Genetics of Hemostatic Disorders

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Disclosures

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Consultancy	Swiss Federal Office of Public Health
Speakers bureau	Roche, Sobi, Sanofi, Takeda
Other	Interprofessional hemophilia care EHCCC Inselspital Bern: Bayer, CSL Behring, NovoNordisk, Octapharma, Sobi, Roche

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ISTH 2007

Answering T.T.P. Thrombotic Thrombocytopenic Purpura Foundation

BloodCenter of Wisconsin Part of Versiti

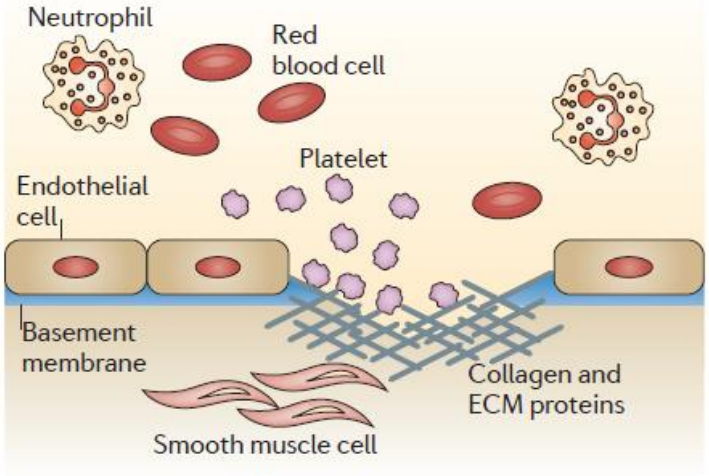
Baxter Baxalta

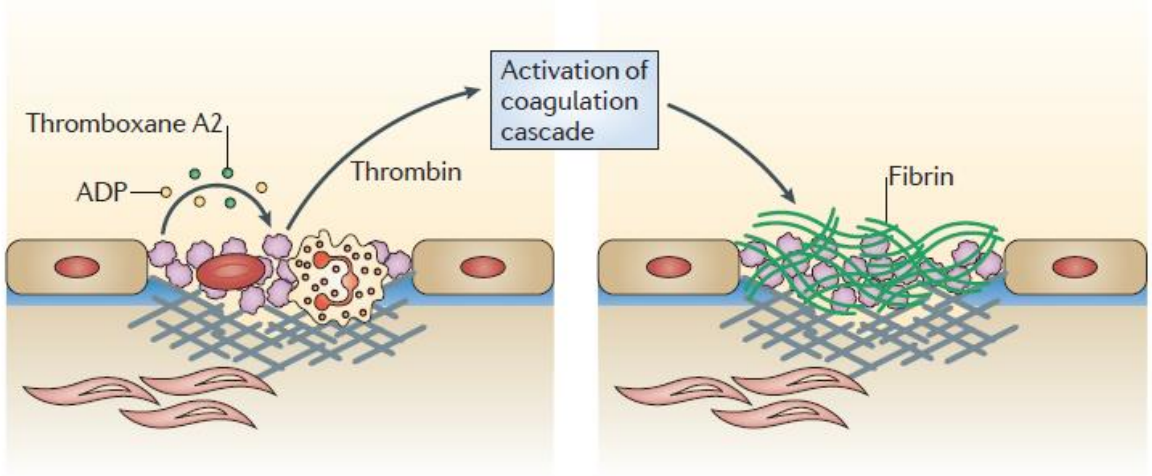
Mach-Gaensslen Stiftung Schweiz

Agenda

1. Elements of Hemostasis
2. Examples of
    1. Disorders of coagulation
    2. Disorders of platelet number and function
3. Summary

HEMOSTASIS in vivo





Primary Hemostasis

Platelets interact w. vessel wall (VWF, GPIb on Tc; collagen; etc.)

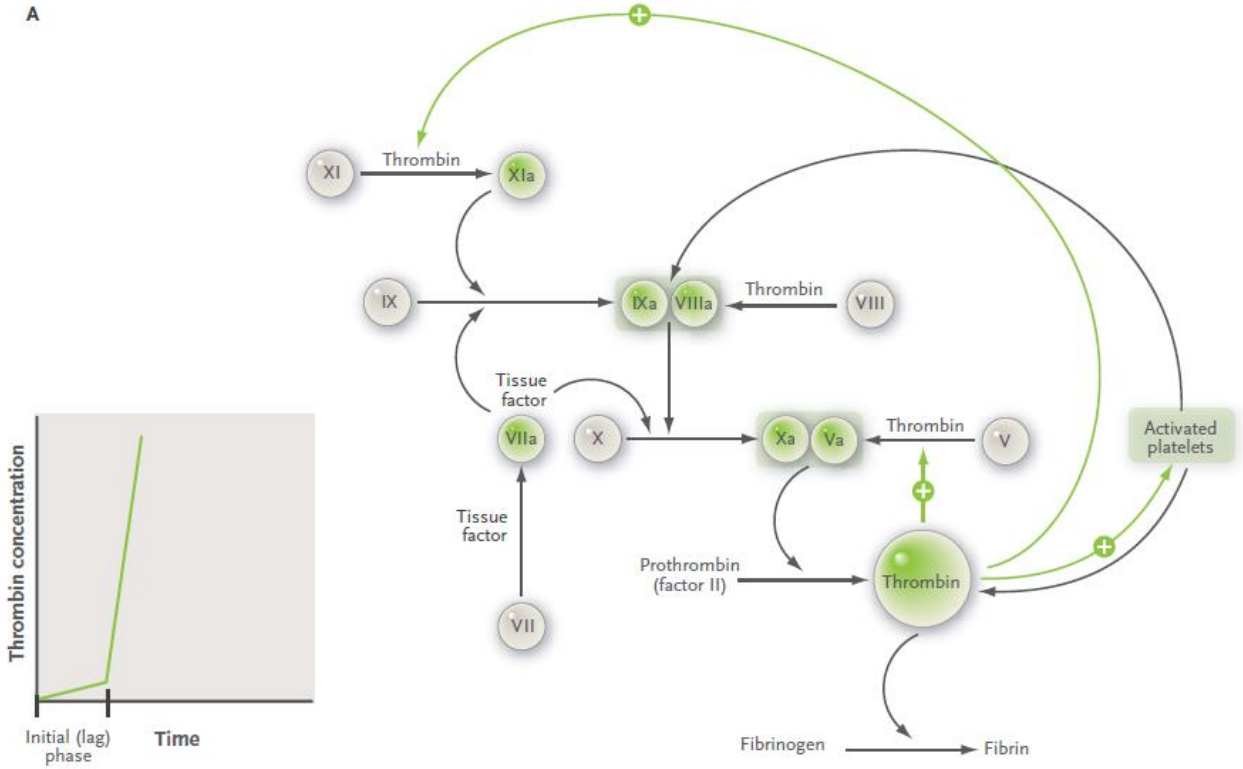
Secondary Hemostasis

Platelet aggregation and activation

Coagulation cascade activation > thrombin generation, fibrinogen > fibrin (strands)

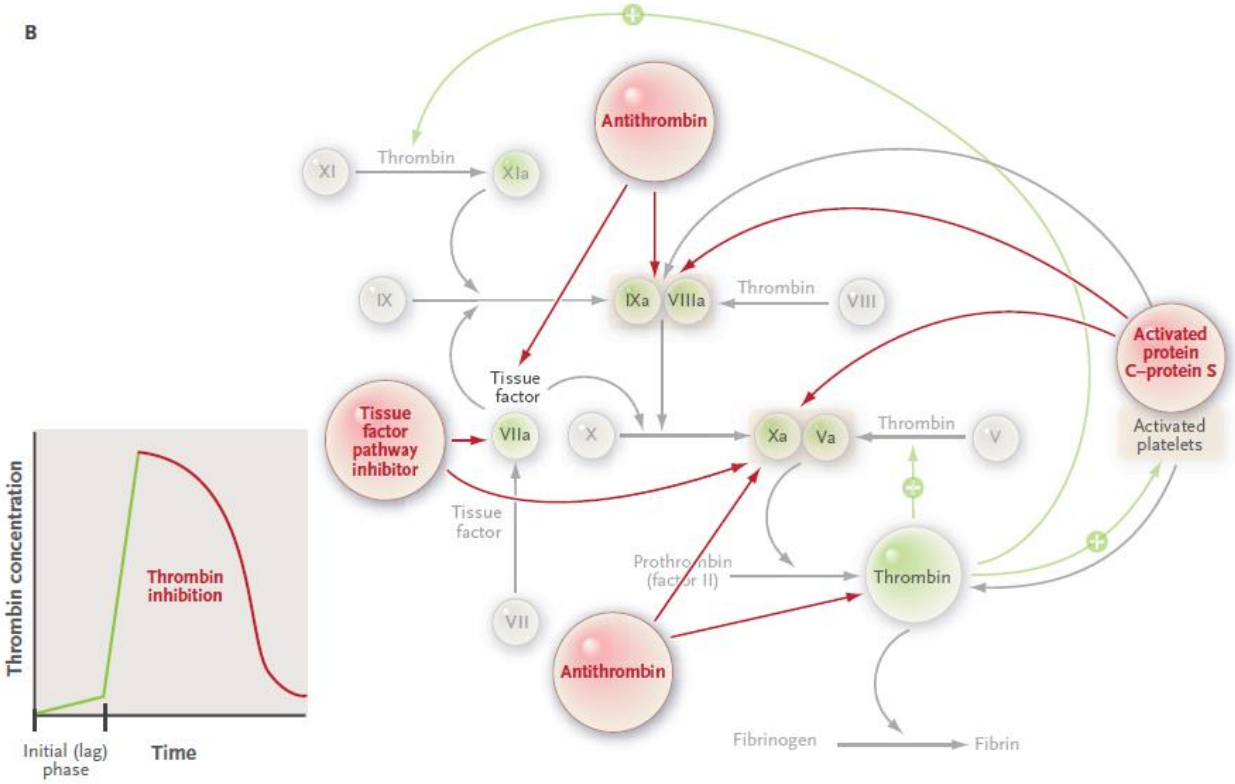
Adapted from Semple JW *et al.* Nat Rev Immunol 2011;11:264ff

