Duplications Xq28 and Haemophilia are questionable for genetic counselling

U955 équipe 11

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Introduction

•Severe haemophilia A is frequently caused by inversions involving intronic sequence of the *F8* gene (int22h1) and one of two remote, distal copies (int22h2 and int22h3) by intrachromosomal recombination.

• This rearrangement is usally explored by Long range PCR (Liu et al 1999) in one tube or Southern blot (Lakich et al 1993).

• Reciproqual large deletion and duplication of F8 gene have been suggested involving these copies. Though several F8 gene deletions have been described, the few duplications reported are most of the time small and associated with moderate haemophilia.

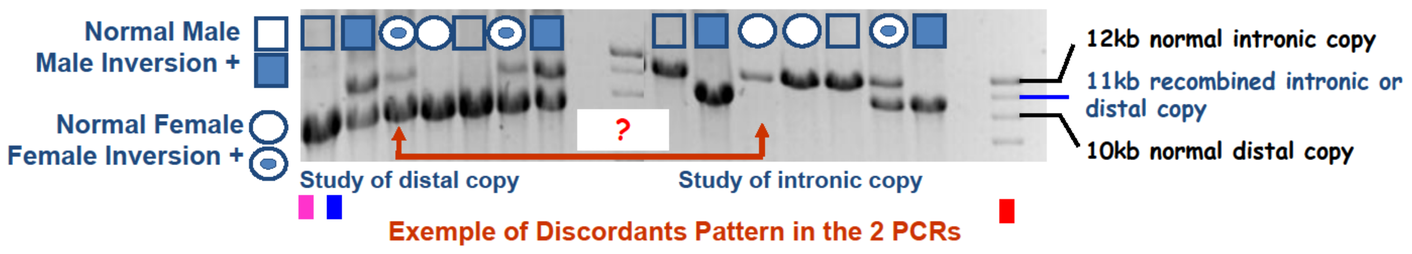
•Why such large duplications are not identified whereas commercial kit are now largely used? Why are they identified in severe haemophilia and intellectual disability ? Which consequences on genetic counselling?

Strategy of identification

Search for *F8* gene intron 22 inversion by **modified standard long-range-PCR (PCR-LR) assay in 2 tubes.**

Another molecular mechanism is suspected because of discordant patterns in the 2 tubes.

Remark, the use of the standard test is misleading, because in one tube it would show a **false positive inversion result**.



Exemple of Discordants Pattern in the 2 PCRs

Unusual patterns are observed in 6 non related families ➔ leading to extensive analysis of *F8* gene including sequence analysis of 26 exons and search for *F8* gene duplication by MLPA.

Identification and characterization of duplications

1-Conventional Study by PCR-LR et MLPA

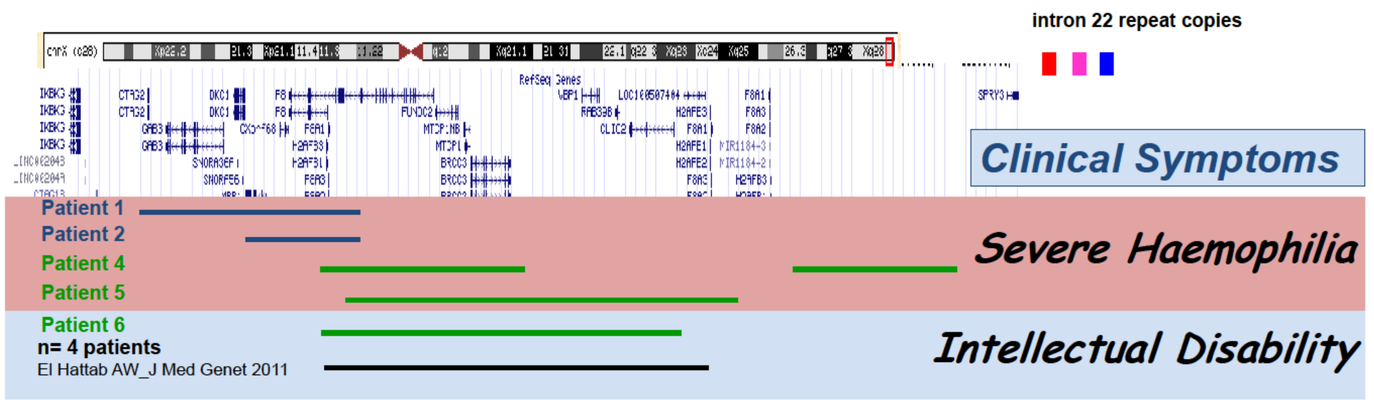
Patients	1	2	3	4	5	6	C+	C-
Recombined intronic copy	-	-	-	+	-	+	+	-
Recombined distal copy	+	+	+	-	+	-	+	-

