The Role of Genetic Factors in Determining Phenotype, and what are the Laboratory Markers which Correlate Best with Clinical Severity

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Phenotype Genotype Determinants for Phenotype

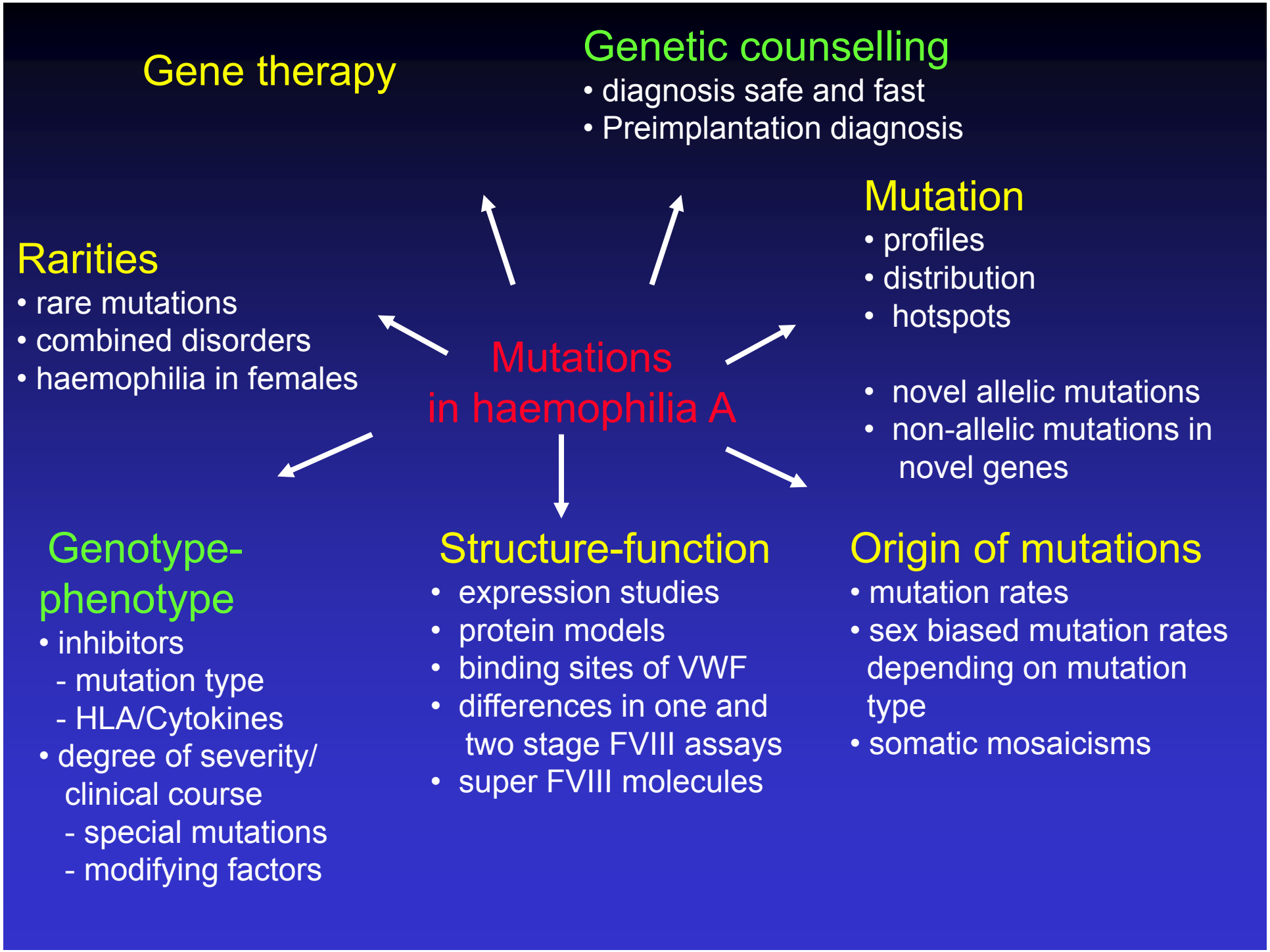
- Inhibitor formation
- Severe haemophiliacs with mitigated bleeding phenotype
- VWD
- Assay Discrepancies between one- and two stage FVIII-Assays

Phenotype vs Genotype

Phenotype
- Variable over time
- actual situation
- Fast (minutes to hours)
- Few costs
- Monitoring therapy
  e. g. substitution, anticoagulation
- Quantitative, complex information
  sometimes high variance

Genotype
- Do not change during live
- result may have no actual correlat
- Fast (hours to days)
- Still more cost intensive
- Risk assessment of relatives

- Qualitative, Yes or No


Gene therapy
Genetic counselling
• diagnosis safe and fast
• Preimplantation diagnosis
Mutation
• profiles
• distribution
• hotspots
• novel allelic mutations
• non-allelic mutations in novel genes
Rarities
• rare mutations
• combined disorders
• haemophilia in females
Mutations in haemophilia A
Genotype-phenotype
• inhibitors
- mutation type
- HLA/Cytokines
• degree of severity/ clinical course
- special mutations
- modifying factors
Structure-function
• expression studies
• protein models
• binding sites of VWF
• differences in one and two stage FVIII assays
• super FVIII molecules
Origin of mutations
• mutation rates
• sex biased mutation rates depending on mutation type
• somatic mosaicisms