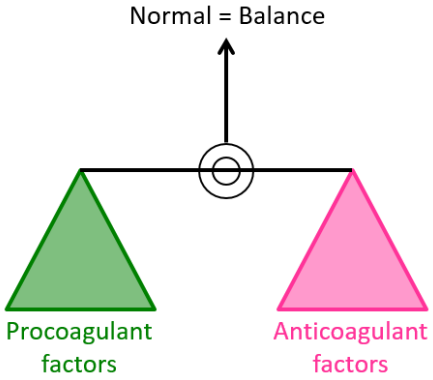
Coagulation in vivo



Tripodi A & Mannucci PM. NEJM 2011;365:147ff

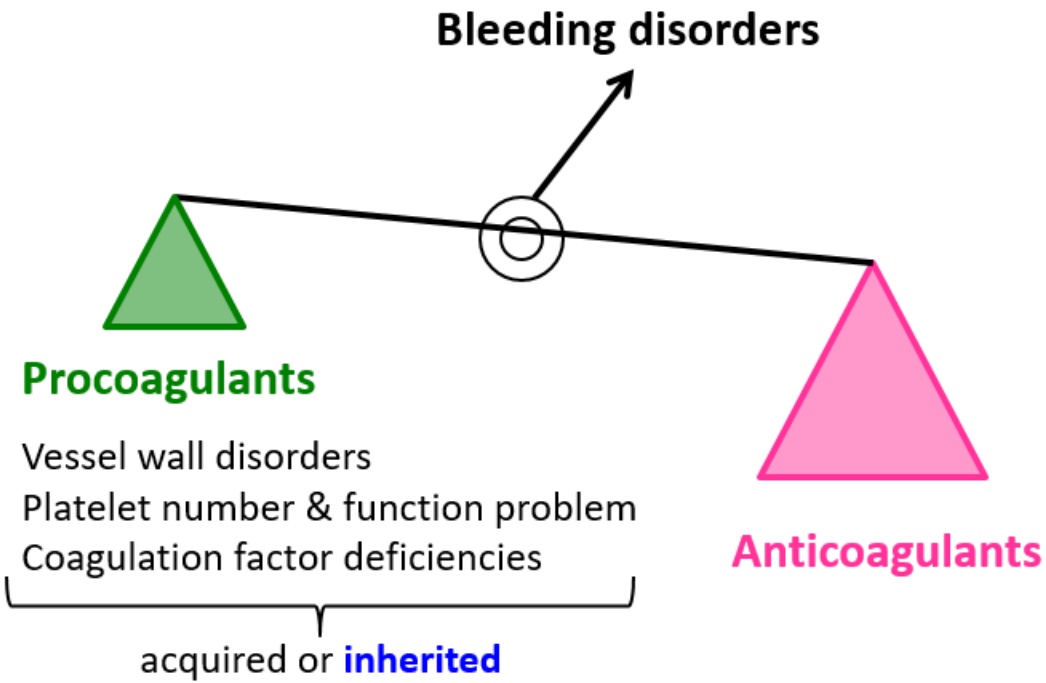
Coagulation in vivo





Tripodi A & Mannucci PM. NEJM 2011;365:147ff

DISORDERS of HEMOSTASIS



Adapetd from Lisman T *et al*. J Hepatol 2010;53:362ff

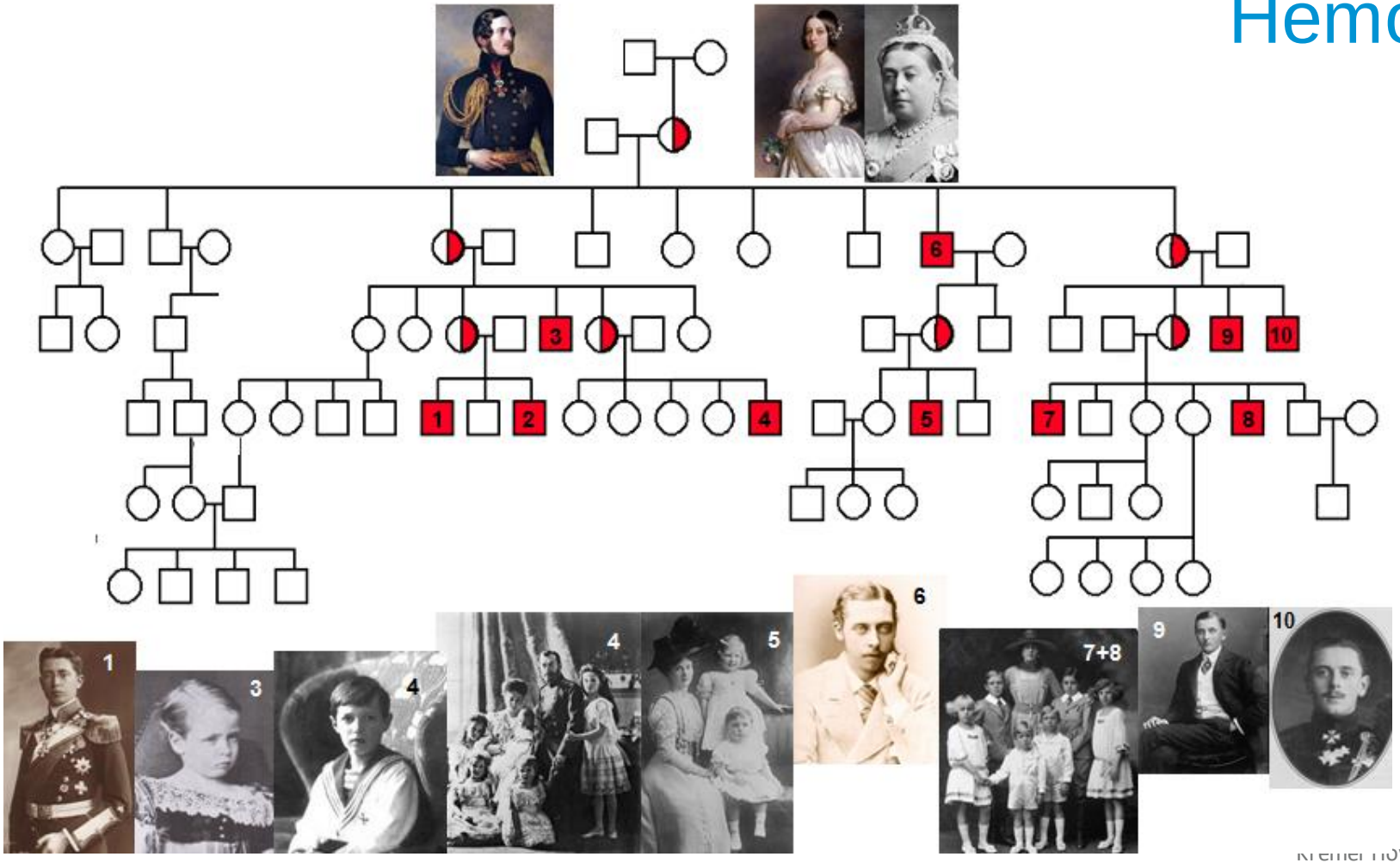
Bleeding Phenotypes = possible first clue

Table 48.1 Main specific clinical differences between diseases of coagulation factors and platelet disorders.

Findings	*Disorders of coagulation*	*Platelets/vessels*
Onset of bleeding	Delayed after trauma	Spontaneous or immediately after trauma
Mucosal bleeding	Rare	Common
Petechiae	Rare	Characteristic
Deep haematomas	Characteristic	Rare
Ecchymoses	Large and solitary	Small and multiple
Haemarthrosis	Characteristic	Rare
Bleeding from superficial cuts and scratches	Minimal	Persistent; often profuse
Sex of patient	80–90% male ?	Equal

Hoffbrand *et al*. Postgraduate Hematology

Hemophilia



Hemophilias

- Hemophilia A – Factor VIII deficiency
  - *F8* on X-chromosome; 1:5’000 male newborns
- Hemophilia B – Factor IX deficiency
  - *F9* on X-chromosome; 1:25’000-50’000 male birth
  - Tenna family
- (Hemophilia C) – Factor XI deficiency
  - *F11* on chromosome 4q32-35; both sexes, in <1% of population, 8-10% in Ashkenazi Jews
- Acquired Hemophilia A (auto-immune disease)

Hemophilia A – molecular defects

40/45 families w. sporadic HA
F8 mutation occurred in the last two generations
82% carrier mothers had de novo *F8* mutation
74% of mutated X-chrom was of paternal origin

242 mothers of sons w. HA / HB
133 (55%) obligate carriers
94 (39%) carrier status confirmed, fam. hist. neg.
8 (3%) non-carrier
7 (3%) unknown

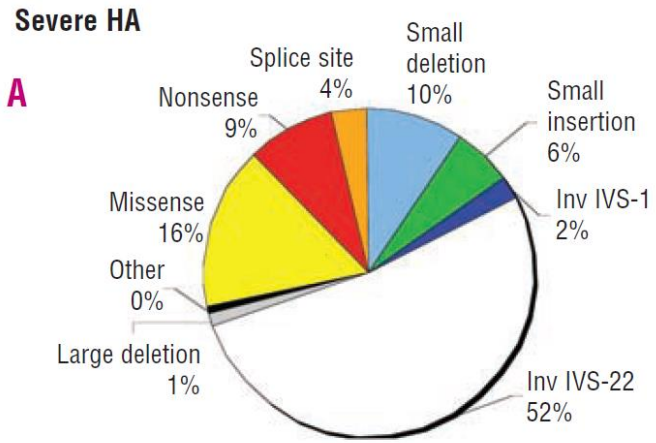
In ~80% of «new carrier mothers» *F8/F9* mutation on paternal X-chrom
- Always youngest daughter
- Father ≥40y

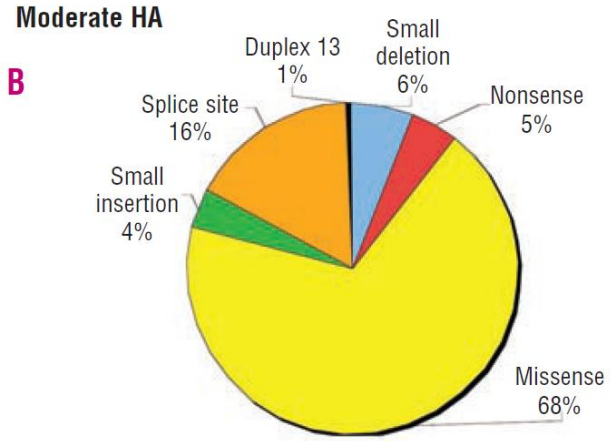
«Hemophilia starts in girls (not mothers)»

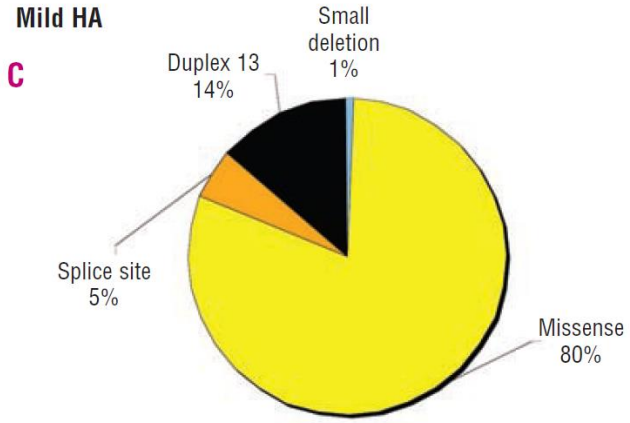
Mårtensson A *et a*l. Clin Genet 2016; 90:63ff

Carcao M *et a*l.
personal communication 9th BIC2017

Hemophilia A – molecular defects







Severe FVIII:C <1%
Moderate FVIII:C 1% - <5%
Mild FVIII:C 5% - 40%

Margaglione *et al*. Haematologica 2008;93:722ff

Hemophilia A – FVIII inhibitors

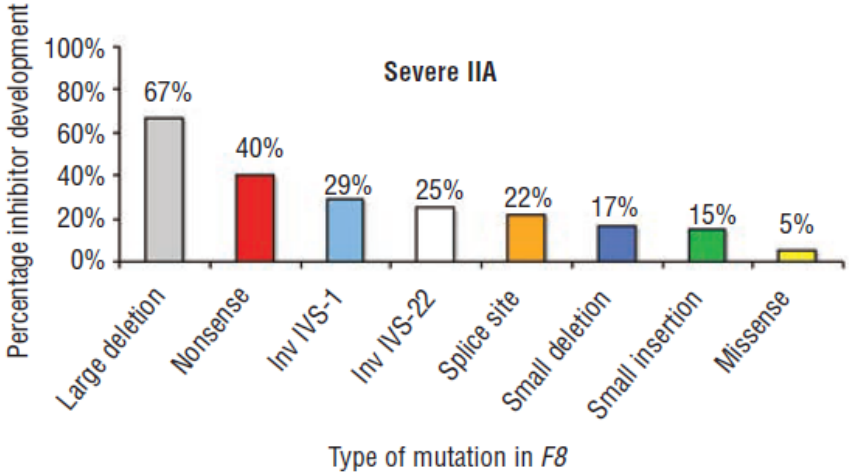


Figure 2. Incidence of inhibitor occurrence in patients with different types of *F8* mutations.

