Laboratory diagnosis and classification of Factor XIII deficiency

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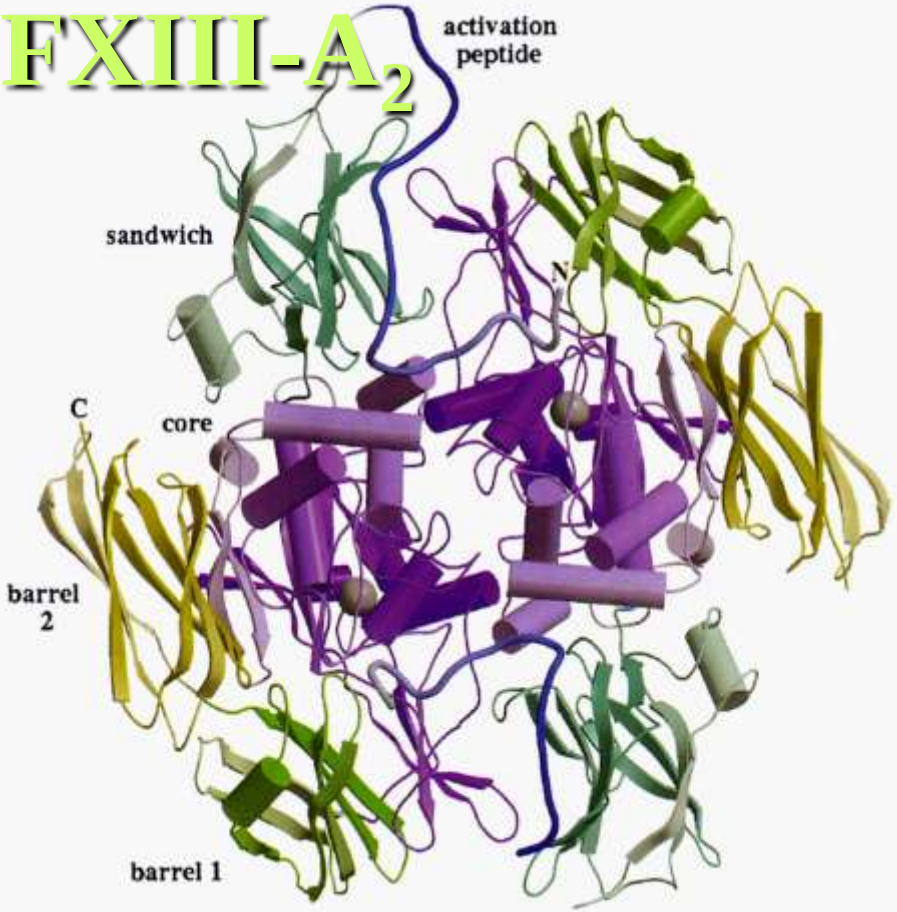
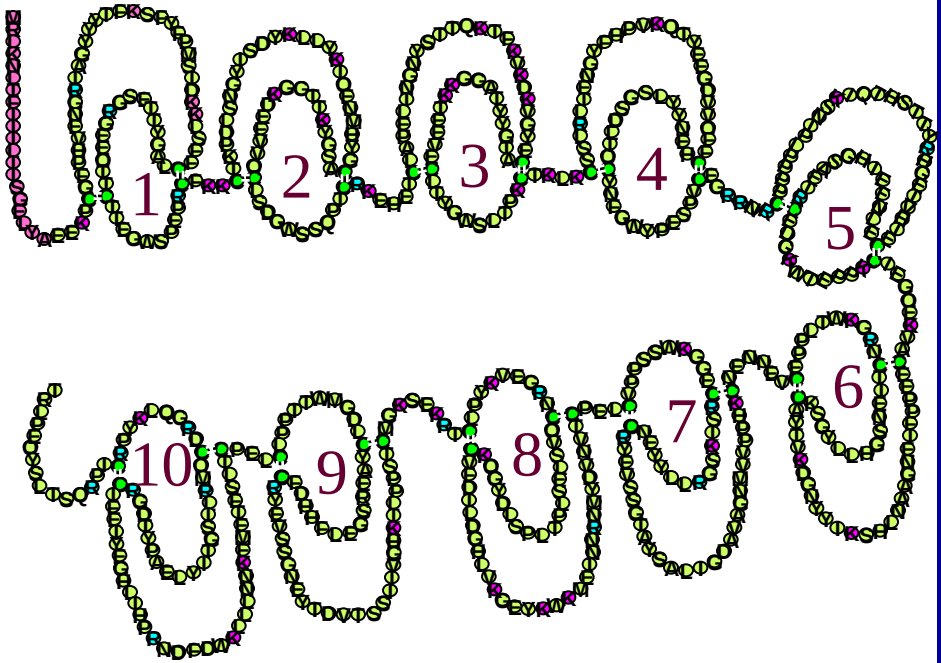
Plasma FXIII: A_2B_2 (B subunit in excess) 14-28 mg/L, associated to fibrinogen

