Analysis of CGG variation through 642 meioses in Fragile X families

M.Rifé1,3, C.Badenas1,4, Ll.Quintó2, E.Puigoriol2, B.Tazón1, L.Rodriguez-Revenga1,3, L.Jiménez7, A.Sánchez1,3 and M.Mila1,3,5

1Servei de Genètica, Centre de Diagnòstic Biomèdic, Hospital Clínic, Barcelona, 2Servei d’Epidemiologia, Hospital Clínic, Barcelona, 3IDIBAPS (Institut d’Investigacions Biomèdiques August Pi i Sunyer), Barcelona and 4Fundació Clínic per a la Recerca Biomèdica, Barcelona, Spain

Fragile X syndrome is the commonest familial form of inherited mental retardation. The molecular defect is an expansion of the CGG trinucleotide repeats in the 5’ untranslated region of the FMR1 gene that is inherited in an unstable fashion in fragile X families. In an attempt to provide more information about the CGG tract intergenerational variation, we have evaluated 642 transmissions in 175 Fragile X families. PCR and Southern blot (StB12.3) was used to analyse the CGG number. Among premutated alleles, 90.2% showed expansion, two-thirds to a full mutation while the rest remained in the premutation range, 5.5% of alleles did not vary and finally 4.3% of them reduced in size. Premutated females showed an increased risk of expansion to the full mutation depending on the CGG tract. The estimated risk for 80 triplets is more than seven times that of a woman carrying 59 CGG, the risk being 100% for alleles of >100 repeats. Fifty-nine repeats was the smallest allele that expanded to full mutation. Contractions were detected more frequently in males than in females, being statistically significant. This study contributes to the literature by increasing the data available regarding transmissions in Fragile X families and it allows us to perform more precise genetic counselling for women with the CGG repeat in the premutation range.

Key words: CGG transmission/contraction/expansion/FMR1/Fragile X syndrome

Introduction

Fragile X syndrome (FXS) is the commonest familial form of mental retardation. The inheritance pattern of this syndrome is unique due to the dynamic mutation that causes 98% of Fragile X cases. This mutation consists of an expansion of the CGG trinucleotide repeat in the 5’-untranslated region of the FMR1 gene that is inherited in an unstable fashion in fragile X families (Fu et al., 1991). In the general population, individuals carry 6–52 repeats and the triplet number is usually stably transmitted. Individuals with alleles between 53 and 200 CGG repeats are called premutated carriers. This range of repeats is unstable through transmission to the next generation and they tend to expand. Affected individuals carry alleles with >200 repeats (full mutation) that are generally hypermethylated, together with the surrounding region that includes the CpG island. This hypermethylation causes the block of the FMR1 gene transcription, leading to the absence of the Fragile X mental retardation 1 protein (FMRP) in affected individuals (Pieretti et al., 1991). Moreover, there is a fourth category called ‘grey zone’ (alleles between 45–55 CGG repeats). These alleles, named intermediate, are occasionally unstable through transmission and they are potential precursors of a premutation in subsequent generations.

Since the discovery of the FMR1 gene, the general mechanism by which the unstable region is transmitted to the next generation has been established: full mutation expansion from premutated alleles is only acquired via maternal meiosis, with an increased risk for the larger premutation-sized alleles. On the other hand, paternal transmissions always remain in the premutated range, from both premutated and full-mutated fathers. Although the molecular basis of the FXS is understood, the causes of the triplet expansion, the different behaviour of CGG repeat ranges and other questions related to the expansion mechanism remain unanswered.

In an attempt to provide more information about the CGG tract intergenerational variation, we have evaluated 642 transmissions in 175 FXS-unrelated families.

Materials and methods

Subjects

We selected 175 unrelated FXS families from the Genetics Service of the Hospital Clinic of Barcelona, in which CGG transmission could be evaluated. In total, we studied 642 CGG transmissions from 264 progenitors carrying the following FXS genotypes: 14 normal transmitting males (NTM, males carrying premutation), two high functioning males (HFM, these are males carrying full mutations but with the CpG island and the CGG repeats unmethylated, resulting in a normal FMR1 gene function), 205 premutated females and 43 full-mutated females.

Additionally, 100 mother–son/daughter or father–daughter transmissions from non-FXS mentally retarded individuals were also studied.

Materials and methods

DNA was isolated from peripheral blood or from chorionic villi samples in prenatal cases. Molecular analysis of the FMR1 CGG repeat region was...
performed by PCR and Southern blotting. PCR amplification was carried out using primers \( f \) and \( c \) previously described by Fu et al., (1991). Radiolabelled PCR products were separated on a 5% denaturing acrylamide/urea gel. Premutation alleles of \(<100\) repeats are accurately sized using this methodology. Double restriction digestion with \( \text{EcoRI} \) and \( \text{EagI} \) and Southern blotting with probe \( Sbh12.3 \) was used to estimate the size of alleles of \( >100 \) repeats (larger premutations and full mutations). Allele sizes were estimated using the Sequaid II\textsuperscript{TM} version 3.81 program.

