Molecular and clinical profile of von Willebrand disease in Spain (PCM-EVW-ES): comprehensive genetic analysis by next-generation sequencing of 480 patients

Nina Borràs,[1,2] Javier Batlle,[3] Almudena Pérez-Rodríguez,[3] María Fernanda López-Fernández,[3] Ángela Rodríguez-Trillo,[3] Esther Lourés,[3] Ana Rosa Cid,[4] Santiago Bonanad,[4] Noelia Cabrera,[4] Andrés Moret,[4] Rafael Parra,[1,5] María Eva Mingot-Castellano,[6] Ignacia Balda,[7] Carme Altisent,[5] Rocío Pérez-Montes,[8] Rosa María Fisac,[9] Gemma Iruín,[10] Sonia Herrero,[11] Inmaculada Soto,[12] Beatriz de Rueda,[13] Víctor Jiménez-Yuste,[14] Nieves Alonso,[15] Dolores Vilariño,[16] Olga Arija,[17] Rosa Campos,[18] María José Paloma,[19] Nuria Bermejo,[20] Rubén Berrueco,[21] José Mateo,[22] Karmele Arribalzaga,[23] Pascual Marco,[24] Ángeles Palomo,[6] Lizheidy Sarmiento,[25] Belén Iñigo,[26] María del Mar Nieto,[27] Rosa Vidal,[28] María Paz Martínez,[29] Reyes Aguinaco,[30] Jesús María César,[31] María Ferreiro,[32] Javier García-Frade,[33] Ana María Rodríguez-Huerta,[34] Jorge Cuesta,[35] Ramón Rodríguez-González,[36] Faustino García-Candel,[37] Rosa Cornudella,[38] Carlos Aguilar,[39] Francisco Vidal*[1,2,40] and Irene Corrales*[1,2]

[1]Banc de Sang i Teixits, Barcelona; [2]Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona (VHIR-UAB); [3]Complexo Hospitalario Universitario A Coruña, INIBIC; [4]Hospital Universitario y Politécnico La Fe, Valencia; [5]Hospital Universitari Vall d'Hebron, Barcelona; [6]Hospital Regional Universitario de Málaga; [7]Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria; [8]Hospital Universitario Marqués de Valdecilla, Santander; [9]Salud Castilla y León, Segovia; [10]Hospital Universitario Cruces, Barakaldo; [11]Hospital Universitario de Guadalajara; [12]Hospital Universitario Central de Asturias, Oviedo; [13]Hospital Universitario Miguel Servet, Zaragoza; [14]Hospital Universitario La Paz, Madrid; [15]Hospital Infanta Cristina, Badajoz; [16]Complejo Hospitalario Universitario Santiago de Compostela; [17]Hospital Universitario Lucus Augusti, Lugo; [18]Hospital Jerez de la Frontera, Cádiz; [19]Hospital Virgen del Camino, Pamplona; [20]Hospital San Pedro de Alcántara, Cáceres; [21]Hospital Sant Joan de Deu, Barcelona; [22]Hospital de la Santa Creu i Sant Pau, Barcelona; [23]Hospital Universitario Fundación de Alcorcón, Madrid; [24]Hospital General de Alicante; [25]Hospital Universitario 12 de Octubre, Madrid; [26]Hospital Clínico San Carlos, Madrid; [27]Complejo Hospitalario de Jaén; [28]Fundación Jiménez Díaz, Madrid; [29]Hospital Nuestra Señora de Sonsoles, Ávila; [30]Hospital Universitario de Tarragona Joan XXIII; [31]Hospital Ramón y Cajal, Madrid; [32]Hospital Montecelo, Pontevedra; [33]Hospital del Río Hortega, Valladolid; [34]Hospital Gregorio Marañón, Madrid; [35]Hospital Virgen de la Salud, Toledo; [36]Hospital Severo Ochoa, Madrid; [37]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia; [38]Hospital Clínico Universitario Lozano Blesa, Zaragoza; [39]Hospital Santa Bárbara, Soria and [40]CIBER de Enfermedades Cardiovasculares, Spain

**IC and FV contributed equally to this work*

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Correspondence: icorrales@bst.cat or fvidal@bst.cat

SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

The VWD classification used in this paper was based in Sadler proposal.[1] Type 1 VWD diagnosis is based VWF:Ag, VWF:RCo or/and VWF:CB <30% at the recruitment moment of the study. The qualitative type 2 VWD was defined as VWF:RCo/VWF:Ag ratio <0.7. A minor modification was the inclusion of subtype 1H (historical) [2] to patients that were diagnosed as type 1 VWD, but at the time of enrollment, their central laboratory findings show a very mild decrease or even a normal VWF plasma level.

Bleeding score and central phenotypic assessment

Bleeding score in each patient was calculated using the ISTH Bleeding Assessment Tool.[3]

The central phenotype characterization was based on laboratory assays including FVIII:C, VWF:Ag, VWF:RCo, VWF collagen binding (VWF:CB) and multimeric pattern. The VWF capacity to bind exogenous FVIII (VWF:FVIIIB) was assessed in patients who presented a FVIII:C/VWF:Ag ratio <1, and in those in whom a type 2N mutation was found. Detailed information is available in a previous report.[4]

Sample collection

Genomic DNA was obtained from peripheral blood samples by using the QIAsymphony DNA Midi Kit on a QIAsymphony SP instrument (Qiagen). DNA was quantified by absorbance at 260 nm with a NanoDrop Lite spectrophotometer (Thermo Scientific), and concentration was adjusted to a range of 25 to 50 ng/µL.

Access Array amplification and sequencing

The Access array was primed in a pre-PCR IFC controller AX (Fluidigm). The chip was loaded with samples and PCR reagents and was transferred to the Fluidigm FC1 Cycler using the Fluidigm AA 48x48 Standard v1 protocol (Supplemental Table 1) for a Fusion PCR. Pooled amplicons from each DNA template were harvested in a post-PCR IFC controller AX (Fluidigm), visualized on agarose gel (Figure S1) and quantified by densitometric analysis using ImageJ software (National Institutes of Health). The final pools of up to 192 samples (4 Access Array fusion libraries) were treated for loading onto a MiSeq Reagent Kit v2 cartridge (Illumina). A paired-end, 500 (2x250)-cycle sequencing run was performed on the MiSeq system, following the Illumina standard protocol.

CLC Bio Data analysis parameters

Primer sequences and low-quality regions in specific patients were trimmed and then aligned against the human genome sequence (hg19), masked for the amplified regions of *VWF* and *VWFP.* Since PCR conditions were not stringent enough to completely avoid *VWFP* amplification, double alignment allowed elimination of reads corresponding to the pseudogene (Figure 1). Read mapping was performed with specific parameter settings (mismatch count, 2; indel count, 3; length fraction, 0.7; similarity fraction, 0.9). The Quality-based Variant detection algorithm from the CLC Genomic Workbench was applied for SNV-calling. Analysis parameters were adjusted to obtain optimal performance and maximize sensitivity and specificity in mutation detection. Final analytical settings were established: minimum coverage=10, minimum variant frequency=25%, minimum variant count=5.

VariantStudio data analysis parameters

The following analytical parameters were used in the VariantStudio software to identify putative mutations: 1) frequency, based on minor allele frequency (MAF) from 1000 Genomes (April 2012 v.3) ≤1% for the Global, African, American, Asian and European populations; 2) location, referring to the Variant Effect Predictor v.2.8 database (intronic mutations located >250 bp into the flanking intronic sequence of each exon were discarded); and 3) variant frequency from our local NGS variant database ≤5% .

Nomenclature used and classification of variant pathogenicity

Amino acid numbering and nomenclature was performed in accordance with international recommendations for the description of sequence variants from the Human Genome Variation Society (HGVS; http://www.HGVS.org), applied to genetic variants in hemostasis.[5, 6] Genetic nomenclature remains controversial in many ways, and it is important to specify the exact meaning of the terms used in each case. We have used the term “mutation” to indicate a disease-causing change and the term “variant” or “variants of unknown significance” (VUS) to indicate changes with an unknown functional effect. The term “polymorphism” was used to indicate both a non-disease-causing change and a change found at a frequency of 1% or higher in any population included in the SNP databases.

The following criteria established the pathogenic nature of the variants found: 1) stop/frameshift variants were considered likely to be disease-causing, 2) co-segregation in the family with VWD phenotype, 3) variant identified as disease-causing in the VWD literature or international databases (VWF EAHAD Coagulation Factor Variant Database compiled in LOVD [EAHAD-VWD-LOVD]),[7] 4) *in silico* evaluation findings, and 5) presence of a second mutant

allele in cases of autosomal recessive inheritance.

In addition to mutations that have a very evident effect (eg, stop, frameshift, acceptor or donor splice site) and missense mutations, whose effects are more unpredictable, we have maintained within the PCM-EVW-ES registry all variants in intronic regions and synonymous changes whose MAF in the general population and in the 1000 Genomes project is below 1%.

SUPPLEMENTAL RESULTS

Of the 556 samples analyzed, 1 failed during access array amplification, and 5 resulted in too few reads in the sequencing process, precluding correct analysis. These 6 samples and 9 additional samples with borderline quality values were successfully amplified in a second access array. Moreover, in patients 30035 and 30037, analyzed in the same access array chip and run, mutations were not detected by the standard method because the percentage of reads corresponding to the mutated allele were below the selected threshold (20%) established in the variant-calling algorithm. In both patients, an identical mutation (p.Arg1779Leu) was discovered in further analysis using an NGS custom gene panel.

Type 1H VWD

Six type 1H and three type 1 VWD patients with borderline VWF:Ag levels (mean 38.6; range 13-72) presented the controversial p.Arg924Gln variant, described as a polymorphism and also reported in type 1 and 2N VWD. However, in type 1 patients, p.Arg924Gln was combined with the novel mutation p.Gly2083Asp in 2 patients (21003 and 21005) or with the synonymous mutation c.3390C>T (36008), whereas in type 1H patients, the mutation was always found alone. This suggests that some clinical signs (BS) are not completely reflected in the laboratory parameters or that plasmatic levels fluctuate with the age of patient. Moreover, although *in silico* analysis did not predict a deleterious effect (global score 1; Supplemental Table 11), RNA studies have revealed activation of a cryptic splice in exon 28 and generation of a premature stop codon.[8] Finally, the p.Arg924Gln was found in a type 3 patient (36007) in compound heterozygous state with the missense mutation p.Gly967Val and the intronic VUS c.6799-27C>T. Although we are unable to determine the haplotype, it is likely that the type 3 phenotype may be due to the resulting null allele carrying p.Arg924Gln in combination with the deleterious effect of p.Gly967Val, predicted by *in silico* analysis (score=5). This is a further example illustrating that certain mutations cannot be linked to only a single VWD type,

especially in quantitative defects. Furthermore, the same mutation may cause different phenotypes depending on whether it is alone or combined with others.

Type 2A and 2B VWD reclassification

With the solely phenotypic data, it is difficult to correctly subclassify VWD type 2 because of the heterogeneity of the phenotype, some intrinsic subjectivity in the interpretation of the multimeric pattern and, specially, when RIPA assay is impracticable (since must be performed with a freshly obtained sample).

Along this line, fifty-nine patients were initially classified as **type 2A VWD** due to central phenotypic results. However, after genetic analysis, 2 patients were excluded because the diagnosis of one AVWS (03006) and the reclassification into type 2B of patient 01065. Otherwise, after genetic analysis, 54 patients were included in this subgroup based on the identified mutation: 45 patients were previously unclassified because it was not possible to establish a definite diagnosis in basis of phenotypic study; 2 patients previously misclassified as type 2B due to local erroneous RIPA assay were reclassified as type 2A after molecular analysis; 6 patients misdiagnosed as type 2A/2M or type 2M VWD due to unclear multimeric pattern were finally reclassified as type 2A VWD as all of them presented the previously described p.Arg1374His and; one patient is the discrepant 42010, previously excluded because the laboratory levels were above 30%, but the genetic analysis and the absence of HMWM confirmed a VWD type 2A.

Twenty-three patients were diagnosed as **type 2B VWD** based on the central phenotypic assessment. After genetic analysis, 2 patients were excluded of this subgroup due to local erroneous RIPA assay and the identification of the type 2A mutation p.Arg1374His. Among the 14 patients included in type 2B VWD after genetic analysis, RIPA had been only assayed in one of them (01065) with an observed aggregation at >0.8 mg/mL but not at 0.4 mg/mL, leading to a type 2A classification. Nevertheless, this patient presented the widely described p.Arg1308Cys 2B mutation. A similar weird effect on the aggregation was also previously reported in a family with the type 2B mutation p.Val1316Met.[9] Among the remaining 13 patients 11 presented a lack of the HMWM and were previously unclassified because it was not possible to distinguish, without the molecular data, between 2A and 2B subtypes. Finally, 2 patients with normal multimeric pattern were initially misclassified as type 1 VWD presented the type 2B Malmö/New York mutation (p.Pro1266Gln) and thus were reclassified into this subtype.

These results support the importance of the genetic study as a powerful tool to orient physician in the establishment of a definite sub-classification of type 2 patients.

SUPPLEMENTAL TABLES

Table S1. Thermal cycling protocol (Fluidigim 'AA 48x48 Standard v1') for fusion PCR protocol

PCR Stages		Number of Cycles
50°C	2 minutes	1
70°C	20 minutes	1
95°C	10 minutes	1
95°C 60°C 72°C	15 seconds 30 seconds 1 minute	10
95°C 80°C 60°C 72°C	15 seconds 30 seconds 30 seconds 1 minute	2
95°C 60°C 72°C	15 seconds 30 seconds 1 minute	8
95°C 80°C 60°C 72°C	15 seconds 30 seconds 30 seconds 1 minute	2
95°C 60°C 72°C	15 seconds 30 seconds 1 minute	8
95°C 80°C 60°C 72°C	15 seconds 30 seconds 30 seconds 1 minute	5

Table S2. Sinopsis of outputs per MiSeq Run

Fluidigm AA	Run MiSeq	Density (K/mm2)	Cluster PF (%)	Phas/Prephas (%)	Reads (M)	Reads PF (M)	% >= Q30	Total samples	Samples from registry	First Sequencing Samples
FL1	**Rxn1**	966 +/- 20	84.67 +/- 2.83	0.108 / 0.129	18.05	15.29	78.92	48	48	48
FL2	**Rxn2**	1,248 +/- 58	76.64 +/- 3.46	0.137 / 0.122	20.57	15.79	72.32	96	85	84
FL3										
FL4	**Rxn3**	893 +/- 44	84.02 +/- 4.20	0.116 / 0.077	15.39	12.96	74.48	192	192	191
FL5										
FL6										
FL7										
FL8	**Rxn4**	1,047 +/- 32	75.75 +/- 7.96	0.066 / 0.089	19.77	14.96	72.64	192	189	189
FL9										
FL10										
FL11										
FL12	**Rxn5**	1,018 +/- 43	82.30 +/- 4.21	0.085 / 0.072	18.75	15.45	61.07	192	57	44
FL13										
FL14										
FL15										
Total		**1034.4**	**80.67**		**18.51**	**14.89**	**71.89**	**720**	**571**	**556**

AA indicates Access Array; PF, passing filter; FL, fluidigm; and Rxn, run number.

Table S3. Genotype-phenotype correlation in type 1 and 1H VWD patients

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1	0103	01006	c.7730-56C>T			45	intronic	het	39	F	O+	11	27	32	72	33
	0106	01010	c.4751A>G	**p.Tyr1584Cys**	28		A2	het	55	M	O+	5	27	30	51	32
	0111	01019	c.3223-7_3236dup	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro	25		D3	het	52	F	O+	12	17	21	49	20
		01021	c.3223-7_3236dup	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro	25		D3	het	11	F	O+	2	18	19	36	19
		01050	c.3223-7_3236dup	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro	25		D3	het	9	M	O+	8	29	27	56	24
		01051	c.3223-7_3236dup	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro	25		D3	het	36	M	O+	7	31	24	57	25
	0113	01025*	ND						54	M	AB-	5	27	36	45	25
	0114	01028	c.4751A>G	**p.Tyr1584Cys**	28		A2	het	8	M	O+	7	21	33	34	30
			c.5312-19A>C			30	intronic	het								
	0115	01030*	c.2561G>A	p.Arg854Gln	20		D'	het	12	F	O+	2	23	33	51	33
	0119	01037	c.3426T>C	c.3426T>C (p.=)	26		D3	het	16	M	O-	14	22	32	28	22
			c.3485_3486delinsTG	p.Pro1162Leu	26		D3	het								
	0201	02001	c.3614G>A	**p.Arg1205His**	27		D3	het	63	F	A+	14	9.7	2.6	19	6
		02014	c.3614G>A	**p.Arg1205His**	27		D3	het	72	M	A+	12	7.1	5	19	7.3
		02015	c.3614G>A	**p.Arg1205His**	27		D3	het	26	F	A+	8	4.9	8.4	31	4.5
		02016	c.3614G>A	**p.Arg1205His**	27		D3	het	67	M	A+	5	16	9	34	16
		02017	c.3614G>A	**p.Arg1205His**	27		D3	het	56	F	B+	12	5.9	6.5	20	5.4
		02018	c.3614G>A	**p.Arg1205His**	27		D3	het	24	F	O+	8	2.3	0.8	12	2.8
			c.8254-5T>G			51	intronic	het								
		02019	c.2586G>T	c.2586G>T (p.=)	20		D'	het	21	M	AB-	3	6.6	7.3	18	6.2
			c.3614G>A	**p.Arg1205His**	27		D3	het								
		02053	c.3614G>A	**p.Arg1205His**	27		D3	het	58	F	O+	9	10	7.2	18	8.8
		02054	c.3614G>A	**p.Arg1205His**	27		D3	het	26	F	AB-	7	11	8.1	20	10
	0206	02023	c.3614G>A	**p.Arg1205His**	27		D3	het	28	F	O+	18	5.5	8	11	4.2
		02055	c.3614G>A	**p.Arg1205His**	27		D3	het	51	F	B+	14	7.2	6.4	17	7.5
		02056	c.3614G>A	**p.Arg1205His**	27		D3	het	24	F	B-	8	8.8	6.6	18	7.3
		02057	c.3614G>A	**p.Arg1205His**	27		D3	het	53	M	B+	6	6.7	5	11	5.7
	0215	02042	c.1109G>A	p.Cys370Tyr	9		D1	het	41	M	O+	1	25	21	77	27
	0218	02047	c.5471C>A	**p.Pro1824His**	32		A3	het	71	M	O+	9	9.3	6.4	30	6.1
		02048	c.5471C>A	**p.Pro1824His**	32		A3	het	45	F	B+	14	8.3	6.5	39	6.1
		02049	c.5471C>A	**p.Pro1824His**	32		A3	het	43	M	A+	4	11	6.6	37	8
		02050	c.5471C>A	**p.Pro1824His**	32		A3	het	16	M	A+	7	10	6.9	37	7.1
	0220	02052	c.1625C>G	p.Ala542Gly	14		D2	het	25	M	A+	11	15	12	20	16
			c.6187C>T	p.Pro2063Ser	36		D4	het								
	0221	02058	c.3614G>A	**p.Arg1205His**	27		D3	het	64	F	A+	19	7.3	5.9	19	6.3
	0222	02059	c.1946-16_1946-15insCTC			15	intronic	het	38	M	A+	12	5.4	6.1	11	4.5
			c.3614G>A	**p.Arg1205His**	27		D3	het								
		02060	c.3614G>A	**p.Arg1205His**	27		D3	het	67	F	A+	9	8.1	6.1	15	7.2
			c.7438-169G>A			43	intronic	het								
	0223	02061*	ND						29	M	O+	7	18	15.7	41	16
		02062*	ND						24	M	O+	3	16	14.7	49	17

