

Autosomal dominant C1149R von Willebrand disease: phenotypic findings and their implications

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ABSTRACT

Background

Mutation C1149R in the von Willebrand factor (VWF) gene has been thought to cause autosomal dominant severe type 1 von Willebrand disease (VWD).

Design and Methods

Eight patients from three unrelated families with this mutation were included in the present study who had distinct VWF abnormalities, not described in earlier studies.

Results

The patients showed notably low levels of VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), VWF collagen binding (VWF:CB), and a reduced ristocetin-induced platelet aggregation (RIPA). VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios were lower than 0.7. At basal conditions, all the VWF multimers were decreased in plasma, with a clearly lower relative proportion of the high molecular weight VWF multimers (HMWM). In high-resolution agarose gels, a large decrease in the relative proportions of the satellite bands was seen. The patients had a brief good response to desmopressin (DDAVP) administration, but the released VWF half-life was shorter than normal, indicating an accelerated clearance of their VWF. Platelet VWF was abnormal.

Conclusions

We conclude from the results obtained in these patients for plasma phenotypic data that this mutation should be classified as a VWD type 2A (IIE). DDAVP therapy may be somewhat helpful for this mutation, at least for mild to moderate bleeding. These data provide evidence that for VWD classification factors other than basal VWF, such as DDAVP response and platelet VWF, should be considered.

Key words: C1149R von Willebrand disease, platelet von Willebrand factor, VWD type 2A (IIE), desmopressin, von Willebrand factor.

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Introduction

Von Willebrand disease (VWD) is a bleeding disorder caused by inherited defects in the concentration, structure, or function of the von Willebrand factor (VWF).^{1,2} According to the updated revision for VWD classification, three categories are distinguished in a primary hierarchical level.³ Type 1 includes partial quantitative deficiencies, type 2 includes qualitative defects, and type 3 includes virtually complete deficiency of VWF.

VWD type 1 plasma may contain mutant subunits of VWF, but has normal functional activity relative to antigen level. The proportion of large multimers is not decreased significantly. VWD type 2 is divided on a secondary level in four VWD types according to different pathophysiological mechanisms: VWD types 2A, 2B, 2M and 2N.

Type 2A includes variants with decreased platelet adhesion caused by selective deficiency of high-molecular-weight VWF multimers (HMWM). Type 2B includes variants with increased affinity for platelet glycoprotein Ib. Type 2M includes variants with markedly defective platelet adhesion despite a relatively normal size distribution of VWF multimers. Type 2N includes variants with markedly decreased affinity for factor VIII (FVIII). Finally, additional tertiary information does mention the subtypes. Such information may include a VWF multimer pattern that suggests a specific disease mechanism (e.g. IIA, IIB, IIC, IID, IIE).

When sensitive assay methods are used, many patients with VWD type 1 have mild abnormalities of multimer structure or distribution. In most cases, therefore, a complex phenotype with features of both VWD type 2 and type 1 should be classified under type 2 in order to preserve a correlation with the response to desmopressin. In clinical practice, distinctions among these VWD types are not always easily made and the boundary between normal and abnormal phenotypes may be controversial or not sharply defined.⁴ The VWD caused by mutation C1149R was initially described as a

dominant severe VWD type 1.^{5,6} The ISTH-SSC (International Society on Thrombosis and Haemostasis-Scientific and Standardization Committee) database for VWF mutation includes this mutation in the type 1 group (<http://www.vwf.group.shef.ac.uk/>).

Our study refers to 8 patients from three unrelated families with the autosomal dominant C1149R mutation who showed distinct abnormalities of VWF in platelets and in plasma following DDAVP administration. They had not been described in earlier studies and it reinforces the criteria that should be considered to be VWD type 2A (IIE).^{7,8}

Design and Methods

Patients and controls

Eight patients from three unrelated families with the C1149R VWD mutation were studied. Fifty healthy individuals of either sex, with no known bleeding problems, and of an age range similar to the enrolled family members were used as control. All of the patients gave their informed consent for this study. The study was approved by the local Research Ethics Committee. The desmopressin response was tested in the 8 patients by intravenous administration of 0.3 µg/kg of DDAVP. Plasmas from patients with VWD types 2A (IIA) heterozygous for the I1568N mutation in exon 28 and patients with VWD type 2A (IIC) homozygous and heterozygous for the mutation F404insNP in exon 11 were included as controls.^{9,10}

Bleeding score

Clinical bleeding history assessment was carried out by interview and completion of a questionnaire based on that originally proposed by the European Study MCMDM-1VWD¹¹ (Table 1).