Cellular FXIII: A_2 in platelets 46-82 fg/platelet, in monocytes/macrophages

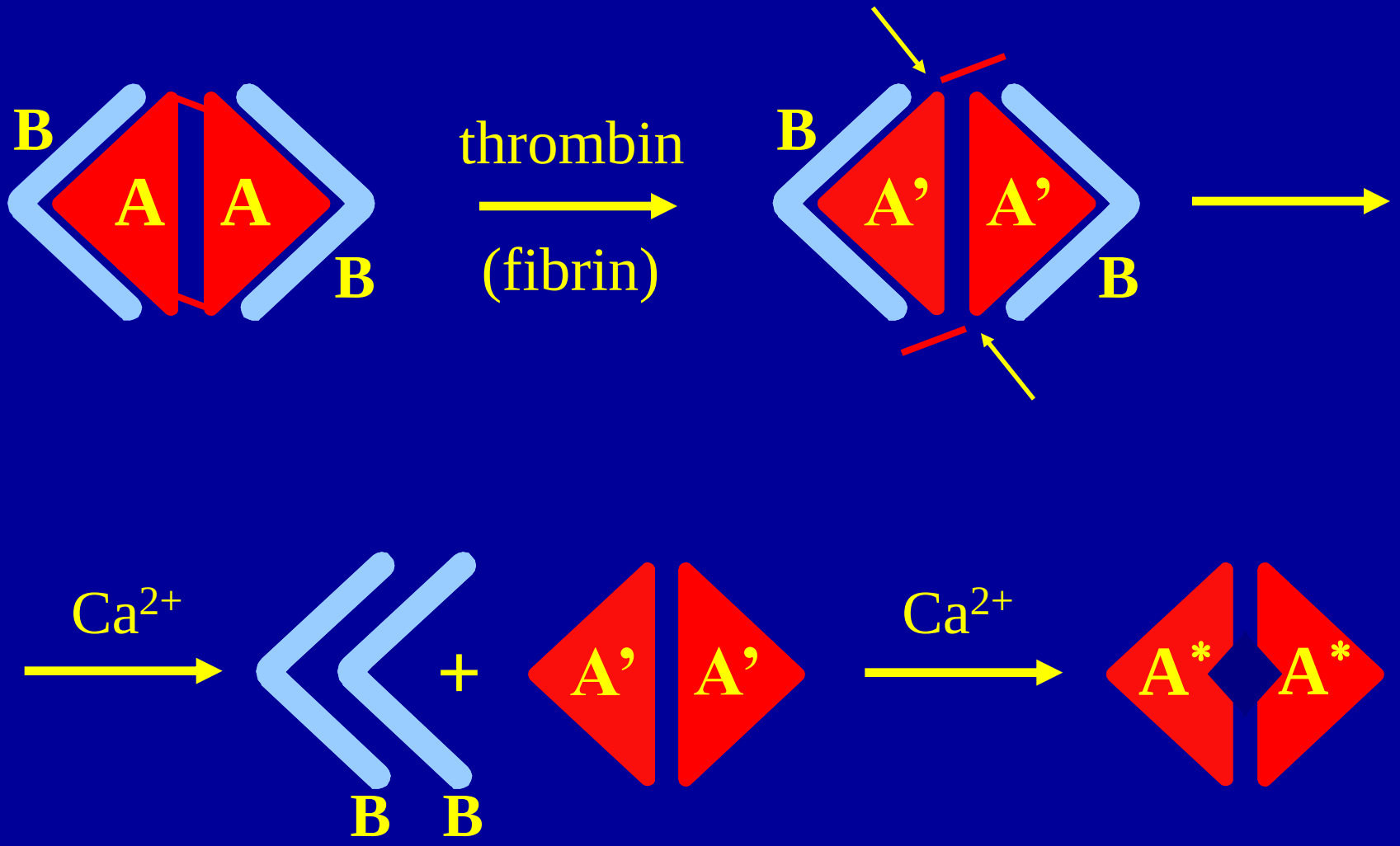
FXIII-A_2



FXIII-B

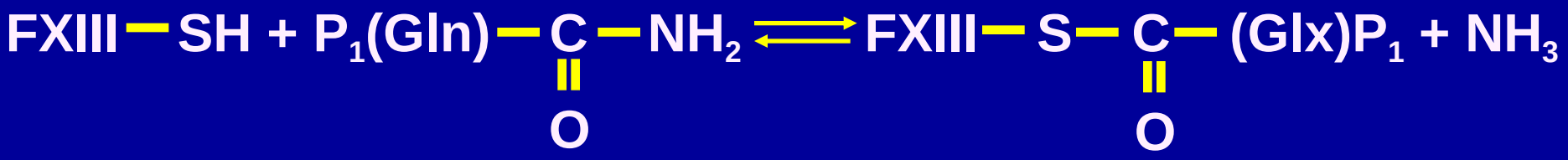


Activation of plasma FXIII

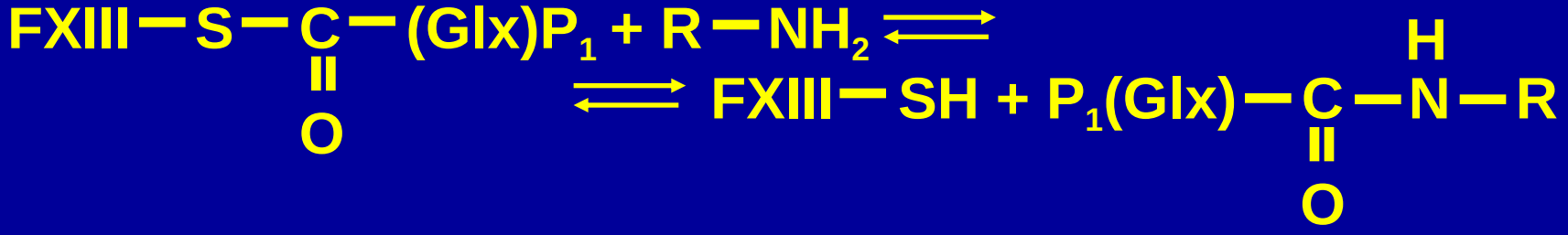


Reaction catalyzed by transglutaminases