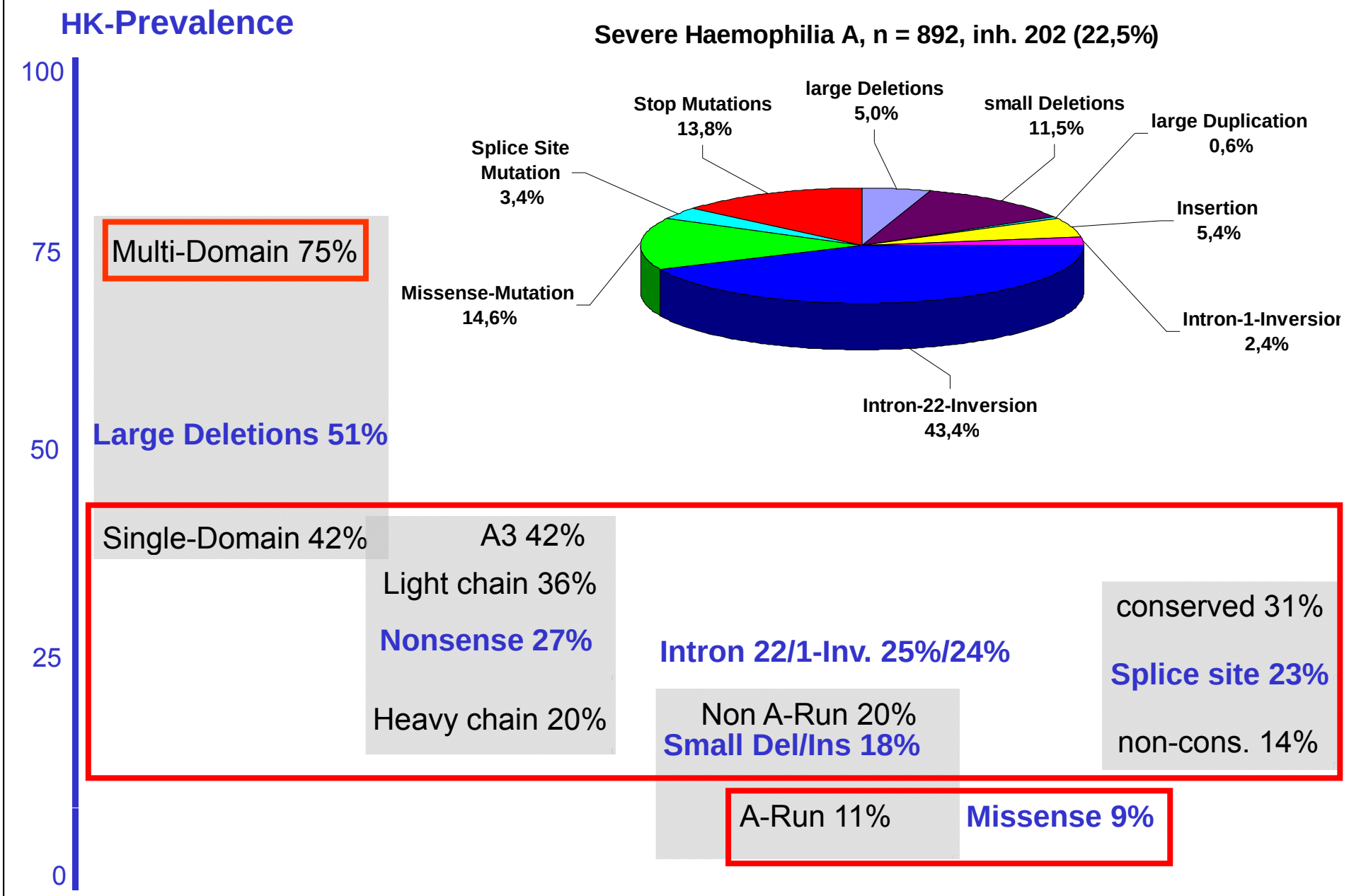
GENOTYPE – PHENOTYPE

Development of Inhibitors in Haemophilia A

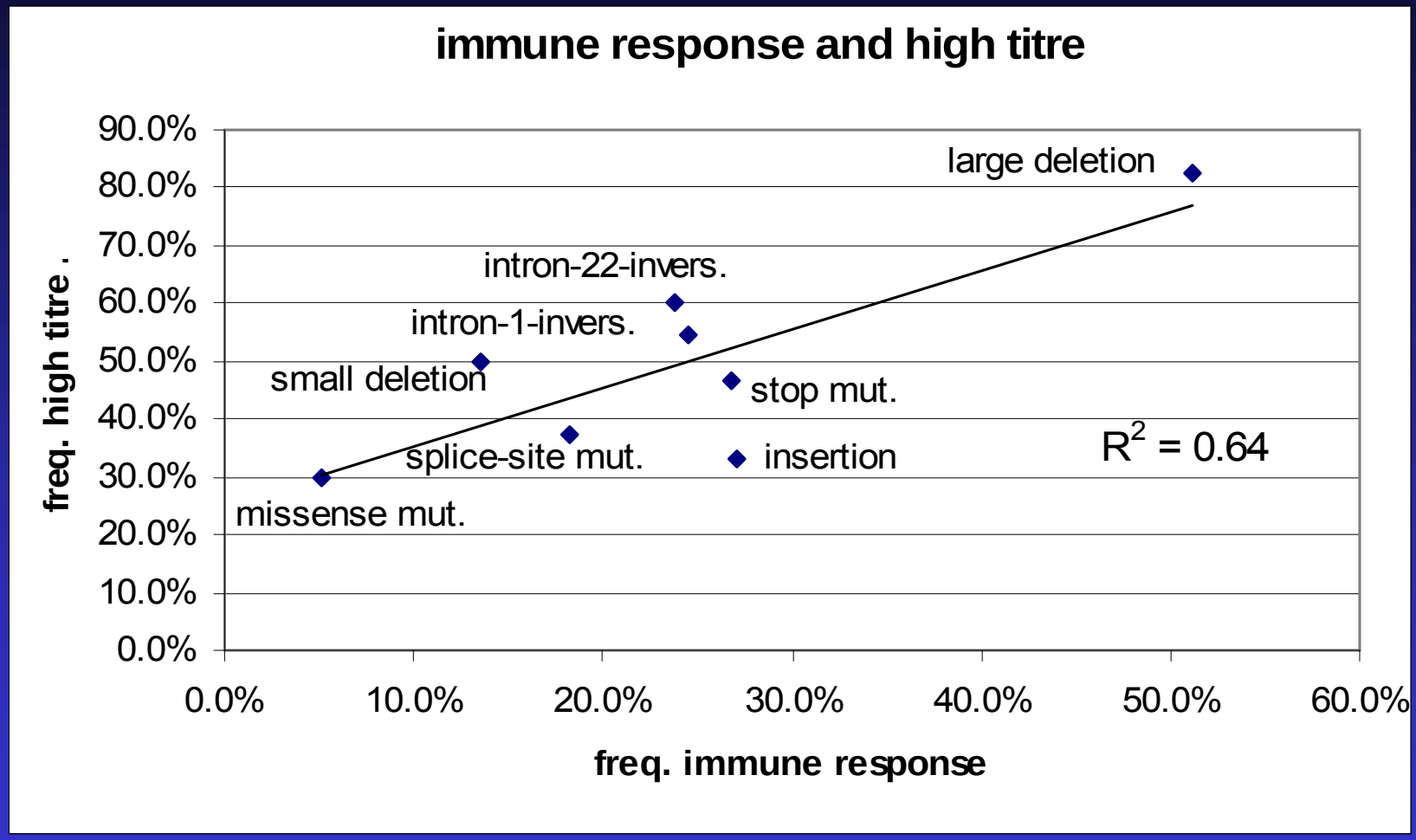
- Most severe and frequent complication of treatment
- 20-30% of severe haemophilia A patients
- neutralisation of substituted FVIII
- alternative treatment options are limited, increased morbidity and mortality
- eradication of the inhibitor (very expensive)

Are inhibitors predictive?
Are they fate or preventable?

