World Journal of Hematology

Online Submissions: http://www.wjgnet.com/esps/
wjhematol@wjgnet.com
doi:10.5315/wjh.v2.i4.99

World J Hematol 2013 November 6; 2(4): 99-108
ISSN 2218-6204 (online)

EDITORIAL

Pseudohemophilia of Erik von Willebrand caused by homozygous one nucleotide deletion in exon 18 of the VW-factor gene

Alain Gadisseur, Zwi Berneman, Wilfried Schroyens, Jan Jacques Michiels

Alain Gadisseur, Hemostasis, Unit, Department of Hematology, University Hospital Antwerp, Wilrijkstraat 10, B-2650, Edegem, Belgium
Zwi Berneman, Wilfried Schroyens, Jan Jacques Michiels, Department of Hematology, University Hospital Antwerp, Wilrijkstraat 10, B-2650, Edegem, Belgium
Jan Jacques Michiels, Goodheart Institute and Foundation, Bloodcoagulation and Vascular Medicine Center, Erasmus Tower, 3069 AT Rotterdam, The Netherlands
Author contributions: Michiels JJ, Berneman Z and Schroyens W analysed the clinical features of congenital severe type 1 and 3 VWD and obligate heterozygous carriers; Gadisseur A and Michiels JJ analysed the molecular characteristics of severe type 1 and 3 VWD patients and wrote the manuscript.
Correspondence to: Jan Jacques Michiels, Professor, Goodheart Institute and Foundation, Bloodcoagulation and Vascular Medicine Center, Erasmus Tower, Veenmos 13, 3069 AT Rotterdam, The Netherlands. goodheartcenter@upcmail.nl
Telephone: +32-3-8213915 Fax: +32-3-8214286
Received: March 4, 2013 Revised: July 30, 2013
Accepted: August 4, 2013
Published online: November 6, 2013

Abstract

The original description of a novel severe bleeding disorder as "Hereditary Pseudohemophilia" by Erik von Willebrand can currently be labelled as von Willebrand disease (VWD) type 3. VWD type 3 is autosomal recessive caused by homozygous or double heterozygous null mutations in the von Willebrand factor (VWF) gene and typically characterized by prolonged bleeding time and APTT, FⅧ: C levels below 2%, undetectable VWF: Ag, VWF: RCo and VWF: CB and absence of ristocetin induced platelet aggregation (RIPA). Autosomal recessive von Willebrand disease type 3 VWD with virtual complete VWF deficiency are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA). Reports on severe recessive VWD compound heterozygous for a null allele and a missense mutation and homozygous or double heterozygous for missense mutations are associated with very low but measurable FⅧ and VWF: Ag and should be reclassified as severe recessive type 1 VWD. Homozygous missense or compound missense/null mutations related to recessive severe type 1 VWD have been indentified in the VWF prosequence D1 and D2 domains, the D4, B1-3, C1-2 domains, and only a very few in the dimmerization site (D3 domain). The detection of even tiny amounts of VWF: Ag after desmopressin acetate (DDAVP) or in hidden sites like platelets allows the differentiation between patients with VWD type 3 and homozygous or double heterozygous recessive severe type 1. Carriers of a null allele related to VWD type 3 or a missense mutation related with severe recessive type 1 VWD may present with mild VWD with low penetrance of bleeding in particular when associated with blood group O. Heterozygous obligatory carriers (OC) of a null mutation or a missense mutation related to recessive VWD type 3 or severe type 1 both present with asymptomatic or mild VWD type 1 in particular when associated with blood group O. The response to DDAVP of OC of either a nonsense or a missense mutation appears to be abnormal and diagnostic with a 3-times higher response of FⅧ: C as compared to VWF: Ag. In contrast, the responses to DDAVP of F Ⅷ: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

Key words: Autosomal recessive von Willebrand disease type 3 and 1; Molecular etiology; Carrier of von

Willebrand disease null or missense allele; Desmopressin acetate responses

Core tip: The novel lethal bleeding disorder described as "Hereditary Pseudohemophilia by von Willebrand (VW) in 1926 is caused by a homozygous nonsense mutation (one nucleotide deletion in exon 18) of the VW-factor gene consistent with autosomal recessive VW disease (VWD) type 3. Heterozygous carriers presented with VWD type 1 with variable penetrance of mild mucocutaneous bleeding manifestations. The present editorial reviews the clinical, laboratory and molecular features of severe recessive type 1 and 3 VWD and obligate heterozygous carriers of VWF nonsense and missense mutations.

Gadisseur A, Berneman Z, Schroyens W, Michiels JJ. Pseudohemophilia of Erik von Willebrand caused by homozygous one nucleotide deletion in exon 18 of the VW-factor gene. *World J Hematol* 2013; 2(4): 99-108 Available from: URL: http://www.wjgnet.com/2218-6204/full/v2/i4/99.htm DOI: http://dx.doi.org/10.5315/wjh.v2.i4.99

INTRODUCTION

In 1926 Erik von Willebrand first described a novel severe bleeding disorder which he named "Hereditary Pseudohemophilia" in at least 4 affected members of the original large family S, living on the Föglo Aland Island[1-5]. In this report we review the available clinical, laboratory and molecular features of the original family S, which can now be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nucleotide deletion of exon 18) and of mild VWD type 1 with variable penetrance of bleeding manifestations in heterozygous carriers.

CLINICAL FEATURES

The pedigree of family S, originally described by Erik von Willebrand in 1926, has been updated and numbered by Blomback in 1999 (Figure 1)[4].

The proband Hjördis S, case 16, aged 5 years was admitted on April 29 1924 to the hospital Diakonianstalten in Helsinki, Finland. At the age of 1 year, her bleeding tendency was observed after falling and hurting her nose and bled unusually long. At 3 years of age, she fell and had a deep cut in the upper lip. She bled heavily for 3 d and became bloodless and almost unconscious. She had to be confined to bed for 10 wk before recovering. After this she had frequent bruising, and regular episodes of epistaxis and gingival bleeding. An ankle distortion was followed by a severe articular bleeding with intense pain for some weeks (hemarthrosis). When a bleeding time according to the Duke method was performed she continued to bleed for 2 h and this had to be stopped by compression. Erik von Willebrand never visited the Aland Islands himself. Eight years later at the age of 13, Hjördis bled to death during her fourth menstrual period.

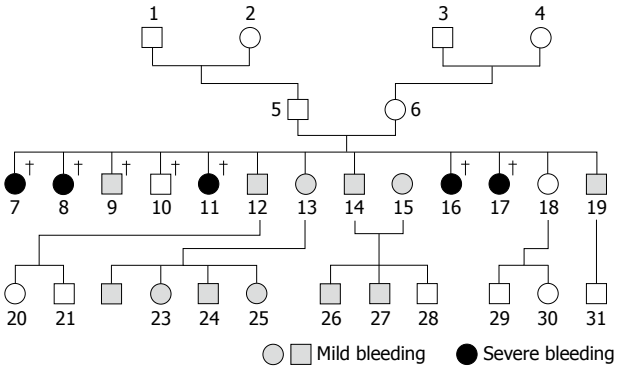

Figure 1 Pedigree of the original family S described by von Willebrand updated by Blombäck and Nilsson in the 1990s.