Table 3. Proportions of patients with severe hemophilia A and different *F8* mutations who developed inhibitors.

	Italy (n=870)	*Germany*[15] *(n=753)*	*HAMSTeRS (n=845)*
Large deletions	7/13 (67%)	41%	46%
Nonsense mutations	31/77 (40%)	31%	35%
IVS-1 inversions	6/19 (29%)	17% (26%)[a35]	n.a.
IVS-22 inversions	113/451 (25%)	21%	n.a.
Splicing-site mutations	7/3 (22%)	17%	8%
Small deletions	14/84 (17%)	16%[b]	17%
Small insertions	8/52 (15%)		19%
Missense mutations	7/143 (5%)	5%	10%

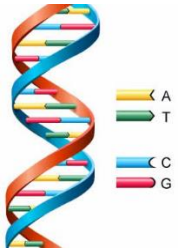
n.a.: not available. [a]1127 patients investigated; [b]small deletion plus small insertion.

Margaglione *et al*. Haematologica 2008;93:722ff

Hemophilia A – FVIII inhibitors

Patient-related factors

- *F8* gene mutation
- Positive family history
- Ethnicity
- Polymorphisms
  - Immune-regulating genes (TNF α, IL-10, CTLA-4,…)
  - MHC class II
- Age
- Infections /Vaccinations
- Intervention



Treatment-related factors

- Intensity & mode of FVIII exposure
- Source of FVIII product (plasma-derived *vs.* recombinant)
- Switching between products (?)
- Extravasation of FVIII
- continuous infusion of FVIII

Adapted from Textbook of Hemophilia, 3rd Edition

Gene therapy

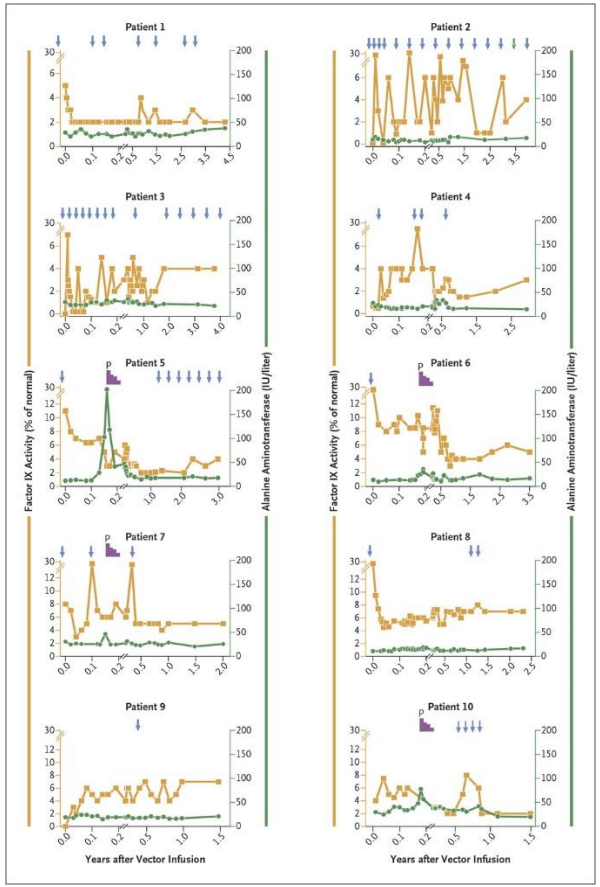
The NEW ENGLAND JOURNAL of MEDICINE

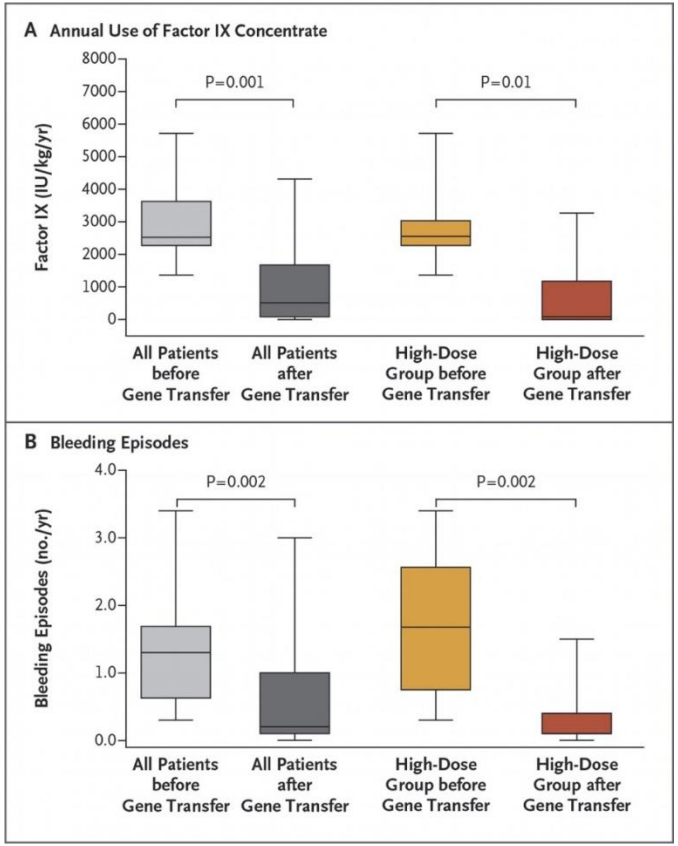
ESTABLISHED IN 1812 DECEMBER 22, 2011 VOL. 365 NO. 25

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

Gene therapy





Nathwani *et al*. N Engl J Med 2014;371:1994ff

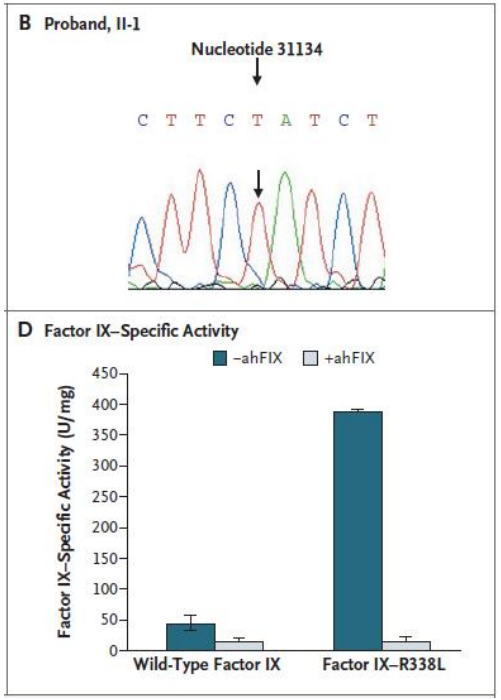
Gene therapy – Codon optimized

- Male 26y; spontaneous VTE, thrombophilia screening negative

Table 1. Clinical Characteristics and Laboratory Data from the Family Members.*

Subject	Sex	Age (yr)	Activated Partial-Thromboplastin Time (sec)†	Factor IX Antigen (% of normal level)	Factor IX Activity (% of normal level)	Factor IX Activity-to-Antigen Ratio
II-1, proband	M	23	25.7	92	776	8.4
I-1	M	53	35.2	105	127	1.2
I-2	F	46	28.2	94	337	3.5
II-2	M	21	33.4	116	123	1.0
II-3	M	11	29.1	64	551	8.6

* II-1 refers to the proband, I-1 to his father, I-2 to his mother, II-2 to the older of his younger brothers, and II-3 to the youngest brother.
† The normal range for activated partial-thromboplastin time is 30 to 40 seconds.



F9 gene Arg 338 Leu = FIX Padua
Arg 338 Gln = hemophilia B
„Pharma“ Arg 338 Leu > rFIX:C 5-10x of wt

Simioni *et al*. NEJM 2009:361:1671ff

Acquired hemophilia B of genetic origin

31y. Male, Swiss origin

- Deep vein thrombosis left leg (Snowboard accident)
- Family history: known Antithrombin deficiency
- Antithrombin 48%; *SERPINC1 splice site* Mutation

➢ Anticoagulation w. Fondaparinux → Vitamin K antagonist

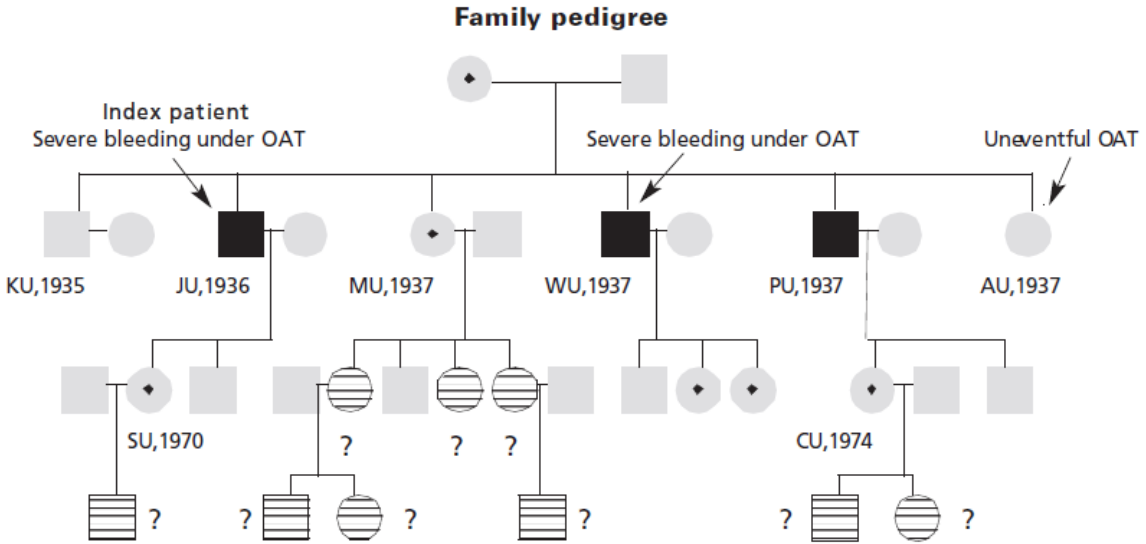
➢ Went, despite anticoagulation, again snowboarding

➢ Fall and large/extreme muscle hematoma (reminiscent of hemophilia)