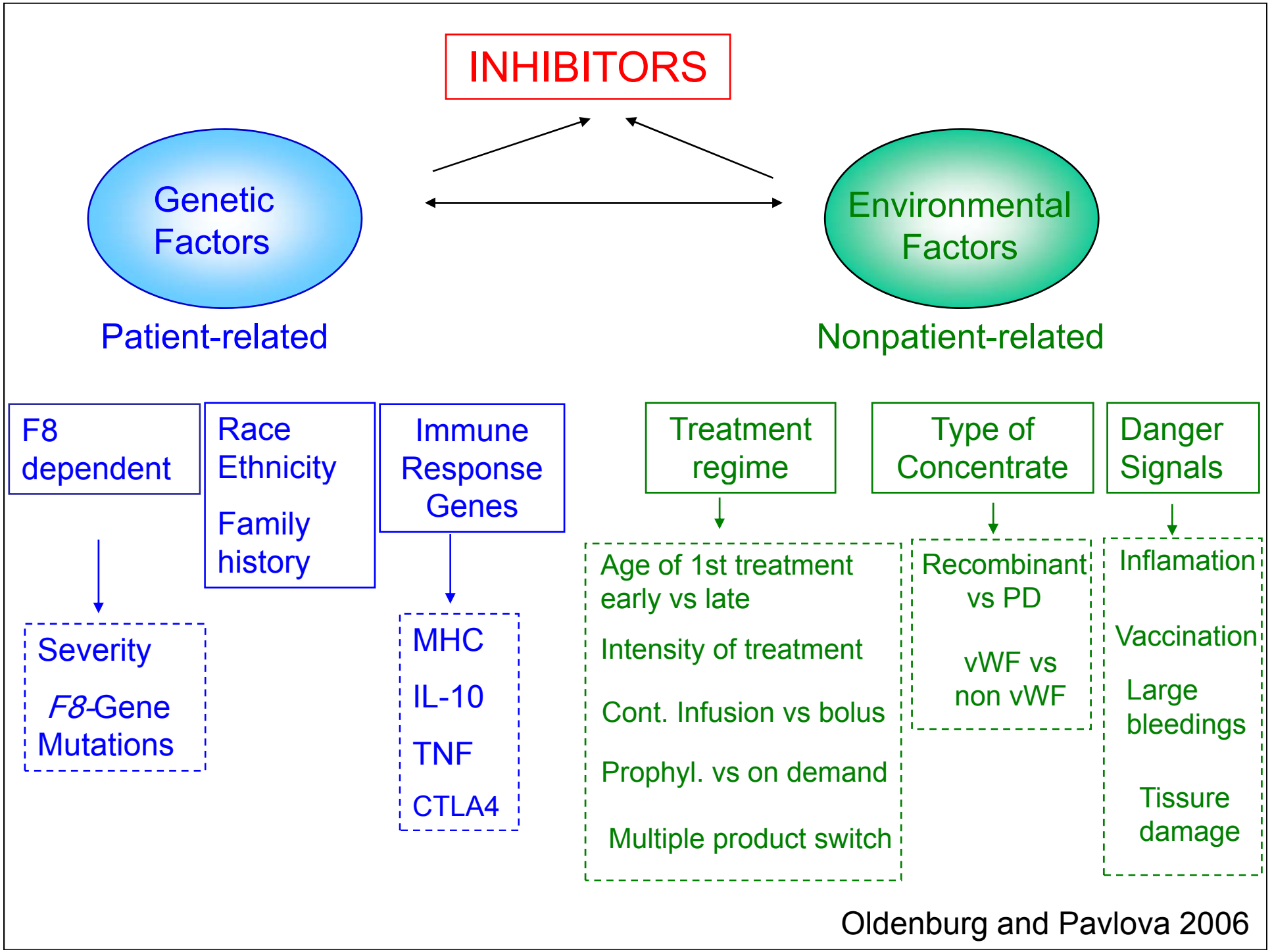
Mutation type and inhibitor prevalence



Mutation Type and Inhibitor Titre



Oldenburg et al. unpublished data


INHIBITORS
Genetic Factors
Environmental Factors
Patient-related
Nonpatient-related
F8 dependent
Race Ethnicity
Family history
Immune Response Genes
Treatment regime
Type of Concentrate
Danger Signals
Severity
F8-Gene Mutations
MHC
IL-10
TNF
CTLA4
Age of 1st treatment early vs late
Intensity of treatment
Cont. Infusion vs bolus
Prophyl. vs on demand
Multiple product switch
Recombinant vs PD
vWF vs non vWF
Inflamation
Vaccination
Large bleedings
Tissure damage
Oldenburg and Pavlova 2006

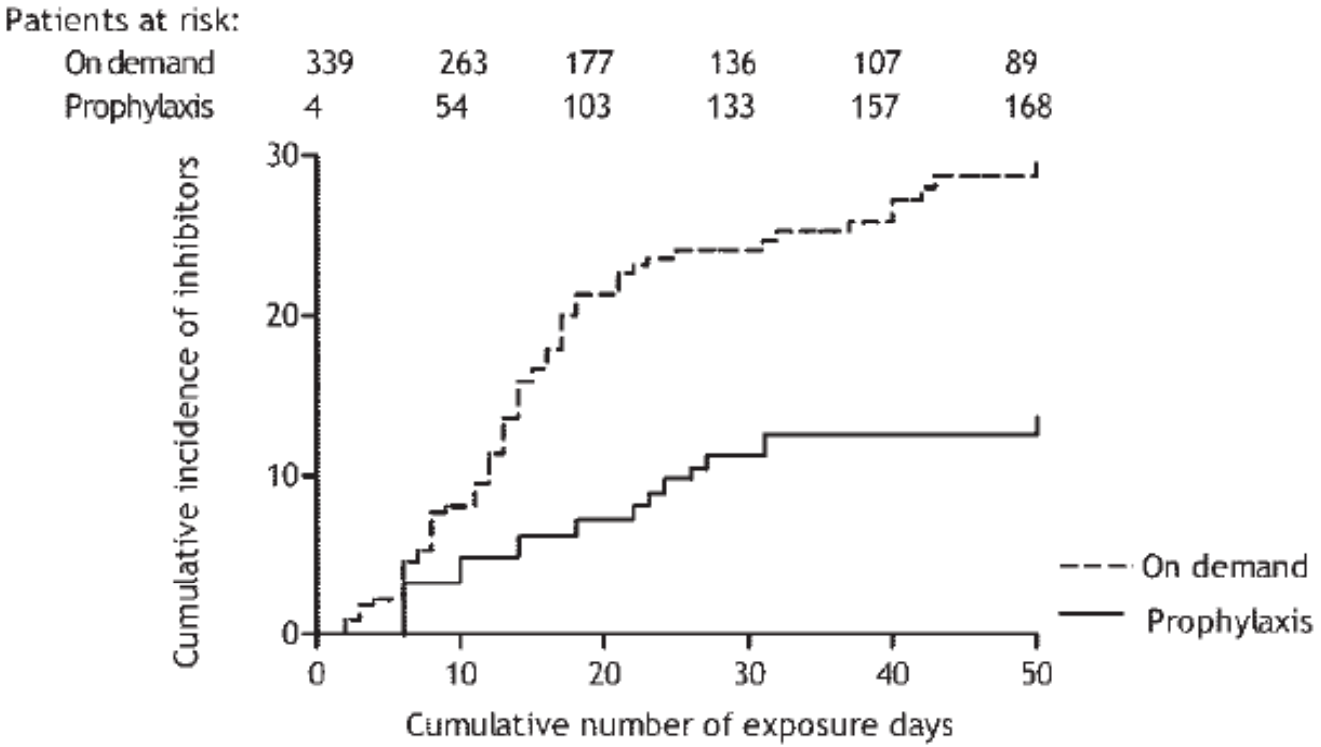
Peak treatment moments may trigger inhibitor formation