Her mother, Mrs Augusta, case 6, aged 44 years, had a history of frequent and persistent nose bleedings during her entire youth, but not lately. Menstruation (menarche) begun at age 16 years and had a duration of 6 d or more, always copious, especially lately. She had experienced normal deliveries without severe bleeding. The bleeding time was normal. Augusta, the mother of Hjördis, (case 4, Figure 1), and four of 10 siblings in the family had an increased bleeding tendency.

Her father Mr Oskar S, case 5, aged 48 years, had rather sturdy nose bleedings, when he was young, and did not bruise more easily than other people. Neither of his parent (cases 1 and 2) had been bleeders. One of his sisters and several of her children (family E) had a moderate bleeding tendency.

Her oldest sister, Dagny S, case 7, had her first severe nose bleeding after a slight trauma at the age of 1 year. After this she had several nose bleedings and died from intestinal bleeding at the age of 2 years.

Her sister Anna S, case 8, began to have frequent nose bleeds from the age of 1 year. When she was 4 years old, she fell and two teeth penetrated her tongue. She had a bleeding that could not be stopped and died.

Her sister Dagny S, case 11, had thrush when she was a few weeks old. When her mother tried to loosen the membranes, there was a bleeding that almost would not stop. She bled much after insect bites and died at the age of 2 years from intestinal bleeding.

Her younger sister, case 17, aged 3 years, bruised easily since the age of 1 year and experienced heavy nose bleeds for the first time at age 1.5 years. Thereafter nose bleeds fairly often recurred. The bleeding often started spontaneously, and once went on for a whole week. The bleeding time was considerable prolonged. She had prolonged bleedings after insect bites. At the age of 5 she developed influenza, started to vomit blood and died within 20 h.

Her younger brother, case 19 (proven heterozygous for del 18 in 1990s), suffered in his youth from a slightly increased bleeding tendency, although he did not report

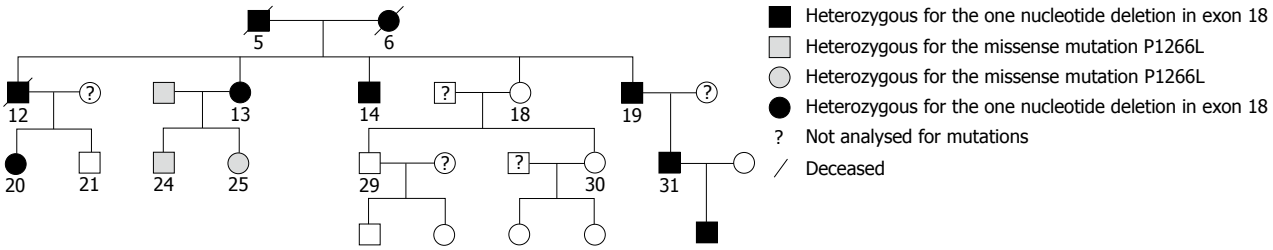

Figure 2 Results of molecular investigations in the original family S in the 1990s reveal a heterozygous nonsense mutation in seven affected members with mild bleeding diathesis[4].

Table 1 FⅧ: C and von Willebrand factor levels in family S together with finding of heterozygous non-sense mutation, deletion exon 18, consistent with mild von Willebrand disease type 1

Family S, Number	Mutation	FⅧ: C IU/dL	VWF: Ag IU/dL	VWF: RCo IU/dL	RCo/Ag Ratio
5		0.93			
6		0.51			
12	del 18	0.81	0.80	0.73	0.91
13	del 18	0.53	0.56	0.46	0.82
14	del 18	0.35	0.35	0.32	0.91
18	negative	0.96	0.86	0.65	0.76
19	del 18	1.10	0.67	0.81	1.20
22	unknown	0.50	0.38	0.25	0.66
23	unknown	0.73	0.39	0.39	1.00
24	P1266L	0.39	0.34	0.42	1.23
25	del 18/P1266L	0.69	0.20	0.30	1.5

VWF: von Willebrand factor; VWD: von Willebrand disease; FⅧ: Factor Ⅷ.

any problems in 1992.

LABORATORY FEATURES

The inheritance of the disease in Family S could be followed through four generations[1-5]. In the female bleeders, the bleeding diathesis is manifested in a mild form or in a severe form, whereas the males show only the mild form of bleeding diathesis (Figure 1). Among female affected members with severe bleeding five deaths in one generation had occurred, four in childhood and one shortly after menarche (Figure 1). The women are of two types, those with a single and those with double trait. The former (heterozygotes) may had a milder form of bleeding, the latter (homozygotes) a severe lethal form. There was no opportunity to study the so-called homozygote women because they died from fatal bleeding before the reproductive age and long before FⅧ and the von Willebrand factor were identified as causes of hemophilia and VWD more than 3 decades later in the late 1950s. This means that data on the level of F Ⅷ: C and VWF parameters are lacking in those deceased women with the double trait (Table 1). This may explain why Jurgens *et al*[6] have falsely interpreted the bleeding disorder as a constitutional thrombopathy mainly based on the very prolonged bleeding times. Jurgens *et al*[7] mentioned several years later a deficiency of an antihemophilic factor (FⅧ) in heterozygous affected family members with mild VWD, but the cases with severe bleeding could not studied anymore at that time.

Studies by Nilsson *et al*[5] showed decreased values for VWF: Ag and VWF: RCo and normal ratios for VWF: RCo/VWF: Ag in the affected heterozygous members of family S with mild VWD type 1 (Table 1) leaving the molecular etiology of the "homozygotes" elusive at that time.

MOLECULAR STUDIES

The DNA samples were screened for mutations with PCR, followed by direct sequencing in the "hot spot" regions in exons 18, 28, 32, 43 and 45 found in the Swedish-Finnish patients[8,9]. In the original family S one nucleotide deletion in exon 18 was identified in heterozygous carriers, which had been found to be a "hot spot" in the majority of VWD type 3 patients in Sweden[9,10]. This mutation interrupts the reading frame leading to an early translational stop (null allele). Five individuals (numbers 13, 14, 19, 20 and 25 in Figure 2 and Table 1) all having VWD type 1 were found to be heterozygous for the deletion. The deceased family member 12 must also have carried the same deletion, as his daughter is a carrier. In the third generation, at least four individuals (numbers 12, 13, 14, 19, Figure 2) carry the deletion. These results indicated that the deletion originated from the parents of family S (number 5 and 6, Figures 1 and 2) who are thought to be heterozygous. All five daughters, who died from uncontrolled bleedings, very likely would have been homozygous for the deletion in exon 18 consistent with pseudohemophilia, now called VWD type 3.

Table 2 Reports of autosomal recessive severe type 1 von Willebrand disease caused by homozygous missense or double heterozygous missense/null mutations in the D1 or D2 domain

Mutation	F/M (yr)	BT (min)	Ⅷ: C (U/dL)	VWF: Ag (U/dL)	VWF: RCo (U/dL)	Domain/VWF	VWD type
D141Y/null[19]	F/63	> 30	0.03	< 1	< 1	D1	Severe 1
C275S/null[19]	F/26	> 30	0.03	< 1	< 1	D1	Severe 1
R273W/R273W[20]	Boy	15	0.20	0.06	0.06	D1	Severe 1
R273W/R273W	Boy	15	0.33	0.09	0.04	D1	Severe 1
R273W/R273W	Boy	> 20	0.09	< 0.01	< 0.01	D1	Severe 1
W377C/W377C[12]	Child	> 20	0.02	0.03	0.03	D1	No data
C570S/C570S[21]	Boy	↑↑	0.12	0.05	0.05	D2	Severe 1
Q77X/splice site Intron[24]		> 30	0.20-0.31	0.04-0.06	0.03-0.06	D1/D2	Severe 1

VWF: von Willebrand factor; VWD: von Willebrand disease.

