Acquired von Willebrand syndrome type 2A in a JAK2-positive essential thrombocythaemia-affected member of a large von Willebrand disease family with a novel autosomal dominant A1716P mutation

Silvia Giannini1; Maria Solimando2; Tiziana Fierro1; Luciano Baronciani2; Augusto B. Federici2,3; Paolo Gresele1

1Division of Internal and Cardiovascular Medicine, Department of Internal Medicine, University of Perugia, Perugia, Italy; 2Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine and Medical Specialties, IRCCS Maggiore Hospital and University of Milan, Milan, Italy; 3Division of Hematology and Transfusion Medicine, L. Sacco University Hospital, Milan, Italy

Dear Sirs,

Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder occurring in subjects with no personal or family history of haemorrhage and is similar to inherited VWD in terms of laboratory findings and clinical manifestations (1). Von Willebrand factor (VWF) is synthesised normally in most AVWS and the defect is caused by an accelerated removal of VWF from plasma due to the presence of auto-antibodies, to the adsorption onto malignant cell clones, to the loss of high-molecular-weight (HMW) VWF multimers upon exposure to very high shear stress or due to the increased proteolytic degradation of VWF by circulating proteases (2). In particular, according to the international registry on acquired von Willebrand syndromes, AVWS is associated with chronic myeloproliferative neoplasms (MPN) in 11–15% of cases (3, 4). Most of these patients (52%) do not have a bleeding history while 48% have a bleeding diathesis: all of them have reduced VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag and 86% have a defect in HMW VWF multimers (3, 5). Due to the reduction of HMW VWF multimers by proteolysis, the patients’ phenotype is similar to congenital type 2A VWD (VWD2A), i.e. a normal or mildly reduced VWF antigen (VWF:Ag) but a clearly reduced VWF activity, with VWF:RCo/Ag and VWF:CB/Ag ratios lower than 0.7 (5). Different enzymes, including platelet-derived calcium-activated neutral proteases, are held responsible for HMW VWF proteolysis (6). Moreover, the interaction between VWF and platelets under high shear stress conditions may contribute to the relative depletion of HMW VWF multimers in plasma (7). An acquired VWF defect in MPN patients has been described in particular in those with a very high platelet count (8) and it normalises upon reappearance of HMW VWF multimers in plasma when the platelet count decreases after cytostatic treatment (7, 8).

We have characterised a patient with type 2A AVWS associated with a JAK2-positive essential thrombocythaemia (ET) in whom, during follow-up, we identified an inherited type 1 VWD associated with a novel autosomal dominant A1716P mutation of the VWF gene. Our observation may be important for haematologists who follow patients with ET because they should always thoroughly evaluate these patients with appropriate tests to exclude inherited defects underlying AVWS.

The patient is a 41-year-old male who came to our attention for the occasional finding of an increased platelet count (6.5x10^8 /ml). He met World Health Organisation (WHO) criteria for ET, i.e. a sustained increase of platelet count, a bone marrow biopsy showing an increased number of megakaryocytes, the presence of the JAK2 somatic mutation (V617F), BCR/ABL not rearranged and no criteria for other MPN or for myelodysplasia; abdominal ultrasonography showed a mildly enlarged spleen.

Platelet aggregation studies (9), performed at the identification of thrombocytosis, revealed a mild platelet function defect with impaired epinephrine-induced aggregation, quite frequent in MPN (10),
but a normal response to collagen, adenosine diphosphate and arachidonic acid. A prolongation of the PFA-100 C/Epi closure time (>300 seconds) (11) and a reduced ristocetin-induced platelet aggregation (RIPA) were also observed (Table 1). Given the latter findings, VWF:Ag, VWF:RCo, VWF:CB and the binding of VWF to the patient’s platelets as assessed by flow cytometry (11) were also evaluated and found to be decreased (Table 1). Recent bleeding history was mild. These data were considered to be compatible with an AVWS associated with MPN. The patient was classified as acquired VWD2A due to the reduced value of VWF:RCo/VWF:Ag (0.34, normal 0.9 ± 0.2) and VWF:CB/VWF:Ag (0.48, normal 0.9 ± 0.1) (2, 5). In the same period, by accident, a nephew of the proband came to our attention for a mild bleeding diathesis and he turned out to be affected by type 1 VWD (Fig. 1A). All the other 16 family members were thus investigated for VWD by bleeding history and laboratory tests (Fig. 1, together with 34 healthy controls. The bleeding severity score (BSS) was calculated using the same questionnaire previously tested in patients with inherited VWD1 (12). All patients studied gave their informed, written consent; all studies were carried out in conformity to the declaration of Helsinki.