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1	0224	02064	c.3614G>A	**p.Arg1205His**	27		D3	het	32	M	O-	13	4.7	5	16	6
		02065	c.3614G>A	**p.Arg1205His**	27		D3	het	40	M	A-	5	7.2	5.6	24	9.5
		02066	c.3614G>A	**p.Arg1205His**	27		D3	het	66	M	A+	1	7.5	6.1	30	9.7
		02067	c.3614G>A	**p.Arg1205His**	27		D3	het	68	M	A-	4	7.5	5.6	29	10.3
	0225	02068	c.6352C>T c.8155+3G>C	p.Arg2118Trp	37	 50	D4 intronic	het het	58	F	O+	13	11	9.2	46	14
	0226	02069	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	het het	45	F	O+	5	30	27	81	40
		02070	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	het het	18	F	A+	5	33	29.6	79	44
		02073	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	het het	44	F	O+	7	22	16.8	53	26
	0228	02074	c.3426T>C c.6254G>T c.3485_3486delinsTG	c.3426T>C (p.=) p.Cys2085Phe p.Pro1162Leu	26 36 26		D3 D4 D3	het het het	37	F	A+	3	24	21	111	29
	03150	03035	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	het het	51	M	ND	4	34	26	43	33
	03230	03044	c.6699_6702dup	p.Cys2235ArgfsTer8	38		null allele	het	27	F	ND	3	22	18	29	17
	0399	03015*	ND						37	F	O+	0	17	17.7	15	16
	0507	05008	c.3614G>A	**p.Arg1205His**	27		D3	het	30	M	A+	2	5.8	5	7.4	6.1
	0511	05012	c.2119T>C	p.Cys707Arg	16		D2	het	10	F	O+	4	15	12.3	45	25
	0602	06002	c.2685G>C c.4751A>G	p.Gln895His **p.Tyr1584Cys**	20 28		D3 A2	het het	39	F	O+	8	25	19.1	88	24
		06003	c.2685G>C c.5665-36T>C	p.Gln895His	20	 33	D3 intronic	het het	74	M	O+	17	31	30	100	33
	0606	06007	c.1156+2T>C			10	intronic	het	54	M	O+	5	28	26.1	56	23
		06008	c.1156+2T>C			10	intronic	het	20	F	A-	5	24	27	54	24
	0607	06009	c.2435del	**p.Pro812ArgfsTer31**	18		null allele	het	60	F	O+	2	23	25	83	23
	0609	06012	c.3363G>T	p.Arg1121Ser	25		D3	het	10	M	O+	10	13	14	40	13
		06013	c.3363G>T	p.Arg1121Ser	25		D3	het	40	M	A+	0	23	23.1	57	23
		06017	c.3363G>T	p.Arg1121Ser	25		D3	het	4	M	A-	8	7.4	6.5	27	9.1
	0617	06024	c.7300C>T	p.Arg2434Ter	43		null allele	het	36	F	O+	1	35	25	69	27
	0703	07003	c.5170+10C>T c.8254-5T>G			29 51	intronic intronic	het het	29	F	O+	5	25	24.9	50	28
	1002	10002	c.3614G>A c.-2077A>T	**p.Arg1205His**	27		D3 upstream	het het	71	M	O+	1	7.8	7.1	8.1	6.4
		10003	c.3614G>A	**p.Arg1205His**	27		D3	het	33	M	A+	1	7.4	7.2	11	6.3
	1011	10013	c.5198T>C	p.Leu1733Pro	30		A3	het	23	F	O+	3	7.2	5.6	19	6.5
	1301	13001	c.7985A>G	p.Lys2662Arg	48		C6	hom	61	F	O+	27	11	9.7	36	12
	1304	13006	c.3614G>A	**p.Arg1205His**	27		D3	het	66	M	A-	8	10	6	16	6.1
	1308	13010	c.1472G>C c.546G>A	p.Arg491Pro c.546G>A (p.=)	13 6		D2 D1	het het	37	F	O+	18	16	15.1	45	14
		13011	c.1472G>C c.7730-177G>T	p.Arg491Pro	13	 45	D2 intronic	het het	39	M	ND	4	25	28.7	81	27

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1	1309	13012	c.3614G>A c.533-43A>C	**p.Arg1205His**	27	 5	D3 intronic	het het	42	M	A+	7	14	9	20	11
	1311	13014	c.4751A>G	**p.Tyr1584Cys**	28		A2	het	20	M	O+	6	24	22.5	40	20
	1314	13018	c.4238C>T	**p.Pro1413Leu**	28		A1	het	5	M	ND	0	18	21	39	15
	1402	14002*	ND						32	M	A+	1	28	23	71	25
	1403	14003	c.221-70G>T c.478G>A	 p.Gly160Arg	 5	3	intronic D1	het het	40	F	O+	6	28	20	61	18
	1616	16001	c.1110-26T>A c.5695T>C	 p.Cys1899Arg	 34	9	intronic D4	het het	50	F	A+	7	21	16	108	20
		16002	c.1110-26T>A c.5695T>C	 p.Cys1899Arg	 34	9	intronic D4	het het	27	F	A+	2	22	16	96	22
	1801	18001	c.3614G>A c.6989T>G	**p.Arg1205His** p.Val2330Gly	27 41		D3 C1	het het	50	M	B+	7	10	6.1	17	8.2
		18002	c.3614G>A c.6989T>G	**p.Arg1205His** p.Val2330Gly	27 41		D3 C1	het het	17	M	AB+	0	12	7	16	9.5
		18003	c.3614G>A c.6989T>G	**p.Arg1205His** p.Val2330Gly	27 41		D3 C1	het het	15	M	O+	5	8.6	6.5	12	6.1
	1903	19005*	ND						44	F	O-	8	48	35	50	28
	2103	21003	c.2771G>A c.6248G>A	**p.Arg924Gln** p.Gly2083Asp	21 36		D3 D4	het het	74	M	A+	10	15	14.2	46	13
		21004	c.55+69A>G c.6248G>A	 p.Gly2083Asp	 36	2	intronic D4	het het	66	F	O+	13	23	19	56	20
		21005	c.2771G>A c.6248G>A	**p.Arg924Gln** p.Gly2083Asp	21 36		D3 D4	het het	63	M	A+	5	13	13	37	12
		21006	c.6248G>A	p.Gly2083Asp	36		D4	het	40	M	O+	8	11	10	29	10.3
	2111	21011	c.246_247insT	p.Ser83Ter	4		null allele	het	54	M	A+	5	12	20.3	33	16
	2120	21023	c.1110-26T>A c.2103C>T c.6248G>A	 c.2103C>T (p.=) p.Gly2083Asp	 16 36	9	intronic D2 D4	het het het	48	F	O+	2	7.6	6	11	3.9
	2201	22001	c.5170+10C>T c.7730-1G>C			29 45	intronic intronic	het het	41	F	O+	14	18	17.5	35	17
	2206	22006	c.1001G>A c.2561G>A	p.Gly334Glu p.Arg854Gln	9 20		D1 D'	het het	58	M	O+	7	27	20	12	22
		22007*	ND						11	M	O+	1	42	36	53	29
	2207	22011	c.6799-47G>A c.7464C>T c.998-46C>T	 c.7464C>T (p.=)	 44	38 8	intronic C3 intronic	hom hom hom	67	M	A-	7	18	16	46	14
	2308	23011	c.3614G>A c.-2077A>T	**p.Arg1205His**	27		D3 upstream	het het	64	M	O+	10	7.5	6.6	10	6.9
		23012	c.3614G>A	**p.Arg1205His**	27		D3	het	32	F	O+	12	7	5.3	6.5	6
	2309	23013	c.6911G>A	**p.Cys2304Tyr**	40		C1	het	46	F	O+	0	33	27.5	56	26
	2310	23015	c.2546+132G>A c.3614G>A	 **p.Arg1205His**	 27	19	intronic D3	het het	74	M	A+	3	18	17.3	19	13
	2703	27030	**c.1533+15G>A**			13	intronic	het	23	M	O+	2	26	28.6	81	30

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1	2707	27012	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	het het	24	F	O+	0	33	29	91	32
	2709	27018*	ND						31	M	O+	2	27	28.5	59	25
	2713	27024	c.3467C>T c.-3151T>G	**p.Thr1156Met**	26		D3 upstream	hom hom	56	F	A+	3	5.3	6	38	4.2
	2716	27032	c.2518G>T	p.Glu840Ter	19		null allele	het	49	F	O+	8	31	22.7	72	38
	2902	29002	c.3223-7_3236dup c.5277C>T	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro c.5277C>T (p.=)	25 30		D3 A3	het het	38	F	O+	7	22	17	54	17
	3005	30006	c.5336G>T	p.Arg1779Leu	31		A3	het	70	F	A-	17	24	29.4	30	28
	3006	30008	c.6536C>T	**p.Ser2179Phe**	37		D4	het	24	F	A+	12	18	19.3	35	21
		30009	c.6536C>T	**p.Ser2179Phe**	37		D4	het	8	M	O+	2	9.4	8	12	9.3
	3012	30035	c.5336G>T	p.Arg1779Leu †	31		A3	het	55	F	A+	1	25	16	38	19
	3013	30037	c.5336G>T	p.Arg1779Leu †	31		A3	het	69	M	O+	1	20	17	25	13
	3202	32002	c.7408C>T	p.Gln2470Ter	43		null allele	het	49	F	A+	3	34	30	53	29
	3204	32004	c.2821-123A>C			21	intronic	het	65	F	A-	19	9.8	4	6.3	5
	3205	32005	c.2546+132G>A c.4751A>G	 **p.Tyr1584Cys**	 28	19	intronic A2	het het	28	F	O-	4	30	25	25	32
	3210	32010	c.3931C>T c.55G>A	p.Gln1311Ter **p.Gly19Arg**	28 2		null allele SigNDl peptide	het het	21	M	ND	12	7.2	5.7	21	6.8
	3501	35001	c.5198T>C c.6187C>T c.7771-87G>A	p.Leu1733Pro p.Pro2063Ser	30 36	 46	A3 D4 intronic	het het het	48	F	O+	1	6.6	8	31	8.1
		35004	c.5198T>C c.6187C>T	p.Leu1733Pro p.Pro2063Ser	30 36		A3 D4	het het	16	M	A+	6	5.6	7.9	19	7.8
		35005	c.5198T>C c.6187C>T	p.Leu1733Pro p.Pro2063Ser	30 36		A3 D4	het het	13	F	A+	5	9.6	8.3	25	10
		35006	c.5198T>C c.6187C>T	p.Leu1733Pro p.Pro2063Ser	30 36		A3 D4	het het	19	F	A+	2	9	7.9	21	9.4
	3502	35002	c.3614G>A c.7730-177G>T	**p.Arg1205His**	27 	 45	D3 intronic	het het	51	M	A+	8	7	6	15	10
		35003	c.3614G>A	**p.Arg1205His**	27		D3	het	75	F	A-	2	6.6	8.5	13	11
	3503	35008	c.1625C>G c.5198T>C c.6187C>T	p.Ala542Gly p.Leu1733Pro p.Pro2063Ser	14 30 36		D2 A3 D4	het het het	64	M	A+	2	5.3	5	10	4.9
	3505	35011	c.5198T>C c.6187C>T	p.Leu1733Pro p.Pro2063Ser	30 36		A3 D4	het het	46	M	O+	2	18	14	42	12
		35012	c.5198T>C c.6187C>T	p.Leu1733Pro p.Pro2063Ser	30 36		A3 D4	het het	13	F	O+	1	25	13	52	20
		35013	c.1293+109T>C c.5198T>C c.6187C>T	 p.Leu1733Pro p.Pro2063Ser	 30 36	11	intronic A3 D4	het het het	9	M	O+	1	12	10	25	10
	3601	36001	c.5170+10C>T c.7730-1G>C			29 45	intronic intronic	het het	5	M	A+	2	33	26.4	88	58

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1	3603	36004	c.2546+55G>T			19	intronic	het	69	F	O-	3	22	18.5	19	19
	3606	36008	c.2771G>A	**p.Arg924Gln**	21		D3	het	34	F	O+	8	34	14	37	25
			c.3390C>T	c.3390C>T (p.=)	26		D3	het								
	3702	37002	c.324-2_326dup	p.Ser110GlufsTer13	5		null allele	het	42	F	O+	3	36	35	49	27
	3705	37005	c.2878C>T	p.Arg960Trp	22		D3	het	39	F	O+	9	26	18	44	18
	3706	37006	c.3426T>C	c.3426T>C (p.=)	26		D3	het	45	F	B+	5	7.6	5	8.9	6.9
			c.3614G>A	**p.Arg1205His**	27		D3	het								
			c.7771-49G>A			46	intronic	het								
			c.3485_3486delinsTG	p.Pro1162Leu	26		D3	het								
	3805	38010	c.2878C>T	p.Arg960Trp	22		D3	het	14	F	O+	11	43	27.5	86	36
	3709	37010*	ND						52	F	O+	14	37	27.4	46	27
	3908	39017	c.3390C>T	c.3390C>T (p.=)	26		D3	het	23	F	A+	4	26	17	44	19
		39019	c.3390C>T	c.3390C>T (p.=)	26		D3	het	19	F	O+	4	23	12	30	16
	4001	40001	c.3363G>T	p.Arg1121Ser	25		D3	het	39	F	ND	10	28	14.2	37	21
	4002	40002	c.5198T>C	p.Leu1733Pro	30		A3	het	39	M	O+	9	9.2	6.7	26	7.4
			c.6187C>T	p.Pro2063Ser	36		D4	het								
		40003	c.5198T>C	p.Leu1733Pro	30		A3	het	10	M	ND	8	17	8.9	28	13
			c.6187C>T	p.Pro2063Ser	36		D4	het								
	4501	45001	c.3614G>A	**p.Arg1205His**	27		D3	het	92	F	ND	9	11	6.6	16	9.4
1H	0117	01034	c.3379+1G>A			25	intronic	het	32	M	O+	2	36	49	101	46
			c.-2627C>T				upstream	het								
	0133	01064	c.7082-13G>C			41	intronic	het	23	F	B+	0	47	41	66	37
		01068	c.7082-13G>C			41	intronic	het	16	F	O+	2	31	31	43	26
	0223	02063	c.2771G>A	**p.Arg924Gln**	21		D3	het	56	M	O+	1	35	36	71	38
	03186	03031	c.1625C>G	p.Ala542Gly	14		D2	het	29	F	O+	0	49	38	56	33
			c.533-2A>G			5	intronic	het								
	0616	06023	c.2771G>A	**p.Arg924Gln**	21		D3	het	27	F	O+	-2	44	40	53	33
	0902	09002	c.2771G>A	**p.Arg924Gln**	21		D3	het	30	M	A+	1	104	80	65	77
			c.5312-138G>A			30	intronic	het								
	1204	12008	c.2771G>A	**p.Arg924Gln**	21		D3	het	14	F	O+	2	72	72	80	65
	1211	12018	c.4747C>T	**p.Arg1583Trp**	28		A2	het	73	F	ND	0	63	49	51	42
			c.7730-238G>A			45	intronic	het								
	1702	17004*	ND						52	M	A+	0	46	39	57	30
	1904	19006	c.7408C>T	p.Gln2470Ter	43		null allele	het	41	F	O+	3	46	38	74	31
	2109	21009*	ND						41	F	O+	3	31	40	58	44
	2112	21014	c.1446C>G	p.Ile482Met	13		D2	het	22	F	A+	6	38	40	43	44
	2117	21019	c.2771G>A	**p.Arg924Gln**	21		D3	het	45	M	O+	10	57	50	59	39
	2703	27004	**c.1533+15G>A**			13	intronic	het	25	M	O-	1	37	36	46	36
		27005	**c.1533+15G>A**			13	intronic	het	55	F	O-	-2	47	60.8	90	55
		27006	**c.1533+15G>A**			13	intronic	het	18	F	O+	2	46	44	175	47
		27026	**c.1533+15G>A**			13	intronic	het	59	M	B-	1	37	33	104	43
		27027	**c.1533+15G>A**			13	intronic	het	53	M	A-	2	50	64	129	59
	2709	27017*	ND						31	M	O+	3	33	38,5	66	31

	Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
1H	2717	27035	c.7888-65C>A			47	intronic	het	29	M	O+	7	32	46	73	45
	2718	27036*	ND						55	F	O+	2	41	36	40	30
	2903	29003	c.5170+10C>T			29	intronic	het	29	M	O+	4	58	51	48	56
			c.5278G>A	**p.Val1760Ile**	30		A3	het								
		29004	c.5278G>A	**p.Val1760Ile**	30		A3	het	30	F	B+	7	59	48	42	41
	3207	32007	c.1446C>G	p.Ile482Met	13		D2	het	29	F	O+	4	69	35	46	34
	3607	36009	c.4751A>G	**p.Tyr1584Cys**	28		A2	het	30	F	O+	2	56	48	47	46
			c.5456-62A>G			31	intronic	het								
	3908	39018	c.3390C>T	c.3390C>T (p.=)	26		D3	het	47	F	A+	7	53	43	69	35
	4202	42002	c.2771G>A	**p.Arg924Gln**	21		D3	het	31	F	O	11	186	140	88	130
			c.3390C>T	c.3390C>T (p.=)	26		D3	het								
			c.7771-86G>A			46	intronic	het								
	4203	42003	c.4751A>G	**p.Tyr1584Cys**	28		A2	het	37	F	O-	3	60	48	50	41
	4205	42005	c.3692A>G	p.Asn1231Ser	28		D3	het	38	M	O-	3	48	40.7	36	31

In bold, mutations previously described in type 1 VWD. NI indicates not identified; and ND, not determined.* Patients with a discrepant phenotype-genotype correlation. † Mutation non detected by Fluidigim method due to an imbalance of alleles reads and detected a posteriori by a NGS custom gene panel.