Phenotypic analysis

Closure times (CT) were determined using the PFA-100® system (Platelet Function Analyzer, Dade

Table 1. Phenotypic results of patients from families 1, 2 and 3.

Family	Patient	B.G.	B.S.	FVIII:C (IU dL ⁻¹)	VWF:Ag (IU dL ⁻¹)	VWF:RCo (IU dL ⁻¹)	VWF:CB (IU dL ⁻¹)	VWFpp (IU dL ⁻¹)	VWFpp/ VWF:Ag	VWF:RCo/ VWF:Ag	VWF:CB/ VWF:Ag	RIPA 2/1.5/0.5 (mg/mL)	PFA100 (C/ADP) CT (sec)	PFA100 (C/Epi) CT (sec)
Family														
1	P1	O	6	15.3	15.5	8.0	7.4	70	4.5	0.52	0.48	41/6/0	>258	>299
	P2	A	8	21.0	13.0	7.7	8.7	68	5.2	0.59	0.67	37/6/0	>183	>241
Family														
2	P3	B	12	35.0	15.3	9.2	9.0	74	4.8	0.60	0.58	39/22/3	>279	>300
	P4	B	3	17.0	11.0	6.0	8.0	62	5.6	0.54	0.72	29/17/2	>300	>300
Family														
3	P5	A	9	18.1	8.0	5.0	5.0	52	6.5	0.62	0.62	40/4/0	>300	>300
	P6	A	12	27.7	8.6	5.2	4.7	50	5.8	0.60	0.55	37/7/0	>300	>300
	P7	A	7	17.6	8.6	5.0	5.6	57	6.6	0.58	0.65	37/6/0	>300	>300
	P8	A	10	18.5	12.0	6.0	6.0	60	5.0	0.50	0.50	34/12/0	>300	>300
N.V.			(-3 to 4)	57-145	43-147	49-147	51-115	60-140	0.8-1.5	>0.7	>0.7	99-110/ 56-117/<20	46-104	88-147

BG: blood group; BS: bleeding score; FVIII:C: procoagulant factor VIII; VWF:Ag: VWF antigen; VWF:RCo: VWF ristocetin cofactor activity; VWF:CB: VWF collagen binding; VWFpp: VWF propeptide; RIPA: ristocetin-induced platelet agglutination; PFA: platelet function analyzer; CT: closure time; (C/ADP): collagen/adenosine diphosphate; (C/Epi): collagen/Epinephrine; NV: normal value.

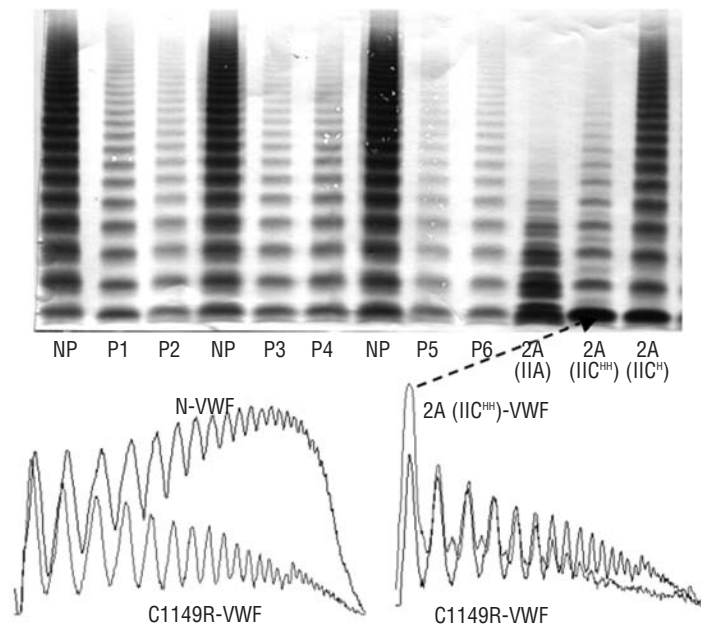


Figure 1. Multimeric analysis of plasma von Willebrand factor (VWF). Upper panel: multimeric analysis of VWF in low resolution (1.2%) SDS-agarose gel in plasmas of a normal subject (NP), patients with the missense C1149R mutation (P1 to P6), and in plasma of a patient with VWD type 2A(IIA) VWD and patients with VWD type 2A(IIC) homozygous (^H) and heterozygous (^H). Lower panel: densitometric analysis comparison of the profiles from the upper panel normal. A large decrease in relative proportion of high molecular weight VWF multimers (HMWM) is evident in all the patients with this mutation. Densitometric profiles of the remaining patients are similar to that of P1. The type 2A (IIC) pattern shows an aberrant multimeric pattern with a clear increase in the relative proportion of the smallest oligomer (discontinuous arrow), not present in the C1149R VWF.

