Unrevealing the Complexities of Type 1 von Willebrand Disease Diagnosis

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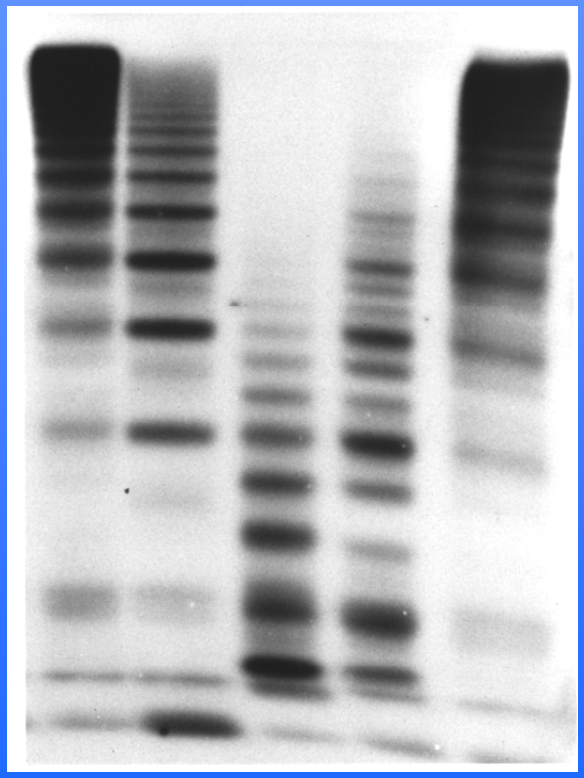
Early Classification of VWD

