td>N</td>
<td>5-20</td>
<td>5-20</td>
<td>5-20</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
<tr>
<td>Vicenza</td>
<td>N</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>&lt;15</td>
<td>N</td>
<td>Moderate</td>
<td>Single Missense</td>
</tr>
</tbody>
</table>

VWF: von Willebrand factor; VWD: von Willebrand disease; RIPA: Ristocetin induced platelet aggregation.

Table 7 Response of FⅧ: C and von Willebrand factor parameters to DDAVP (0.3 pg/kg) in an obligatory carriers of a null allele heterozygous for the nonsense splice site mutation IV7 + 1G > A in intron 7, (0874 + 1G > A) in intron 7

<table>
<thead>
<tr>
<th>DDAVP</th>
<th>Before</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>H post-DDAVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FⅧ: C</td>
<td>0.84</td>
<td>5</td>
<td>5.4</td>
<td>5.3</td>
<td>4.9</td>
<td>IU/mL</td>
</tr>
<tr>
<td>VWF: Ag</td>
<td>0.64</td>
<td>1.3</td>
<td>1.7</td>
<td>1.5</td>
<td>1.4</td>
<td>IU/mL</td>
</tr>
<tr>
<td>VWF: RCo</td>
<td>0.67</td>
<td>1.8</td>
<td>2</td>
<td>1.35</td>
<td>1.2</td>
<td>IU/mL</td>
</tr>
<tr>
<td>FⅧ: C/VWF: Ag ratio</td>
<td>1.3</td>
<td>3.8</td>
<td>3.1</td>
<td>3.5</td>
<td>3.5</td>
<td>Carrier of null allele</td>
</tr>
<tr>
<td>VWF: RCo/Ag ratio</td>
<td>1.05</td>
<td>1.38</td>
<td>1.17</td>
<td>0.9</td>
<td>0.86</td>
<td>Mild type 1 VWD</td>
</tr>
</tbody>
</table>

VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.

Figure 4 Good response of FⅧ: C and restricted response of von Willebrand factor: RCo to desmopressin acetate (0.3 pg/kg) in a carrier of a null allele (Q2470X/normal). VWF: von Willebrand factor; DDAVP: Desmopressin acetate.

Similarly, the C1584 variant of mild WVD type 1 is associated with a slight decrease of VWF and FⅧ: C levels, especially in combination with bloodgroup O.

We studied a consanguineous family with type 3 VWD. The propositus was a boy with VWD type 3, who presented with mucocutaneous bleeding and recurrent hemarthrosis of an ankle. Laboratory analysis found FⅧ: C < 1% and an absence of VWF: Ag due to the homozygous nonsense splice site mutation IV7 + 1G > A in intron 7 (0874 + 1G > A)

From this analysis of the literature and personal experiences in VWD we conclude that heterozygous carriers of a null allele had normal FⅧ: C levels, while VWF: Ag levels were 1.3 before DDAVP but more than 3 after DDAVP consistent with a carrier of a VWF null allele (Table 7). The VWF: RCo/Ag ratio was normal before and after DDAVP consistent with true congenital type 1 VWD disease (Table 7). This demonstrates that an increased ratio FⅧ: C/VWF: Ag ratio after DDAVP is typically and diagnostic for true VWF deficiency type 1 heterozygous for a null allele. This important diagnostic clue to true congenital type 1 VWD has also been demonstrated by Lethagen et al. in a carrier of a null allele (Q2470X/normal, Figure 4).
higher response of FVIII: C as compared to VWF: Ag. In contrast, the responses to DDAVP of FVIII: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of Erik von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

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