X-inactivation patterns in carriers of X-linked myotubular myopathy


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Abstract

X-linked myotubular myopathy is a rare severe muscle disorder in affected male neonates. Most female carriers are free from symptoms. Skewed X inactivation has been proposed to be responsible for the affected phenotype seen in some carriers. We have compared the X inactivation patterns in blood DNA with the clinical phenotype in carriers of X-linked myotubular myopathy. The X-inactivation analysis was performed using Hpa II predigestion of DNA followed by polymerase chain reaction of the highly polymorphic CAG repeat of the androgen receptor (AR) gene. The frequency of skewed X inactivation was similar in the X-linked myotubular myopathy carriers (22%) and in 235 controls (18%). Three overtly affected carriers had skewed X inactivation with the mutated X as the predominantly active X in at least two of them. Four females with mild symptoms had random X inactivation. The unaffected X-linked myotubular myopathy carriers had either skewed X inactivation in favour of expression from the normal X or random X-inactivation. Thus, there was a tendency for females with a more severe phenotype to have a skewed pattern of X inactivation, while females with an intermediate phenotype had a random pattern of X-inactivation.

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1. Introduction

X-linked myotubular myopathy (XLMTM) is a rare congenital muscle disorder caused by mutations in the MTM1 gene on Xq28 [1]. Most affected boys die within the first year of life because of respiratory failure [2–4]. Female carriers are usually asymptomatic or have only mild weakness [2,4–6], and inheritance has thus been regarded as X-linked recessive. However, females with severe manifestations of the disease have been reported [7–11]. One of the two X chromosomes in female mammalian cells is inactivated in early embryonic life. Females are therefore mosaic for two different cell types, cells with the paternally inherited X chromosome as the active X, and cells with the maternally inherited X chromosome as the active X. The distribution of the two cell types normally approximates 50:50. Any marked deviation (≠ 80:20) from this distribution is called skewed X inactivation. Older females have a higher frequency of skewed X inactivation in blood cells, probably due to a selection process [12,13].

Phenotypic variation in carriers of X-linked disorders has been attributed to a variable pattern of X-chromosome inactivation, and females affected with XLMTM have been reported showing skewed X-inactivation patterns [7,8,10,11]. In order to further investigate the relationship between X-inactivation patterns and phenotypes in carriers of XLMTM, we analysed the X-inactivation patterns in a
population of XLMTM carriers, non-carriers from the same families and controls.

2. Material and methods

2.1. Subjects

The series consisted of 18 XLMTM families with 43 carriers identified by mutation detection, or known by family history to be obligate carriers, and 15 non-carriers. One affected female carrier was aged 7 years. The remaining carriers were 16–71 years of age (median 37 years). Fifteen families (36 carriers) were ascertained after identification of a male patient, and three families (seven carriers) were ascertained through an affected female. Clinical information was available for 20 of the carriers (Table 1). Seven females were characterised as unaffected by history, six as unaffected by clinical examination, four as mildly affected (no history of symptomatic muscle weakness but mild weakness on examination in one or a few muscle groups, or a history of easy fatigability). Three females from three different families were overtly affected (definite subjective and objective muscle weakness for which the patient had sought medical attention) and more extensive details have been published previously [8,9,11]. The controls consisted of 235 blood donors of the same age group.

2.2. DNA isolation

Blood was obtained for carrier testing after informed consent and DNA was extracted from peripheral blood cells according to a standard procedure, using the automated phenol extraction method (Nucleic Acid Extractor 340A, Applied Biosystems) or using QIAGEN Maxi DNA extraction kit (QIAGEN).

2.3. X-inactivation analysis

X-inactivation pattern was determined by PCR of the highly polymorphic CAG repeat in the first exon of the androgen receptor (AR) gene [14]. Methylation of HpaII sites in close proximity of this repeat correlates with X inactivation. After digestion with the methylation sensitive enzyme HpaII, a PCR product is obtained from the inactive X chromosome only. PCR products from both digested and undigested DNA were separated on an ABI 373 automated sequencer, and analysed by GeneScan software (Applied Biosystems). The X-inactivation patterns were classified as random (ratios 50:50–<80:20) or skewed (≥80:20). For XLMTM carriers with skewed X inactivation, the direction of skewing could be distinguished in carriers who had inherited the mutation from their mother. In these carriers, the maternal X is carrying the mutation while the paternal X is carrying the wild-type gene.

2.4. Statistical methods

The Pearson chi-squared test was used for testing categorical variables. The Fisher two-tailed exact test was used where appropriate. P values of less than 0.05 were taken as indicating statistical significance.

3. Results

The results of X-inactivation analysis in the carriers and controls are summarised in Table 1. There was no difference in the frequency of skewed X inactivation in carrier females and controls. Two overtly affected carriers were excluded from this analysis, since the families were ascertained through these affected females. Of the carriers, therefore, nine out of 41 (22%) had a skewed X inactivation as did 42 out of 235 (18%) of the controls. The non-carriers had similar X-inactivation patterns to those of the controls.