- Type 1 - Mild
- Type 2 - Moderate to severe
- Type 3 - Severe

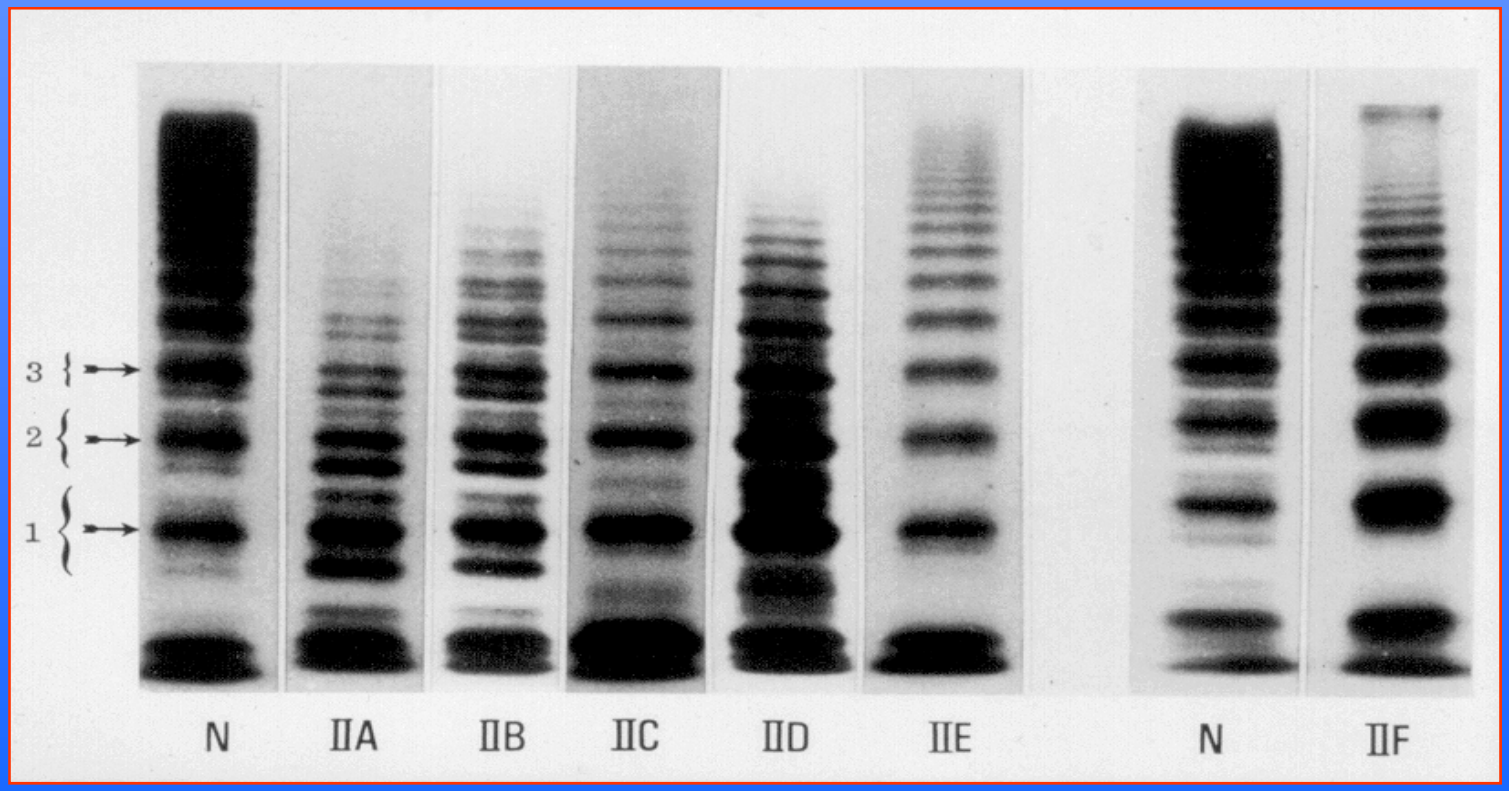
Multimer analysis of VWF



NP 2A (IID) 2A 2B NP

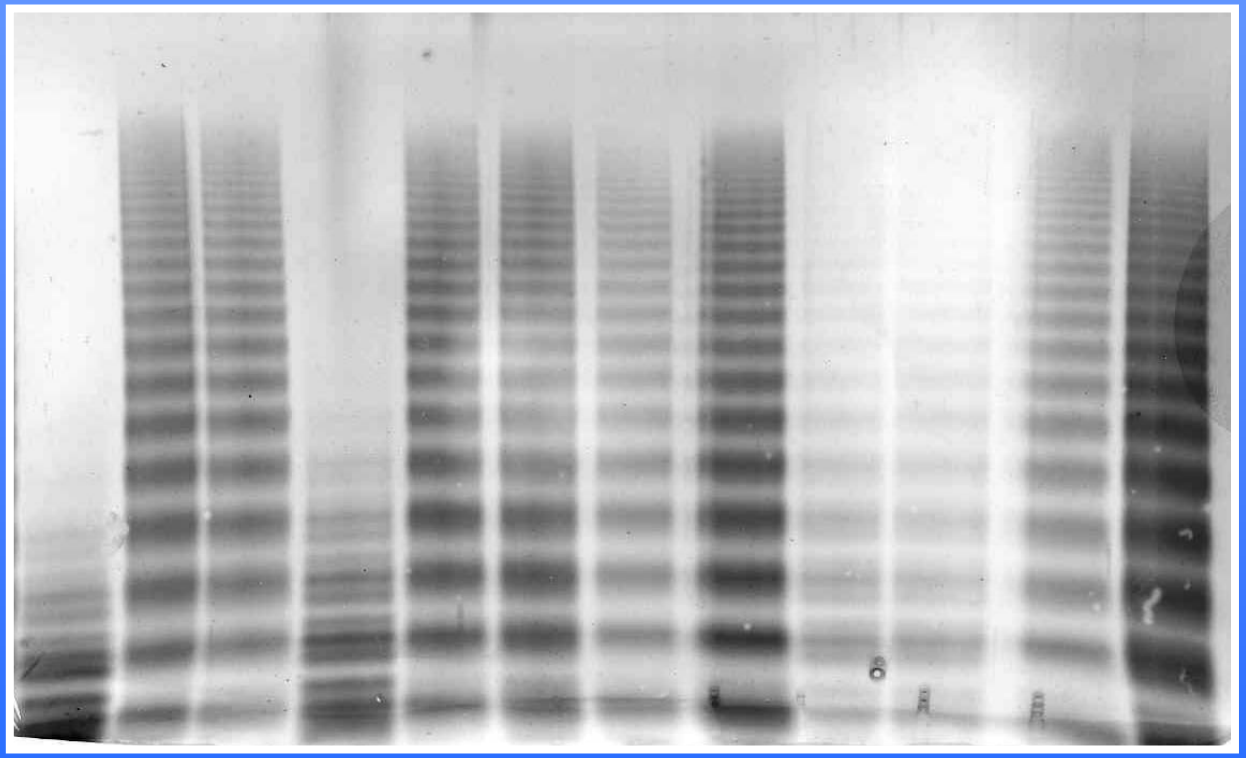
Band visualization with autoradiography

VWF Multimer pattern in different types of VWD



Band visualization with autoradiography

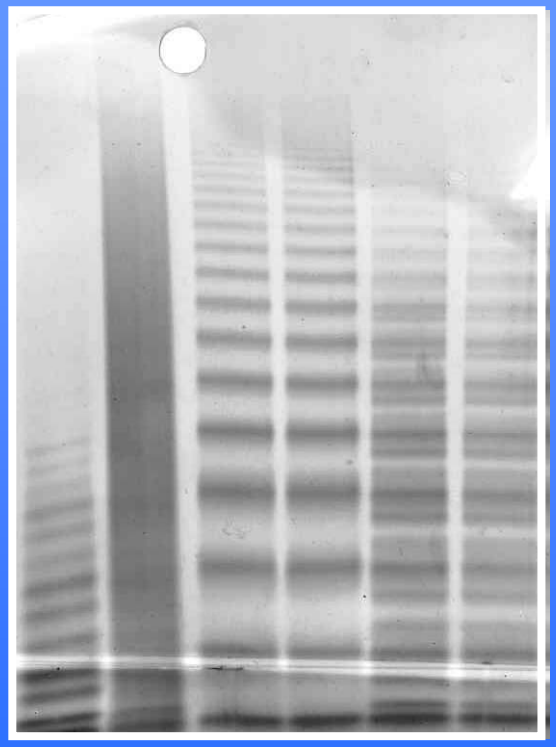
1.2% agarose gel



2A NP 1 2A NP NP 1 NP 2B 2B 1 NP

Band visualization with non-radioactive staining method

1.1% agarose



2A 2E? 2E 2B P-VWD

unclassified

Recent classification of VWD

- Type 1
- Quantitative deficiency of VWF with clinically mild to moderately severe bleeding.
- Reduced (by how much?) VWF:Ag, activity and Factor VIII.
- Normal VWF:Ag multier.
- Autosomal dominant inheritance with variable penitrance and expression.

