von Willebrand Disease in Siriraj Hospital: Where Are We Now?

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Von Willebrand disease (vWD), was first described by a Finnish physician, Erik A. von Willebrand[1] and since 1924, and has been the most common hereditary bleeding disorder in clinical practice. The index case was a 5-year-old girl presenting with epistaxis and bleeding in her gum. Three of her sisters experienced hypermenorrhea and gastrointestinal bleeding. The pathogenesis of the disease was uncertain for more than fifty years until the discovery of von Willebrand factor (vWF) by Zimmerman TS[2] in 1973 and later four independent groups of researchers[3,4,5,6] were simultaneously able to clone cDNA of vWF in 1985. Even though there is no certain incidence in the general population in Thailand, the incidence of vWD in Thai healthy blood donors[7] is similar to that of Western countries[8] (0.8−1.2% of the general population). So there would be approximately 600,000 people with vWD in our nation.

Biosynthesis of vWF

The vWF gene is located on the short arm of chromosome 12. Even though the vWF gene presents in every organ, the synthesis of vWF is exclusively expressed in endothelial cells and megakaryocytes. The vWF gene is composed of 52 exons, whose size ranges from 40 base pairs to 1.4 kilo bases. After translation, the pre-pro vWF is modified in the rough endoplasmic reticulum. Firstly the signal peptide (the first 18 amino acid residues in the amino terminal end of the pre-pro vWF) is cleaved out. The remaining peptide, namely pro vWF, is glycosylated and sulfated (formed disulfide bonds between C terminus of individual pro vWF molecules) resulting in pro vWF dimers whose molecular weight is 500 kilo Daltons. Prior to releasing into the circulation, the vWF mature subunits are generated by cleaving the propeptide from pro vWF dimers and finally they are multimerized by sulfation in the golgi apparatus.

Hemostatic Function of vWF

The vWF multimer takes an important part in both primary (promote platelet adhesion to denuded subendothelial area) and secondary (proteolytic protection of plasma FVIII) hemostatic functions. As depicted in Fig 1, the mature subunit of vWF is divided into four different domains, named A, B, C and D. There are three A domains, A1, A2 and A3, according to the repeated amino acid residues sequence. The A1 domain posses the binding site of glycoprotein Ib/IX/V complex on the platelet surface. After exposure to connective tissues, the A1 domain is activated and promptly binds to the platelet, causing platelet plug or primary hemostatic plug formation. Besides connective tissues, many substrates or conditions can activate the A1 domain such as ristocetin, botrocetin, high shear and high flow rate (more than 1,500 sec^{-1}). In addition the abnormally large vWF multimer, found in conditions associated with the deficiency of ADAMTS 13 either hereditary or acquired, can spontaneously bind to the platelet. Although the A2 domain does not contain hemostatic function like other domains, the A2 domain contains the binding site of ADAMTS 13 which is the cleaving enzyme of vWF. Some type 2A vWDs are caused by the genetic mutations in this domain resulting in increased proteolysis of the vWF multimer. The last important domain is the D domain which responds for FVIII binding. After released from endothelial cells and megakaryocytes, approximately 100 molecules of vWF in the circulation will bind to one molecule of FVIII. The vWF protects FVIII from proteolysis. The disorders in the D domain result in a very low FVIII level due to shortening of FVIII half life from 12 to 2 hours[9].

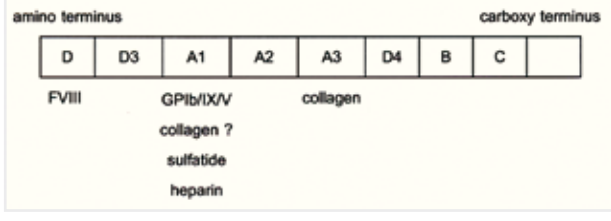

Fig 1. Functional Domain of Mature subunit of vWF.

Although the disorders of A3 domain can cause bleeding, unfortunately the classification of this particular type is at present not available.

Clinical Manifestation

Despite the inherited trait, most of the patients experience bleeding problems after teenage. Only type 3 vWD presents in the early years of life because of severe bleeding. With primary hemostatic defects, the most common bleeding symptoms are mucocutaneous bleeding such as epistaxis, bleeding in the gums, hypermenorrhea and ecchymoses. Presenting with hypermenorrhea, women are more commonly diagnosed with vWD than men. The others suffer from bleeding after and operation. Like hemophilia, besides mucocutaneous bleeding, bleeding from secondary hemostatic defects such as hemarthrosis and muscle hematoma can also be found in type 3 vWD. Recently a systematic bleeding history taking is set by scoring techniques, namely bleeding score. The bleeding scores (in 0-3 scoring techniques) above 3 in men and 5 in women are suggestive of vWD. The use of bleeding score[10] is not only for the diagnostic purpose, but also for the discrimination among normal individuals, carrier of type 1 and carrier of type 3 vWD.

