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ARTICLE

Systematic analysis of X-inactivation in 19 XLMR families: extremely skewed profiles in carriers in three families

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It has been demonstrated in several X-linked disorders, both with and without mental retardation, that the X-inactivation process plays a significant role in the expression of X-linked diseases in females. Moreover, in some disorders extremely skewed inactivation of the X chromosome is constant in carriers, and this is thought to result from a proliferation or a survival advantage for cells expressing the normal allele at this locus over cells expressing the mutated allele. X-linked mental retardation (XLMR) is heterogeneous, and cloning and characterization of the mutated genes are in progress. XLMR can be expressed in carrier females but often with milder manifestations. We report the systematic study of the X-inactivation profile of obligate carriers and other females in 19 multiplex XLMR pedigrees, using leucocyte-extracted DNA. Extremely skewed profiles were observed in carriers in three of 19 families. *European Journal of Human Genetics* (2000) **8**, 253–258.

Keywords: skewed X-inactivation; XLMR carriers; mental retardation

Introduction

XLMR disorders include specific conditions (MRXS), which are characterised by consistent neuromuscular, metabolic or physical abnormalities, and non-specific conditions (MRX) in which mental retardation (MR) is the only trait.[1] XLMR accounts for the excess of males in mentally deficient individuals[2] but some carrier females are also affected in MRX and MRXS families.[1,3] There is strong evidence that the penetrance of mental retardation in carrier females may be related to the randomness or skewing of X-inactivation in critical tissues[4] but systematic evaluation of X-inactivation profiles has not so far been reported in XLMR families.

X-inactivation profiles have been evaluated in several X-linked disorders with or without mental retardation in order to understand the female phenotypes (reviewed by Belmont[5]). We report the systematic study of the X-inactivation profile of obligate carriers and other females in 19 multiplex XLMR pedigrees. This study could explain the phenotypes in females and provide information about the mutated gene expression and normal gene function compared with other X-linked conditions.

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Materials and methods

Clinical and linkage analysis and X-chromosome inactivation profile study were performed on blood leukocyte-extracted DNA in 143 females from 19 large XLMR multiplex families (at least two affected males born of two female-related siblings). All females in each family, whether obligate carriers (50 females) or of unknown status, were investigated for X-inactivation when DNA was available. Lymphoblastoid cell line-extracted DNA was not used for the X-inactivation PCR assays because the cultured cell population may not reflect the *in vivo* cell population. X-chromosome inactivation analysis was performed using two PCR assays: *Hpa*II digestion and either androgen-receptor (*AR*) gene polymorphic trinucleotide[6] of FMR 1 variable CGG repeat.[7] The density of the bands was quantitated visually and by image analysis (software package Bioprofil, Vilber Lourmat system: Vilber Lourmat, Marne-La-Vallée, France). In some cases,