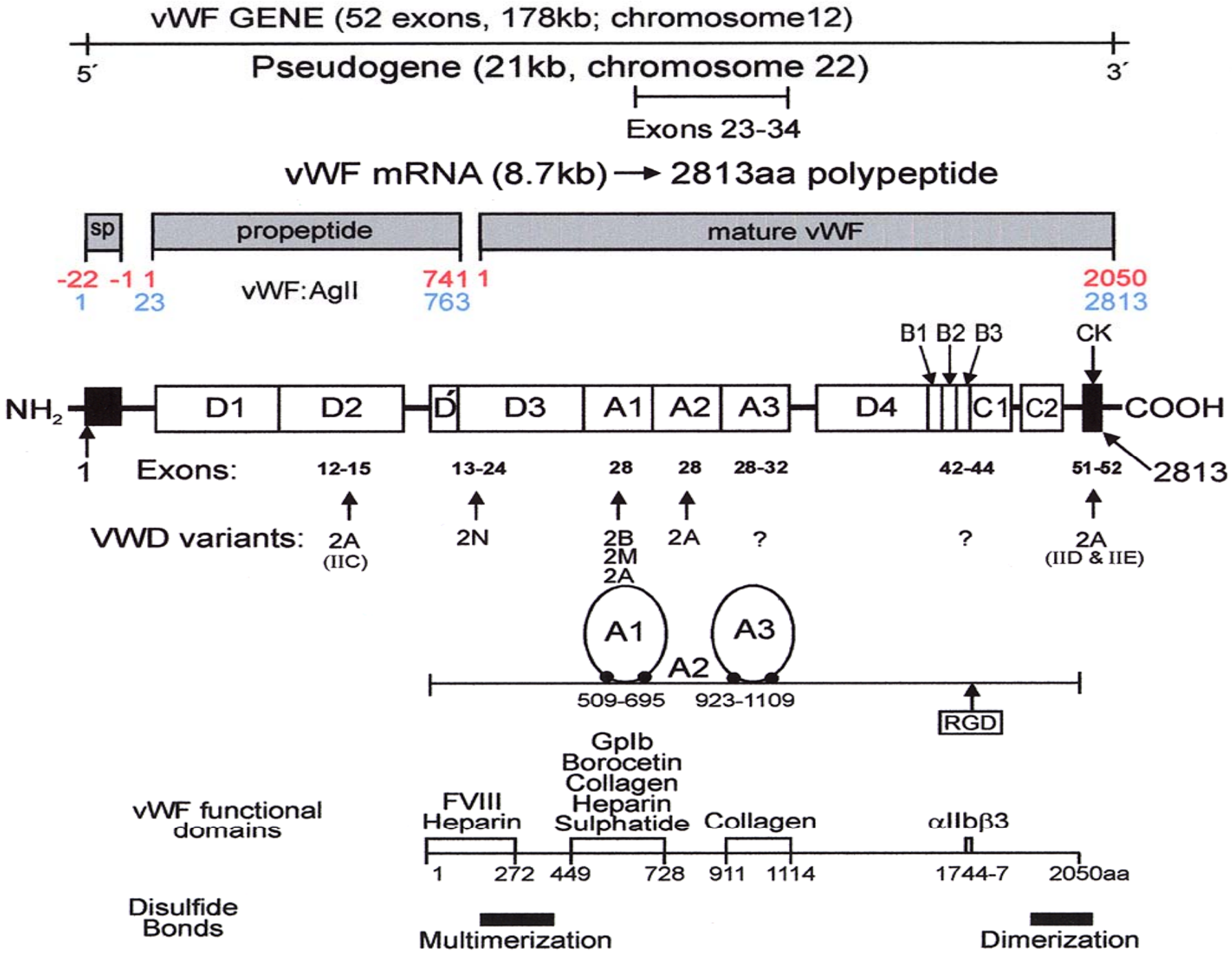
- Type 3
- Severe deficiency or absence of platelet and plasma VWF:Ag and VWF activity. Clinically severe.
- Usually autosomal recessive inheritance.

Recent classification of VWD

Type 2 patients have a qualitative defect of VWF:

1. Type 2A: Reduced VWF dependent platelet function (VWF:RCo) due to the loss of the HMW VWF multimers.
2. Type 2M: Reduced VWF:RCo, but with normal VWF multimers.
3. Type 2B: Enhanced VWF binding activity to platelet, resulting in loss of HMW multimers.
3. Type 2N: Reduced binding capacity of VWF for Factor VIII.

"Vicenza" VWD (type 1, 2M?) and the unclassified VWD (2M?)


vWF GENE (52 exons, 178kb; chromosome12)
5´
Pseudogene (21kb, chromosome 22)
3´
Exons 23-34
vWF mRNA (8.7kb) → 2813aa polypeptide
sp
propeptide
mature vWF
-22 -1 1
1 23
vWF:AgII
741 1
763
2050
2813
B1 B2 B3
CK
NH2
D1
D2
D´
D3
A1
A2
A3
D4
C1
C2
COOH
1
Exons:
12-15
13-24
28
28
28-32
42-44
51-52
2813
VWD variants:
2A (IIC)
2N
2B
2M
2A
2A
?
?
2A (IID & IIE)
A1
A3
A2
509-695
923-1109
RGD
vWF functional domains
FVIII Heparin
GpIb Borocetin Collagen Heparin Sulphatide
Collagen
αIIbβ3
1
272
449
728
911
1114
1744-7
2050aa
Disulfide Bonds
Multimerization
Dimerization

Role of Genotyping dilemma in VWD

Is genotype investigation in VWD only a complimentary test to the phenotype tests

or

it is an essential test for a definitive diagnosis and classification of VWD patients?