Light-transmission aggregometry and the PFA-100 C/Epi closure time were measured as previously described (9, 11, 13). RIPA, VWF:Ag, VWF:RCo, VWF:CB and VWF binding to patients’ platelets as measured by flow cytometry were also evaluated by previously reported methods (11, 14).

Genomic DNA was extracted from peripheral blood using standard methods. Polymerase chain reaction (PCR) was performed as previously reported (15) to amplify the whole VWF gene-coding region of the propositus. The patient’s relatives were investigated only for the mutation identified in the propositus. Direct DNA sequence analysis was made using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) after purification of PCR products with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Purification by MultiScreen HTS (Millipore, Billerica, MA, USA) and Sephadex G-50 (Sigma-Aldrich, Steinheim, Germany) was performed before loading the sample on an automated Electrophoresis Capillary Sequencer (ABI Prism 3130 Genetic Analyzer, Applied Biosystems).

Our patient was initially classified as AVWS type 2A due to the reduced VWF:RCo/VWF:Ag ratio (0.34, normal 0.9 ± 0.2) and VWF:CB/VWF:Ag ratio (0.48, normal 0.9 ± 0.1) and the concomitant MPN (2, 5). Upon identification of type 1 VWD in a nephew of the proband, 16 members of his family were studied, and six of them were found to be affected by type 1 VWD (Table 1). Thus, a congenital VWD in the proband was supposed that, however, differed from his relatives for phenotype, being a type 2A and not type 1.

Indeed, sequencing analysis of the VWF gene revealed a previously undescribed missense mutation in exon 29 (c.5146 GCT > CCT) leading to an alanine to proline substitution in the collagen-binding domain (A3) of VWF (A1716P) in the proband and all the other affected family members.

This novel mutation has recently been reported under abstract form by Castaman et al. (16). We concluded that our patient, originally an undiagnosed congenital type 1 VWD, had turned into an AVWS-type 2A in concomitance with the development of ET. Considering the young age, the absence of other cardiovascular risk factors, the platelet count only moderately increased and the VWF defect, the patient was initially left untreated. However, shortly after diagnosis he suffered a myocardial infarction (MI), diagnosed according to the European Society of Cardiology (ESC)-defined criteria (17); he was therefore started on antiplatelet and cytostatic therapy (aspirin 100 mg/day, hydroxyurea 500 mg/day). Following treatment his platelet count normalised (3.5x10^11/ml) and upon further study it resulted that the VWD phenotype had turned into type 1 VWD (Table 1). Plasma VWF multimers were evaluated and the affected family members revealed a normal distribution of VWF multimers, as usual in type 1 VWD. On the contrary, the proband showed a mild reduction of HMW VWF multimers at the

Table 1: Platelet count and VWD-related laboratory parameters in the proband, before and after cytoreductive therapy, and in three affected family members.