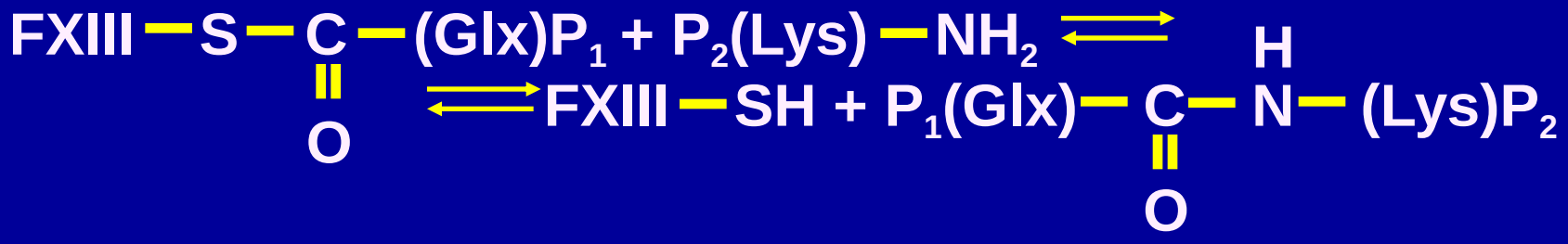
1.



2a.



2b.



Main functions of FXIII

By cross-linking of fibrin γ and α-chains, and α_2 plasmin inhibitor to fibrin α-chains it produces mechanically stronger clot and protects it from fibrinolysis.

Maintaining pregnancy.

Involved in wound healing and angiogenesis.