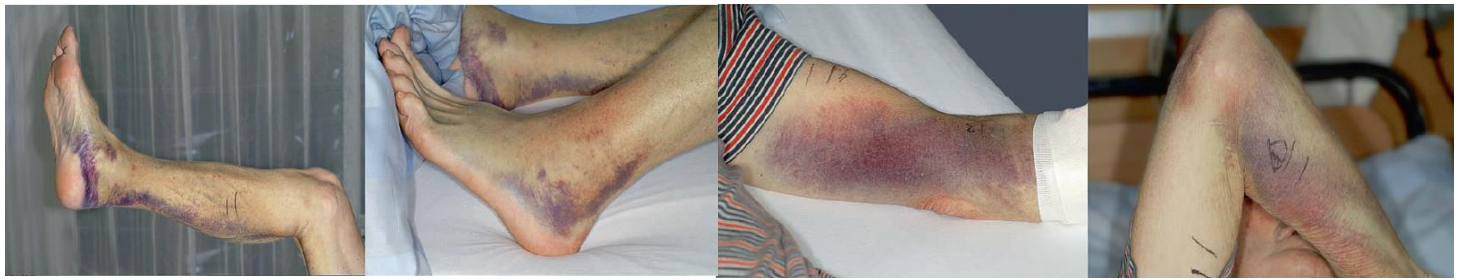
- Lab – coagulation tests:
  - INR 1.96; aPTT 97.3 sec (normal 25-36sec)
  - Extended lab: FII 46%, FVII 30%, FX 19%; FIX <1%,
  - *F9* propeptide mutation p.Ala 37 Thr

Jahns *et al.* Thromb Haemost. 2011;106:381f

F9 propeptide mutation



OAT = oral anticoagulation treatment



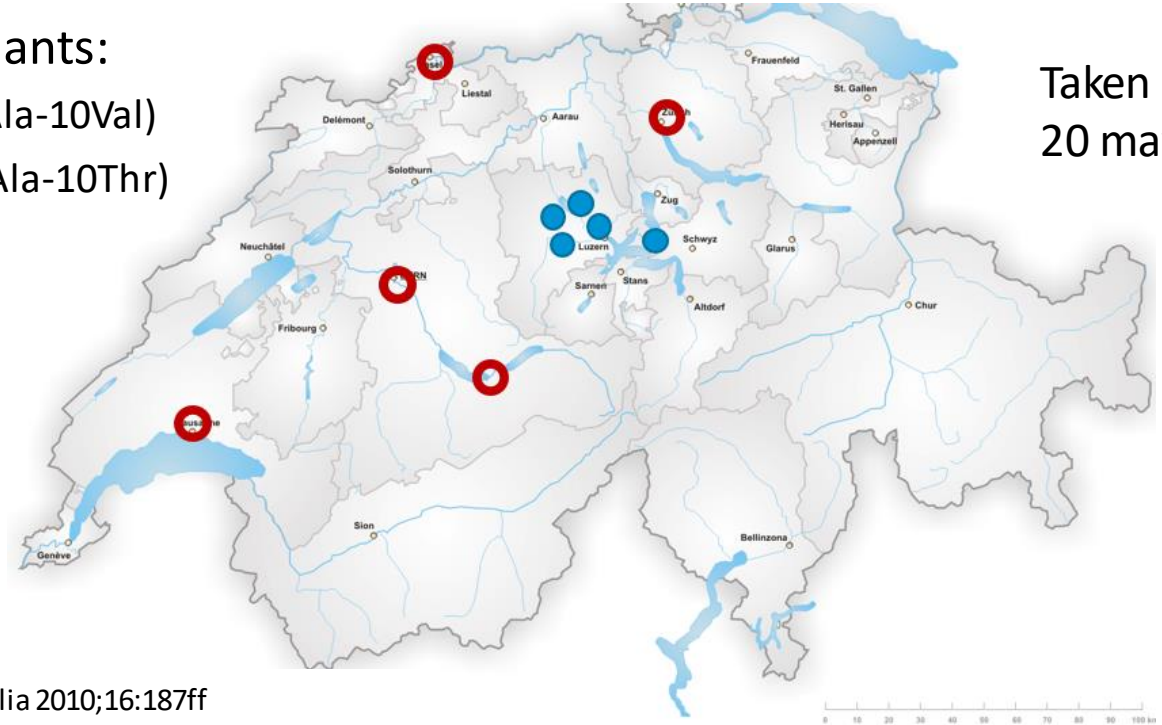
Ulrich *et al*. Swiss Med Weekly 2008;138:100ff

F9 propeptide mutation – a Swiss problem

F9 propeptide variants:

p.Ala37Val (legacy Ala-10Val)

p.Ala37Thr (legacy Ala-10Thr)

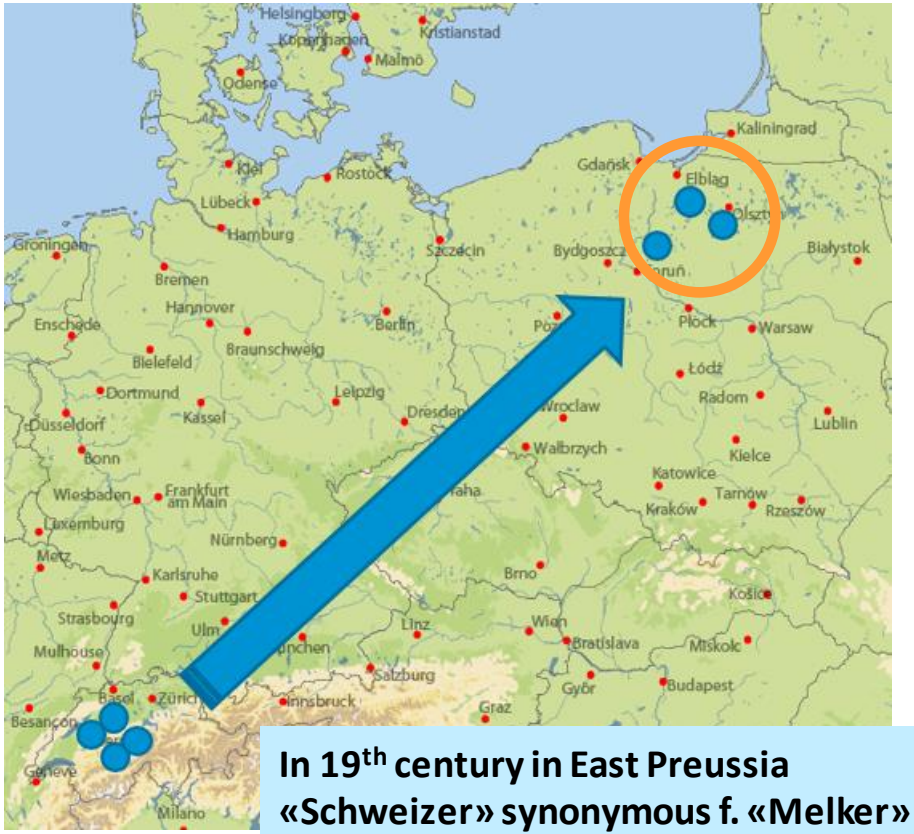


Taken together
20 maybe more

BASEL 1: Holbro *et al*. Haemophilia 2010;16:187ff
BERN 1&2: Oldenburg *et al*. T&H. 2001;85:454ff
BERN 3: Jahns *et al*. T&H. 2011;106:381f
LAUSANNE 1: Gavillet l*et al*. J Thromb Thrombol. 2011;32:232f
ZüRICH 1: Oldenburg *et al*. T&H. 2001;85:454ff
ZüRICH 2: Ulrich *et al*. SMW 2008;138:100f

……..and many more:
Pezeshkpoor *et al*. Ann Hemtol 2018:67:1091f

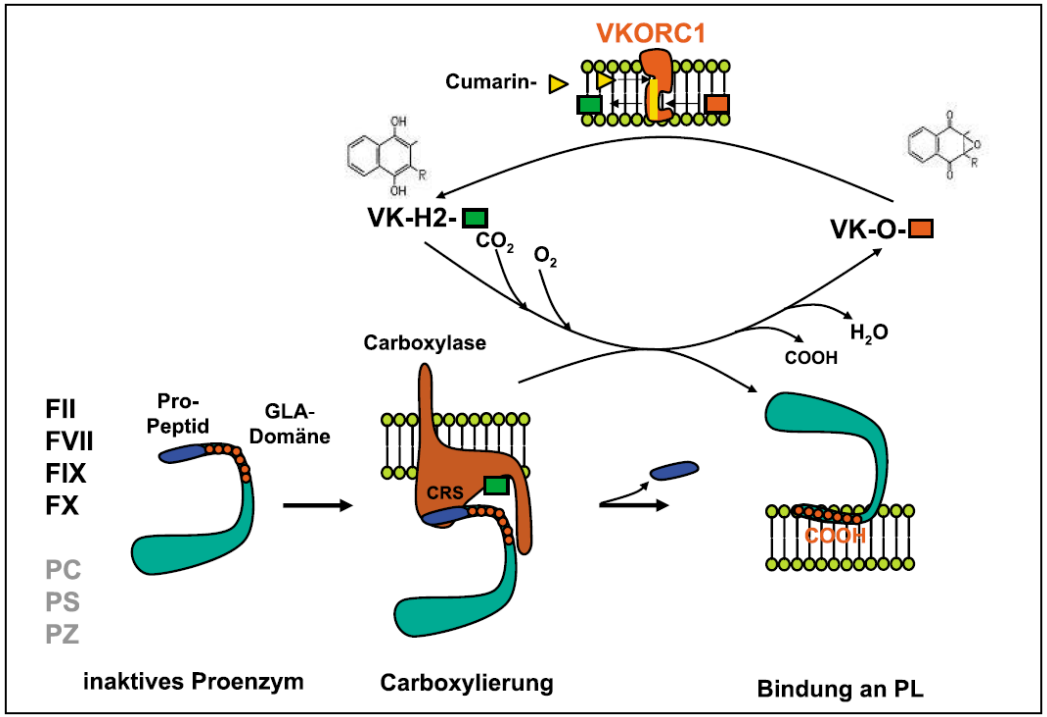
F9 propeptide mutation – a Swiss problem



In 19th century in East Preussia «Schweizer» synonymous f. «Melker»

Here,
first cases
were detected

F9 propeptide mutation – mechanism



γ-Carboxylation

in presence of:

- unlimitted Vit K = normal
- limited Vit K / competition (Vit K Antagonists, i.e. Phen-procoumon, etc.) reduced, because reduced affinity / not well fitting into enzymatic pocket

Oldenburg *et al*. Haemostaseologie 2008;28:44ff

Rare bleeding disorders

Table 42.1 General features of autosomal recessive deficiency of coagulation factors.

Deficiency	*Estimated prevalence**	*Gene (chromosome)*
Fibrinogen	1 in 1 million	*FGA*, *FGB*, *FGG* (all on 4q28)
Prothrombin	1 in 2 million	*F2* (11p11–q12)
Factor V	1 in 1 million	*F5* (1q24.2)
Combined factor V and VIII	1 in 1 million	*LMAN1* (18q21.3–q22) *MCFD2* (2p21–p16.3)
Factor VII	1 in 500 000	*F7* (13q34)
Factor X	1 in 1 million	*F10* (13q34)
Factor XI	1 in 1 million	*F11* (4q35.2)
Factor XIII	1 in 2 million	*F13A1* (6p24–p25) *F13B* (1q31–q32.1)
Vitamin-K dependent coagulation factors	Reported in about 30 families	*GGCX* (2p12) *VKORC1* (16p11.2)

*Including dysfunctional proteins.