Table S4. Genotype-phenotype correlation in type 3 patients

Family	Patient	Phenotype	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
0121	01040	3	c.970C>T	**p.Arg324Ter**	8		null allele	hom	34	M	A+	26	0	4	3.9	0
0122	01041	3	c.970C>T	**p.Arg324Ter**	8		null allele	hom	48	M	B-	16	0	4	5.4	0
0124	01059	3 (carrier)	c.6187C>T	**p.Pro2063Ser**	36		D4	het	34	M	AB	0	53	45	75	44
			c.970C>T	**p.Arg324Ter**	8		null allele	het								
	01060	3 (carrier)	c.970C>T	**p.Arg324Ter**	8		null allele	het	59	F	O+	2	143	114	100	98
0126	01049	3	c.5170+10C>T			29	intronic	hom	27	F	A+	19	4	1	3.9	0
			c.7730-1G>C			45	intronic	hom								
0202	02004	3 (carrier)	c.2685G>C	p.Gln895His	20		D3	het	73	M	O+	8	69	67.6	146	71
	02007	3 (carrier)	c.2685G>C	p.Gln895His	20		D3	het	8	M	A+	4	53	54.8	115	41
	02009	3 (carrier)	c.2685G>C	p.Gln895His	20		D3	het	7	M	O-	1	61	71	111	46
0204	02020	3	c.3931C>T	**p.Gln1311Ter**	28		null allele	hom	23	M	AB-	24	0	4	2.6	0
0215	02039	3	c.1109G>A	p.Cys370Tyr	9		D1	hom	49	F	O+	23	1.2	6	11	0
	02040	3 (carrier)	c.1109G>A	p.Cys370Tyr	9		D1	het	23	F	O+	0	41	43	104	41
	02041	3 (carrier)	c.1109G>A	p.Cys370Tyr	9		D1	het	20	F	O+	0	35	41	93	33
	02043	3 (carrier)	c.1109G>A	p.Cys370Tyr	9		D1	het	85	F	O+	-2	36	36.5	103	37
	02044	3 (carrier)	c.1109G>A	p.Cys370Tyr	9		D1	het	78	M	O+	-1	40	39	105	42
0217	02046	3	c.4975C>T	**p.Arg1659Ter**	28		null allele	hom	47	F	A-	18	0	4	4.3	0
0219	02051	3	c.3931C>T	**p.Gln1311Ter**	28		null allele	hom	35	F	B+	21	0	4	3.7	0
0301	03018	3	c.546G>A	c.546G>A (p.=)	6		D1	het	20	F	O+	14	0	5	2.9	0
			c.7082-2A>G			41	intronic	het								
			c.8155+3G>C			50	intronic	het								
0302	03016	3	c.5170+10C>T			29	intronic	hom	48	F	A+	13	1.6	5	4.5	0
			c.7730-1G>C			45	intronic	hom								
03127	03037	3	c.3931C>T	**p.Gln1311Ter**	28		null allele	hom	5	M	A	10	0	5	1.9	0
	03038	3 (carrier)	c.3931C>T	**p.Gln1311Ter**	28		null allele	het	32	M	ND	0	67	37	82	56
	03039	3 (carrier)	c.3931C>T	**p.Gln1311Ter**	28		null allele	het	29	F	ND	1	66	34	68	47
0314	03041	3	c.3931C>T	**p.Gln1311Ter**	28		null allele	hom	25	M	O+	7	0	5	2.3	0
03150	03034	3	c.3467C>T	p.Thr1156Met	26		D3	het	6	M	A+	4	3	5	12	2
			c.5170+10C>T			29	intronic	het								
			c.7730-1G>C			45	intronic	het								
			c.-3151T>G				upsteram	het								
	03036	3 (carrier)	c.5170+10C>T			29	intronic	het	40	M	A+	-1	83	65	103	61
			c.7730-1G>C			45	intronic	het								
03167	03032	3	c.2540dupA	**p.Asn847LysfsTer18**	19		null allele	hom	31	M	A+	8	0	4	2	0
	03033	3 (carrier)	c.2540dup	**p.Asn847LysfsTer18**	19		null allele	het	35	M	A+	0	37	30.7	61	29
			c.7771-29G>A			46	intronic	het								
0318	03001	3	c.3835_4105conNG_001212.3:g.6566 6836	p.[(V1279I;Q1311*;I1343V;V1360A;F1369I)]	28		A1	hom	27	F	AB+	8	1	1	4	ND
0321	03046	3	c.100C>T	**p.Arg34Ter**	3		null allele	hom	29	F	O+	12	0	5	3	0
0325	03021	3	c.449T>C	**p.Leu150Pro**	5		D1	het	15	M	A	10	3.7	5	6.7	1.9
			c.7082-2A>G			41	intronic	het								
			c.7730-177G>T			45	intronic	het								
	03022	3 (carrier)	**c.7082-2A>G**			41	intronic	het	48	M	ND	0	88	46	85	48
			c.7730-177G>T			45	intronic	het								
0391	03002	3	c.1625C>G	p.Ala542Gly	14		D2	hom	43	F	A+	8	0	4	5.7	0
			c.533-2A>G			5	intronic	hom								

Family	Patient	Phenotype	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
0501	05001	3	c.1533+1G>A			13	intronic	het	34	M	A+	9	0	5	1.5	0
			c.6256+2dup			36	intronic	het								
0506	05007	3	c.2561G>A	p.Arg854Gln	20		D'	het	14	F	O+	20	2.9	5	5.8	2.7
			c.3319T>C	p.Tyr1107His	25		D3	het								
			c.4146G>T	c.4146G>T (p.=)	28		A1	het								
			c.6197A>G	p.Asn2066Ser	36		D4	het								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	het								
0508	05009	3	c.1992dupC	p.Cys665LeufsTer13	16		null allele	het	9	F	B+	12	0	5	3.6	0
			c.4146G>T	c.4146G>T (p.=)	28		A1	het								
			c.6197A>G	p.Asn2066Ser	36		D4	het								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	het								
0509	05010	3 (carrier)	c.3735G>A	c.3735G>A (p.=)	28		D3	het	12	F	B+	4	37	40.6	69	41
			c.3797C>T	p.Pro1266Leu	28		D3	hom								
			c.6187C>T	**p.Pro2063Ser**	36		D4	het								
0510	05011*	3	c.5837G>T	p.Cys1946Phe	34		D4	het	9	F	A+	13	0	5	3.3	0
0514	05015	3 (carrier)	c.1533+15G>A			13	intronic	het	43	F	O+	4	59	60	109	54
0515	05016	3	c.100C>T	**p.Arg34Ter**	3		null allele	hom	47	F	B-	13	0	5	12	0
	05017	3	c.100C>T	**p.Arg34Ter**	3		null allele	hom	47	F	B-	18	0	5	4.1	0
0803	08003	3 (carrier)	c.6187C>T	**p.Pro2063Ser**	36		D4	het	23	F	O+	6	76	78	108	80
	08004	3 (carrier)	c.6187C>T	**p.Pro2063Ser**	36		D4	het	16	F	A+	6	33	39	75	40
1001	10001	3	c.7664_7665insAG	**p.Cys2557SerfsTer8**	45		null allele	hom	53	M	O+	2	5.1	5	13	4.3
1004	10005	3	c.1534_1536del	p.Leu512del	14		D2	het	26	F	O-	4	0	5	3.5	0
			c.4146G>T	c.4146G>T (p.=)	28		A1	het								
			c.6197A>G	p.Asn2066Ser	36		D4	het								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	het								
1005	10006*	3	c.7408C>T	p.Gln2470Ter	43		null allele	hom	14	M	B+	8	68	29	59	41
1007	10008	3	c.1093C>T	**p.Arg365Ter**	9		null allele	hom	29	M	O+	2	3.2	5	10	2.5
1009	10011*	3	c.5455+1G>A			31	intronic	het	33	F	O+	1	0	5	4.4	0
1014	10016	3	c.7118C>T	p.Pro2373Leu	42		C2	het	19	M	A+	4	0	5	3	0
			c.7408C>T	p.Gln2470Ter	43		null allele	het								
			c.8347C>T	p.Gln2783Ter	52		null allele	het								
1217	12024	3 (carrier)	c.6187C>T	**p.Pro2063Ser**	36		D4	het	31	M	O+	0	56	40	59	41
1306	13008	3	c.4146G>T	c.4146G>T (p.=)	28		A1	hom	39	F	A+	18	3	4	2.7	0.7
			c.6197A>G	p.Asn2066Ser	36		D4	hom								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	hom								
2001	20001	3	c.4146G>T	c.4146G>T (p.=)	28		A1	hom	9	F	O-	6	0	5	1.6	0
			c.6197A>G	p.Asn2066Ser	36		D4	hom								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	hom								
	20003	3 (carrier)	c.4146G>T	c.4146G>T (p.=)	28		A1	het	46	M	A+	0	45	38	63	34
			c.6197A>G	p.Asn2066Ser	36		D4	het								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	het								
2101	21001	3	c.4162C>T	p.Gln1388Ter	28		null allele	hom	45	M	ND	14	0	5	2.5	0
2102	21002	3	c.3426T>C	c.3426T>C (p.=)	26		D3	hom	41	F	B+	12	0	5	6.8	0
			c.3485_3486delinsTG	p.Pro1162Leu	26		D3	hom								
2104	21007	3	c.5311G>A	p.Gly1771Arg	30		A3	het	51	F	A+	16	2.3	5	10	0
			c.818G>C	p.Arg273Pro	7		D1	het								
2113	21015	3	c.4162C>T	p.Gln1388Ter	28		null allele	het	39	M	A+	16	0	5	6.4	0
			c.7583G>A	p.Cys2528Tyr	45		C4	het								

Family	Patient	Phenotype	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:CB
2505	25005	3	c.(1945+1_1946-1)_(7437+1_7438-1)del		16-43		D2-C3	hom	32	M	O+	11	0	4	2.4	0
2716	27034	3 (carrier)	c.2518G>T	p.Glu840Ter	19		null allele	het	16	F	A+	3	42	32	78	54
2720	27038	3 (carrier)	c.6187C>T	**p.Pro2063Ser**	36		D4	het	51	M	A+	-1	68	52	67	59
2721	27041	3 (carrier)	c.2944G>C	p.Val982Leu	22		D3	het	43	F	A+	4	42	25	66	48
			c.4146G>T	c.4146G>T (p.=)	28		A1	het								
			c.6197A>G	p.Asn2066Ser	36		D4	het								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	het								
3001	30001	3	c.4975C>T	**p.Arg1659Ter**	28		null allele	het	60	F	O+	26	0	5	2.2	0
			c.8347C>T	p.Gln2783Ter	52		null allele	het								
3210	32011	3 (carrier)	c.3931C>T	**p.Gln1311Ter**	28		null allele	het	31	F	A+	3	51	23.5	88	21
3401	34001	3 (carrier)	c.1209C>G		11		null allele	het	42	M	O-	3	41	35	48	28
			c.2821-88A>G			21	intronic	het								
			c.425G>A	p.Gly142Asp	5		D1	het								
			c.7771-82G>A			46	intronic	het								
3605	36007	3	c.2771G>A	p.Arg924Gln	21		D3	het	52	M	A-	10	4.1	5	5.7	0
			c.2900G>T	p.Gly967Val	22		D3	het								
			c.6799-27C>T			38	intronic	het								
3806	38008	3	c.2025G>A	c.2025G>A (p.=)	16		D2	het	12	F	ND	11	4.3	4	13	3.7
			c.3797C>T	p.Pro1266Leu	28		D3	het								
			c.3835G>A	p.Val1279Ile	28		A1	het								
			c.5198T>C	p.Leu1733Pro	30		A3	het								
3905	39009	3	c.6598+1G>A			37	intronic	het	37	M	B-	19	0	4	2.3	0
			c.8347C>T	p.Gln2783Ter	52		null allele	het								
	39010	3 (carrier)	c.6598+1G>A			37	intronic	het	76	F	B-	-3	104	92	128	57
	39014	3	c.6598+1G>A			37	intronic	het	37	M	B-	20	0	4	2.5	0
			c.8347C>T	p.Gln2783Ter	52		null allele	het								
4401	44001	3	c.4146G>T	c.4146G>T (p.=)	28		A1	hom	23	M	ND	21	0	4	1.3	0
			c.6197A>G	p.Asn2066Ser	36		D4	hom								
			c.375_376delinsC	**p.Tyr126ThrfsTer49**	5		null allele	hom								

In bold, mutations previously described in type 3. ND indicates not determined.* Patients with a discrepant phenotype-genotype correlation.

Table S5. Genotype-phenotype correlation in type 2A patients

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
0101	01001	c.3445T>C	**p.Cys1149Arg**	26		D3	het	44	F	B+	7	↓HMWM	10	6.8	18	0.68	5.8
	01045	c.3445T>C	**p.Cys1149Arg**	26		D3	het	49	F	B+	12	↓HMWM	13	7.7	20	0.59	8.7
		c.7070A>T	p.Asn2357Ile	41		C2	het										
0105	01008	c.4121G>A	**p.Arg1374His**	28		A1	het	68	F	O+	8	↓HMWM	20	4	45	0.20	13
		c.-1875G>A				upstream	het										
0108	01012	c.1946-17_1946-16insTTT			15	intronic	het	45	F	B+	16	↓HMWM	16	4	36	0.25	1.6
		c.3815G>C	**p.Cys1272Ser**	28		A1	het										
0110	01018	c.4196G>A	p.Arg1399His	28		A1	het	31	F	A-	12	↓HMWM	8.3	4	18	0.48	3.4
		c.4517C>T	**p.Ser1506Leu**	28		A2	het										
0123	01043	c.3445T>C	**p.Cys1149Arg**	26		D3	het	25	F	B+	7	↓HMWM	9.5	4	11.6	0.42	6.3
	01046	c.3445T>C	**p.Cys1149Arg**	26		D3	het	65	M	A+	9	↓HMWM	8	5	18	0.63	5
	01047	c.3445T>C	**p.Cys1149Arg**	26		D3	het	40	M	A+	12	↓HMWM	8.6	5.2	14	0.60	4.7
	01053	c.3445T>C	**p.Cys1149Arg**	26		D3	het	36	M	A+	10	↓HMWM	15	8.1	20	0.54	6.1
0125	01048	c.3445T>C	**p.Cys1149Arg**	26		D3	het	25	F	B+	18	↓HMWM	11	7.6	15	0.69	5.1
	01061	c.3445T>C	**p.Cys1149Arg**	26		D3	het	53	M	B+	2	↓HMWM	17	7.9	27	0.46	8.2
0127	01069	c.4517C>T	**p.Ser1506Leu**	28		A2	het	17	F	O+	13	↓HMWM	19	4	30	0.21	6.3
		c.7344C>T	c.7344C>T (p.=)	43		C3	het										
	01070	c.4517C>T	**p.Ser1506Leu**	28		A2	het	38	F	O+	21	↓HMWM	38	7.2	59	0.19	12
0130	01055	c.3445T>C	**p.Cys1149Arg**	26		D3	het	18	F	O+	6	↓HMWM	12	7.9	20	0.66	5.2
	01056	c.3445T>C	**p.Cys1149Arg**	26		D3	het	38	F	A+	8	↓HMWM	13	7.3	23	0.56	6.1
	01057	c.3445T>C	**p.Cys1149Arg**	26		D3	het	71	F	O+	10	↓HMWM	9.6	4	13	0.42	4.4
0135	01072	c.4789C>T	**p.Arg1597Trp**	28		A2	het	48	M	O	7	↓HMWM	37	22	51	0.59	15
0203	02010	c.4789C>T	**p.Arg1597Trp**	28		A2	het	54	F	O+	21	↓↓HMWM	25	6.5	91	0.26	9.4
	02011	c.4789C>T	**p.Arg1597Trp**	28		A2	het	31	F	O+	13	↓↓HMWM	27	8	122	0.30	8.9
	02012	c.4789C>T	**p.Arg1597Trp**	28		A2	het	27	F	ND	8	↓↓HMWM	33	10.2	45	0.31	12
	02013	c.4789C>T	**p.Arg1597Trp**	28		A2	het	18	M	ND	11	↓↓HMWM	16	8.4	49	0.53	8.1
0205	02021	c.4883T>C	**p.Ile1628Thr**	28		A2	het	44	F	A-	17	↓↓HMWM	38	6	61	0.16	6.6
	02022	c.4883T>C	**p.Ile1628Thr**	28		A2	het	14	F	A-	8	↓↓HMWM	43	7.3	64	0.17	7.4
		c.5191T>A	p.Ser1731Thr	30		A3	het										
0209	02028	c.4825G>A	**p.Gly1609Arg**	28		A2	het	47	F	A-	18	↓↓HMWM	35	7.8	67	0.22	8.6
		c.6187C>T	p.Pro2063Ser	36		D4	het										
	02029	c.4825G>A	**p.Gly1609Arg**	28		A2	het	14	F	A+	7	↓↓HMWM	31	7.2	46	0.23	13
		c.6187C>T	p.Pro2063Ser	36		D4	het										

