Von Willebrand's disease: a novel mutation, P1824H and the incidence of R1205H defect among families with dominant quantitative von Willebrand factor deficiency

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To date, few mutations associated with a dominant quantitative deficiency of von Willebrand factor (VWF) and a high penetrance have been reported. This phenotype was confirmed in seven unrelated families of several patients diagnosed with von Willebrand's disease out of 70 who requested genetic studies of the *VWF* gene. The mutations linked to this type were identified: R1205H in five families; T1156M in one family; and the new P1824H alteration in one other family. The R1205H mutation linked to the different haplotypes might well be frequent among this variant. The P1824H in the A3 domain is associated with very low VWF levels and with a moderate-to-severe bleeding tendency, unlike the other mutations reported in this domain.

Key words: dominant type 1 VWD, quantitative VWF deficiency, VWF gene, von Willebrand's disease.

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The current classification of von Willebrand's disease (VWD) considers that this bleeding disorder is caused by mutations at the *VWF* locus and comprises six types.[1] Specifically, type 1 VWD is caused by partial quantitative deficiencies of von Willebrand factor (VWF), and has been considered as the most common variant to date. However, this type of VWD is often difficult to diagnose. Molecular-based knowledge has increased vastly in the last decade, especially concerning the functional variants since analytical data have been directed at searching for mutations in specific regions of the *VWF* gene. Most mutations registered in the current VWF database are associated with types 2A, 2B and 2N VWD. Type 2M VWD includes variants with functional deficiency of VWF dependent on the platelet, in the presence of all the plasma multimers. According to the current classification, a small number of type 1 families could be re-classified as having type 2M VWD; furthermore defects in the A1 domain have been associated with this phenotype.[2-4] Presently, only three missense mutations are reported as being responsible for dominant type 1 VWD.[5-7] Segregation analyses with *VWF* gene markers allowed us to determine whether the bleeding phenotype was linked to this gene.[8]

Design and Methods

Patients

Seventy unrelated families were recruited in our Center for genetic studies of VWD. Sixty-three of these families were included in the search for mutations in the *VWF* gene; the families had the following types of VWD: type 1 (n=25), type 2A (n=6), type 2B (n=9), type 2M (n=7), type 2N (n=3) , type 1/2 (n=2) , type 3 (n=9) and unclassified (n=2). Seven families comprising 89 individuals, 46 classified as having type 1 VWD in accordance with the Consensus Criteria for the diagnosis of type 1 VWD,[9] were involved in this study. Different family members gave their consent to investigation after they were informed of the kind of study they were to take part in. The phenotype laboratory assays and segregation polymorphism analyses were performed as previously described.[2,8] The collagen binding assay was performed using a Gradipore kit, with equine collagen type 3 as a substrate.

Mutation detection

A search for mutations was performed by single strain conformation polymorphism analysis (SSCP) in fragments of about 300 bp, while larger fragments were analyzed by a modified conformation sensitive gel electrophoresis (CSGE) analysis with a commercial polymer. The polymerase chain reaction (PCR) products with an abnormal electrophoretic pattern were sequenced. Mutations were confirmed in another PCR sample and in the family study either by sequencing or restriction analysis. At least 100 normal chromosomes were also examined for the new mutations.

Results and Discussion

The phenotype studies showed VWF deficiency in all the patients included. The multimeric structure was considered normal in

Table 1. Phenotype and genotype data.

Family	*Patient*	*Sex*	*Age*	*BG*	*VWF:Ag (IU/dL)*	*VWF:RCo (IU/dL)*	*VWF:CB (IU/dL)*	*Ratio CB/Ag*	*FVIII:C (IU/dL)*	*Mutation*	*Affected members*	*Non-affected members*
F1	IV:12	F	55	A+	8.7±2.3	6.2±2.7	7.2±2.5	0.83	17±3	3614G→A (R1205H)	16	12
F26	IV:1	M	17	A-	34 ± 5	2±5	27	0.79	68±11	3467C→T (T1156M)	7	7
F3	II:1	F	16	AB+	7.2±2.0	6	5.7±0.3	0.79	13± 1	3614G→A (R1205H)	9	9
F4	III:1	F	37	B+	11±3	8.4±4.7	4.8±1.3	0.44	32±10	5471C→A(P1824H)	5	8
F5	III:3	F	34	A+	6.8±2.6	7	5.8±0.8	0.85	12± 2	3614G→A (R1205H)	3	1
F6	I:1	F	51	–	4.5±1.5	< 7	5.4±1.6	1.20	10±3	3614G→A (R1205H)	2	4
F7	III:1	M	32	–	12±2	10±1	13±5	0.92	23±6	3614G→A (R1205H)	4	2
F1	IV:12		1 hour		63	88	57	0.90	93			
			2 hours		41	45	38	0.93	65			
			4 hours		18	13	14	0.78	29	DDAVP test-infusion		
			6 hours		15	< 7	8	0.53	16			

