Thursdays Webinars

EuroBloodNet

# Genetic counseling of hemophilia

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# Layout

- Heredity in hemophilia
- Genetic counseling
- The consultation of genetic counseling
- Prenatal diagnosis and preimplantation genetic diagnosis

# Heredity in hemophilia

# HEMOPHILIA: rare hereditary bleeding disorders

Hemophilia **A** (FVIII) 80-85%: 1/5,000 males

Hemophilia **B** (FIX) 15-20%: 1/25,000-30,000 males

2 genes and 2 proteins

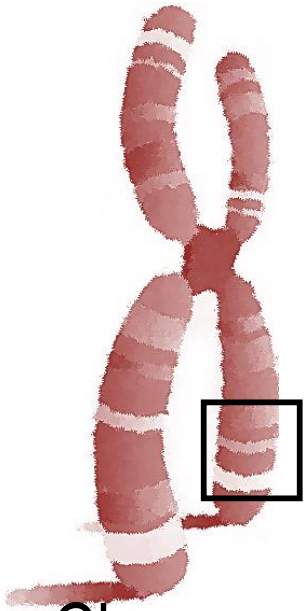
But same clinical manifestations and genetic transmission



Castaman G, Matino D. Hemophilia A and B: molecular and clinical similarities and differences. Haematologica. 2019 Sep;104(9):1702-1709.

# **HEMOPHILIA:** heredity

Hemophilia A or B

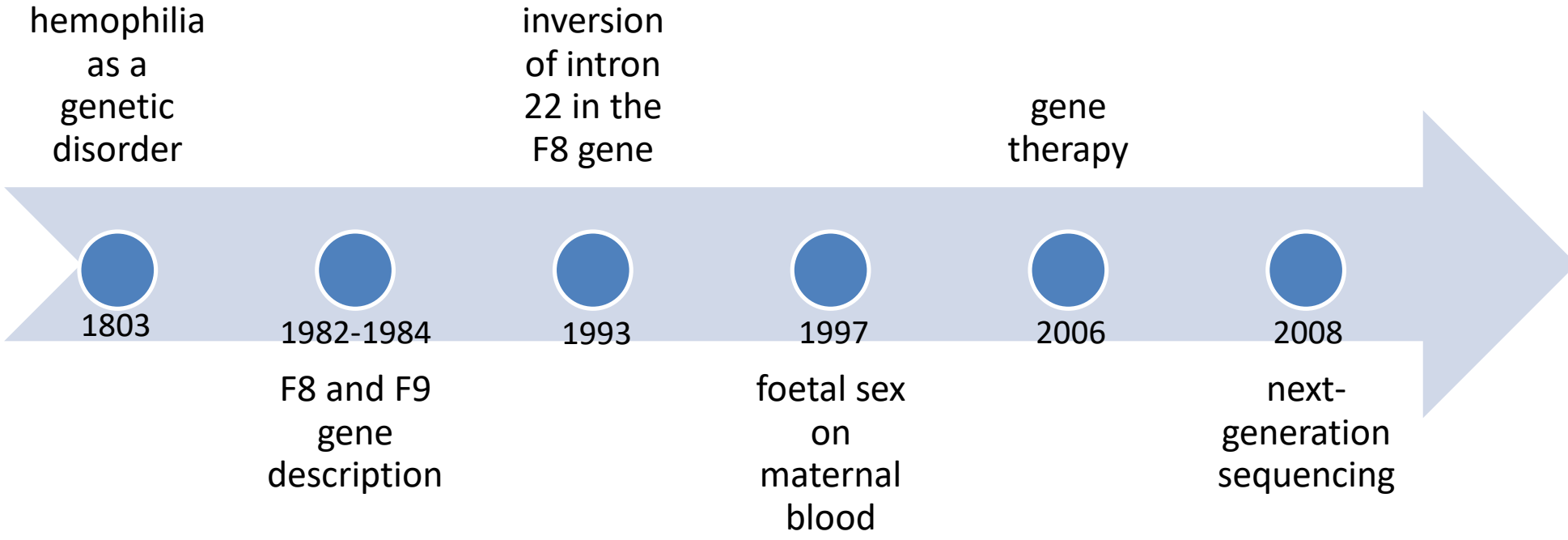


Chromosome **X**

## X-linked transmission

- males : affected
- females: “carriers”

# Main events in the genetic field of hemophilia





- Choo, K., *et al.* Molecular cloning of the gene for human anti-haemophilic factor IX. *Nature* **299,** 178–180 (1982).
- Gitschier, J., et al. Characterization of the human factor VIII gene. *Nature* 312: 326-330, 1984.
- Lakich, D., et al. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nature Genet. 5: 236-241, 1993.
- Lo, Y.M.D., et al. (1997) Presence of fœtal DNA in maternal plasmaand serum.Lancet,350, 485.

# Genetic counseling

# What is Genetic Counseling ?

Genetic counseling is the process through which knowledge about the genetic aspects of illnesses is shared by trained professionals with those who are at an increased risk of either having a heritable disorder or of passing it on to their unborn offspring.