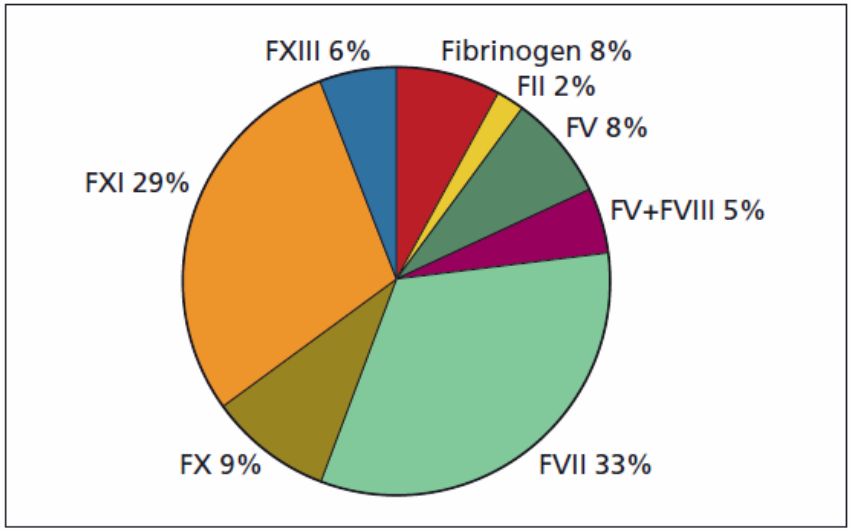


Figure 42.1 Worldwide distribution of rare bleeding disorders derived from two main large data collections (WFH and RBDD).

Hoffbrand *et al*. Postgraduate Hematology

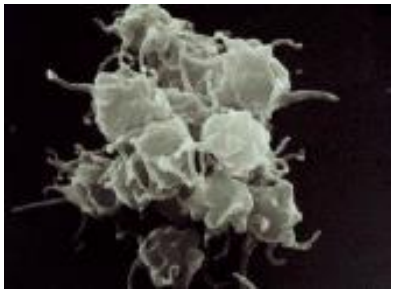
Platelet disorders

Table 48.2 Classification of congenital platelet disorders.

Thrombocytopenia
Non-inherited thrombocytopenia
Drugs and chemical agents
Isoimmune thrombocytopenia
Infiltration of bone marrow
Infections
Other causes
Inherited thrombocytopenias
Thrombocytopenias with reduced platelet size
Thrombocytopenias with normal platelet size
Thrombocytopenias with increased platelet size
Thrombocytopathies
Disorders of platelet adhesion
Disorders of platelet signalling transduction
Disorders of platelet aggregation

Hoffbrand *et al*. Postgraduate Hematology

Resting platelets



Activated platelets

Platelet morphology and disease

C. Bernard Soulier syndrome
D. May Hegglin anomaly
E. Epstein syndrome
F. Gray platelet syndrome

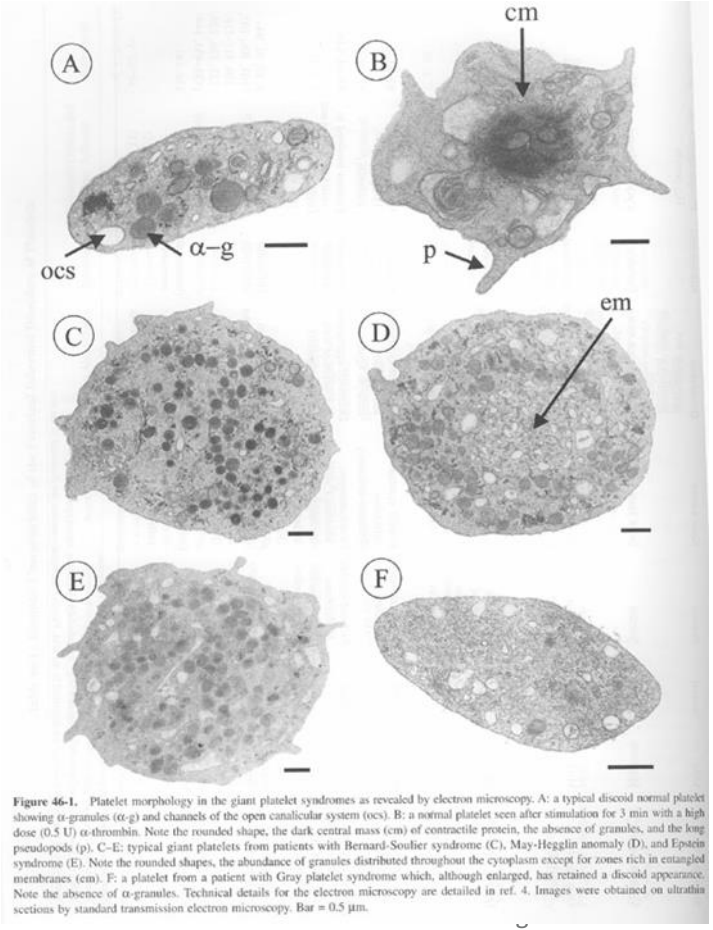
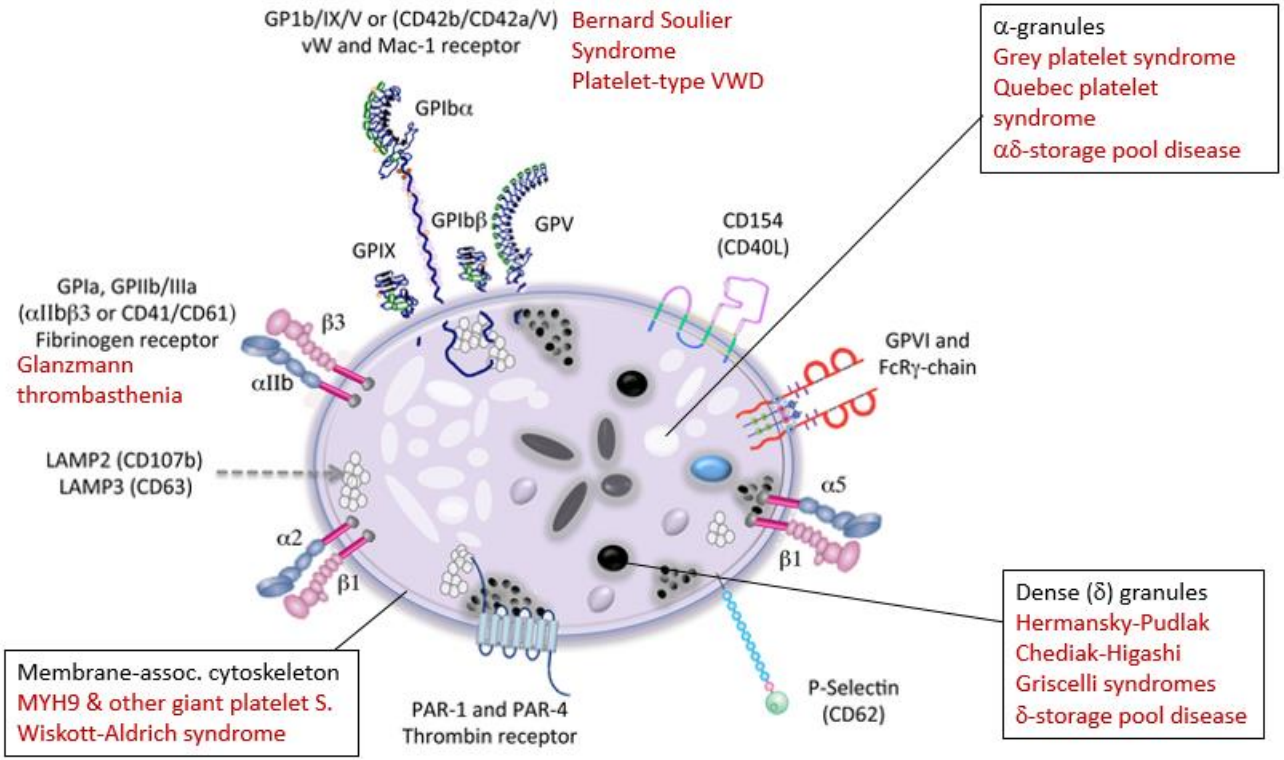


Figure 46-1. Platelet morphology in the giant platelet syndromes as revealed by electron microscopy. A: a typical discoid normal platelet showing α-granules (α-g) and channels of the open canalicular system (ocs). B: a normal platelet seen after stimulation for 3 min with a high dose (0.5 U) α-thrombin. Note the rounded shape, the dark central mass (cm) of contractile protein, the absence of granules, and the long pseudopods (p). C–E: typical giant platelets from patients with Bernard-Soulier syndrome (C), May-Hegglin anomaly (D), and Epstein syndrome (E). Note the rounded shapes, the abundance of granules distributed throughout the cytoplasm except for zones rich in entangled membranes (em). F: a platelet from a patient with Gray platelet syndrome which, although enlarged, has retained a discoid appearance. Note the absence of α-granules. Technical details for the electron microscopy are detailed in ref. 4. Images were obtained on ultrathin sections by standard transmission electron microscopy. Bar = 0.5 μm.

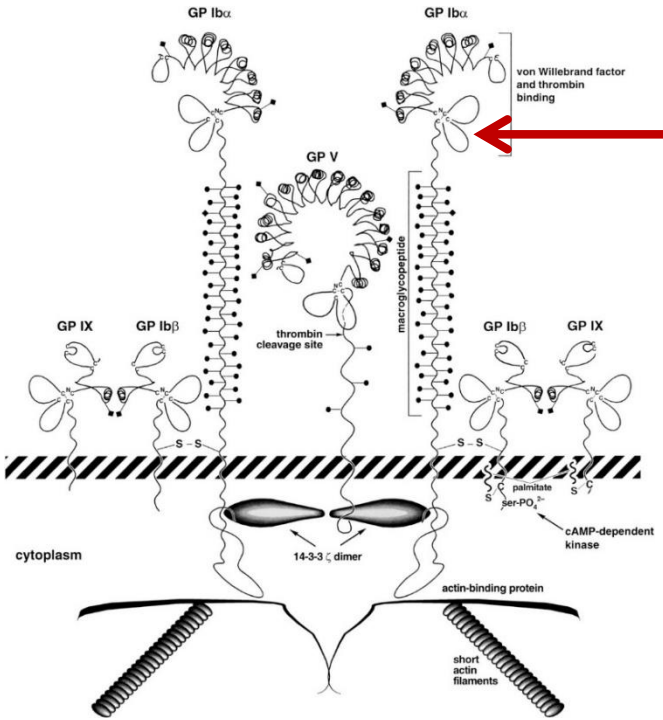
Major platelet receptors and their ligands



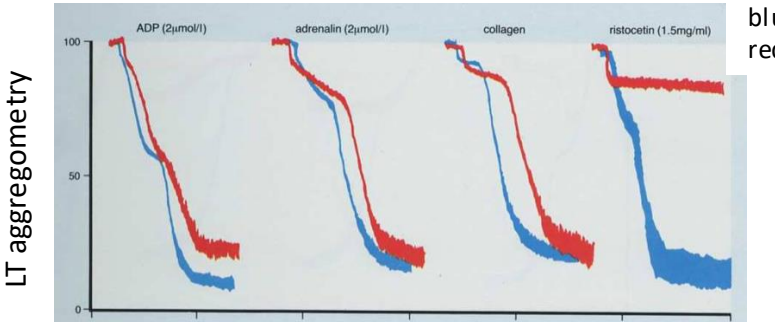
Adapted from Zapata *et al*. PLOS Neglected Tropical Diseases 2014;8(6):e2858

