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LETTER TO THE EDITOR

Clinical profile of the association of P.R1205h and P.R924q in a patient with von Willebrand's disease

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von Willebrand's disease (VWD)-type Vicenza is characterized by a mild–moderate bleeding tendency, presence of ultralarge multimers (ULVWF), low von Willebrand's factor (VWF) and factor VIII (FVIII:C) [1], attributed to reduced VWF survival [2], and the propeptide/VWF ratio (VWFpp/VWF:Ag) is increased [2]. Patients with p.R1205H had a 5–10-fold increase in FVIII [3] and 5-fold increase in VWF:RCo after DDAVP, although their levels did not reach normal values [4]. p.R924Q was previously reported as a polymorphism (SNP) [5], in association with type 1 VWD [6], responsible of VWD2N phenotype by us [7], and the Brazilian group (Hemocentro UNICAMP, www.vwf.group.shef.ac.uk), as a marker of null allele [8], and as responsible for reductions in VWF and FVIII levels, particularly in combination with O blood group, and with a second mutation [9]. We report an 18-year-old female at the moment of her first consultation in our Institution. She presented with a history of easy bruising and gum bleeding during childhood, and one episode of post-traumatic gingival and lip bleeding at her 9 years of age requiring replacement therapy. Her bleeding score was 5, with normal menstrual cycles (score = 40; normal Pictorial Bleeding Assessment Chart score $\leq$185). On admission, her laboratory tests revealed normal platelet count and bleeding time (BT), prolonged activated partial thromboplastin time (aPTT) (63 s; normal range [nr] 34–45 s), with no inhibitory activity detected, reduced FVIII:C (14 IU dL^{-1}), VWF:RCo (<10 IU dL^{-1}), VWF:Ag (9 IU dL^{-1}) and FVIII binding/VWF:Ag ratio (VWF:FVIIIB/VWF:Ag) (0.31; nr $\geq$ 0.8), normal platelet VWF:Ag (0.29 IU 10^{-9}plt; nr 0.18–1.02 IU 10^{-9}plt), increased VWFpp/VWF:Ag (11.3; nr 0.91–2.14) and presence of ULVWF. At her 18 years, DDAVP response was evaluated before, 60 and 120 min (a single intravenous dose of 0.3 μg kg^{-1} body weight of DDAVP, in 50 mL saline solution over 20 min). FVIII:C, VWF:Ag and VWF:RCo showed 2-, 3.1- and ≈4-fold increases respectively at 60 min, and 1.75-, 2.15- and ≈2.5-fold at 120 min. Their levels at 120 min were lower than at 60 min, suggesting reduced survival of VWF and FVIII:C (Table 1). The mother suffered epistaxis that required tamponades and cauterization during childhood, menorrhagia (score = 248), easy bruising and bleeding in four pregnancies at first trimester and in three abortions (bleeding score = 4), with no bleeding in deliveries and episiotomies. Her laboratory tests revealed normal platelet count and BT, prolonged aPTT (53 s) (no inhibitory activity detected), reduced FVIII:C (40 IU dL^{-1}), normal VWF:RCo (66 IU dL^{-1}), VWF:Ag (75 IU dL^{-1}) and VWF:FVIIIB/VWF:Ag (1.0) and absence of ULVWF. The father only suffered ecchymoses after nose plastic surgery (bleeding score = 2). He did not have neither bleeding after tooth extractions nor familial bleeding history. The sister did not have bleeding symptoms. Both the father and sister had normal laboratory tests. Maternal grandparents were asymptomatic; therefore we excluded the patient's mother as a carrier of haemophilia A. Patient and relatives had Caucasian origin and O blood group.

Table 1. Patient's response to DDAVP test.

	BT (min)	aPTT (s)	FVIII:C IU dL^{-1}	VWF:Ag IU dL^{-1}	VWF:RCo IU dL^{-1}	Platelet count per μL
Basal	2.5	62*	20	13	<10*	283 000
60 min	2	49*	40 (2)	40 (3.07)	40 (≈4)	250 000
120 min	1.5	52*	35 (1.75)	28 (2.15)	25 (≈2.5)	283 000

*no inhibitory activity was detected. In brackets: increase from baseline.

Table 2. VWF haplotype analysis of kindred using markers within the VWF gene.

Sequence variant	Allele frequency*	Patient	Mother	Father	Sister
Exon 13 g.1451A>G	0.43/0.57 0.62/0.38	A/G	G/G	A/A	A/G
Exon 18 g.2365A>G	0.66/0.34	A/A	A/G	A/G	A/A
Exon 28 g.4141A>G	0.38/0.62	G/G	G/G	A/G	G/G
g.4641T>C	0.42/0.58	C/C	C/C	T/C	C/C

*Caucasian frequency, reported on http://www.vwf.group.shef.ac.uk

Genomic DNA was extracted from peripheral blood leukocytes. Exons 17–24, and 27 of VWF gene were amplified by polymerase chain reaction (PCR), screened by CSGE and sequenced. When using CSGE, exons 21 and 27 showed double-band patterns in the proband. In the mother, only exon 21 showed double band. No double bands were observed in the remaining exons of the patient or her relatives. The proband was shown to be compound heterozygous for p.R924Q and p.R1205H. The mother was heterozygous for the p.R924Q. The father and sister showed normal sequencing of the exons considered. Haplotype analysis of the proband and relatives was determined by analysis of four SNP with known population frequencies: rs1800378, rs1063856, rs216311 and rs216310 in three polymorphic regions (exons 13, 18 and 28) within the VWF gene. This analysis revealed that the p.R1205H mutation had arisen *de novo* in the second generation (the proband) (Table 2). This is the first report of a case of a *de novo* p.R1205H mutation in combination with p.R924Q, in heterozygosity, present in a symptomatic patient with implications in her phenotypic expression of VWD. Firstly, she was diagnosed as having VWD-type Vicenza according to her phenotypic profile. In addition, the increase in FVIII:C was lower than expected in Vicenza patients; it made us think of the possibility of another defect, confirmed by the low VWF:FVIIIB/VWF:Ag and the presence of p.R924Q. We agree with Hickson [9] in considering that the association of p.R924Q with a second mutation

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and with O blood group reinforces the decrease in FVIII:C and VWF. This could be the case in our patient, who showed moderate–severe symptoms, but not in her mother, who only showed mild symptoms and the presence of p.R924Q associated with O blood group. In conclusion, we report here an association of VWD-type Vicenza with p.R924Q in a girl with a moderate–severe bleeding phenotype, probably induced by the association of R924Q and O blood group. This is the first report of clinical description for this association.

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Authorship

AIW performed the research, collected the data and wrote the manuscript. AIW, ACK, ASL and MAL designed the research study. AIW, ACK, JCC and SHG contributed essential reagents or tools. ASL and MAL analysed the data. AIW, ACK, ASL and MAL reviewed the manuscript and all authors approved the final version of the manuscript.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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