Classification of FXIII deficiencies

I. Inherited FXIII deficiency

- **FXIII-A deficiency:**
  - **Type I**
  - **Type II**
- **FXIII-B deficiency**

II. Acquired FXIII deficiency

- **Auto-antibody against FXIII-A:**
  - **Neutralizing antibody**
  - **Non-neutralizing antibody**
- **Auto-antibody against FXIII-B**
- **Consumption, decreased synthesis (moderate deficiencies)**

Inherited FXIII deficiencies

FXIII-A deficiency:

Frequency: 1:2.000.000, over 70 mutations,

FXIII activity is usually <3%,

FXIII-B ~ 50%,

In normal conditions low FXIII level (>5%) is sufficient to maintain hemostasis.

Do heterozygotes have a mild bleeding tendency?

ETRO Registry of FXIII deficient patients.

Ivaskevicius et al. Thromb Haemost 2007; 97: 914-21

FXIII-B deficiency:

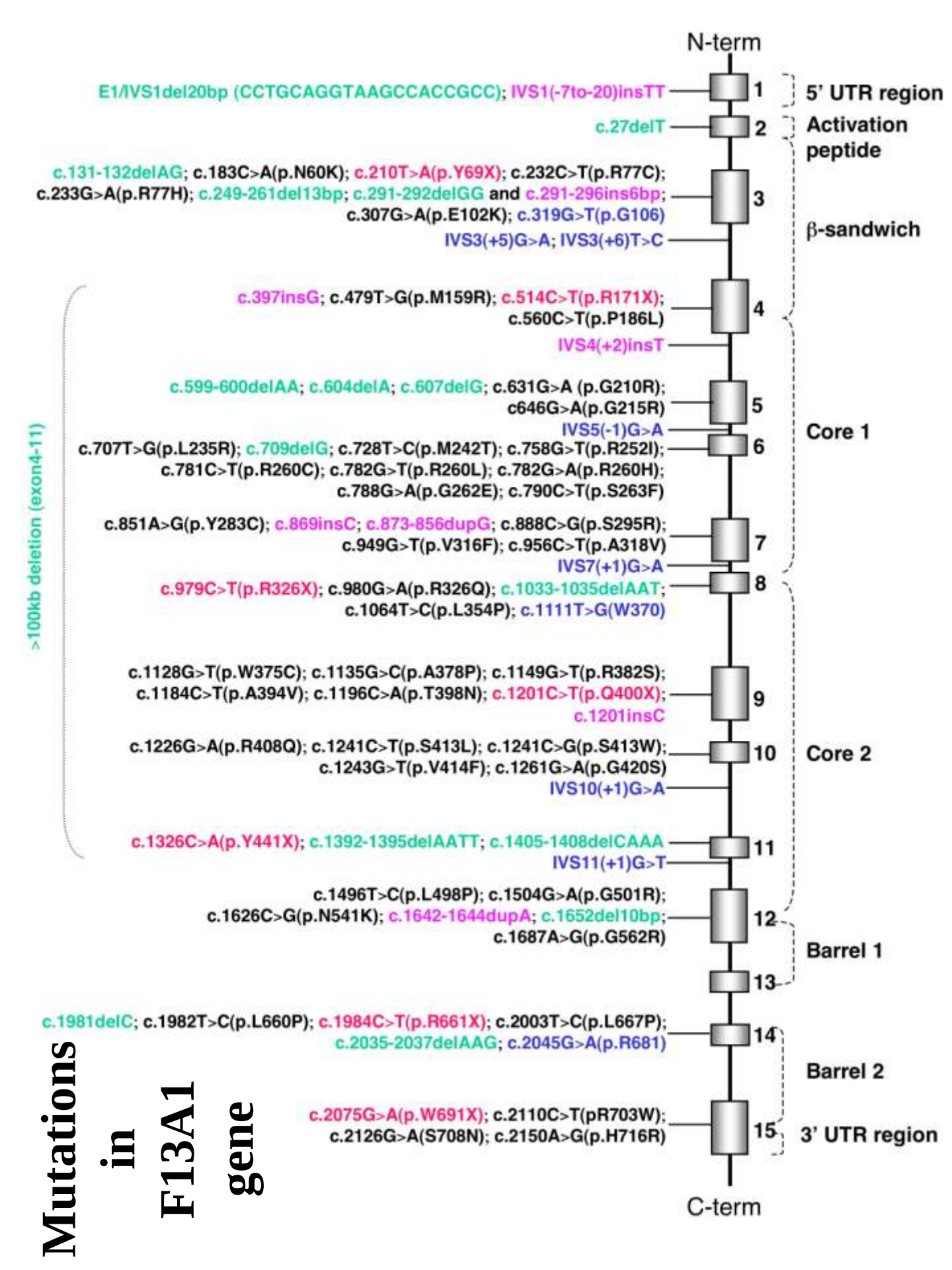
Very low FXIII A_2B_2 and FXIII-B antigen,