Bernard Soulier syndrome

platelet GP Ib-IX-V complex

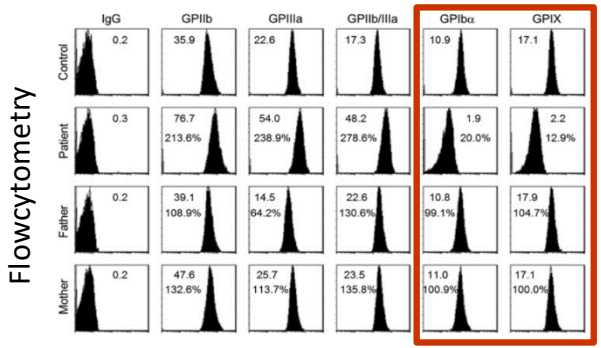


Caveat:
gain of function mutation in *GP1BA*
lead to «Platelet type VWD»



blue – control
red – BSS patient

BSS w.
mutation in *GP9*



López *et al*. Blood 1998;91:4397ff
https://practical-haemostasis.com/Platelets/platelet_function_testing_lta.html
Kanda *et al*. Human Genome Variation 2017;4:17030

Bernard Soulier syndrome

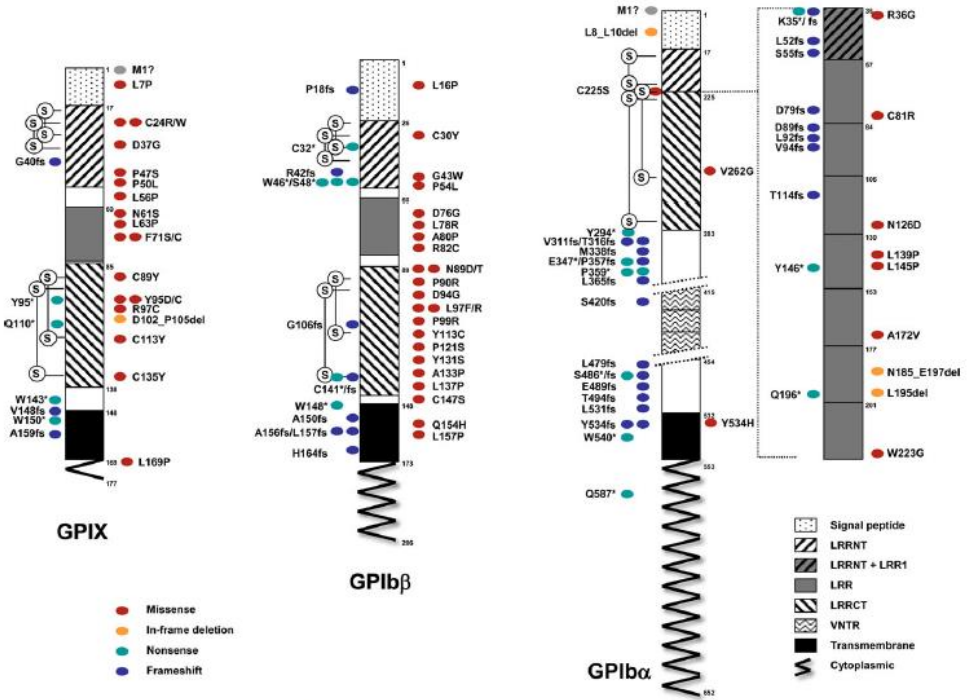


Figure 1. Schematic representation of GPIbα, GPIbβ, and GPIX showing the different domains. Positions are according to UniProt accession numbers P07359, P13224, and P14770, respectively. Positions of LRRNT, which also contains LRR1, LRR2–8, and LRRCT of GPIbα are according to Blenner et al. (2014). Putative disulfide bonds in GPIX are based on sequence conservation of GPIX with GPIbβ as indicated by McEwan et al. (2011).

Autosomal recessive disorder

usually

- Severe forms 2 mutations in *GPIBB* and/or *GPIBA*
- Milder forms 2 mutations in *GP9 (> European founder mutation)*

Monoallelic Bernard Soulier syndrome

- one mutation in *GPIBB* or *GPIBA; Bolzano mutation*
- Mean Platelet Volume (MPV) between normal and ar BSS

Savoia *et al*. Hum Mutat. 2014;35:1033ff

Bernard Soulier syndrome – GP9 mutation

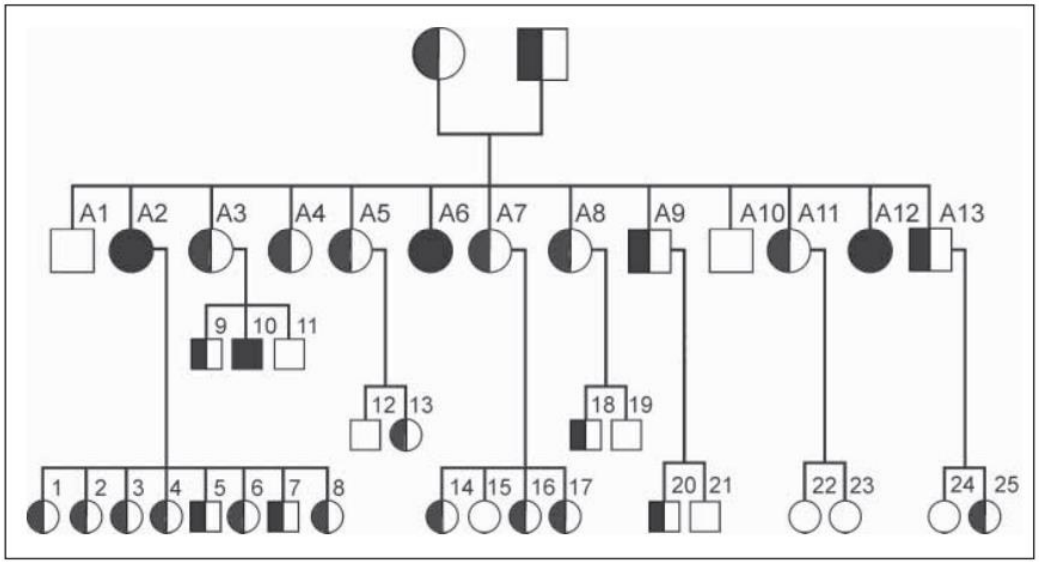


Fig. 1 Pedigree of the first (A1–A13) and second generation (1–25)
■● patients homozygous for Bernard-Soulier syndrome: A1829G mutation in the GPIX gene;
◧◗ heterozygous family members

Large Swiss family

- European founder mutation in GP9
- A2: transfusion of Ec after C-section
- A6: index case, recognized during preoperative evaluation
- A12: splenectomized in childhood when misdiagnosed as iTP

Zieger *et al*. Haemostaseologie 2009;29:161ff

Bernard Soulier syndrome – GP9 mutation

parameter		haemo-globin [g/l]	leuko-cytes [g/l]	throm-bocytes [g/l]	MPV [fl]	CD42b [%]*	CD41 [%]*	CD42a [%]*	genotype
patient	A1	142	6.1	181	11.4	-	-	-	wildtype
	A2	143	6.1	48	18.4	4	188	-	homozygous
	A3	131	5.9	348	10.2	-	-	-	hetero-zygous
	A4	131	4.6	179	11.4	-	-	-	
	A5	113	6.2	179	12.0	-	-	-	
	A6	120	7.2	73	18.4	7	89	-	homozygous
	A7	132	5.4	258	11.0	-	-	-	hetero-zygous
	A8	135	5.1	290	10.3	-	-	-	
	A9	150	5.1	272	10.2	-	-	-	
	A10	140	5.5	115	11.7	-	-	-	wildtype
	A11	129	7.0	252	10.7	-	-	-	heterozygous
	A12	125	6.0	33	17.4	3	225	-	homozygous
	A13	146	6.0	194	11.1	-	-	-	hetero-

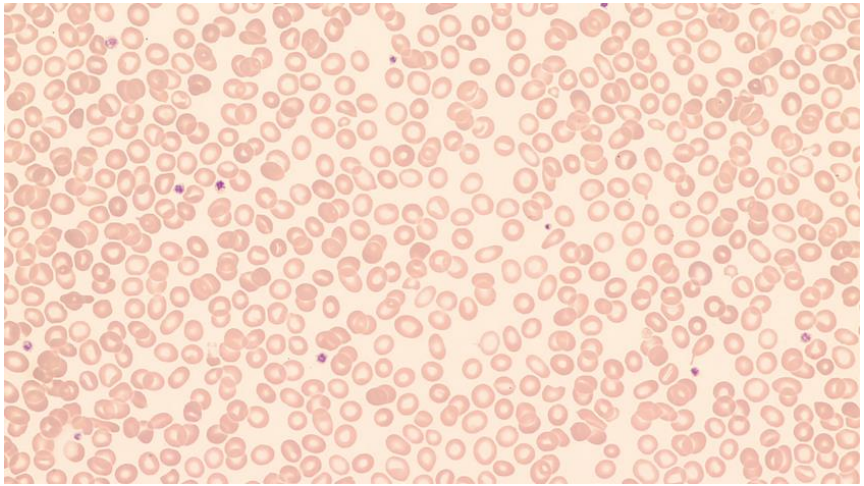
Zieger *et al*. Haemostaseologie 2009;29:161ff

Glanzmann Thrombasthenia

19y. male,

- Refugee from Afghanistan
- recurrent Epistaxis, possible abdominal manifestations > Suspicion of Hereditary Hemorrhagic Telangiectasia

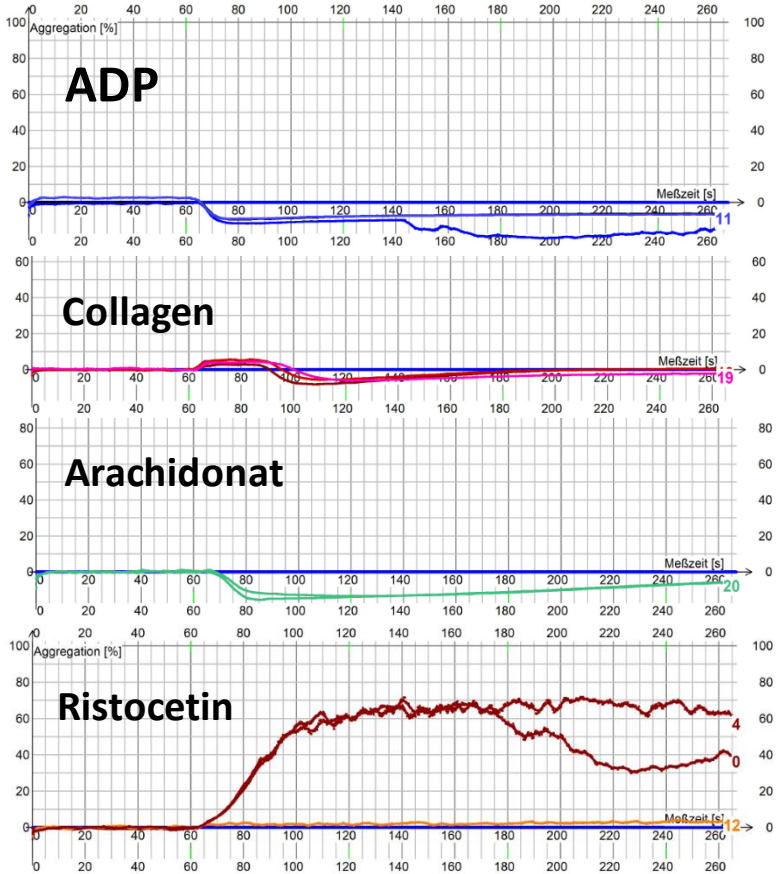
- Hb 82g/L
  - MCV 61 fl
  - MCH 18 pg
- Tc 162 G/l
- CRP <3mg/L
- Trf-Saturation 6%
- Ferritin 14µg/L