International, Miami, USA) for collagen/ADP (C/ADP) and the collagen-epinephrine (C/Epi) cartridges. Procoagulant factor VIII activity in plasma was measured using a chromogenic assay (FVIII:C) and VWF antigen (VWF:Ag) levels were determined by ELISA. The von Willebrand factor ristocetin cofactor activity (VWF:RCo) test was performed on lyophilized platelets (Helena Biosciences Europe, Sunderland, United Kingdom) using an aggregometer. Ristocetin induced platelet agglutination (RIPA) was carried out in platelet-rich-plasma in an aggregometer (Helena Biosciences Europe, Sunderland, United Kingdom) with final concentrations of ristocetin of 2, 1.5 and 0.5 mg/mL. The VWF:CB level was determined with an in-house ELISA method that utilized Type I collagen purchased from Nycomed (Nycomed, Linz, Austria). VWF multimeric distribution was analyzed using high- and low-resolution SDS-agarose gel electrophoresis. Both the low-resolution and the high-resolution gels were run in an LKB Multiphor system, essentially as previously described.^{12,15} VWF multimers were transferred to Immobilon P membrane (Millipore, MA, USA) by electroblotting and the multimers were visualized using Extravidin-Alkaline Phosphatase-conjugated immunopurified anti-VWF polyclonal antibodies (Dako, Glostrup, Denmark). Densitometric analysis was performed using the ImageQuant program (General Electric, Fairfield, CT, USA). Densitometric profiles were compared between lanes located in the same gel. VWF propeptide (VWFpp) concentrations were determined with an ELISA test kit (SANQUIN, Amsterdam, The Netherlands).

Levels of FVIII:C, VWF:RCo, VWF:Ag, VWFpp, CT PFA-100 with C/ADP and C/Epi were tested before and after administration of DDAVP. Platelet VWF was measured as previously described,¹⁴ modified by using citrated blood in the presence of inhibitors (Protease Inhibitor

Cocktail, Sigma-Aldrich, St. Louis, MO, USA) instead of acid-citrate-dextrose (ACD) anticoagulant and lysing with 1:40 v/v of 20% Triton X-100 as previously published¹⁵ instead of sonication. The half-life ($t_{1/2}$) of VWF:Ag and VWFpp was determined according to a previously published method.¹⁶⁻¹⁸

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes (Invisorb Spin Blood Mini Kit, Nucliber, Berlin, Germany). The promoter, all 52 exons and intron-exon boundaries were amplified by polymerase chain reactions (PCR) in a GeneAmp PCR System 9700 (Applied Biosystem, Forster City, CA, USA). The primers used for this study were those described in the MCMMDM-1VWD Study.¹⁹ The oligonucleotide primers used for polymerase chain reaction PCR amplification of exon 26 were CAA CAT TAT CTC CAG ATG GC (forward primer) and TTG CAG GTC AGA GAT AGG AC (reverse primer). The PCR products were purified using the Qiaquick PCR purification kit (QIAGEN, Hilden, Germany). Both forward and reverse strands were directly sequenced by using an automated Applied Biosystems 3130 sequencer (Applied Biosystem, Forster City, CA, USA).

Results

All 8 patients showed notably decreased VWF antigen and activity as well as reduced RIPA and VWF:RCo levels (Table 1). The VWF:RCo/VWF:Ag ratio and the VWF:CB/VWF:Ag ratio were lower than the 0.7 normal range indicating a VWD type 2. Baseline data showed that all the plasma VWF multimers were decreased, with a clearly lower relative proportion of HMWM,

