Iceland Liechtenstein Norway grants

"Working together for
a green, competitive and inclusive Europe"

"HE-RO-IS strategic cooperation in hematology"

F SEE 2014-2021 No. 19-COP-0031

Curricula in hemophilia

Disclaimer: This curricula was realised with the EEA Financial Mechanism 2014-2021 financial support. Its content (text, photos, videos) does not reflect the official opinion of the Programme Operator, the National Contact Point and the Financial Mechanism Office. Responsibility for the information and views expressed therein lies entirely with the authors.

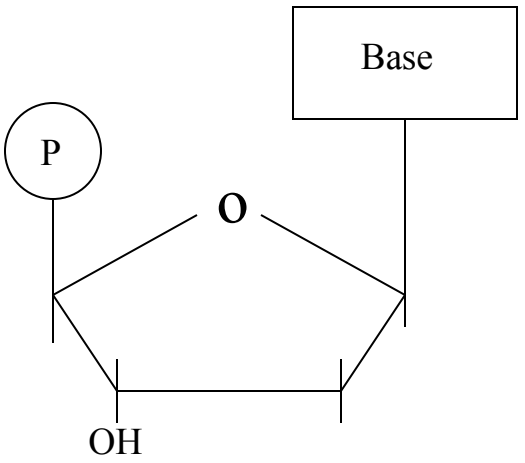
Genetics of hemophilia

The message ……..

The mutation should be characterized in all patients with hemophilia A or B regardless of the clinical severity since …..

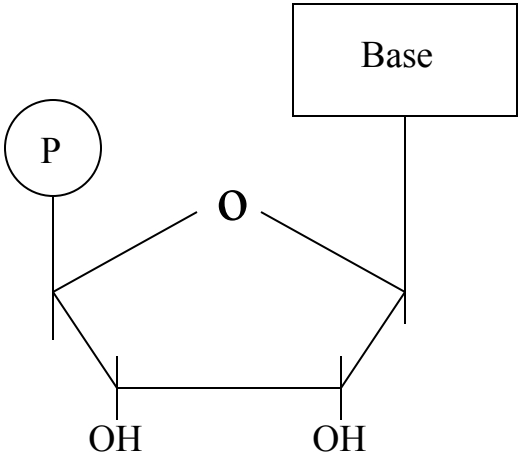
- It predicts the risk of developing an inhibitor and may thus have an impact on the clinical management
- It allows carrier- and prenatal diagnosis in the family
- It predicts anaphylactoid reactions in hemophilia B
- It is needed for research purposes

DNA:



A
T
C
G

RNA:

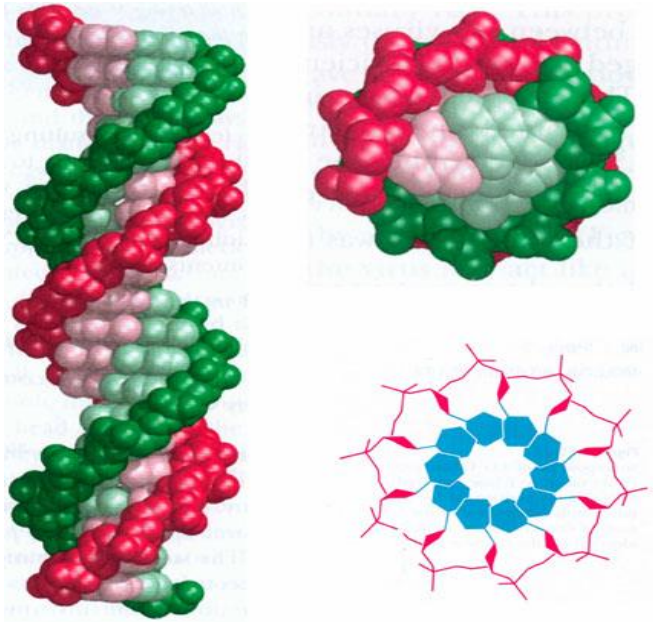


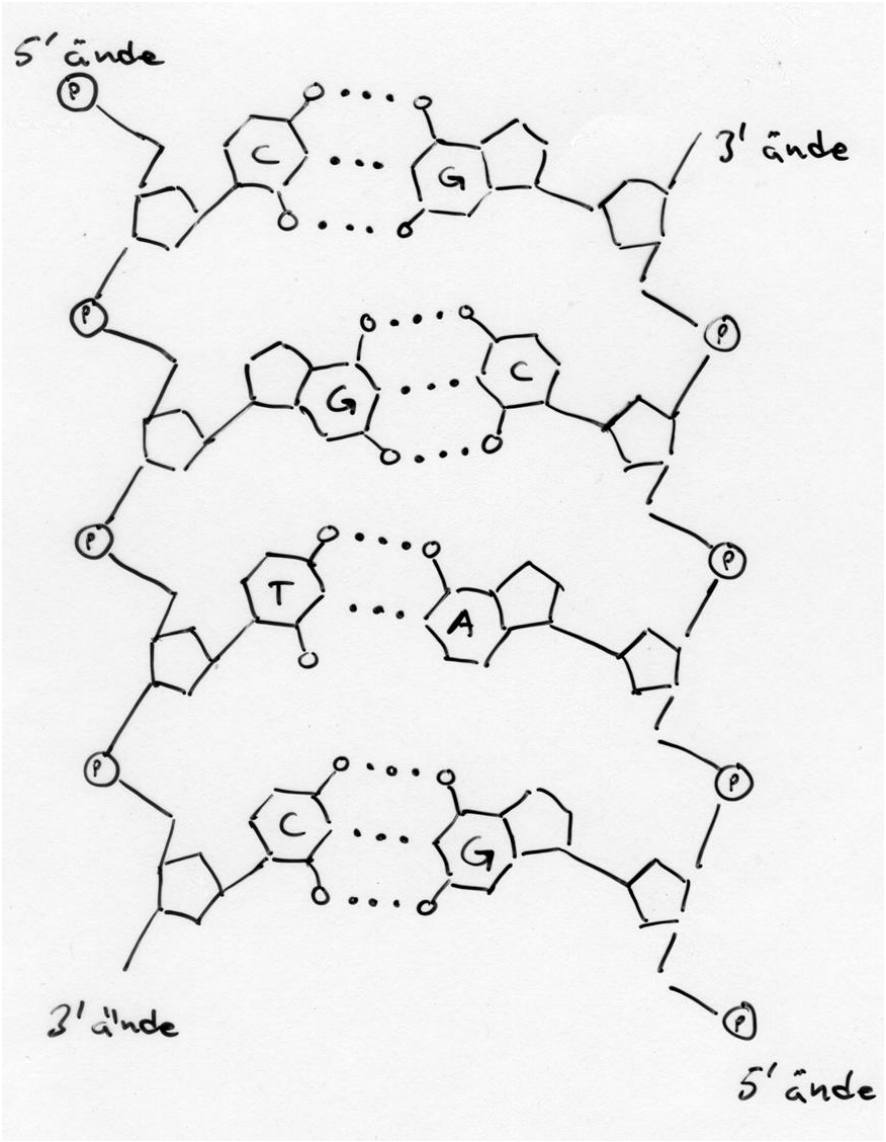
A
U
C
G

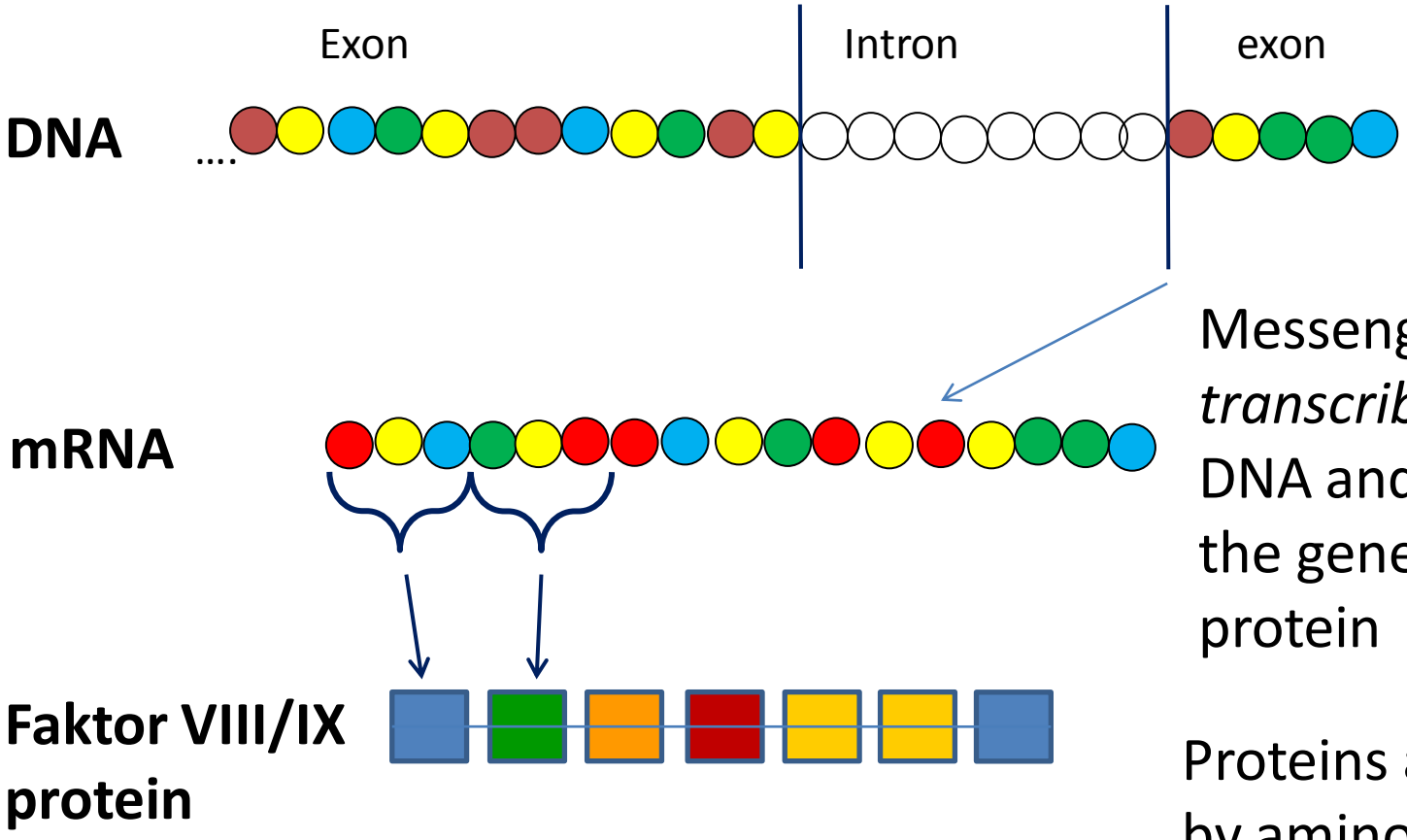
Double stranded DNA

Two antiparallel strands which can be 'melted' and 'reannealed'