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
0227	02071*	c.2926C>T	p.Arg976Cys	22		D3	het	34	F	O+	3	NORMAL	27	23.6	67	0.87	36
		c.6187C>T	p.Pro2063Ser	36		D4	het										
	02072*	c.2926C>T	p.Arg976Cys	22		D3	het	62	F	O+	5	NORMAL	45	46	114	1.02	57
		c.6187C>T	p.Pro2063Ser	36		D4	het										
0231	02081	c.4885G>A	**p.Gly1629Arg**	28		A2	het	43	M	O-	9	↓↓HMWM	35	6.4	30	0.18	15
0304	03007	c.3426T>C	c.3426T>C (p.=)	26		D3	het	50	M	A+	8	↓HMWM	18	6.4	30	0.36	15
		c.4121G>A	**p.Arg1374His**	28		A1	het										
		c.8156-42C>T			50	intronic	het										
		c.3485_3486delinsTG	p.Pro1162Leu	26		D3	het										
	03008	c.1156+42C>T			10	intronic	het	23	F	ND	3	↓HMWM	14	7	26	0.50	14
		c.4121G>A	**p.Arg1374His**	28		A1	het										
03152	03012*	c.1157_5620del	p.Gly386_Ser1873del	11-32		D2-D3	het	62	F	O+	10	↓HMWM	42	20	70	0.48	21
03231	03048	c.3426T>C	c.3426T>C (p.=)	26		D3	het	15	F	O+	0	↓HMWM	117	6.5	90	0.06	30.8
		c.8318G>C	**p.Cys2773Ser**	52		CK	het										
		c.3485_3486delinsTG	p.Pro1162Leu	26		D3	het										
0329	03026	c.4883T>C	**p.Ile1628Thr**	28		A2	het	73	M	A+	8	↓HMWM	56	9.1	29	0.16	11
	03027	c.4883T>C	**p.Ile1628Thr**	28		A2	het	42	F	O+	5	↓HMWM	41	8.7	29	0.21	7
	03028	c.4883T>C	**p.Ile1628Thr**	28		A2	het	9	M	O+	10	↓HMWM	38	7.4	22	0.19	7.7
	03029	c.4883T>C	**p.Ile1628Thr**	28		A2	hom	44	F	O+	6	↓HMWM	47	6	32	0.13	8.9
0336	03009	c.4678_4680dup	p.Asp1560dup	28		A2	het	45	F	O+	5	↓HMWM	19	5	35	0.26	13
	03010	c.4678_4680dup	p.Asp1560dup	28		A2	het	25	F	ND	5	↓HMWM	49	5.3	44	0.11	18
0347	03011	c.1625C>G	p.Ala542Gly	14		D2	het	80	M	A+	4	↓HMWM	41	12.1	89	0.30	15
		c.1721C>G	p.Pro574Arg	14		D2	het										
		c.533-2A>G			5	intronic	het										
0351	03040	c.4883T>C	**p.Ile1628Thr**	28		A2	het	35	F	A+	7	↓HMWM	43	6.3	39	0.15	8.2
		c.7344C>T	c.7344C>T (p.=)	43		C3	het										
0505	05005	c.4889T>G	p.Val1630Gly	28		A2	het	47	M	AB+	8	↓HMWM	19	5	36	0.26	8.5
	05006	c.4889T>G	p.Val1630Gly	28		A2	het	9	F	B-	4	↓HMWM	16	6.2	43	0.39	6.8
0615	06021	c.4121G>A	**p.Arg1374His**	28		A1	het	36	F	O+	3	↓HMWM	13	5.7	31	0.44	13
		c.6187C>T	p.Pro2063Ser	36		D4	het										
	06022	c.4121G>A	**p.Arg1374His**	28		A1	het	11	M	O-	0	↓HMWM	16	6.1	30	0.38	16
0702	07002	c.4840G>A	p.Asp1614Asn	28		A2	het	39	F	O+	15	↓HMWM	18	6.4	24	0.36	8.9
	07005	c.4840G>A	p.Asp1614Asn	28		A2	het	13	F	O+	11	↓HMWM	19	8.3	27	0.44	13
	07006	c.4840G>A	p.Asp1614Asn	28		A2	het	13	F	O+	11	↓HMWM	17	4	26	0.24	12
0804	08005	c.4499C>A	**p.Ala1500Glu**	28		A2	het	8	F	A+	9	↓↓HMWM	15	5	34	0.33	9.4
		c.4620G>C	c.4620G>C (p.=)	28		A2	het										

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
1006	10007	c.4883T>C	**p.Ile1628Thr**	28		A2	het	49	F	A+	1	↓↓HMWM	56	5	59	0.09	11
	10009	c.4883T>C	**p.Ile1628Thr**	28		A2	het	43	M	A+	3	↓↓HMWM	48	5	47	0.10	9.9
1303	13004	c.4883T>C	**p.Ile1628Thr**	28		A2	het	42	M	A+	17	↓↓HMWM	26	5	31	0.19	7
1310	13013	c.4517C>T	**p.Ser1506Leu**	28		A2	het	37	M	A+	20	↓↓HMWM	18	5	29	0.28	7.2
1312	13015	c.4622A>T	p.Gln1541Leu	28		A2	het	48	F	B+	22	↓↓HMWM	30	9.3	43	0.31	10.5
		c.6182del	p.Phe2061SerfsTer38	36		D4	het										
1404	14004	c.4825G>A	**p.Gly1609Arg**	28		A2	het	63	F	B+	9	↓↓HMWM	78	15	62	0.19	18
1405	14005	c.4789C>T	**p.Arg1597Trp**	28		A2	het	10	F	O+	11	↓↓HMWM	24	5	35	0.21	6.6
2002	20002	c.4517C>T	**p.Ser1506Leu**	28		A2	het	22	F	O-	13	↓HMWM	12	6.1	19	0.51	6
2116	21018	c.250C>T	p.Leu84Phe	4		D1	het	41	F	B+	14	↓HMWM	16	6.2	44	0.39	11
		c.4739T>C	**p.Leu1580Pro**	28		A2	het										
		c.6187C>T	p.Pro2063Ser	36		D4	het										
2202	22002	c.4960T>C	**p.Phe1654Leu**	28		A2	het	19	F	A+	9	↓HMWM	35	7.3	50	0.21	18
		c.5368C>T	p.Pro1790Ser	31		A3	het										
2203	22003	c.4790G>A	**p.Arg1597Gln**	28		A2	het	72	F	A+	3	↓HMWM	34	7.5	66	0.22	16
		c.6976+111G>A			40	intronic	het										
	22008	c.4790G>A	**p.Arg1597Gln**	28		A2	het	70	M	ND	11	↓HMWM	39	6.8	40	0.17	13
		c.6976+111G>A			40	intronic	het										
	22009	c.4790G>A	**p.Arg1597Gln**	28		A2	het	41	F	ND	14	↓HMWM	53	8.2	48	0.15	17
	22010	c.4790G>A	**p.Arg1597Gln**	28		A2	het	38	M	A+	10	↓HMWM	66	10.6	51	0.16	22
2205	22005	c.4241T>G	p.Val1414Gly	28		A1	het	31	F	O+	10	↓HMWM	12	6.9	47	0.58	11
2301		c.2821-123A>C			21	intronic	het										
	23001	c.4960T>C	**p.Phe1654Leu**	28		A2	het	19	F	A+	11	↓HMWM	48	6.5	43	0.14	18
		c.6187C>T	p.Pro2063Ser	36		D4	het										
	23002	c.4960T>C	**p.Phe1654Leu**	28		A2	het	42	F	A+	14	↓HMWM	66	7.1	44	0.11	20
	23014	c.1446C>G	p.Ile482Met	13		D2	het	21	M	A+	4	↓HMWM	82	7.2	45	0.09	24
		c.4257T>G	p.His1419Gln	28		A1	het										
		c.4960T>C	**p.Phe1654Leu**	28		A2	het										
2702	27003	c.2771G>A	p.Arg924Gln	21		D3	het	75	M	ND	9	↓↓↓HMWM	40	9.9	43	0.25	10
		c.4943C>G	**p.Pro1648Arg**	28		A2	het										
2705	27008	c.4735G>A	**p.Gly1579Arg**	28		A2	het	56	F	A+	6	↓↓HMWM	43	9	45	0.21	10
3002	30003	c.4517C>T	**p.Ser1506Leu**	28		A2	het	49	M	A+	10	↓↓HMWM	18	5.9	16	0.33	7.1
		c.7393G>A	p.Val2465Met	43		C3	het										

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
3003	30004	c.2821-78A>C			21	intronic	het	30	F	B+	6	↓HMWM	16	5.4	26	0.34	13
		c.3920T>C	**p.Leu1307Pro**	28		A1	het										
	30007	c.3920T>C	**p.Leu1307Pro**	28		A1	het	61	F	B+	22	↓HMWM	13	5.9	24	0.45	9.9
		c.-1650G>C				upstream	het										
	30010	c.3920T>C	**p.Leu1307Pro**	28		A1	het	66	M	A+	13	↓HMWM	11	5.6	21	0.51	9.2
3004	30005	c.4121G>A	**p.Arg1374His**	28		A1	het	67	F	O+	20	NORMAL	22	5.8	26	0.26	15
		c.5277C>T	c.5277C>T (p.=)	30		A3	het										
	30011	c.2685+147G>A			20	intronic	het	38	M	O-	9	NORMAL	18	5.8	26	0.32	15
		c.4121G>A	**p.Arg1374His**	28		A1	het										
	30014	c.4121G>A	**p.Arg1374His**	28		A1	het	49	M	O+	16	↓HMWM	15	4	14	0.27	6.7
	30015	c.4121G>A	**p.Arg1374His**	28		A1	het	18	M	A+	2	NORMAL	31	5	29	0.16	14
	30016	c.2821-148G>A			21	intronic	het	14	M	O-	9	NORMAL	22	5	26	0.23	12
		c.4121G>A	**p.Arg1374His**	28		A1	het										
	30019	c.4121G>A	**p.Arg1374His**	28		A1	het	75	F	O+	15	NORMAL	26	5	21	0.19	12
	30020	c.4121G>A	**p.Arg1374His**	28		A1	het	71	F	O+	11	NORMAL	62	5.6	45	0.09	27
		c.5277C>T	c.5277C>T (p.=)	30		A3	het										
	30021	c.4121G>A	**p.Arg1374His**	28		A1	het	60	F	A+	14	NORMAL	35	5.8	33	0.17	17
		c.5277C>T	c.5277C>T (p.=)	30		A3	het										
	30022	c.4121G>A	**p.Arg1374His**	28		A1	het	62	F	O+	16	NORMAL	30	5.6	25	0.19	14
	30024	c.4121G>A	**p.Arg1374His**	28		A1	het	18	M	A+	7	NORMAL	25	5.4	19	0.22	14
	30025	c.4121G>A	**p.Arg1374His**	28		A1	het	47	M	A+	12	NORMAL	26	4	25	0.15	11
	30026	c.4121G>A	**p.Arg1374His**	28		A1	het	37	F	A+	10	NORMAL	33	4	27	0.12	18
	30029	c.4121G>A	**p.Arg1374His**	28		A1	het	48	F	A+	10	NORMAL	19	5.7	19	0.30	13
	30031	c.4121G>A	**p.Arg1374His**	28		A1	het	36	F	A+	8	NORMAL	22	4	21	0.18	11
	30032	c.4121G>A	**p.Arg1374His**	28		A1	het	69	F	A+	6	NORMAL	57	9	41	0.16	29
	30034	c.4121G>A	**p.Arg1374His**	28		A1	het	8	F	A+	12	NORMAL	21	5	21	0.24	9.8
	30036	c.4121G>A	**p.Arg1374His**	28		A1	het	24	M	O+	2	NORMAL	23	5	30	0.22	11
	30038	c.4121G>A	**p.Arg1374His**	28		A1	het	24	F	A+	6	↓HMWM	29	5	24	0.17	12
	30039	c.4121G>A	**p.Arg1374His**	28		A1	het	33	M	A+	3	↓HMWM	21	5	20	0.24	7.5
	30040	c.4121G>A	**p.Arg1374His**	28		A1	het	33	F	A+	10	↓HMWM	22	6.9	33	0.31	11
3009	30017	c.2821-123A>C			21	intronic	het	17	M	O-	7	↓↓HMWM	17	5	12	0.29	4.3
		c.4789C>T	**p.Arg1597Trp**	28		A2	het										
		c.5001G>A	c.5001G>A (p.=)	28		A2	het										
3701	37001	c.4789C>G	**p.Arg1597Gly**	28		A2	het	36	F	A+	15	↓↓HMWM	41	5	40	0.12	15
	37007	c.4789C>G	**p.Arg1597Gly**	28		A2	het	3	F	AB+	3	↓↓HMWM	48	8.8	33	0.18	21
3708	37009	c.4825G>A	**p.Gly1609Arg**	28		A2	het	41	F	O-	6	↓HMWM	60	6.6	29	0.11	18
3802	38002	c.4883T>C	**p.Ile1628Thr**	28		A2	het	13	M	O+	23	↓HMWM	57	4	50	0.07	15

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
3902	39001	c.3433C>T	p.Arg1145Cys	26		D3	het	70	M	O+	10	↓HMWM	18	5	35	0.28	17
		c.4121G>A	**p.Arg1374His**	28		A1	het										
		c.-1875G>A				upstream	het										
	39004	c.4121G>A	**p.Arg1374His**	28		A1	het	33	M	A+	11	ND	13	6.7	29	0.52	15
		c.-1875G>A				upstream	het										
3903	39005	c.4121G>A	**p.Arg1374His**	28		A1	het	35	M	A-	8	↓HMWM	13	7.1	26	0.55	14
		c.-2076A>G				upstream	het										
	39006	c.4121G>A	**p.Arg1374His**	28		A1	het	59	F	A+	15	↓HMWM	12	4.7	23	0.39	14
3906	39011	c.4517C>T	**p.Ser1506Leu**	28		A2	het	62	F	A+	11	↓↓HMWM	17	7.7	31	0.45	21
	39012	c.4517C>T	**p.Ser1506Leu**	28		A2	het	41	M	O+	15	↓↓HMWM	8.2	5	13	0.61	6.9
	39013	c.4517C>T	**p.Ser1506Leu**	28		A2	het	31	M	B+	12	↓↓HMWM	21	6.8	30	0.32	11
3907	39016	c.4517C>T	**p.Ser1506Leu**	28		A2	het	17	F	A-	11	↓↓HMWM	37	7.7	36	0.21	14
		c.5277C>T	c.5277C>T (p.=)	30		A3	het										
4201	42001	c.4121G>A	**p.Arg1374His**	28		A1	het	70	M	O+	8	↓HMWM	27	4	24	0.15	14
4210	42010*	c.4580G>A	**p.Arg1527Gln**	28		A2	het	9	M	O+	2	↓HMWM	69	58.5	52	0.85	45
4403	44004	c.4892G>A	p.Gly1631Asp	28		A2	het	19	F	A+	14	↓HMWM	51	5	54	0.10	12
4502	45002	c.4789C>T	**p.Arg1597Trp**	28		A2	het	16	F	ND	6	↓HMWM	22	5	27	0.23	8
	45003	c.4789C>T	**p.Arg1597Trp**	28		A2	het	48	M	O+	9	↓HMWM	26	5	30	0.19	9.3
4503	45004	c.3944G>T	**p.Arg1315Leu**	28		A1	het	49	M	ND	11	↓HMWM	21	5	31	0.24	14

In bold, mutations previously described in type 2A. ND indicates not determined; and HMWM, high molecular weight multimers. *Patients with a discrepant phenotype-genotype correlation.

Table S6. Genotype-phenotype correlation in type 2A/2M patients

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
0102	01002	c.4120C>T	**p.Arg1374Cys**	28		A1	het	51	F	O+	11	SMEAR	16	4.4	34	0.28	9.2
		c.6187C>T	p.Pro2063Ser	36		D4	het										
	01003	c.4120C>T	**p.Arg1374Cys**	28		A1	het	58	F	A	15	SMEAR	20	6.7	45	0.34	13
	01014	c.4120C>T	**p.Arg1374Cys**	28		A1	het	55	F	O	19	SMEAR	15	4.5	29	0.30	11
	01020	c.4120C>T	**p.Arg1374Cys**	28		A1	het	66	F	O+	12	SMEAR	32	11	47	0.34	19
		c.6433C>T	p.Pro2145Ser	37		D4	het										
	01022	c.4120C>T	**p.Arg1374Cys**	28		A1	het	80	M	A	11	SMEAR	54	18	59	0.33	30
	01035	c.4120C>T	**p.Arg1374Cys**	28		A1	het	38	F	A-	18	SMEAR	14	6.7	25	0.48	15
	01039	c.2771G>A	p.Arg924Gln	21		D3	het	18	M	A+	8	SMEAR	23	12	24	0.52	15
		c.4120C>T	**p.Arg1374Cys**	28		A1	het										
0104	01007	c.4120C>T	**p.Arg1374Cys**	28		A1	het	66	M	AB+	6	SMEAR	25	7.8	38	0.31	15
	01009	c.3835G>A	p.Val1279Ile	28		A1	het	88	F	A+	9	SMEAR	17	4	52	0.24	12
		c.4120C>T	**p.Arg1374Cys**	28		A1	het										
	01015	c.4120C>T	**p.Arg1374Cys**	28		A1	het	39	F	AB	12	SMEAR	19	7.8	36	0.41	11
	01023	c.4120C>T	**p.Arg1374Cys**	28		A1	het	38	M	AB+	7	SMEAR	30	13	27	0.43	18
	01026	c.3835G>A	p.Val1279Ile	28		A1	het	90	M	A+	16	SMEAR	18	4	37	0.22	18
		c.4120C>T	**p.Arg1374Cys**	28		A1	het										
	01027	c.4120C>T	**p.Arg1374Cys**	28		A1	het	11	F	B+	8	SMEAR	11	6.4	28	0.58	10
		c.4255C>A	p.His1419Asn	28		A1	het										
	01029	c.4120C>T	**p.Arg1374Cys**	28		A1	het	60	F	AB+	10	SMEAR	18	10	34	0.56	19
		c.6798+32G>A			38	intronic	het										
	01031	c.4120C>T	**p.Arg1374Cys**	28		A1	het	16	M	A+	15	SMEAR	12.3	5.5	21	0.45	10
		c.4255C>A	p.His1419Asn	28		A1	het										
	01033	c.4120C>T	**p.Arg1374Cys**	28		A1	het	19	F	B+	10	SMEAR	27	10	32	0.37	16
0112	01024	c.4120C>T	**p.Arg1374Cys**	28		A1	het	33	F	O	10	SMEAR	12	4	24	0.33	11
		c.7730-4C>G			45	intronic	het										
0116	01032	c.4120C>T	**p.Arg1374Cys**	28		A1	het	10	M	A+	6	SMEAR	15.7	4.5	19	0.29	11
		c.7393G>A	p.Val2465Met	43		C3	het										
	01042	c.4120C>T	**p.Arg1374Cys**	28		A1	het	79	F	O+	0	SMEAR	27	4.9	26	0.18	17
	01071	c.4120C>T	**p.Arg1374Cys**	28		A1	het	50	M	O+	12	SMEAR	13	6.6	17	0.51	6.7
		c.4196G>A	p.Arg1399His	28		A1	het										
0120	01038	c.3943C>T	**p.Arg1315Cys**	28		A1	het	59	F	O+	7	SMEAR	20	5.3	20	0.27	13
		c.5842+31C>T			34	intronic	het										
		c.-2627C>T				upstream	het										
0208	02026	c.3943C>T	**p.Arg1315Cys**	28		A1	het	55	M	O-	10	SMEAR	21	7.4	44	0.35	8.8
		c.-1896C>T				upstream	het										
	02027	c.3943C>T	**p.Arg1315Cys**	28		A1	het	31	M	O+	16	SMEAR	11	7.1	28	0.65	6.4
		c.-1896C>T				upstream	het										