Table 1 Clinical and linkage findings on the 19 XLMR families

RC Family	Description	NA	NC	NF	Zmax/theta=0 at marker	Flanking recombinant markers	Ref
T49 1624	MRX 60. Extreme variability of MR severity. Mutation 1578 del in the oligophrenin 1 gene	5	3	10	3.01 at AR (Xq12)	OTC–DXS 981 Xp21.1–Xp12	10 (family 4); 11
T3 15901	Severe MR, spastic paraplegia, seizures, lethal in a few months	4	4	6	2.36 at DXS1003 (Xp11.3), DXS991 (Xp11.21), DXS1275 (Xq12) and DXS56 (Xq13.3)	MAOB–DXS3 Xp11.3–Xq21.33	
T4 20127	Non-specific MR, seizures	5	2	3	1.2 from DXS1224 (Xp22.31) to DXS1237 (Xp21.1)	DXS996–5′DYSII Xp22.32–Xp21.1	
/ 18540	Non-specific MR	2	2	3	mapping in progress		
T6 5199	Severe MR, refractory seizures, muscular hypodevelopment	5	5	8	2.9 at DXS1052 and DXS451 (Xq22.13)	DXS7163–DXS1238 Xp22.13–Xp21.2	10 family 1); 9
T50 24688	MR, obesity, dental weakness	3	1	5	1.33 at DXS1213 (Xq11.2), DXS986 (Xq21.1), DXS3 (Xq21.33) and DXS458 (Xq21.33)	DXS426–DXS1106 Xp11.3–Xq22.2	
T9 5721	MRX 16. Variable severity in affected males	8	4	15	5.43 at DXS1108 (Xq28)	DXS1113–tel Xq28–Xqter	10 (family 5); 12
T12 19086	Non-specific MR. One affected female	5	1	3	1.67 at DXS3 (Xq21.33)	DXS1002–DXS1106 Xq21.2–Xq22.2	
T13 14029	MRX 62. DNA available for only four affected males	6	1	7	2.23 at DXS178 (Xq22.1) and DXS1001 (Xq24)	DXS1002–DXS737 Xq21.2–Xq25	
T4 19147	Non-specific MR	3	2	6	1.2 at DXS426 (Xp11.3), AR1 (Xq12), DXS3 (Xq21.33) and DXS8088 (Xq23)	MAOB–DXS425 Xp11.3–Xq25	
T15 14292	Non-specific mild to moderate MR	5	2	4	1.93 at DXS556 (Xp11.4) and AlaS2 (Xq11.21)	DXS164–DXS441 Xp21.1–Xq13.2	
/ 27414	Non-specific severe MR	4	1	4	mapping in progress		
T16 13671	Non-specific MR. Figure 2	6	4	17	2.74 at DXS993, DXS8085, DXS8054 (Xp11.4)	DXS8025–DXS1003 Xp11.3–Xp11.4	10 (family 3)
T18 22192	MRX 66	6	3	10	2.36 at COL4A5 (Xq22.3), DXS456 (Xq22.3)	DXS458–DXS424 Xq21.33–Xq23	
T40 18529	Non-specific MR. Figure 3	4	2	7	1.51 at MAOA (Xp11.4), AR1 (Xq12) and DXS424 (Xq23)	DXS556-DXS1001 Xp11.4–Xq24	
T22 20113	MRX 63. Figure 1. One affected female	4	4	11	2.14 at DXS1001 (Xq24)	DXS990–DXS1227 Xq21.33–Xq27.1	
T19 19254	MRGH. Short stature, microcephaly, particular facial traits Growth hormone deficiency. Heterozygote manifestations	8	5	9			8
T34 11391	MR, coloboma, microphthalmia	3	2	10	1.11 at DXS297 (Xq27.3)	DXS8028–DXS998 Xq27.3–Xq27.3	
/ 5781	Occipital horn syndrome (MIM 304150) C2055T transition in exon 8 of the ATP7A gene	5	2	5			13

RC=Reference in the XLMR Consortium; NA=No. affected subjects; NC=No. obligate carriers and/or affected females studied; NF=No. females studied

allelic fragments differed by one repeat and the additional bands that resulted from DNA strand slippage during amplification made it impossible to analyse the relative intensity (NA) of each fragment. In others the microsatellite was not informative (NI). After systematic exclusion of FRAXA, FRAXE and ATRX syndromes linkage analysis was performed as previously reported.[8,9]

Results

Clinical and linkage information on each of the 19 families is schematically set out in Table 1. Linkage to the other X chromosome regions was excluded. Three MRX families of the 19 XLMR families showed extremely skewed inactivation profiles (85% : 15%–100% : 0%) in obligate carriers, a pattern that was found only in three of 32 female controls analysable for the AR assay, and the maternally-inherited X chromosome was always the inactive one. The three pedigrees are shown in Figures 1, 2 and 3. Clinical and neuropsychological data will be reported elsewhere. Mild to moderate mental retardation was not associated with any biochemical, morphological or neurological abnormalities. A single female was retarded in one family (III2 in Figure 1). Clinical and linkage data are summarized in Table 1 and comparison with haplotypes in the localisation area can be seen with pedigrees in Figures 1, 2 and 3.