FXIII activity and FXIII-A decreased to a great extent, but detectable,

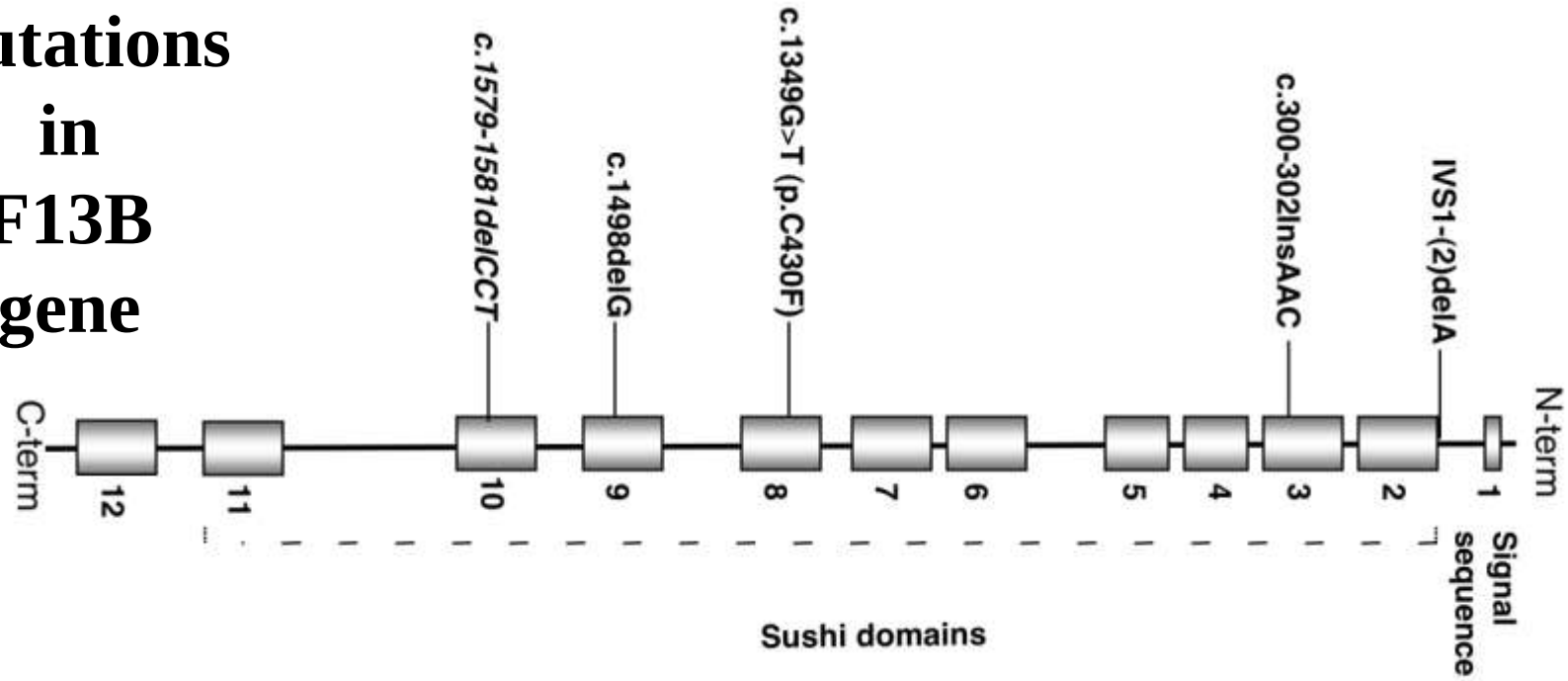
Moderate bleeding tendency, only five reported families.