Laboratory Tests

At present, many sophisticate laboratory tests are developed to improve not only the sensitivity of the diagnosis but also the characterization of diseases. The tests are divided into screening and specific tests. Being available in most of the hospitals, screening tests such as complete blood count, bleeding time and activated partial thromboplastin time are not sensitive. Specific tests are designed to analyze vWF in terms of quantity and quality. According to a variety of vWF functions, there are many tests, including vWF: Ag, vWF: Rco, vWF: CB, vWF: FVIIIB and vWF: Multimer, which have been developed to classify all types of vWD. If the degree of suspicion is high, specific tests are needed despite the normal result of screening tests.

Complete blood count (CBC)

According to long-term bleeding tendency, iron deficiency anemia from chronic blood loss may be found. Most of the patients have normal platelet number except type 2B in which thrombocytopenia is the characteristic finding.

Bleeding time (BT)

BT is a global study of both platelet and vascular function. Prolonged bleeding time, resulting from impaired platelet binding against collagen or connective tissue beneath the denuded endothelium is exclusively found in severe cases.

Activated partial thromboplastin time (aPTT)

Protecting FVIII from proteolysis, vWF can influence aPTT. The FVIII binding defects of vWF will somehow cause low FVIII level resulting in prolonged aPTT. Similar to bleeding time, aPTT is not a sensitive test in the diagnosis of vWD.

vWF: Antigen (vWF: Ag)

vWF: Ag, measured by the ELISA technique, of normal individual varies from 50-100 IU/dl. Many physiologic and pathologic conditions such as blood group, age, infection etc have an effect on plasma vWF: Ag level. The elderly have a higher level of vWF: Ag. Individuals with blood group O have a lower vWF: Ag level than those with non-blood group O. Being one of the acute phase reactant proteins, vWF is elevated during inflammation or infection. Therefore repeated examinations of vWF: Ag are required prior to making the definite diagnosis.

vWF: Ristocetin cofactor (vWF: Rco)

With different techniques such as test tube agglutination, platelet aggregation by aggregometer or microtiter plate assay, ristocetin is used to demonstrate the platelet binding ability of the A1-domain by using patient plasma and formaldehyde-fixed platelets from a normal blood group O donor. The normal value of vWF: Rco in plasma is varies from 50-100 IU/dl.

RIPA (Ristocetin-induced platelet aggregation)

The principle of the test is similar to vWF: Rco but both plasma and platelet used in the test system are derived from the patient. Normally platelets can aggregate with ristocetin of 0.6-1.2 mg/dl for final concentration. A low dose RIPA (ristocetin 0.3 mg/dl for final concentration) is positive in solely type 2B vWD, not in normal persons.

vWF: Collagen Binding (vWF: CB)[11]

To study the A3 domain function, collagen is coated to the microtiter plate and the binding between vWF and collagen is evaluated by ELISA techniques. Whereas bleeders with a low vWF: CB are infrequently detected, to date there is no classification of vWD for this particular abnormality.

vWF: Factor VIII Binding (vWF:FVIIIB)[12]

The binding between the D domain of vWF and FVIII can be evaluated by vWF: FVIIIB with ELISA techniques. Purified FVIII is coated to the microtiter plate and afterwards patient's and normal plasma are added to the plate. The amount of vWF left on the plate represents the FVIII binding capacity. The test was developed to diagnose type 2N vWD and to differentiate type 2N vWD from mild hemophilia A. Besides different inherited pattern, vWF: FVIIIB can aid in the definite diagnosis.

vWF: Multimer

Prior to releasing into the circulation, mutimerization of vWF is desirable for adhesive function. The presence of vWF: mutimer can be demonstrated by electrical separation. Loss of vWF: multimer can be found in type 3, type 2B, and type 2A vWD. In type 3 vWD, the multimer is absent, because of a complete inability to generate vWF. Two crucial mechanisms of decreased vWF: multimer in type 2A vWD are the assembly and proteolytic defects. The abnormal assembly during the post-modification process in the rough endoplasmic reticulum and golgi apparatus causes depletion of vWF: multimer both in plasma and in the platelet pallet while the abnormality of the A2 domain results in the increase of proteolysis of vWF immediately after release into the circulation. In the later mechanism, the vWF: multimer in plasma is depleted but the vWF: multimer in platelet pallet is still present. As a result of hyperfunction of the mutant vWF in type 2B vWD, the mutant vWF is used to bind to the platelet, resulting in thrombocytopenia and very low plasma vWF: multimer.