The X-inactivation profiles in the remaining 16/19 XLMR families were random or moderately skewed. Inactivation studies of two of the 16 families have previously been reported, along with the clinical and linkage findings.[8,9]

Discussion

As explained by Martinez *et al*,[14] the probability of extremely skewed X-inactivation occurring by chance in four, five or six females in the same family for the chromosome associated with the disease is very weak. X-autosome translocation and large deletions were excluded by karyotype for the three families. Two main explanations for extremely skewed X-inactivation therefore remain to be discussed. First, skewed

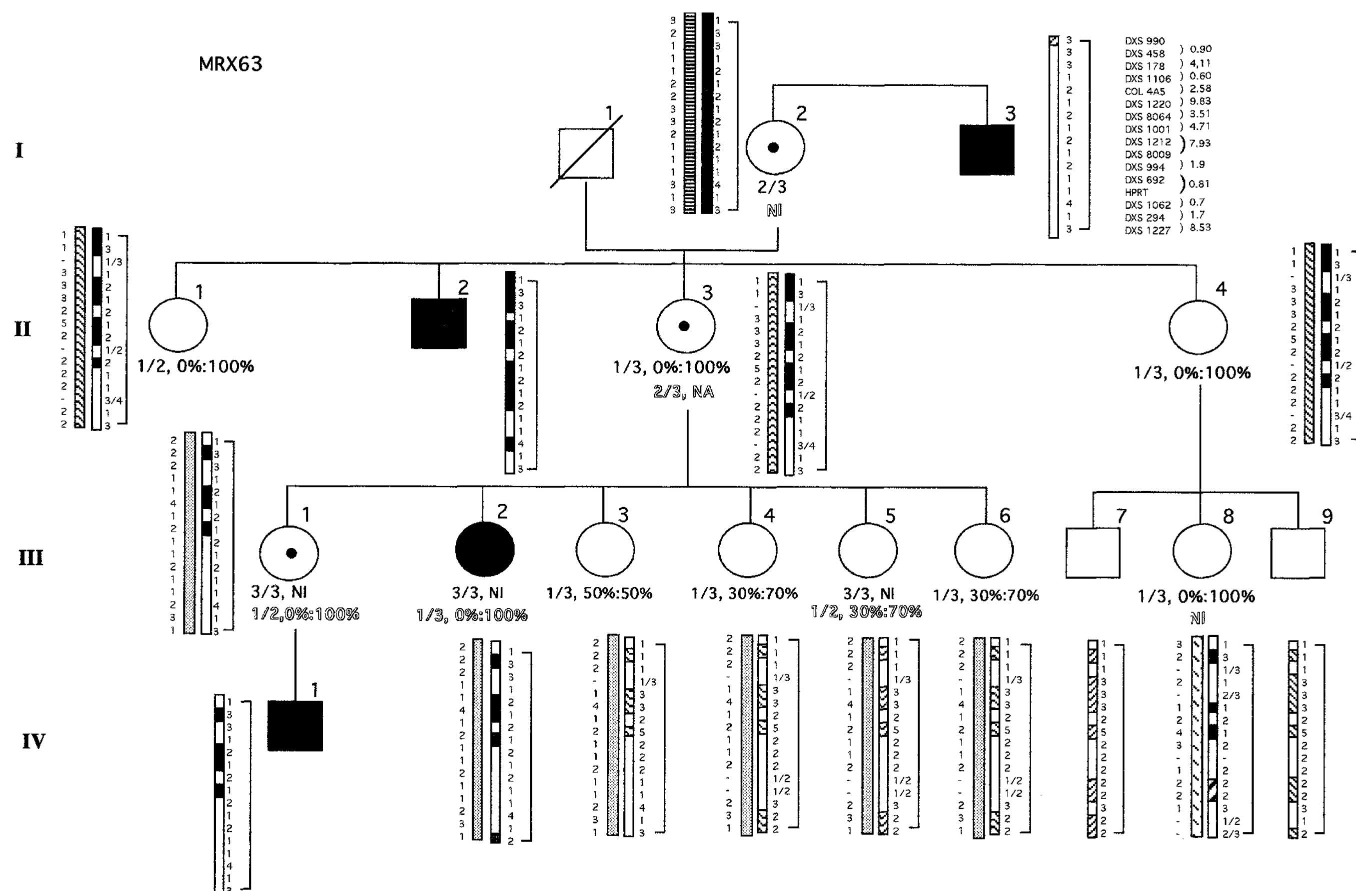

Figure 1 Family pedigree and haplotypes for the polymorphic markers corresponding to the linkage region. Black symbols denote affected subjects. Standard nomenclature is used for all symbols. Bold type: AR/HpaII analysis; outline type: FMRI/HpaII analysis; NI: not informative; NA: not analysable (allelic fragments differing by only one repeat). Alleles are numbered arbitrarily in each family. Numbers are followed by the proportions of density in each allele. The localisation area between the flanking recombinant markers is showed by a line. Genetic distances (GDB 1998) are behind the markers.

