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Identification of a novel candidate splice site mutation (0874+1G>A) in a type 3 von Willebrand disease patient

Alain P. Gadisseur1,2, Inge Vrelust1, Inge Vangenechten2,3, Reinhard Schnepfenheim4, Marc Van der Planken2,3

1Department of Hematology, Antwerp University Hospital, Edegem, Belgium; 2Hemostasis Unit, Antwerp University Hospital Antwerp, Edegem, Belgium; 3Laboratory of Hematology and Hemostasis, University Hospital, Edegem, Belgium; 4Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Von Willebrand disease (VWD) is a congenital bleeding disorder which results from quantitative or qualitative defects of the von Willebrand factor (VWF) produced by endothelial cells and megakaryocytes. It is the most commonly inherited bleeding disorder in humans with published prevalence figures ranging from 3–4 per 100,000 to 1.3% of the population (1). More than 20 subtypes of VWD have been described, although current classification consists of 6 distinct types (2). Types 1 and 3 are qualitative VWF defects while the four type 2 variants are quantitative defects. The underlying genetic lesions and associated molecular pathology have been identified in many cases of type 2A, 2B, 2M, 2N and type 3 VWD. However, in the most common variant, type 1 VWD, the causative molecular defect is unknown in a large majority of cases (3–5), but has been addressed recently by two large multi center studies (6, 7).

The gene encoding VWF is located on the short arm of chromosome 12 (12p13.3), consists of 52 exons and is approximately 178kb kb in length. The signal peptide and propeptide (VW antigen II) are encoded by 16 exons (approximately 80kb kb), while the mature subunit of VWF and 3’ non-coding region are encoded by 35 exons in the remaining 100 kb of the gene (8). Exon 1 is untranslated.

The mature VWF protein consists of different domains which are involved in multimerisation, and binding to heparin, clotting factor VIII (FVIII), glycoprotein Ib (GpIb), collagen, and glycoprotein IIb/IIIa (GpIIb/IIIa) (9).

Type 1 VWD (60–80% of all VWD cases) is a quantitative defect (heterozygous for the defective gene) often without overtly impaired clotting, with most patients usually leading a relatively normal life. Complications may arise in the form of bleeding following surgery (including dental procedures), noticeable easy bruising, and/or menorrhagia. Decreased levels of VWF are detected (10–45% of normal). In type 3 VWD patients (homozygous for the defective gene) there is an almost total absence of VWF and, correspondingly, a low level of FVIII. These patients may present with severe mucosal bleeding and spontaneous haemarthroses similar to patients with haemophilia (10, 11).

A six-year-old male child (FB) of Turkish origin presented at the outpatient department because of easy bruising. There was no clear family history of an obvious bleeding tendency. The parents (father EB, mother SB) were consanguineous: first cousins, the issue of brothers.

In this child (FB) VWF:Ag (Elisa) was undetectable (<1%) with collagen binding activity <2%, VWF:RCo <1% and FVIII at 1%, which led to the phenotypic diagnosis of a type 3 VWD. On multimer analysis only a tiny signal in the region of the protomer was detectable (Fig. 1). Later in the year the patient returned with recurrent haemarthrosis of the left ankle after trauma and was put on prophylactic treatment three times weekly with FVIII/VWF concentrate (Haemate-P®, CSL Behring, Bern, Switzerland).

Laboratory testing of both parents (EB and SB) showed levels of VWF:Ag at 56% and 40%, VWF:RCo at 38% and 36% and FVIII levels of 73% and 64%, respectively (Table 1). Neither parent had a history of severe bleeding problems. A tentative diagnosis of VWD type 1 was made in both parents and also in one other child (HaB), while the third child was normal (HyB). Multimer analysis of both parents and the two siblings of the patient showed a normal pattern with presence of the high-molecular-weight multimers and the typical triplet structure (Fig. 1). The findings were discussed with the parents and, with informed consent, molecular studies were carried out in all family members to clarify the diagnosis.