FVIII: C

The vWF prevents FVIII from proteolysis, so the abnormality of vWF will somehow cause a varied low FVIII: C. FVIII: C lower than 10 IU/dl can be found in type 3 and type 2N.

vWD Classification[13,14]

Type 1 is the majority of vWD found all around the world. It is transmitted by an autosomal dominant trait. As the severity of the disease is mild, the type 1 vWD usually presents after teenage. For women, hypermenorrhea is the most common presenting symptom. Superficial ecchymosis, epistaxis and bleeding per gum are also found. Despite no sex selection, most patients are women. Some patients are undiagnosed until they experience profound bleeding after surgery or dental extraction.

Type 2A

The incidence is rare. The patients have normal vWF: Ag but impaired vWF: Rco. No vWF multimer is demonstrated in the plasma. Two main mechanisms are vWF assembly defects and the defects of the A2 domain resulting in increased proteolysis of vWF multimer. Besides plasma vWF multimer assay, platelet vWF multimer assay is needed to characterize the mechanism.

Type 2B

The mutation of type 2B vWD results in gain of function in platelet binding. The characteristic of this type is thrombocytopenia. The patients may be misdiagnosed as idiopathic thrombocytopenic purpura without response to steroids for years. A low dose ristocetin-induced platelet aggregation test is highly recommended if the type 2B vWD is suspected. Normally the final concentration of ristocetin causing platelet aggregation is 0.6-1.2 mg/dl. However, 0.3 mg/dl for the final concentration of ristocetin can induce platelet aggregation in type 2B vWD.

Type 2M

Although the abnormalities of both type 2M and type 2B vWD are within the same domain (A1 domain), the laboratory results are contrary. Mutant vWF of type 2M is unable to bind to the platelet and subsequently leads to severe bleeding. Both vWF: Ag and vWF: multimer are normal, whereas the functional assay including vWF: Rco and RIPA are obviously impaired.

Type 2N

"N" stands for Normandy where the first reported case came from. The abnormality is located in the D domain, resulting in the impaired binding between FVIII and vWF. The FVIII: C level is varied. Some patients have a slightly lower than normal FVIII:C value, while the others have a considerably lower valve as found in hemophilia (5-20 IU/dl). The other tests of vWF such as vWF: Ag, vWF: Rco, RIPA and vWF: CB usually are normal. The pattern of inherited transmission is essential for distinguishing the type 2N vWD from hemophilia.

Type 3

Even though the incidence is very low (1: 1,000,000 in the general population), type 3 vWD is the most severe form. The genetic mutations are either compound heterozygosity or homozygosity. The common mutations are large gene deletions, nonsense mutations and frameshift mutations. Patients usually experience bleeding since the early year of life. Besides mucocutaneous bleeding, musculoskeletal bleeding and hemarthrosis are frequently found. The diagnosis is not difficult because of the obvious abnormalities of all tests of vWF assays such as Ag, Rco and CB which usually are lower than 5 IU/dl with the absence of vwF: multimer.