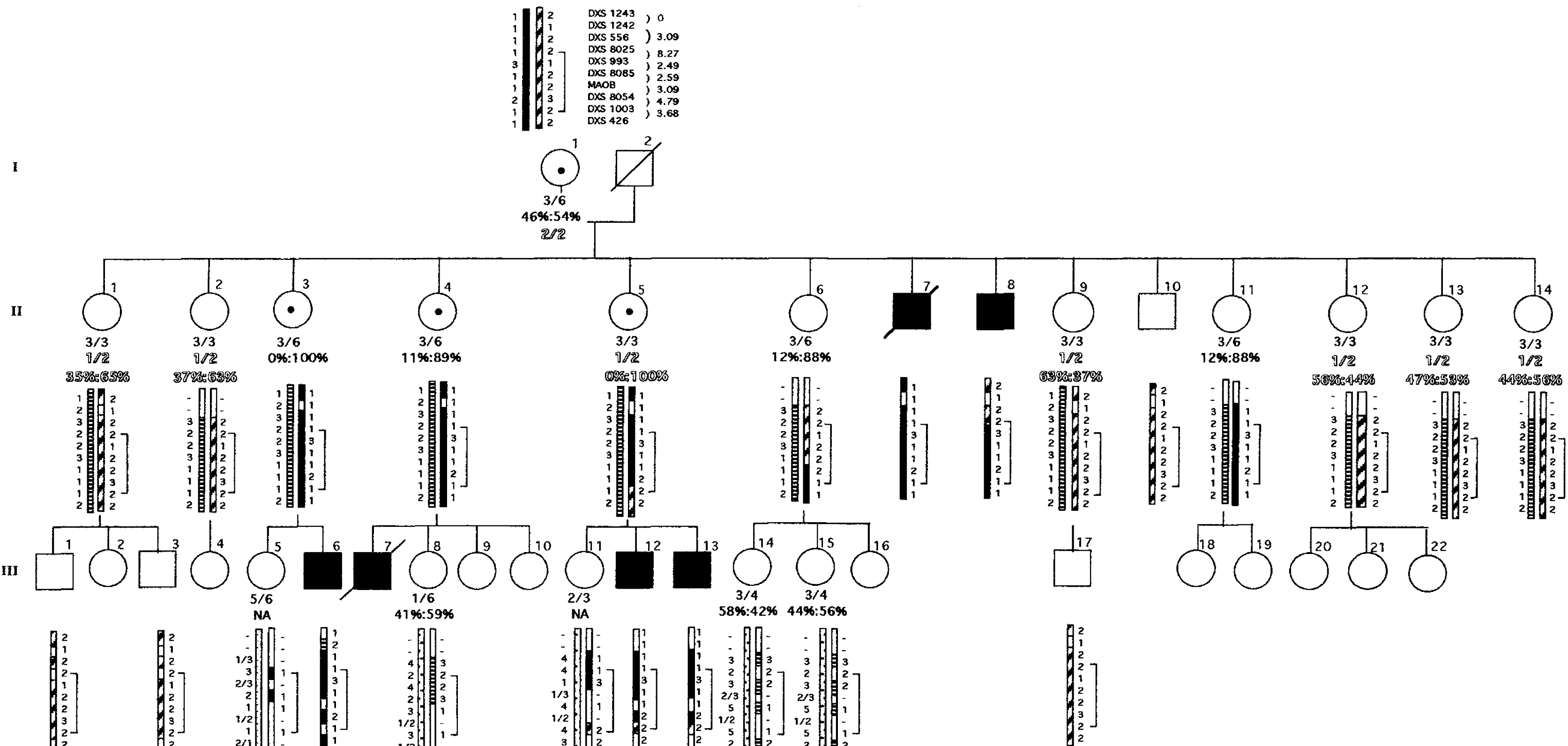

Figure 2 Family pedigree and haplotypes for the polymorphic markers corresponding to the linkage region. Black symbols denote affected subjects. Standard nomenclature is used for all symbols. Bold type: AR/HpaII analysis; outline type: FMRI/HpaII analysis; NI: not informative; NA: not analysable (allelic fragments differing by only one repeat). Alleles are numbered arbitrarily in each family. Numbers are followed by the proportions of density in each allele. The localisation area between the flanking recombinant markers is showed by a line. Genetic distances (GDB 1998) are behind the markers.

