

Case Report

R924Q substitution encoded within exon 21 of the von Willebrand Factor gene related to mild bleeding phenotype

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Von Willebrand disease (VWD) type 2 comprises the qualitative defects of VWF molecule (1). Among them, VWD 2N is the result of mutations in the D' or D3 domains in the N-terminal region that cause VWF with a defective binding capacity to FVIII, resulting in the premature degradation of FVIII (2).

Heterozygous individuals are generally asymptomatic, but homozygotes and compound heterozygotes have phenotypes mimicking hemophilia A (3). The presence of confounding genetic factors, such as those conferred by VWD type 1, would explain the variability in phenotype.

Thirty-nine mutations (exons 18–21 and 24) in VWD 2N were reported to the von Willebrand disease database of the International Society on Thrombosis and Haemostasis (4). In addition, a mutation in exon 25 causing defective binding and multimerization has been described recently (5–8).

The R924Q substitution, encoded within exon 21 of VWF gene, was described as a polymorphism. Using site-directed mutagenesis and transient expression in COS-7 cells, Hilbert et al. (9) showed that R924Q substitution was a polymorphism because the VWF:FVIII of the mutated recombinant VWF was similar to that of wild type recombinant VWF. However, the patient displayed moderately decreased VWF: FVIII, VWF and FVIII levels. The authors considered the symptoms related to other novel mutations, Q1053H and C1060R.

We report the R924Q substitution in two members of a family and in an unrelated man, with mildly reduced VWF: FVIII and a bleeding tendency.

Case reports

The proband (P1) was a 48-year-old man with a previous diagnosis of sporadic hemophilia A. He suffered from epistaxis, hematomas, bleeding after tooth extraction, and major hemorrhages related to surgical procedures that required treatment with FVIII concentrates. He presented several episodes of hemarthrosis, all post-traumatic. The first, at the age of 10 years, was in the knee. Other joint bleedings were tarsus in 1999, left wrist and scapulo-clavicular in 2002. In addition, he suffered from a cephalohematoma in 2002. All episodes required FVIII concentrates. The response to desmopressin infusion was inadequate because FVIII did not reach hemostatic levels after the infusion of the drug (Table 1). His 24-year-old son (P2) suffered from bleeding after tooth extraction that required FVIII concentrates. His daughter (P3, 26-year-old) suffered from easy bruising but no other bleeding symptoms; she had an uneventful pregnancy and delivery. His granddaughter (P4, 2-year-old, daughter of P3) had no hemorrhagic episodes.

A non-related 61-year-old man (P5) with gingival bleeding and severe epistaxis during childhood that required nasal packing was evaluated as well. His family history was not significant, except for his brother who had epistaxis but could not be studied.

One hundred eighty-three unrelated blood donors (366 alleles) were controls.

Blood samples

VWF:Ag was assayed by enzyme-linked immunosorbent assay (10); FVIII activity was measured by one-stage method (11), and VWF:RCo was determined by an aggregometer method (12). FVIII binding to immobilized VWF was performed by an ELISA method (13). Multimeric analysis was assayed by immunoenzymatic stain using avidin-biotin peroxidase complex (14). The evaluation was performed applying scanning densitometry using

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Table 1: Proband's response to desmopressin test.

	BT (min)	APTT (sec)	FVIII (%)	VWF:Ag (%)	VWF:RCo (%)
Basal	5	66	20	170	165
60 minutes	8	59	30	257	216
120 minutes	6	58	30	220	195
24 hours	10	68	16	135	160

(*) no inhibitory activity was detected. N/D: not done

Table 2: Phenotypic data.

Parameters	Normal value	P1	P2	P3	P4	P5
Blood group	-	O+	A+	O+	N/D	O+
Ivy's BT (min)	≤4.5	5.5	6.0	3.5	N/D	N/D
APTT (seg)	34–45	54 (*)	45 (*)	48 (*)	45 (*)	45 (*)
FVIII (IU/dL)	50–150	21	50	43	50	50
VWF:Ag (IU/dL)	55–223	100	97	75	67	107
FVIII/VWF:Ag	≥0.6	0.21	0.51	0.57	0.74	0.46
VWF:RCO (IU/dL)	50–150	74	74	75	66	87
VWF:FVIII/VWF:Ag	≥0.8	0.70	0.61	1.0	0.95	0.82
Bands in CSGE	Single	Double	Double	Single	Single	Double
Sequencing of Exons 17–24	-	Heterozygous R924Q	Heterozygous R924Q	Normal	Normal	Heterozygous R924Q

(*) no inhibitory activity was detected. N/D: not done.

a Sharp scanner (JX 330, Hamburg, Germany) employing ImageMaster software (Pharmacia, Newcastle, England).

Coding regions, intron/exon boundaries and 5' and 3' regions of exons 17–24 of VWF gene were amplified. Each PCR consisted of the following: 1 µg of genomic DNA diluted in a final volume of 100 µl containing 200 µM dNTPs, 300 ng of each primer, 1 U Taq DNA polymerase, 67 mM Tris-HCl pH 8.8 and 1.5 mM MgCl₂.

Heteroduplex analysis and Conformation-Sensitive Gel Electrophoresis (CSGE) were performed as previously described (15, 16). Amplified DNA fragments were purified with the use of a GFX kit (Amersham Pharmacia Biotech). Sequence analysis of amplified DNA fragments was performed with the use of a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences). Automated sequencing of PCR fragments was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

All samples were screened by CSGE and sequenced.

Results

Table 2 summarizes the phenotypic data. P1, P2 y P5 showed a decreased FVIII/VWF:Ag. P1 and P2 presented mildly reduced VWF: FVIII, while P5 had a normal binding capacity. The three of them were heterozygous for the substitution R924Q encoded within exon 21 of the VWF gene. P3 and P4 were homozygous for R924. No other mutations were detected by CSGE screening or in the DNA sequencing studies.

Clinical penetrance in this family was 100% because all the carriers of the mutation were symptomatic.

R924Q substitution was not found in the 366 alleles from normal controls. Multimeric pattern was normal both in patients and in normal controls.

Discussion

Our observations suggest that R924Q might have implications in phenotypic expression of VWD because we have found the

R924Q mutation in symptomatic patients but not in asymptomatic family members nor in the general population. Moreover, the European Study Molecular and Clinical Markers for the Diagnosis and Management of Type 1 VWD (MCMDM-1VWD) reported that missense mutations predominated in the study population, among them R924Q was found in four patients with a clinical penetrance of 78% (17, 18), similar to our patients. However, the contribution of the mutation to the phenotype deserves further investigation.

Because mutations in exons 17 and 18 change the consensus sequence for the specific Furin/PACE enzyme abolishing the cleavage of VWF pro-peptide leading to a decreased capacity of VWF to bind FVIII in plasma (8, 19), we extended our sequencing strategy to include exon 17 but detected no changes there. However, even though unlikely, we cannot exclude any other alteration located outside exons 17–24 that may affect the VWF: FVIII and the bleeding tendency.

Some authors have proposed that the genotype would determine the response or the lack of response to desmopressin in these patients (20–23). Our patient did not achieve an adequate response to DDAVP, because FVIII levels did not reach hemostatic values. However, we cannot exclude nor assess the role of R924Q in the response to DDAVP.

In summary, bleeding tendency, a decreased FVIII/VWF:Ag and R924Q substitution were concomitantly present in two members of this family and in an unrelated man, all of them symptomatic, but not in the general population or in asymptomatic family members. However, further reports are required to show the role of R924Q both in phenotype and in the response to desmopressin.

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