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
0212	02034	c.2446C>T c.3943C>T	p.Arg816Trp **p.Arg1315Cys**	19 28		D' A1	het het	63	F	O+	16	SMEAR	26	8.4	23	0.32	14
	02035	c.3943C>T	**p.Arg1315Cys**	28		A1	het	35	M	O+	5	SMEAR	12	6.5	31	0.54	9.2
0376	03017	c.4120C>T	**p.Arg1374Cys**	28		A1	het	65	M	O+	9	SMEAR	44	11.8	39	0.27	23
1307	13009	c.3943C>T	**p.Arg1315Cys**	28		A1	het	40	F	O+	19	SMEAR	9.5	5	20	0.53	5.8
	13016	c.3943C>T	**p.Arg1315Cys**	28		A1	het	11	M	ND	8	SMEAR	11	5	21	0.45	6.7
3007	30012*	c.2289G>C	p.Arg763Ser	18		D2	het	33	F	O-	15	SMEAR	32	28	18	0.88	19
	30030*	c.2289G>C	p.Arg763Ser	18		D2	het	53	M	O-	6	SMEAR	40	25.4	22	0.64	29
3008	30013*	c.2289G>C	p.Arg763Ser	18		D2	het	79	M	A+	4	SMEAR	78	31.8	53	0.41	34
3703	37003	c.7471T>C	p.Cys2491Arg	44		C3	het	31	F	O+	0	SMEAR	30	25	44	0.83	20
	37011	c.7471T>C	p.Cys2491Arg	44		C3	het	37	F	O+	7	SMEAR	34	17.7	43	0.52	24
3904	39008	c.4120C>T c.7771-40G>A	**p.Arg1374Cys**	28	46	A1 intronic	het het	73	M	A+	6	SMEAR	15	7.7	33	0.51	17

In bold, mutations included in EAHAD-VWD-LOVD. ND indicates not determined. *Patients that present an altered VWF:FVIII:B 30012=0.28; 30003=0.37 and 30013=0.42 leading to a slightly 2N phenotype due to the presence of the new mutation p.Arg763Ser.

Table S7. Genotype-phenotype correlation in type 2B patients

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
0134	01065	c.3922C>T	**p.Arg1308Cys**	28		A1	het	40	F	A+	13	↓HMWM	63	19	50	0.30	23
0207	02024	c.3922C>T	**p.Arg1308Cys**	28		A1	het	53	F	O-	7	↓HMWM	21	9	37	0.43	6.3
	02025	c.2771G>A	p.Arg924Gln	21		D3	het	30	M	O+	7	↓HMWM	25	8.8	33	0.35	5.7
		c.3922C>T	**p.Arg1308Cys**	28		A1	het										
	02078	c.3922C>T	**p.Arg1308Cys**	28		A1	het	76	F	AB-	16	↓HMWM	34	24	55	0.71	21
0210	02030	c.4010C>T	**p.Pro1337Leu**	28		A1	het	50	M	A+	15	↓HMWM	75	35.4	134	0.47	50
		c.6237A>G	c.6237A>G (p.=)	36		D4	het										
0213	02036	c.3797C>T	**p.Pro1266Leu**	28		D3	het	34	F	O+	10	NORMAL	31	33	45	1.06	28
	02037	c.3797C>T	**p.Pro1266Leu**	28		D3	het	58	F	O-		NORMAL	67	65	114	0.97	67
0214	02038	c.3916C>T	**p.Arg1306Trp**	28		A1	het	45	F	A+	12	↓HMWM	31	21	75	0.68	18
		c.8366C>G	p.Thr2789Ser	52		CK	het										
0516	05018	c.3916C>T	**p.Arg1306Trp**	28		A1	het	82	M	A+	13	↓HMWM	119	72.8	63	0.61	71
0611	06015	c.3916C>T	**p.Arg1306Trp**	28		A1	het	67	M	O	11	↓HMWM	42	23	60	0.55	27
	06016	c.3379+27A>T			25	intronic	het	40	F	O-	8	↓HMWM	49	31	58	0.63	32
		c.3916C>T	**p.Arg1306Trp**	28		A1	het										
		c.7548+22G>A			44	intronic	het										
0614	06020	c.3922C>T	**p.Arg1308Cys**	28		A1	het	28	F	A+	20	↓HMWM	66	18	55	0.27	22
0806	08006	c.3916C>T	**p.Arg1306Trp**	28		A1	het	7	F	A+	11	↓↓HMWM	27	5	30	0.19	9.1
1012	10014	c.3946G>A	**p.Val1316Met**	28		A1	het	53	F	O+	4	↓HMWM	40	18	50	0.45	14
1208	12015	c.3917G>A	**p.Arg1306Gln**	28		A1	het	61	F	O+	13	↓HMWM	56	45	48	0.80	42
1302	13002	c.3922C>T	**p.Arg1308Cys**	28		A1	het	23	M	A+	11	↓↓HMWM	42	13.2	38	0.31	15
	13003	c.3922C>T	**p.Arg1308Cys**	28		A1	het	54	M	A+	8	↓↓HMWM	39	12.2	49	0.31	17
	13005	c.3922C>T	**p.Arg1308Cys**	28		A1	het	61	M	O+	8	↓↓HMWM	26	8.6	42	0.33	14
1305	13007	c.3922C>T	**p.Arg1308Cys**	28		A1	het	41	M	A+	24	↓↓HMWM	63	14	33	0.22	15
		c.-2692C>T				upstream	het										
1313	13017	c.3922C>T	**p.Arg1308Cys**	28		A1	het	50	M	O+	3	↓↓HMWM	21	14	30	0.67	0.61
1401	14001	c.3916C>T	**p.Arg1306Trp**	28		A1	het	28	M	O+	10	↓↓HMWM	9	6	15	0.67	7.6
1905	19007	c.1432+166C>A			12	intronic	het	38	F	O+	15	↓HMWM	32	18.7	32	0.58	17
		c.3426T>C	c.3426T>C (p.=)	26		D3	het										
		c.3946G>A	**p.Val1316Met**	28		A1	het										
		c.5278G>A	p.Val1760Ile	30		A3	het										
		c.3485_3486delinsTG	p.Pro1162Leu	26		D3	het										
2119	21022	c.3916C>T	**p.Arg1306Trp**	28		A1	het	42	F	A+	4	↓HMWM	44	20.5	37	0.47	19
2708	27013	c.3916C>T	**p.Arg1306Trp**	28		A1	het	59	F	O-	11	↓↓HMWM	22	10.3	45	0.47	12
	27014	c.3916C>T	**p.Arg1306Trp**	28		A1	het	32	M	O-	8	↓↓HMWM	25	10.8	38	0.43	14
	27015	c.3916C>T	**p.Arg1306Trp**	28		A1	het	67	M	O-	3	↓↓HMWM	33	18.5	63	0.56	25
	27016	c.3916C>T	**p.Arg1306Trp**	28		A1	het	62	F	O-	13	↓↓HMWM	29	14.1	40	0.49	19
		c.5277C>T	c.5277C>T (p.=)	30		A3	het										
3203	32003	c.3946G>A	**p.Val1316Met**	28		A1	het	28	M	B+	13	↓↓HMWM	36	12.2	32	0.34	15
		c.4751A>G	p.Tyr1584Cys	28		A2	het										
3208	32008	c.3917G>A	**p.Arg1306Gln**	28		A1	het	34	F	AB+	9	↓HMWM	116	46	50	0.40	44

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB
3504	35009	c.3922C>T	**p.Arg1308Cys**	28		A1	het	40	F	O	10	↓↓HMWM	23	18	24	0.78	16
	35010	c.3922C>T	**p.Arg1308Cys**	28		A1	het	10	M	O-	5	↓↓HMWM	25	14	41	0.56	12
3602*	36002	c.3946G>A	**p.Val1316Met**	28		A1	het	58	F	O+	14	NORMAL*	57	21	70	0.37	30
	36003	c.3946G>A	**p.Val1316Met**	28		A1	het	37	M	O+	12	↓HMWM*	58	23	97	0.40	40
4101	41001	c.3797C>A	**p.Pro1266Gln**	28		D3	het	17	M	O+	3	NORMAL	41	36	48	0.88	30
	41002	c.3797C>A	**p.Pro1266Gln**	28		D3	het	20	M	O+	0	NORMAL	39	31	41	0.79	30

In bold, mutations previously described in type 2B. ND indicates not determined.; and HMWM, high molecular weight multimers. * The family 3602 had the mutation p.Val1316Met related with Montreal platelet syndrome, detected in both related patients, but results in different multimeric patterns, as previoulsy described by Rendal *et al*.

Table S8. Genotype-phenotype correlation in type 2M patients

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB	VWF:CB(VI)
0109	01013	c.220+100G>A c.4273A>T c.7549-80T>A	**p.Ile1425Phe**	28	3 44	intron A1 intron	het het het	15	M	O+	12	15	8	47	0.53	13	
0118	01036	c.4196G>A	p.Arg1399His †	28		A1	het	60	F	O+	7	16	24	17	1.50	20	1.48
0128	01052*	c.5837G>T	p.Cys1946Phe	34		D4	het	51	F	O-	16	30	18	68	0.60	18	
0132	01066	c.4244G>A	p.Gly1415Asp	28		A1	het	20	F	O+	17	16	4	26	0.25	11	
	01067	c.4244G>A	p.Gly1415Asp	28		A1	het	53	M	O+	8	22	4	35	0.18	16	
0216	02045	c.3961T>A	p.Tyr1321Asn	28		A1	het	63	F	A+	12	71	18.2	97	0.26	54	
0230	02079	c.4133C>T	p.Ser1378Phe	28		A1	het	50	M	A-	3	46	8.6	68	0.19	42	
	02080	c.4133C>T	p.Ser1378Phe	28		A1	het	15	M	ND	2	40	8.6	72	0.22	32	
0332	03023	c.4225G>T c.6187C>T	**p.Val1409Phe** p.Pro2063Ser	28 36		A1 D4	het het	48	F	A+	12	12	4.4	21	0.37	7.7	
	03024	c.4196G>A c.4225G>T	p.Arg1399His † **p.Val1409Phe**	28 28		A1 A1	het het	22	M	ND	5	25	7.2	26	0.29	12	0.1
	03025	c.4225G>T	**p.Val1409Phe**	28		A1	het	77	F	O+	8	109	17	65	0.16	42	
0382	03045*	c.3788C>T	p.Ser1263Leu	28		D3	het	24	M	O-	3	78	48	20	0.62	62	
0512	05013	c.4196G>A	p.Arg1399His †	28		A1	het	41	F	O-	3	50	47.6	78	0.95	39	6.14
0705	07007	c.4196G>A c.6847T>C	p.Arg1399His † p.Cys2283Arg	28 39		A1 C1	het het	27	F	O+	10	32	39	97	1.22	24	0
1213	12020	c.4196G>A c.7730-177G>T	p.Arg1399His †	28	 45	A1 intron	het het	28	M	O-	0	84	55	67	0.65	53	4.59
1216	12023	c.4183C>T c.533-43A>C	p.Arg1395Trp ‡	28	 5	A1 intron	het het	71	M	ND	8	272	186	213	0.68	178	
2307	23010*	c.1110-26T>A c.1156+27C>T c.7773C>T	 c.7773C>T (p.=)	 47	9 10 	intron intron C5	het het het	39	F	O-	4	44	29	70	0.66	36	
2601	26001	c.4121G>T	p.Arg1374Leu	28		A1	het	37	M	B+	9	12	5	38	0.42	11	
	26002	c.4121G>T c.6068C>T	p.Arg1374Leu p.Thr2023Met	28 36		A1 D4	het het	7	M	B+	-1	30	12.2	62	0.41	26	
	26003	c.4121G>T	p.Arg1374Leu	28		A1	het	40	F	B+	13	15	6.8	37	0.45	14	
	26004	c.4121G>T	p.Arg1374Leu	28		A1	het	13	M	O+	10	8.3	5	38	0.60	9	
2706	27009	c.4145T>C	**p.Leu1382Pro**	28		A1	het	39	M	O-	2	5.4	4	15	0.74	5.6	
	27010	c.4145T>C c.4196G>A	**p.Leu1382Pro** p.Arg1399His †	28 28		A1 A1	het het	44	M	O-	1	6.8	4	18	0.59	4.3	ND
	27011	c.4145T>C	**p.Leu1382Pro**	28		A1	het	73	M	O-	18	32	14	70	0.44	26	
2712	27022*	c.5191T>A c.-1873A>G	p.Ser1731Thr §	30		A3 upstream	het het	19	F	O+	1	38	35.5	65	0.93	41	72.2
2901	29001	c.4244G>A	p.Gly1415Asp	28		A1	het	35	M	A-	10	29	10.4	46	0.36	22	
3010	30027	c.5336G>T	p.Arg1779Leu	31		A3	het	38	F	O-	11	36	16	34	0.44	20	
	30028	c.5336G>T	p.Arg1779Leu	31		A3	het	15	F	A-	0	28	13.6	19	0.49	16	

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB	VWF:CB(VI)
3608	36010	c.4157C>A	p.Ala1386Asp	28		A1	het	40	M	O-	1	30	6	33	0.20	21	
		c.6433C>G	p.Pro2145Ala	37		D4	het										
3803	38003	c.4313T>G	p.Phe1438Cys	28		A1	het	15	F	O+	21	28	18	30	0.64	24	
		c.-2077A>T				upstream	het										
3901	39002	c.4222_4224del	**p.Lys1408del**	28		A1	het	66	F	A+	19	21	9	43	0.43	27	
	39003	c.4222_4224del	**p.Lys1408del**	28		A1	het	39	F	A-	9	25	10	50	0.40	30	
	39007	c.4222_4224del	**p.Lys1408del**	28		A1	het	32	M	A+	7	17	5.4	33	0.32	17	
4402	44002	c.4244G>A	p.Gly1415Asp	28		A1	het	43	F	ND	5	13	5	20	0.38	11	
	44003	c.2561G>A	p.Arg854Gln	20		D'	het	6	F	ND	4	16	5	11	0.31	11	
		c.4244G>A	p.Gly1415Asp	28		A1	het										
4406	44007	c.4225G>T	**p.Val1409Phe**	28		A1	het	51	F	O-	3	28	13	37	0.46	21	
	44008	c.4225G>T	**p.Val1409Phe**	28		A1	het	56	M	O+	7	28	6.2	23	0.22	19	
	44009	c.2771G>A	p.Arg924Gln	21		D3	het	22	M	O+	9	46	10.4	60	0.23	32	
		c.4225G>T	**p.Val1409Phe**	28		A1	het										
	44010	c.2771G>A	p.Arg924Gln	21		D3	het	19	M	O+	2	33	7.4	33	0.22	21	
		c.4225G>T	**p.Val1409Phe**	28		A1	het										

In bold, mutations previously described in type 2M. ND indicates not determined. *Patients with a discrepant phenotype-genotype correlation. † Mutations causing a defective binding to collagen type VI. ‡ Mutation causing a defective binding to collagen type IV. § Mutation causing a defective binding to collagen types I,III.

Table S9. Genotype-phenotype correlation in type 2N patients

Family	Patient	Phenotype	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	VWF:Ag	VWF:RCo	FVIII:C	VWF/FVIIIB
0107	01011	2N	c.2446C>T	**p.Arg816Trp**	19		D'	hom	56	F	A+	8	59	86	4.5	0.00
	01016	2N (Carrier)	c.2446C>T	**p.Arg816Trp**	19		D'	het	25	F	A-	1	74	95	124	0.56
0124	01044	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	40	M	A+	0	52	82	46	0.65
			c.6187C>T	p.Pro2063Ser	36		D4	het								
	01058	2N	c.2561G>A	**p.Arg854Gln**	20		D'	het	31	M	O+	9	42	40	12	0.02
			c.970C>T	p.Arg324Ter	8		null allele	het								
0131	01063	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	42	F	O+	3	62	61	46	0.65
0202	02002	2N	c.2561G>A	**p.Arg854Gln**	20		D'	het	44	F	O+	8	33	33.6	14	0.05
			c.2685G>C	**p.Gln895His**	20		D3	het								
	02003	2N	c.2561G>A	**p.Arg854Gln**	20		D'	het	45	F	O+	6	32	31.5	16	0.06
			c.2685G>C	**p.Gln895His**	20		D3	het								
	02005	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	68	F	B+	0	128	149	110	0.52
	02006	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	12	F	O+	0	67	71	114	0.49
	02008	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	7	F	O-	0	73	101	71	0.66
			c.7438-31T>C			43	intronic	het								
			c.7936C>A	p.Pro2646Thr	48		C5	het								
	02031	2N	c.2561G>A	**p.Arg854Gln**	20		D'	het	41	M	O+	6	44	56	24	0.11
			c.7672_7676del	p.Pro2558GlyfsTer7	45		null allele	het								
0211	02032	2N (Carrier)	c.1654G>A	p.Ala552Thr	14		D2	het	13	F	ND	1	82	84.8	108	0.63
			c.2561G>A	**p.Arg854Gln**	20		D'	het								
			c.3538+20G>A			26	intronic	het								
	02033	2N (Carrier)	c.2546+97C>G			19	intronic	het	9	M	ND	0	65	80	111	0.62
			c.2561G>A	**p.Arg854Gln**	20		D'	het								
0229	02075	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	52	M	O+	5	55	54.4	54	0.7
			c.3144C>T	c.3144C>T (p.=)	24		D3	het								
			c.546G>A	c.546G>A (p.=)	6		D1	het								
	02076	2N (Carrier)	c.2561G>A	**p.Arg854Gln**	20		D'	het	18	M	ND	0	34	34.4	54	0.57
			c.3144C>T	c.3144C>T (p.=)	24		D3	het								
0319	03014	2N	c.2446C>T	**p.Arg816Trp**	19		D'	hom	67	F	A+	11	274	171	5.1	0.00
0513	05014	2N	c.2446C>T	**p.Arg816Trp**	19		D'	hom	39	F	A+	11	51	48.8	5.2	0.00
1406	14006	2N	c.2561G>A	**p.Arg854Gln**	20		D'	hom	17	F	ND	16	102	110	3	0.1
2105	21008	2N	c.2446C>T	**p.Arg816Trp**	19		D'	hom	44	M	O+	7	114	93	7.5	0.00
3604	36006	2N (Carrier)	c.1293+86C>T			11	intronic	het	4	M	A+	0	75	60	57	0.44
			c.2561G>A	**p.Arg854Gln**	20		D'	het								

In bold, mutations previously described in type 2N. ND indicates not determined.