https://www.who.int/genomics/professionals/counseling/en/

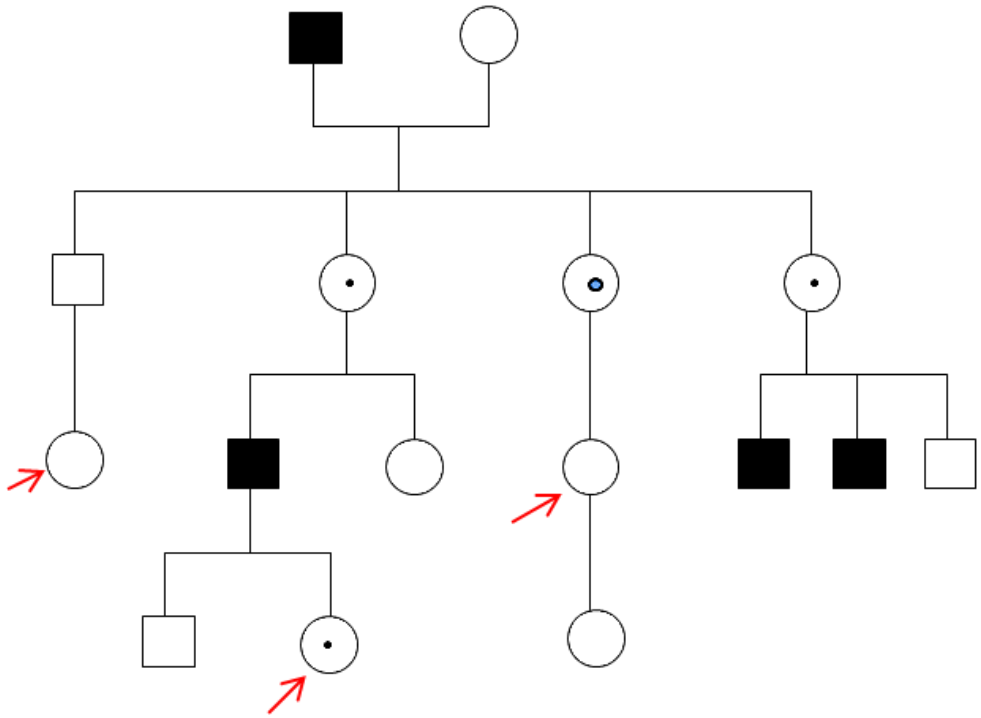
# Indications of genetic counseling in hemophilia

- Identification of the carrier status ➢woman/girl
- Information on the disorder and its consequences for the offspring ➢men or woman
- Prenatal diagnosis ➢couple/woman
- Preimplantation genetic diagnosis ➢couple/woman

# Genetic counseling in hemophilia

## 1st consultation

1. Evaluation of the type of hemophilia (A or B) and clinical severity
   - Other pathology ?…
2. Drawing the pedigree +++
3. Evaluation of the risk of the consultant
   - Affected male ?
   - Female:
     - Not a carrier
     - Obligate carrier ?
     - Potential carrier?
4. Laboratory tests
   - Coagulation tests
   - Genetic tests



# 1- What is the type of hemophilia?

- Hemophilia A (Factor VIII activity)

or

- Hemophilia B (Factor FIX activity )

# 1- What is the clinical severity?

| Type | factor (VIII or IX) activity |
|---|---|
| • Severe | <1% |
| • Moderate | 1-5% |
| • Mild | >5% - 40% |