The three siblings, Harald, Sylvia and Runar, (cases 12, 13 and 14, proven heterozygous for del 18) had more less severe nose bleeds, especially in their youth but never experienced a pronounced bleeding tendency during life-long follow-up. These data demonstrate that the original family S, described by Erik von Willebrand as pseudohemophilia A, has to be diagnosed as autosomal recessive VWD type 3 caused by a homozygous null mutation (one nuclotide deletion of exon 18). In the family S in addition to the deletion in exon 18, two mutations at S1263 and P1266 in exon 28 were identified in two siblings (numbers 24 and 25, Table 1, Figure 2) with an unrelated and clinically normal father, who married into the family S. The transition G→A at S1263 is neutral, and the other C→T at P1266L results in an amino acid substitution of proline to leucin (P1266L). P1266L is a frequent mutation in Sweden and has been described as VWD type 1 Malmö with increased ristocetin induced platelet aggregation (RIPA) (mild 2B).

DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE TYPE 3 VWD

The inheritance of a pronounced bleeding tendency in subsequent reports of families with VWD type 3 is autosomal recessive[11-15]. Patients with VWD type 3 are typically characterized by prolonged bleeding time (BT) and APTT, FⅧ: C levels below 2%, undetectable VWF: Ag, VWF: RCo and VWF: CB levels before and after desmopressin acetate (DDAVP) and absence of RIPA[15]. VWD type 3 patients with FⅧ: C levels above 2% and detectable levels of VWF: Ag and response of FⅧ: C to DDAVP should be reclassified as severe recessive type 1 VWD caused by double heterozygous for a nonsense/missense mutation or homozygous or double heterozygous missense mutation causing a severe secretion defect[12,13].

In 31 cases diagnosed as VWD type 3, (age 2 to 80, median 15 years) described by Schneppenheim *et al*[12] bleeding manifestations were recorded as easy bruising and prolonged epistaxis in 31 (100%), spontaneous joint bleeding in 23 (76%), muscle bleeding in 7 (22%) and gastrointestinal bleedings in 3 (10%). The bleeding manifestations and complications of childbirth have been nicely evaluated in 385 Iranian patients diagnosed as autosomal recessive type 3 VWD (SSC-ISTH classification) and compared to age matched severe hemophilia A[14]. Among patients with type 3 VWD the incidences of spontaneous hemarthrosis (37%) and muscle bleedings (52%) are lower most likely because FⅧ: C levels are higher (1%-9%) as compared to severe hemophilia A (≤ 1%).

Type 3 VWD with virtual complete VWF deficiency (severe VWD) and absence of FⅧ: C (pseudohemophilia) are homozygous or compound heterozygous for two null alleles (gene deletions, stop codons, frame shift mutations, splice site mutations, and absence of mRNA) in the majority and rarely compound heterozygous for a null allele and a missense mutation or homozygous for a missense mutation[11-18]. The null alleles are located all over the VWF gene in nearly all exons 3-52[18]. The data base of the SSC of the ISTH reports 58 null alleles and 14 missense alleles involved in the etiology of type 3 VWD[18]. Missense mutations related to severe recessive VWD type 1 are mainly located in the D1-D2 domains (D47H, S85P, Y87S, D141Y, D141N, C275S, W377C, I427N, and in the D4, B1-3, C1-2, CK domains (P2063S, C2174G, C2362F, N2546Y, C2671Y, C2754W, and C2804Y), but not in the D3, A1 and A2 domains except one (C1071F/null)[18]. Consequently, some so-called type 3 VWD patients, who are compound heterozygous for a null allele and a missense mutation and may have detectable but very low VWF levels, are incorrectly diagnosed as VWD type 3 and should be reclassified as recessive severe type 1 VWD[19-27].

DIAGNOSIS AND MOLECULAR BIOLOGY OF RECESSIVE SEVERE TYPE 1 VWD

A considerable number of missense mutations related to autosomal recessive severe type 1 VWD have been identified in the VWF prosequence (D1 and D2 domains),) and the D4, B1-3, C1-2 and CK (dimerization) domains), but only a very few in the dimerization site (D3 domain (Tables 2 and 3)[19-26]. There are two reports on double heterozygous missense/null mutation D141Y/

Table 3 Laboratory features of recessive severe type 1 due to a double heterozygous missense mutation in the CK domain of the von Willebrand factor gene

Mutation	Age (yr)	Gender	BT	FⅧ: C	VWF: Ag	VWF: RCo	VWF: RCo/Ag	RIPA	VWD type
C2754W/C2754W[31]	13	F	> 20	0.12	< 0.05	< 0.05	-	nt	3
Father C2754W[31]	-	M	5	0.54	0.33	0.38	1.15	nt	Mild 1
Mother C2754W[31]	-	F	5	0.55	0.38	0.43	1.13	nt	Mild 1

VWF: von Willebrand factor; VWD: von Willebrand disease; M: Male; F: Female; FⅧ: Factor Ⅷ; RIPA: Ristocetin induced platelet aggregation.

null and C275S/null associated with VWD severe type 1 and not type 3 with documented hemarthos in one of them (Table 2)[19]. Expression studies the missense mutation D141Y and C275S showed a severe secretion defect of mainly dimers while higher molecular weight bands like tetramers and hexamers were barely detectable[26]. Homozygotes for the missense mutations W377C[12] and for R273W[20] in the propeptide D1 domain have been described to be associated with autosomal recessive severe type 1 (not type 3) VWD phenotype (Table 2). Homozygous missense mutation C570S in the D2 domain has been described as the cause of recessive severe type 1 VWD laboratory phenotype mimicking a type 2C (ⅡC) VWF multimers[21].

The multimeric pattern of homozygous R273W and C570S clearly showed the absence of high molecular weight multimers and a pronounced monomeric band mimicking type 2C (ⅡC) subtype VWD[20]. Expression studies of recombinant R273W, W377C and C570S showed a severe secretion defect mainly consisting of dimers and failed to form intermediate and high molecular weigth multimers[19-21]. These findings demonstrate that mutations in the D1 and D2 VWFpp domain completely abolishes multimerization of VWF. Heterozygous asymptomatic carriers of such missense mutation are asymptomatic or diagnosed as mild type 1 VWD with borderline values of VWF parameters around 0.50 U/dL. Heterozygotes for a missense mutation in the D1 or D2 domain typically show a pronounced VWF dimer band.