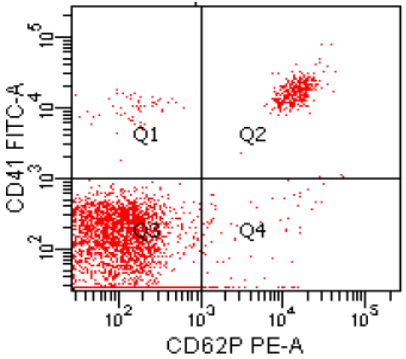


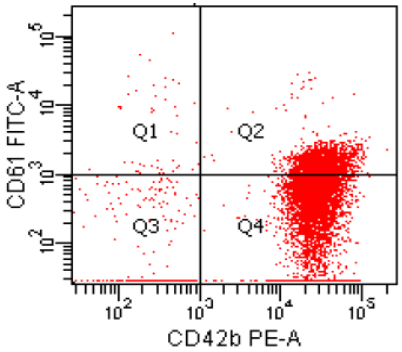
WES performed:

ITGA2B
c.3092 delT, homozygous

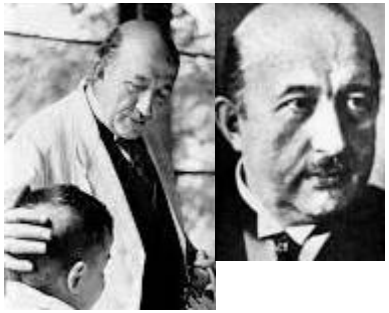
Glanzmann Thrombasthenia







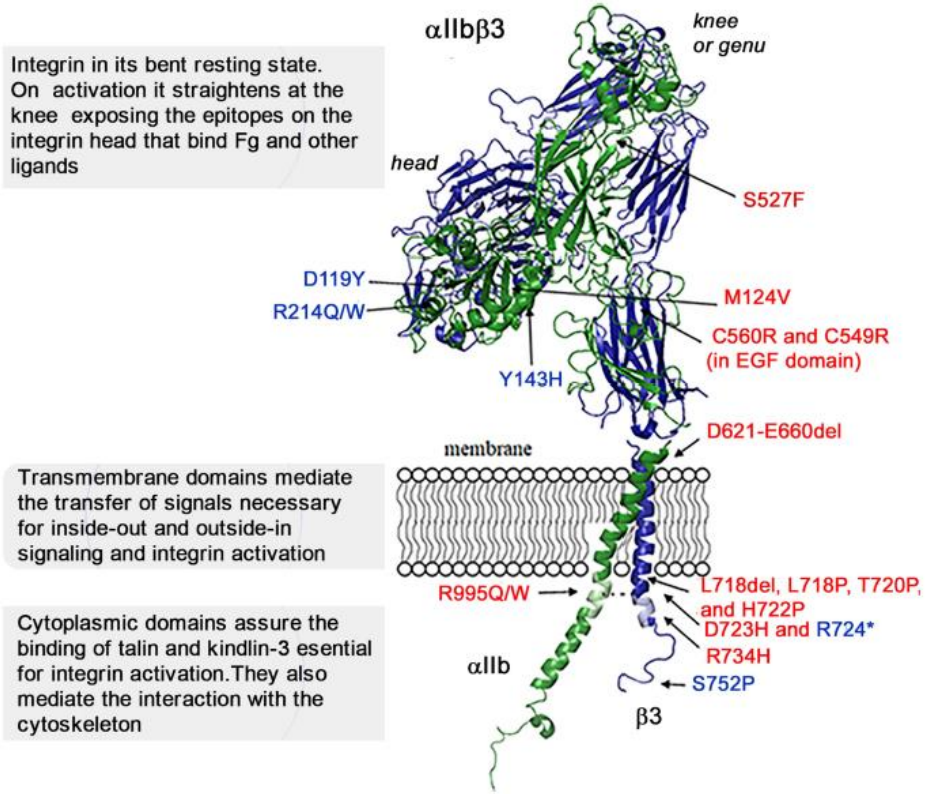
Glanzmann Thrombasthenia



Eduard GLANZMANN
(*12.4.1887 – †2.2.1959)
1912 Medical exam in Bern
1929 Habilitation
1932 Assoc. Prof. Pediatrics
1939-1952 full Prof. of Pediatrics and Director Kinderklinik, Universität Bern

- Rare, prevalence unknown
  - GPIIb (*IGTA2B*), GPIIIa (*IGTB3*) Chr. 17q21.31-32
  - Autosomal recessive inheritance
- OMIM: #273800 (*IGTA2B*) & #619267 (*IGTB3*)
- mainly muco-cutaneous bleeding
- Platelet count and morphology normal
- (massively) increased bleeding time
- No platelet-aggregation after stimulation w. ADP, collagen, epinephrine, thrombin, Arachidonat
- Flowcytometry: no /severely reduced Expression of CD 41 / CD 61 on platelet surface

Glanzmann Thrombasthenia



Loss-of-function mutations (blue)

- In the β3 extracellular head prevent binding of Fbg
- in the β3 cytoplasmic tail prevent binding of kindlin-3 and/or talin and block steps essential for integrin activation

Gain-of-function mutations (red)

- ➢ at least partial activation of αIIbβ3
- often associated with macro-thrombocytopenia
- variable loss of αIIbβ3 function

Nurden. Journal of Blood Medicine 2021;12:581ff

Glanzmann Thrombasthenia

Type I Subgroup	
- Absence of platelet aggregation and little or no clot retraction. Levels of αIIbβ3 <5% or absent. Platelet Fg storage pool lacking or negligible. AR inheritance.	- The most common type of GT, given by defects in *ITGA2B* and *ITGB3* genes. With *ITGA2B* defects αvβ3 may still be present and functional. Patients susceptible to form isoantibodies reactive with αIIbβ3 and/or αvβ3 after blood transfusion or pregnancy.
Type II Subgroup	
- Absence of platelet aggregation but clot retraction can be partial or normal. Residual αIIbβ3 historically defined as 5–15% of normal levels. Platelet Fg pool can be substantial. AR inheritance.	- Frequency variable within populations but usually less than 20% of the patients. Given by defects in *ITGA2B* and *ITGB3*. Clot retraction defects and the platelet Fg storage capacity are mutation dependent.
Variant Forms	
- Absence of platelet aggregation but clot retraction and Fg storage highly variable. Residual αIIbβ3 mainly >50% or even normal but non-functional with little or no activation-dependent Fg binding as also shown by a lack of PAC-1 binding. AR inheritance.	- Rare. Can be given by defects in *ITGA2B* but mostly by *ITGB3* variants. Extracellular mutations directly or indirectly abrogate Fg-binding sites. Intracellular mutations stop signals for αIIbβ3 activation. Clot retraction and Fg storage are mutation dependent. Can be confused with defects in *FERMT3* and *RASGRP2* that prevent kindlin-3 (LAD-III disease) and CalDAG-GEFI signaling.
Upregulated αIIbβ3 and Macrothrombocytopenia (MTP)	
- Much reduced platelet aggregation with clot retraction and Fg storage again variable. Residual αIIbβ3 normally >30% but with spontaneous binding of PAC-1 (but rarely Fg). MTP mostly moderate with subpopulations of enlarged even giant platelets. AD inheritance.	- Rare. Patients with up-regulated αIIbβ3 interfering with megakaryocyte maturation and platelet biogenesis with enlarged platelets in variable numbers. Bleeding mostly due to defective αIIbβ3 function. Single allele mutations on *ITGA2B* but mostly *ITGB3*. Often these affect cytoplasmic domains.

Nurden. Journal of Blood Medicine 2021;12:581ff

GT & alloantibodies

Total patients (*n*)	Patients screened (*n*)	Positive cases [*n* (%)]	Methods
177	Not specified	6 (3·5%)	Not specified
59	54	21 (39%)	MAIPA; other immunological methods; methods based on inhibition of normal platelet aggregation by the patient's plasma
17	16	2 (12·5%)	MAIPA / ELISA using the commercial plate (PAK2-LE)
24	24	13/16 (81%) of the French Gypsy patients and 2/8 (25%) of the patients with other GT mutations	MAIPA
83	Not specified	20 (24%)	Not specified
218	Not specified	47 (22%)	Not specified

rf: - Having had an anti-αIIbβ3
- GT Typ 1
- Bi-allel. Null-Mutations (*IGTA2B/IGTB3*)
- sex
- ethnicity

A - George *et al.* Blood 1990;75:1383–1395
B - Poon *et al.* JTH 2004;2:1096–1103
C - Santoro *et al.* Haemophilia 2010;16:805–812
D - Fiore *et al.* Haemophilia 2012;18:e201–e209
E - Nurden *et al.* Human Mutation 2015;36:548–561
F - Poon *et al.* Transfus Med Rev 2016;30:92-9

Fiore *et al.* Br J Haematol 2018; 181:173ff

“Modern approach” to chronic Immune Thrombopenia (ITP)

20y. female,

- CBC at age 10 y: Hb 139 g/L, MCHC 345 g/L, MCV 78 fl, MCH 27 pg, Lc 7.6 G/L, normal distribution, Tc 109 G/L, MPV 12.6 fl.
- BMP (11y): *compatible with chronic ITP*
- Diagnosis of chron. ITP at age 11y

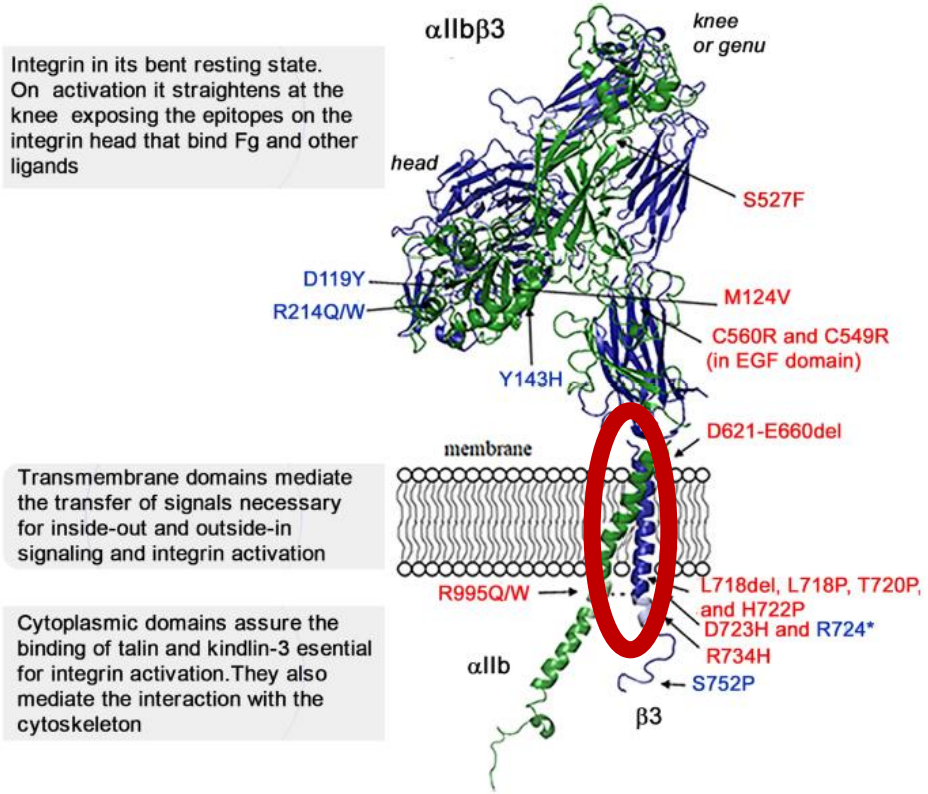
➢ Now Reevaluation

“Modern approach” to chronic ITP (2)