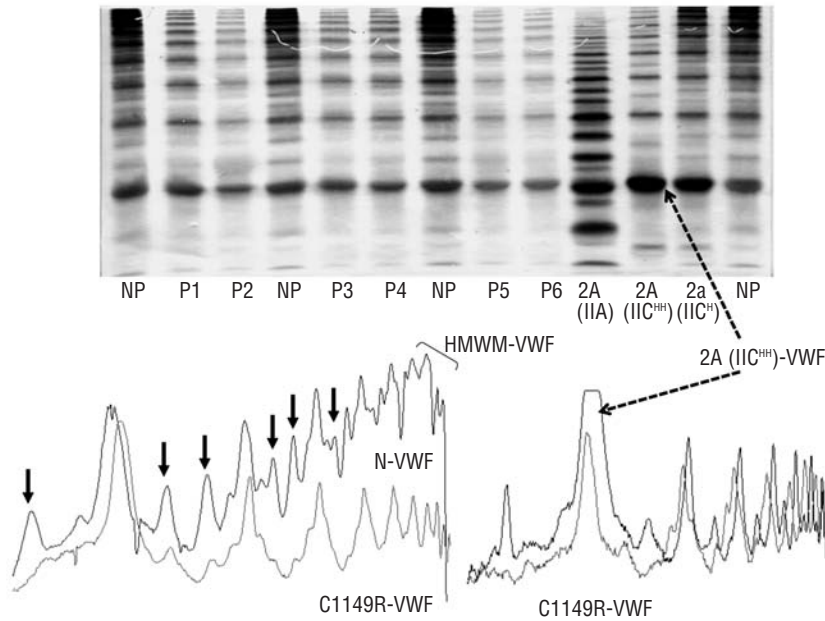


Figure 2. Multimeric analysis of plasma von Willebrand factor (VWF). Upper panel: multimeric analysis of VWF in higher resolution (2%) SDS-agarose gel in plasmas of a normal subject (NP), patients with the missense C1149R mutation (P1 to P6), a patient with VWD type 2A(IIA) VWD and patients with VWD type 2A(IIC) homozygous (^H) and heterozygous (^H). Lower panel: densitometric analysis comparison of: to the left, a normal VWF and the C1149R VWF; to the right, the 2A(IIC^H) and the C1149R VWF. A large decrease in relative proportion of the outer satellite bands is noticeable in all the patients compared to the normal subject (indicated by arrows). The type 2A (IIC) pattern shows an aberrant multimeric pattern with a clear increase in the relative proportion of the smallest oligomer (discontinuous arrow), not present in the C1149R VWF.