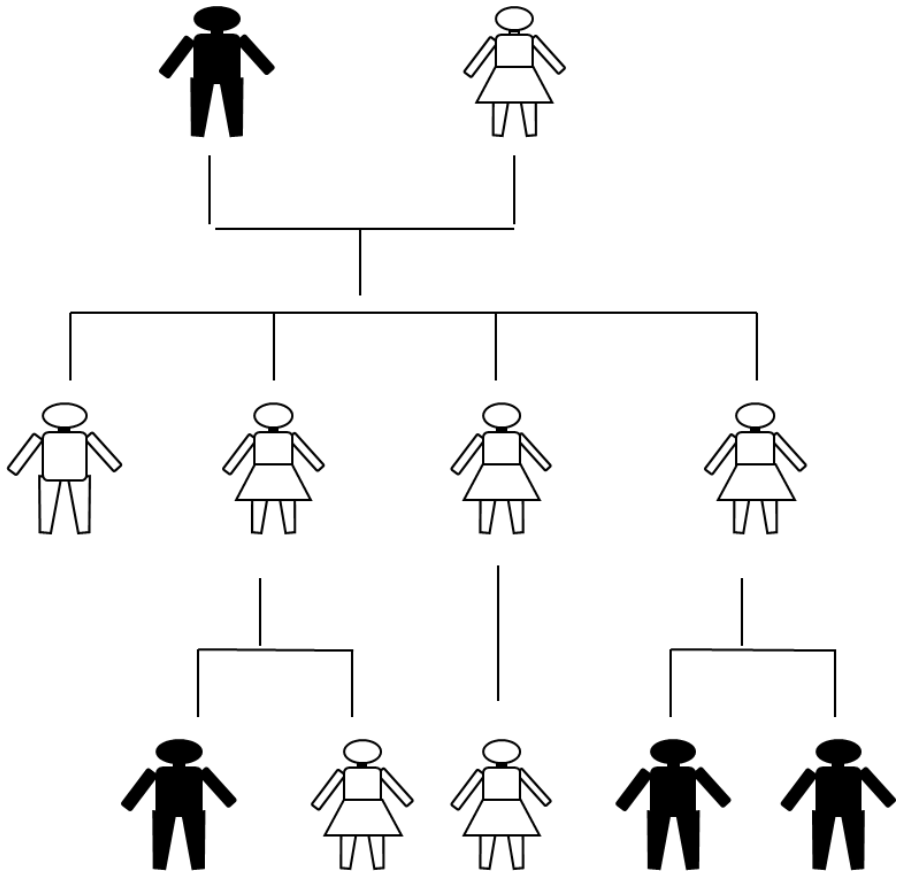
# 2- Drawing the pedigree

# HEMOPHILIA: transmission

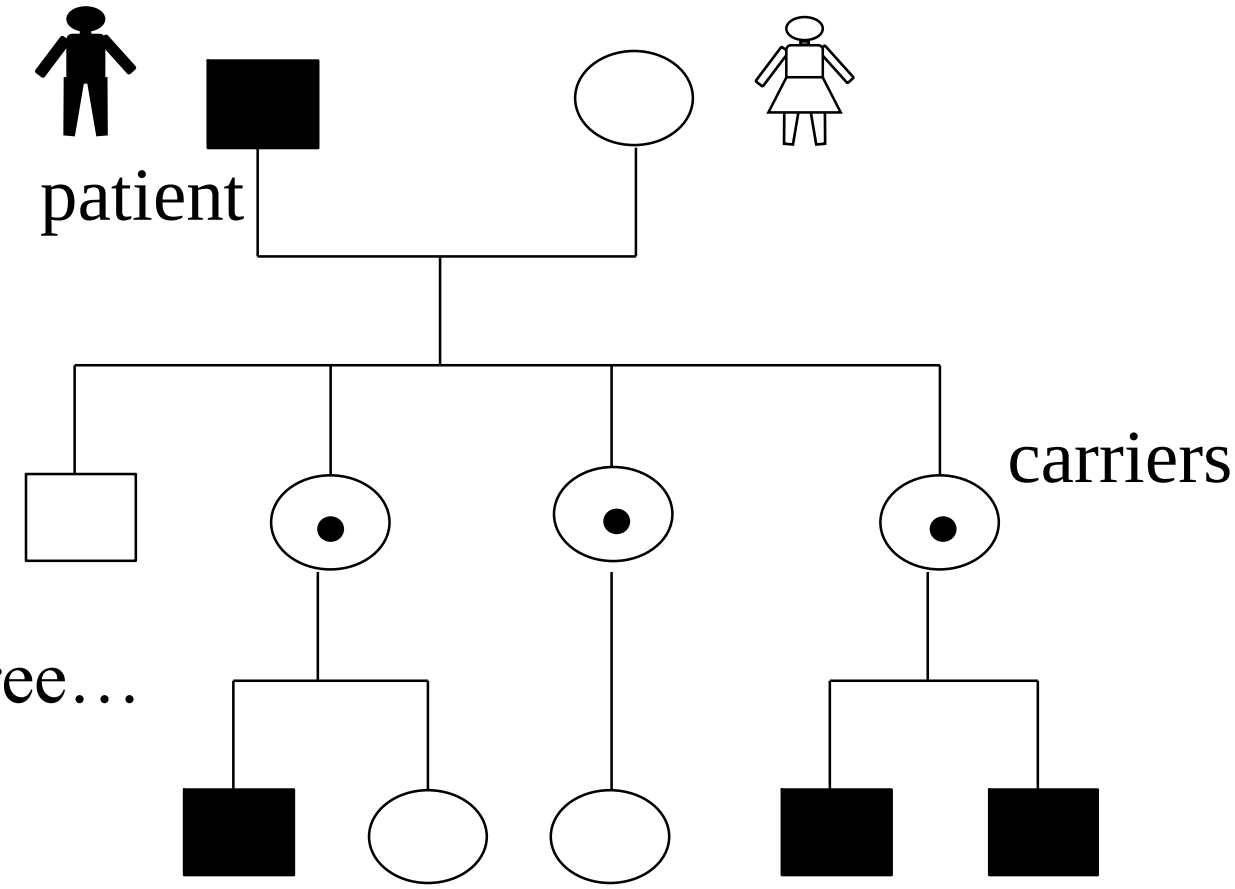
- Family forms
- Sporadic forms (30%)

# Family form

- Several hemophilia patients in different generations of the pedigree



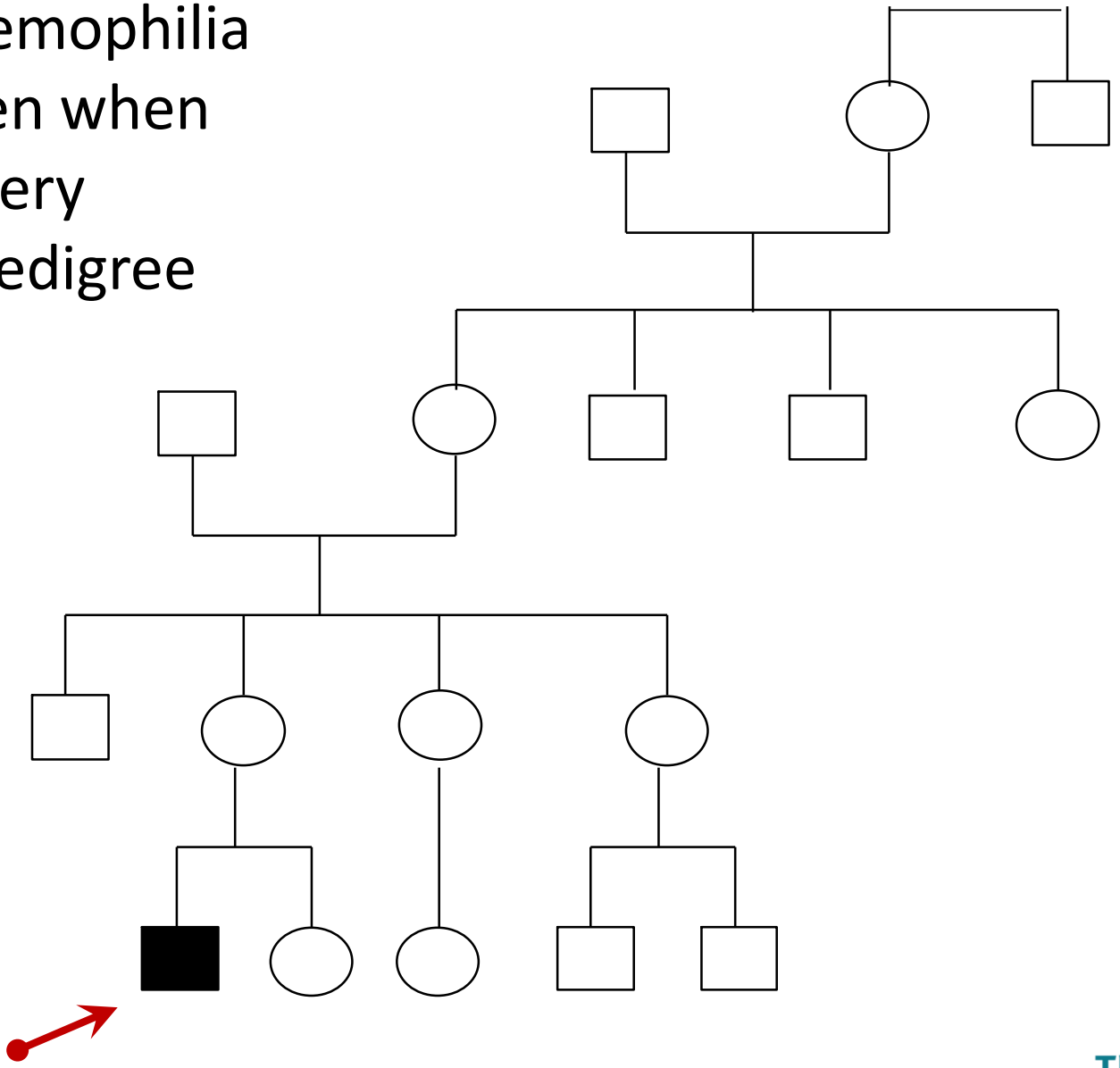
# Family form




Drawing the pedigree…

# Sporadic form

- Only one hemophilia patient, even when drawing a very extensive pedigree



# 3- Evaluation the risk

Carrier or not a carrier ?

