Biogenesis of Weibel-Palade bodies in von Willebrand's disease variants with impaired von Willebrand factor intrachain or interchain disulfide bond formation

Jiong-Wei Wang,[1] Dafna J. Groeneveld,[1] Guy Cosemans,[1] Richard J. Dirven,[1] Karine M. Valentijn,[2] Jan Voorberg,[3] Pieter H. Reitsma,[1] and Jeroen Eikenboom[1]

[1]Einthoven Laboratory for Experimental Vascular Medicine, Department of Thrombosis and Hemostasis, Leiden University Medical Center, The Netherlands; [2]Department of Molecular Cell Biology, Leiden University Medical Center, The Netherlands; and [3]Department of Plasma Proteins, Sanquin-AMC Landsteiner Laboratory, Amsterdam, The Netherlands

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Online Supplementary Table S1. Expression of VWF variants in transfected HEK293 cells.

Variant	Transfection	VWF:Ag* Medium		VWF:Ag* Lysate		Ratio†
		mU	%	mU	%	
WT-VWF		74.1 ± 4.7	100	23.8 ± 3.2	100	3.2 ± 0.2
p.Cys1130Phe	Single transfection	41.3 ± 2.7	56.4 ± 6.4	59.2 ± 11.1	255.9 ± 56.7	0.7 ± 0.1‡
	Co-transfection	63.7 ± 4.8	86.3 ± 4.1	44.0 ± 5.8	186.3 ± 9.9	1.5 ± 0.1‡
p.Cys2671Tyr	Single transfection	55.2 ± 5.8	76.0 ± 12.7	45.5 ± 4.8	194.3 ± 18.2	1.2 ± 0.2‡
	Co-transfection	65.8 ± 4.4	89.0 ± 4.1	32.2 ± 3.2	136.5 ± 5.9	2.1 ± 0.1‡
p.Cys2773Ser	Single transfection	90.8 ± 10.7	124.0 ± 18.2	35.7 ± 2.1	154.1 ± 16.1	2.5 ± 0.2§
	Co-transfection	84.1 ± 11.6	114.7 ± 17.7	33.6 ± 4.1	142.1 ± 5.9	2.5 ± 0.4§

* VWF:Ag (mU) was produced by about 7×10^5 cells in the medium or lysate. In parallel, VWF:Ag is expressed as percentage relative to the amount of VWF:Ag in the medium and lysate of cells expressing WT-VWF. Each value represents the mean ± SEM of three independent experiments in duplicate.

† Ratio of the absolute amount of VWF:Ag in medium to VWF:Ag in lysate.

‡ Compared with WT-VWF $P<0.01$.

§ Compared with WT-VWF $P>0.05$.

Online Supplementary Table S2. VWF cysteine mutations identified in VWD*.

Loss of cysteine				*Gain of cysteine*			
Domain	**Mutation**	**VWD type**	**VWF level**	**Domain**	**Mutation**	**VWD type**	**VWF level**
D1	p.Cys57*	3	↓	D1	p.Trp377Cys	3	↓
D1	p.Cys263_E270del	3	↓	D2	p.Arg760Cys	2N	↓
D1	p.Cys275(Ser/Arg)†	1 or 3	↓	D'	p.Tyr795Cys	2N	↓
D2	p.Cys570Ser	2A	↓	D3	p.Tyr1107Cys	Unclassified	↓
D2	p.Cys623Trp	2A	↓	D3	p.Tyr1146Cys	1	↓
D'	p.Cys709Leufs*3	2A	↓	A1	p.Arg1308Cys	2B	N.A.
D'	p.Cys788(Arg/Tyr)	2N	↓	A1	p.Trp1313Cys	2B	N.A.
D'	p.Cys804Phe	2N	↓	A1	p.Arg1315Cys	1 or 3	↓
D'	p.Cys858Phe	2N	↓	A1	p.Arg1342Cys	1	↓
D3	p.Cys996Glu	1	↓	A1	p.Arg1374Cys	1 or 2A or 2M	↓
D3	p.Cys1060(Arg/Tyr)	1 or 2N	↓	A1	p.Arg1379Cys	1 or Unclassified	↓
D3	p.Cys1071Phe	3	↓	A1	p.Arg1399Cys	Unclassified	N.A.
D3	p.Cys1099Tyr‡§	IIC Miami	=/↑	A2	p.Phe1514Cys	2A	N.A.
D3	p.Cys1101(Arg/Trp)	Unclassified	N.A.	A2	p.Tyr1584Cys	1	↓
D3	p.Cys1111Tyr	1	↓	A3	p.Trp1745Cys	2M	↓
D3	p.Cys1130(Phe/Gly/Arg)	1	↓	B1-B3	p.Arg2379Cys	1	↓
D3	p.Cys1149Arg	1	↓	C2	p.Gly2441Cys	1	↓
D3	p.Cys1157Phe	Unclassified	↓	C2	p.Arg2464Cys	1 or unclassified	↓
D3	p.Cys1190Arg	1	↓				
D3	p.Cys1196Arg	2M or 3	↓				
D3	p.Cys1225Gly	2N	↓				
D3	p.Cys1227Arg	1	↓				
D3	p.Cys1234Trp	Unclassified	↓				
A1	p.Cys1272(Phe/Gly/Arg/Ser)	2A	↓				
A1	p.Cys1458Tyr	2A	N.A.				
D4	p.Cys2174Gly	3	↓				
D4	p.Cys2257Ser	1	↓				
B1-B3	p.Cys2304Tyr	1	↓				
B1-B3	p.Cys2340Arg	1	↓				
B1-B3	p.Cys2362Phe	1 or 3	↓				
C2	p.Cys2477(Ser/Tyr)	1	↓				
C2	p.Cys2533*	Unclassified	↓				
C2	p.Cys2557Serfs*8	3	↓				
C2-CK¶	p.Cys2671Tyr	3	↓				
C2-CK¶	p.Cys2693Tyr	1	↓				
CK	p.Cys2739Tyr	3	↓				
CK	p.Cys2754Trp	3	↓				
CK	p.Cys2771(Ser/Tyr)‡	2A(IID)	=/↑				
CK	p.Cys2773(Arg/Ser)‡	2A(IID)	=/↑				
CK	p.Cys2804(Tyr/fs)	1 or 3	↓				
CK	p.Cys2806Arg	Unclassified	↓				

* Summarized from the ISTH-SSC VWF mutation database up to 2010 (www.vwf.group.shef.ac.uk/).

† When there are multiple mutations reported at a position the various amino acid substitutions are indicated in brackets.

‡ Cysteines involved in the interchain disulfide bonds in VWF; N.A., data not available.

§ Not yet in the ISTH-SSC VWF mutation database.[35]

¶ The connecting area between C2 and CK domain.