Homozygous missense mutation C2364F in the B1-3 domain and double heterozygous C2364F/null has been reported to be associated with severe type 1 VWD featured by FⅧ: C levels of 12 to 32 U/L, very low but detectable VWF: Ag and undetectable VWF: RCo[22-26]. C2364F heterozygous carriers were asymptomatic, had normal or slightly prolonged BT, subnormal values for VWG: Ag and VWF: RCo with a normal VWF: RCo/Ag ratio, and a normal VWF multimeric pattern in a low 0.8% or 0.9% agarose resolution gel (asymptomatic "dominant" VWD type 1)[22]. However, analysis of VWF in plasma from cases with severe autosomal recessive VWD homozygous for a missense mutation C2362F or compound C2362F/null (exon 42 of the B1-3 domain) as well as heterozygous carrier of C2364F all showed a heightened proteolytic pattern with marked increase of 176 and 140 kDa degradation products mimicking type 2A (ⅡA) VWD[26]. Other causes of severe autosomal recessive type 1 VWD include homozygous C2364Y (B1-3 domain) or double heterozygous C2364Y/intron 13 splice site[23], homozygous C2671Y (exon 49) or double heterozygous missense mutation C2671Y/del (exon 49) of the VWF gene[27]. DDAVP in recessive VWD severe type 1 induces a poor for VWF: Ag and VWF: RCo but significant increase of FⅧ: C levels. In some cases of autosomal recessive severe type 1 VWD patients FⅧ: C, VWF: Ag and VWF: RCo reached values of > 0.50, 0.11 and 0.09 U/L respectively after DDAVP[22].

RECESSIVE SEVERE TYPE 1 VWD DUE TO MUTATIONS IN THE CK DOMAIN

The replacement of cysteine residues in the CK dimerization domain of the VWF gene causes two completely different laboratory phenotypes of VWD either severe VWD type 3 or VWD type 2D (ⅡD)[28-36]. Homozygous or double heterozygous loss of cysteines mutations C2739Y, C2754W, C2804 and C2806 results in severe autosomal recessive type 1 VWD with nearly complete absence of VWF[29,35]. Homozygous C2754W mutation is associated with VWD severe type 1 and a mild type 1 VWD in heterozygous carriers (Table 3). Expression studies of C2754W show intracellular production of mainly monomers and dimmers (indicating a dimerization defect) with no secretion of mutant VWF indicating that homozygous C2754W mutation indeed will lead to severe type 1 or 3 VWD[35].

Experimental and clinical data are in line with the concept that loss of a single disulfide band in the CK domain of VWF leads to a recessive quantitative VWF deficiency with very low VWF: Ag (VWD type 3) if an intrachain disulfide band is involved (C2739Y or C2754W), and to a dominant-negative qualitative defect of VWF with abnormal multimers if an interchain-disulfide bond is involved, which leads to the characteristic type dominant type VWD type 2D (ⅡD) multimeric pattern (Figure 3)[35,36].

RECESSIVE TYPE 1 VWD DUE TO A HOMOZYGOUS MISSENSE MUTATION IN THE D2 DOMAIN

The 1534C > A mutation in the consensus splicing site of intron 13 (D2 domain) induces exon 14 skipping with the introduction of a premature termination after codon 586, resulting in a truncated VWF[37]. Moreover, the 1534C > A mutation induces the activation of a cryptic

Patients	Blood group	[1]BT min	aPPT s	[2]RIPA	FⅧ (U/dL)	VWF: Ag (U/dL)	VWF: RCo (U/dL)	VWF: CB (U/dL)	Plat. VWF: Ag (U/dL)	Mutation
Ⅰ-1 (father)	O	7	34.3	89%	120	101.2	70	82.9	48.1	1543-3C > A/N
Ⅰ-2 (mother)	O	-	31.2	87.5%	123	64.6	44	56.4	56.4	1543-3C > A/N
Ⅱ-1 (proband)	O	20	37.8	5.4%	51	14.5	12.5	9.8	9	1543-3C > A/1543-3C > A
Ⅱ-2 (sister)	O	6	34.2	85.6%	146	76.5	77	86.7	56.8	1543-3C > A/N
Normal range		2-9	30-40	60%-84%	60-160	60-160	60-130	65-150	70-140	

[1]BT (bleeding time) was performed using Ivy method; [2]RIPA was performed with 1.2 mg/mL ristocetin; RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; aPPT: activated partial thromboplastin time.