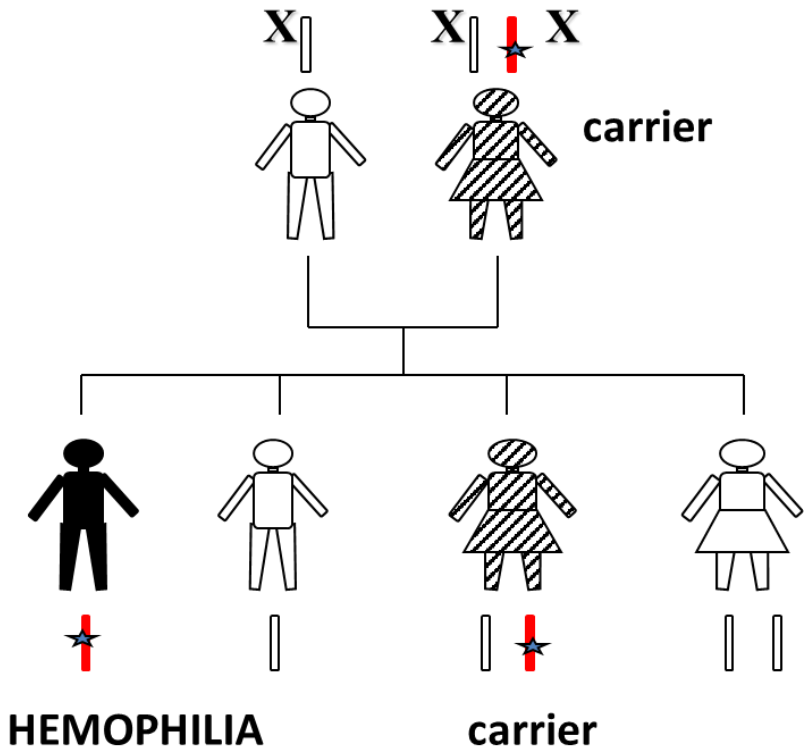
# HEMOPHILIA: carriers

- **A carrier is a girl having:**
  - one X chromosome with a sequence variant on the factor VIII or IX gene
  - and one, with a normal X chromosome

Consequences for
- the **offspring**
- the carrier

# Consequences for the offspring of a carrier mother

- ½ affected males
- ½ carrier females



# HEMOPHILIA: carriers

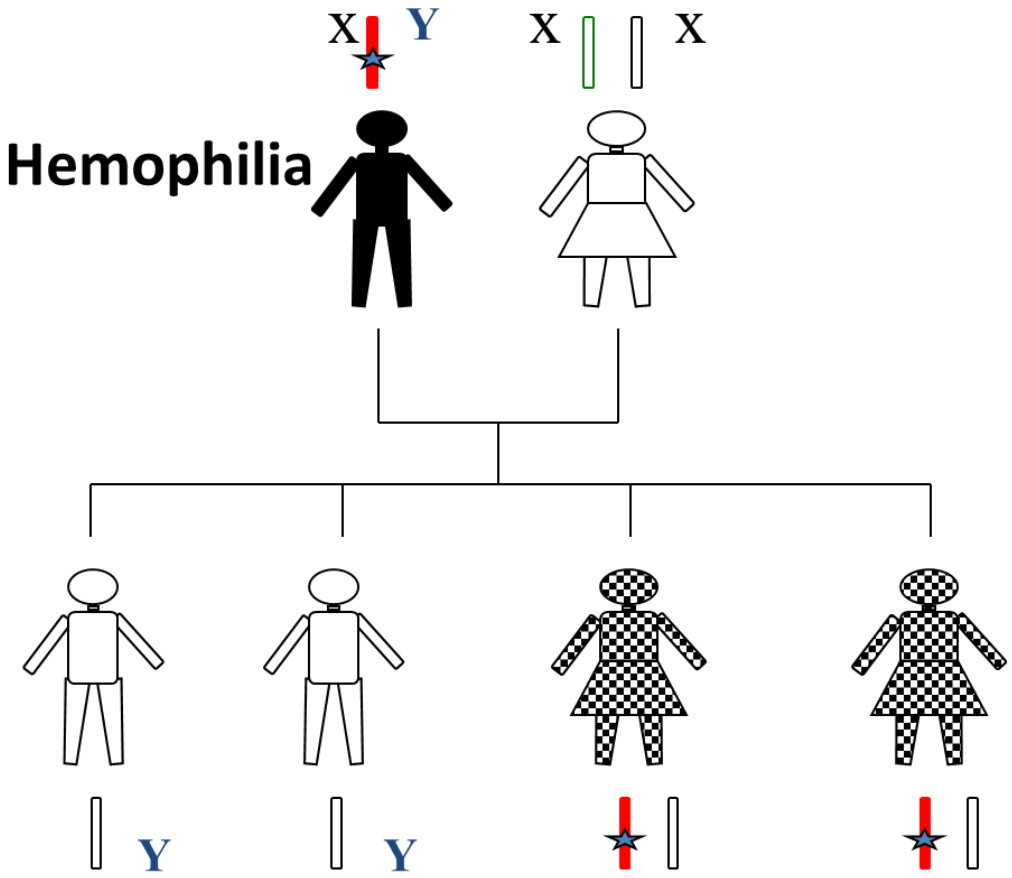
- Obligate carriers
- Potential carriers

# Obligate carrier

A woman

- having ≥ 2 sons with hemophilia
- having a son and a close family member with hemophilia (uncle, grandfather, nephew, brother, first male cousin)
- daughter of an hemophilia patient…