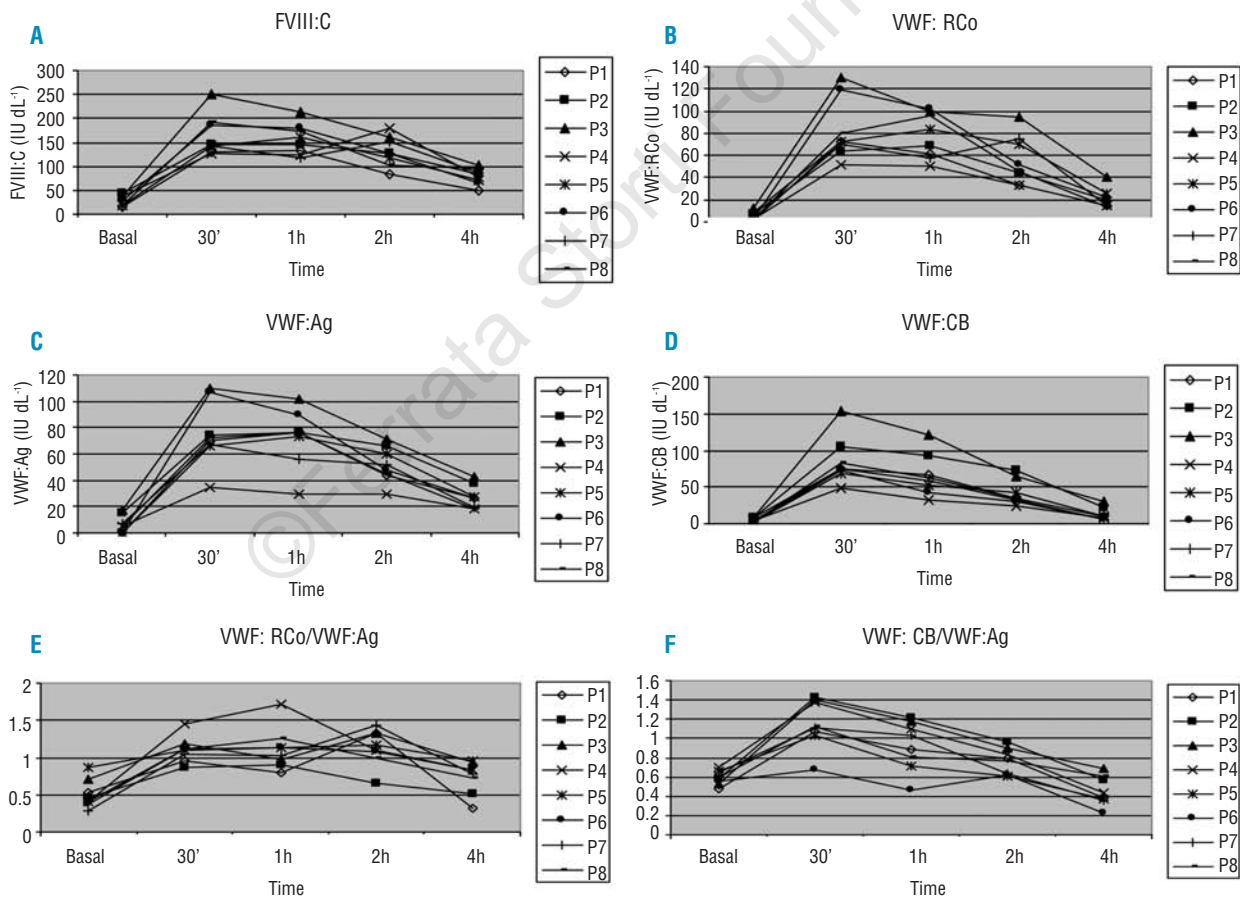


Figure 3. Responses to desmopressin (DDAVP) in 8 patients with the von Willebrand factor (VWF) C1149R mutation. Changes are shown for each patient before and at 30 min, one hour, two hours, and four hours (30', 1h, 2h, 4h) after the end of infusion of DDAVP. Panel A: procoagulant factor VIII (FVIII:C) (IU dL⁻¹). Panel B: von Willebrand factor ristocetin cofactor activity (VWF:RCo) (IU dL⁻¹). Panel C: von Willebrand factor antigen (VWF:Ag) (IU dL⁻¹). Panel D: von Willebrand factor collagen binding (VWF:CB) (IU dL⁻¹). In all patients, FVIII:C, VWF:RCo, VWF:Ag and VWF:CB levels rose significantly 30 min after DDAVP administration. Panel E: A significant rise in the VWF:RCo/VWF:Ag ratio is seen at one hour after DDAVP administration in all patients. This increase was much more pronounced in patient P4. Panel F: it is noticeable that VWF:CB behaves quite similarly to VWF:RCo after DDAVP administration.

Table 2. Factor VIII (FVIII) and von Willebrand factor (VWF) measurements before and after desmopressin (DDAVP) treatment in patient P1. DDAVP induced a 5-fold increase of VWF:Ag, a 10-fold increase of VWF:RCO and VWF:CB and a 6.4 to 9.2 fold increase of FVIII:C followed by decreased half-life times of all VWF parameters and FVIII:C consistent with or even pathognomonic for VWD type 2A (IIE) . There is change from type 2 to type 1 phenotype with transient correction of PFA CT using sensitive tests for functional VWF tests and platelet function.

Time after DDAVP	FVIII:C (IU dL-1)	VWF:Ag (IU dL-1)	VWF:RCo (IU dL-1)	VWF:CB (IU dL-1)	VWFpp (IU dL-1)	VWFpp/VWF:Ag	VWF:RCo/VWF:Ag	VWF:CB/VWF:Ag	PFA100 (C/ADP) CT (sec)	PFA100 (C/Epi) CT (sec)
Basal	14.0	13.6	7.2	7.0	66	4.8	0.53	0.51	>258	>29
30 min	90/129	70	73	75	298	4.3	1.04	1.07	69	70
1 h	91/134	76	61	67.2	315	4.1	0.80	0.88	74	95
2 h	59/84	44.6	33.5	35	187	4.2	0.75	0.78	162	188
4 h	36/51	26	14.2	10	127	4.9	0.54	0.38	>300	>224

FVIII:C, procoagulant factor VIII; VWF:Ag, VWF antigen; VWF:RCo, VWF ristocetin cofactor activity; VWF:CB, VWF collagen binding; VWFpp, VWF propeptide; PFA (Platelet Function Analyzer); CT, closure time; (C/ADP), collagen/adenosine diphosphate; (C/Epi), collagen/Epinephrine.

compared to normal plasma (Figure 1).