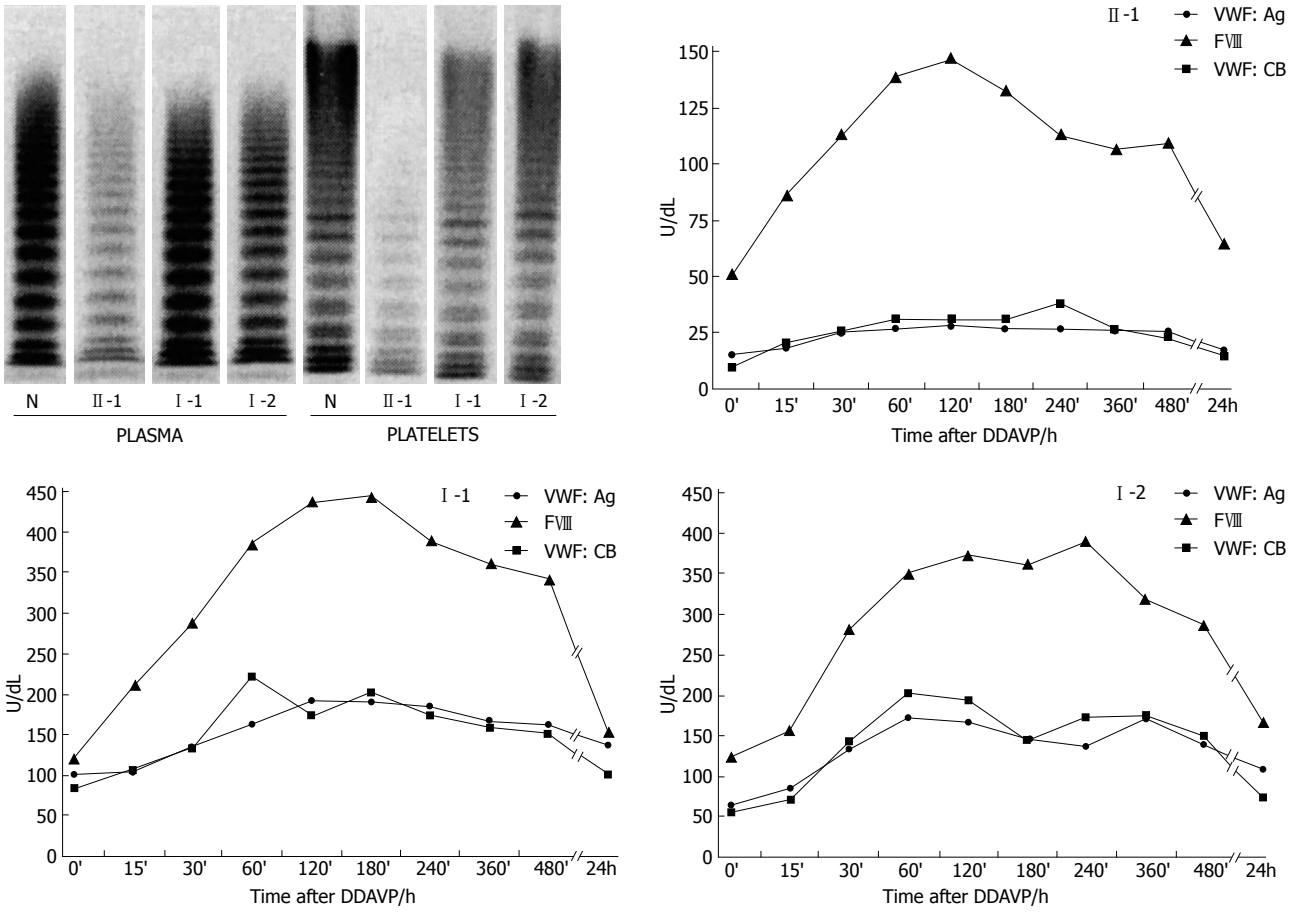

Figure 3 State of the Art characterization of a family with autosomal recessive von Willebrand disease type 1 by Casonato and co-workers using a complete set of laboratory assays related to von Willebrand disease diagnosis, von Willebrand factor multimeric analysis in plasma and platelets and the response of FⅧ, VWF: Ag and VWF: RCo to desmopressin acetate before and at several time points after desmopressin acetate according to standardized recommendations anno 2006. RIPA: Ristocetin induced platelet aggregation; VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.

splicing site, 62 nucleotides upstream from the normal site[37]. The spliceosome produces a normal transcript of normal VWF. Gallinaro described a family with autosomal recessive type 1 VWD caused by homozygous intron 13 splicing site mutation 1534-3C > A (Figure 3)[17]. The proband, a 34-year old man had a history of severe epistaxis requiring blood transfusion and recurrent mild epistaxis and gingivorrhagia not requiring medical attention. The proband had VWF values between 10 to 15 IU/dL with normal ratios of VWF: RCo/VWF: Ag (0.86), decreased VWF: CB/VWF: Ag ratio (0.6), and increased ratio of FⅧ/VWF: Ag (3.5) indicating a secretion defect. (dot) The plasma VWF multimeric pattern showed a homogenous decrease in all oligomers with a subtly loss of large VWF multimers and a more pronounced loss of platelet VWF multimers (VWD type 1 plasma low/platelet low). The response to DDAVP was restricted for VWF parameters but good for FⅧ: C thereby confirming a severe secretion defect. After DDAVP VWF: Ag increased from 15 to 28 IU/dL (1.9x) and FⅧ from 51 to 146 IU/dL (2.9x) followed by normal half life times for VWF and FⅧ (Figure 3). The father (Ⅰ-1) and sister (Ⅱ-2) heterozygous for the 1534-3C > A mutation never bled whereas the heterozygous mother suffered from mild menorrhagia, hematomas and bleeding after delivery. The plasma VWF values of affected family members were in the low normal range and relatively decreased as compared to FⅧ (Figure 3). Interestingly the response of FⅧ: C to DDAVP was rather good but severely restricted for all VWF parameters as compared the completely normal increase of FⅧ to values above 3.0 IU/dL consistent with carrier state of a missense mutation related to a secretion defect (Figure 3)[37].

Table 4 Laboratory phenotype and clinical symptoms in 69 patients with true von Willebrand factor deficiency type 1 heterozygous for the von Willebrand factor null allele (parents of type Ⅲ von Willebrand disease)

Author		Zhang *et al*[13]		Eikenboom *et al*[15]		
Number of patients		25	17	14		6
Blood Group		A	O	A		O
FⅧ: C (%)	Mean	81	74	93		81
	Range	37-121	11-128	69-138	58-93	
VWF: Ag	Mean	45	32	61		
	Range	13-94	12-70	37-98	40-66	52
VWF: RCo	Mean	-	-	56		53
	Range	-	-	30-92	39-68	
Mild bleedings[1]		13	11	1		1
		52%	65%	7%		17%

[1]Mild bleeding was defined by one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations without abnormal bleeding after both extraction or surgy, hemarthros or muscle bleeding. VWF: von Willebrand factor.

Table 5 Von Willebrand factor antigen (VWF: Ag) levels in heterozygous carriers for a null allele related to pseudohemophilia A-von Willebrand disease type 3 and for the mutation C2364F related to severe recessive type 1 von Willebrand disease

Carriers	Number of patients	VWF: Ag mean ± SD (IU/dL)	VWF: Ag range (IU/dL)
Null allele:			
Blood group O	15	43.2 ± 10.8	30-66
Blood group non-O	15	61.3 ± 23.6	25-98
C2364F:			
Blood group O	8	35.2 ± 16.2	25-55
Blood group non-O	15	61.5 ± 26.6	30-140

VWF: von Willebrand factor; VWD: von Willebrand disease.

DETECTION OF SYMPTOMATIC AND ASYMPTOMATIC OBLIGATORY CARRIERS OF RECESSIVE VWD TYPE 3 AND SEVERE TYPE 1

The only objective and correct way to characterise true type 1 VWF deficiency heterozygous for the VWF null allele or missense mutation is to analyse the bleeding manifestations and FⅧ: C/VWF parameters in obligate heterozygous parents of recessive type 3 or severe type 1 VWF patients. In the study of 27 patients with congenital type 1 VWF deficiency associated with one null allele analysed by Schneppenheim *et al*[12], 20 were asymptomatic and only 7 presented very mild bleeding, mainly bruising and epistaxis. All except one, had a normal BT. The mean values for FⅧ: C, VWF: Ag, and VWF: RCo were 0.76, 0.39 and 0.39 U/mL respectively with an increased FⅧ: C/VWF: Ag ratio of 1.9 and a normal VWF: RCo/Ag ratio of 1 consistent with true type 1 VWF deficiency. In the study of Zhang *et al*[13] including 25 patients heterozygous for the VWF null allele and blood group non-O, 12 had no history of bleeding and 13 presented with very mild bleeding (one or two bleeding symptoms mainly epistaxis, bruises and/or prolonged menstruations with no abnormal bleeding after tooth extraction). The mean values for FⅧ: C and VWF: Ag were 0.81 and 0.45 respectively with an increased ratio for FⅧ: C/VWF: Ag of 1.8 (Table 4). In the same study of Zhang *et al*[13] out of 17 patients heterozygous for the VWF null allele but having blood group O, 8 had no bleeding history and 11 presented minor bleedings (65%, Table 4). The mean values for FⅧ: C and VWF: Ag were 0.74 and 0.32 respectively with an increased ratio for FⅧ: C/VWF: Ag of 2.3 (Table 2). In the study of Eikenboom the values VWF: Ag and VWF: RCo in carriers of a null allele ranged from 0.30 to 0.98 IU/mL (Table 4)[15]. In these studies[12,13,15], there is a wide range of values from 0.11 to 1.28 U/mL for FⅧ: C, from 12 to 0.94 for VWF: Ag, with ratios of FⅧ: C/VWF: Ag ranging from normal to increased above 2 indicating the difficulty to distinguish true congenital type 1 VWF deficiency from VWF deficiency related to blood group O.

Using the recently developed sensitive bleeding score assessment, Castaman *et al*[38] compared the severity of bleeding symptoms in 70 OC of recessive type 3 VWD, 42 OC of recessive type 1 VWD and in 215 normal controls. OC of VWD type 3 with a null mutation had clearly less severe bleeding than patients diagnosed as type 1 VWD. OC of type 1 VWD with a missense mutation were distinct from normal controls, presenting more epistaxis, cutaneous bleeding and usually did not significantly bleed after surgery, further pointing to the wide heterogeneity of VWD as a heterozygous congenital disorder of the VWF gene mutations (Table 5).