One strand contains the information to create a complete DNA copy







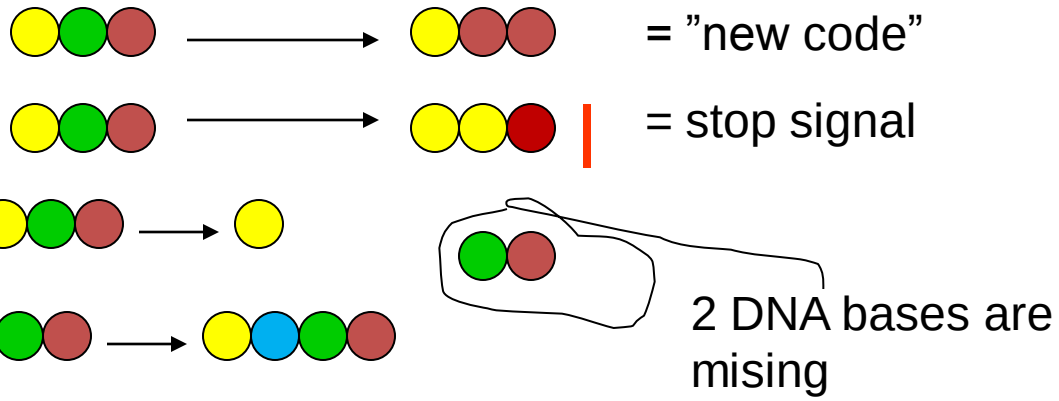
F8 or *F9* gene

Messenger RNA is *transcribed* from DNA and *translates* the genetic code to protein

Proteins are built by amino acids

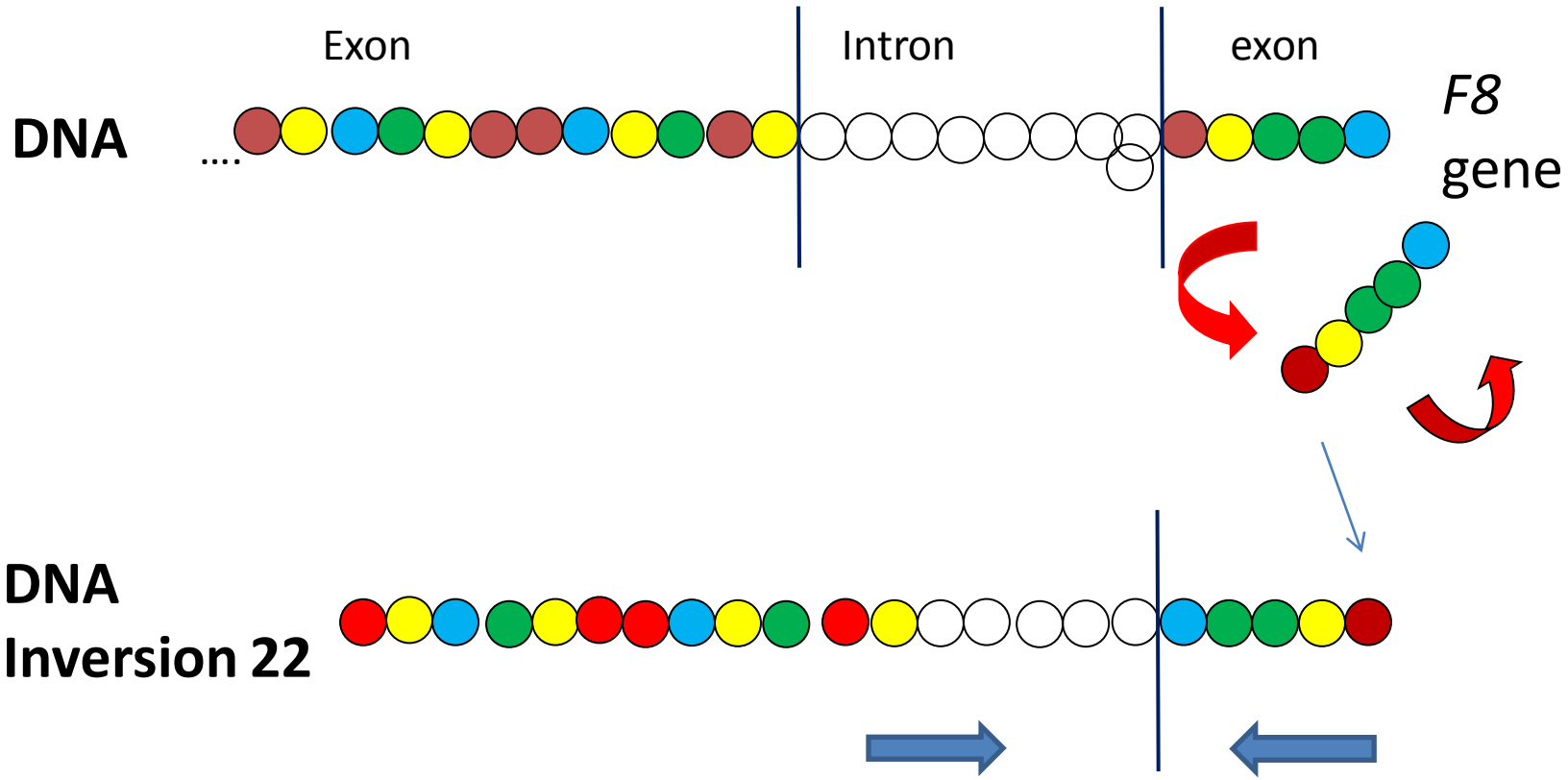
Mutation = sequence change in DNA

- Pointmutation
  - ”missense”
  - ”non-sense”
- Deletion
- Insertion

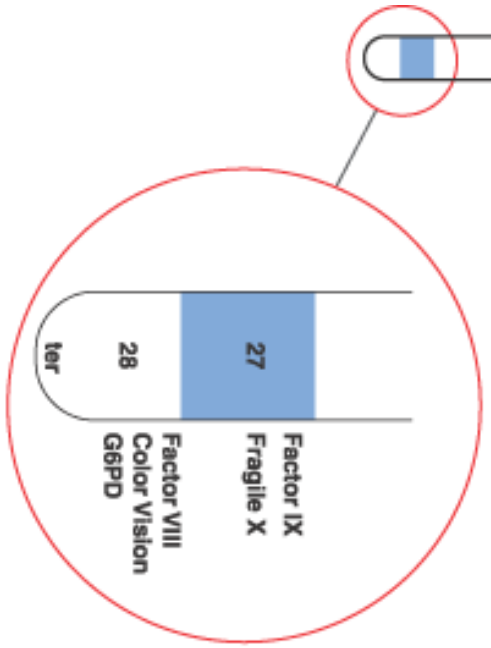


”Null mutations” = will cause disease

”Point mutations” = severe/mild disease or neutral polymorfism



Approx. 40% of patients with severe hemophilia A have inversion 22 – no FVIII protein can be produced although nothing is missing in the F8 gene on sequencing.



Factor VIII – Hemophilia A
- gene 26 exons
- protein 2332 aa

FVIII

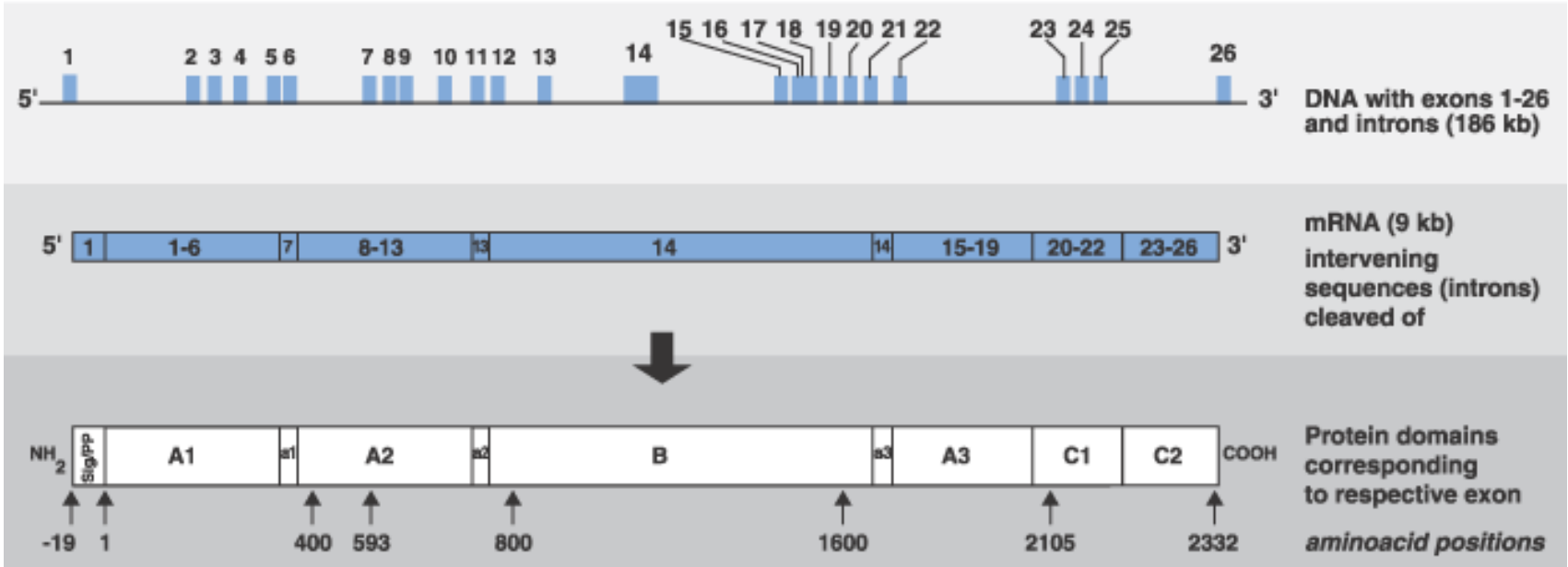
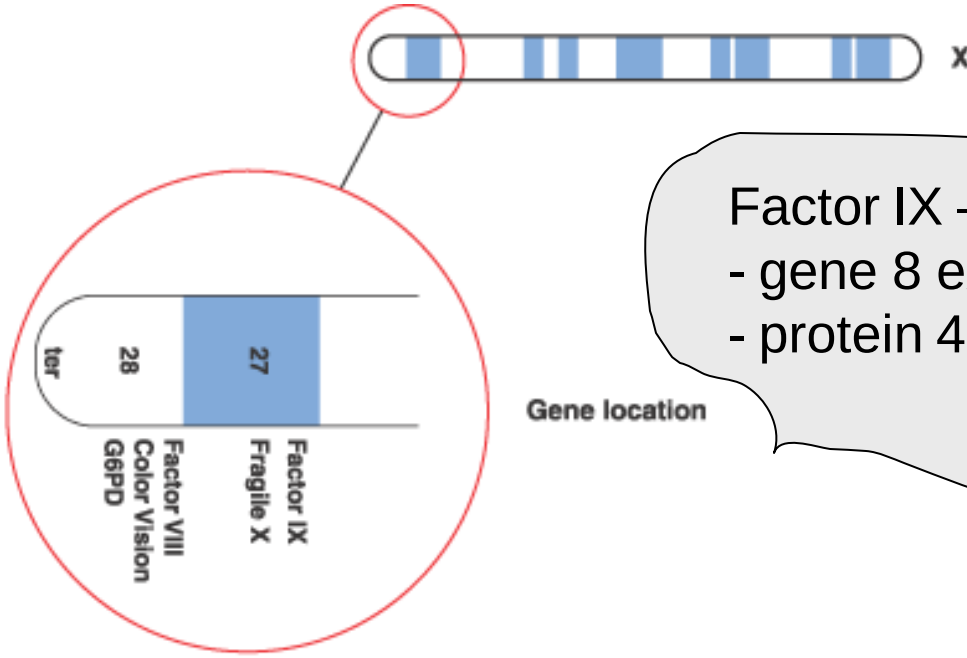
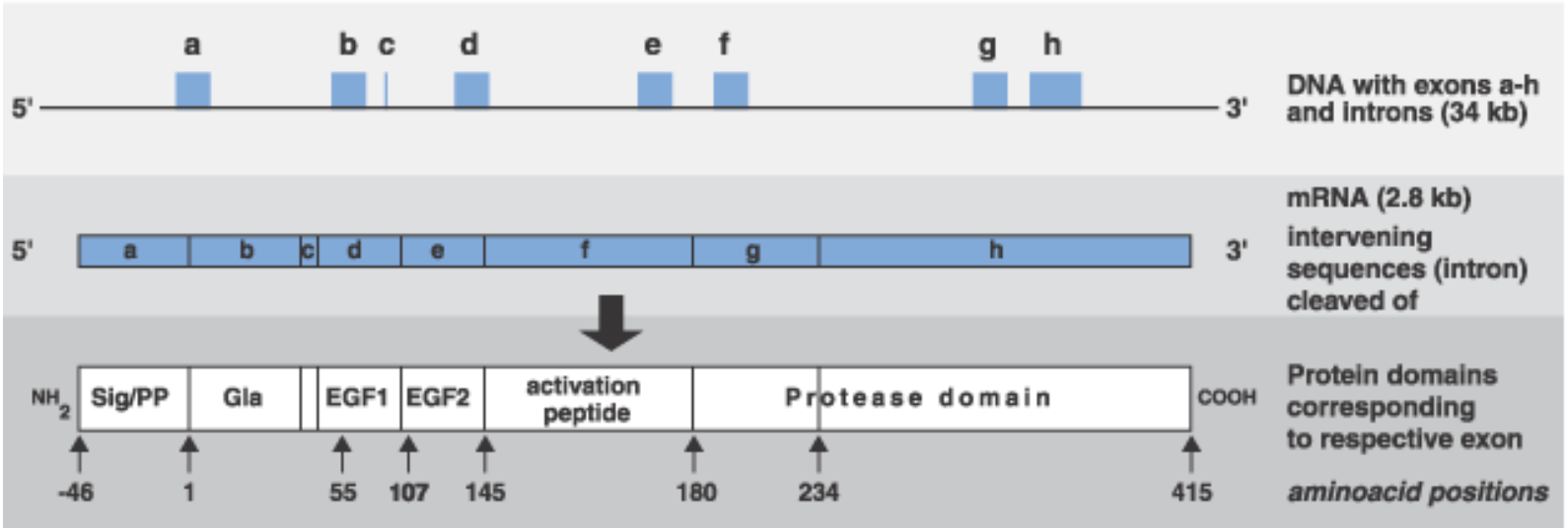