MLPA *F8* gene — Dup exons 23-26 — Dup exons 1-22 — Dup exons 14-22

•Duplications are identified in all our patients and involve one of the multiple copies of F8 gene.

•Are other genes duplicated?

2-CGH-array in our patients and in literature

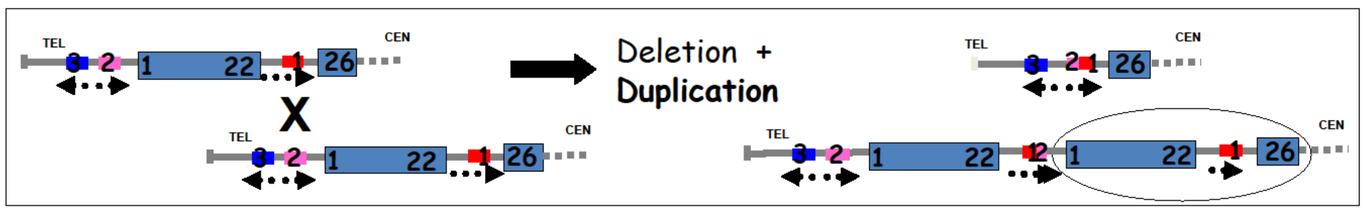


Size of duplications in 10 unrelated patients

•The analysis revealed in 3 patients with different clinical symptoms a telomeric duplication that covers 0.5Mb, affects several genes and part of the *F8* gene from the int22h1 copy to a locus lying between the distal int22h2 and int22h3 copies.

•This same duplication has been recently described in 4 patients with intellectual disability (ID).

What is the mechanism of this rearrangement?



The molecular mechanism is probably related to the repeat copies of intron 22 sequence ▪▪▪ and confirm the hypothesis that interchromosomal recombination should produce more deletions and duplications than inversion rearrangement.

Discussion

- **We show here a Xq28 duplication involving the *F8* gene is associated :**

-with 2 different clinical phenotypes:

❖ intellectual disability (ID) without low factor VIII levels in 5 patients

❖ isolated haemophilia A in 2 patients.

-severe form of haemophilia contrary to the initial hypothesis that *F8* gene duplication has no consequences.

- ***F8* gene duplication might be thought to be associated with normal phenotype as at least one normal copy is present. However these observations suggest that *F8* gene duplication is associated to severe phenotype.**

A possible hypothesis is that the rearrangement could have a positionning effect and could lead to disrupt *cis*-regulatory elements or increase the physical distance with the *F8* gene and other non identified genes

Conclusion

F8 gene duplications have been predicted without consequences. However they are identified in patients with ID without low factor VIII levels and conversely in severe HA patients without ID.
If the exact molecular mechanism is still not identified this duplication must be considered as disease associated or causing of haemophilia.

These data are important and must be taking account for genetic counselling. Indeed, this is of particular interest when such duplication is identified in pregnant woman with low factor VIII level suggesting of haemophilia carrier and no family history of the disease or conversely in woman with history of ID.

WFH2012 140--Mo
Molecular Genetics of Bleeding Disorders
Catherine Costa
DOI: 10.3252/pso.eu.WFH2012.2012
Poster presented at: WFH 2012 World Congress Paris, France Sunday, July 8 - Thursday, July 12
PosterSessionOnline.com