On high-resolution agarose gel, a markedly decreased proportion of the satellite bands was observed (Figure 2). The increase in the relative proportion of the first oligomer characteristic of VWD type 2A (IIC) was not seen in the patients. Two hours after the administration of DDAVP, all plasma levels of FVIII:C, VWF:RCo, VWF:Ag and VWF:CB had increased more than three-fold over baseline (Figures 3A, B, C and D). The $t_{1/2}$ of released VWF was shorter (range 1-1.5 h) than normal (Table 2). Interestingly, the mean $t_{1/2}$ value of VWFpp after DDAVP administration in the patients was 100 min (range: 85-115 min) which is shorter than that previously reported in normal individuals (2-3 h).¹⁶⁻¹⁸ Some temporary correction of VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios was observed (Figures 3E and F). Noticeable quantitative differences were seen among the three families of patients. The platelet VWF (VWF:Ag and VWF:RCo) content in the 8 patients studied was within the normal range (0.14-0.50 IU/10⁹ platelets). All the control subjects analyzed displayed normal VWF multimers in platelet VWF with super-large VWF multimer present (Figure 4). In contrast, patients with this mutation had a qualitatively abnormal content of VWF showing a very decreased relative proportion of the super-large HMWM.

Following DDAVP administration, the relative proportion of HMWM normalized temporarily in the low resolution gels was seen. However, it should be emphasized that following its administration super-large HMWM were not seen in any of the patients (Figure 4) in contrast to their appearance in normal subjects as previously described.²⁰ In the high resolution gels, a temporary increase in the relative proportion of the satellite bands was discernable, although striking differences from the response seen in normal subjects was evident. Thus, after DDAVP administration in the patients the inner VWF multimeric satellite bands showed an increased relative proportion higher than that seen in normal subjects (Figure 5).

All affected individuals from the three families had an increased VWFpp/VWF:Ag ratio before and after DDAVP administration (Table 1 and 2).

We determined the entire coding sequence of the VWF gene²¹ in the 8 patients. In addition to several polymorphisms, we identified only one candidate mutation,

3445T>C in exon 26, which predicts an amino acid change, Cys1149Arg. Both nucleotides T and C were detected in this position, indicating that only one allele was the carrier for the mutation, as would be expected for a heterozygous state.

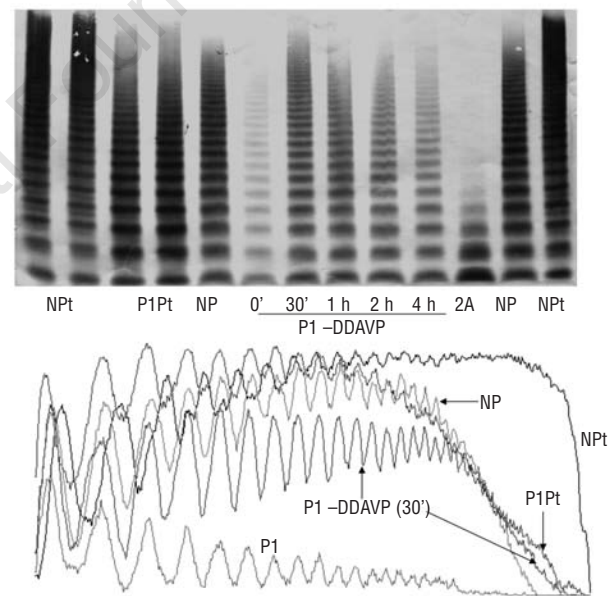


Figure 4. Multimeric analysis of von Willebrand factor (VWF) in platelets and plasma of a patient with C1149R mutation. DDAVP response. Upper panel: multimeric pattern of VWF in low resolution (1%) SDS-agarose gel in platelet lysates of a normal individual (NPt) and patient (P1Pt), as well as in plasma in a normal subject (NP) and in the patient before (0') and after DDAVP administration at 30 min, one hour, two hours, four hours (30', 1h, 2h, 4h). Plasma of a patient with 2A VWD (IIA) is included as a control. Platelet VWF in the patient shows an absence of the super-large HMWM of VWF decrease in the relative proportion of HMWM compared with a normal subject. Plasma VWF from the patient at basal state presents a decreased relative proportion of the HMWM compared to patient platelet VWF. Lower panel: densitometric analysis of the lanes from the gel. Super-large high molecular weight multimers HMWM (bar with arrow) are present in normal platelets but not in the corresponding normal plasma. After DDAVP therapy, a transient increase of the HMWM is observed in the patient plasma, with no appearance of the super-large multimers HMWM of VWF. The remaining patients showed a similar response and pattern. Type 2A (IIA) VWF shows the lack of HMWM.