Fig 12



Factor IX – Hemophilia B
- gene 8 exons
- protein 415 aa

FIX



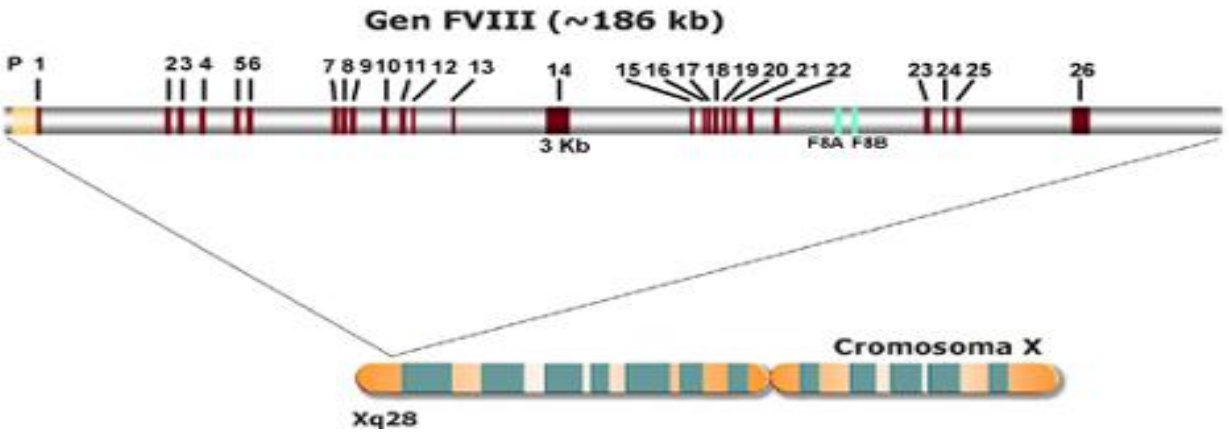
Diagnostic approach *F8* gene:

Inv 22 ➡ inv 1 ➡ sequening all 26 exons ➡

MLPA | ➡ vWD2N ➡ ”new inversion” | ➡

mRNA / cDNA ➡ LR-PCR ➡ NGS of the whole *F8* gene/exome/genome

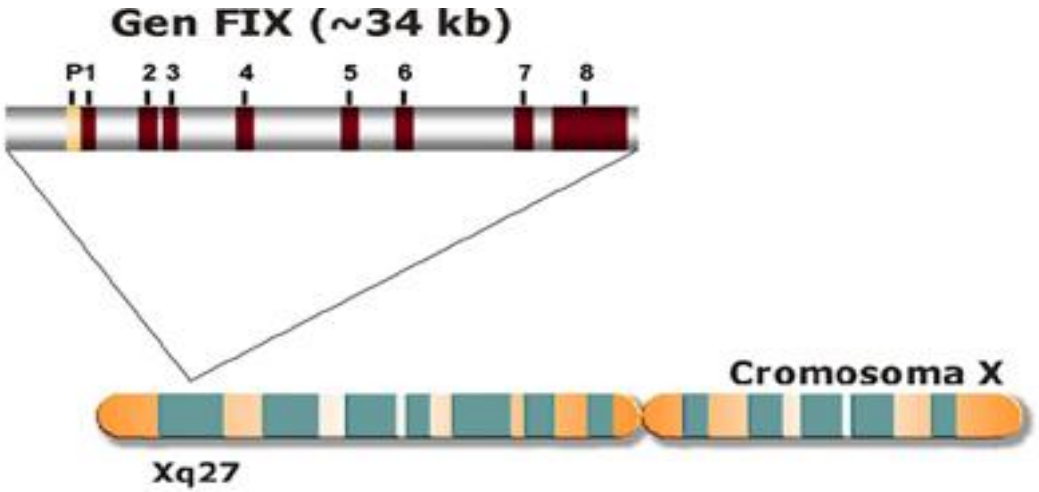
=routine =research



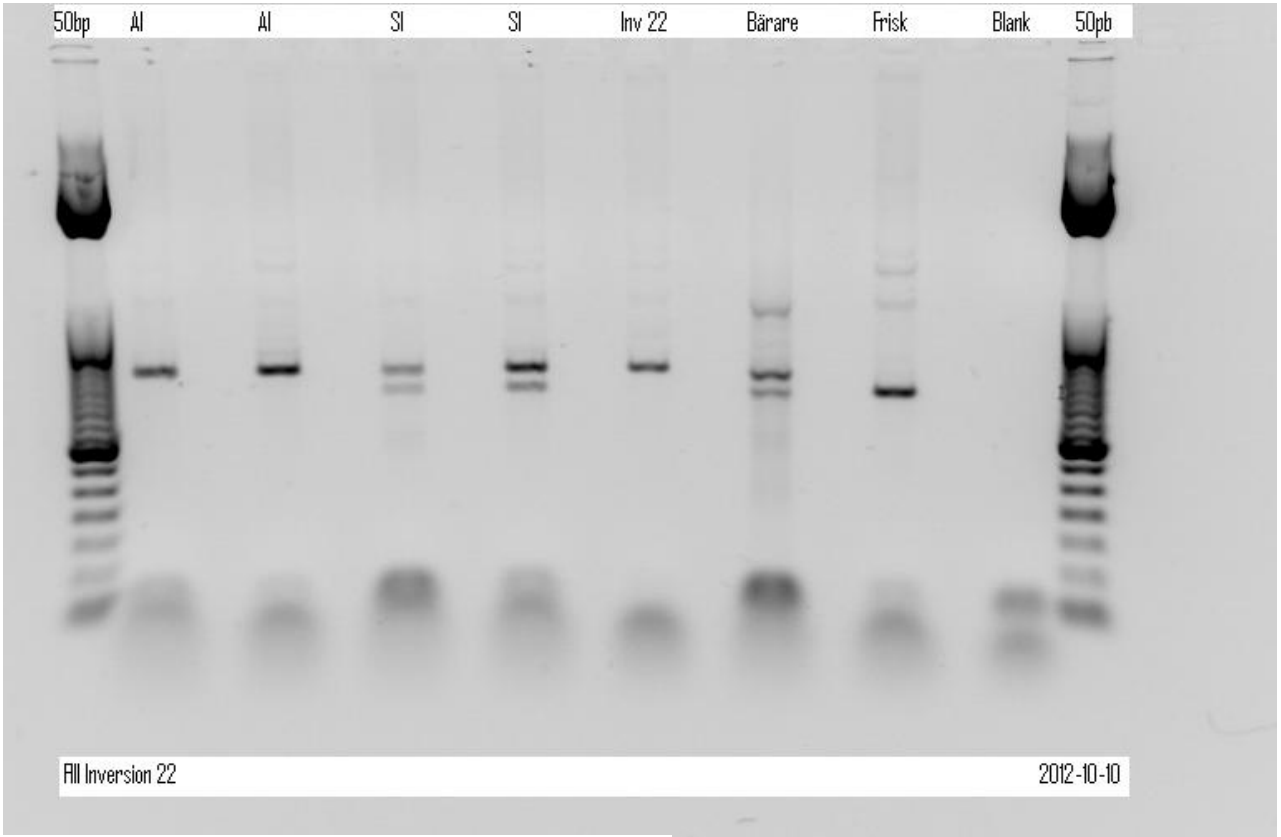
Diagnostic approach *F9* gene

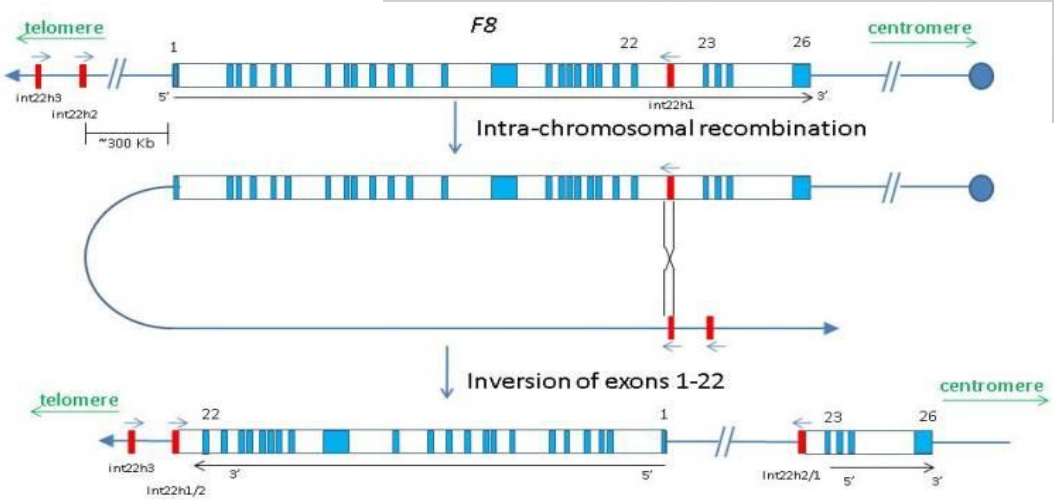
Sequencing all 8 exons (a-h) in the *F9* gene