Clinical symptoms in severe inherited FXIII-A deficiency

Bleeding:	**100%**
Delayed umbilical	**80%**
Superficial bruising	**60%**
Subcutaneous hematoma	**55%**
Intracranial	**30%**
Intramuscular hematoma	**27%**
Poor wound healing	**13%**
Habitual abortion	**close to 100%**


Mutations in F13A1 gene
N-term
C-term
E1/IVS1del20bp (CCTGCAGGTAAGCCACCGCC); IVS1(-7to-20)insTT
1
5' UTR region
c.27delT
2
Activation peptide
c.131-132delAG; c.183C>A(p.N60K); c.210T>A(p.Y69X); c.232C>T(p.R77C);
c.233G>A(p.R77H); c.249-261del13bp; c.291-292delGG and c.291-296ins6bp;
c.307G>A(p.E102K); c.319G>T(p.G106)
3
β-sandwich
IVS3(+5)G>A; IVS3(+6)T>C
c.397insG; c.479T>G(p.M159R); c.514C>T(p.R171X);
c.560C>T(p.P186L)
4
IVS4(+2)insT
c.599-600delAA; c.604delA; c.607delG; c.631G>A (p.G210R);
c646G>A(p.G215R)
5
IVS5(-1)G>A
Core 1
c.707T>G(p.L235R); c.709delG; c.728T>C(p.M242T); c.758G>T(p.R252I);
c.781C>T(p.R260C); c.782G>T(p.R260L); c.782G>A(p.R260H);
c.788G>A(p.G262E); c.790C>T(p.S263F)
6
c.851A>G(p.Y283C); c.869insC; c.873-856dupG; c.888C>G(p.S295R);
c.949G>T(p.V316F); c.956C>T(p.A318V)
7
IVS7(+1)G>A
c.979C>T(p.R326X); c.980G>A(p.R326Q); c.1033-1035delAAT;
c.1064T>C(p.L354P); c.1111T>G(W370)
8
>100kb deletion (exon4-11)
c.1128G>T(p.W375C); c.1135G>C(p.A378P); c.1149G>T(p.R382S);
c.1184C>T(p.A394V); c.1196C>A(p.T398N); c.1201C>T(p.Q400X);
c.1201insC
9
c.1226G>A(p.R408Q); c.1241C>T(p.S413L); c.1241C>G(p.S413W);
c.1243G>T(p.V414F); c.1261G>A(p.G420S)
10
Core 2
IVS10(+1)G>A
c.1326C>A(p.Y441X); c.1392-1395delAATT; c.1405-1408delCAAA
11
IVS11(+1)G>T
c.1496T>C(p.L498P); c.1504G>A(p.G501R);
c.1626C>G(p.N541K); c.1642-1644dupA; c.1652del10bp;
c.1687A>G(p.G562R)
12
Barrel 1
13
c.1981delC; c.1982T>C(p.L660P); c.1984C>T(p.R661X); c.2003T>C(p.L667P);
c.2035-2037delAAG; c.2045G>A(p.R681)
14
Barrel 2
c.2075G>A(p.W691X); c.2110C>T(pR703W);
c.2126G>A(S708N); c.2150A>G(p.H716R)
15
3' UTR region

Mutations in F13B gene



Databases for FXIII mutations:

www.f13-database.de,
www.med.unc.edu/isth/mutations-databases,
www.hgmd.cf.ac.uk

Auto-antibody against FXIII-A:

36 reported cases (3 cases in inherited FXIII deficiency)

Neutralizing antibody: inhibits FXIII actvity, activation or fibrin binding.

Non-neutralizing antibody: one or two cases

Associations: 1:3 cases with SLE, prolonged drug therapy therapy, idiopathic in elderly patients

Leading clinical symptomes: intramuscular haemorrhage, intracranial bleeding (50% with lethal outcome)

Auto-antibody against FXIII-B:

A single most recent report.

Ajzner et al. Blood 2008; doi:10.1182/blood-2008-09-179333

Life-threatening intramuscular and subcutaneous bleeding and impaired wound healing in an SLE patient.