Discussion

The mutation Cys1149Arg (3445T>C) of the VWF gene was first described as causing severe VWD type 1 with high penetrance.⁵ The ISTH VWF mutation database (www.vwf.group.shef.ac.uk) still includes this mutation as VWD type 1. A study of the expression of the corresponding mutant recombinant VWF (C386R) demonstrated a reduced secretion and intracellular retention of normal VWF, creating a dominant negative defect.^{5,6} Defects in multimer assembly can be caused by heterozygous mutations in the D3 domain that interfere with intersubunit disulfide bond formation in the Golgi. This phenotype was initially designated VWD type IIE, first described in a family with aberrant multimeric pattern of VWF. It is like IIC but a marked increase in the smallest oligomer, a hallmark of type IIC, was not present (Figures 1 and 2).^{9,22} VWD type 2A (IIE) share in common with VWD type 2A (IIC) a reduced proteolysis of plasma VWF with a large decrease in the relative proportion of the outer satellite bands. By contrast, VWD type 2A (IIA) has an intense proteolysis of VWF visible by a higher proportion of the outer satellite bands of the triplet structure (Figures 2 and 5).

In our study the mutation C1149R was identified in 8 patients from three unrelated families and we found some different phenotypic results from those previously reported. Baseline plasma levels of VWF:Ag were diminished, with VWF:RCo/VWF:Ag and VWF:CB/VWF:Ag ratios <0.7 and decreased platelet adhesion caused by selective deficiency of HMWM and intermediate molecular weight VWF multimers (IMWM). This suggests a VWD type 2A (Figure 1). A clearly relevant decrease in the relative proportion of the outer satellite bands was also evident (Figure 2), with a pattern of VWD type 2A (IIE). A qualitative abnormal platelet VWF was observed in contrast with the results of previous expression studies that showed a synthesis of an almost fully normal VWF^{5,8} (Figure 4). However, the quantitative platelet VWF content seen in this study may support the platelet retention of this molecule suggested by these earlier expression studies.

Accelerated clearance can also be involved in dominant VWD type 1 contributing to the low plasma VWF levels. According to the criteria recommended by Federici *et al.*^{23,24} the patients in this study had a good response to DDAVP infusion, although the $t_{1/2}$ of VWF was short (range 1-1.5 h) when compared with normal controls, as already reported by other authors.^{7,8,25-27} These patients also exhibited an increased plasma VWFpp/VWF:Ag ratio (a surrogate marker for VWF clearance), indicating an accelerated clearance of the endogenously produced VWF (Table 1).^{17,18,28} DDAVP was clinically effective in those patients, although we did not have the opportunity to evaluate the efficacy of DDAVP therapy in situations with a high risk of bleeding.

An intriguing aspect of the patients presented here is the temporary correction of the VWF:RCo/VWF:Ag ratio and the low resolution multimeric pattern of VWF after DDAVP, which in fact would suggest more a type 1 characteristic not described in classical type 2A,