MLPA NGS

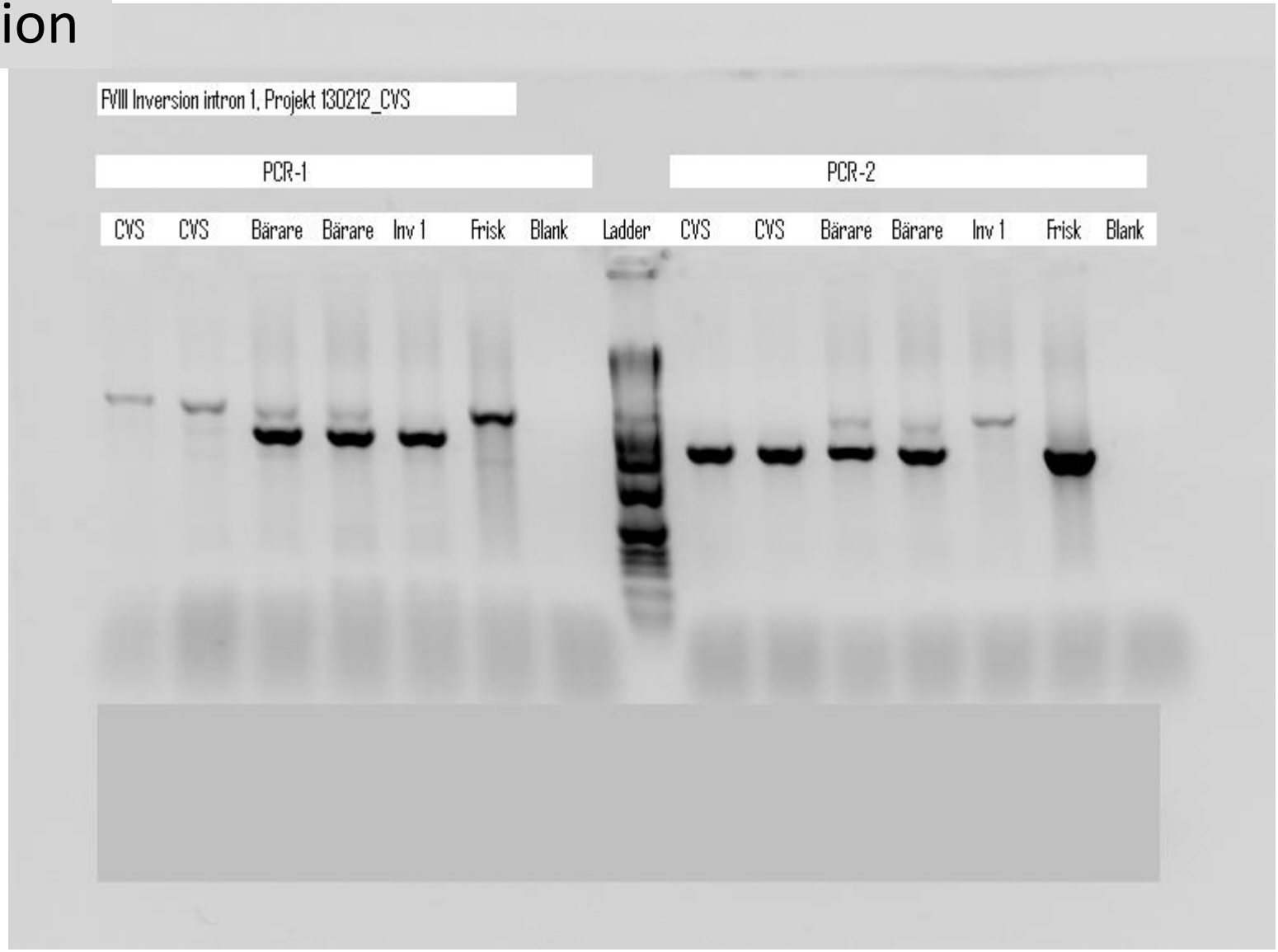


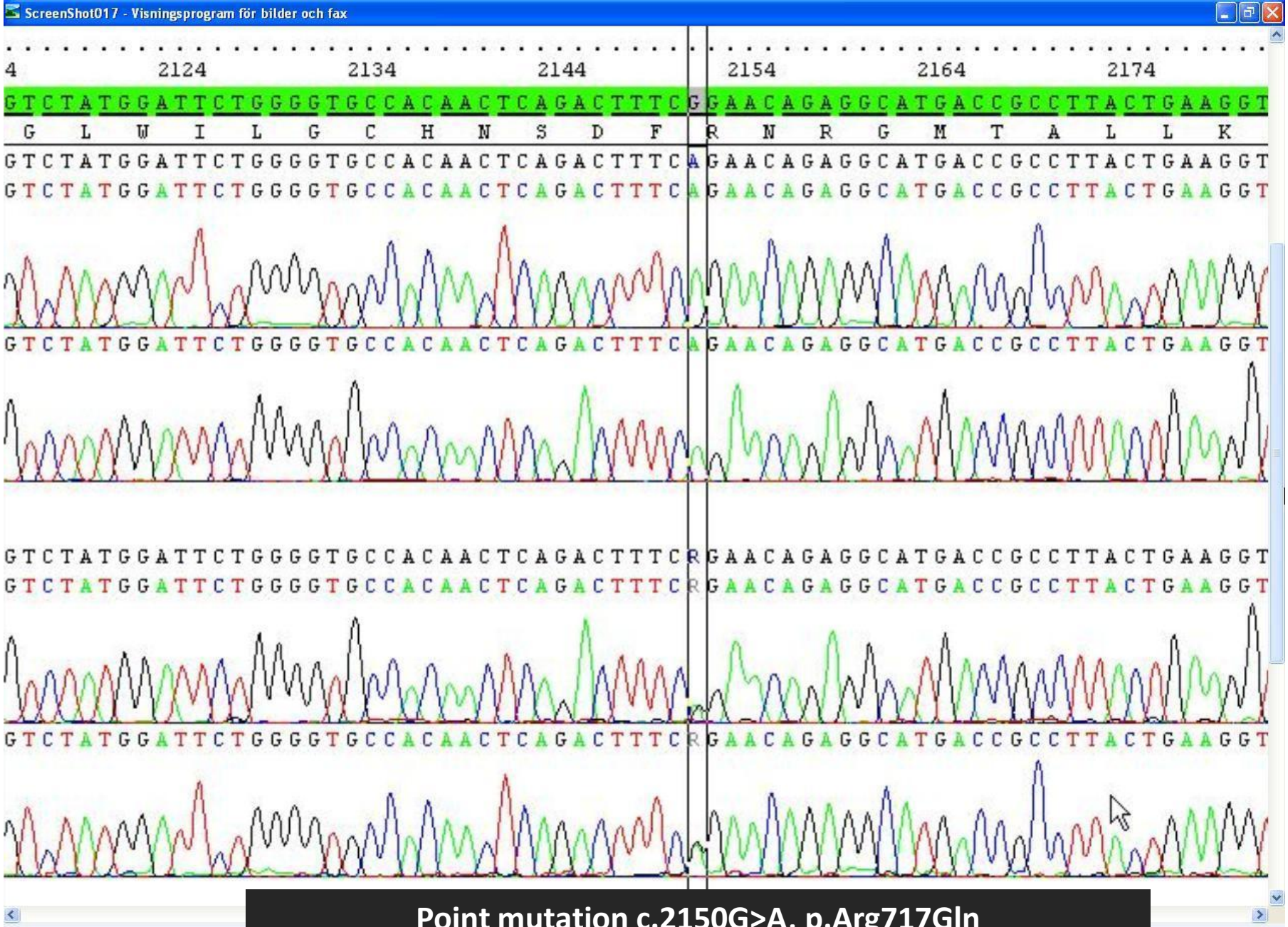
Intron 22 inversion

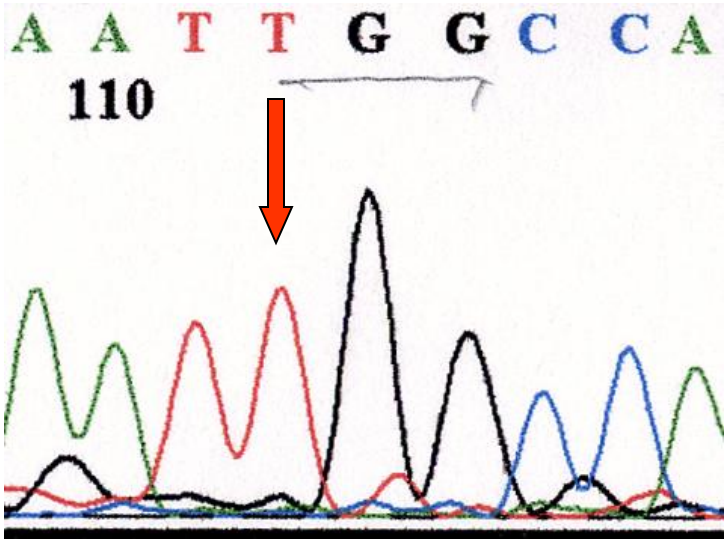




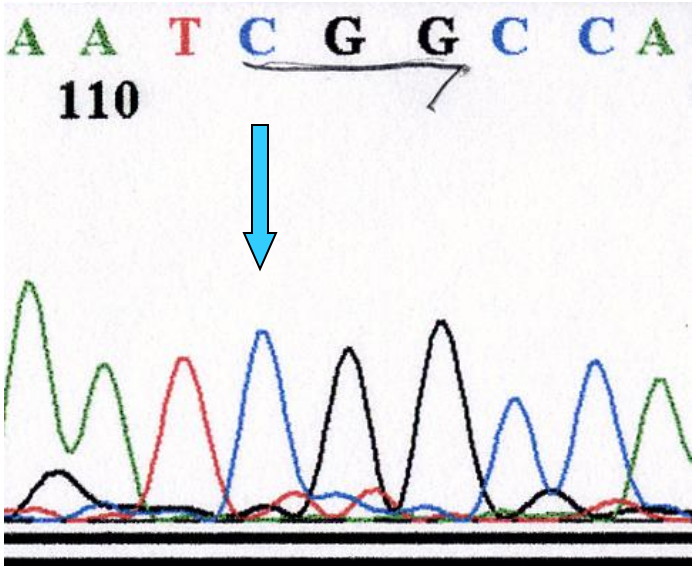
Intron 1 inversion



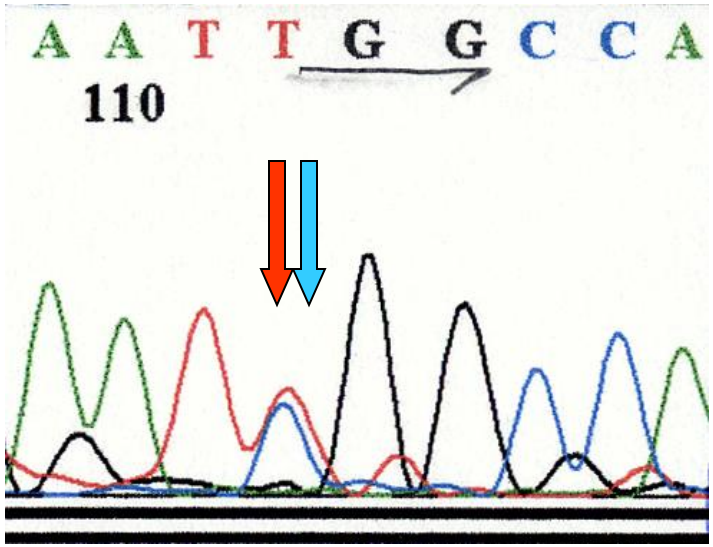

ScreenShot017 - Visningsprogram för bilder och fax
4
2124
2134
2144
2154
2164
2174
G T C T A T G G A T T C T G G G G T G C C A C A A C T C A G A C T T T C G G A A C A G A G G C A T G A C C G C C T T A C T G A A G G T
G L W I L G C H N S D F R N R G M T A L L K
Point mutation c.2150G>A, p.Arg717Gln
index och carrier



