Diagnosis and Management of von Willebrand Disease

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Aland Islands from Space:  6,500 islands
Ted Zimmerman  1937 - 1988
Immunologic differentiation of classic hemophilia (factor 8 deficiency) and von Willebrand’s disease, with observations on combined deficiencies of antihemophilic factor and proaccelerin (factor V) and on an acquired circulating anticoagulant against antihemophilic factor

Zimmerman TS, Ratnoff OD, Powell AE.

J Clin Invest. 1971 Jan;50(1):244-54
Determination of the von Willebrand’s disease antigen (factor VIII-related antigen) in plasma by quantitative immunoelectrophoresis

Zimmerman TS, Hoyer LW, Dickson L, Edgington TS.

VWF Gene: Chromosome 12p

175 kb
von Willebrand Factor Structure

VWFpp
740 AA

VWF mature subunit
2050 AA

D Assembly
Composition

VWD-C8-TIL-E

The Mature VWF Subunit with Associated Ligands

- FVIII
- P-selectin
- VWFpp
- β2 integrins
- ADAMTS13
- αIIbβ3
- αvβ3

Lenting et al. JTH 2012

D’D
A1
A2
A3
D4
C1
C2
C3
C4
C5
C6
C K

GPIbα
Collagen VI
OPG
PSGL-1
β2GPI
Collagen I
Collagen III
TSP1

β2 integrins
ADAMTS13
VWFpp
P-selectin
FVIII

VWF Functions

- Atherothrombosis
- Platelet adhesion/aggregation
- Venous thrombosis
- Inflammation
- Cell proliferation/apoptosis
- Angiogenesis

Lenting et al. JTH 2012
Von Willebrand Disease Classification
(ISTH 2006)

- Qualitative Variants
  - Type 1
  - Type 2
  - Type 3

- Quantitative Variants
Von Willebrand Disease Classification (ISTH 2006)

Qualitative Variants

Type 1

Type 2

Type 3

20-35%

65-80%

1 per million

Quantitative Variants
ISTH 2006 VWD Classification

Type 2 - qualitative traits

\[2A, 2B, 2M, 2N\]

\(~20-35\%\)
The Diagnosis of von Willebrand Disease

• Personal History of excessive mucocutaneous bleeding

• Laboratory findings of
  • VWF deficiency
  • VWF dysfunction

• Family history of von Willebrand disease
Recent Evolution of Bleeding Assessment Tools

- 2005 Vicenza
  - 0 to +3
  - 40 min

- 2006 MCMDDM-1VWD
  - -1 to +4
  - 40 min

- 2008 Condensed MCMDDM-1VWD
  - -1 to +4
  - 10 min

- 2009 PBQ
  - -1 to +4
  - 20 min

- 2010 ISTH BAT
  - 0 to +4
  - 20 min

Rydz and James Nov 2012 JTH
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epistaxis</td>
<td>No or trivial (less than 5)</td>
<td>&gt; 5 or more than 10'</td>
<td>Consultation only</td>
<td>Packing or Cauterization or Antifibrinolytic</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>No or trivial (&lt;1 cm)</td>
<td>&gt; 1 cm and no trauma</td>
<td>Consultation only</td>
<td>Surgical hemostasis</td>
</tr>
<tr>
<td>Bleeding minor wounds</td>
<td>No or trivial (less than 5)</td>
<td>&gt; 5 or more than 5'</td>
<td>Consultation only</td>
<td>Surgical hemostasis or Antifibrinolytic</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>No</td>
<td>Referred at least one</td>
<td>Consultation only</td>
<td>Surgical hemostasis or Antifibrinolytic</td>
</tr>
<tr>
<td>GI bleeding</td>
<td>No</td>
<td>Associated with ulcer, portal hyp., hemorrhoids, angiodysplasia</td>
<td>Spontaneous</td>
<td>Surgical hemostasis, Blood transf, Replacement therapy, Desmopressin</td>
</tr>
<tr>
<td>Tooth extraction</td>
<td>No bleeding in at least 2 extraction</td>
<td>None done or no bleed. in 1 extraction</td>
<td>Referred in &lt;25% of all procedures</td>
<td>Resuturing or packing</td>
</tr>
<tr>
<td>Surgery</td>
<td>No bleeding in at least two surgeries</td>
<td>None done or no bleed. in 1 surgery</td>
<td>Referred in &gt;25% of all procedures, no intervention</td>
<td>Surgical hemostasis or Antifibrinolytic</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>-</td>
<td>No</td>
<td>Consultation only</td>
<td>Antifibrinolytics, Pill use</td>
</tr>
<tr>
<td>Post-partum hemorrhage</td>
<td>No bleeding in at least two deliveries</td>
<td>No deliveries or no bleeding in 1 delivery</td>
<td>Consultation only</td>
<td>D &amp; C, Iron therapy</td>
</tr>
</tbody>
</table>