If there is a relationship between the XLMTM carrier phenotype and X-inactivation pattern, a tendency to more skewed X-inactivation patterns would be expected in carriers with more extreme phenotypes, i.e. in overtly affected carriers or in completely asymptomatic carriers. Similarly, a tendency to a more random pattern was expected in carriers with an intermediate phenotype, i.e. carriers with only slight symptoms. Such a tendency was found in our series, since none of the four mildly affected females had skewed X inactivation, whereas seven out of 16 (44%) of the overtly affected and unaffected females had skewed X-inactivation patterns. This difference, however, was not significant (P = 0.25).

The direction of skewing was known for two of the

<table>
<thead>
<tr>
<th>X inactivation pattern</th>
<th>Carriers (%)</th>
<th>Carriers with clinical information</th>
<th>Non-carriers (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unaffected (%)</td>
<td>Mildly affected (%)</td>
<td>Overly affected (%)</td>
</tr>
<tr>
<td>Random (50:50– &lt; 80:20)</td>
<td>32 (74)</td>
<td>9 (69)</td>
<td>4 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Skewed (≥80:20)</td>
<td>11 (26)</td>
<td>4 (31)</td>
<td>0 (0)</td>
<td>3 (100) [8,9,11]</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>13</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>
overtly affected females with skewed X-inactivation patterns. They had inherited the mutation from their mother as well as having the maternal, disease-carrying X chromosome, as the preferentially active X [8,9]. One of these patients had in another laboratory previously shown to have a random pattern (60:40) [9] but had a mean ratio of 83:17 in four repeated assays of the same sample in the present study. The direction of skewing was not known for the third overtly affected female [11].

One of the unaffected carriers with skewing was the mother of one of the affected females with skewing [8]. In this female, in contrast to that seen in her affected daughter, the direction of skewing was favourable, since the mutated gene was most probably on the inactive X chromosome. This was also the case for the two unaffected sisters with skewed X inactivation. They had inherited the mutation from their mother but had the paternally inherited X chromosome as the preferentially active X. The direction of skewing was not known for the other unaffected carrier with a skewed pattern.

4. Discussion

Skewed X inactivation may occur due to chance (few cells at the time of X inactivation), to genetic factors influencing the X inactivation process, or to a selection process [15,16]. Carriers of several severe X-linked disorders, such as Wiskott–Aldrich syndrome, the ATR-X syndrome, some of the X-linked immunodeficiency syndromes and Barth syndrome have skewed X inactivation patterns [17–20], presumably as a result of selection against cells with the mutated gene on the active X [21]. In such disorders, female carriers will have a completely normal phenotype. Over expression of the mutated gene may cause a lethal phenotype, which may explain why affected carriers with an unfavourably skewed X inactivation have not been reported.

In many other serious X-linked disorders, including Duchenne muscular dystrophy and haemophilia, affected females have been reported with an unfavourably skewed X inactivation, with the mutated gene on the predominantly active X chromosome [22,23]. In these disorders, it is expected that no selection process takes place and that a skewed pattern occurs by chance, and is in favour either of the mutated or the normal X. Thus, under these conditions, a difference between the X inactivation pattern of carriers and controls is not expected. In the present study, no such difference was found between carriers of MTM1 mutations and controls, which is in agreement with absence of a selection against cells expressing the X chromosome carrying the mutated MTM1 gene.

All three overtly affected females included in this study had skewed X inactivation. Furthermore, two of them showed skewing in favour of activity of the mutated X [8,9]. The direction of skewing was not known for the third overtly affected female [11]. We also found a random X inactivation pattern in the four carriers with mild symptoms, and skewed or random patterns in the unaffected females. For three of the four carriers with skewing and a normal phenotype, the direction of skewing was known, and was in a favourable direction.

Our findings are therefore in agreement with the proposed correlation between the X inactivation pattern and phenotype. The correlation was not significant. However, this could be due to the small number of carriers of this very rare disorder with clinical information available. However, the report of a female with XLMTM and random X inactivation both in blood and muscle DNA indicates a complex relationship between X inactivation and phenotype [10]. This has been observed in carriers of Duchenne muscular dystrophy and haemophilia also. Both skewed and random patterns were found in muscle DNA in five symptomatic carriers of Duchenne muscular dystrophy [22], and no relationship was found between the X inactivation pattern in blood and phenotype in proven carriers of haemophilia [24].

Skewed X inactivation may be due to genetic factors [15,25], and Mendelian inheritance of skewed X inactivation has been reported in families without known X-linked disorders [26,27]. Families with X-linked disorders have also been reported who have been ascertained through an affected female, where other females, both carriers and non-carriers, also had a skewed X inactivation [23,28]. This is in agreement with the findings in our study where a pair of unaffected carrier sisters both showed evidence of favourable skewed X inactivation. Furthermore, the finding of an opposite skewing pattern in an unaffected mother and her affected daughter, both of whom carry the same MTM1 mutation, supports the existence of genetic factors influencing X inactivation patterns.

X inactivation occurs at different times in different tissues, and X inactivation patterns are therefore expected to vary between tissues [29,30]. We studied X inactivation in blood. It is possible that a more appropriate tissue, such as muscle cells, would reveal a closer correlation between X inactivation and phenotype in carriers of XLMTM.

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References