Hemophilia B
CGG - TGG

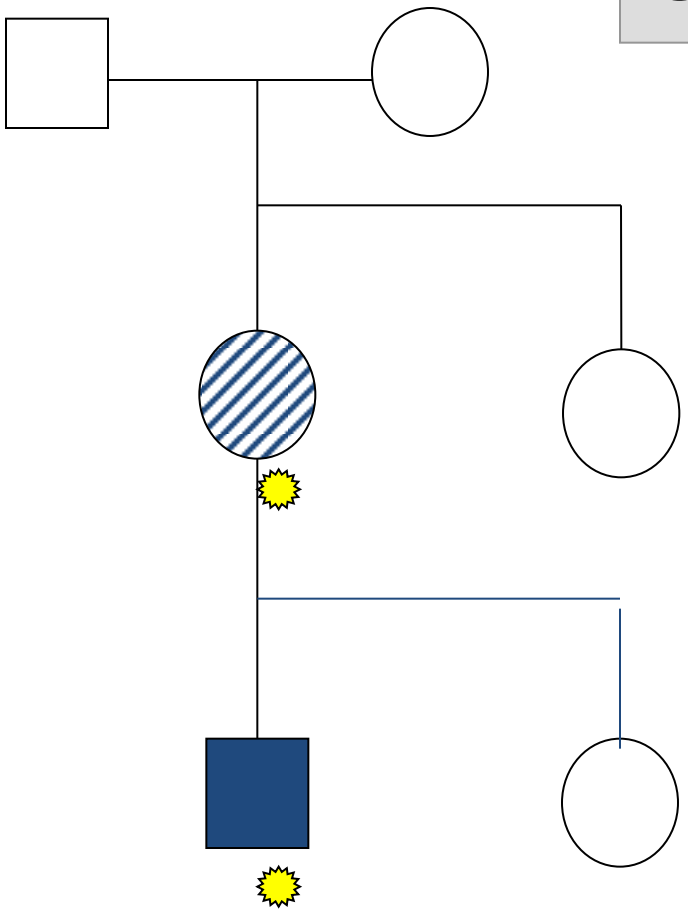


His sister does not carry mutation – non-carrier

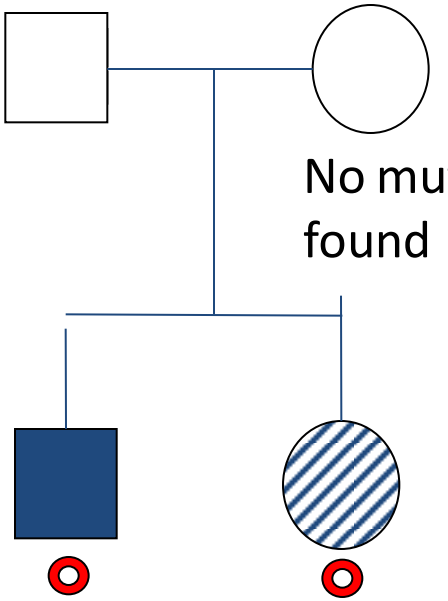


His mother carries both the mutant and normal allele – *i.e.* she is carrier

Sporadic cases of hemophilia



Carrier ?

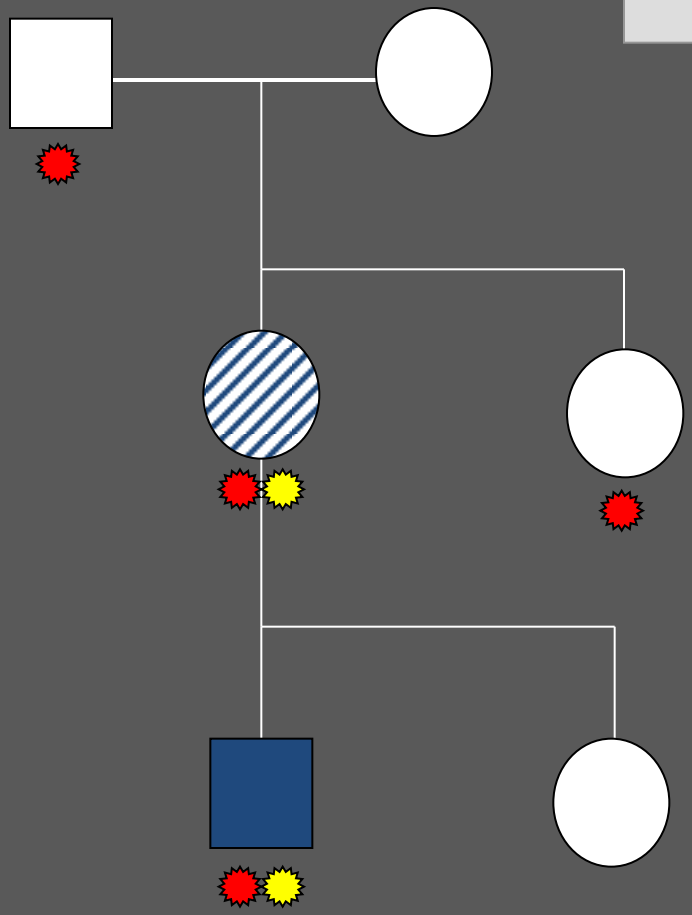


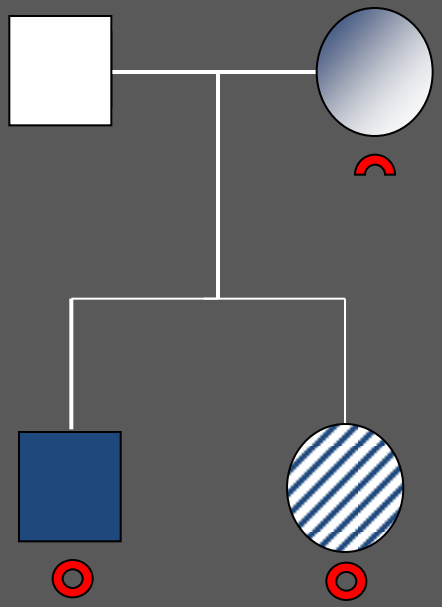
No mutation found

p.His257Tyr

p.Arg2228Gln

Sporadic cases of hemophilia





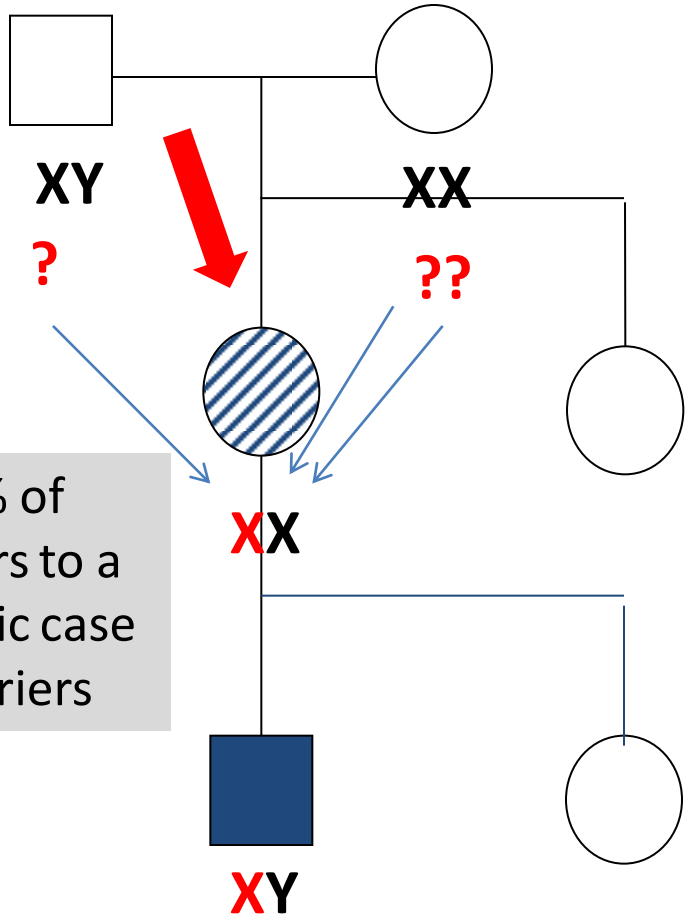
mosaik

Arg 116 stop

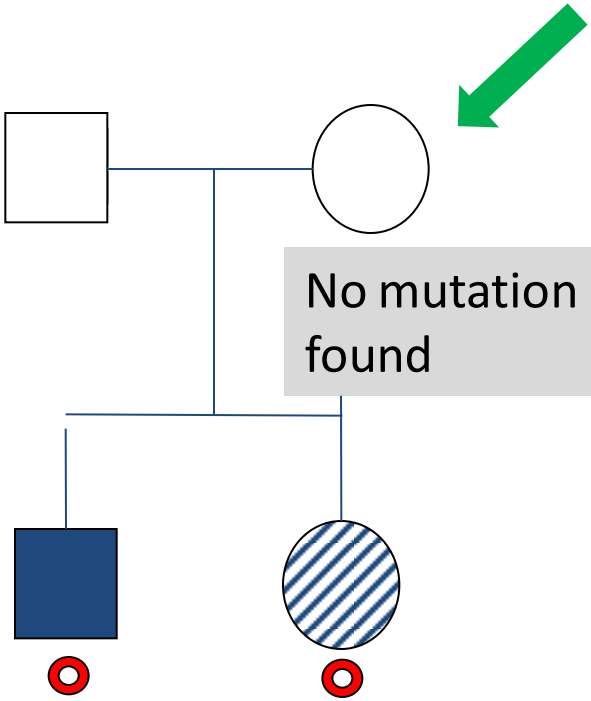
His 257 Tyr

~50% of new cases are sporadic !

The origin of mutation in sporadic cases of hemophilia?