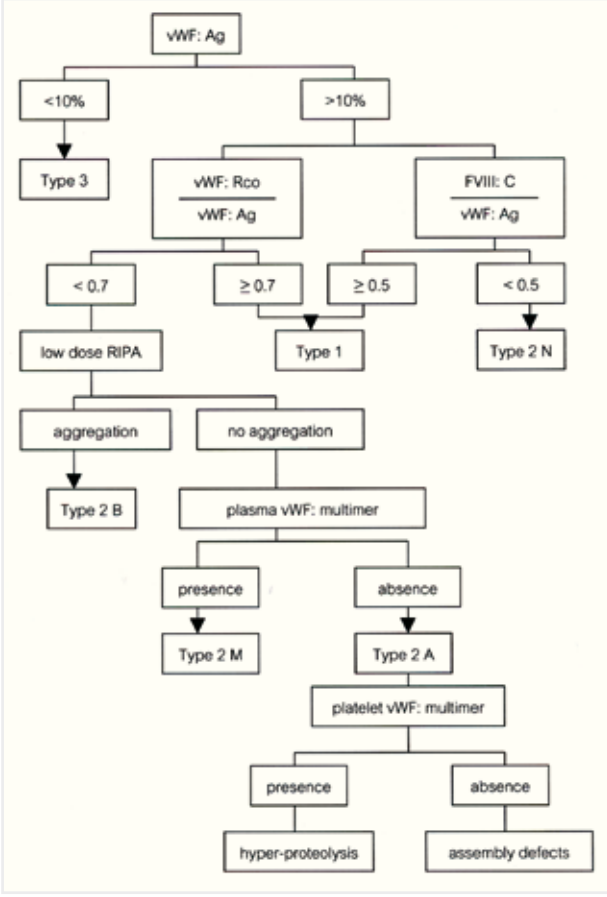

Fig 2. Diagnostic Flow Chart of vWD.

The Division of Hematology, Department of Medicine has set up the vWF: Ag, vWF: Rco and vWF: CB assays since 2002, 2003 and 2007, respectively. Using the cut-off level below 40 IU/dl[15] of vWF: Rco and/or vWF: CB, twenty five of 696 cases (3.6%) with bleeding consultation during 2002-2009, were definitely diagnosed vWD. Twelve of 25 cases were analyzed. All were women with the age of 16-48 years (mean 30). The age of first presentation ranged from 1-26 years of age. Blood groups were O, A and B in 9, 1 and 2 cases respectively. The most common bleeding manifestations were ecchymosis and hypermenorrhea. With the complete set of vWF test panel, three patients were in type 1, five were in type 2, three were in type 3 vWD and one was not able to be characterized, respectively as shown in Table 2.

The first three type 3 vWD patients have experienced severe bleeding since the early years of life and had both a very high bleeding score and the obvious abnormal laboratory results. In case 4, despite a high bleeding score of 9, representing in severe bleeding

TABLE 1. Summary of the laboratory results in different types of vWD.

Type	FVIII:C	vWF: Ag	vWF: Rco or vWF: CB	RIPA	vWF: Multimer
1	Decreased	Decreased	Decreased	Decreased	Presence of all multimer but decreased in concentration
2 A	Normal or decreased	Normal or decreased	Disproportionately decreased when compared to vWF: Ag	Disproportionately decreased when compared to vWF: Ag	Absence
2 B	Normal or decreased	Normal or decreased	Normal or decreased	Platelet aggregation with low dose ristocetiin	Absence
2 M	Normal or decreased	Normal or decreased	Disproportionate decreased compared to vWF: Ag	Disproportionate decreased compared to vWF: Ag	Normal
2 N	Moderately decreased	Normal	normal	Normal	Normal
3	Lower than 10 IU/dl	Absence	Markedly decreased	Markedly decreased	Absence

vWF: Ag, von Willebrand factor antigen; vWF: Rco, von Willebrand factor ristocetin cofactor; vWF: CB, von Willebrand factor collagen binding assay; RIPA, ristocetin induced platelet aggregation assay

symptoms, the vWF: Ag and vWF: Rco were nearly normal. However vWF: CB result revealed a very low level. Without vWF: CB in the panel of tests, this particular type of disease will not be diagnosed. Besides type 3 vWD, very low FVIII: C could be found in another two siblings that were definitely not type 3 vWD (case 11 and 12). Both patients had normal vWF: Ag. The ratio of FVIII: C to vWF: Ag ratio was lower than 0.5, suggestive of type 2N vWD. Persistent unexplained low vWF: Rco were revealed in both cases. Therefore, their mother (case 10) was tested and, similar to her offspring, a low vWF: Rco was detected. It is possible that she was type 2A vWD and her offspring were the combination of type 2A and type 2N vWD. Unfortunately, her husband's blood is not available for the vWF test panel. The vWF: FVIIIB test and the analysis of genetic mutations were further needed to characterize the type of vWD in this family.

CONCLUSION

To date, the roles of vWF are crucial not only in vWD but also in many acquired diseases or conditions such as thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) and atherosclerosis. The knowledge of vWD is in progress and has been more elucidated in terms of pathogenesis, diagnosis, classification and treatment during the past eighty years. Careful and systematic bleeding history assessment with bleeding score and the full panel of investigations will provide the definite diagnosis and type of disease. The higher incidence of vWD including type 3 in our institute than those previously reported in the general population implies the effect of high pre-test probable subjects selected for the study.

