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Mosaicism in men in haemophilia : is it exceptional? Impact on genetic counselling.

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Haemophilia A is an X-linked bleeding disorder caused by a wide range of mutations in the factor VIII (*F8*) gene [1]. About one third of cases are due to a *de novo* mutation. The majority are thought to occur in a single germ cell but some, occurring during early embryogenesis, produce a germline and/or somatic mosaic. In haemophilia, somatic mosaicism has been generally observed in women and seems to represent a fairly common event [2]. We report here a case of exceptional mosaicism in the asymptomatic maternal grandfather of a haemophilia A patient.

The proband has severe haemophila A with factor (F)VIIIc levels <1% and no previous family history of the disorder. Gene mutation studies were performed in order to identify the deleterious mutation and offer genetic counselling to the mother and the family. The mutation p.Arg336X in exon 8 was identified in the proband by direct sequencing and subsequently searched for in the mother and maternal grandmother. It was found only in the mother, suggesting a *de novo* germline mutation in one of the grandparents or a *de novo* somatic mutation early during embryogenesis in the mother. The maternal aunt, who had not been tested, was at first reassured as being probably not a carrier. Several years later, when undergoing medically assisted procreation because of the infertility of her partner, a genetic test was performed. Unexpectedly, the mutation p.Arg336X was identified, leading to a modification of her status as being a carrier of severe haemophilia A. The presence of the mutation in the two sisters thus first suggested the grandmother was a carrier with a somatic mosaicism. The absence of the mutation in her peripheral blood as well as in her buccal and uroepithelial cells, which have different embryological origins, then raised the question of the mechanism of occurrence of this mutation. Linkage analysis, using intragenic and extragenic markers linked to the *F8* gene, actually showed that the deleterious allele originated from the asymptomatic maternal grandfather whose FVIIIc was normal FVIIIc=96% (Fig1).

Somatic mosaicism in the grandfather was then hypothesized. However, it is well known that somatic mosaicism may be difficult to detect with conventional methods such as direct sequencing. Mutation-enrichment procedures, not used during routine test analyses, are often required [2]. Nowadays, due to technology progress, methods presenting higher sensitivity are available. One of them, denaturing-high-liquid-pressure-chromatography (DHPLC) was used in this family. DHPLC is well known for its efficiency to detect heteroduplexes that are DNA molecules containing mismatched base pairs and created during amplification reaction (PCR) when a mutation is present in heterozygosity. Under partial denaturation, heteroduplexes are eluted from the column by an acetonitrile gradient flow before homoduplexes [3]. Analysis of the grandfather's leucocytes, buccal and uroepithelial cells showed the presence of the mutated allele with a proportion estimated between 15-20% (fig 1). Karyotype analysis showed a normal 46,XY karyotype, ruling out Klinefelter syndrome.

The presence of the mutation in all tested grandpaternal tissues and in his two daughters suggested that the mutation had arisen very early during embryonic development.

The distinction between isolated or sporadic cases is of major importance in genetic counselling. A case may appear to be isolated because family size is small; DNA testing may help for carrier diagnosis but negative results will not rule out the possibility of an occult mosaic. In a recent study only a small number (11%) of maternal grandmothers of isolated cases had the mutation in their white blood cells, while 85% of mothers were carriers, which favours the hypothesis that isolated cases may have originated as a *de novo* germline mutation in one of the grandparents or a *de novo* somatic mutation early during embryogenesis in the proband's mother [2, 4]. Somatic mosaicism has been found in around 10% of mothers of isolated cases [2] and in 13% of patients' mothers and grandmothers in a study which used mutation enrichement procedures [5]. These results indicate that mosaicism is a fairly common event in haemophilia, but is still underestimated due to the limited sensitivities of the

methods for detection of mosaicism and probably also because the distinction between carrier and 50% mosaicism is difficult. It is of note that most of the time, mosaicism has been reported in families with point mutations while only once in an isolated case with intron 22 inversion. [2, 5, 6]. Somatic mosaicism in families with apparent *de novo* mutations is however rarely explored in women, and grandfathers are usually not considered. In this present case we have been questioned because the proband's mother and aunt were carriers while the grandmother was not. In the literature only three cases of mosaicism in men have been reported, all of them in grandfathers in families with point mutations |7-9]. Our case underlies that somatic mosaicism in men is probably underestimated because of the difficulties of obtaining blood sample from grandfathers, and points to the need for testing men in such apparent isolated cases. In these situations and even if the mutation is characterized, linkage analysis remains a precious help to identify the origin of the deleterious allele.

Assessment of mosaicism in mothers of apparent isolated cases is now part of genetic counselling. It also seems important now to take into account the risk of mosaicism in grandfathers as well as grandmothers with a view to the genetic counselling of all their daughters.

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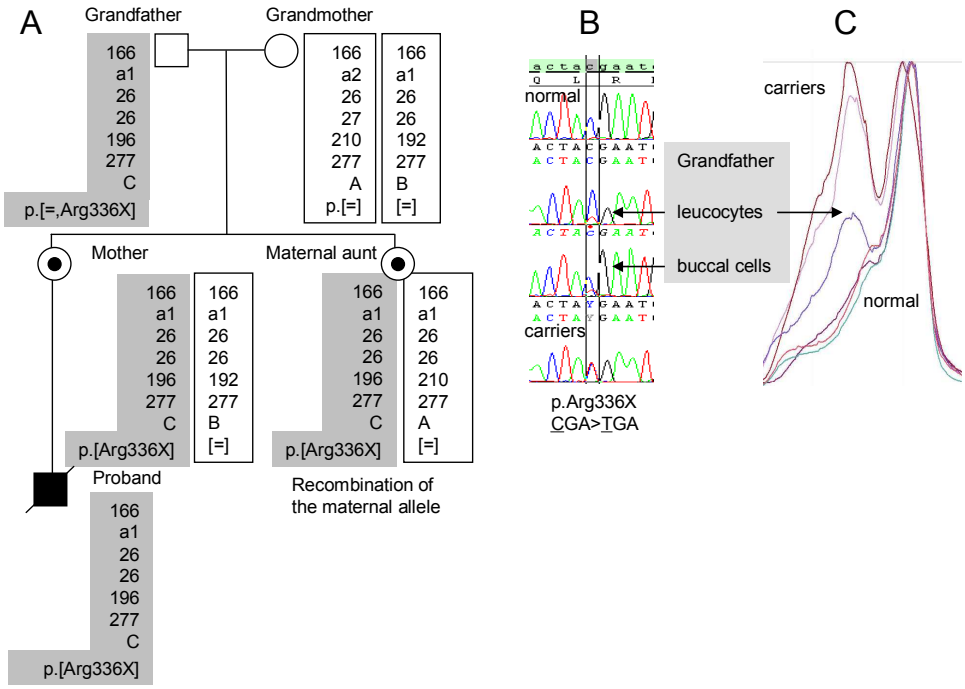
Figure 1



Legend to Figure 1 : A-Family pedigree, haplotypes and mutation studies. B-Electropherogram obtained by direct sequencing. C-Denaturing High Liquid Chromatography (DHPLC) elution profiles of carrier, control and grandfather: in female carriers, heteroduplexes, which contain mismatched base pairs, are eluted first (left peaks) followed by the homoduplexes (right peaks); normal control have only one right peak. Analysis of the grandfather's leucocytes and buccal cells shows a right peak corresponding to the normal allele and a small left peak indicative of the presence of the mutated allele with a proportion estimated between 15-20%.

The mutation is detected with higher sensitivity with DHPLC compared to direct sequencing.