n=366 severe HA patients; 87 [24%] of patients with inhibitor

	Proportion of Inh (%)	Crude RR (CI)	*P* for trend	Adjusted RR (CI)	*P* for trend
At first factor VIII exposure					
None	44/229 (19)	1.0		1.0¶	
3 to 4 days	7/36 (19)	1.0 (0.5-2.3)	.98	1.1 (0.5-2.4)	.87
At least 5 days	32/57 (56)	3.3 (2.1-5.3)	< .001	3.1 (1.9-5.0)	< .001
During first 50 exposure days					
After peak treatment moment compared with before	NA	1.6 (1.0-2.7)	.06	1.5 (0.9-2.5)¶	.14
After major peak treatment moment compared with before	NA	2.0 (1.3-3.1)	.002	1.6 (1.0-2.6)¶	.03
After major surgical procedure compared with before	NA	1.4 (0.8-2.5)	.21	1.3 (0.8-2.3)¶	.32

Gouw et al.: Blood 2007 109:4648

Incidence of inhibitor development according to treatment regime



Regular prophylaxis was associated with a 60% decreased risk of inhibitor development compared with on-demand treatment (RR, 0.4; CI, 0.2-0.8)

Gouw et al 2007

The good risk vs the bad risk patient

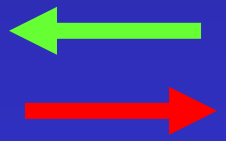
Good Risk Factors

Genetic Background

- Negative family history
- Non-severe haemophilia
- Caucasian origin
- Missense mutation
- IL10 134 negative
- TNF alpha A2 negative
- CTLA4-318 T positive

Environmental

- Early prophylaxis
- Absence of danger signals
- (type of concentrate)



Bad Risk Factors

Genetic Background

- Positive family history
- Severe haemophilia
- African origin
- Null mutation
- IL10 134 positive
- TNF alpha A2 positive
- CTLA4-318 T negative

Environmental

- Early event-based treatment
- Intensive treatment
- Continuous infusion
- Danger signals
- (type of concentrate)

Individualized treament strategy

Inhibitor development on a given genetic background may be not a fate but preventable by the right environmental action

Consequence

- Test for the genetic background (F8 gene mutation)
- Stratify treatment during the first 20-50 exposure days

Which is in case of a F8 gene mutation at high risk

- Avoid peak treatment moments
- Avoid elective surgery
- Avoid danger signals (vaccination)
- Early start of low dose prophylaxis

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Genotype - Phenotype

Why do some patients with laboratory severe haemophilia A show a non-severe clinical course?

(rare bleeders inspite of having severe haemophilia A)

Mutation profile in severe Haemophilia A

(based on the analysis of 635 families)

Mutation Type	Relative %
Intron 22 Inversion	47.7
Intron 1 Inversion	1.2
Stop Mutation	12.4
Small Del./Ins.	13.7
Large Deletions	4.0
Splice Site	3.5
Missense Mutation	12.3
Mutation not found	4.2

Oldenburg and Pavlova 2006

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Small Del./Ins.	13.7
(In two adenine runs of B domain	(3.0)
Large Deletions	4.0
Splice Site	3.5
(at non-conserved positions)	(1,5)
Missense Mutation	12.3
Mutation not found	4.2

GENOTYPE - PHENOTYPE

Patients mutation	FVIII:C (IU/ml)	TEG-r (min)
Group A		
1. Del-ACAC, codon 1187	< 0.01	29
2. Del-A, codon 1192	< 0.01	26
3. Del-A, codon 1192	< 0.01	48
4. Del-A, codon 1192	< 0.01	26
Group B		
1. CGC(Arg) 1689 TGC(Stop)	< 0.01	158
2. TAC(Tyr) 636 TAG(Stop)	< 0.01	134
3. Intron 22 inversion (Prox.)	< 0.01	178
4. Intron 22 inversion (Dist.)	< 0.01	159