Table S10. Patients with uncertain classification

Family	Patient	HGVSc	HGVSp	Exon	Intron	Domain	Genotype	Age	Gender	ABO	BS	Multimeric pattern	VWF:Ag	VWF:RCo	FVIII:C	VWF:RCo/VWF:Ag	VWF:CB	Potential classification*
0109	01017	c.220+100G>A			3	intronic	het	39	F	O+	3	NORMAL	36	49	101	1.36	46	-
		c.7549-80T>A			44	intronic	het											
	01074	c.220+100G>A			3	intronic	het	17	F	O+	2	NORMAL	39	41	96	1.05	41	-
		c.7549-80T>A			44	intronic	het											
0901	09001	c.4775T>A	p.Val1592Asp	28		A2	het	9	M	O	0	NORMAL	104	90	70	0.87	82	-
		c.7888-65C>A			47	intronic	het											
1003	10004	c.3829G>C	p.Asp1277His	28		A1	het	38	F	O+	3	ND	46	19.8	43	0.43	25	2A
1203	12006	c.1110-26T>A			9	intronic	het	29	F	O+	0	NORMAL	42	38.5	55	0.92	33	-
		c.1646T>C	p.Phe549Ser	14		D2	het											
1209	12016	c.2900G>A	p.Gly967Asp	22		D3	het	61	F	O+	0	NORMAL	75	62	48	0.83	56	
1901	19001	c.4627T>C	p.Ser1543Pro	28		A2	het	31	M	O-	9	↓HMWM	34	9.1	55	0.27	17	2A
	19002	c.4627T>C	p.Ser1543Pro	28		A2	het	55	F	O+	17	↓HMWM	25	9.3	51	0.37	14	2A
1902	19003	c.4574T>G	p.Ile1525Ser	28		A2	het	34	F	O+	3	↓HMWM	29	12	41	0.41	17	2A
	19004	c.4574T>G	p.Ile1525Ser	28		A2	het	27	F	O+	0	↓HMWM	46	24	51	0.52	25	2A
2302	23004	c.7493C>A	p.Ala2498Asp	44		C4	het	35	F	O+	3	NORMAL	64	54.5	78	0.85	53	-
2701	27002	c.7025G>A	p.Arg2342His	41		C2	het	32	F	ND	-2	NORMAL	46	42.7	72	0.93	48	-
3002	30018	c.2821-88A>G			21	intronic	het	19	M	A-	5	↓↓HMWM	15	5	15	0.33	4.6	-
		c.-2077A>T				upstream	het											
3905	39015	c.-138A>G		1		upstream	het	44	F	ND	-1	NORMAL	98	97	88	0.99	96	-
		c.3539-35G>C			26	intronic	het											

ND indicates not determined. *Potential classification on the basis of molecular results, even though we preferred achieve further clinical and phenotypic evidence to establish a definite classification. In this line, five patients were potentially classified as 2A since the mutation found in each case was located outside the 16 residues related to type 2B.

Table S11. Missense candidate mutations in silico analysis

HGVSc	HGVSp	Exon	Domain	PolyPhen	Sift	TASTERprediction	Mut Assesor	Provean Prediction (cutoff=-25)	*in silicio* global score	#Patients	#Families	Potencial ancestral origin
c.55G>A	p.Gly19Arg	2	SigNDI peptide	possibly_damaging (0.884)	deleterious (0)	disease causing	medium	Neutral	**4**	1	1	-
c.250C>T	**p.Leu84Phe**	4	D1	probably_damaging (0.985)	tolerated (0.15)	disease causing	low	Neutral	2	1	1	-
c.425G>A	**p.Gly142Asp**	5	D1	possibly_damaging (0.818)	tolerated (0.28)	polymorphism	medium	Neutral	2	1	1	-
c.449T>C	p.Leu150Pro	5	D1	probably_damaging (0.972)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.478G>A	**p.Gly160Arg**	5	D1	possibly_damaging (0.854)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.818G>C	**p.Arg273Pro**	7	D1	probably_damaging (0.963)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.1001G>A	**p.Gly334Glu**	9	D1	probably_damaging (0.998)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.1109G>A	**p.Cys370Tyr**	9	D1	probably_damaging (0.933)	deleterious (0.01)	disease causing	high	Deleterious	**5**	6	1	-
c.1446C>G	**p.Ile482Met**	13	D2	probably_damaging (0.998)	deleterious (0)	disease causing	medium	Neutral	**4**	3	3	NO
c.1472G>C	**p.Arg491Pro**	13	D2	probably_damaging (0.936)	deleterious (0.01)	disease causing	medium	Deleterious	**5**	2	1	-
c.1625C>G	p.Ala542Gly	14	D2	possibly_damaging (0.539)	deleterious (0)	polymorphism	low	Deleterious	**3**	5	5	NO
c.1646T>C	**p.Phe549Ser**	14	D2	probably_damaging (0.998)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.1654G>A	**p.Ala552Thr**	14	D2	benign (0.363)	tolerated (0.3)	polymorphism	low	Neutral	0	1	1	-
c.1721C>G	**p.Pro574Arg**	14	D2	probably_damaging (1)	deleterious (0.01)	disease causing	medium	Deleterious	**5**	1	1	-
c.1847C>T	**p.Ser616Leu**	15	D2	probably_damaging (0.973)	deleterious (0.04)		medium	Deleterious	**5**	1	1	-
c.2119T>C	**p.Cys707Arg**	16	D2	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.2289G>C	**p.Arg763Ser**	18	D2	probably_damaging (0.994)	deleterious (0)	disease causing	medium	Deleterious	**5**	3	2	YES
c.2446C>T	p.Arg816Trp	19	D'	probably_damaging (1)	deleterious (0)	disease causing	medium	Deleterious	**5**	6	5	NO
c.2561G>A	p.Arg854Gln	20	D'	probably_damaging (0.996)	deleterious (0.01)	disease causing	low	Deleterious	**4**	19	11	NO
c.2685G>C	p.Gln895His	20	D3	probably_damaging (0.987)	deleterious (0)	disease causing	medium	Deleterious	**5**	7	2	YES
c.2771G>A	p.Arg924Gln	21	D3	possibly_damaging (0.635)	tolerated (0.36)	polymorphism	low	Neutral	1	15	13	NO
c.2878C>T	p.Arg960Trp	22	D3	probably_damaging (0.997)	deleterious (0.03)	disease causing	medium	Neutral	**4**	2	2	NO
c.2900G>A	p.Gly967Asp	22	D3	probably_damaging (0.991)	tolerated (0.21)	disease causing	low	Neutral	2	1	1	-
c.2900G>T	**p.Gly967Val**	22	D3	probably_damaging (0.998)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.2926C>T	**p.Arg976Cys**	22	D3	probably_damaging (0.939)	deleterious (0.01)	polymorphism	medium	Deleterious	**4**	2	1	-
c.2944G>C	**p.Val982Leu**	22	D3	probably_damaging (0.999)	tolerated (0.06)	disease causing	low	Neutral	2	1	1	-
c.3319T>C	**p.Tyr1107His**	25	D3	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.3363G>T	**p.Arg1121Ser**	25	D3	probably_damaging (0.999)	deleterious (0)	disease causing	high	Deleterious	**5**	4	2	YES
c.3433C>T	**p.Arg1145Cys**	26	D3	probably_damaging (1)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.3445T>C	p.Cys1149Arg	26	D3	probably_damaging (0.999)	deleterious (0)	disease causing	high	Deleterious	**5**	11	4	YES
c.3467C>T	p.Thr1156Met	26	D3	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	7	4	NO
c.3485_3486delinsTG*	p.Pro1162Leu	26	D3	probably_damaging (1)	deleterious (0.01)		medium	Deleterious	**5**	7	7	NO
c.3614G>A	p.Arg1205His	27	D3	probably_damaging (0.998)	tolerated (0.16)	disease causing	medium	Neutral	**3**	35	15	NO
c.3692A>G	p.Asn1231Ser	28	D3	benign (0.083)	tolerated (0.26)	disease causing	low	Neutral	1	2	2	NO
c.3788C>T	**p.Ser1263Leu**	28	D3	benign (0.005)	tolerated (0.66)	polymorphism	low	Neutral	0	1	1	-
c.3797C>A	p.Pro1266Gln	28	D3	probably_damaging (0.999)	deleterious (0.05)	polymorphism	medium	Neutral	2	2	1	-
c.3797C>T	p.Pro1266Leu	28	D3	probably_damaging (0.995)	tolerated (1)	disease causing	medium	Neutral	**3**	4	3	YES
c.3815G>C	p.Cys1272Ser	28	A1	probably_damaging (1)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.3829G>C	**p.Asp1277His**	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.3835G>A	p.Val1279Ile	28	A1	possibly_damaging (0.725)	tolerated (0.07)	disease causing	high	Neutral	**3**	3	2	NO
c.3916C>T	p.Arg1306Trp	28	A1	probably_damaging (0.998)	deleterious (0)	disease causing	high	Neutral	**4**	11	7	NO
c.3917G>A	p.Arg1306Gln	28	A1	possibly_damaging (0.874)	tolerated (0.12)	disease causing	medium	Neutral	**3**	2	2	NO
c.3920T>C	p.Leu1307Pro	28	A1	probably_damaging (1)	deleterious (0.01)	disease causing	high	Neutral	**4**	3	1	-
c.3922C>T	p.Arg1308Cys	28	A1	probably_damaging (0.925)	deleterious (0.02)	disease causing	low	Neutral	**3**	12	7	NO

HGVSc	HGVSp	Exon	Domain	PolyPhen	Sift	TASTERprediction	Mut Assesor	Provean Prediction (cutoff=-25)	*in silicio* global score	#Patients	#Families	Potencial ancestral origin
c.3943C>T	p.Arg1315Cys	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Neutral	**4**	7	4	NO
c.3944G>T	p.Arg1315Leu	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.3946G>A	p.Val1316Met	28	A1	probably_damaging (1)	deleterious (0.01)	disease causing	high	Neutral	**4**	5	4	NO
c.3961T>A	**p.Tyr1321Asn**	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.4010C>T	p.Pro1337Leu	28	A1	probably_damaging (0.998)	tolerated (0.72)	disease causing	medium	Neutral	**3**	1	1	-
c.4120C>T	p.Arg1374Cys	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Neutral	**4**	22	6	NO
c.4121G>A	p.Arg1374His	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Neutral	**4**	30	7	NO
c.4121G>T	p.Arg1374Leu	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Neutral	**4**	4	1	-
c.4133C>T	p.Ser1378Phe	28	A1	probably_damaging (0.982)	deleterious (0.03)	polymorphism	medium	Deleterious	**4**	2	1	-
c.4145T>C	**p.Leu1382Pro**	28	A1	probably_damaging (1)	deleterious (0)	disease causing	high	Neutral	**4**	3	1	-
c.4157C>A	**p.Ala1386Asp**	28	A1	probably_damaging (1)	deleterious (0)	disease causing	medium	Neutral	**4**	1	1	-
c.4183C>T	**p.Arg1395Trp**	28	A1	probably_damaging (0.981)	deleterious (0)	polymorphism	high	Neutral	**3**	1	1	-
c.4196G>A	p.Arg1399His	28	A1	possibly_damaging (0.549)	deleterious (0.05)	disease causing	medium	Neutral	**4**	8	8	NO
c.4225G>T	**p.Val1409Phe**	28	A1	probably_damaging (0.998)	deleterious (0)	disease causing	high	Neutral	**4**	7	2	YES
c.4238C>T	p.Pro1413Leu	28	A1	probably_damaging (0.989)	tolerated (0.11)	disease causing	medium	Deleterious	**4**	1	1	-
c.4241T>G	**p.Val1414Gly**	28	A1	probably_damaging (0.996)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.4244G>A	p.Gly1415Asp	28	A1	probably_damaging (1)	deleterious (0.02)	disease causing	high	Neutral	**4**	5	3	NO
c.4255C>A	**p.His1419Asn**	28	A1	possibly_damaging (0.884)	tolerated (0.73)	polymorphism	high	Neutral	2	2	1	-
c.4257T>G	**p.His1419Gln**	28	A1	possibly_damaging (0.819)	tolerated (0.22)	polymorphism	medium	Neutral	2	1	1	-
c.4273A>T	p.Ile1425Phe	28	A1	probably_damaging (0.996)	deleterious (0.01)	disease causing	high	Deleterious	**5**	1	1	-
c.4313T>G	**p.Phe1438Cys**	28	A1	probably_damaging (0.958)	deleterious (0.01)	disease causing	high	Neutral	**4**	1	1	-
c.4499C>A	p.Ala1500Glu	28	A2	probably_damaging (0.934)	tolerated (0.17)	polymorphism	medium	Neutral	2	1	1	-
c.4517C>T	p.Ser1506Leu	28	A2	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	10	7	NO
c.4574T>G	**p.Ile1525Ser**	28	A2	probably_damaging (1)	deleterious (0)	disease causing	medium	Neutral	**4**	2	1	-
c.4580G>A	p.Arg1527Gln	28	A2	possibly_damaging (0.784)	tolerated (0.5)	polymorphism	low	Neutral	1	1	1	-
c.4622A>T	**p.Gln1541Leu**	28	A2	probably_damaging (1)	deleterious (0.02)	disease causing	high	Deleterious	**5**	1	1	-
c.4627T>C	**p.Ser1543Pro**	28	A2	probably_damaging (0.999)	deleterious (0.01)	disease causing	high	Neutral	**4**	2	1	-
c.4735G>A	p.Gly1579Arg	28	A2	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	1	1	-
c.4739T>C	p.Leu1580Pro	28	A2	probably_damaging (0.99)	tolerated (0.21)	polymorphism	high	Neutral	2	1	1	-
c.4747C>T	p.Arg1583Trp	28	A2	possibly_damaging (0.88)	deleterious (0)	polymorphism	low	Neutral	2	1	1	-
c.4751A>G	p.Tyr1584Cys	28	A2	probably_damaging (0.981)	tolerated (0.09)	polymorphism	high	Neutral	2	8	8	NO
c.4775T>A	**p.Val1592Asp**	28	A2	benign (0.201)	tolerated (0.56)	polymorphism	neutral	Neutral	1	1	1	-
c.4789C>G	p.Arg1597Gly	28	A2	probably_damaging (0.999)	tolerated (0.16)	polymorphism	high	Neutral	2	2	1	-
c.4789C>T	p.Arg1597Trp	28	A2	probably_damaging (1)	deleterious (0)	polymorphism	high	Deleterious	**4**	9	5	NO
c.4790G>A	p.Arg1597Gln	28	A2	probably_damaging (0.984)	tolerated (0.05)	polymorphism	high	Neutral	2	4	1	-
c.4825G>A	p.Gly1609Arg	28	A2	probably_damaging (1)	tolerated (0.23)	polymorphism	high	Neutral	2	4	3	NO
c.4840G>A	**p.Asp1614Asn**	28	A2	probably_damaging (1)	deleterious (0.02)	disease causing	high	Neutral	**4**	3	1	-
c.4883T>C	p.Ile1628Thr	28	A2	probably_damaging (0.998)	deleterious (0.01)	disease causing	high	Neutral	**4**	11	6	NO
c.4885G>A	p.Gly1629Arg	28	A2	probably_damaging (1)	deleterious (0.03)	disease causing	high	Deleterious	**5**	1	1	-
c.4889T>G	**p.Val1630Gly**	28	A2	probably_damaging (1)	deleterious (0)	disease causing	high	Deleterious	**5**	2	1	-
c.4892G>A	**p.Gly1631Asp**	28	A2	probably_damaging (1)	deleterious (0.03)	disease causing	high	Deleterious	**5**	1	1	-
c.4943C>G	p.Pro1648Arg	28	A2	probably_damaging (1)	deleterious (0.01)	disease causing	high	Deleterious	**5**	1	1	-
c.4960T>C	p.Phe1654Leu	28	A2	probably_damaging (0.999)	tolerated (0.06)	polymorphism	high	Neutral	2	4	2	YES
c.5191T>A	p.Ser1731Thr	30	A3	probably_damaging (0.996)	deleterious (0.02)	disease causing	medium	Neutral	**4**	2	2	NO
c.5198T>C	**p.Leu1733Pro**	30	A3	probably_damaging (0.995)	deleterious (0)	disease causing	medium	Neutral	**4**	12	6	NO
c.5278G>A	p.Val1760Ile	30	A3	benign (0.043)	tolerated (0.77)	polymorphism	low	Neutral	0	3	2	NO
c.5311G>A	**p.Gly1771Arg**	30	A3	possibly_damaging (0.774)	deleterious (0.03)	disease causing	medium	Deleterious	**4**	1	1	-