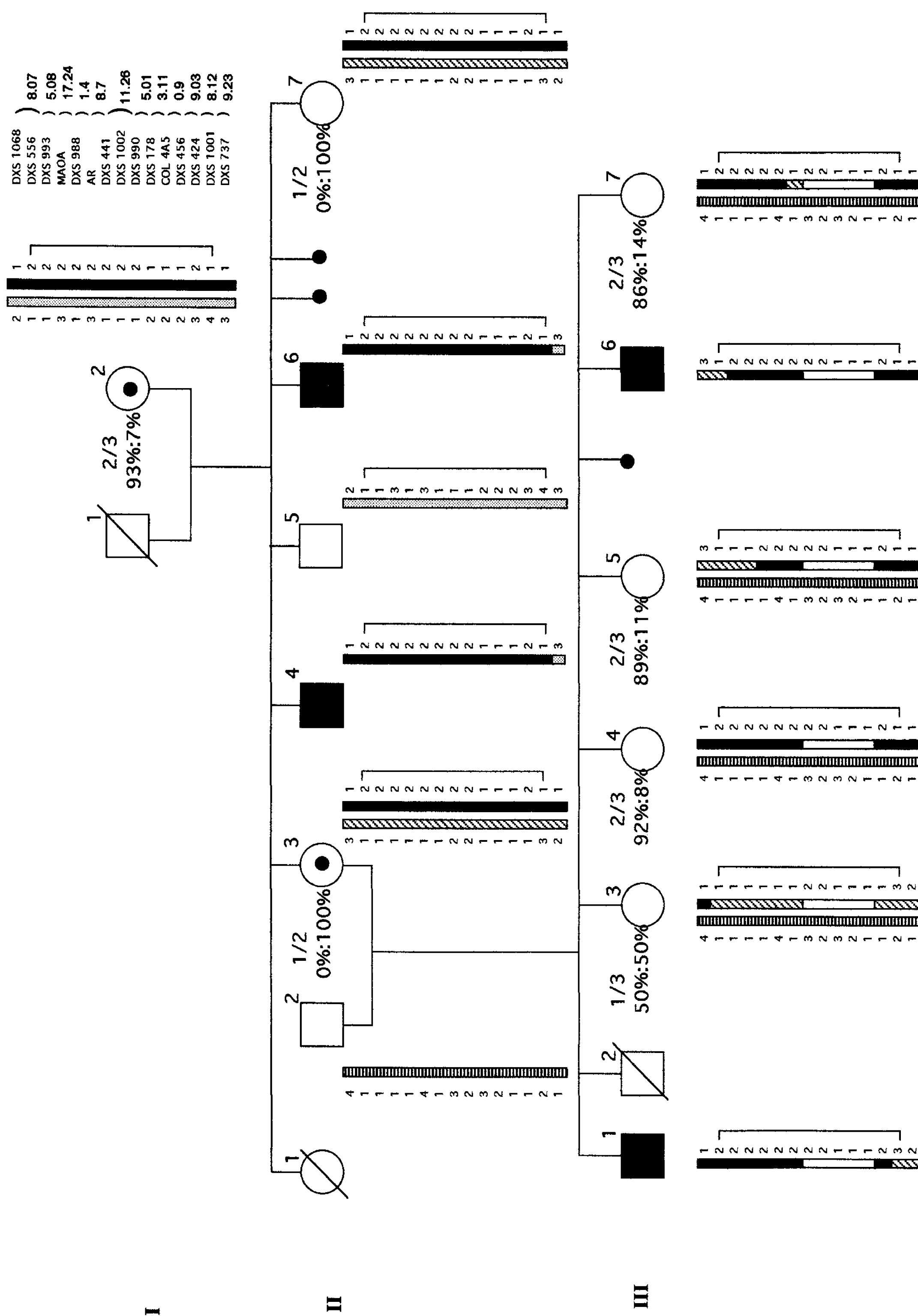

Figure 3 Family pedigree and haplotypes for the polymorphic markers corresponding to the linkage region. Black symbols denote affected subjects. Standard nomenclature is used for all symbols. Bold type: AR/HpaII analysis; outline type: FMRI/HpaII analysis; NI: not informative; NA: not analysable (allelic fragments differing by only one repeat). Alleles are numbered arbitrarily in each family. Numbers are followed by the proportions of density in each allele. The localisation area between the flanking recombinant markers is showed by a line. Genetic distances (GDB 1998) are behind the markers.

X-inactivation may be a familial trait reflecting inheritance of a genetic factor that controls the randomness or non-randomness of X-inactivation. Alternatively, skewed inactivation may reflect the segregation of a defect influencing cell survival or proliferation (the effect may be limited to leucocyte cells). The precise cosegregation of the skewed X-inactivation profile in leukocytes and the MRX carrier trait in the three families reported here makes the second explanation more likely. The suspected defect that gives a proliferative or survival advantage to cells might be the disease gene itself or, less likely, a contiguous gene that might have been removed by a submicroscopic deletion.

A random pattern of X-chromosome inactivation was observed for the oldest carrier female in one of our three families reported above (I1 in Figure 2, 66 years old). Conflicting results have been reported about the stability of X-inactivation in females > 60 years old. Further studies should clarify this. Identification of carriers among potential female carriers in X-linked disorders is not always possible, even when the mutated gene has been mapped by linkage analysis, because of the possible occurrence of recombinations in the localisation area itself (eg recombination in DXS692 for female III8 in Figure 1 and recombination in MAOB for II6 in Figure 2). The X-inactivation profile can be taken into account in evaluating the carrier risk for these females, as in ATRX syndrome.