Mutations in different types of VWD

VWD database: www.vwf.group.shef.ac.uk

- Type 3 VWD

  >80 Large & small deletions, missense mutations

- Homozygous pseudogene conversion or compound heterozygous mutations for

  1+1=3, 2M+2M=3, 2N+others=3

Mutations in different types of type 2 VWD

- **Type 2A (IIA)** — **>50 missense mutations in A2 domain (3' end of ex 28). Mutations for the other subtypes (phenotypes IIC, D, E) of 2A VWD are in the other exons.**
- **Type 2B** — **>20 missense mutations in A1 domain (5' end of ex 28).**
- **Type 2M** — **>21 mutations in A1 domain (5' end of ex 28).**
- **Type 2N** — **>20 mutations in D' and D3 domain (ex 13-24 mostly in ex 18-20).**
- **Other types** — **1. Vicenza mutation in ex 27 (& Ex17).**

  2. Several mutations for unclassified VWD in the 5' end of ex 28.

Differences in genetic investigation of haemophilia and VWD

- **In haemophilia genetic investigation is primarily for carrier detection, genetic counselling and patients' treatment.**

- **In VWD mutation detection is mainly for the patient's diagnosis confirmation, choice of the treatment and to help genetic counselling in some severe (type 3) cases.**

Role of mutation in treatment of type 2 VWD patients

- Response to DDAVP in type 2M VWD patients with different mutations.
- Role of the two different groups (I & II) mutations in treatment of type 2A (IIA) patients.

To conclude

Genotyping i.e. correct identification of the causative mutation and its tracking in VWD families:

1. Can provide an accurate diagnosis and classification.

2. Can help in better patient management.

3. Facilitate genetic counselling.

4. However, there are some type 2 VWD patients in whom no mutation have been detected.

Any classification of VWD should answer the followings:

- Can it distinguish between 2 sets of the patients with low levels of VWF:Ag & VWF:RCo.
- Can such a classification provide adequate information to clinicians and genetic counselors to manage these patients.

The most recent classification of type 1 VWD suggests that:

- Only patients <20 IU dL^{-1} VWF:Ag should be diagnosed type 1.
- Those between 35-50 IU dL^{-1} VWF:Ag should be considered to have a risk factor for bleeding rather than a disorder.

What do the presenting individual want to know?

- Am I at increased risk of bleeding ?
- Are any of my relatives at increased risk of bleeding?
- What treatment , if any, is required?

Investigation for type 1 VWD diagnosis and classification

- Three major studies in the last seven years:
EU, Canadian and UK National study

Molecular basis of type 1 VWD
Recent international studies

- Canadian population-based study of molecular basis of type 1 VWD

  James *et al*, Blood 2007, 109, 145-54

- EU study “Molecular and clinical markers for diagnosis and management of type 1 von Willebrand disease”(MCMDM-1VWD)

  Goodeve *et al*, Blood 2007, 109, 112-21

- UK study “Molecular pathogenesis of type 1 von Willebrand disease”

  Cumming *et al*, Thromb Haemostas 2006, 96, 630-41

Haemophilia (2008), **14**, 685–696

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REVIEW ARTICLE

Type 1 von Willebrand disease: application of emerging data to clinical practice

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Summary. There has been much recent data published on type 1 von Willebrand disease (VWD) predominantly from three multi-centre cohort studies. These data have influenced a revision of the classification of type 1 VWD and have important implications for the management of this disorder. Patients with low von Willebrand factor (VWF) levels tend to have *VWF* mutations and VWD is transmitted predictably within families. In patients with VWF levels close to the lower end of the normal range, candidate mutations are found less often, ABO blood group is a more important factor and the disease has variable heritability within families. The importance of bleeding symptoms, in addition to VWF levels, in the diagnosis of type 1 VWD has been highlighted.

Keywords: bleeding, diagnosis, management, mutation analysis, von Willebrand disease

Usefulness of Bleeding Score (BS)

- BS was designed using a complex questioner.
- BS was as good as VWF:RCO. e.g. at predicting bleeding after dental extraction.
- BS was better at predicting post operative bleeding.
- But its use is questionable in children and it is very long and comprehensive.