HGVSc	HGVSp	Exon	Domain	PolyPhen	Sift	TASTERprediction	Mut Assesor	Provean Prediction (cutoff=-25)	*in silicio* global score	#Patients	#Families	Potencial ancestral origin
c.5336G>T	**p.Arg1779Leu**	31	A3	possibly_damaging (0.609)	tolerated (0.11)	disease causing	medium	Deleterious	**4**	3	2	YES
c.5368C>T	**p.Pro1790Ser**	31	A3	benign (0.045)	tolerated (0.07)	disease causing	medium	Neutral	2	1	1	-
c.5471C>A	p.Pro1824His	32	A3	probably_damaging (0.955)	deleterious (0)	disease causing	medium	Deleterious	**5**	4	1	-
c.5695T>C	**p.Cys1899Arg**	34	D4	benign (0.358)	deleterious (0)	disease causing	medium	Deleterious	**4**	2	1	-
c.5837G>T	**p.Cys1946Phe**	34	D4	benign (0.214)	deleterious (0)	disease causing	medium	Deleterious	**4**	2	2	YES
c.6068C>T	**p.Thr2023Met**	36	D4	benign (0.032)	tolerated (0.23)	polymorphism	low	Neutral	0	1	1	-
c.6187C>T	p.Pro2063Ser	36	D4	benign (0.372)	deleterious (0.02)	disease causing	medium	Deleterious	**4**	28	18	NO
c.6197A>G	p.Asn2066Ser	36	D4	possibly_damaging (0.534)	deleterious (0.03)	disease causing	medium	Neutral	**4**	8	7	NO
c.6248G>A	**p.Gly2083Asp**	36	D4	probably_damaging (0.986)	deleterious (0)	disease causing	medium	Deleterious	**5**	5	2	YES
c.6254G>T	**p.Cys2085Phe**	36	D4	probably_damaging (0.978)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.6352C>T	**p.Arg2118Trp**	37	D4	benign (0.199)	deleterious (0.01)	polymorphism	neutral	Neutral	2	1	1	-
c.6433C>G	**p.Pro2145Ala**	37	D4	benign (0)	tolerated (0.74)	polymorphism	neutral	Neutral	1	1	1	-
c.6433C>T	p.Pro2145Ser	37	D4	benign (0)	tolerated (0.61)	polymorphism	neutral	Neutral	1	1	1	-
c.6536C>T	p.Ser2179Phe	37	D4	possibly_damaging (0.876)	deleterious (0)	disease causing	medium	Neutral	**4**	2	1	-
c.6847T>C	**p.Cys2283Arg**	39	C1	benign (0.326)	deleterious (0)	disease causing	medium	Deleterious	**4**	1	1	-
c.6911G>A	p.Cys2304Tyr	40	C1	probably_damaging (0.936)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.6989T>G	**p.Val2330Gly**	41	C1	benign (0.001)	tolerated (0.23)	polymorphism	medium	Neutral	1	3	1	-
c.7025G>A	**p.Arg2342His**	41	C2	benign (0.001)	tolerated (0.23)	polymorphism	neutral	Neutral	1	1	1	-
c.7070A>T	**p.Asn2357Ile**	41	C2	possibly_damaging (0.533)	tolerated (0.67)	polymorphism	low	Neutral	0	1	1	-
c.7118C>T	**p.Pro2373Leu**	42	C2	possibly_damaging (0.636)	tolerated (0.21)	disease causing	medium	Deleterious	**4**	1	1	-
c.7393G>A	**p.Val2465Met**	43	C3	probably_damaging (0.96)	tolerated (0.16)	disease causing	low	Neutral	2	2	2	YES
c.7471T>C	p.Cys2491Arg	44	C3	possibly_damaging (0.876)	deleterious (0)	disease causing	medium	Deleterious	**5**	2	1	-
c.7493C>A	**p.Ala2498Asp**	44	C4	possibly_damaging (0.795)	tolerated (0.1)	disease causing	medium	Neutral	**3**	1	1	-
c.7583G>A	**p.Cys2528Tyr**	45	C4	possibly_damaging (0.49)	deleterious (0)	disease causing	medium	Deleterious	**5**	1	1	-
c.7936C>A	**p.Pro2646Thr**	48	C5	benign (0.235)	deleterious (0.01)	disease causing	medium	Neutral	**3**	1	1	-
c.7985A>G	**p.Lys2662Arg**	48	C6	benign (0.301)	tolerated (0.09)	disease causing	medium	Neutral	2	1	1	-
c.8318G>C	p.Cys2773Ser	52	CK	benign (0.004)	deleterious (0)	disease causing	medium	Deleterious	**4**	1	1	-
c.8366C>G	**p.Thr2789Ser**	52	CK	benign (0.054)	tolerated (0.53)	disease causing	medium	Neutral	2	1	1	-

Mutations In bold were not described previously in EAHAD-VWD-LOVD. The *in silico* global score is based on the number of *in silico* algorithms that predicted a deleterious effect. In silico global score >2 are highlighted in bold.

*The c.3485_3486delinsTG (p.Pro1162Leu) was also aNDlysed by localSpliceEffect program with a prediction of cryptic acceptor strongly activated site.

Table S12. Nonsense mutations

HGVSc	HGVSp	Exon	Domain	Consequence	#Patients	#Families	Potencial ancestral origin
c.100C>T	p.Arg34Ter	3	D1	stop gained	3	2	NO
c.246_247insT	**p.Ser83Ter**	4	D1	frameshift variant, feature elongation	1	1	-
c.324-2_326dup	**p.Ser110GlufsTer13**	5	D1	frameshift variant, feature elongation	1	1	-
c.375_376delInsC	**p.Tyr126ThrfsTer49**	5	D1	frameshift variant, feature truncation	8	7	NO
c.970C>T	p.Arg324Ter	8	D1	stop gained	5	3	NO
c.1093C>T	p.Arg365Ter	9	D1	stop gained	1	1	-
c.1209C>G	**p.Tyr403Ter**	11	D2	stop gained	1	1	-
c.1992dupC	**p.Cys665LeufsTer13**	16	D2	frameshift variant, feature elongation	1	1	-
c.(1945+1_1946-1)_(7437+1_7438-1)del	**-**	16-43	D2-C3	frameshift variant	1	1	-
c.2435delC	p.Pro812ArgfsTer31	18	D'	frameshift variant, feature truncation	1	1	-
c.2518G>T	**p.Glu840Ter**	19	D'	stop gained	2	1	-
c.2540dupA	**p.Asn847LysfsTer18**	19	D'	frameshift variant, feature elongation	2	1	-
c.3835_4105conNG_001212.3:g.6566_6836	**p.[(V1279I;Q1311*;I1343V;V1360A;F1369I)]**	28	A1	gene conversion	1	1	-
c.3931C>T	p.Gln1311Ter	28	A1	stop gained	8	6	NO
c.4162C>T	**p.Gln1388Ter**	28	A1	stop gained	2	2	YES
c.4975C>T	p.Arg1659Ter	28	A2	stop gained	2	2	NO
c.6182delT	p.Phe2061SerfsTer38	36	D4	frameshift variant, feature truncation	1	1	-
c.6699_6702dup	**p.Cys2235ArgfsTer8**	38	D4	frameshift variant, feature elongation	1	1	-
c.7300C>T	p.Arg2434Ter	43	C3	stop gained	1	1	-
c.7408C>T	p.Gln2470Ter	43	C3	stop gained	4	4	YES
c.7664_7665insAG	p.Cys2557SerfsTer8	45	C4	frameshift variant, feature elongation	1	1	-
c.7672_7676del	**p.Pro2558GlyfsTer7**	45	C4	frameshift variant, feature truncation	1	1	-
c.8347C>T	**p.Gln2783Ter**	52	CK	stop gained	4	3	NO

In bold, mutations not described previously in EAHAD-VWD-LOVD. Since all are null alleles, suffering potential NMD, domains are only indicated for informative positioNDl purposes.

Tabla S13. In frame candidate mutations

HGVSc	HGVSp	Exon	Domain	Genotype	Combined with		#Patients	#Families	Potencial ancestral origin
c.1157_5620del	p.Gly386_Ser1873del	11-32	D2-A3	het	-	2A	1	1	-
c.1534_1536del	p.Leu512del	14	D2	het	p.Tyr126ThrfsTer49 and p.Asn2066Ser	3	1	1	-
c.3223-7_3236dup	p.Pro1079_Tyr1080insLeuGlnValAspProGluPro	25	D3	het	-	1	5	2	YES
c.4222_4224del	p.Lys1408del	28	A1	het	-	2M	3	1	-
c.4678_4680dup	p.Asp1560dup	28	NP	het	-	2A	2	1	-

All mutations are new.

Tabla S14. Splicing candidate mutations

HGVSc	Intron	wtGSScore	varGSScore	wtSSFScore	varSSFScore	wtHSFScore	varHSFScore	HSF score variation (%)	wtNNSScore	varNNSScore	wtMaxEntScore	varMaxEntScore	Combinen HSF&MaxEnt conclusion	#Patients	#Families	Potencial ancestral origin
c.533-2A>G	5	8.48	NP	88.84	NP	96.35	67.4	30.05 WT site broken	0.90	NP	9.11	1.15	Alteration of the WT donor site. most probably affecting splicing.	3	3	YES
c.874+8G>A	7	-	-	-	-	43.86	72.8	+65.98 new aceptor site creation	-	-	-	-	No significant splicing motif alteration detected.	1	1	-
c.1156+2T>C	10	8.55	NP	84.14	82.05	89.49	62.65	29.99 WT site broken	0.95	NP	9.88	2.12	Alteration of the WT donor site. most probably affecting splicing.	2	1	-
c.1533+1G>A	13	14.21	NP	89.61	NP	90.71	63.87	29.59 WT site broken	0.98	NP	10.29	2.10	Alteration of the WT donor site. most probably affecting splicing.	1	1	-
c.3379+1G>A	25	7.25	NP	70.36	NP	81.32	54.48	33.01 WT site broken	0.90	NP	8.56	0.38	Alteration of the WT donor site. most probably affecting splicing.	1	1	-
c.5455+1G>A	31	5.57	NP	73.31	NP	81.38	54.55	32.97 WT site broken	0.89	NP	5.96	-2.21	Alteration of the WT donor site. most probably affecting splicing.	1	1	-
c.6256+2dupT	36	2.02	NP	83.11	61.39	86.69	74.37	18.41 WT site broken	0.99	0.002	8.24	-4.77	Alteration of the WT donor site. most probably affecting splicing.	1	1	-
c.6598+1G>A	37	8.03	NP	84.07	NP	92.33	65.5	29.06 WT site broken	0.99	NP	8.95	0.76	Alteration of the WT donor site. most probably affecting splicing. Activation of an intronic cryptic donor site 3 nt upstream.	3	1	-
c.7082-2A>G	41	3.41	NP	79.01	NP	88.28	59.34	32.78 WT site broken	0.69	NP	6.68	-1.27	Alteration of the WT donor site. most probably affecting splicing.	3	2	NO
c.7730-4C>G	45	13.44	14.55	87.12	87.12	91.73	91.11	-0.68. New site 3 nt upstream	0.99	0.99	11.71	13.00	Activation of an intronic cryptic acceptor site. Potential alteration of splicing.	1	1	-
c.7730-1G>C	45	13.44	NP	87.12	NP	91.73	62.79	31.55 WT site broken	0.99	NP	11.71	3.65	Alteration of the WT donor site. most probably affecting splicing.	6	5	NO
c.8155+3G>C	50	1.81	NP	83.02	77.50	85.46	78.65	-7.97	0.94	0.21	7.83	2.02	Alteration of the WT donor site. most probably affecting splicing.	2	2	YES
c.8254-5T>G	51	15.94	12.23	93.49	89.36	92.50	88.88	-3.91	0.99	0.94	12.47	10.09	No significant splicing motif alteration detected. This mutation has probably no impact on splicing.	2	2	YES

A splice site effect was considered as potentially deleterious when a variation between the NDtive and the mutation score of more than 10% was observed in both algorithms. In bold, mutations not described previously in EAHAD-VWD-LOVD. GS indicates GeneSplicer; SSF, Splicing Sequences Finder; HSF, Human Splicing Finder; NNS, Neural Network Splice; MaxEnt, Maximum Entropy; NP, not predicted; and WT, wild type.

Table S15. Upstream variants

HGVSc	Genotype		Combined with	#Patients	#Families	Comments	Potencial ancestral origin
c.-3151T>G	het	1	p.Thr1156Met	5	3	-	YES
	het	3	p.Thr1156Met and c.7730-1G>C	1	1		
	hom	1	p.Thr1156Met	1	1		
c.-2692C>T	het	2B	p.Arg1308Cys	1	1	-	-
c.-2627C>T	het	1	c.3379+1G>A	1	1	-	NO
		2A/2M	p.Arg1315Cys	1	1		
c.-2077A>T	het	1	p.Arg1205His	2	2	-	NO
		2M	p.Phe1438Cys	1	1		
		UC	None	1	1		
c.-2076A>G	het	2A	p.Arg1374His	1	1	Within the 20 nt close to the second cluster for DNDse I	-
c.-1896C>T	het	2A/2M	p.Arg1315Cys	2	1	-	-
c.-1875G>A	het	2A	p.Arg1145Cys and p.Arg1374His	1	1	Within the 10 nt close to the first DNDse I cluster	YES
			p.Arg1374His	2	2		
c.-1873A>G	het	2M	p.Ser1731Thr	1	1	Within the 10 nt close to the first DNDse I cluster	-
c.-1650G>C*	het	2A	p.Leu1307Pro	1	1	Within the first Pol 2 and DNDse I cluster	-
c.-138A>G	het	UC	None	1	1	Within uORF mRND regulador element, and in Exon enhancer	-

ENCODE project datasets was used to determine the colocalization of these upstream mutations with a well defined regulatory elements (DNDseI Hypersensitivity Clusters, TF motif sequences determined by ChIPseq high throughput sequencing) in the genome. In bold, mutations not described previously in EAHAD-VWD-LOVD. Of interest, except for 3 patients (2 with uncertain classification and 1 type 3), all upstream mutations were combined with another mutation that could explained the phenotypes. uORFs indicates upstream open reading frames defined as a major gene expression regulatory elements; and UC indicates uncertain classification.* This mutation is located into the consensus sequence for the zinc finger transcription factor GATA binding protein 2 (GATA2).

Tabla S16. Synonymous candidate mutations

HGVSc	HGVSp	Exon	Domain	Adjacent nucleotides	phastCons	LocalSpliceEffect	wtHSFScore	varHSFScore	HSF score variation (%)	wtMaxEntScore	varMaxEntScore	HSF&MaxEnt conclusion	#Patients	#Families	Potencial ancestral origin
c.546G>A	**p.Ser182**	6	D1	tcGga>tcAga	0.13	New Acceptor Site	57.31	86.26	50.51 New site	NP	NP	Activation of an exonic cryptic acceptor site. with presence of one or more cryptic branch point (s). Potential alteration of splicing.	3	3	NO
c.2025G>A	**p.Pro675**	16	D2	ccGga>ccAga	1.00	New Acceptor Site	59.55	88.5	48.61 New site	1.26	9.22	Activation of an exonic cryptic acceptor site. with presence of one or more cryptic branch point (s). Potential alteration of splicing.	1	1	-
c.2103C>T	**p.Cys701**	16	D2	tgCgt>tgTgt	0.91		44.46	71.29	60.35 New site	NP	NP	Activation of an exonic cryptic donor site. Potential alteration of splicing.	1	1	-
c.2586G>T	**p.Val862**	20	D'	gtGtg>gtTtg	0.99		70	43.16	38.34 Site broken	NP	NP	Activation of an exonic ESE site. Potential alteration of splicing.	1	1	-
c.3144C>T	p.Asn1048	24	D3	aaCaa>aaTaa	0.51		NP	NP	NP	NP	NP	-	2	1	-
c.3390C>T	p.Cys1130	26	D3	tgCga>tgTga	0.98	New Donor Site	52.69	79.53	50.94 New site	NP	NP	Activation of an exonic cryptic donor site. Potential alteration of splicing.	5	3	NO
c.3426T>C	**p.Cys1142**	26	D3	tgTga>tgCga	0.95		82.16	55.33	32.66 Site broken	6.96	0.79	Activation of an exonic ESE site. Potential alteration of splicing.	7	7	NO
c.3735G>A	**p.Val1245**	28	D3	gtGcc>gtAcc	0.07		71.51	72.67	1.62	0.91	3.07	Activation of an exonic ESE site. Potential alteration of splicing.	1	1	-
c.4146G>T	p.Leu1382	28	A1	ctGct>ctTct	1.00	Cryptic Acceptor Strongly Activated	NP	NP	NP	NP	NP	Creation of an exonic ESS site. Potential alteration of splicing.	8	8	NO
c.4620G>C	**p.Leu1540**	28	A2	ctGca>ctCca	0.99		79.7	80.33	0.79	NP	NP	Alteration of an exonic ESE site. Potential alteration of splicing.	1	1	-
c.5001G>A	**p.Gln1667**	28	A2	caGag>caAag	0.98	Cryptic Donor Strongly Activated	84.98	56.04	34.06 Site broken	4.83	3.91	Creation of an exonic ESS site. Potential alteration of splicing.	1	1	-
c.5277C>T	p.Asp1759	30	A3	gaCgt>gaTgt	0.13	Cryptic Acceptor Strongly Activated	79.7	80.41	0.89	3.72	5.00	Creation of an exonic ESS site. Potential alteration of splicing.	6	4	NO
c.6237A>G	**p.Ser2079**	36	D4	tcAaa>tcGaa	0.00		81.02	80.95	-0.09	NP	NP	Activation of an exonic ESE site. Potential alteration of splicing.	1	1	-
c.7344C>T	p.Cys2448	43	C3	tgCga>tgTga	0.00		40.38	67.21	66.44 New site	NP	NP	Activation of an exonic cryptic donor site. Potential alteration of splicing.	2	2	NO
c.7464C>T	**p.Gly2488**	44	C3	ggCga>ggTga	0.79	New Donor Site	71.05	97.89	37.78	2.72	10.47	No significant splicing motif alteration detected. This mutation has probably no impact on splicing.	1	1	-
c.7773C>T	**p.Pro2591**	47	C5	ccCgg>ccTgg	0.09		69.47	69.05	-0.6	7.77	6.90	Creation of an exonic ESS site. Potential alteration of splicing.	1	1	-

In bold, mutations not described previously in EAHAD-VWD-LOVD. NP indicates not predicted; and HSF, Human Splicing Finder.

