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Genotypic classification of von Willebrand disease

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A B S T R A C T

Many mutations in the von Willebrand factor (VWF) gene associated with von Willebrand disease (VWD) have been reported. To examine the profile of mutations reported for each VWD type, two sources of information were consulted. The International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC) on von Willebrand factor has an electronic database to list VWF mutations; www.shef.ac.uk/vwf to which all researchers are invited to submit mutations that they have identified. The 307 mutations submitted to the database were classified by VWD type, VWF location, and by mutation type to seek common features within each disease type and characteristics discriminating the different disease types. Type 2 VWD subtypes are characterized by missense mutations in discrete areas of VWF, whereas 80% of type 3 VWD mutations are predicted to result in non-expressed alleles located throughout VWF. Remaining type 3 mutations are missense and these are predominantly located in the D1 and D4-CK domains, none are to date reported in the A domains. Few mutations associated with type 1 VWD have been submitted to the database. The European Union collaborative study on type 1 von Willebrand disease; Molecular and Clinical Markers for the Diagnosis and Management of type 1 VWD (MCMDM-1VWD) has investigated 153 index cases originally classified as having type 1 VWD for mutations in the VWF gene. 80% of the mutations are missense, these are located throughout VWF. Common features of mutation type and location in each VWD type can therefore be identified and may be useful for directed mutation analysis

Key words: von Willebrand factor, von Willebrand disease, classification.

Von Willebrand disease (VWD) is a common autosomally inherited bleeding disorder that results from quantitative or qualitative deficiency of von Willebrand factor, a large multimeric plasma glycoprotein. VWF plays two important roles in haemostasis. Firstly, it promotes platelet adhesion to exposed subendothelium at high shear stress and secondly, it acts as a carrier for coagulation factor VIII (FVIII), protecting it from proteloytic degradation. These important functions may be disrupted by mutation in the VWF gene. Clinically significant forms of VWD affect at least 100 per million of the population (reviewed in Castaman *et al.*[1] VWD is classified into three types.[2] Type 1 VWD is a partial quantitative deficiency, generally demonstrating dominant inheritance and type 3 VWD is a virtually complete quantitative deficiency of VWF, inherited recessively. Type 2 VWD comprises qualitative VWF deficiencies and is divided into four subtypes; 2A, B, M and N. Type 2A shows a loss of high molecular weight (HMW) multimers associated with decreased platelet dependant function; type 2B is associated with enhanced interaction with platelet GpIb; type 2M VWD has reduced GpIb interaction with no loss of HMW multimers. In these three disease subtypes, except for a small subgroup of type 2A VWD, inheritance is dominant. In 2N VWD, the interaction of VWF with FVIII is affected. The disease is recessively inherited and one or both alleles express VWF that has an absent or markedly reduced FVIII binding capacity.

The VWF gene covers 178kb of genomic DNA on chromosome 12. The 52 exon gene encodes an 8.8 kb mRNA which is translated into a 2813 amino acid (aa) pre-pro VWF. During processing, the 22 aa signal peptide and 741 aa propeptide are cleaved from the 2050 aa mature VWF. Molecular analysis of VWD began in the late 1980s as soon as cDNA and partial genomic DNA sequences

for VWF became available.[3,4] An extensive literature on characterisation and *in vitro* expression of VWF mutations now exists and to collate this information, the ISTH SSC on VWF established an electronic database to list mutations and polymorphisms in the mid 1990s. The database now holds several hundred entries on VWF sequence alterations associated with all three types of VWD. Type 1 VWD has until recently remained largely uncharacterised at the molecular level, however, several current multi-centre studies are beginning to elucidate the molecular basis of type 1 VWD.

This article will analyse the nature and location of reported VWF mutations to seek common features within each VWD type and to seek differences discriminating the different VWD types. It will examine the profile of mutations submitted to the ISTH VWF SSC database and summarise properties of mutations identified in the European Union multi-centre study; *Molecular and clinical markers for the diagnosis and management of type 1 von Willebrand disease (MCMDM-1VWD)* in patients diagnosed with type 1 VWD.