Blood transf or Replacement therapy or Desmopressin
Utility of Bleeding Assessment Tools

1. Facilitate caregiver communication concerning severity of bleeding phenotype.

2. Justification for intensity of laboratory investigation.
The Diagnosis of von Willebrand Disease

Standardized measurement of

VWF:Ag

VWF:RCo
The Diagnosis of von Willebrand Disease

VWF Measurement

1. Use of appropriate plasma standard

2. May often require repeat testing
Hemostasis Laboratory Diagnosis of VWD

- VWF:Ag
- VWF:RCo
- FVIII:C

1. VWF multimer analysis
2. RIPA
3. VWF:F8
4. VWF:CB
5. VWFpp
Platelet Binding VWF Mutants

1. Loss-of function Type 2A (abnormal multimers)

2. Loss-of function Type 2M (normal multimers)

3. Gain-of-function Type 2B
VWF Multimer Analysis
Type 2A von Willebrand Disease

Loss of Platelet-dependent Function
( abnormal multimers)

>55 missense mutations

\[ \text{VWF:RCo/VWF:Ag} < 0.6 \]
Location of Type 2 von Willebrand Disease Mutations
Type 2A VWD – Treatment Options

DDAVP trial justified

but responsiveness < 10%

(include a 4 hr time point – accelerated proteolysis)
Location of Type 2 von Willebrand Disease Mutations
Location of Type 2 von Willebrand Disease Mutations

A3 Domain: Collagen Binding Mutants

- S1731T
- W1745C
- S1783A
- H1786D

Ribba et al., Thomb Haemost 2001
Riddell et al., Blood 2009
Flood et al., JTH 2010
Type 2M VWD

18 A1 domain missense mutations
Differentiation of Type 2M and Type 1 VWD

VWF:RCo : VWF:Ag

0.3 0.5 0.7 1

Type 2M VWD
A1 domain missense mutns
DDAVP response less likely

Type 1 VWD
Heterogeneous mutns
DDAVP response likely
Type 2B VWD

24 A1 domain missense mutations
Type 2B VWD Phenotypic Heterogeneity
(Federici et al Blood 2009)

67 type 2B VWD patients

11 type 2B A1 domain missense mutations

30% resting thrombocytopenia - ~60% with “stress”

Bleeding score
Clinical bleeding

Inverse relation to platelet count
Type 2N von Willebrand Disease

VWF:Ag 0.52 IU/mL
VWF:RCo 0.48 IU/mL
FVIII:C 0.22 IU/mL
Genetic Mechanisms in Type 2N VWD

Missense 1 + Missense 1

Missense 1 + Missense 2

Missense 1 + Null

Homozygosity

Compound Heterozygosity

Compound Heterozygosity
Location of Type 2 von Willebrand Disease Mutations
Type 2N von Willebrand Disease

Recurrent Type 2N VWD Mutations

R854Q \( \gg \) R816W, T791M

\[
\begin{align*}
FVIII:C & \sim 20\% \quad \text{(DDAVP +ve)} \\
FVIII:C & \sim 10\% \quad \text{(DDAVP -ve)}
\end{align*}
\]
Type 3 VWD - Autosomal Recessive Trait
Type 3 VWD - Autosomal Co-Dominant
Genetic Patterns in von Willebrand Disease

Type 2A, 2B and 2M VWD:

- Dominant traits
- Monogenic – VWF gene
- Fully penetrant
- Type 2B phenocopy: PT-VWD - GPIBA
Type 3 and 2N VWD:

- Recessive traits
- Monogenic – VWF gene
Genetic Patterns in von Willebrand Disease

Type 1 VWD:

- Dominant trait
- Oligogenic – \textit{VWF}, \textit{ABO}, other loci
- Variable penetrance and expressivity
Proportion of Type 1 VWD with a Candidate VWF Gene Mutation

- MCMDM-1VWD: 105/150, 70%
- Canada: 78/123, 63%
- UKHCDO: 19/32, 59%
- ZPMCB-VWD: 111/179, 62%
- University Clinic Bonn: 19/28, 68%

332/512, 65%
Results from Recent Type 1 VWD Studies

1. “Candidate” VWF mutations in 65% of index cases

2. >100 different VWF gene mutations

3. Missense mutations predominate

4. ~15% of cases have >1 mutation
R924Q – Confused Identity

Polymorphism

Type 2N
Type 2M
Type 1

Pathogenic Mechanism??
VWF Sequence Variants: ? Functional Significance

In vitro evaluation
(biosynthesis/storage(secretion)

- heterologous (HEK293 – pseudo WPBs)
- autologous (BOECs)