Oldenburg et al. 1997 Thromb & Haem

Series of adenine nucleotides

B-domain

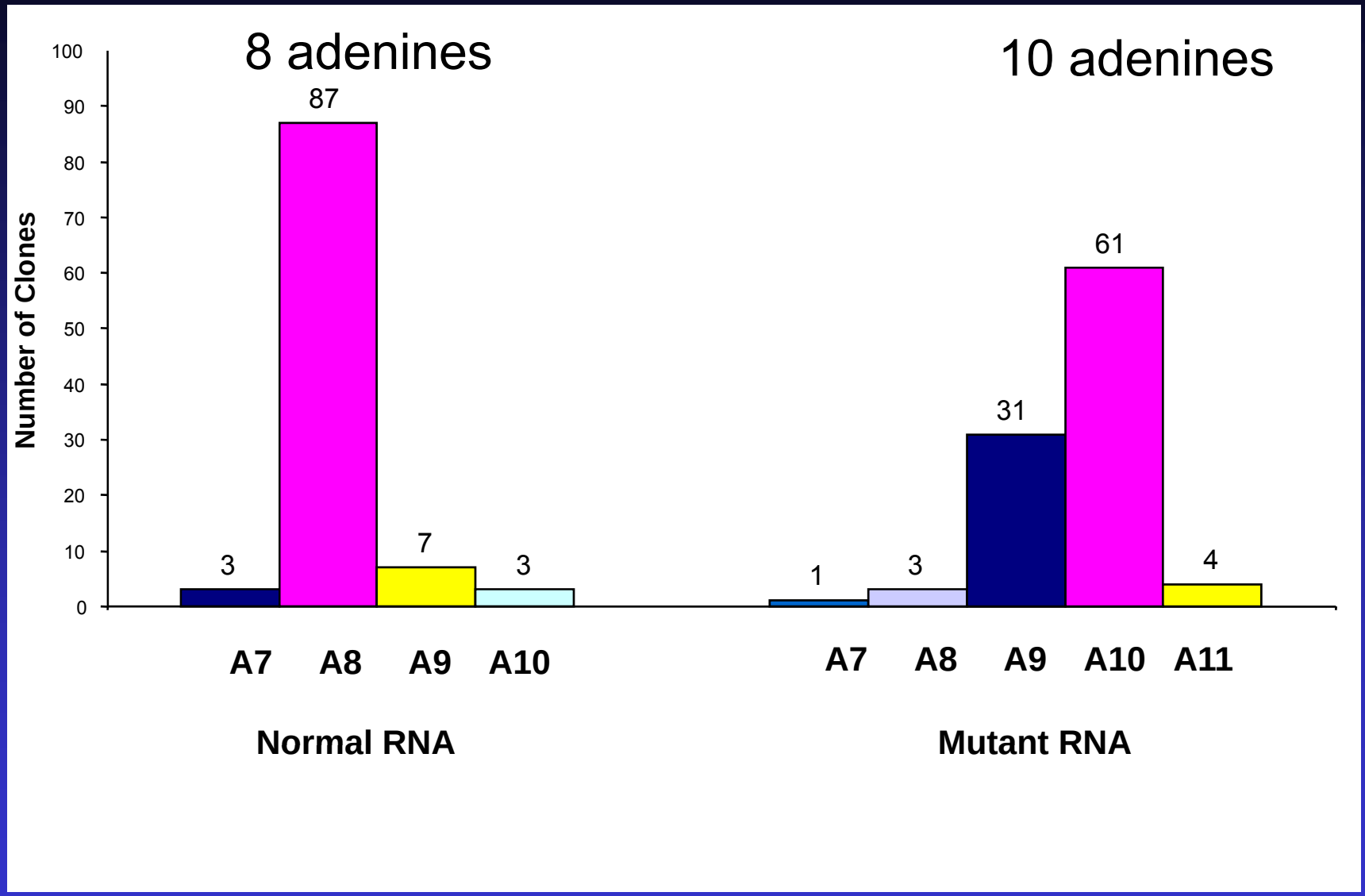
CAA	GAA	AAA	AAA	ATT	CAG
Gln	Glu	Lys	Lys	Ile	Gln
1190	1191	1192	1193	1194	1195

GCC	AAA	AAA	AAT	AAC	CTT
Ala	Lys	Lys	Asn	Asn	Leu
1438	1439	1440	1441	1442	1443

20% of all small deletions/insertions of the FVIII gene are located at one of these two sites

Oldenburg et al. 1997 Thromb & Haem

Partial correction of a frame shift T-deletion in an adenine run (A8TA2)



(Young et al. 1997, Am J Hum Gen)

Journal of Thrombosis and Haemostasis, 3: 2730–2737

ORIGINAL ARTICLE

Factor V Leiden improves *in vivo* hemostasis in murine hemophilia models

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Haemophilia and FV Leiden / Prothrombin Mutation

	No thrombophilia	*With thrombophilia*	*p value**
Year of birth	1990 [1991-1999]	1991 [1982-1999]	0.54
Age at first bleeding: years [median/range]	0.9 [0.1-4.0]	1.5 [0.5-7.1]	0.009
Therapy given: number [%]			
on demand	58 [63.0]	8 [53.3]	0.67
prophylaxis	34 [37.0]	7 [46.7]	
Start of prophylactic regimen: Median/range values (years)	1.3 [0.1-6.7]	1.9 [0.8-7.0]	0.44
Factor concentrates used [%]			
pdFVIII	27.3	33.3	0.33
rFVIII	48.5	55.5	
vWFVIII	24.2	11.2	
Annual bleeding frequency	6 [0-30]	1.8 [0-7]	0.012

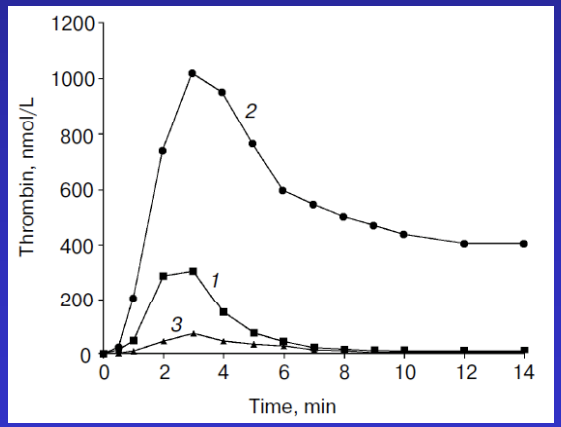
Kurnik et al. 2007 Haematologica

Laboratory Assessment of the Bleeding Phenotype

Single Factor activity (FVIII:C)

Thrombelastography

Thrombin generation assays



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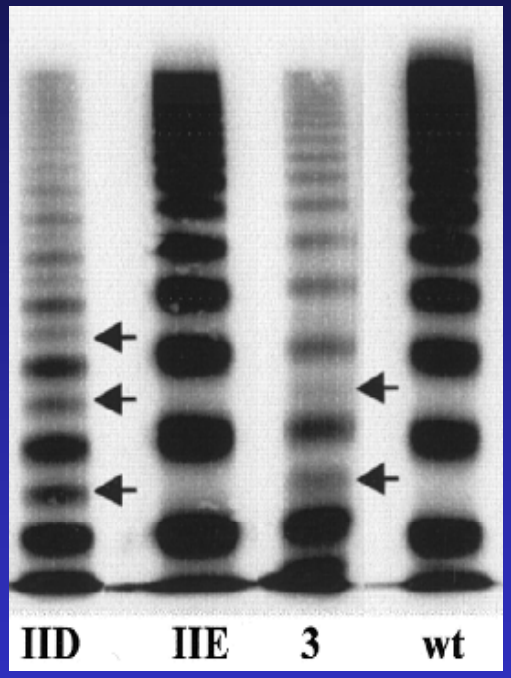
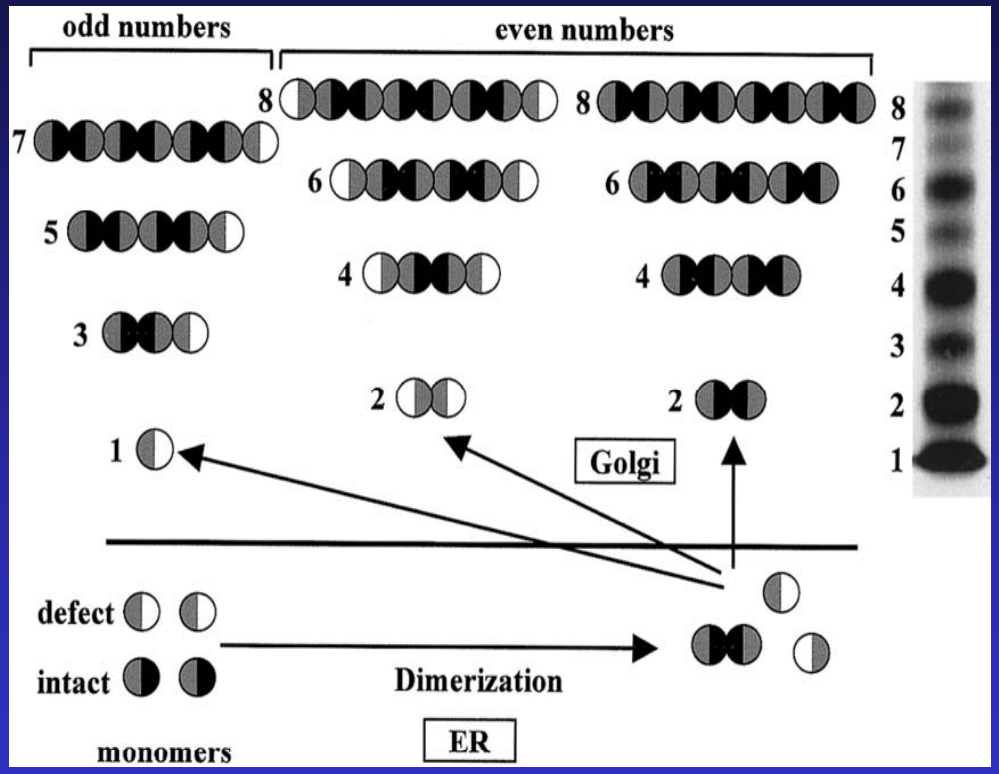
VWD Type 2