70-80% of mothers to a sporadic case are carriers



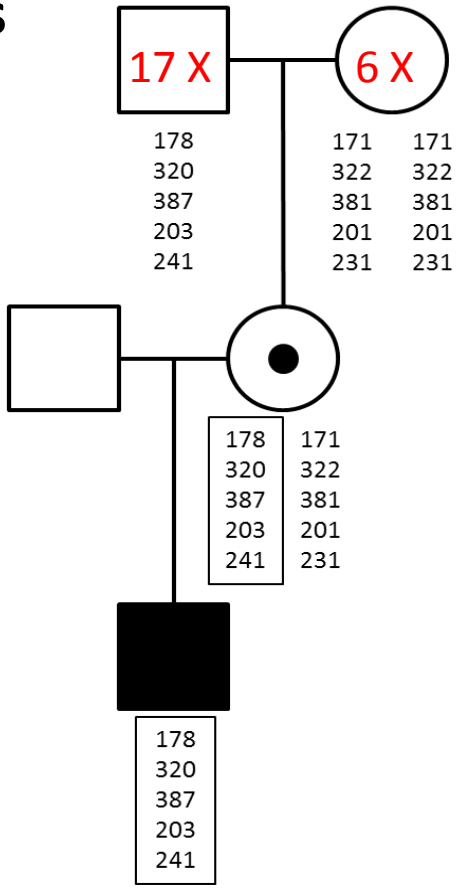
No mutation found

20-30% of mothers to a sporadic case are not carriers …. But may be a mosaic = partial carrier

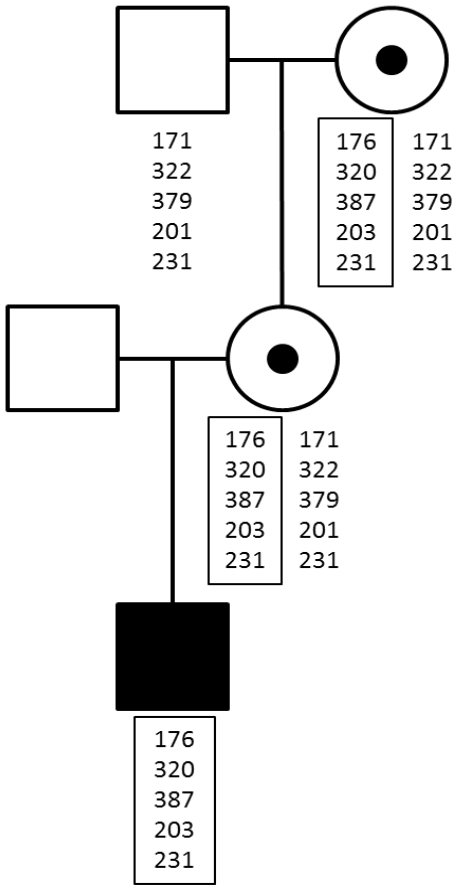
= identical mutation

28/45 mothers were carriers

23/28



5/28



In 17/23 mothers carrying a *de novo* mutation the mutation was of grandpaternal origin (8/17 were inversion 22).

Mårtensson et al., Clin Genet 2015; Dec 10 [Epub ahead of print]

17/45 (38%) of the mothers of a *true sporadic* case of haemophilia A did not carry the mutation…

Is this true or might the mother be a mosaic?

Previous studies – with insensitive techniques – have shown:

13% (*Becker et al., Am J Hum Genet 1996; 58: 657)*

27% (*Ljung et al., Br J Haematol 1999; 106: 870)*

19% (*Leuer et al., Am J Hum Genet 2001; 69: 75)*

of mothers being mosaics. Depends on type of mutation?!

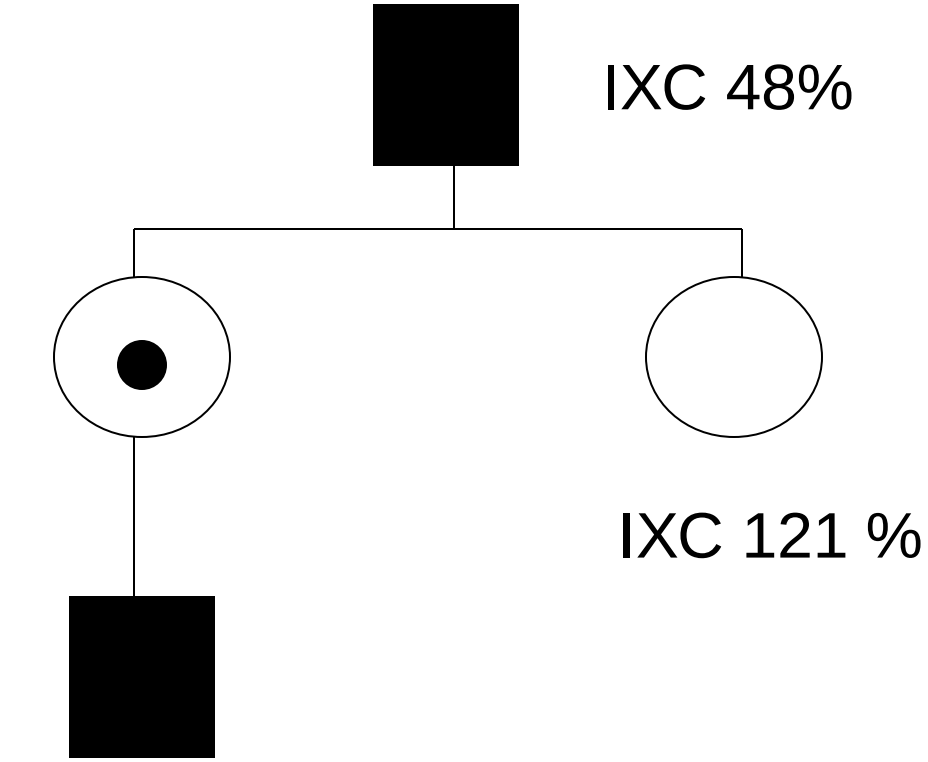
What is the risk of mosaicism in hemophilia B ?

- ”a non-carrier mother of a sporadic child with haemophilia B should have a risk < 6.2% of manifesting gonadal mosaicism by transmission of the mutation to a second child”

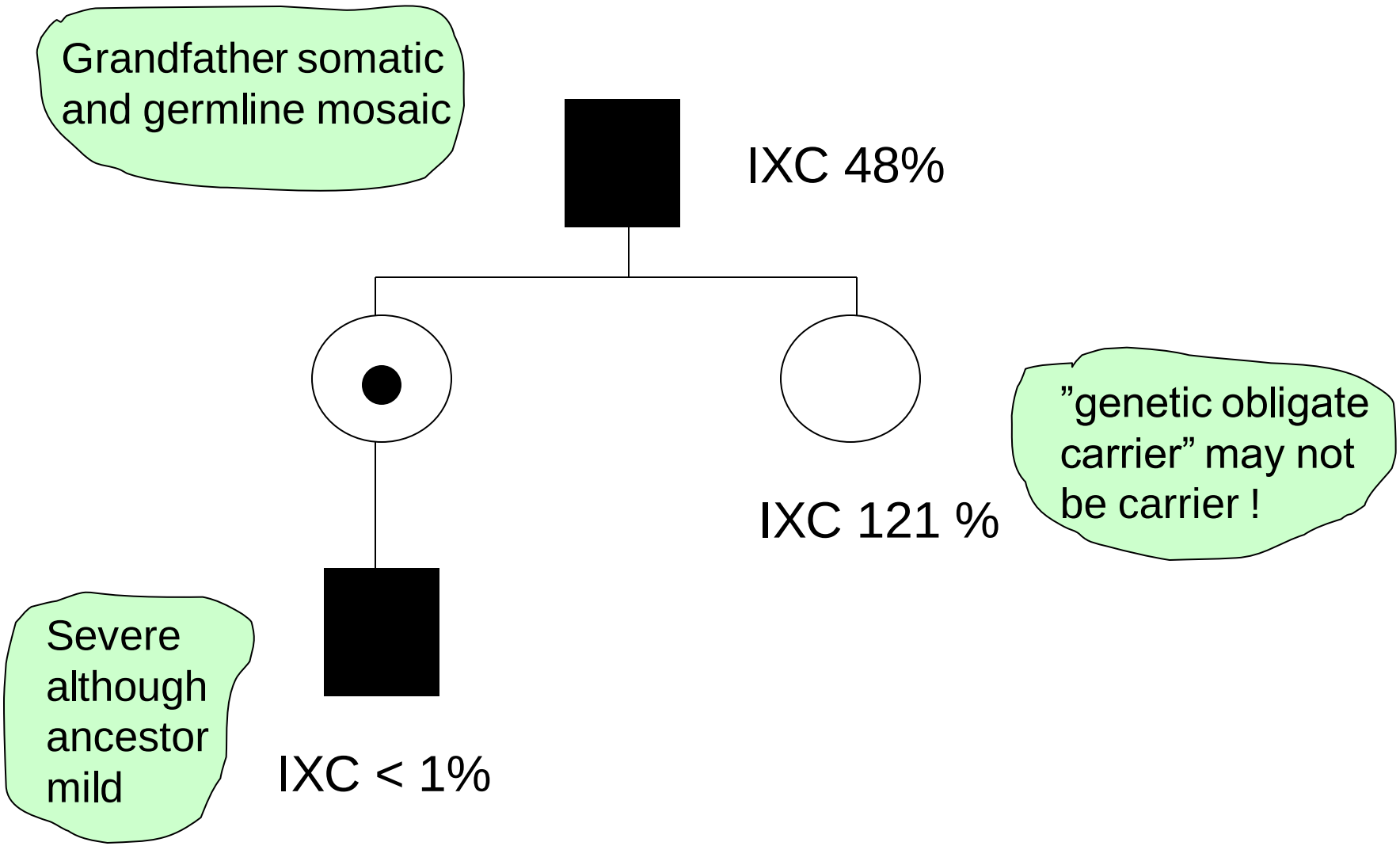
(Green et al, Am J Hum Genet 1999, 65: 1572)