VWF Sequence Variants: ? Functional Significance

In vivo assessment

Animal models
i) Dog
ii) Mouse
iii) Zebrafish

Patient studies
i) Pt. numbers
ii) Genetic modifiers
iii) Ethics
Blood Outgrowth Endothelial Cell (BOEC)
R924Q VWF Variant in Patient-derived BOECs
Type 1C von Willebrand Disease

1. Dominant (highly penetrant)

2. ~15% of Type 1 VWD

2. Missense mutations (eg. R1205H)

2. Role of VWFpp/VWF:Ag testing -> 3.0

3. Excellent, but short lived DDAVP response
Type 1C Comprises the Majority of Severe Type 1 VWD Cases

- Reduced Secretion
- Increased Clearance

76% 38% 7%

%-age of quadrant consistent with type 1C phenotype
Penetrant VWF Mutations

Incompletely Penetrant VWF Mutations

ABO Blood Group

VWF SNP Combinations

Major Effect on VWF Level

Minor Effect on VWF Level

Prevalence of Variant

Rare

Frequent
Quantitative VWF Defects

- Gene expression
- Biosynthetic processing
- Constitutive secretion
- Storage and regulated secretion
- VWF clearance
  a) Differential glycan additions
  b) VWF misfolding
- Receptor-mediated clearance
VWD Mutation Database
(www.vwf.group.shef.ac.uk)

399 unique VWF mutations

VWF mutation detection rate

Type 1 ~65%  Type 2 A,B,M,N ~90%, Type 3 ~85%

127  type 1
75   type 2A
25   type 2B
29   type 2M
31   type 2N
112  type 3

181 VWF polymorphisms
VWF mutations and new sequence variations identified in healthy controls are more frequent in the African-American population.


184 Healthy Controls

i) 118 Whites
ii) 66 African-Americans

35 sequence variants identified (21 new)

34% of this healthy population had VWF variations

65% of African-Americans (63% >1)
17% of Whites (10% >1)
Ethnic variances: mutation vs polymorphism

Penetrant VWF Mutations

Incompletely Penetrant VWF Mutations

+ Additional, non-VWF loci

ABO Blood Group

VWF SNP Combinations

Major

Effect on VWF Level

Minor

Rare

Frequent

Prevalence of Variant
Novel Associations of Multiple Genetic Loci With Plasma Levels of Factor VII, Factor VIII, and von Willebrand Factor

The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium

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Novel Genetic Loci Associated with VWF

**Vesicular trafficking and exocytosis**

STXBP5  Syntaxin binding protein 5  
STX2     Syntaxin 2

**Receptor proteins**

SCARA5  Scavenger receptor class A member 5  
STAB2    Stabilin 2  
CLEC4M   C-type Lectin Type 4 Member M
Treatment of von Willebrand Disease

1. Desmopressin
   - Therapeutic trial (1, 2 and 4 hrs)
   - Genetic prediction of response

1. VWF concentrates
   - pd-VWF/FVIII concentrates
   - rVWF
von Willebrand Disease: Treatment

1. Desmopressin
2. pd-VWF/FVIII concentrates
3. Antifibrinolytics
4. High purity pd-VWF concentrates
5. rVWF/rFVIII (2011 - )
## pd-VWF/FVIII Concentrates Licensed in Europe

<table>
<thead>
<tr>
<th>Product</th>
<th>VWF:RCo/Ag</th>
<th>VWF:RCo/FVIII</th>
</tr>
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<tbody>
<tr>
<td>Alphanate</td>
<td>0.47</td>
<td>0.91</td>
</tr>
<tr>
<td>Factor 8Y</td>
<td>0.29</td>
<td>0.81</td>
</tr>
<tr>
<td>Fanhdhi</td>
<td>0.47</td>
<td>1.04</td>
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<tr>
<td>Haemate P</td>
<td>0.59</td>
<td>2.45</td>
</tr>
<tr>
<td>Immunate</td>
<td>0.47</td>
<td>1.1</td>
</tr>
<tr>
<td>Wilate</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>Wilfactin</td>
<td>~0.95</td>
<td>~50</td>
</tr>
</tbody>
</table>

Castaman et al. Haematologica 2013
Recombinant VWF (Baxter BioScience)

1. CHO cell derived
2. VWF:RCo/FVIII:C ratio  1.3:1

1st in-human clinical study of the safety, tolerability and pharmacokinetics of rVWF

32 subjects  (Mannucci et al. Blood June 18th 2013)
VWF:FVIII Concentrates

All safe and effective

Things we don’t know -

• Influence of different VWF:FVIII ratios?

• HMW multimer content differences?

• Dosing by FVIII or VWF:RCo?

• Effect of transient supranormal FVIII levels?

• Optimal prophylaxis regimens?