Indications of laboratory investigation for FXIII deficiency

Clinical history of bleeding diathesis,

APTT, PT, TT are normal, vWD, platelet disorders excluded

APTT and/or PT and/or TT are prolonged, but the diagnosed abnormality does not explain the clinical symptoms

Tests to be carried out:
α_2 plasmin inhibitor, FXIII activity

Algorithm for the laboratory diagnosis and classification of FXIII deficiency

I. Screening for FXIII deficiency

1/ Quick functional (kinetic photometric) assay for the determination of plasma FXIII activity

2/ If FXIII activity is <3-5%, further functional test for the assessment of FXIII activity in the low activity range (amine incorporation assay, fibrin cross-linking by SDS PAGE)

II. Classification of FXIII deficiencies

1/ Determination of FXIII A_2B_2 complex (ELISA),

2/ If the concentration of the complex decreased determination of individual FXIII subunits

3/ FXIII activity and FXIII-A concentration in platelets

III. Detection of autoantibodies against FXIII

1/ Mixing study for the detection of neutralizing antibody

2/ Binding assays for the detection of non-neutralizing antibodies

IV. Molecular genetic investigations

Diagnosis/classification of FXIII deficiencies

	Plasma FXII				Platelet FXIII	
	Act.	A_2B_2	A_2	B	Act.	A_2
Inherited deficiency						
FXIII-A def.						
type I	↓↓↓	↓↓↓	↓↓↓	>30%	↓↓↓	↓↓↓
type II	↓↓↓	↓-n	↓-n	>30%	↓↓↓	↓-n
FXIII-B def.	↓↓	↓↓↓	↓↓	↓↓↓	n	n
Autoantibody						
Anti-FXIII-A Ab						
Neutralizing	↓↓↓	↓↓↓-n	↓↓↓-n	>30%	n	n
Non-neutralizing	↓↓↓	↓↓↓	↓↓↓	>30%	n	n
Anti-FXIII-B Ab						
Non-neutralizing	↓↓↓	↓↓↓	↓↓↓	↓↓↓	n	n

The traditional qualitative fibrin solubility test for FXIII activity

Fibrin solubility test:

- **in 5M or 8M urea**
- **in 1% or 2% monochloroacetic acid**
- **in 2% acetic acid**

Problems with the fibrin solubility tests

1/ It is not a screening test

2/ It detects the most severe FXIII deficiencies only (< 0.5-2% activity)

3/ Poorly standardized, its sensitivity depends on:

on features and concentration of the solubilizing agent

on the concentration of fibrinogen

4/ It cannot be used in most acquired deficiencies

Solubility tests must not be used as screening tests

Commercially available quantitative FXIII activity assays

1. Ammonia release assays:

Berichrom FXIII

(Dade Behring, Marburg, Germany)

TECHNOCHROM FXIII

Technoclone, Vienna, Austria)

REA-chrom FXIII,

(Reanalker, Budapest, Hungary)

2. Amine incorporation assay

Pefakit Factor XIII

Pentapharm, Basel, Switzerland

A. FXIII assays monitoring ammonia release

1. *Activation during the lag phase:*

$$FXIII \xrightarrow[Ca^{2+}]{thrombin} FXIIIa$$

Inhibition of fibrin polymerization by GPRP(A)-NH_2

2. *Transglutaminase reaction:*

$$Gly\text{-}OEt + Q\text{-}peptide \xrightarrow{FXIIIa} Q\text{-}peptide\text{-}Gly\text{-}OEt + NH_3$$

3. *Monitoring the release of ammonia:*

$$NH_3 + \alpha\text{-}ketoglutarate + NAD(P)H \xrightarrow{GlDH} glutamate + NAD(P)$$

<u>Berichrom:</u> NADH in the detection reaction, Q-peptide:
Leu-Gly-Pro-Gly-Gln-Ser-Lys-Val-Ile-Gly
<u>REA-chrom, TECHNOCHROM:</u> NADPH in the detection reaction, Q-peptide: Asn-Gln-Glu-Gln-Val-Ser-Pro-Leu-Thr-Leu-Leu-Lys

B. Amine incorporation FXIII assay

1. *Activation before or during the reaction:*

$$\text{FXIII} \xrightarrow[Ca^{2+}]{\text{thrombin}} \text{FXIIIa}$$

2. *Transglutaminase reaction:*

$$\text{amine*} + \text{Q-protein} \xrightarrow{\text{FXIIIa}} \text{Q-protein-amine*} + NH_3$$

separation of unbound and protein-linked amine

3. *Determination of incorporated amine:*

Measurement of Q-protein-amine* by spectrofluorimeter, β scintillation, or enzyme-labeled streptavidin