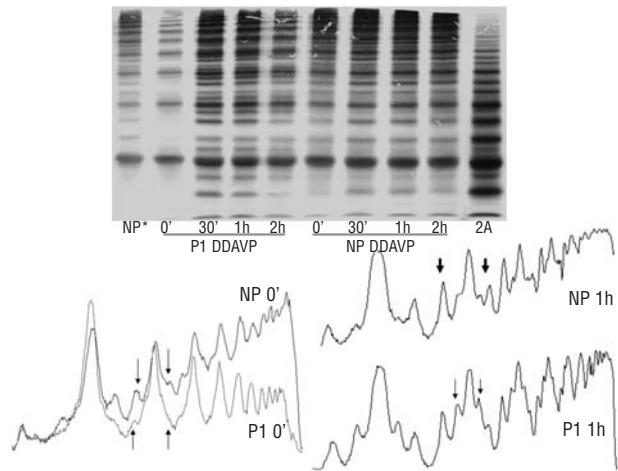


Figure 5. DDAVP responses in plasmas of a normal subject and a patient with C1149R mutation. Upper panel: multimeric pattern of VWF in high resolution (2%) SDS-agarose gel in plasmas of a normal individual (NP) and a patient (P1) before (0') and after desmopressin (DDAVP) administration at 30 min, one hour, two hours (30', 1h, 2h), respectively. Lower panel: densitometric analysis of the multimeric profiles from the upper panel, before and after DDAVP administration. Before DDAVP (left) a marked relative decrease in the proportion of outer satellite bands is noticeable in the patient compared to the normal subject (indicated by thin arrows). After DDAVP (right) a clear increase in the relative proportion of the inner satellite bands (thin arrows) is visible in the patients, in contrast to the increase of the outer satellite bands seen in the normal subject (thick arrows). Again, the remaining patients showed a similar response and pattern. Type 2A VWF shows the characteristic increase of the outer satellite bands. It clearly shows a change in VWF multimers after DDAVP consisting of a mixture of normal VWF and mutant VWF, but the combination, a mixture of mutant and normal VWF, is featured by rapid clearance *in vivo*. This observation is pathognomonic for VWD 2A (IIE).

although it occurs very briefly becoming abnormal when VWF:Ag is still higher than the basal values. Furthermore, the abnormal proteolytic pattern seen after DDAVP administration reinforces its consideration as VWD type 2. However, it must be emphasized that platelet VWF is not completely normal as it occurs in VWD type 1, because it shows a lack of the super-large multimers not previously reported.⁵

We believe that this response is a key observation VWD type 2A (IIE). However, no super-large multimers appear in their plasma. In fact, in patients and after DDAVP, the multimeric pattern was closer to that of normal plasma (baseline values) and to that seen in patients' platelets (Figure 4). Furthermore, satellite bands, as seen in normal subjects,²⁰ were observed in the affected individuals, indicating degradation of the newly secreted VWF by ADAMTS13. However, the relative distribution of the satellite bands was quite different from that in normal subjects (Figure 5), with a predominant increase of the relative proportion of the inner satellite bands in contrast with the predominant increase of the outer bands observed in normal subjects (Figure 5). It suggests that the molecular abnormality present in the patients favors a proteolytic fragmentation different from that occurring for normal VWF and for VWD type 2A (IIA).²⁹

These findings are in agreement with the potential

intracellular retention of VWF, an increased clearance and/or degradation of plasma VWF as dual mechanisms involved in C1149R mutation processing. The absence of satellite bands in baseline data suggests that the clearance is not only due to the proteolytic action of ADAMTS13. Other mechanisms could explain this clearance.³⁰⁻³²

Several mutations previously diagnosed as type 1 in the European Study MCMDM-1VWD were reclassified as VWD type 2A (IIE) after detailed multimeric analysis.^{19,25,26} C1149R mutation was not found in the European study. Once again, it demonstrates the problem of VWD type 1 classification, which has been widely investigated in three different international studies.³³⁻³⁵ It is very well known in routine daily practice that distinction between type 1 or 2 in cases of VWD 2A(IIE) and 2M is frequently not possible due to the limited test set used by laboratories and to the lower limit of sensitivity for these assays including VWF multimeric analysis, VWF:Ag and VWF:RCO^{36,37} as recommended by the ISTH-SSC.³⁸ The present data clearly demonstrates that VWD type 2A (IIE) as a distinct VWD phenotype truly exists if sensitive functional tests such as VWF:RCO, VWF:CB and PFA100^{39,40} plus DDAVP response curves and VWFpp/VWF:Ag

ratios are used. Our findings also confirm recent data in defining the VWD 2A type (IIE) in other missense mutations in the D3 domain of the VWF gene as a defect of VWF clearance.^{17,48}

In conclusion, our data demonstrate that this mutation causes dominant VWD type 2A (IIE), which is clearly different from the other 2A subtypes defined by the ISTH-SSC classification. They also support the importance of studying platelet VWF and DDAVP response to provide complementary information when classifying VWD cases.

Authorship and Disclosures

AP, EL, AG and AR performed the experimental part of the study; JB and MFL performed the clinical studies; JB, AP, AG and MFL designed the research, analyzed the results and wrote the paper. None of the co-authors has any financial or other conflict of interests related to this research project. The present manuscript was reviewed and approved by the Research Committee of C.H.U. de La Coruña, Spain.

The authors reported no potential conflicts of interest.

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