Identification of the mutation in carrier diagnosis - limitations

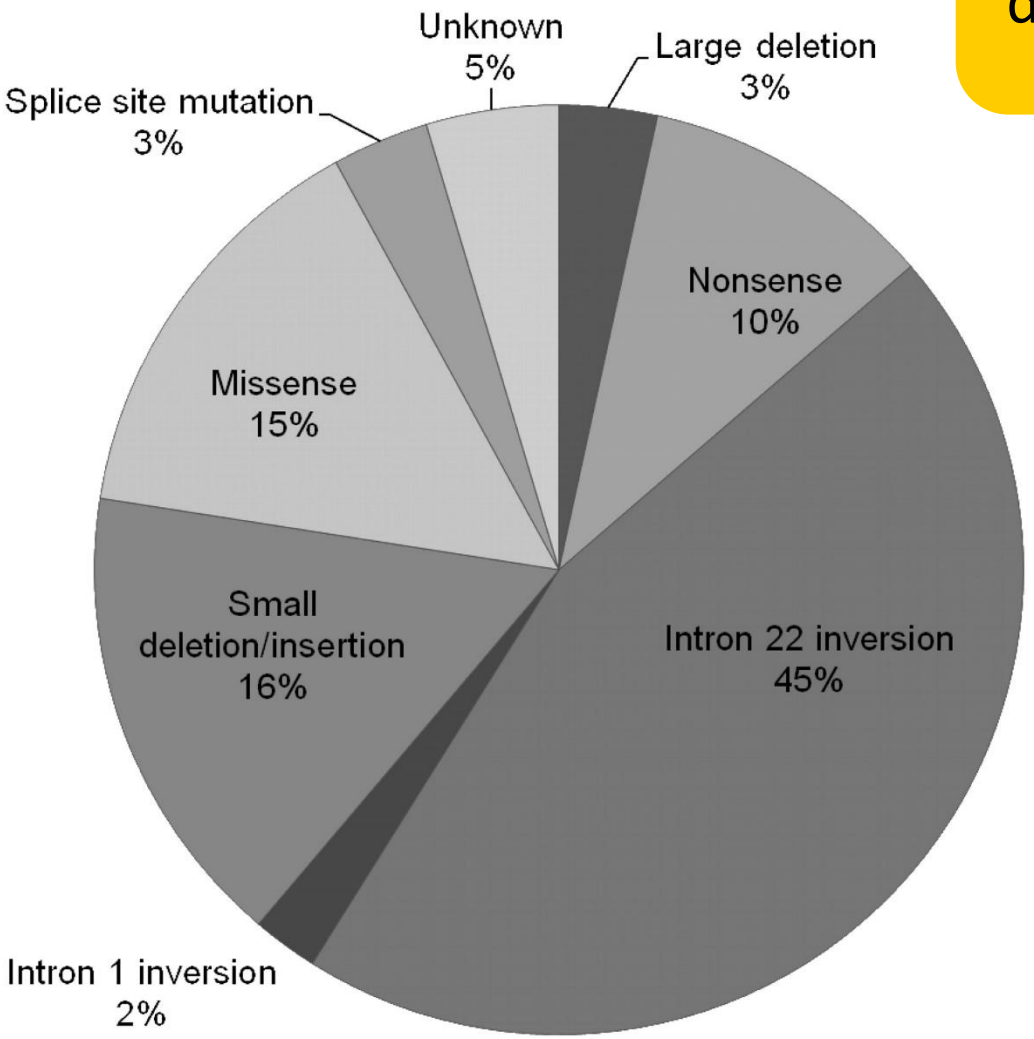
- neutral mutation
- mosaicism
- total deletions



Cutler et al. Am J Med Genet 2004, 129A, 13.


Grandfather somatic and germline mosaic
IXC 48%
”genetic obligate carrier” may not be carrier !
IXC 121 %
Severe although ancestor mild
IXC < 1%

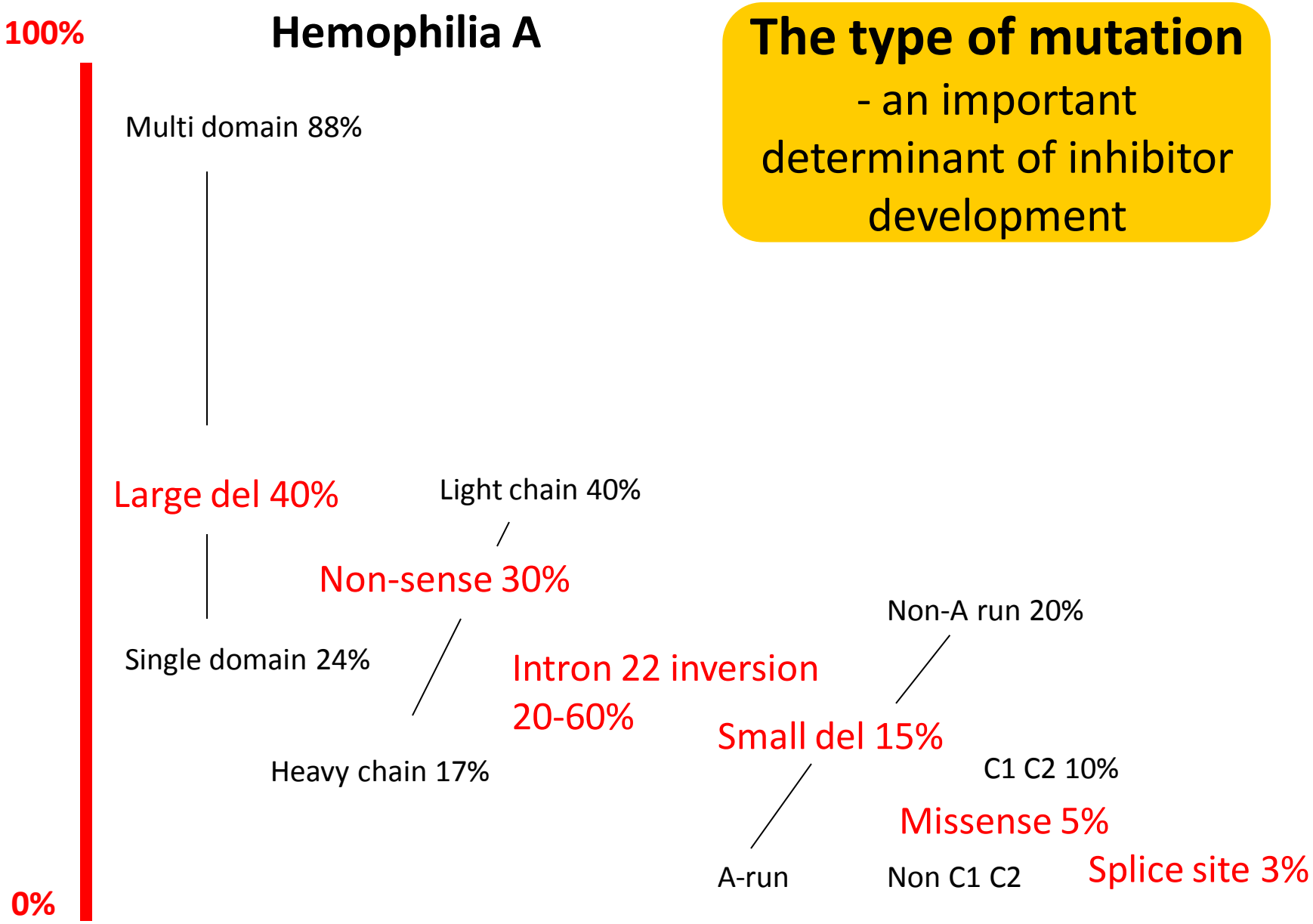
Distribution of F8 genotypes



The type of mutation
- An important determinant of inhibitor development

75% are ”null mutations”

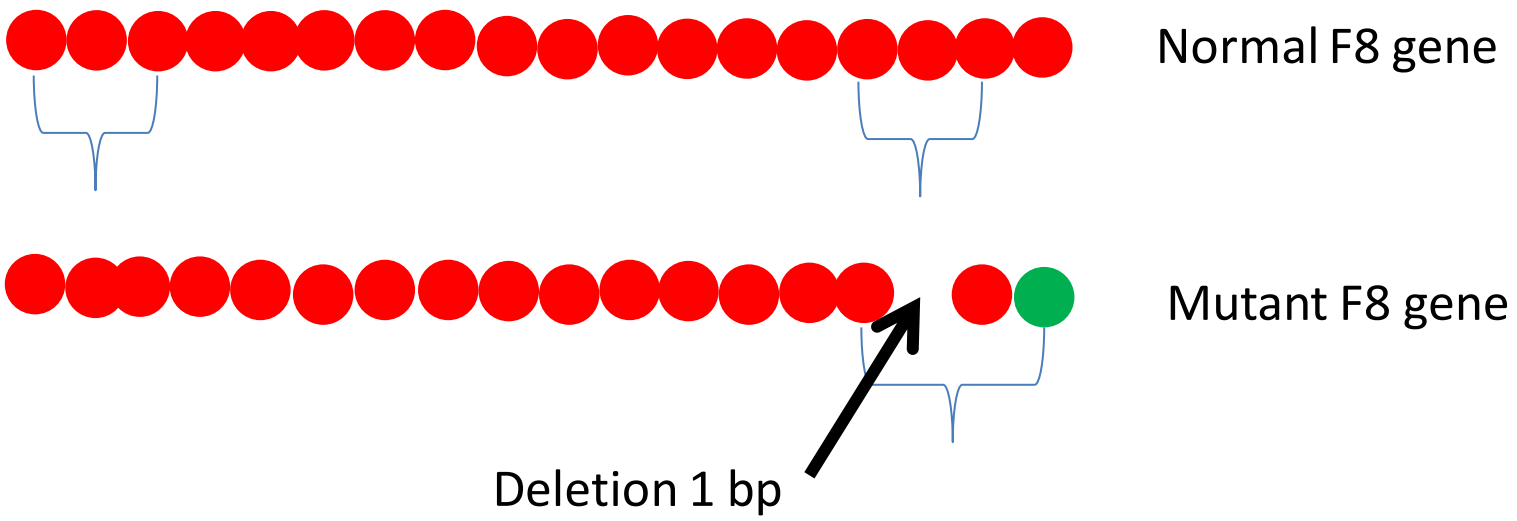
Gouw S C et al. Blood 2012;119:2922-2934



The type of mutation
- an important determinant of inhibitor development

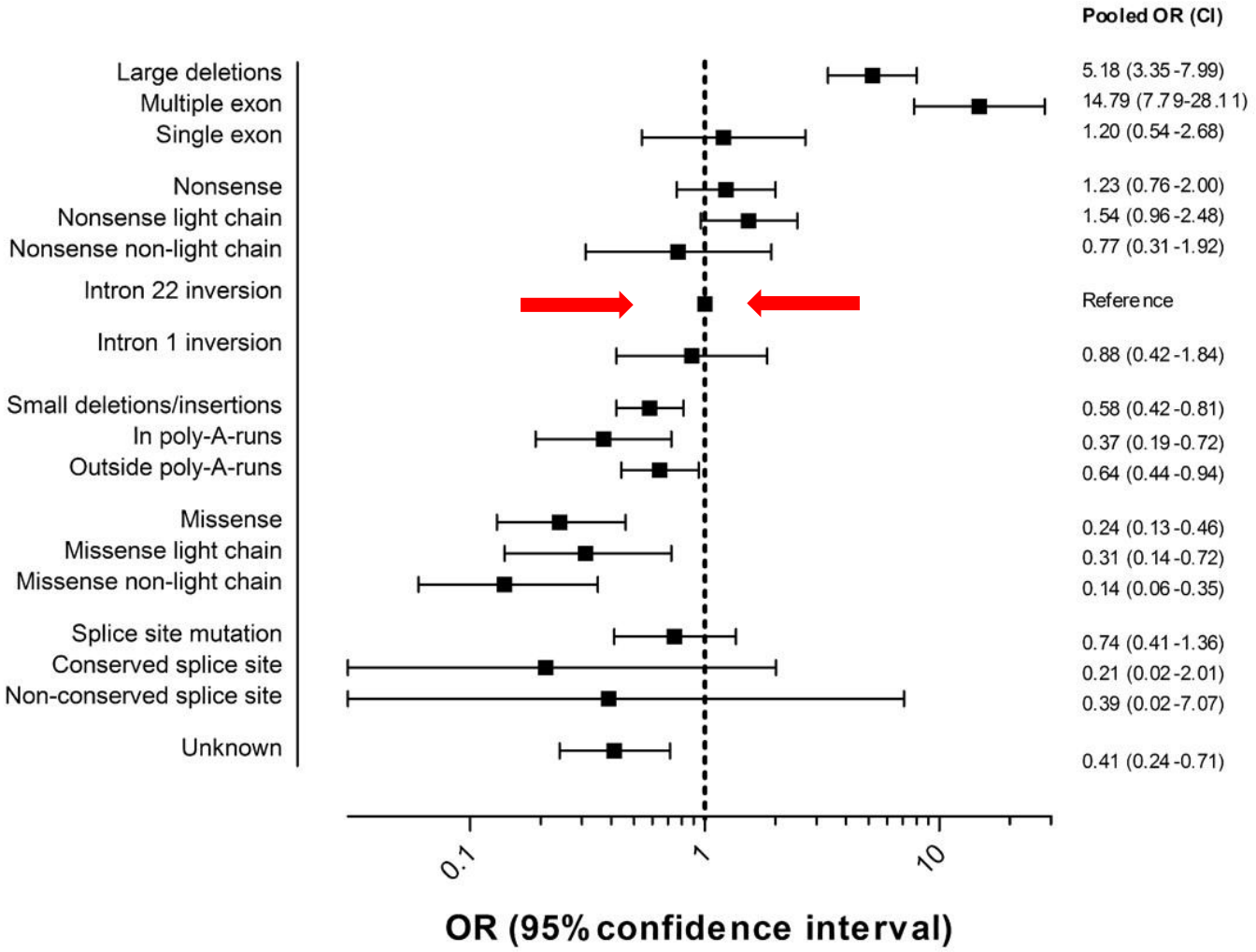
Oldenburg et al. Haematologica, 2000, HAMSTeRS database hemophilia A mutations

Why is the risk for inhibitors very low when mutation is a small deletion in a ”poly-A run”?