# Obligate carrier




**Obligate carriers**

# Potential carrier

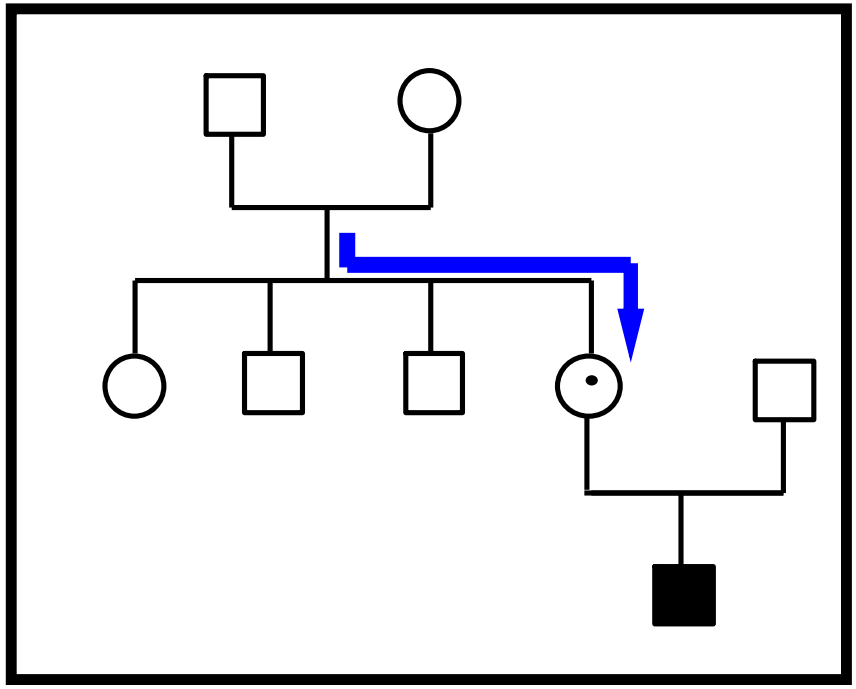
A woman

– having only 1 son with hemophilia

– sister of an hemophilia patient

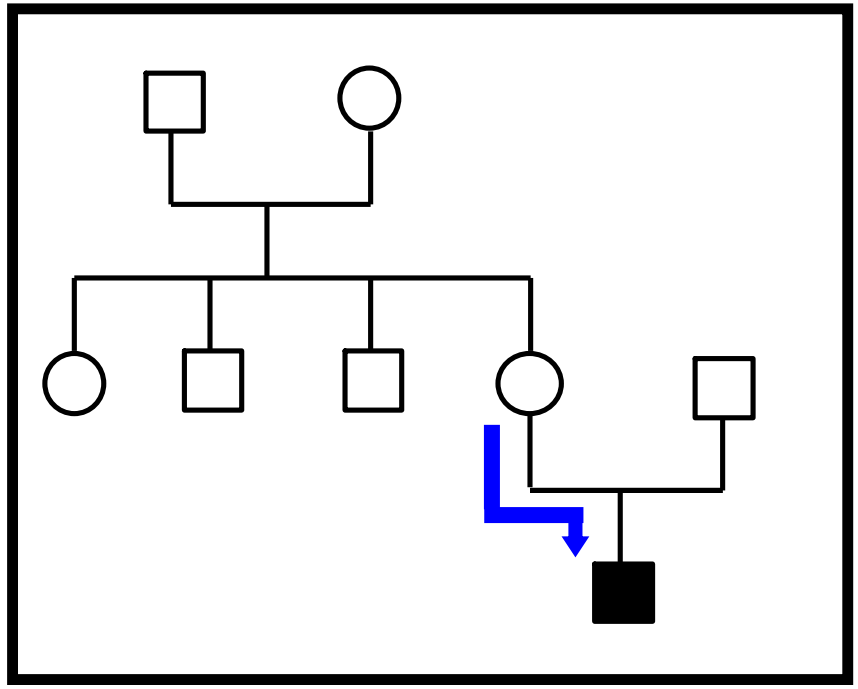
– aunt or cousin of an hemophilia patient (maternal side)

# Sporadic forms: carrier or not carrier ?



carrier mother

70%



non-carrier mother

30%

# Consequences for the hemophilia carrier

- total absence of clinical manifestations
- until « hemophilia phenotype »

  main risk : surgery/delivery

# HEMOPHILIA in females

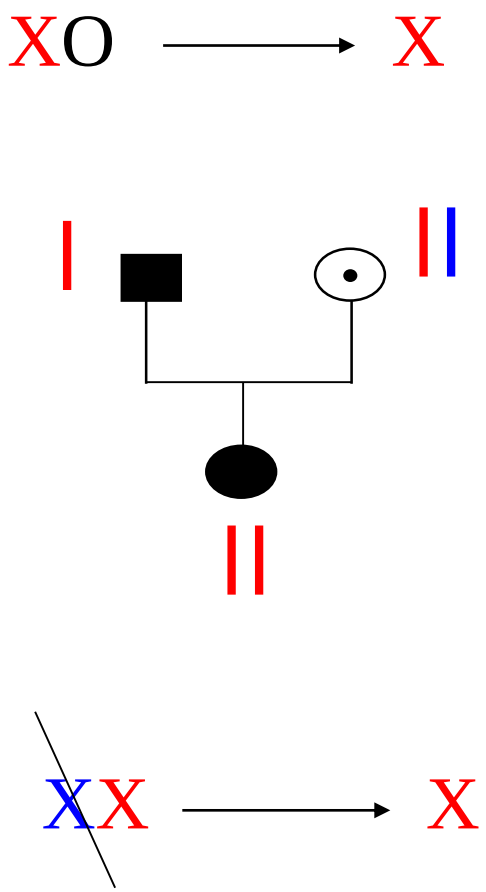
*Anecdotal:*

- **Turner Syndrome: XO**

- **2 X chromosomes with mutated *F8 or F9* genes** (or both)

*Frequent:*

- **Skewed X inactivation +++**



# 4-Role of laboratory tests

In the carrier’s diagnosis

# HEMOPHILIA: carrier diagnosis

| | Hemophilia patient | Carrier |
|---|---|---|
| Clinic | +++ | +/- |
| Biology (coagulation) | +++ (dosage F8 ou F9) | +/- |
| Genetics | +/- | +++ |

