Various genetic mechanisms resulting in phenotypic expression of haemophilia A in three females

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Introduction:

Haemophilia A (HA) is a recessive X-linked disease that affects 1 in 5000 males. Females are carriers of the disease and usually exhibit normal or slightly decreased factor VIII activity (FVIII:C>25%). Haemophilia A has occasionally been described in females and various genetic circumstances can explain such phenotype. We report three cases of mild to severe HA in females with underlying different genetic mechanisms.

Case 1

The first case occurred in an 18-month-old baby girl suffering an unexplained knee haemarthrosis without any family bleeding disorder (Figure 1). Blood coagulation study disclosed a prolonged activated partial thromboplastin time (APTT: 91s, N: 28-38s) and a severe factor VIII deficiency (FVIII:C<1%) with normal vWF:Ag and vWF:Rco levels (respectively 138% and 104%). A type 2N von Willebrand disease was excluded, as the binding capacity of FVIII by vWF was normal. Factor VIII inhibitor screening was negative.

The FVIII:C level was normal in her parents, her 7-year-old brother and her 4-year-old sister. Her karyotype was 46XX. Molecular analysis showed **heterozygous intron 22 inversion inherited from her mother.**
The entire sequencing of *F8* coding regions and flanking splicing sites were normal. Multiplex ligation-dependent probe amplification (MLPA) failed to identify any large deletion or duplication. X chromosome inactivation analysis revealed a **complete skewed X inactivation of the paternal X chromosome** (100% / 0%) (Figure 2).

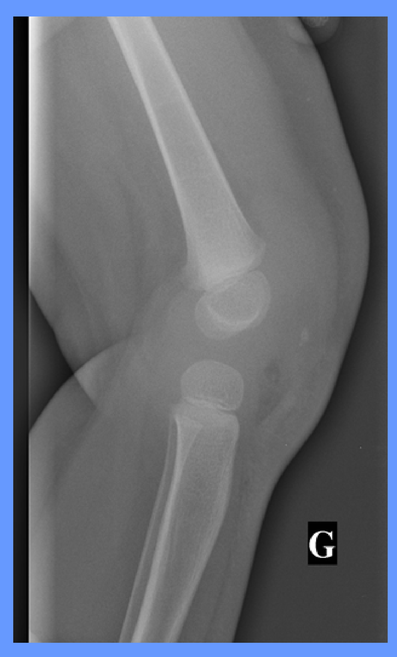

Figure 1. Lateral radiograph of the left knee

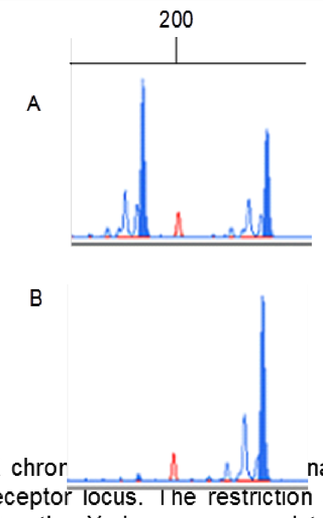

Figure 2. X chror nalysis at the androgen receptor locus. The restriction sites on the methylated-inactive X chromosome resist cleavage by *Hpa*II. (A) Profile obtained after PCR amplification without *Hpa*II digestion showing the two alleles were informative. (B) After *Hpa*II digestion, only one allele was amplified, corresponding to the methylated allele, indicating complete skewed inactivation.

Case 2

The second case was a 24-year-old woman who had a maternal male cousin with severe HA. She only reported haemorrhagic tooth extraction. The FVIII:C was 6-14%. Her sister presented with the same profile. Family study disclosed an unknown mild HA in her father (FVIII:C=25%) in whom the p.Ser2011Asn mutation in exon 19 was identified. Both sisters were **compound heterozygous**: they inherited the missense mutation from their father and the abnormal X chromosome from their mother, with a preferential X inactivation of the paternal X chromosome (Figure 3). However, thorough molecular analysis (including detection of the intron 1 and 22 inversion, sequencing of *F8* coding regions and flanking splicing sites, MPLA and cGH arrays) in the maternal family failed to identify the underlying molecular mechanism responsible for the severe HA.
DDAVP test permitted a factor VIII increase from 6% to 40% after 1 hour and 29% after 2 hours. During her unique pregnancy, factor VIII level showed no increase. Analysis of RFLP on the trophoblast biopsy performed at 14 weeks of gestation diagnosed a female baby carrying severe haemophilia A. Delivery occurred without bleeding complication and DDAVP was administrated intravenously after baby extraction.

