Short Report

Female factor IX deficiency due to maternally inherited X-inactivation


X-chromosome inactivation is normally a random event that is regulated by the X chromosome itself. Rarely, females are affected by X-linked disorders from extremely skewed X-chromosome inactivation. Here, we report a family with hemophilia B with female expression through inherited X skewing that appears to be independent of either X chromosome. This finding suggests the possibility of a dominant autosomal contribution to inherited skewed X inactivation.

Conflict of interest

The authors have no conflict of interests to disclose.

Hemophilia B is an X-linked recessive bleeding disorder caused by a deficiency or functional defect in clotting factor IX, with a prevalence of approximately 1 in 25,000 male births. Normal plasma levels of factor IX range from 50% to 150%. Severity of bleeding tendency generally tracks with the degree of factor deficiency, with levels of 6–30% defined as mild disease (1). Because of random X-chromosome inactivation, asymptomatic female carriers normally exhibit factor IX (or VIII) levels at or below the normal lower limits, usually between 30 and 70% (2).

Rarely, females are affected by X-linked disorders resulting from several distinct mechanisms, such as extremely skewed X-chromosome inactivation. Female carriers manifesting hemophilia A (factor VIII deficiency) and hemophilia B through skewed X-chromosome inactivation are rare but well documented (3). Here, we describe a woman with factor IX deficiency from extremely skewed X-inactivation. Notably, one of her four daughters inherited both the factor IX deficiency and the skewed X-inactivation, but inheritance appears not to have been from the affected X chromosome.

Methods

This research was performed in conformity with policies of Institutional Review Boards for Columbia and Johns Hopkins Universities.

Case history

The proband is a 38-year-old woman who first presented to our Pediatric Hematology service at age 14 years with multiple episodes of moderately severe bleeding, including hemarthroses. Evaluation had revealed a prolonged activated partial thromboplastin time (aPTT) of 43.1 s (upper limit of normal is 37.9 s) and a factor IX level of 17%. Evaluation of factor VIII activity, circulating factor IX inhibitor, von Willebrand factor and other laboratory evaluation
for bleeding disorders were normal. Bleeding episodes were resolved with factor IX replacement. She experienced several more episodes of bleeding, including postpartum hemorrhage requiring red blood cell transfusions and factor IX replacement therapy. Prophylactic factor IX replacement therapy at subsequent deliveries prevented further bleeding complications. There was no history of miscarriages.

Her father also had hemophilia and succumbed to its complications during his daughter’s childhood. Her mother did not have any excess clinical bleeding nor was there any known consanguinity. The proband has four daughters, each with a different father (Fig. 1). One of her daughters has mild increased bleeding. Evaluation of this symptomatic daughter revealed an aPTT of 41.1 s and a factor IX level of 16%. Evaluation for factor VIII deficiency, von Willebrand disease and a circulating inhibitor were negative. Two of the other daughters have low normal factor IX activity, as expected for asymptomatic carriers; another daughter has normal levels.

The proband has a full sister and two nephews. Her sister has menorrhagia, but no other excessive bleeding history. One of her two nephews has hemophilia B, with moderate bleeding and a factor IX level of 14% (Table 1). This nephew has one full brother, who is without any bleeding history and presumably unaffected.

Factor IX gene sequencing
Bidirectional sequencing of the entire factor IX coding region, intron–exon boundaries and the proximal promoters was performed by ARUP laboratories, Salt Lake City, UT.

Karyotype
Karyotype analysis was performed on peripheral blood from the proband and each of her daughters by standard analysis. Each has a normal karyotype of 46, XX.

X-inactivation studies
X-inactivation patterns were assessed for skewing with the human androgen-receptor locus (HUMARA) using the standard methylation assay (performed by Greenwood Genetic Center, Greenwood, SC). Extracted genomic DNA was digested with the methylation-sensitive restriction endonuclease, HpaII. The HUMARA polymorphic CAG repeats were amplified by polymerase chain reaction (PCR) and used to determine X-inactivation status. Random inactivation ratios are defined by the testing laboratory as less than 80:20. Ratios between 80:20 and 90:10 are considered moderately skewed, while ratios >90:10 are highly skewed (4).

Results and discussion
In this unusual family, the rare condition of mild hemophilia B is expressed in the proband and in one of her four daughters, with two others genotypically and clinically assessed as typical carriers, and a fourth daughter unaffected (Table 1). The affected male nephew has 14% factor IX activity, showing that the specific variant is causal for mild hemophilia B, and is not associated with other detected abnormalities. The proband and her daughters are each normal