REFERENCES

1. von Willebrand EA. Hereditär pseudohamofili. Fin Lakaresallsk Handl. 1926;67: 7-12.
2. Zimmerman TS, Edgington TS. Factor VIII coagulant activity and factor VIII-like antigen: independent molecular entities. J Exp Med. 1973 October 1;138(4):1015-20.
3. Lynch DC, Zimmerman TS, Collins CJ, Brown M, Morin MJ, Ling EH, et al. Molecular cloning of cDNA for human von Willebrand factor: authentication by a new method. Cell. 1985 May;41(1):49-56.
4. Ginsburg D, Handin RI, Bonthron DT, Donlon TA, Bruns GA, Latt SA, et al. Human von Willebrand factor (vWF): isolation of complementary DNA (cDNA) clones and chromosomal localization. Science. 1985 Jun 21;228(4706):1401-6.
5. Sadler JE, Shelton-Inloes BB, Sorace JM, Harlan Jm, Titani K, Davie EW. Cloning and characterization of two cDNAs coding for human von Willebrand factor. Proc Natl Acad Sci U S A. 1985 Oct;82(19): 6394-8.
6. Verweij CL, Hofker M, Quadt R, Briet E, Pannekoek H. RFLP for a human von Willebrand factor (vWF) cDNA clone, pvWF1100. Nucleic Acids Res. 1985 Nov 25;13(22):82-89.

TABLE 2. Demographic information of the documented 12 cases of vWD.

Case	Age (yr)	BS	FVIII:C	vWF: Ag	vWF: Rco	FVIII: C vWF: Ag	vWF: Rco vWF: Ag	vWF: CB	Type
1	18	12	9	2	3	4.5	1.5	3	3
2	35	7	1	4	5	0.3	1.3	5	3
3	39	10	2	3	9	0.7	3.0	13	3
4	34	9	41	35	35	1.2	1.0	1	NA
5	30	4	36	11	14	3.3	1.3	21	1
6	23	8	45	21	35	2.1	1.7	27	1
7	27	8	46	40.6	47	1.1	1.2	40	1
8	44	5	42	44	20	0.9	0.5	36	2
9	38	4	60.5	68.7	39.2	0.9	0.6	NA	2
10	48	0	96	78.6	34.3	1.2	0.4	136	2A
11	17	9	3.4	44.8	18.6	0.1	0.4	60	2N
12	16	2	4.3	41.2	25.8	0.1	0.6	72	2N

BS, bleeding score; NA, not available

7. Rojnuckarin P, Akkawat B, Intragumtornchai T. Von Willebrand factor (vWF) antigen levels and function in healthy Thais. Southeast Asian J Trop Med Public Health. 2005 Sep;36(5):1292-7.
8. Werner EJ, Broxson EH, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: a multiethnic study. J Pediatr. 1993 Dec;123(6):893-8.
9. Vlot AJ, Koppelman SJ, Bouma BN, Sixma JJ. Factor VIII and von Willebrand factor. Thromb Haemost. 1998 Mar;79(3):456-65.
10. Rodeghiero F, Castaman G, Tosetto A, Batlle J, Baudo F, Cappelletti A, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. J Thromb Haemost. 2005 Dec;3(12):2619-26.
11. Favoloro EJ, Grispo L, Exner T, Koutts J. Development of a simple collagen based ELISA aids in the diagnosis of and permits sensitive discrimination between type 1 and type 2 von Willebrand diseases. Blood Coagul Fibrinolysis. 1991 Apr;2(2):285-91.
12. Favaloro EJ, Mohammed S, Koutts J. Identification and prevalence of von Willebrand disease type 2N (Normandy) in Australia. Blood Coagul Fibrinolysis. 2009 Oct 3.
13. Sadler JE, Budde U, Eikenboom JC, Favaloro EJ, Hill FG, Holmberg L, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost. 2006 Oct;4(10):2103-14.
14. Ginsburg D. Molecular Genetics of von Willebrand Disease. Thromb Haemost. 1999 Aug;82(2):585-91.
15. Tosetto A, Rodeghiero F, Castaman G, Bernardi M, Bertoncello K, Goodeve A, et al. Impact of plasma von Willebrand factor levels in the diagnosis of type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1VWD). J Thromb Haemost. 2007 Apr;5(4):715-21.