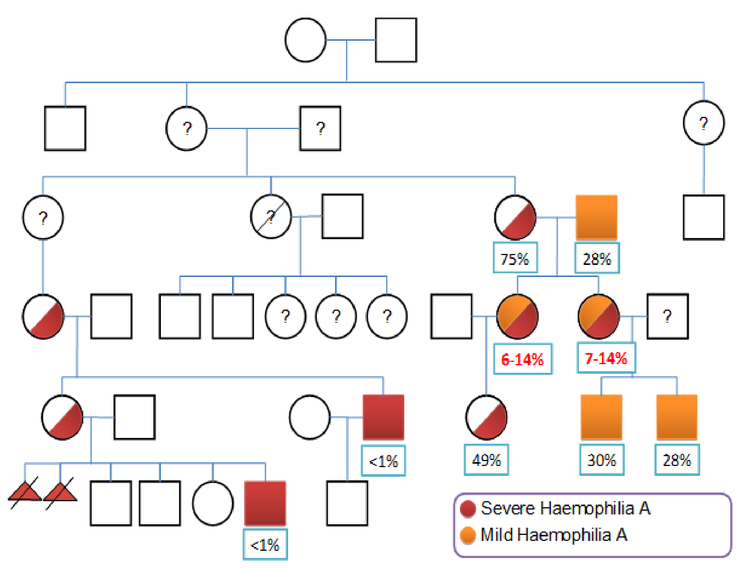

Figure 3. Family pedigree of the HA female patient analyzed in case 2

Case 3

The third case was a 28-year-old woman. Her male cousin was recently diagnosed with a mild HA. She suffered bruising and epistaxis. In her childhood, surgery of the tympanic membrane for otitis was complicated by haematoma. Laboratory studies showed a prolonged APTT, normal vWF:Ag and vWF:Rco levels (respectively 79% and 74%). The FVIII:C level was 19%. Her karyotype revealed the **X-monosomy of a Turner syndrome with a 45,X0/46,XY mosaicism**. The hemizygous p.Ser2011Asn mutation in exon 19 was found to be responsible of the Haemophilia A phenotype.

Conclusion:

In most cases, females with isolated decreased factor VIII level are carriers of haemophilia A. When factor VIII clotting activity is lower than approximately 25%, thorough molecular analysis has to be performed to detect a combination of several mechanisms (Figure 4).

These cases of HA in females resulted from various underlying mechanisms which need to be highlighted in order to provide an adapted genetic counselling.

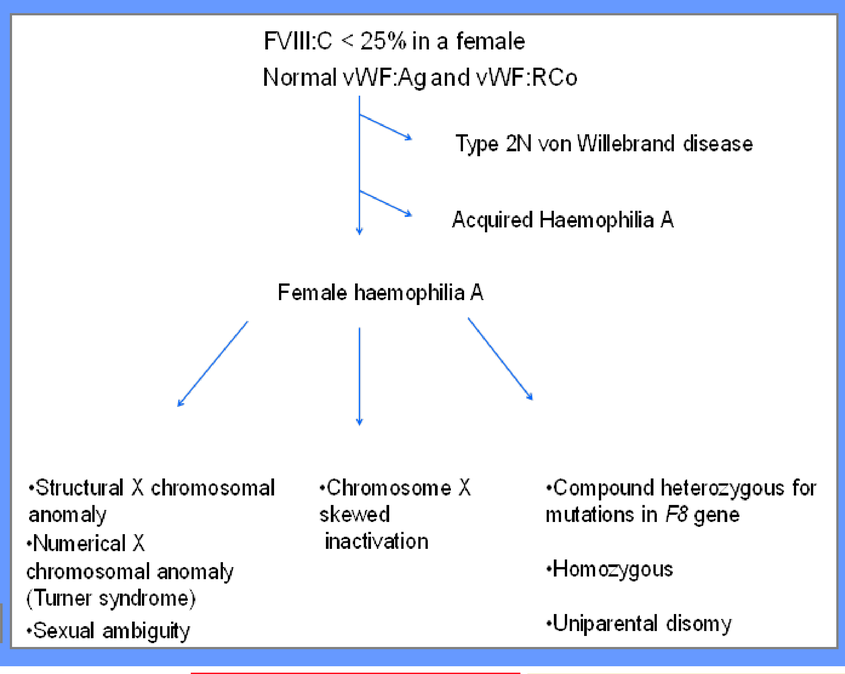

Figure 4.

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