Schneppenheim et al. Blood 2001

VWD Multimer Diagnostic

Large Multimers

Thrombocytes dependent VWF-function → **Type 2A**
- Defect intracellular Multimers IIC, Dimers IID
- Increased sensitivity for plasma Metalloprotease IIA

Type 2

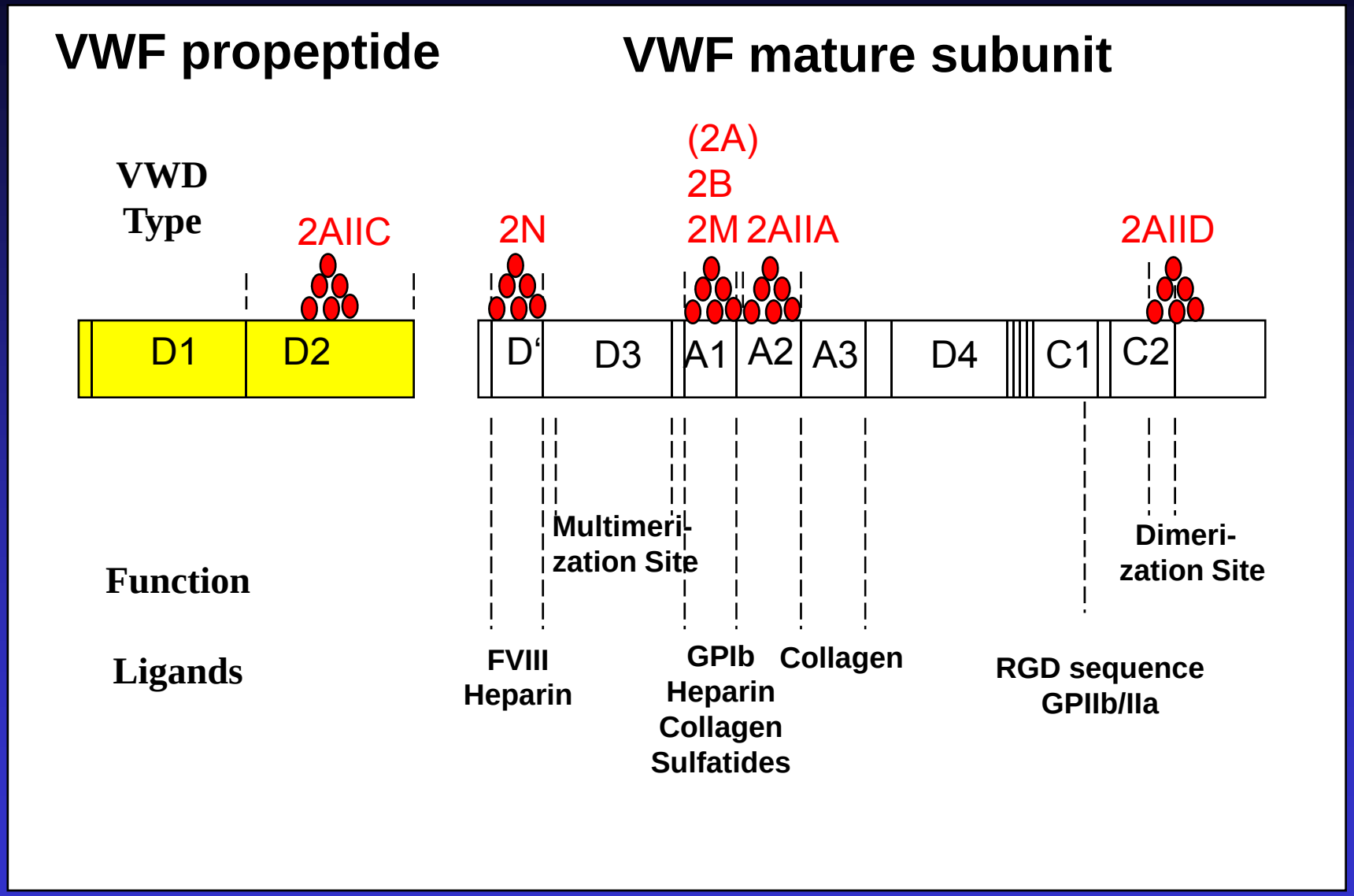
Thrombocytes dependent VWF-function → **Type 2B**
- Increased interaction with GPIb
- Mutations in A1

Normal Multimers

Thrombocytes dependent VWF-function → **Type 2M (Multimers)**
- Increased interaction witht GPIb
- Mutations in A1

FVIII-Binding → **Type 2N (Normandy)**
- Decreased FVIII-binding
- Mutations in D‘ und D3

Genotype - Phenotype - Correlation



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Discrepancies

Up to 1/3 of cases with non severe haemophilia A

show inherited discrepancies

FVIII:C one-stage > FVIII:C chromogenic

One-stage : two-stage ratios ≥2.0

or

FVIII:C one-stage < FVIII:C chromogenic

One-stage : two-stage ratios ≤0.5

– fail to diagnose some cases of mild haemophilia

– which level to assign for diagnosis

– which level to refer for dosing during treatment

Which assay is giving the true FVIII:C?