Tabla S17. Intronic variants

HGVSc	Intron	LocalSpliceEffect	#Patients	#Families
c.55+69A>G	2		1	1
c.220+100G>A	3		3	1
c.221-70G>T	3		1	1
c.533-43A>C	5		3	3
c.998-46C>T	8		1	1
c.1110-26T>A	9		8	6
c.1156+27C>T	10		2	2
c.1156+42C>T	10		1	1
c.1293+86C>T	11	New Donor Site	1	1
c.1293+109T>C	11		1	1
c.1432+166C>A	12		1	1
c.1533+15G>A	13	New Acceptor Site	7	2
c.1946-17_1946-16insTTT	15		1	1
c.1946-16_1946-15insCTC	15		1	1
c.2546+55G>T	19	Cryptic Acceptor Strongly Activated	1	1
c.2546+97C>G	19		1	1
c.2546+132G>A	19		2	2
c.2685+147G>A	20		1	1
c.2821-148G>A	21		1	1
c.2821-123A>C	21		3	3
c.2821-88A>G	21		2	2
c.2821-78A>C	21		1	1
c.3379+27A>T	25		1	1
c.3538+20G>A	26	Cryptic Donor Strongly Activated	1	1
c.3539-35G>C	26		1	1
c.5170+10C>T	29	New Donor Site	8	7
c.5312-138G>A	30		1	1

HGVSc	Intron	LocalSpliceEffect	#Patients	#Families
c.5312-19A>C	30		1	1
c.5456-62A>G	31		1	1
c.5665-36T>C	33		1	1
c.5842+31C>T	34		1	1
c.6798+32G>A	38		1	1
c.6799-47G>A	38		1	1
c.6799-27C>T	38		1	1
c.6976+111G>A	40		2	1
c.7082-13G>C	41		2	1
c.7438-169G>A	43		1	1
c.7438-31T>C	43		1	1
c.7548+22G>A	44	New Acceptor Site	1	1
c.7549-80T>A	44	New Acceptor Site	3	1
c.7730-238G>A	45		1	1
c.7730-177G>T	45	Cryptic Acceptor Strongly Activated	5	4
c.7730-56C>T	45		1	1
c.7771-87G>A	46	Cryptic Donor Strongly Activated	1	1
c.7771-86G>A	46	Cryptic Donor Strongly Activated	1	1
c.7771-82G>A	46	Cryptic Donor Strongly Activated	1	1
c.7771-49G>A	46		1	1
c.7771-40G>A	46		1	1
c.7771-29G>A	46		1	1
c.7888-65C>A	47		2	2
c.8156-42C>T	50		1	1

In bold, variants/putative mutations not described previously in EAHAD-VWD-LOVD.

SUPPLEMENTAL FIGURES

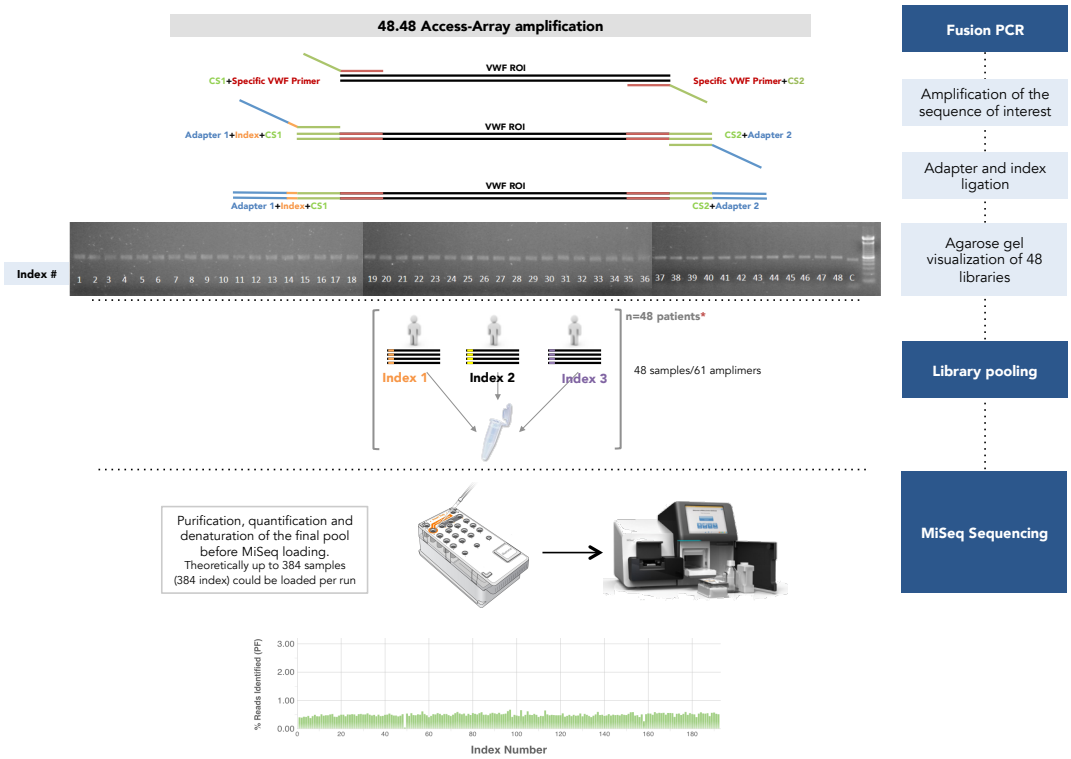

Figure S1. Schematic representation of *VWF* amplification by the Fluidigim Access Array and sequencing on an Illumina MiSeq. The Access Array method amplifies the VWF regions of interest (ROI: exons 1 to 52, intronic flanking regions, and approximately 1,300 bp of the promoter region, resulting in 25,625 bp per patient) by 61 custom primer pairs (red). Each primer pair contains common flanking sequences (CS1 and CS2, green) that permit attachment of a platform-specific barcode index (orange) and sequencing adaptors (blue) in a fusion PCR. The outcome, a pool of 61 amplicons indexed per patient, is known as a library. Each library is visualized on an agarose gel (C is the control size of a library without the adapters, hence the final libraries must be larger than the C band size). As the concentrations of the 48 PCR pools from the same array were highly uniform, a final pool containing a mixture of all samples in the same proportion was created. The final pool of up to 192 samples (4 Access Array) was purified with Agencourt AMPure XP beads (Beckman Coulter Genomics), adjusted to 4 nM, and denatured in 0.2 N NaOH before loading onto a MiSeq Illumina instrument.

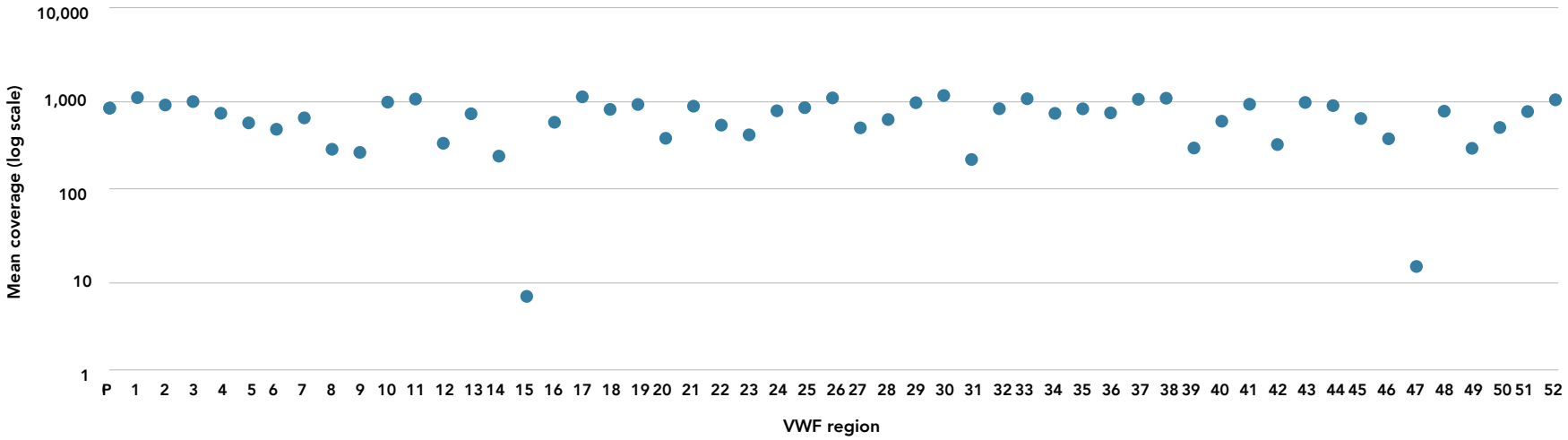

Figure S2. Mean exon coverage graphical representation. Homogeneous output is shown for the promoter and the 52 exons except for exon 47 and exon 15 where amplification failed in a significant number of arrays (undoubtedly due to their high GC content). P indicates promoter region.

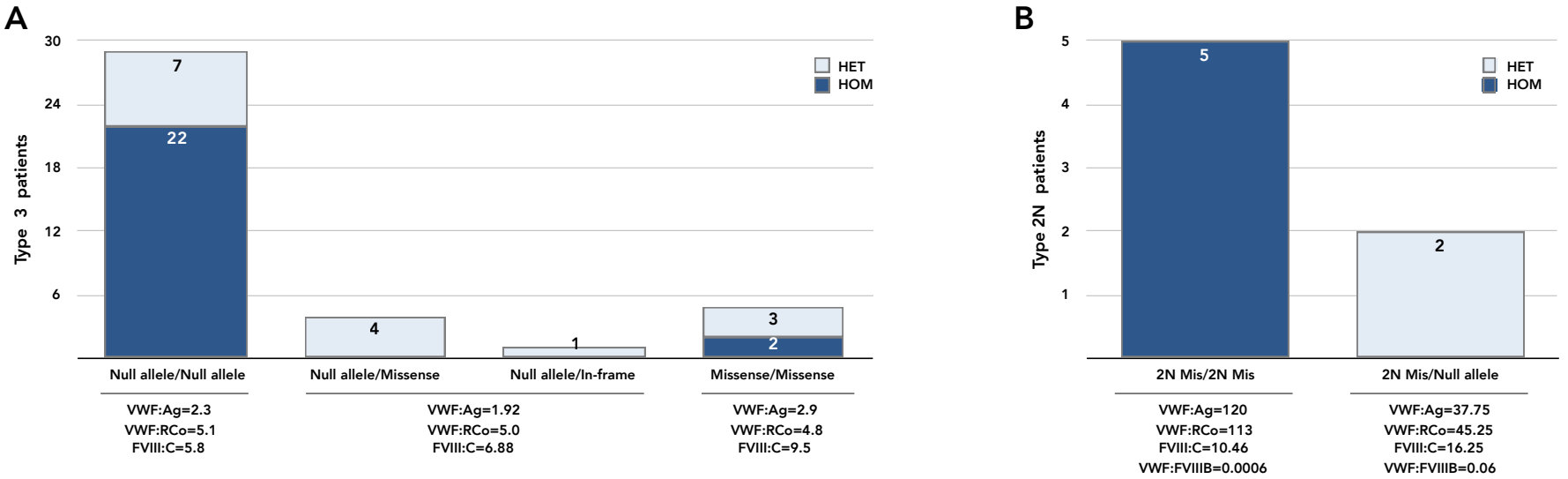

Figure S3. Graphical representation of combined mutations found in recessive VWD subtypes in all phenotype-genotype concordant patients. (A) Mutation combinations in type 3 VWD patients. The VWF:Ag, VWF:RCo and FVIII:C levels observed were below the technical error of the determination employed methods, precluding the application of statistical analyses to unravel differences between groups. (B) Mutation combinations in type 2N VWD patients. Laboratory values were found to be different (although not statistically tested) between patients with 2N mutations in both alleles (all homozygous in our cohort) versus those with a 2N mutation *in trans* with a second mutation. Patients with two 2N mutations, despite having normal VWF levels, showed a FVIII binding inability and a reduction of FVIII:C, resulting in a more severe phenotype. Null allele indicates nonsense mutations, frameshift indels and ±3 intronic mutations; HET, compound heterozygous; and HOM, homozygous.

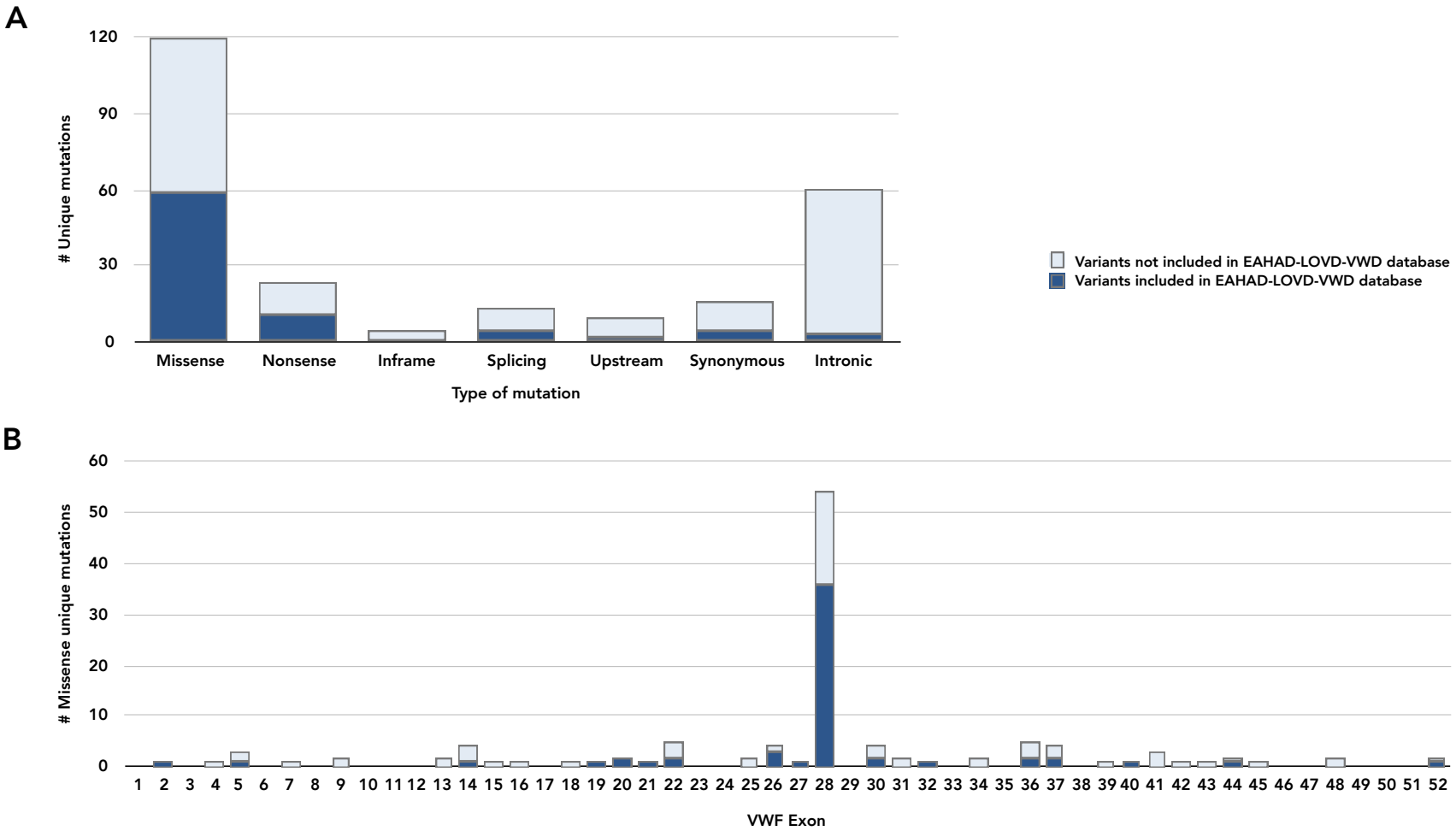

Figure S4. Graphs showing the mutation types identified in the cohort, highlighting variants that are not included in EAHAD-LOVD-VWD. (A) Number of unique mutations by type. In all cases the number of variants that are not included in EAHAD-LOVD-VWD is higher than those previously described. (B) Distribution of the 120 unique missense mutations identified along the *VWF*. Note that exon 28, the most often analyzed by Sanger sequencing, has the largest number of variants described in EAHAD-LOVD-VWD. Nonetheless, novel variants not included in EAHAD-LOVD-VWD were also found in this exon.

APPENDIX: Study group members

The members of the PCM-EVW-ES Study Group are:

Nina Borràs, Barcelona, Spain; Javier Batlle, A Coruña, Spain; Almudena Pérez-Rodríguez, A Coruña, Spain; María Fernanda López-Fernández, A Coruña, Spain; Ángela Rodríguez-Trillo, A Coruña, Spain; Esther Lourés, A Coruña, Spain; Ana Rosa Cid, Valencia, Spain; Santiago Bonanad, Valencia, Spain; Noelia Cabrera, Valencia, Spain; Andrés Moret, Valencia, Spain; Rafael Parra, Barcelona, Spain; María Eva Mingot-Castellano, Málaga, Spain; Ignacia Balda, Las Palmas de Gran Canaria, Spain; Carme Altisent, Barcelona, Spain; Rocío Pérez-Montes, Santander, Spain; Rosa María Fisac, Segovia, Spain; Gemma Iruín, Vizcaya, Spain; Sonia Herrero, Guadalajara, Spain; Sonia Herrero, Guadalajara, Spain; Inmaculada Soto, Asturias, Spain; Ángeles Rodríguez, Asturias, Spain; Nuria Fernández Mosteirín, Zaragoza, Spain; Beatriz de Rueda, Zaragoza, Spain; Víctor Jiménez-Yuste, Madrid, Spain; María Teresa Álvarez, Madrid, Spain; Nieves Alonso, Badajoz, Spain; Dolores Vilariño, Santiago de Compostela, Spain; Olga Arija, Lugo, Spain; Rosa Campos, Cádiz, Spain; María José Paloma, Navarra, Spain; Nuria Bermejo, Cáceres, Spain; Teresa Toll, Barcelona, Spain; Rubén Berrueco, Barcelona, Spain; José Mateo, Barcelona, Spain; Karmele Arribalzaga, Madrid, Spain; Pascual Marco, Alicante, Spain; Ángeles Palomo, Málaga, Spain; María Ángeles Martín, Madrid, Spain; Lizheidy Sarmiento Serrato, Madrid, Spain; María Paz Maluenda, Madrid, Spain; Belén Iñigo, Madrid, Spain; María del Mar Nieto, Jaén, Spain; Rosa Vidal, Madrid, Spain; María Paz Martínez, Ávila, Spain; Reyes Aguinaco, Tarragona, Spain; Jesús María César(†), Madrid, Spain; María Ferreiro, Pontevedra, Spain; Javier García-Frade, Valladolid, Spain; Ana María Rodríguez-Huerta, Madrid, Spain; Jorge Cuesta, Toledo, Spain; Ramón Rodríguez-González, Madrid, Spain; Faustino García-Candel, Murcia, Spain; Rosa Cornudella, Zaragoza, Spain; Carlos Aguilar, Soria, Spain; Francisco Vidal, Barcelona, Spain; and Irene Corrales, Barcelona, Spain.

(†) Jesús María César died on August 25th, 2014

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