Table 1. Characterization of family members

<table>
<thead>
<tr>
<th></th>
<th>Proband</th>
<th>Daughter 1</th>
<th>Daughter 2</th>
<th>Daughter 3</th>
<th>Daughter 4</th>
<th>Sister</th>
<th>Nephew 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IX activity</td>
<td>17%</td>
<td>133%</td>
<td>16%</td>
<td>42%</td>
<td>64%</td>
<td>69%</td>
<td>14%</td>
</tr>
<tr>
<td>(86–176%)</td>
<td>Hetero</td>
<td>WT</td>
<td>Hetero</td>
<td>Hetero</td>
<td>Hetero</td>
<td>Hetero</td>
<td>Affected</td>
</tr>
<tr>
<td>Factor IX genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>100:0</td>
<td>95:5</td>
<td>73:27</td>
<td>68:32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-inactivation</td>
<td>Extremely skewed</td>
<td>Extremely skewed</td>
<td>Random</td>
<td>Random</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(normal &amp; &lt;=80%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bleeding history</td>
<td>Mild-moderate bleeding</td>
<td>Normal</td>
<td>Mild bleeding</td>
<td>Normal</td>
<td>Normal (at one year of age)</td>
<td>Mild bleeding</td>
<td>Moderate bleeding</td>
</tr>
</tbody>
</table>

JHU, Johns Hopkins University; NA, not applicable; WT, wild type.
Female factor IX deficiency

in appearance and function and have normal karyotypes. She and three of her four daughters bear the identical heterozygous missense mutation in their factor IX gene (nucleotide mutation c.301C->G), resulting in amino acid substitution (p.Pro101Ala). Extreme X-chromosome skewing of the proband and one of her daughters caused hemophilia B, with factor IX levels similar to that of an affected male relative (Table 1).

Factor IX sequencing and X-chromosome inactivation studies showed that disease in the proband’s daughter was likely inherited through extreme skewing and not other apparent mechanisms.

That one of four daughters is also affected by extreme skewing is consistent with a dominant inheritance. Normal human females have a reported incidence of >90:10 skewing of 1.8% and >95:5 skewing of 0.8%. (4). The probability of coincidental skewing of 95:5 or more in both the proband and one daughter is estimated as (0.08)^2 to (0.18)^2, or 6.4 × 10^{-5} to 3.3 × 10^{-4}. Hence, the likelihood of random extreme skewing in this family is quite low.

Uneven X-inactivation among normal human females is uncommon, estimated as 8% with >80:20 skewing and only 1–2% with 90:10 or more, with some differences by age and tissue of origin (4, 5). Female expression of X-linked disorders is often avoided or dampened by disadvantageous growth, viability or development of cells expressing the affected allele. Occasionally, non-random X-inactivation leads to expression of X-linked recessive or co-expressed alleles, and can result in the manifestation of X-linked disease in females, e.g. hemophilia A (3). Thus, clinically a highly skewed X-chromosome inactivation ratio suggests a biologic preference for one X chromosome (4). Such preferences are manifested through an underlying genetic process or through positive or negative selection of cells with biased X-chromosome expression at the embryonic or tissue level. For example, skewing of 80:20 or more against the affected X allele is common in female carriers with X-linked mental retardation syndromes (6). Rarely, female disease occurs in certain X-linked disorders or cytogenetic abnormalities, such as translocations or Turner syndrome in the extreme. Female carriers for factors VIII or IX nearly always show skewing of <95:5 (2).

Mechanisms described for X inactivation and skewing to date are primarily attributed to DNA elements on the X chromosome itself (7–9). The process of X-inactivation begins early in embryogenesis, when non-coding Xist RNA stably accumulates on the X chromosome to be inactivated (Xi). Inactivation is regulated by the complex genetic locus of the X-inactivation center, consisting of sequences near the Xist gene on the chromosome to be inactivated. Stable accumulation of non-coding RNA on the Xi leads to chromosome-wide gene silencing. Studies of females with X-linked dystrophinopathies may suggest a role of inherited non-X loci (5). Dominant autosomal modifiers of X chromosome choice have been described in mice (10). The potential for epigenetic regulation of X-chromosome inactivation exists through mechanisms involving allele-specific chromatin modulation (11, 12).

There is no evidence that skewed inactivation in this family was caused by or is associated with either inherited X chromosome, such as an interaction between each pair of X chromosomes, as each of the maternal alleles shows a non-skewed pattern among the other three daughters. Gene-environmental selection of one X chromosome, such as through HPRT-selectivity (13), is also unlikely because the proband maintained normal uric acid levels between and during pregnancies, reported no variability between pregnancies in intake of prenatal vitamins, did not take any antimetabolites, nor did she travel or change her place of residence. Two distinct tissues showed skewing: liver (site of factor IX synthesis) and lymphocytes (HUMARA assay), rendering tissue mosaicism or tissue-specific selection unlikely explanations. Absence of sons could raise the question of reduced viability of male offspring, despite multiple different paternities. However, this explanation is highly unlikely, as there is one otherwise healthy affected nephew; she had four healthy term pregnancies and no miscarriages; the factor IX mutation is described in the mutation database in other males as causative for mild hemophilia B; neither this mutation nor others are known to be reasons for negative selection of the X chromosome.

We conclude that one of the proband’s four daughters appears to have maternally inherited extreme skewing of X-chromosome inactivation through an autosomal dominant mechanism. The possible transacting mechanism(s) involved are yet unknown.

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References