All data presented in this study have been performed and evaluated in our centre and following the same procedures, avoiding possible inter-centre variations.

**Statistical analysis**

Summary measures of all variables are reported in terms of percentage for categorical and medians for continuous variables. Logistic regression models are used for the estimation of the odds ratio for contraction or full mutation. Multivariate models are calculated using a forward stepwise method using \( P = 0.05 \) as an enter criterion and a \( P = 0.1 \) as a remove criterion. All comparisons are made using a significance level of 5%, and 95% confidence intervals are calculated for all estimations. Software for analysis is STATA (StataCorp. 1999; Stata Statistical Software: Release 7.0. College Station, USA).

**Results**

From the 642 transmissions analysed, 244 individuals inherited the normal allele (38%) and 398 the FRAXA allele (62%). Among the premutated alleles, 359 (90.2%) showed expansion, two-thirds to a full mutation while the rest remained in the premutation range, 22 (5.5%) did not vary and 17 (4.3%) reduced in size.

All male transmissions (24 from 14 NTM and four from two HFM) remained at the premutation range in their offspring, as expected. Most transmissions from NTM expanded but we also found four contractions and three alleles that did not change their size through transmission. Two HFM had two descendants each with a premutation (Figure 1a).

Premutated female descendants inherited both premutated and full-mutated alleles (Table I and Figure 1b). There was an increased risk of expansion to full mutation showing a positive correlation with the size of CGG tract, being 100% for alleles of \( >100 \) repeats. The evaluation of these transmissions allowed us to calculate the risk of instability in our population (summarized in Table I). The estimated risk for expansion to full mutation of \( >80 \) triplets is more than seven times that of a woman carrying 59 CGG.

The sex of the parent also appears to be a determining factor in contractions. Contractions were detected more frequently in males than in females, 16.7% (4/24) in males and 1.6% (6/374) in females, this being statistically significant.

If we take into account the sex of the offspring, there are statistical differences between postnatal but not prenatal samples. In postnatal samples there is an excess of affected males (202 males and 68 females), but not in the prenatal samples (16 males and 13 females). These results reaffirm that expansion does not depend on the sex of the offspring.

Regarding the 100 transmissions studied from MR (Mentally retarded) patients, but non-FXS families, 91 parents carried alleles from 19 to 40 repeats and nine parents between 41–50 repeats. In contrast with the FXS alleles, 99 normal alleles were inherited in

**Figure 1.** (a) Expansions, no changes and contractions [from premutated and high frequency males (HFM)] in CGG repeat length through male transmissions. (b) Expansions, no changes, contractions and deletions (affecting the CGG tract) through female transmissions.
Table I. Risk of CGG expansion for Fragile X carrier females

<table>
<thead>
<tr>
<th>CGG repeat size (mother)</th>
<th>Full mutation</th>
<th>Total %</th>
<th>Risk of expansion</th>
<th>(95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53–59</td>
<td>10</td>
<td>2</td>
<td>16.6</td>
<td>1</td>
</tr>
<tr>
<td>60–69</td>
<td>26</td>
<td>7</td>
<td>21.2</td>
<td>1.35 (0.24; 7.61)*</td>
</tr>
<tr>
<td>70–79</td>
<td>30</td>
<td>21</td>
<td>41.1</td>
<td>3.50 (0.69; 17.64)*</td>
</tr>
<tr>
<td>80–89</td>
<td>31</td>
<td>45</td>
<td>59.2</td>
<td>7.26 (1.49; 35.44)</td>
</tr>
<tr>
<td>90–99</td>
<td>1</td>
<td>25</td>
<td>96.1</td>
<td>125.0 (10.16; 1538.07)</td>
</tr>
<tr>
<td>100–109</td>
<td>0</td>
<td>21</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>110–119</td>
<td>–</td>
<td>23</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>120–129</td>
<td>–</td>
<td>8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>130–139</td>
<td>–</td>
<td>13</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>140–149</td>
<td>–</td>
<td>12</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>150–159</td>
<td>–</td>
<td>7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>160–169</td>
<td>–</td>
<td>14</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>170–179</td>
<td>–</td>
<td>2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>180–189</td>
<td>–</td>
<td>3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>190–199</td>
<td>–</td>
<td>3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>&gt;200</td>
<td>–</td>
<td>66</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

The risk of expansion considers the first range (53–59) as a unit.

*Not significant.

a stable manner and only one was unstably transmitted belonging to a female with 50 CGG repeats, which increased to 52 in the next generation.

During the revision we have detected three individuals carrying deletions involving the CGG repeat region and three families in which both parents were carriers of the premutation.