Obligatory carriers (OC) of a nonsense mutation related to VWD type 3 and OC of missense mutation related to severe recessive VWD type 1 in the population are asymptomatic or manifest mild bleeding, and have VWF levels at 50% of normal (true type 1 VWD according to the law of Mendel). Such OC of a null allele or missense mutation may become more symptomatic when associated with blood group O or another modifier of the VWF level. Castaman and Eikenboom demonstrated that ABO blood group significantly influences the VWF: Ag levels in OC of a null allele related to VWD type 3 or the missense mutation C2364F related to severe recessive VWD type 1 (Table 5)[39]. From a genotypic point of view, OC of a null allele in type 3 VWD are very similar to asymptomatic or mild type 1 VWD patients with a single missense allele.

Based on careful analysis of reports on recessive VWD we proposed in 2006 the Antwerp Classification of recessive VWD type 3, recessive severe type 1 VWD, and true type 1 VWD heterozygous for a null allele or missense mutation with variable penetrance of bleeding manifestations but symptomatic when associated with blood group O (Table 6)[40]. In subsequent studies the variable penetrance of bleeding manifestations mild VWD type 1 is clearly related to blood group O[41,42].

Table 6 The 2006 Antwerp Classification of recessive von Willebrand disease type 3, recessive severe on Willebrand disease type 1 and obligatory carriers of a null or missense allele with asymptomatic or mild on Willebrand disease type 1 and variable penetrance of bleeding tendency

Category VWD	BT	FVIII: C (%)	VWF (%) Ag	RCo	RIPA	Bleeding type	VWF gene mutation
Severe type 3 Recessive	↑↑↑	1-9	zero	zero	zero	Severe Hemophilia	Double Nonsense
Severe type 1 Recessive VWD	↑↑↑	9-40	1-10	0-6	zero	Moderate Severe	Double Missense
Blood group O (30-32) (Pseude-VWD)	N	35-150	35-150	35-150	N	Asymp Very mild	None
Carrier type 3 Minor influence (-10%) of bloodgroup O	N↑	30-140	15-90	15-90	N	Asymp Very mild	Single Non-sense (null allele)
Carrier type 1 (polymorphism)	N	N	N	N	N	Asymp	Single Missense
Mild type 1 Recessive or variable penetrance and multigenetic backgroud	N↑	20-80	20-50	20-50	N	Mild	Mis/Non-sense or Y1584C/ Bloodgroup O[19]
Dominant type 1	N↑	20-80	10-40	0-30	N	Mild	Single
Secretion defect Dominant type 1	↑/↑↑	5-20	5-20	5-20		Moderate	Missense
Vicenza	N/↑	< 15	< 15	< 15		Moderate	Single^{R120SH} Missense

VWF: von Willebrand factor; VWD: von Willebrand disease; RIPA: Ristocetin induced platelet aggregation.

Table 7 Response of FVIII: C and von Willebrand factor parameters to DDAVP (0.3 ug/kg) in an obligatory carriers of a null allele heterozygous for the nonsense splice site mutation IV7 + 1G > A in intron 7. (0874 + 1G > A) in intron 7

DDAVP	Before	1	2	4	6	H post-DDAVP
FVIII: C	0.84	5	5.4	5.3	4.9	IU/mL
VWF: Ag	0.64	1.3	1.7	1.5	1.4	IU/mL
VWF: RCo	0.67	1.8	2	1.35	1.2	IU/mL
FVIII: C/VWF: Ag ratio	1.3	3.8	3.1	3.5	3.5	Carrier of null allele
VWF: RCo/Ag ratio	1.05	1.38	1.17	0.9	0.86	Mild type 1 VWD

VWF: von Willebrand factor; VWD: von Willebrand disease; DDAVP: Desmopressin acetate.

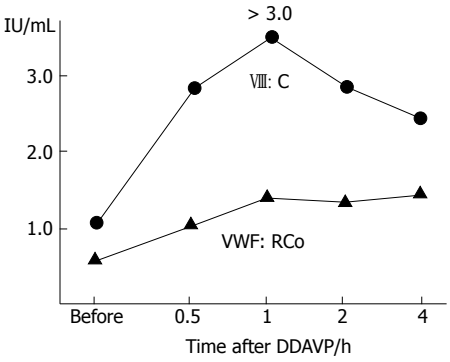

Figure 4 Good response of FVIII: C and restricted response of von Willebrand factor: RCo to desmopressin acetate (0.3 ug/kg) in a carrier of a null allele (Q2470X/normal). VWF: von Willebrand factor; DDAVP: Desmopressin acetate.

Similarly, the C1584 variant of mild VWD type 1 is associated with a slight decrease of VWF and FVIII: C levels, especially in combination with bloodgroup O[43,44].

We studied a consanguineous family with type 3 VWD. The propositus was a boy with VWD type 3, who presented with mucocutaneous bleeding and recurrent hemarthrosis of an ankle. Laboratory analysis found F VIII: C < 1% and an absence of VWF: Ag due to the homozygous nonsense splice site mutation IV7 + 1 G>A in intron 7 (0874 + 1G > A)[45]. Both parents were heterozygous for the nonsense mutation and completely asymptomatic with near normal to normal values for F VIII: C, VWF parameters and normal ratios for FVIII: C/VWF: Ag (Table 7)[45]. After DDAVP, the FVIII: C levels rose to much higher levels as compared to VWF: Ag, VWF: RCo and VWF: CB levels. FVIII: C/VWF: Ag ratios were 1.3 before DDAVP but more than 3 after DDAVP consistent with a carrier of a VWF null allele (Table 7). The VWF: RCo/Ag ratio was normal before and after DDAVP consistent with true congenital type 1 VWD disease (Table 7). This demonstrates that an increased ratio FVIII: C/VWF: Ag ratio after DDAVP is typically and diagnostic for true VWF deficiency type 1 heterozygous for a null allele. This important diagnostic clue to true congenital type 1 VWD has also been demonstrated by Lethagen *et al*[46] in a carrier of a null allele (Q2470X/normal, Figure 4).

From this analysis of the literature and personal experiences in VWD we conclude that heterozygous carriers of a null mutation related to VWD type 3 and a missense mutation related to recessive VWD severe type 1 both do present with asymptomatic or mild VWD type 1 in particular when associated with blood group O. The response to DDAVP of OC of either a nonsense or a missense mutation related to VWD type 3 or severe type 1 appears to be abnormal and diagnostic with a 3-times

higher response of FⅧ: C as compared to VWF: Ag. In contrast, the responses to DDAVP of FⅧ: C and VWF: Ag are equally good in individuals with low VWF levels related to blood group O and a normal VWF gene and protein (pseudo-VWD). These observations are completely in line with and extend the original observations of Erik von Willebrand in a large family with VWD type 3 and asymptomatic or mild true type 1 VWD in OC.