For the sequencing of the gene, the VWF cDNA nucleotides are numbered from the A of the initiator ATG site as +1. Amino acid residues are numbered from the ATG initiation codon (residue 1) to the carboxy-terminal lysine (residue 2813) of pro-pro-VWF. Correspondence with residues in the mature VWF sequence is obtained by subtracting 763 from the residue number of pre-pro-VWF. High-molecular-weight genomic DNA was prepared from leukocyte buffy coats by standard techniques and was used for the amplification of VWF coding exons 2 through 52 by PCR as previously described (12). PCR products were sequenced by the “ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit” on an ABI Prism 310 (ABI, Foster City,
Candidate mutations were confirmed by sequencing both strands. By analysing the complete coding sequence of the VWF gene, including 51 exons and the exon-intron boundaries, a candidate splice site mutation 0874+1G>A according to ISTH-SSC-VWF nomenclature (13) (IVS7+1G>A according to Human Gene Nomenclature Working Group [14]) could be identified in intron 7 for which the patient FB proved homozygous, with both parents (EB and SB) and another child (HaB) heterozygous, while the third child (HyB) proved to be homozygous wild type. No other mutations could be identified, and there was no evidence for the presence of a co-existing mutation in the FVIII gene.

In-silico analysis by the splice site prediction programme (NetGene2 URL: http://www.cbs.dtu.dk/services/NetGene2) predicts the loss of the original donor splice site, since the original probability score of 0.97 was reduced to nil after introducing the respective base exchange. Exon 7 skipping could be the possible consequence of 0874+1G>A. This would result in a frameshift, generation of a nonsense codon at position 456 in exon 12 and subsequently with either a severely truncated protein with no VWF function or with an unstable RNA due to nonsense mediated decay. However, due to the lack of patient's RNA we could not experimentally confirm the pathogenic mechanism of this mutant.

Mutations that destroy or create mRNA splice sites are associated with variable severity of disease; this very much depends on whether some correct transcripts can be processed (mild to moderate disease) or whether there is a complete loss of correct mRNA processing (severe disease). In our case the homozygous candidate splice site mutation is linked to a severe phenotype. Heterozygosity for this mutation seems to lead to a quantitative reduction in plasma VWF with conservation of the normal multimeric pattern resulting in a mild type 1 VWD with few bleeding symptoms. In view of the mild bleeding symptoms most heterozygous carriers of this mutation may not be diagnosed as having VWD type 1, as was the case in our patient family. The discussion concerning the classification of these kind of patients as having VWD type 1 or simply low VWF is still ongoing in the literature (15, 16). The presence of the 0874+1G>A mutation in intron 7 of the VWF gene may only lead to the formal diagnosis of a bleeding disorder when a homozygous patient arises. To our knowledge, 0874+1G>A is a novel candidate splice site mutation which has not been reported before in the literature or in the ISTH-SSC-VWF database registry of known VWD mutations.

**Table 1: Laboratory and clinical patient data.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>APTT (sec)</th>
<th>FVIII:C (%)</th>
<th>VWF:Ag (%)</th>
<th>VWF:Rco (%)</th>
<th>PFA COL/EPI (sec)</th>
<th>PFA COL/ADP (sec)</th>
<th>Bleeding symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td></td>
<td>30 – 39</td>
<td>60 – 150</td>
<td>60 – 160</td>
<td>60 – 150</td>
<td>95 – 160</td>
<td>70 – 110</td>
<td></td>
</tr>
<tr>
<td>FB</td>
<td>6y</td>
<td>62.0</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>Ecchymoses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haemarthrosis (traumatic)</td>
</tr>
<tr>
<td>EB</td>
<td>30y</td>
<td>31.2</td>
<td>73</td>
<td>56</td>
<td>38</td>
<td>267</td>
<td>169</td>
<td>None</td>
</tr>
<tr>
<td>SB</td>
<td>29y</td>
<td>32.1</td>
<td>64</td>
<td>40</td>
<td>36</td>
<td>270</td>
<td>165</td>
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</tr>
<tr>
<td>HaB</td>
<td>4y</td>
<td>33.3</td>
<td>60</td>
<td>45</td>
<td>38</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>None</td>
</tr>
<tr>
<td>HyB</td>
<td>3y</td>
<td>34.5</td>
<td>72</td>
<td>94</td>
<td>88</td>
<td>144</td>
<td>92</td>
<td>None</td>
</tr>
</tbody>
</table>

**Figure 1: VWF multimers (medium resolution gel).** 1 + 5 + 10: normal plasma. 2 + 3: EB (father), 4: FB (patient), 6 + 7: SB (mother), 8 + 9: HaB (brother).
References