<table>
<thead>
<tr>
<th></th>
<th>Plt count (x10^8/ml)</th>
<th>WBC count (x10^9/ml)</th>
<th>PFA-100 C/Epi CT (seconds)</th>
<th>RIPA (mg/ml)</th>
<th>VWF:Ag (U/dl)</th>
<th>VWF:RCo (U/dl)</th>
<th>VWF:CB (U/dl)</th>
<th>VWF:RCo/Ag (ratio)</th>
<th>VWF:CB/Ag (ratio)</th>
<th>VWF binding to plts (MFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=34)</td>
<td>1.5–4.0</td>
<td>4.0–10.0</td>
<td>117.1–137.2</td>
<td>1.0</td>
<td>92.5</td>
<td>83.0</td>
<td>79.0</td>
<td>&gt;0.7</td>
<td>&gt;0.7</td>
<td>13.5 (10.5–17.0)</td>
</tr>
<tr>
<td>Proband pre</td>
<td>6.5</td>
<td>6.77</td>
<td>&gt;300</td>
<td>1.4</td>
<td>44.0</td>
<td>15.0</td>
<td>21.5</td>
<td>0.34</td>
<td>0.48</td>
<td>1.8</td>
</tr>
<tr>
<td>Proband post</td>
<td>3.5</td>
<td>8.06</td>
<td>&gt;300*</td>
<td>2.1</td>
<td>30.0</td>
<td>29.0</td>
<td>27.0</td>
<td>0.96</td>
<td>0.89</td>
<td>1.7</td>
</tr>
<tr>
<td>Pt 1</td>
<td>2.9</td>
<td>4.80</td>
<td>&gt;300</td>
<td>1.5</td>
<td>7.7</td>
<td>12.0</td>
<td>9.5</td>
<td>1.54</td>
<td>1.23</td>
<td>2.7</td>
</tr>
<tr>
<td>Pt 2</td>
<td>2.6</td>
<td>5.20</td>
<td>&gt;300</td>
<td>1.3</td>
<td>12.0</td>
<td>15.5</td>
<td>17.5</td>
<td>1.27</td>
<td>1.45</td>
<td>5.0</td>
</tr>
<tr>
<td>Pt 3</td>
<td>2.6</td>
<td>4.90</td>
<td>&gt;300</td>
<td>1.7</td>
<td>7.8</td>
<td>12.5</td>
<td>8.5</td>
<td>1.60</td>
<td>1.09</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Plt = platelet; WBC = white blood cell; Pt = patient (affected family members); MFI = mean fluorescence intensity. Normal values for healthy controls are reported as means (95% CIs). Pre= at diagnosis; post= after cytoreductive therapy. *under aspirin treatment.
time of diagnosis which normalised after cytostatic therapy (Fig. 1B).

Recurrent bleeding in AVWS is usually associated with very high platelet counts (>10x10^9/ml), and the reduction of the number of circulating platelets is associated with a decrease in the frequency of bleeding episodes (18). Our patient at presentation showed mild mucocutaneous bleeding symptoms despite a platelet count lower than 10x10^9/ml, probably due to the concomitant inherited VWD, and despite of this he developed a MI with normal coronaries at angiography. An elevated white blood cell (WBC) count has recently been suggested to be a risk factor for arterial thrombosis in patients with MPN (19); however, our patient had normal WBC both immediately before and after developing acute MI. A slightly elevated white blood cell count was indeed observed in the hours following acute MI (14.0x10^9/ml), but this is likely to be a manifestation of the haematological stress syndrome accompanying acute MI (20). Our patient had a JAK2 V617F-positive ET (21): although the exact role of mutated JAK2 in the risk of thrombosis is still controversial (22), several studies have suggested it to be associated with an increased risk of thrombosis (21). It is suggestive that our patient, a young previously healthy male without cardiovascular risk factors and with a congenital mild form of VWD, suffered a MI soon after diagnosis of a JAK2-positive ET. The alternative hypothesis, that the previously undescribed inherited VWF mutation found in our patient facilitated the thrombotic event by favouring the binding of...

Figure 1: Family pedigree and plasma VWF multimers. A) Family pedigree with identification of the patients carrying the alanine-to-proline A1716P substitution in the collagen binding domain (A3) of VWF. Bleeding severity scores (BSS) as well as VWF:Ag, VWF:RCO and VWF:CB are also reported. The propositus is indicated by an arrow. B) Plasma VWF multimers of the proband, pre- and post-cytoreductive treatment, and of two family members (Pt 3, Pt 1) (also shown in Table 1) carrying the A1716P mutation.
HMW VWF multimers to platelets, seems to be excluded by the finding that VWF:RCo, VWF binding to platelets by flow cytometry, and RIPA did not show enhanced affinity of VWF for platelets. The present case is interesting for several reasons. First, because it represents a paradigmatic case of AVWS occurred in concomitance with the development of a MPN, and reverted upon normalisation of the platelet count and of the VWF multimer pattern after cytoreductive therapy; second, because it shows that when AVWS is diagnosed in a patient with a MPN, the possibility of a concomitant inherited VWD must be considered, given the not negligible prevalence of VWD in the general population (23); third, because it indicates that the phenotype of an inherited VWD can be modified by an acquired condition due to the consumption of HMW VWF multimers; fourth, because it suggests that a defect in VWF does not necessarily reduce thrombotic risk in patients with JAK2-positive ET. In conclusion, AVWS is a complex and heterogeneous disorder with a multifactorial aetiology. Early and thorough characterization of AVWS and of its cause, including the study of possible underlying inherited coagulation defects, is mandatory to provide adequate therapy.

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