# 1- Coagulation tests in hemophilia A carriers (phenotype)

- **FVIII determination is not reliable for the diagnosis of carriers**

- **It is increased :**
  - During pregnancy
  - With oestroprogestative treatments
  - Chronic inflammation …

- **It is decreased** (plasma FVIII 25% lower) in blood group O

- Only 10% of carriers are expected to have plasma FVIII<35%
- Most obligate female carriers have normal FVIII levels

# 2- Molecular diagnosis in hemophilia

- Indications :
  1) Carrier diagnosis
  2) Prenatal diagnosis
  3) Pre-implantation genetic diagnosis

  4) Characterization of the genetic variant (prognosis, management…)

# Determination of the carrier's status

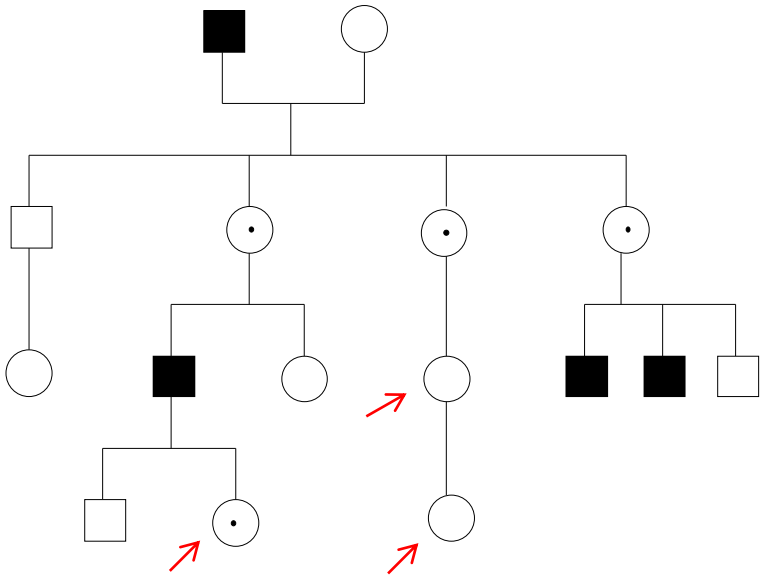
## Before any pregnancy

- Informed consent
- Mainly direct molecular diagnosis (genetic variant identified)
- Very rarely today: indirect diagnosis using haplotyping (segregation of polymorphic markers)

# Genetic counseling in hemophilia

## 2nd consultation

1. Announcement/confirmation of the diagnosis :
   - non carrier (control on a second sample can be requested)
   - carrier
2. Explanation about the “genetic risk”
   - Transmission to the offspring
   - Available reproductive options for the couple (PND, PIGD…)
3. Personal bleeding risk for carrier women
   - Carriers with low coagulation factor VIII or IX levels
4. Genetic risk for relatives and information (depending on national regulations)



# Some difficulties in hemophilia carrier testing

- Testing children and adolescents
  - national regulation, international recommendations
- Skewed X inactivation and female hemophilia patients
- Mosaicism (role of NGS)*
- Genetic associations of bleeding disorders
  - HA and HB
  - Association to von Willebrand disease (frequency)
  - Other genetic associations and differential diagnosis (eg. : combined FV and VIII deficiency)
- Incidental findings … (i.e. anomalies of other genes found with NGS testing)



*Lannoy, N, Hermans, C. Genetic mosaicism in haemophilia: A practical review to help evaluate the risk of transmitting the disease. Haemophilia. 2020; 26: 375– 383

# Prenatal and preimplantation genetic diagnosis in hemophilia

# 1- PRENATAL DIAGNOSIS in hemophilia

- Indication: *severe* hemophilia
- Mother is a known carrier
- Genetic counseling mandatory (*in France)*
- Multidisciplinary decision (*in France)*
- Final decision is up to the woman /couple .
- It depends mainly on the personal/family history



Punt, MC, Aalders, TH, Bloemenkamp, KWM, et al. The experiences and attitudes of hemophilia carriers around pregnancy: A qualitative systematic review. J Thromb Haemost. 2020; 18: 1626– 1636.

# Evolution of the demand of PND or PIGD with time

- Evolution of the diagnostic strategies
  - From cord blood sampling - chorionic villus sampling… maternal blood…
- Of the molecular biology knowledge
  - From haplotyping to direct genetic diagnosis
- Terrible time of the HIV contamination
- Therapeutic progress :
  - Better and safer coagulation factors
  - Gene  therapy