<u>Pefakit</u> biotinamidopentylamine (BAPA), Q-protein: fibrinogen adsorbed to a microtiter plate, detection: streptavidin linked to alkaline phosphatase, washing, alkaline phosphatase determination

Advantages and disadvantages of commercial ammonia release assays

Advantages:

1. True kinetic assays, both amine and glutamine substrates can be used at saturating concentration. Single point calibration.
2. Quick, one-step reactions, ideal screening tests
3. Reference interval established according to CLSI guidelines is available (Kárpáti et al. Clin Chem 2000; 46: 1946-55)

Disadvantages:

Relatively insensitive in the lowest range of FXIII activity (<3-5%).

Requirement of plasma blank in ammonia release FXIII assays

Side reactions resulting in the FXIIIa-independent decrease of OD at 340 nm:

1/ NADH consuming reactions, like the effect of LDH on pyruvate present in the plasma (NADPH does not participate in this reaction)

2/ Ammonia producing reaction: deamidation of glutamine by γ-glutamyl transferase

FXIIIa-independent reactions can be measured in the presence of FXIIIa inhibitor (iodoacetamide) and deducted from the results (plasma blank).

Blank reagent is included in the REA-chrom, TECHNOCHROM kits, in the case of Berichrom assay the user has to prepare it.

Over-estimation of FXIII activity by the Berichrom assay when plasma blank is not determined and deduced

Sample	FXIII activity	
	without blank	with blank
1.	3.9%	0.0%
2.	9.6%	4.3%
3.	12.6%	6.6%
4.	13.7%	6.7%
5.	134.6%	108.0%
6.	141.9%	120.2%

Ajzner and Muszbek J Thromb Haemost 2004;2:2075-7

Advantages and disadvantages of commercial amine incorporation assay

Advantage:

High sensitivity even in the low activity range

Disadvantages:

1. **Not a kinetic assay, the glutamine substrate is not present in saturating concentration, results are not a linear function of FXIII concentration. Multipoint calibration.**
2. **Relatively time-consuming**
3. **As low thrombin concentration is used in the assay, FXIII activation does not go to completion, and the rate of FXIII activation depends on FXIII-A Val34Leu polymorphism.**
4. **Different "expected" intervals for different FXIII-A Val34Leu genotypes.**

Commercially available FXIII antigen assays

FXIII-A_2B_2 antigen assay (ELISA)

R-ELISA FXIII (Reanalker, Budapest, Hungary)
TECHNOZYM FXIII (Technoclone, Vienna, Austria)
Monoclonal antibodies against FXIII-A and FXIII-B
Matched pair antibody set (polyclonal anti-FXIII-A and anti-FXIII-A_2B_2) from Affinity Biologicals (Ancaster, Canada)

FXIII-A antigen assay

Latex-enhanced immunoprecipitation assay (Instrumentation Laboratory, Milan, Italy)

Two important aspects

1/ Commercial reference plasmas should be calibrated against WHO-ISTH international plasma standard available from the National Institute for Biological Standards and Control, Potters Bar, UK (Raut et al. J Thromb Haemost 2007; 5: 1923-9).

2/ In the nomenclature of FXIII the recommendations of ISTH SSC is to be followed (Muszbek et al. 2007; 5: 181-3).

Prophylaxis and treatment of FXIII deficiencies

Preferred treatment: FXIII concentrate
Fibrogammin-P highly purified, heat-treated plasma preparation (Dade Behring , Marburg, Germany)
Prophylaxis: 10-20 U/kg, in 4-week intervals
Target value for prophylaxis: >5% before the next dose.
Target value during pregnancy: >10%
Target value before surgery and labor: >30%
In the case of autoantibodies much higher doses could be required; plasmapheresis, immunosuppression with cyclophosphamide, cyclosporine or combinations, anti-CD20, and intravenous gammaglobulins are recommended as additional treatments.

Karimi M, Bereczky Z, Cohan N, Muszbek L. Factor XIII deficiency: clinical manifestations and laboratory diagnostics. Semin Thromb Hemost in press

Any question concerning FXIII deficiency:

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