Patients with abnormal multimer

- Excluded in UK and Canadian studies.
- In most of the patients with severe BS type 2 VWD mutations were identified.
- A small group of patients with mutations outside exon 28 can have real type 1 VWD.

Influence of ABO blood group

- VWF is 25% lower in O individuals than in A group.
- Mechanism?
- O group is over represented in cohorts of type 1 VWD compared to other blood groups.
- In index patients of 3 studies, VWF:Ag of above 30 IU $dL^{-1,}$ 66% had O bg and those below had 50% and similar to local population (46%).
- Synergistic affect of O bg with Y1584 mutation in clearance of VWF.

Summary of major genetic findings from the EU and Canadian type 1 VWD studies

In total of 273 Type 1 VWD family/index cases:

- Linkage to the *VWF* gene in ~70% of families.
- *VWF* gene candidate mutations in ~65% of index cases.
- >100 different candidate *VWF* mutations documented.
- 80% of candidate mutations are missense substitutions.
- In 10-20% of cases there is more than 1 candidate VWF mutation.

Mutations in type 1 VWD

- Before these international studies - 14 different *VWF* gene mutations were reported in association with type 1 VWD

  in the VWD Database
- As of September 2007, 117 different mutations or candidate mutations.
- BUT, 1/3 of patients mutations are for type 2 VWD

  1/3 of patients mutations are polymorphisms

  1/3 of patients no mutation detected

Some previously identified mutations: 1. Y1584C in exon 28

- (8-25%) prevalence and shown to be associated with a 26% decrease in VWF:Ag. Together with blood group O would cause a fall of 39%.
- Its association with ADAMST 13 in clearance of VWF and increased risk of bleeding

Some previously identified mutations: 2. R1205H "Vicenza" in exon 27

- Causes highly penetrnat moderate to sever type 1 VWD caused by markedly increased clearance of VWF.
- About 5.5% frequency in all studies.
- Ultra high molecular weight multimer?

Some previously identified mutations:
3. R854Q in exon 20 & R924Q in exon 21

- Are these 2 real mutations or polymorphism?
- R854Q causes severe VWD in compound heterozygous form, particularly type 2N VWD.
- Very little evidence of pathogenesis associated with R924Q.

Some previously identified mutations: 4.