[15] Moreover, taking into account the linkage data from females with known status, with a calculated probability from their X-inactivation profile, may reduce the localisation area to the region that they share with affected males and carrier females in the family. For the family in Figure 1, the localisation area would be DXS990–DXS692 (Xq21.33–Xq26.1) instead of DXS990–DXS1227 (Xq21.33–Xq27.1). For the family in Figure 2 it would be MAOB–DXS1003 (Xp11.3–Xp11.4) instead of DXS8025–DXS1003 (Xp11.3–Xp11.4).

The skewed X-inactivation in leucocyte DNA provided evidence that the gene co-segregating with mental retardation in these three families (the *MR* gene itself or the contiguous gene) is expressed in leukocytes as a cell population.

In the 16/19 XMLR families with random or moderately skewed profiles, with possible or constant expressing carriers, the X-inactivation profiles in leukocytes were not correlated with phenotypes, as observed by Des Portes *et al*[3] in MRX48. Moreover, despite extremely skewed inactivation in leukocyte cells, like the other obligate carriers from family MRX63, III2 was affected. The X-inactivation profile may be different in some critical tissue and this may explain the unexpected phenotypes. Another explanation for the phenotype differences in females that are not correlated with the X-inactivation profile is the genetic background and the influence of the other genes in each individual. Variable severity is often observed in males in XLMR families.

Lastly, why a disorder that is not very severe in males and limited to mental retardation, as observed in our three families with extremely skewed X-inactivation in females, is responsible for a selection mechanism in female cells remains to be understood.

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