F: female. M: male. BG: blood group. The mean and standard deviation are shown when three or more determinations were performed, and the results were over the technique detection limit which was between 5-7 IU/dL for VWF:RCo.

all affected individuals. The RIPA analyses were normal or low. Laboratory data from one patient of each family are shown in Table 1. Patients from families F1, F3 and F5 to F7 had a moderate-to-mild bleeding tendency despite the significant decreases in the VWF levels. Responses to a DDAVP test-infusion were good, although the VWF levels soon decreased (Table 1). The F4 propositus had easy bruising with minimal or no apparent trauma, and required a blood transfusion to treat heavy menorrhagia when she was a teenager. She had two vaginal deliveries, under prophylactic treatment with FVIII/VWF concentrates (Haemate-P), without bleeding complications. Her father had prolonged bleeding after tooth extractions. Her grandmother died after a post-partum hemorrhage.

Linkage analysis showed that the phenotypes were linked to the *VWF* locus; the results from families 1-4 have been previously described.[8] SSCP analysis was proved to be useful in searching mutation patterns (Figure 1) in patients from all families, except family 4, in which CSGE analysis was used to that end. Mutations were characterized by sequencing. Remarkably, the missense mutation R1205H in exon 27 was detected in five families. The presence of an M740I mutation in exon 17, which was also carried by four families from the Vicenza area (Italy),[10] was excluded. In F2, the T1156M change segregates with the mild phenotype.[6] A new mutation, 5471C→A(P1824H), was detected in exon 32 of the *VWF* gene, which codes for part of the A3 domain and gives rise to a restriction site for the *XcmI* enzyme. The presence of the 5471C→A mutation could, therefore, be confirmed by restriction analysis in the five F4 patients (Figure 2). Restriction analysis was also used to show that this mutation was not present in 100 normal chromosomes.

Mutations causing dominant type 1 VWD with a high penetrance were first described in cysteine residues 1149 and 1130 of the D3 domain. The studies with recombinant VWF suggested that dimers with C1149R subunits were retained and degraded in the endoplasmatic reticulum, and that only the wild-type subunits were transported to the Golgi apparatus to be secreted normally.[5] A possible cluster of dominant quantitative variants, in this region, as occurring for other qualitative variants, has not been confirmed. The R1205H mutation was described in eight Italian families and one German family with type 2M VWD.[11,10] It had also been reported in 18 unclassified VWD French patients from ten families,[12] in two Hungarian families with dominant type 1 VWD,[13] and was frequent among the families included in the international multi-center study of type 1 VWD, as well as in other direct genetic studies.[14,15] It was also identified in five families from our Center, who showed a similar phenotype to the Vicenza one, the only exception being the presence of extra-large multimers, which were only occasionally detected in our series. The segregation analyses showed that the VWD phenotype was linked to distinct haplotypes in these families, and this was also confirmed in the whole *VWF* gene screening. Thirty-four patients out of 46 affected members included in this series carried this mutation, which represents more than 70% of the studied patients. Our Center's 2005 Census showed that we have data on 286 patients with a VWD phenotype, when all the variants are included, 135 of them are compatibly diagnosed with type 1 VWD. Therefore, the R1205H mutation is the cause of the bleeding disorder in approximately one quarter of our patients classified as having type 1 VWD, and in more than 10% of all VWD patients. In addition, the nucleotide change 3614G→A occurs in a dinucleotide CG. Consequently, we believe the R1205H mutation might well be frequent in the dominant type 1 VWD with a high penetrance. The expression studies of this mutation are more difficult to interpret than those in the case of the more classic qualitative variants. An *in vivo* study had conclud-

Figure 1. Pedigree from family 1 with the segregation of the R1205H mutation. Solid symbols represent affected members, slashed symbols represent deceased members, and the arrow represents the index case. The SSCP analysis of the exon 27 fragment is shown at the bottom. The normal pattern from sample III:4 was confirmed in another gel.