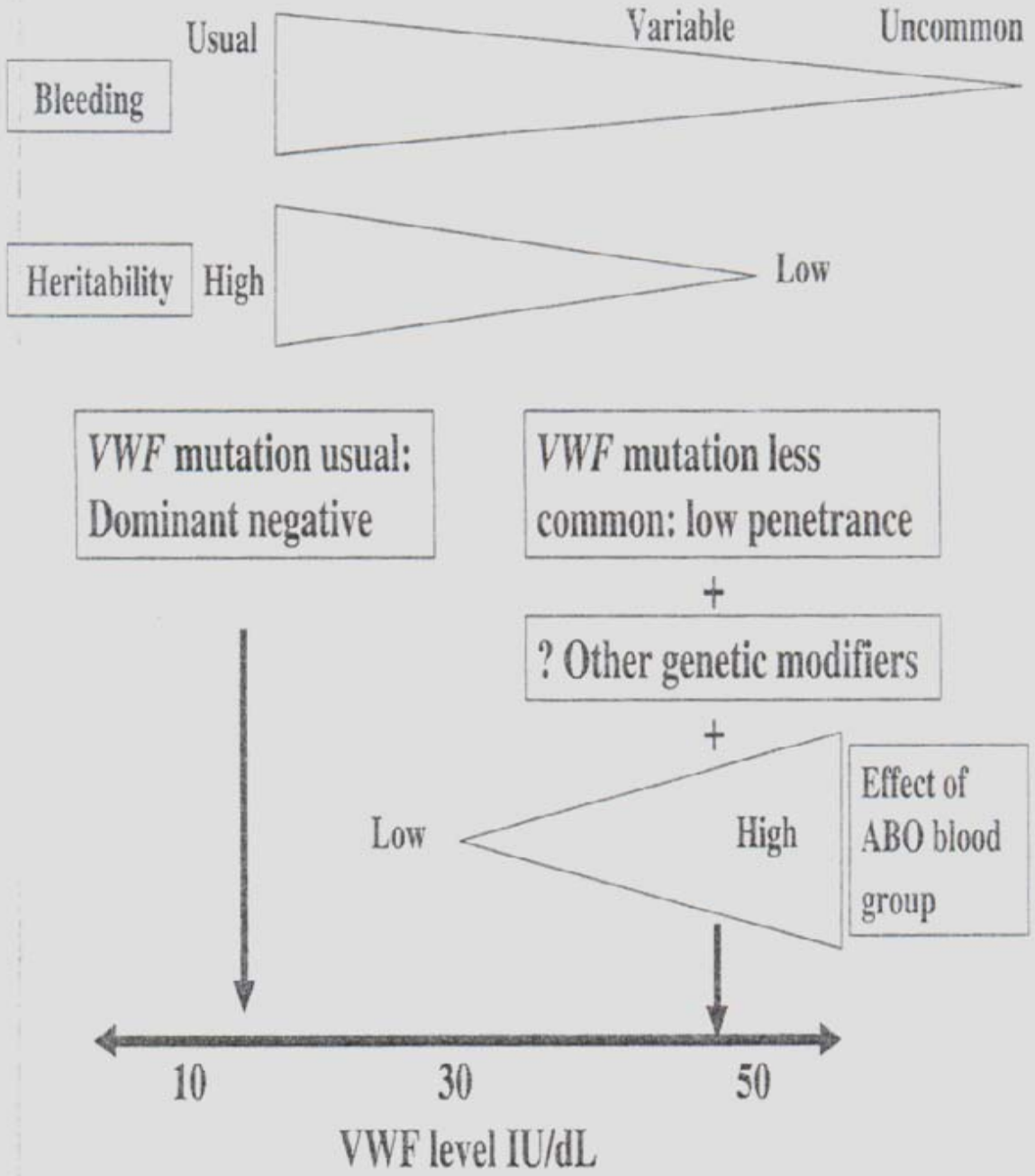
- R1315C, R1315L, R1374C, R1374H, I1416N in exon 28 causing type 2M VWD by reducing VWF function.
- Other mutations at Cysteine residue.

DDAVP response and mutation

- Direct association in groups with mutations at codon 1130 and 1205 reducing DDAVP's half life.
- Less influential in groups with mutations in the A1-A3 domains or abnormal multimer pattern.
- No clear information in clinical practice.

Type 1 VWD diagnosis and molecular characterization

VWF level <25-30%	**VWF level 30-40%**	**VWF level 40-50%**
Significant bleeding not unusual	May have significant bleeding	Mild or doubtful bleeding
Diagnosis is more straightforward	These are the most of cases seen in clinics. With variable ease of diagnosis	ABO blood group have a major effect on this group
Disease is highly heritable. **Often associated with dominant *VWF* mutation**	Molecular basis is only identified **in some of the cases** **Mutation ??**	Low heritability. VWF levels often don't segregate with bleeding or with *VWF* gene. *VWF* mutations **infrequently** identified. Do they have VWD?


Usual
Variable
Uncommon
Bleeding
Heritability
High
Low
VWF mutation usual:
Dominant negative
VWF mutation less
common: low penetrance
+
? Other genetic modifiers
+
Low
High
Effect of
ABO blood
group
10
30
50
VWF level IU/dL

Conclusions

1. The 3 recent studies have improved our knowledge of the pathogenesis of type 1 VWD and management and differentiation of people with low levels of VWF.

Conclusions

2. Improved laboratory assays, use of the bleeding scores (suitable for adults and children) and the influence genes other than VWF e.g. ABO blood group is likely to be important in understanding of the control of VWF level and improving management of type 1VWD patients.

Conclusions

3. Although these 3 studies have provided a sound basis for our better understanding of the mechanisms of type 1 VWD, much additional work is needed to be done before we can claim to truly comprehend the genetic pathology of this disease.

Conclusions

4. While genetic testing is useful in some of the VWD sub-types, the obvious genetic complexity associated with type 1 VWD, currently precludes the use of this methodoloyg as a complementary diagnostic aid.