REFERENCES

1 **Von Willebrand EA**. Hereditär pseudohemofili. *Finska Läkarsällskapets Handl* 1926; **67**: 7-112
2 **Von Willebrand EA**. Ueber hereditäre Pseudohemophilie. *Acta Med Scand* 1931; **67**: 521-550
3 **Von Willebrand EA**. Hereditary pseudohaemophilia. *Haemophilia* 1999; **5**: 223-231; discussion 222 [PMID: 10444294 DOI: 10.1046/j.1365-2516.1999.00302.x]
4 **Blombäck M**. Scientific visits to the Aland Islands. *Haemophilia* 1999; **5** Suppl 2: 12-18 [PMID: 23401895 DOI: 10.1046/j.1365-2516.1999.0050s2012.x]
5 **Nilsson IM**. The history of von Willebrand disease. *Haemophilia* 1999; **5** Suppl 2: 7-11 [PMID: 23401894 DOI: 10.1046/j.1365-2516.1999.0050s2007.x]
6 **Jurgens R**, Forsius H. On Willebrand-Jürgens constitutional thrombopathy in the Aland Islands. *Schweiz Med Wochenschr* 1951; **81**: 1248-1253 [PMID: 14913085]
7 **Jurgens R**, Lehmann W, Wegelius O, Eriksson AW, Hiepler E. Antihemophilic globulin (factor Ⅷ) deficiency in Aland (Willebrand-Jürgens) thrombopathy. *Thromb Diath Haemorrh* 1957; **1**: 257-260 [PMID: 13580992]
8 **Zhang ZP**, Lindstedt M. Falk G. Blombäcl M, Egberg N, Anvrt M. Nonsense mutations of the von Willebrand factor gene in patients with von Willebrand disease. *Am J Hum Genet* 1992; **51**: 858-855
9 **Zhang ZP**, Falk G, Blombäck M, Egberg N, Anvret M. A single cytosine deletion in exon 18 of the von Willebrand factor gene is the most common mutation in Swedish vWD type Ⅲ patients. *Hum Mol Genet* 1992; **1**: 767-768 [PMID: 1302613 DOI: 10.1093/hmg/1.9.767]
10 **Zhang ZP**, Blombäck M, Nyman D, Anvret M. Mutations of von Willebrand factor gene in families with von Willebrand disease in the Aland Islands. *Proc Natl Acad Sci USA* 1993; **90**: 7937-7940 [PMID: 8367445 DOI: 10.1073/pnas.90.17.7937]
11 **Eikenboom JC**. Congenital von Willebrand disease type 3: clinical manifestations, pathophysiology and molecular biology. *Best Pract Res Clin Haematol* 2001; **14**: 365-379 [PMID: 11686105]
12 **Schneppenheim R**, Krey S, Bergmann F, Bock D, Budde U, Lange M, Linde R, Mittler U, Meili E, Mertes G. Genetic heterogeneity of severe von Willebrand disease type Ⅲ in the German population. *Hum Genet* 1994; **94**: 640-652 [PMID: 7989040 DOI: 10.1007/BF00206958]
13 **Zhang Z**, Lindstedt M, Blombäck M, Anvret M. Effects of the mutant von Willebrand factor gene in von Willebrand disease. *Hum Genet* 1995; **96**: 388-394 [PMID: 7557958 DOI: 10.1007/BF00191794]
14 **Lak M**, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. *Br J Haematol* 2000; **111**: 1236-1239 [PMID: 11167767 DOI: 10.1046/j.1365-2141.2000.02507.x]
15 **Eikenboom JC**, Castaman G, Vos HL, Bertina RM, Rodeghiero F. Characterization of the genetic defects in recessive type 1 and type 3 von Willebrand disease patients of Italian origin. *Thromb Haemost* 1998; **79**: 709-717 [PMID: 9569178]
16 **Zhang ZP**, Blombäck M, Egberg N, Falk G, Anvret M. Characterization of the von Willebrand factor gene (VWF) in von Willebrand disease type Ⅲ patients from 24 families of Swedish and Finnish origin. *Genomics* 1994; **21**: 188-193 [PMID: 8088787 DOI: 10.1006/geno.1994.1241]
17 **Baronciani L**, Cozzi G, Canciani MT, Peyvandi F, Srivastava A, Federici AB, Mannucci PM. Molecular characterization of a multiethnic group of 21 patients with type 3 von Willebrand disease. *Thromb Haemost* 2000; **84**: 536-540 [PMID: 11057846]
18 ISTH data base of von Willebrand factor mutations. Available from: URL: http//www.shef.ac.uk/vwf
19 **Baronciani L**, Federici AB, Cozzi G, La Marca S, Punzo M, Rubini V, Canciani MT, Mannucci PM. Expression studies of missense mutations p.D141Y, p.C275S located in the propeptide of von Willebrand factor in patients with type 3 von Willebrand disease. *Haemophilia* 2008; **14**: 549-555 [PMID: 18328061]
20 **Allen S**, Abuzenadah AM, Hinks J, Blagg JL, Gursel T, Ingerslev J, Goodeve AC, Peake IR, Daly ME. A novel von Willebrand disease-causing mutation (Arg273Trp) in the von Willebrand factor propeptide that results in defective multimerization and secretion. *Blood* 2000; **96**: 560-568 [PMID: 10887119]
21 **Lanke E**, Kristoffersson AC, Philips M, Holmberg L, Lethagen S. Characterization of a novel mutation in the von Willebrand factor propeptide in a distinct subtype of recessive von Willebrand disease. *Thromb Haemost* 2008; **100**: 211-216 [PMID: 18690339]
22 **Castaman G**, Lattuada A, Mannucci PM, Rodeghiero F. Factor Ⅷ: C increases after desmopressin in a subgroup of patients with autosomal recessive severe von Willebrand disease. *Br J Haematol* 1995; **89**: 147-151 [PMID: 7833254 DOI: 10.1111/j.1365-2141.1995.tb08921.x]
23 **Castaman G**, Eikenboom JC, Lattuada A, Mannucci PM, Rodeghiero F. Heightened proteolysis of the von Willebrand factor subunit in patients with von Willebrand disease hemizygous or homozygous for the C2362F mutation. *Br J Haematol* 2000; **108**: 188-190 [PMID: 10651743 DOI: 10.1046/j.1365-2141.2000.01807.x]
24 **Castaman G**, Novella E, Castiglia E, Eikenboom JC, Rodeghiero F. A novel family with recessive von Willebrand disease due to compound heterozygosity for a splice site mutation and a missense mutation in the von Willebrand factor gene. *Thromb Res* 2002; **105**: 135-138 [PMID: 11958803 DOI: 10.1016/S0049-3848(02)00007-5]
25 **Tjernberg P**, Castaman G, Vos HL, Bertina RM, Eikenboom JC. Homozygous C2362F von Willebrand factor induces intracellular retention of mutant von Willebrand factor resulting in autosomal recessive severe von Willebrand disease. *Br J Haematol* 2006; **133**: 409-418 [PMID: 16643449 DOI: 10.1111/j.1365-2141.2006.06055.x]
26 **Castaman G**, Bertoncello K, Bernardi M, Eikenboom JC, Budde U, Rodeghiero F. Autosomal recessive von Willebrand disease associated with compound heterozygosity for a novel nonsense mutation (2908 del C) and the missense mutation C2362F: definite evidence for the non-penetrance of the C2362F mutation. *Am J Hematol* 2007; **82**: 376-380 [PMID: 17109387 DOI: 10.1002/ajh.20803]
27 **Castaman G**, Giacomelli S, Rodeghiero F. Autosomal recessive vonWillebrand disease type 1 or 2 due to homozygous or compound heterozygous mutations in the von Willebrand factor gene. *Acta Haematol* 2009; **121**: 106-110 [DOI: 10.1159/000214850]
28 **Denis CV**. Molecular and cellular biology of von Willebrand factor. *Int J Hematol* 2002; **75**: 3-8 [PMID: 11843287 DOI: 10.1007/BF02981972]
29 **Katsumi A**, Tuley EA, Bodó I, Sadler JE. Localization of disulfide bonds in the cystine knot domain of human von Willebrand factor. *J Biol Chem* 2000; **275**: 25585-25594 [PMID: 10831592 DOI: 10.1074/jbc.M002654200]
30 **Schneppenheim R**, Brassard J, Krey S, Budde U, Kunicki TJ, Holmberg L, Ware J, Ruggeri ZM. Defective dimerization of von Willebrand factor subunits due to a Cys-& gt; Arg mu-