Poulsen et al. Haemophilia 2009

FVIII one-stage > FVIII chromogenic

Mutation	One-stage	Chromogenic
Ala284Glu	34%	9%
Ala 284Pro	Mazurier et al. 1997	
Ser289Leu	33%	9%
Arg527Trp	27%	13%
Arg531Cys	14-18%	2-9%
Arg531His	42%	11%
Asn694Ile	19%	9%
Arg698Trp	22%	6%
Arg698Leu	42%	16%
Arg1749His	52%	8%
Ser1791Pro	19-32%	5-9%
Leu1932Phe	19%	11%
Met1947Val	93%	23%
His1954Leu	106%	35%
Leu1978Phe	10%	2-4%
Asn2228Lys	Casey et al. unpublished	

Rudziki et al. 1995, Keeling et al. 1999, Schwaab et al. 2000, Pipe et al. 2001, Hill et al. 2005, Lucia et al. 2005, Rodgers et al. 2006, Cid et al. 2008

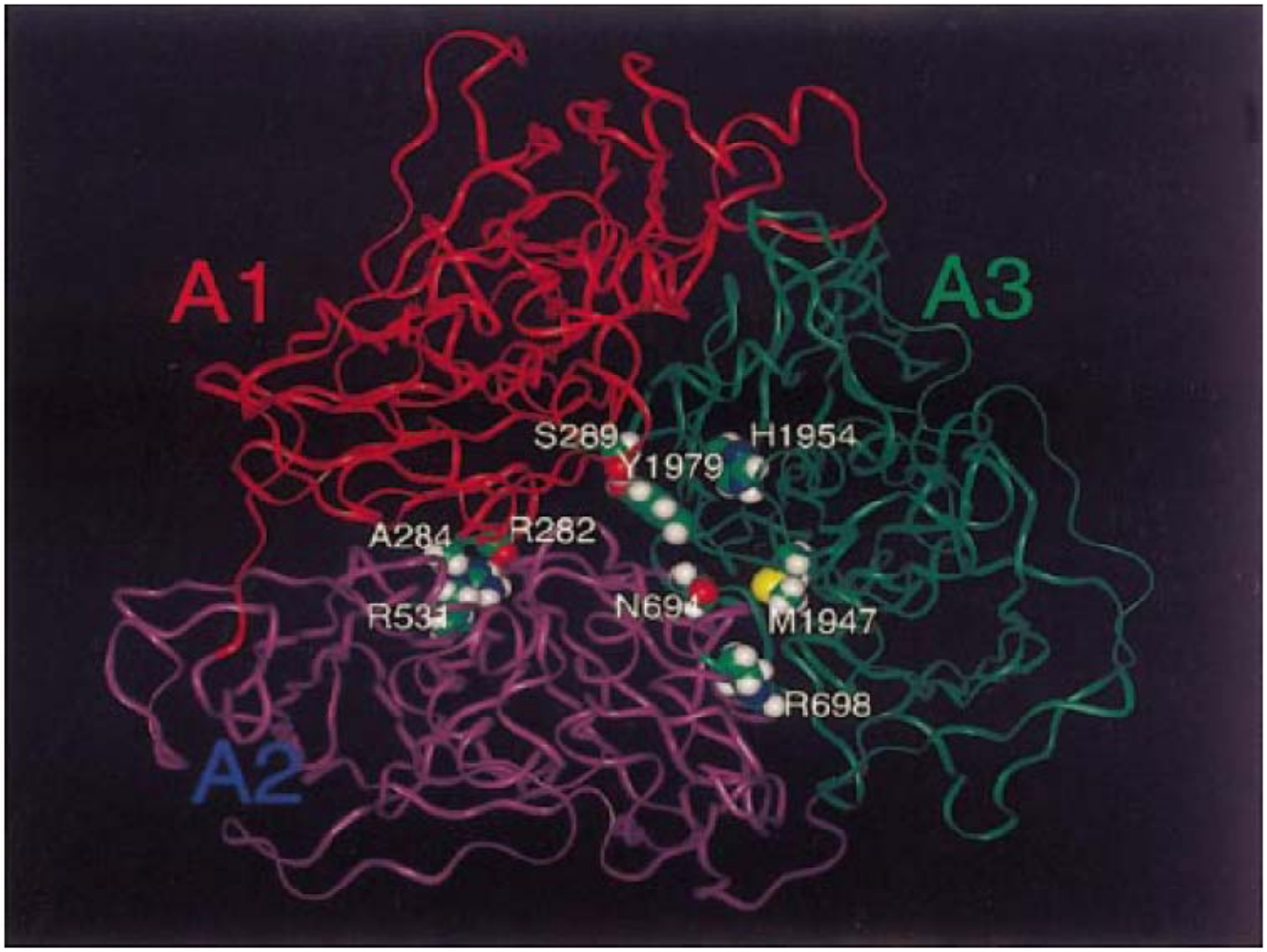


Figure 5. Hemophilic mutations with 1-st/2-st discrepancy are located at or close to the A domain interfaces. Global view of the FVIII A domain model marking the positions of $^{ALA}284$, $^{ARG}531$, and $^{ARG}282$ at the A1-A2 interface; $^{SER}289$ and $^{TYR}1979$ at the A1-A3 interface close to the pseudo-threefold axis; and $^{HIS}1954$, $^{ASN}694$, $^{MET}1947$, and $^{ARG}698$ at, or adjacent to, the A2-A3 interface.