# PND in Hemophilia: how ?

*2 steps :*

1. Fetal sex determination
2. Genetic diagnosis of the *F8* or *F9* gene defect

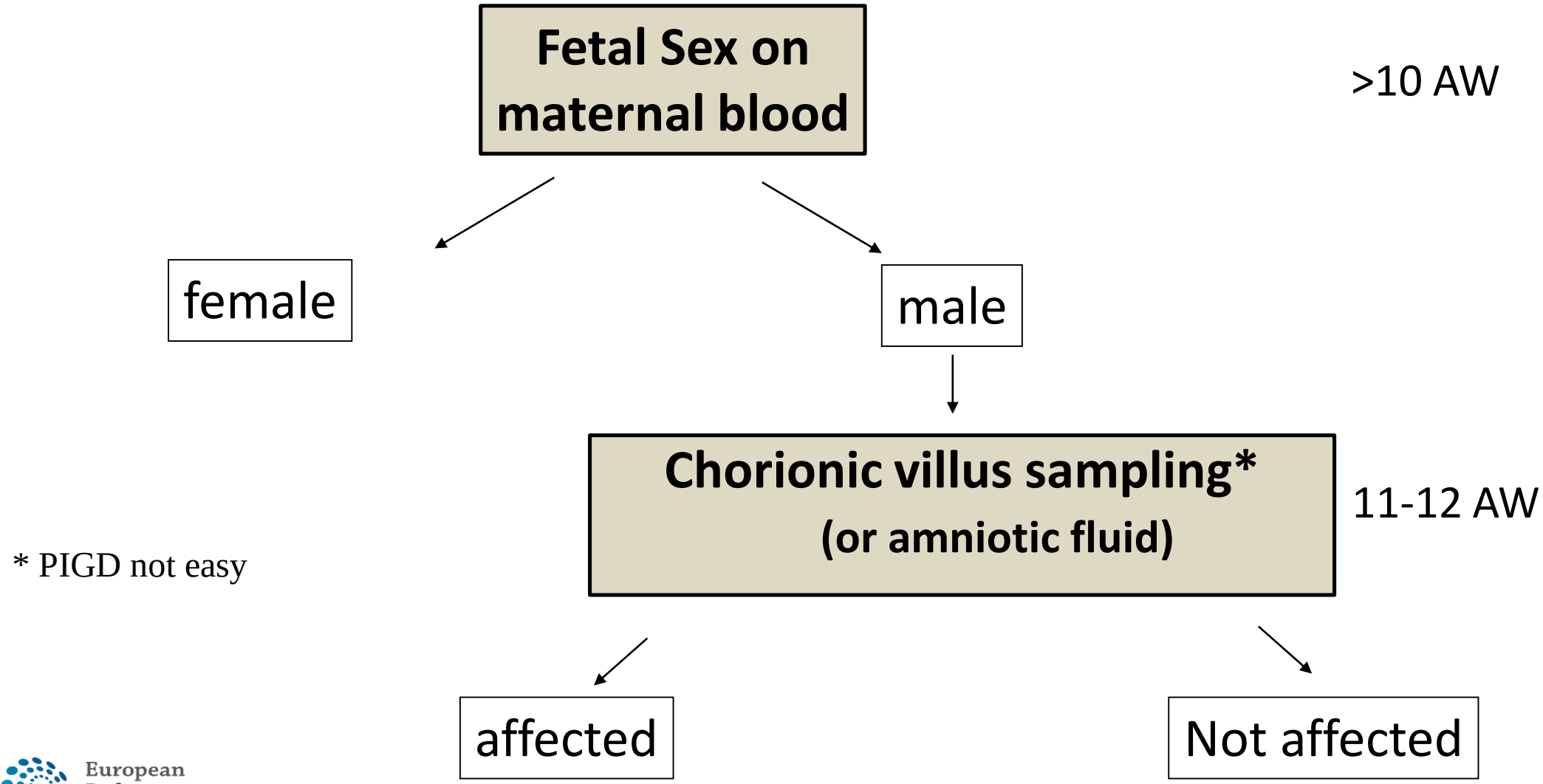
***NB: * coagulation factors testing is possible, but exceptional in countries where molecular diagnosis is available***

# PND of hemophilia: strategy

## 1. Fetal sex determination:

- On maternal blood (SRY sequences of circulating fetal DNA) >10 weeks- result in 2 days
- Karyotypes
- PCR (chrom. Y sequences) on chorionic villus sampling
- Ultrasound examination…

# PND of Hemophilia: testing strategy




* PIGD not easy

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for rare or low prevalence complex diseases
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# PND of Hemophilia: strategy

## 2. Molecular diagnosis

- **Hemophilia A**
  - **> Severe hemophilia**
  1. Intron 22 or intron 1 inversions (50%)
  2. Point mutations
  3. Large genetic rearrangements
- **Hemophilia B**
  1. Mainly point mutations
  2. Rare large rearrangements

# 2- Preimplantation Genetic Diagnosis (PIGD)

- *In vitro* fertilization methods ++
- One embryo cell biopsy (6-8 cell embryo, D**3)**
- Diagnosis made on a single cell DNA
- Transfer of non-affected embryos the same day

Handyside , Nature1990

# Preimplantation Genetic Diagnosis (PIGD)

Method:

- Sex determination by PCR

  +

- Molecular variant previously identified (and/or intragenic markers)

# Conclusions

- Tremendous development of genetic knowledge over the past 3 decades
- Increasing complexity
- Fewer requests of PND
- PIGD remains a heavy strategy
- New therapeutic developments such as gene therapy to change the future of the disorder for patients and their families

# Thank you for your attention!

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Serge Pissard, Paris
Jacques Rochette, Amiens…