tation in type ⅡD von Willebrand disease. *Proc Natl Acad Sci USA* 1996; **93**: 3581-3586 [PMID: 8622978]
31 **Schneppenheim R**, Budde U, Obser T, Brassard J, Mainusch K, Ruggeri ZM, Schneppenheim S, Schwaab R, Oldenburg J. Expression and characterization of von Willebrand factor dimerization defects in different types of von Willebrand disease. *Blood* 2001; **97**: 2059-2066 [PMID: 11264172 DOI: 10.1182/blood.V97.7.2059]
32 **Hommais A**, Stépanian A, Fressinaud E, Mazurier C, Pouymayou K, Meyer D, Girma JP, Ribba AS. Impaired dimerization of von Willebrand factor subunit due to mutation A2801D in the CK domain results in a recessive type 2A subtype ⅡD von Willebrand disease. *Thromb Haemost* 2006; **95**: 776-781 [PMID: 16676067]
33 **Kinoshita S**, Harrison J, Lazerson J, Abildgaard CF. A new variant of dominant type Ⅱ von Willebrand's disease with aberrant multimeric pattern of factor Ⅷ-related antigen (type ⅡD). *Blood* 1984; **63**: 1369-1371 [PMID: 6426551]
34 **Enayat MS**, Guilliatt AM, Surdhar GK, Jenkins PV, Pasi KJ, Toh CH, Williams MD, Hill FG. Aberrant dimerization of von Willebrand factor as the result of mutations in the carboxy-terminal region: identification of 3 mutations in members of 3 different families with type 2A (phenotype Ⅱ D) von Willebrand disease. *Blood* 2001; **98**: 674-680 [PMID: 11468166 DOI: 10.1182/blood.V98.3.674]
35 **Tjernberg P**, Vos HL, Castaman G, Bertina RM, Eikenboom JC. Dimerization and multimerization defects of von Willebrand factor due to mutated cysteine residues. *J Thromb Haemost* 2004; **2**: 257-265 [PMID: 14995987 DOI: 10.1111/j.1538-7836.2003.00435.x]
36 **Tjernberg P**, Vos HL, Spaargaren-van Riel CC, Luken BM, Voorberg J, Bertina RM, Eikenboom JC. Differential effects of the loss of intrachain- versus interchain-disulfide bonds in the cystine-knot domain of von Willebrand factor on the clinical phenotype of von Willebrand disease. *Thromb Haemost* 2006; **96**: 717-724 [PMID: 17139364]
37 **Gallinaro L**, Sartorello F, Pontara E, Cattini MG, Bertomoro A, Bartoloni L, Pagnan A, Casonato A. Combined partial exon skipping and cryptic splice site activation as a new molecular mechanism for recessive type 1 von Willebrand disease. *Thromb Haemost* 2006; **96**: 711-716 [PMID: 17139363]
38 **Castaman G**, Rodeghiero F, Tosetto A, Cappelletti A, Baudo F, Eikenboom JC, Federici AB, Lethagen S, Linari S, Lusher J, Nishino M, Petrini P, Srivastava A, Ungerstedt JS. Hemorrhagic symptoms and bleeding risk in obligatory carriers of type 3 von Willebrand disease: an international, multicenter study. *J Thromb Haemost* 2006; **4**: 2164-2169 [PMID: 16999850 DOI: 10.1111/j.1538-7836.2006.02070.x]
39 **Castaman G**, Eikenboom JC. ABO blood group also influences the von Willebrand factor (VWF) antigen level in heterozygous carriers of VWF null alleles, type 2N mutation Arg854GIn, and the missense mutation Cys2362Phe. *Blood* 2002; **100**: 1927-1928 [PMID: 12211196 DOI: 10.1182/blood-2002-04-1168]
40 **MichielsJJ**, Berneman Z, Gadisseur A, Van Der Planken M, Schroyens W, Van De Velde A, Van Vliet HHDM. Characterization of recessive severe type 1 and 3 von Willebrand disease (VWD), asymptomatic heterozygous carriers versus bloodgroup O-related von Willebrand factor deficiency, and dominant type 1 VWD. *Clin Applied Thromb Hemostas* 2006; **12**: 277-295 [PMID: 16959681]
41 **Collins PW**, Cumming AM, Goodeve AC, Lillicrap D. Type 1 von Willebrand disease: application of emerging data to clinical practice. *Haemophilia* 2008; **14**: 685-696 [PMID: 18510569 DOI: 10.1111/j.1365-2516.2008.01757.x]
42 **Michiels JJ**, Berneman Z, Gadisseur A, van der Planken M, Schroyens W, van Vliet HH. Laboratory diagnosis and molecular basis of mild von Willebrand disease type 1. *Acta Haematol* 2009; **121**: 85-97 [PMID: 19506353 DOI: 10.1159/000214847]
43 **Davies JA**, Collins PW, Hathaway LS, Bowen DJ. Effect of von Willebrand factor Y/C1584 on in vivo protein level and function and interaction with ABO blood group. *Blood* 2007; **109**: 2840-2846 [PMID: 17119126]
44 **Davies JA**, Collins PW, Hathaway LS, Bowen DJ. von Willebrand factor: evidence for variable clearance in vivo according to Y/C1584 phenotype and ABO blood group. *J Thromb Haemost* 2008; **6**: 97-103 [PMID: 17949477 DOI: 10.1111/j.1538-7836.2007.02809.x]
45 **Gadisseur AP**, Vrelust I, Vangenechten I, Schneppenheim R, Van der Planken M. Identification of a novel candidate splice site mutation (0874 + 1G & gt; A) in a type 3 von Willebrand disease patient. *Thromb Haemost* 2007; **98**: 464-466 [PMID: 17721632]
46 **Lethagen S**, Isaksson C, Schaedel C, Holmberg L. Von Willebrand's disease caused by compound heterozygosity for a substitution mutation (T1156M) in the D3 domain of the von Willebrand factor and a stop mutation (Q2470X). *Thromb Haemost* 2002; **88**: 421-426 [PMID: 12353070]

P- Reviewer: Batlle J **S- Editor:** Wen LL
L- Editor: A **E- Editor:** Liu XM

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com