Premature termination codon mutations in the von Willebrand factor gene are associated with allele-specific and position-dependent mRNA decay

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Supplementary Design and Methods

Laboratory assays for VWF quantitation, DNA extractions, and sequencing of the VWF gene were performed as described.[1]

Platelets were obtained from patients' blood as described.[2] Lymphocytes were collected from peripheral blood using Lympholite-H (Cedarlane, Hornby, Canada), following standard protocols. Total RNA was isolated from platelets and lymphocytes by using the RNAgents Total RNA Isolation System (Promega, Madison, USA).

Reverse transcription (RT) was performed using random nonamers and the ImProm-II RT-System kit (Promega). Real time RT-PCR assays were used to confirm the presence of VWF transcripts in lymphocytes (*data not shown*), as it has been demonstrated that RNA derived from lymphocytes contains ectopic transcripts of genes not normally expressed in these cells.[3]

An aliquot (4 μL) of the RT reaction was used to PCR amplify the cDNA regions containing the relevant mutations for each patient: 10 ng of DNA were used as template to amplify genomic fragments containing the same genetic alterations.

Direct sequencing of the amplified products was performed using the BigDye Terminator Cycle Sequencing Kit v1.1 and an automated ABI-3130XL sequencer (Applied Biosystems, Foster City, USA).

Online Supplementary Table S1. Nonsense-mediated mRNA decay in coagulation genes.

Gene	NMD	Experimental evidence	Reference
FGA	No	*In vitro* (transfection of the whole gene in COS1 cells)	Asselta *et al.*[4]
FGG	No	*In vitro* (transfection of a minigene construct in COS7 cells)	Neerman-Arbez *et al.*[5]
FV	Yes	*In vivo* (sequencing of cDNAs from patients carrying one heterozygous PTC-introducing mutation)	Montefusco *et al.*[2]
FVIII	No	*In vitro* (transfection of a minigene construct in CHO cells)	David *et al.*[3]
FXI	Yes	*In vivo* (sequencing of cDNAs from patients carrying one heterozygous PTC-introducing mutation)	Soldà *et al.*[6]
FXIII	Yes	*In vivo* (Northern Blot and solid-phase minisequencing of mRNA from patients)	Mikkola *et al.*[7]

Online Supplementary Table S2. Phenotype and genotypes of the VWD patients and their available family members.

Individual	Mutation	Sex/age (M/F)/ yr	VWD type	Bleeding time (min)	Bleeding severity score	Blood group	FVIII:C (IU/dL)	VWF:Ag (IU/dL)	VWF:RCo (IU/dL)	Platelet VWF:Ag (IU/10^9)	Platelet VWF:RCo (IU/10^9)
P1	p.R854Q/c.2546+3G>C	M/15	VWD2N	nt	4	nt	35	66	57	nt	nt
P1-father	c.2546+3G>C/WT	M/44	Hetero-VWD3	2	1	nt	91	58	44	nt	nt
P2	p.C1927R/c.8155+6T>C	F/59	VWD1	35	18	non-O	27	2	<1***	0.03	<0.06
P2-sister	p.C1927R/WT	F/66	VWD1	8	4	non-O	110	59	57	0.06	<0.06
P2-sister	c.8155+6T>C/WT	F/69	Hetero-VWD3	6	1	O	129	143	120	nt	nt
P3	c.6182delT/WT	F/28	Hetero-VWD3	nt	0	O	153	77	58	nt	nt
P3-father	c.6182delT/c.6182delT	M/55	VWD3	35	29	O	5	<1	<1***	<0.01	<0.06
Normal range	Na	na	na	<7	-1 to 3	na	50-150	40-169* 55-165**	41-160* 53-168**	0.22-0.87	0.15-0.80

*Values are shown as a mean of 3 independent measurements; na, not applicable; nt, not tested; * range values of normal individuals with blood group O. ** range values of normal individuals with blood group non-O. *** VWF:RCo values have been measured by ELISA method.16 Bleeding score is according to the Scientific and Standardization Committee on VWF of the International Society of Thrombosis and Haemostasis (http://www.med.unc.edu/isth/ssc_home.htm).*

References

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