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**Achille Iolascon,** Italy
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Mayka Sanchez, Spain
L Ribeiro, Portugal
Graça Porto, Portugal
MD Capellini, Italy
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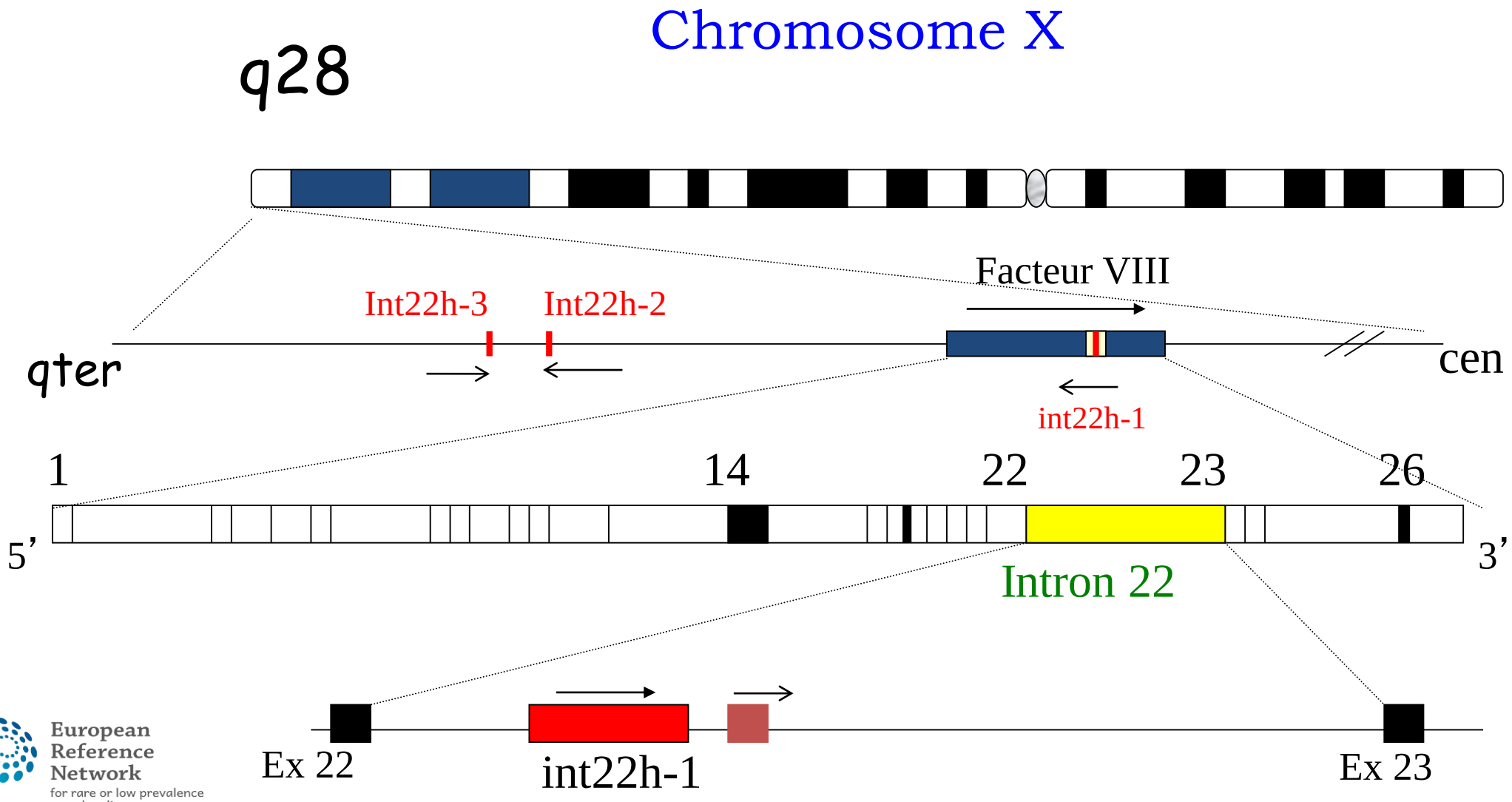
# ANY QUESTIONS ?

# ADDITIONAL SLIDES

# gène FVIII


Chromosome X
q28
Facteur VIII
Int22h-3
Int22h-2
qter
cen
int22h-1
1
14
22
23
26
5'
3'
Intron 22
Ex 22
int22h-1
Ex 23

# hemophilia in females

## Homozygous hemophilia in females

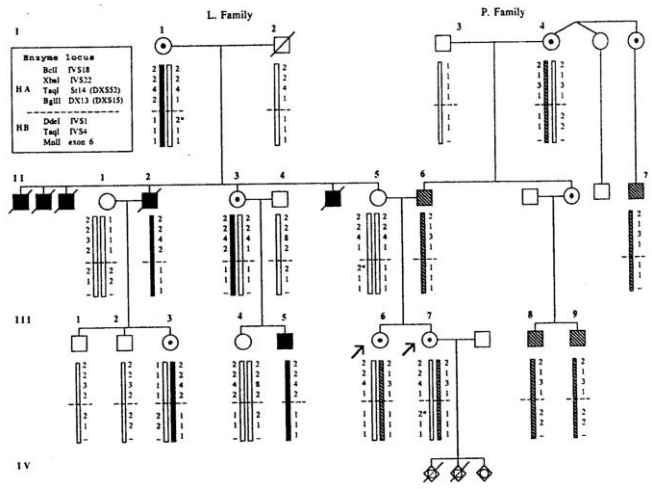
- Morita H, Kagami M, Ebata Y, Yoshimura H. The occurrence of homozygous hemophilia in the female. Acta Haematol. 1971;45(2):112-9

## Turner syndrome and hemophilia : ($9^{th}$ cases reported)

- Berendt A, Wójtowicz-Marzec M, Wysokińska B, Kwaśniewska A. **Severe haemophilia** a in a preterm girl with **Turner syndrome** - a case report from the prenatal period to early infancy (part I). Ital J Pediatr. 2020;46(1):125.

# Coinheritance of HA and HB


- Karch C, Masser-Frye D, Limjoco J, Ryan SE, Fletcher SN, Corbett KD, Johnsen JM, Thornburg CD. The odds and implications of coinheritance of hemophilia A and B. Res Pract Thromb Haemost. 2020 Jul 12;4(5):931-935.
- Roy, N.B.A., Curry, N. and Keeling, D. (2017), Unexpected haemophilia despite pre-natal testing – a combined haemophilia A and haemophilia B family. Br J Haematol, 179: 182-182.
- Aguilar-Martinez P, Navarro R, Schved JF, Gris JC, Bonnet H, Demaille J. Potential co-existence of haemophilia A and B carrier status in two sisters. Prenat Diagn. 1992 Nov;12(11):972-3.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# Combined deficiency of coagulation factors V and VIII

- Combined deficiency of factor V (FV) and FVIII (F5F8D)
- Autosomal recessive (mild) bleeding disorder
- Simultaneous decreases FV and FVIII
- Caused by mutations in the LMAN1 and MCFD2 genes
- Coding for a Ca2+-dependent cargo receptor complex that functions in the transport of FV/FVIII from the endoplasmic reticulum (ER) to the Golgi


- Oeri J, Matter M, Isenschmid H, Hauser F, Koller F. Angeborener mangel an faktor V (parahaemophilie) verbunden mit echter haemophilie A bein zwei brudern. Med Probl Paediatr. 1954;1:575–588.
- Zhang B, Cunningham MA, Nichols WC, Bernat JA, Seligsohn U, Pipe SW, McVey JH, Schulte-Overberg U, de Bosch NB, Ruiz-Saez A, White GC, Tuddenham EG, Kaufman RJ, Ginsburg D. Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. Nat Genet. 2003 Jun; 34(2):220-5.
- Nichols WC, Seligsohn U, Zivelin A, Terry VH, Hertel CE, Wheatley MA, Moussalli MJ, Hauri HP, Ciavarella N, Kaufman RJ, Ginsburg D. Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII.Cell. 1998 Apr 3; 93(1):61-70.