Discussion

Although the unusual inheritance pattern of FXS is now well understood, there is little information about the factors that influence the instability of the CGG tract. The gender of the parent carrying an expanded repeat (maternal imprinting), the number of repeats (dynamic mutation) or the absence of AGG interruptions in long tracts of CGG repeats have been described as the main factors related to this instability.

A bias towards inheriting the mutated allele (62%) compared to non-FXS alleles (38%) is detected in the 642 transmissions evaluated. Nevertheless, it is due to distorted pedigree analysis in which some phenotypically normal family descendants are only occasionally analysed as previously reported by other authors (Nolin et al., 1995; Ashley-Koch et al., 1998).

As expected, all male transmissions analysed in our study are inherited in the premutation range even the two HFM’s descendants. Here we report four more cases in which a male carrying a full mutation only transmits the premutation.

On the other hand, the instability of premutated alleles in females is correlated with the repeat size. The risk of expansion to full mutation increases with the repeat size, being 100% for alleles of >100 repeats. The study of FXS female allele transmissions has allowed us to calculate the risk of instability in our population. The results are displayed in Table I, and they are in agreement with those previously reported by other authors (Fu et al., 1991; Yu et al., 1992; Snow et al., 1993; Nolin et al., 1996, 2003; Ashley-Koch et al., 1998). However, the risk of expansion in our population for the alleles ranging from 60 to 79 is slightly higher than in other populations and lower for alleles from 70 to 89 CGG repeats (Nolin et al., 1996). The minimum premutated allele that expanded to full mutation was of 59 repeats.

These data allow us to provide accurate genetic counselling. Although we always advise prenatal diagnosis to all female carriers, we inform them that women carrying alleles between 53 and 59 repeats have a very low probability of expansion to full mutation.

The offspring gender has not been identified as an influencing factor for inheriting the full mutation. Our results seem to reflect that sex of the offspring could play a role in the inheritance of the expansion as in other previous reports (Rousseau et al., 1994; Loesch et al., 1995). However, this bias is due to the phenotypic selection of family members for diagnosis, as usually only samples from affected members reach Genetics Services. Indeed, in results from prenatal diagnosis (in which no previous phenotypic selection is made) no influence of descendant’s sex in CGG repeat expansion is seen, in agreement with prenatal results from Nolin et al. (2003).

It is well known that contractions are not frequent in FXS alleles. These contractions are not influenced by the offspring’s gender, but there is positive evidence implicating the progenitor’s sex, being lower in females than in males ($P \leq 0.001$) (avoiding HFM transmissions). In our results, both male and female contractions occur within the 70–99 CGG premutation range, while smaller and larger alleles do not show contractions. On the other hand, deletions involving CGG tract are detected in the full mutation range. These data are not in agreement with that reported by Nolin et al. (1996), who observed a positive correlation between paternal premutations and increasing repeat size in the next generation.

Alleles remaining the same size are transmitted both from fathers and mothers. Paternally transmitted alleles without size variation correspond to the 80–89 CGG range (Figure 1a), while maternally transmitted ones are mostly represented in the 53–69 ranges and also in the 70–89 ranges (Figure 1b). We do not have any explanation for the restricted range in males; probably it is a random event more than a structural role of the allele for this CGG range. This effect could be due to the small number of samples analysed.

Despite the low number of non-FXS transmissions analysed, there is evidence of allele stability among non-FXS families. Only one instability was detected (50–52 CGG), but this allele could also be considered to belong to intermediate alleles.

Among 175 unrelated FXS families analysed, some remarkable cases have been detected. First of all, three deletions involving the CGG repeat region; all of them are detected in mosaicism with a full
mutation. Two of them have been previously reported and correspond to affected males (Mila` et al., 1996a). The last one is a large deletion spanning 347 bp detected in a prenatal diagnosis. The deletion affects the CGG tract and the Chi-element, so it was considered an affected fetus. These deletions together with contractions show that slippage is not an infrequent phenomenon within the CGG repeat region of the FMR1 gene.

Moreover, in three families both parents were carriers of a premutation (1.7%). In the offspring of one of them there was one mentally retarded female carrying pre- and full-mutated alleles (Mila` et al., 1996b). In the other two families there were two sisters with no mental impairment carrying two premutated alleles. In spite of the negative genetic counselling, one of the sisters, carrying alleles with 60 and 90 CGG, had a NTM son with an allele of 90 repeats.

This study contributes to the data available regarding transmissions in FXS families and it allows us to perform more precise genetic counselling for women with the CGG repeat in the premutation range.

Acknowledgements
We would like to thank the Fragile X families and the ‘Associació Catalana Síndrome X fràgil’. This work was supported by SAF 2003 00897 (IP-MMR), GIRMGEN (V2003REDG 03–98) and REDGEN (V2003REDC 07) financed by the ‘Fondo de Investigación Sanitaria’.

References


Submitted on June 1, 2004; accepted on July 24, 2004