Pipe, S. W. et al. Blood 2001;97:685-691

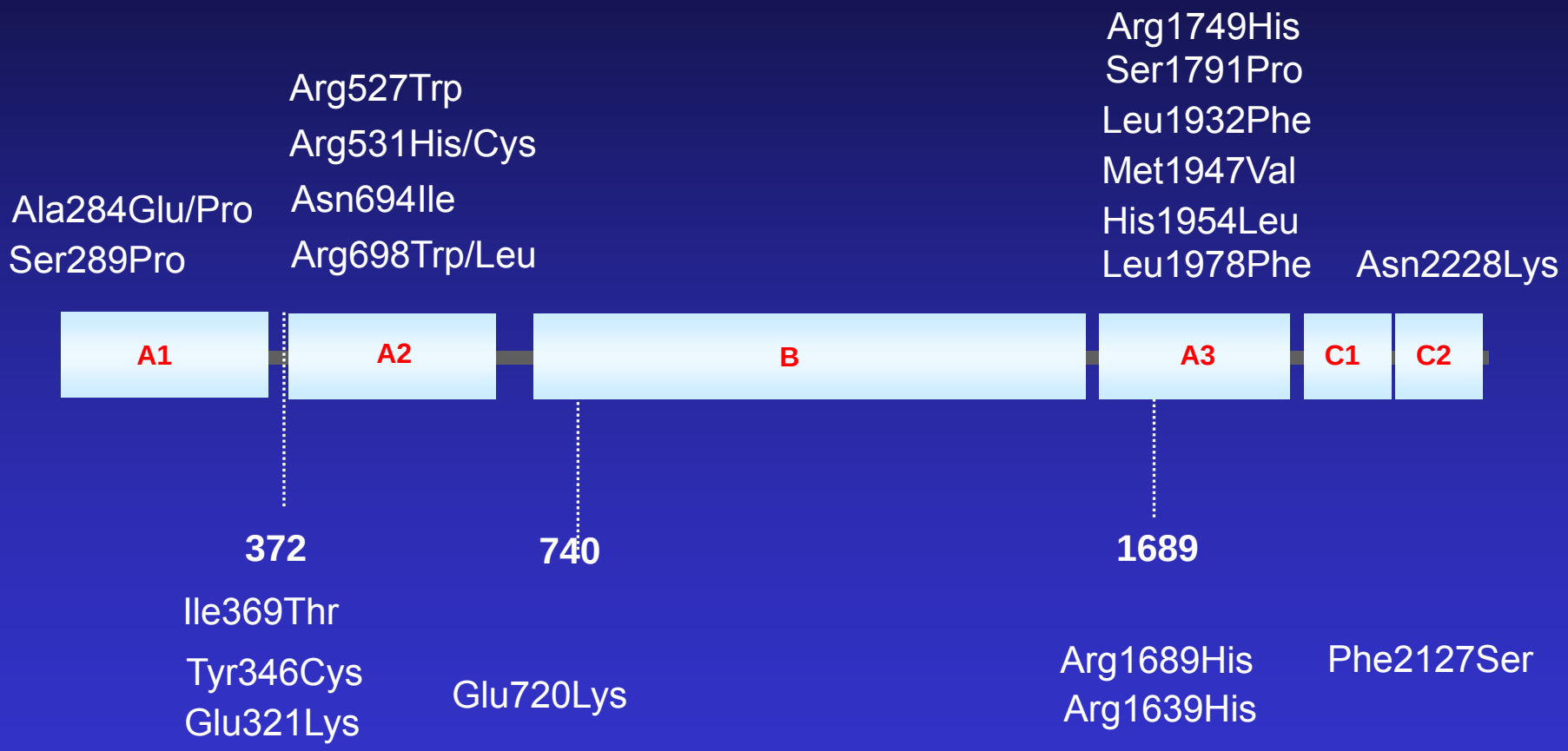
FVIII one-stage < two-stage assay

Mutation	FVIII:C (1-st)	FVIII:C (2-st)	FVIII:C Ag	Severity
Glu321Lys[1]	39	78	-	Mild
Tyr346Cys[2]	34	110	118	Mild
Ile369Thr[3]	14 ± 5	90 ± 15 / 43 ± 9	118 ± 29	Mild
Glu720Lys[4]	10-30	60-90	n.d.	Mild
Arg1639His[5]	24	117	198	Mild
Arg1689His[5]	25-26	99-111	74-160	Mild-Severe
Phe2127Ser[3]	10 ± 4	47 ± 13 / 50 ± 19	58 ± 20	Mild

[1] Goodeve et al. 2001; [2] Mumford et al. 2001/2002; [3] Trossaërt et al. 2007; [4] Roelse et al. 1999; [5] Cid et al. 2008

FVIII one-stage > FVIII chromogenic

Genetic defects are mainly clustered in the A1/A2/A3 domain interfaces



FVIII one-stage < two-stage assay

Genetic defects are mainly clustered around thrombin cleavage sites

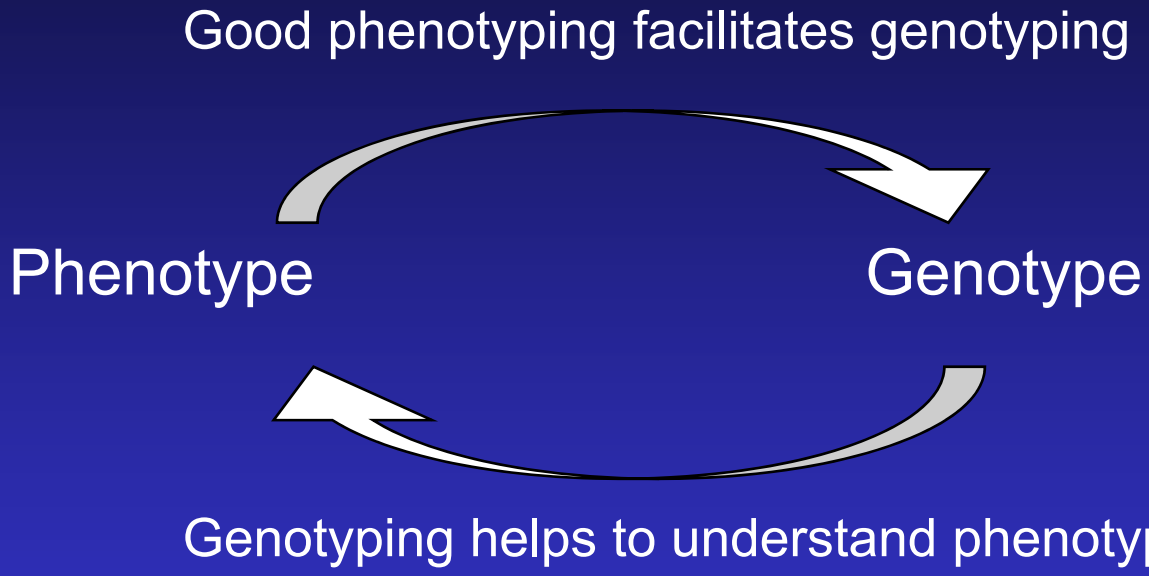
FVIII:C one-stage > FVIII:C chromogenic

- Hereditary discrepancy
- More common
- Facilitated dissociation of A2
- Which FVIII:C to refer?
- Clinically these patients bleed

FVIII:C one-stage < FVIII:C chromogenic

- Hereditary discrepancy
- Less common
- Alteration of thrombin cleavage
- Missing diagnosis with 2-stage
- Clinically patients bleed rare

Phenotype and Genotype assist each other



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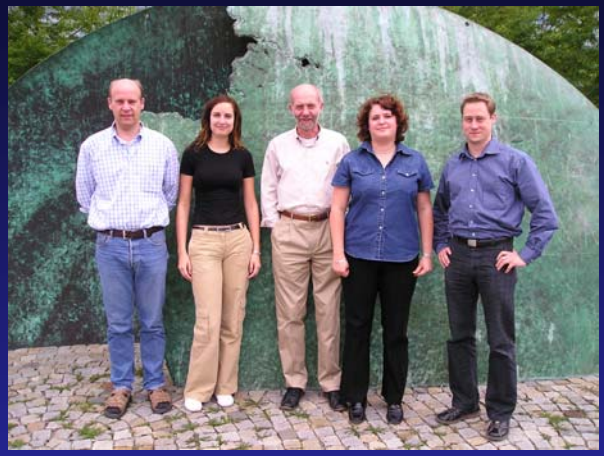

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