		Auftragsnummer:	46608385 N	47261921 N	47406204 N	48275673 N
		Probenabnahme:	15.07.20 13:30	11.10.21 14:50	12.07.22 09:45	30.01.23 09:00
		Auftragseingang:	15.07.20 13:49	11.10.21 15:04	12.07.22 10:05	30.01.23 09:08
		Auftragsabschluss:	15.07.20 19:05	11.10.21 15:29	12.07.22 13:02	30.01.23 09:25
Analyse	Einheit	Referenzber.				
Hämatogramm						
Entnahmeart			venös	venös	venös	venös
Leukozyten	G/L	3.00 - 10.5	6.05	6.32	5.01	4.95
Hämoglobin	g/L	121 - 154	151	165 !	167 !	165 !
Hämatokrit	L/L	0.36 - 0.44	0.42	0.46 !	0.47 !	0.46 !
Erythrozyten	T/L	3.90 - 5.00	5.33 !	5.81 !	5.69 !	5.64 !
MCV	fL	80 - 98	79 !	80	83	82
MCH	pg	27 - 33	28	28	29	29
MCHC	g/L	320 - 360	359	356	352	355
[illegible]						
Thrombozyten	G/L	150 - 450	51 !	38 !	50 !	59 !
MPV	fL	6.7 - 11.0	14.1 !	13.8 !	12.8 !	13.1 !
IPF	%	1.0 - 6.0	14.6 !	22.5 !	17.7 !	15.3 !
Normoblasten maschinell	/100 Leuk	0	0.00	0.00	0.00	0.00
Differenzierung maschinell						
Neutrophile	G/L	1.60 - 7.40	3.53 (1)	3.87	2.81 (1)	2.62
Eosinophile	G/L	0.02 - 0.40	0.05	0.03	0.03	0.05
Basophile	G/L	0.00 - 0.15	0.02	0.02	0.03	0.02
Monozyten	G/L	0.20 - 0.93	0.51	0.66	0.43	0.45
Lymphozyten	G/L	1.10 - 3.50	1.93	1.71	1.68	1.79
Immature Granulozyten	G/L		0.01	0.03	0.03	0.02
Differenzierung visuell						
Stabkernige N.	G/L	0.20 - 1.40	0.06 !		0.13 !	
Stabkernige N.	%	3.0- 18.0	1.0 !		2.5 !	
Segmentkernige N.	G/L	1.60 - 6.00	3.51		2.98	
Segmentkernige N.	%	35.0 - 67.0	58.0		59.5	
Neutrophile Granulozyten	G/L	1.60 - 7.40	3.57		3.11	
Eosinophile	G/L	0.02 - 0.40	0.06		0.03	
Basophile	G/L	0.00 - 0.15	0.06		0.03	
Monozyten	G/L	0.20 - 0.93	0.33		0.35	
Lymphozyten	G/L	1.10 - 3.50	2.03		1.50	

- Thrombopenia/-pathy NGS Panel: heterozygous *ITGA2B* variant, formally a VUS, but…

Glanzmann Thrombasthenia-like syndrome ?



20y. female,

chron. Tc-penia of unknown origin

- *Heterozygous VUS*

  ***ITGA2B* c.2349-3 C>A**

  Protein region AA783 (transmembrane part)

«*ITGA2B / ITGB3*-related Tc-penia»

- Anisocytosis of platelets
- macrothrombocytopenia,
- Tc-function defects (spontaneous binding of PAC-1 but not of Fbg)
- GPIIb/IIIa (CD41/CD61) ↓ but mostly >30%

Nurden. Journal of Blood Medicine 2021;12:581ff

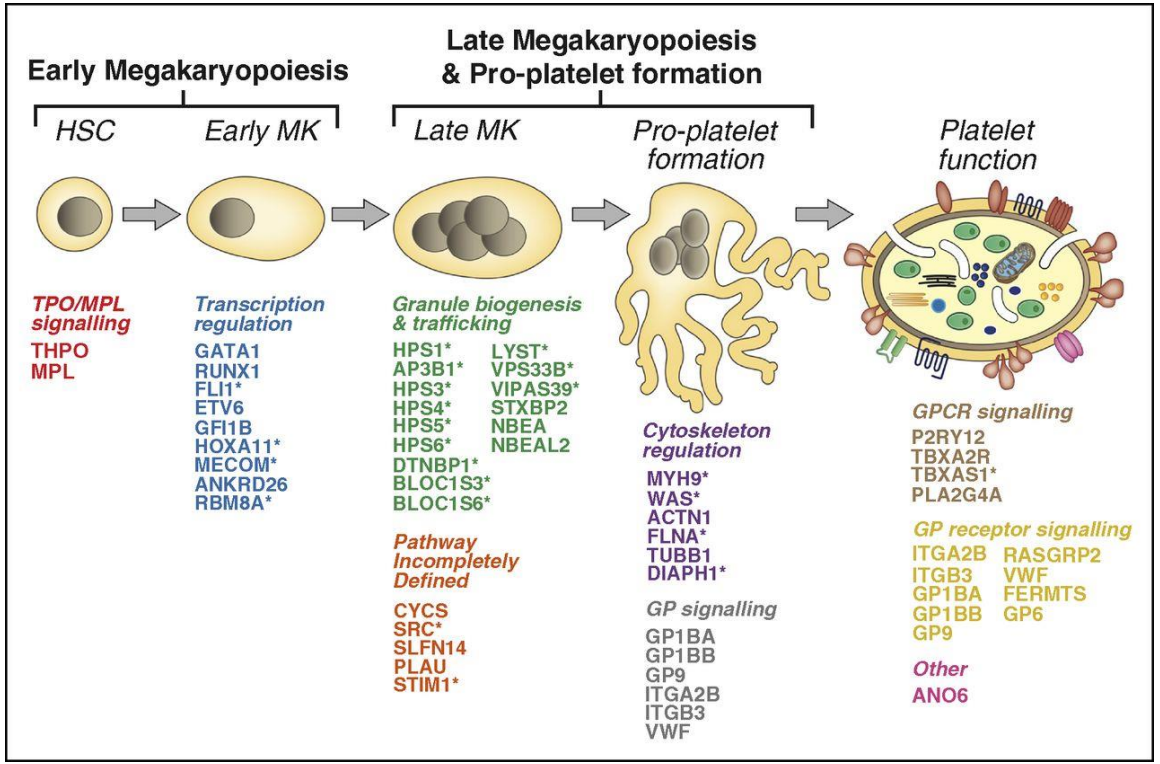
GT-like syndrome ?

	GTLS patients(F1-F10) (n = 33)	Pat.
Bleeding Score (ISTH-BAT)		
BS	4 (0–14)	6/56 points = pathol.
	4±3	
Abnormal BS	10/29 (34%)	
Platelet count, x10^9/L		
150–450	90 (47–194)	
	96±38	
<150	30/33 (91%)	
<100	22/30 (67%)	
<50	3/33 (9%)	25-50
Platelet indexes		
Mean Platelet Volume (MPV), fL		
[7–11]	13 (10–19)	
	13±2	
>11	28/32 (88%)	12.5 – 14.1
Platelet Distribution Width (PDW), %		n.a.
[9–14]	18 (13–35)	
	19±4	
> 14	25/27 (93%)	
Immature Platelet Fraction (IPF), %		
[1–7]	12 (4–36)	
	14±8	
> 7	24/29 (83%)	15 – 22.5

Flowcytometry pending
PAC-1 binding pending

Morais *et al*. PlosONE 2020;15:e0235136

Summary 1



IPDs
within 10 years from <10 to >60 causative genes

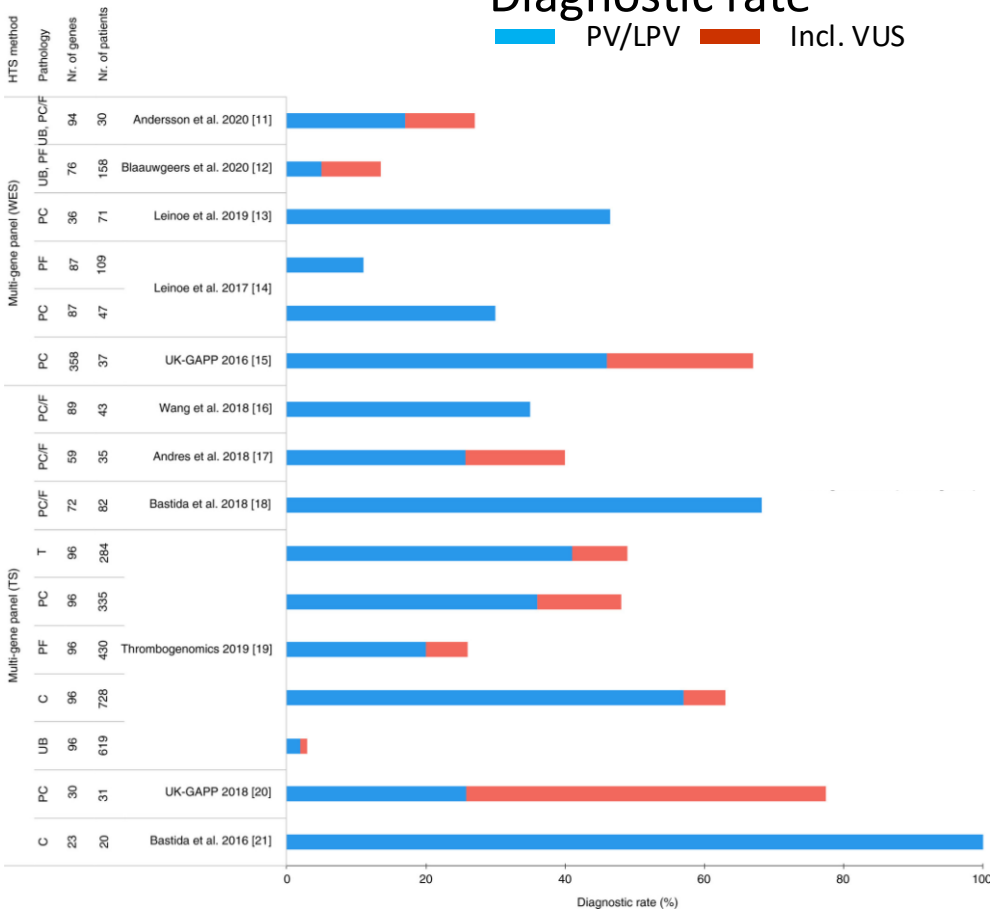
for many no functional test

«Dose-effect» (mono-allelic BSS; GT-like syndrome, etc.)

- Report VUS ?
- Family studies (i.e. when functional tests available)

Lentaigne *et al.* Blood 2016;127:2814ff

Summary 2



Inherited bleeding & platelet disorders =

- heterogeneous group
- High susceptibility of spontaneous and prolonged bleeding
- Specific treatment available for some
  - Associated risk (“inhibitor”)
- Molecular diagnosis important > cannot (always) replace functional investigation
- KVG / health insurance allow only a limited number of investigations
  - Diagnostic glass ceiling
  - No carrier examination (“hemophilia starts in girls” > sisters of patients may be carriers….)

Ver Donck *et al*. JTH 2020;18:1839ff