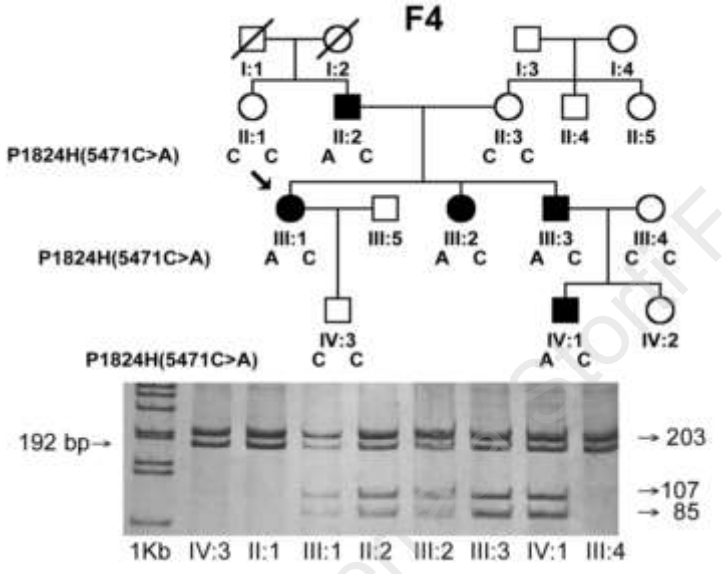

Figure 2. Pedigree from family 4. Mutation restriction analysis in 10% acrylamide/bis gels (29:1) and silver stain: the digestion of the 395 bp fragment with *XcmI* generated 203 and 192 bp fragments from normal alleles, and 203, 107 and 85 from mutated alleles. The restriction analysis for individual II:3 did not appear in this gel, although it was visualized in the simultaneous electrophoresis, and the genotype was also confirmed by sequencing.

ed the R1205H mutation *per se* is associated with an increased clearance rate, which could explain the reduced VWF levels among patients.[16]

The T1156M mutation was detected in the seven affected members of F2; their VWF levels were below borderline, and were quite independent of blood group.[6] No other major abnormalities were identified in the whole gene screening. This mutation was also detected in an heterozygous patient; the *in vitro* expression studies indicate that the T1156M mutation causes intracellular retention, and its heterozygosity is consistent with mild type 1 VWD.[7]

The P1824H mutation was confirmed in the five VWD patients in F4, and no other defects were detected in the *VWF* gene, thus making P1824H a candidate causative mutation for VWD with a dominant negative effect. This is the first time that a missense mutation in the A3 domain of VWF in the binding site to subenthothelium collagen has been reported to be associated with low VWF levels and a moderate-to-severe bleeding history; the other mutations described in this domain (S1731T in two patients,[17] and Q1734H, I1741T, Q1726R in asymptomatic patients) are not associated with this phenotype.[18]

The P1824 residue is located in the main collagen type I and III binding sites of VWF.[19] It is hypothesized that the putative collagen interaction surface is primarily smooth and negatively charged. Binding would be produced through a larger number of low affinity interactions between negatively-charged surfaces in the A3 domains of the VWF multimers and the positively-charged binding sites in the triple collagen helices. P1824 is in the central beta-sheet that contains six strands, surrounded by seven α helices; it is specially

found in the β5 strand between helices α4 and α5, and it does not seem to be essential for collagen binding. Nevertheless, the 5471C→A change predicts the substitution of a non-polar amino acid by another basic positively-charged one, which is likely to produce a change in the conformation of the A3 domain that may, in turn, alter the collagen binding surface. Furthermore, the P1824H mutation might produce another effect which would give rise to low VWF levels in plasma, as seen in the F4 patients. VWF levels are regulated by a balance between VWF biosynthesis, secretion, proteolysis through the protease ADAMTS13, and protein catabolism by means of a still unknown mechanism.[20] It is difficult to determine which of these mechanisms is affected from the laboratory data available on the phenotype. Nonetheless, the data indicate a marked decrease in VWF protein and function with a slight discrepancy in relation to the result of the collagen type III binding assay. Unfortunately, patients are somewhat reluctant to undergo certain analyses, such as the DDAVP test. Future *in vitro* expression studies would enable us to determine the possible mechanism through which this mutation causes a bleeding disorder.

In short, the molecular studies undertaken indicate that the mutation R1205H is the most frequently recurrent mutation among cases of dominant partial quantitative VWF deficiency with high penetrance. A novel mutation in the A3 domain is associated with a moderate-to-severe bleeding tendency, very low VWF levels and a high penetrance. The detection of causative mutations may prove highly useful in health care services as it facilitates and improves VWD management, especially if the effects and the possible physiopathological mechanisms of the mutations can be understood.

PC was responsible for designing the study, performing genetic analyses, analyzing the data and writing the manuscript. NC helped design the study and performed the search for mutations. SH and ARC helped design the study, and collected the clinical and analytical data from the patients. JAA and all authors supervised and revised the final version of the paper. Table 1 and Figure 1 by PC; Figure 2 by NC. We wish to thank all the staff of the Unidad de Coagulopatías Congénitas de la Comunidad Valenciana for their technical and clinical assistance, as well as Dr. MA Dasí from Children's Hospital La Fe, and hematologists from both General Hospital of Valencia and Vinaros Hospital for referring patients. This work was partly supported by FIS grant # 99/0633 and FIS grant # PI020612 (Spain). Manuscript received February 17, 2006. Accepted June 20, 2006.

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