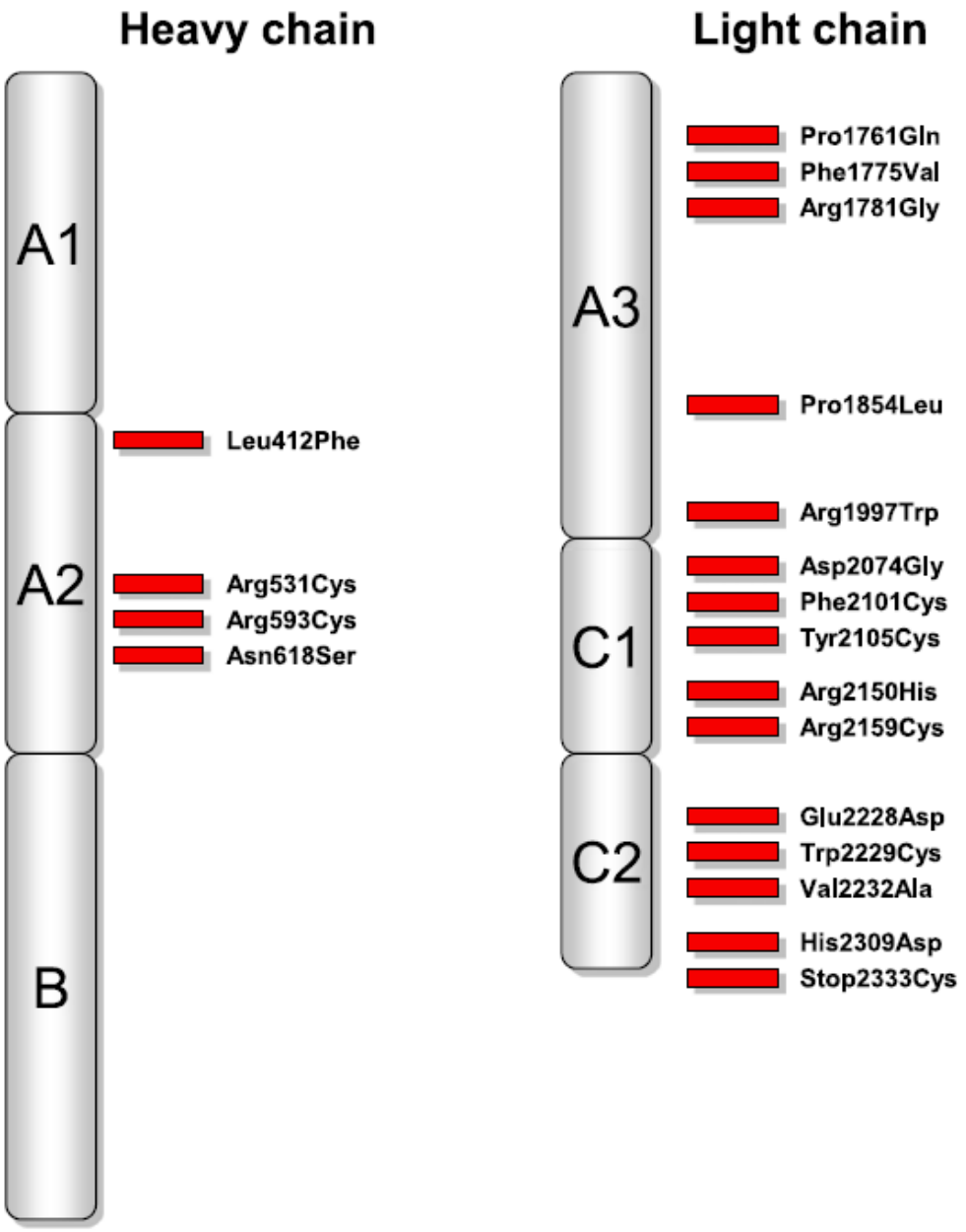


By ”misstake” a ”correct” transcript may occur – translates to a normal FVIII protein which is presented to the immune system.

Pooled ORs of high-titer-inhibitor development according to the F8 genotype
Meta-analysis of 30 studies with 5383 patients incl. 1029 with inhibitors



Gouw S C et al. Blood 2012;119:2922-2934

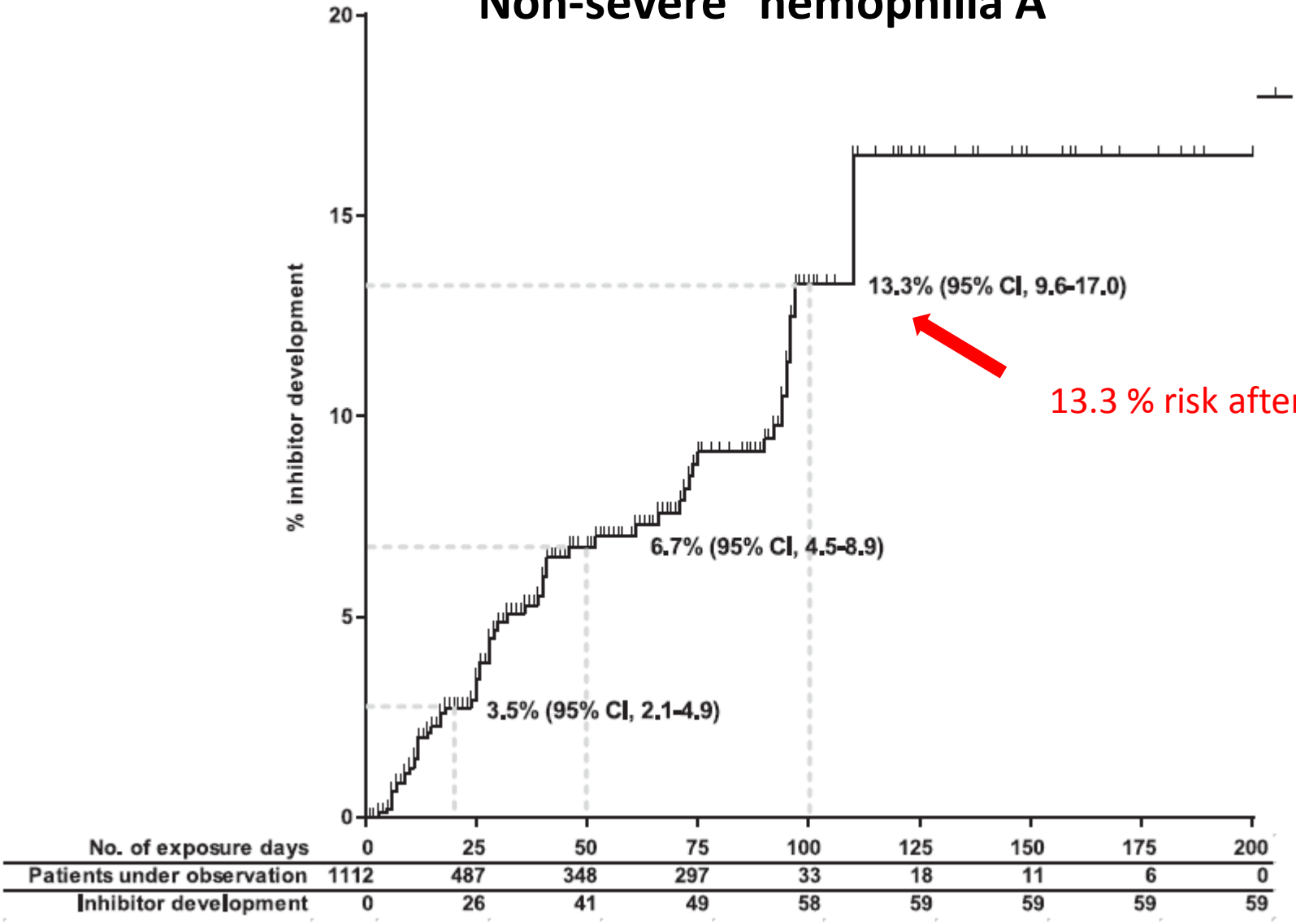


Patients with ***non-severe*** **hemophilia A** (2-40 IU/dL) have a cumulative risk of **5.3%** (95%CI: 4.0-6.6) to develop inhibitor after 26 ED.

Certain mutations causing mild hemophilia show discrepancies in one/two stage FVIII clotting assays!

Eckhardt et el. INSIGHT Study Group, Blood, 2013, 122,1954

”Non-severe” hemophilia A



13.3 % risk after 100 ED

Figure 2. Cumulative inhibitor incidence in 1112 nonsevere hemophiliaA patients, according to cumulative exposure days to factor VIII concentrates.

Eckhardt et el. INSIGHT Study Group, Blood, 2013, 122,1954

Hemophilia B ?

- 2-5% of patients with severe hemophilia B develop inhibitors

- Risk group – deletions, nonsense mutations

In Sweden – approx 20% of severe hemophilia B developed inhibitors due to a high frequency of ’risk mutations’ (large deletions).

Swedish Haemophilia A Registry

212 presumably unrelated families with haemophilia A

- 54% (115/212) had a mutation that was not present in any other family
- 46% (97/212) had a mutation that was also present in another family/ies

Do these 97 families carry mutations? …….

- **IBD** = '**identical by descent',** *i.e.* related without knowing it ('founder effect')?

Or

- **RM** = **'recurrent mutation',** true unique mutations ('independent mutational events')?

F8 gene region was haplotyped in the 97 families using 70 SNPs and 5 microsatellites (M1–M5).
285 healthy controls.

Halldén et al., J Thromb Haemost 2012; 10: 2505.

Of the 97 families with the same mutation…

- 47/97 **(48%) were IBD** (i.e. related to each other)
- 50/97 **(52%) were RM** (i.e. new mutations)

The IBD mutations were 2–35 generations old (700–800 years old) – the older the mutation, the milder the variants

Halldén et al., J Thromb Haemost 2012; 10: 2505.

The message ……..

The mutation should be characterized in all patients with hemophilia A or B regardless of the clinical severity since …..

- It predicts the risk of developing an inhibitor and may thus have an impact on the clinical management
- It allows carrier- and prenatal diagnosis in the family
- It predicts anaphylactoid reactions in hemophilia B
- It is needed for research purposes