Methods

The ISTH VWF SSC electronic database comprises mutation entries submitted by investigators worldwide (*www.shef.ac.uk/vwf*). Some older database entries were submitted on multiple families sharing the same VWF mutation, new entries are encouraged, and should summarize data on a single affected family. 307 mutation entries on the Excel sheet downloaded from the web-site on 1st June 2004 were analysed. In addition, interim mutation data was available from the MCMDM-1VWD study. 153 index cases diagnosed with type 1 VWD were analysed for mutations. 124 candidate mutations were identified and brief details are included in this analysis. Mutations were analysed by type and VWF location, and by associated VWD type. Common features are described below.

Genotypic classification of VWD

A summary of the data in the 307 ISTH VWF database entries is shown in Table 1. Database submissions strongly reflect VWD mutation analysis carried out to date, and the relative frequency of different disease types reported does not correspond with their relative population frequencies.

Type 1 VWD

Mutations in type 1 VWD are poorly represented on the ISTH VWF database to date.[14] Mutations have been submitted, comprising two small deletions resulting in frameshift mutations, one nonsense and 11 missense mutations. Eikenboom and colleagues reported mutations predicted to result in C1130F and C1149R in classic dominantly inherited type 1 VWD.[5] In 2003, O'Brien and colleagues reported that Y1584C, previously reported on the VWF database as a polymorphism was commonly associated with type 1 VWD, and was found in 14% of 70 Canadian families.[6] T1156M is the only mutation reported twice. Missense mutations are reported in D3, A1-2 and CK domains. Of the database entries to date, 80% are missense mutations. This contrasts sharply with type 3 VWD mutation type (*below*).

Table 1. Summary of mutation entries on the ISTH VWF SSC mutation database as of 1st June 2004.

VWD type	*No. entries*	*No. different mutations*	*No. (%) missense mutations*	*Domain(s)*	*Exon(s)*
1	14	13	11 (79)	D3, A2,CK	26,28,52
2A	71	50	66 (93)	D2,A1-A2,CK	12-16,28 52
2B	52	16	51 (98)	A1-A2	28
2M	18	17	17 (94)	D3, A1	27-28
2N	37	16	37 (100)	D'- D3	18-20,24
3	85	72	15 (18)	D1-CK	3-52
U	30	29	27 (90)	D1-CK	4-52
Total	307	213	224 (73)	D1-CK	3-52

Type 2A VWD

2A VWD is the second largest class of mutations on the database. 71 mutations have been submitted, these comprise 66 missense (93%), two small in-frame deletion or insertion mutations and three small deletions resulting in frameshift mutations (two of which are associated with recessively inherited type 2A VWD). Mutations are summarized in Table 2. They occur in very discrete areas of VWF; a small number of reported missense mutations lie in the D2 domain (exons 12-15) and are reported in recessively inherited type 2A VWD (previous subtype IIC). Affected individuals are either homozygous or compound heterozygous, the second allele being null. A second small group of seven mutations lie in the amino terminal of the A1 domain between amino acids 1272 and 1383. The predominant location for mutations is in the A2 domain between amino acids 1503 and 1672 (53 of 71 mutations, 75%). The most common mutations are seen at S1506L (11% of 2A), R1597, where mutation to four different replacement amino acids (G, L, Q and W) has been reported (21%) and G1609R (4%). Together, these mutations represent 37% of type 2A VWD. Three mutations are reported in exon 52 (CK domain) to be

Table 2. Summary of the location of 71 mutations associated with type 2A VWD.

Previous subtype	*Exon*	*Domain*	*Codons*	*No. (%) reports*
IIC	12-15	D2	404-625	8 (11)
	28	A1	1272-1383	7 (10)
	28	A2	1503-1672	53 (75)
IIE	52	CK	2773fs*	1 (1)
IID	52	CK	2773-2801	2 (3)
Total				71

**Frameshift mutation.*

associated with previous subtypes IID and IIE. Type IID results from recessively inherited missense mutations and leads to a dimerisation defect. Type 2A mutations are thus located in four discrete VWF locations and are predominantly dominantly inherited missense mutations, while 10% are associated with recessively inherited VWD.

Type 2B VWD

52 type 2B mutations have been submitted to the database to date. These comprise a very discrete group of mutations; 20 different missense changes plus one in frame insertion are reported, all within the A1 domain affecting amino acids 1266-1461. Changes occur at only 14 different amino acids, highlighting the importance of these residues in the gain of function 2B mutation. A high proportion of mutations affect one of four amino acids; R1306W (19% of 2B), R1308C (12%), V1316M (17%) and R1341Q (13%). Together, these four common mutations comprise 61% of all type 2B mutations. Their occurrence highlights the importance of certain residues, particularly amino acids 1304-1316, where mutations affecting 8 of the 13 residues have been reported. Type 2B mutations are thus dominantly inherited missense mutations located in the VWF A1 domain.

Type 2M VWD

Only 18 2M mutations have been submitted to the database to date, these comprise 17 missense mutations (94%) and one in-frame deletion. Mutations affect codons R1205H (Vicenza mutation) to P1467S and affect the D3, A1 and amino terminal of the A2 domains. There are no particularly common mutations reported to date, mutations only recur at two codons; G1342S/A and two reports of I1425S. Two patients are compound heterozygous, one with a second missense mutation and the other with a null allele. Type 2M mutations are thus largely dominantly inherited missense mutations located in the VWF A1 domain.

Type 2N VWD

37 entries to the database include mutations in exons 18-20 and 24, in the D' and D3 domains, which affect codons 782-1060. The mutations include 16 different missense mutations affecting only 15 different amino acids. Approximately 50% of patients on the database are compound heterozygous for a 2N plus a null allele, but these second mutations are often not identified. The remainder are either homozygous, or compound heterozygous for a second missense FVIII binding mutation. A high proportion of mutations affect only one of three amino acids; T791M (14% of 2N), R816W (11%) and R854Q (32%), these three account for 57% of all database entries. FVIII binding defects therefore result from missense mutations in the D' and amino terminal of the D3 domains.

Type 3 VWD

85 mutations are currently listed on the database, they occur throughout VWF from codons 47-2804. These are predominantly mutations predicted to result in null alleles (82%). Mutations comprise 34% nonsense, 23% small deletion, 11% splice site defects, 7% small insertion, 7% large deletion and 18% missense mutations. The missense mutations are located towards the amino and carboxyl terminals of VWF; 40% in the D1 domain and 47% in D4-CK. To date, no missense mutations lie in the A domains, so it is possible that missense mutation in the A domains will result in a qualitative, rather than quantitative VWF defects. Type 3 VWF mutations are thus predominantly null alleles, which are located throughout VWF.

Unclassified VWD

30 mutations were considered by the investigators who submitted them not to fit the current VWD classification. These comprise 27 missense and three insertion or deletion mutations, two of which are in-frame. Notable groups of mutations include seven affecting the D3 domain (codons 1101-1196), with one of these reporting a IIQ phenotype, two reports of Vicenza mutations (R1205H/L), which should be reported as 2M VWD (2) and five mutations affecting R1374 and resulting in replacement by C/H/L/S. The latter mutations demonstrate pleotrophic features. The unclassified mutations illustrate where the current nomenclature may need a little revision to include them within it.

Molecular and clinical markers for the diagnosis and management of type 1 VWD

124 candidate mutations have been reported in 153 patients originally diagnosed with type 1 VWD to date. No attempt has yet been made to reclassify the patients/mutations into different VWD types. Of these patients, 84 were heterozygous for a single missense mutation (55%) and 19 had more than one mutation (12%), some of which were allelic and some as compound heterozygotes. The remaining 33% lacked a detectable mutation. The 124 candidate mutations included 81% missense changes and 15% possible null alleles. This contrasts with type 3, where only 20% mutations are missense and 80% are null alleles. The majority of type 1 VWD patients thus do not appear to be carriers of type 3 mutations. Missense mutations were found throughout VWF and affected exons 2-52. Unlike type 2 VWD, the majority of missense mutations were not clustered in specific domains.

A number of missense mutations were found recurrently, the most common was Y1584C, seen in 8% of index cases.

Analysis of mutations in the VWF gene associated with the different VWD types therefore enables conclusions to be drawn regarding the type and distribution of mutations. Common features may inform future VWD diagnosis made using molecular characterisation and highlight areas of the VWF gene to be investigated when seeking mutations associated with particular VWD types.

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