Genetic localisation of mental retardation with spastic diplegia to the pericentromeric region of the X chromosome: X inactivation in female carriers

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Abstract

We report on two brothers and one maternal cousin with severe mental retardation, microcephaly, short stature, cryptorchidism, and spastic diplegia. The patients were born to normal and non-consanguineous parents. All other members of the family, almost exclusively females, were clinically normal, suggesting X linked inheritance. By multipoint linkage analysis with markers spanning the whole X chromosome, we have tentatively assigned the underlying genetic defect to Xp11.4-q21, achieving a maximum lod score of 1.3. This localisation overlaps MRXS3, a syndromic form of mental retardation resembling that found in the family described here, although with a milder presentation. We discuss the possibility that both phenotypes might be allelic variants of the same gene localised in the pericentromeric region of the X chromosome.

Analysis of the X inactivation pattern in one potential and three obligate carrier females showed non-random inactivation of the allele linked to the disease. This finding may be interpreted as: (1) a negative selection effect on cells bearing the mutation on the active X chromosome; (2) both the disease causing gene and the X inactivation centre are simultaneously affected by the same alteration, a deletion for instance; or (3) the skewed inactivation is the consequence of an independent event randomly associated with the disease. In any case, the observation of consistent X inactivation supports X linkage of the disease.

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At least 105 X linked disorders have been described in which mental retardation appears as either the primary characteristic or a major component.¹ X linked mental retardation (XLMR) is subdivided into syndromal and non-specific depending on the presence or absence of a characteristic pattern of biochemical, morphological, or neurological anomalies. Gene mapping of these conditions can be performed in large families by linkage analysis. Occasionally, it is also feasible in smaller fami-

lies by using an adequate number of DNA markers situated along the X chromosome, but only when all the disease associated markers are found in a single region.

Here we report on a family segregating severe mental retardation, microcephaly, short stature, cryptorchidism, and spastic diplegia. This condition was tentatively localised following DNA typing of a set of markers spanning the X chromosome.

Materials and methods

CLINICAL FINDINGS

The family pedigree is shown in fig 1. The family includes three male patients in two different kinships, two brothers and a cousin. The family was referred for genetic counselling.

Case 1 (IV.2)

This 14 year old boy is the second child of a healthy, non-consanguineous couple. He was born after an uneventful pregnancy. The mother was delivered in a private clinic and no details of the delivery or perinatal period are available. The family reported abnormal movements of the head, "similar to hiccups", in the first days of life. These "hiccup movements" ceased spontaneously after a few days.

The patient currently shows severe motor and mental retardation. He is able to crawl but not to sit, stand, walk, or speak. He just emits guttural sounds and needs assistance with feeding. He has never developed seizures.

Examination showed length 121 cm (below the 3rd centile), head circumference 49 cm (below the 2nd centile), epicanthus, slender fingers, cryptorchidism, severe hypertonia of the lower limbs, adducted hips, pes equinovarus, and osteotendinous hyperreflexia. MRI and all the laboratory tests performed, such as haemoglobin, copper, caeruloplasmin, and uric acid, were normal, as were cytogenetic and molecular testing for fragile X.

Case 2 (IV.3)

This patient, aged 11 years, is a brother of case 1. No details of the pregnancy or delivery are available as in the brother's case, though no relevant perinatal events were reported by the family except for the same jerking of the head. For this reason, he was admitted to hospital, where only growth delay was diagnosed. All the studies performed, including computed tomography, amino acids in blood and urine, and karyotype were normal.

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Figure 1 Pedigree of the family segregating MRX and diplegia. Black symbols indicate the affected members. The extended haplotypes corresponding to the 15 markers analysed in each family member are shown below. Note that the only region shared by the three patients and their maternal grandmother is also the only region shared by the four females with non-random X chromosome inactivation.

Subsequently, severe motor and mental retardation became evident. He can sit and crawl, but he is unable to walk or speak, except for yells. No seizures have been reported, apart from the above mentioned jerking.

When examined at the age of 11 years, his height was 105 cm (<3rd centile) and head circumference 45 cm (<2nd centile); he had

hypertelorism with epicanthus, antimongoloid eyes with convergent strabismus, low set ears, highly arched palate, and cryptorchidism. He has a tendency to put his fingers into his mouth without self-destructive biting, as well as to paroxysmal bursts of laughter. Spasticity affects the four limbs, predominantly the lower ones.

Figure 2 Multipoint linkage results for the disease locus versus the polymorphic markers. Calculations were performed as described in the text (see Materials and methods). The positions of the markers in the genetic map and their chromosomal localisations are shown below.

Figure 3 X chromosome inactivation assay based on amplification of the polymorphic marker ARA after digestion of the DNA sample with *HpaII* (indicated with an H at the top), and compared with amplification of the same marker after digestion with *RsaI* (indicated with an R) as control.

Case 3 (IV4)

Case 3 is the maternal cousin of cases 1 and 2. He showed the same head jerks as the above cases in the first two days of life, and subsequently manifested severe global developmental retardation.

When examined at the age of 9 years, he was barely able to sit or crawl. He cannot walk or speak except for guttural sounds. Computerised tomography of the brain detected generalised corticosubcortical atrophy. On EEG, reported as normal, only a lack of structure of basal activity was evident, without a clear epileptic pattern. Serum levels of lactic acid, pyruvic acid, and ammonium were normal, as were amino acids and immunoglobulins. Examination of the retina was also normal.

On examination, he shows short stature (103 cm, below the 3rd centile), head circumference 47 cm (below the 2nd centile), no facial dysmorphism except for a thin upper lip, slender fingers, cryptorchidism, and spasticity of the lower limbs more pronounced than in the upper limbs. Like case 2, he tends to laugh for no apparent reason.

DNA ANALYSIS

Genomic DNA was isolated from white blood cells by standard methods. The microsatellite markers (di- or trinucleotide repeats) used in this study were: DXS999, DXS1275, DXS1196, DXS1231, DXS1220, and DXS1192 from Génethon,² DXS237,³ DMD/STR-49,⁴ MAO-A,⁵ SYN/ARAF,⁶ ARA in the androgen receptor,⁷ DXS456 and DXS425,⁸ and p39 at the DXS15 locus.⁹

After PCR amplification following the protocol described for each marker, only slightly modified when necessary, the microsatellites

were run on native polyacrylamide gels and silver stained.¹⁰

Analysis of X chromosome inactivation was performed using PCR analysis of the microsatellite in the first exon of the androgen receptor gene after digestion of the DNA samples with the methylation sensitive enzyme *HpaII* or with *RsaI* as a control for relative intensities of the bands.¹¹ Densitometric analysis of the bands was performed by image analysis (Intelligent Quantifier 2.1 from BI Systems Corporation; Gelstation system from TDI). The intensity ratio of methylated alleles (obtained after *HpaII* digestion) was corrected for the amplification effect by multiplying by the inverse of the intensity ratio in the *RsaI* control lane.

LINKAGE ANALYSIS

The lod scores for linkage between the putative X linked locus in this family and the genetic markers were calculated by six point analysis of the disease locus against different fixed maps of five markers, using the program FASTLINK version 3.0P.^{12,13} The genetic map of the X chromosome (shown in fig 2, taking DXS237 as reference) has been taken from Fain *et al.*¹⁴

Results

After establishment of haplotypes (fig 1), it became evident that both distal short and long arms of the X chromosome present in the three patients were of grandpaternal origin. It was thus possible to eliminate these regions from consideration since the grandfather was clinically unaffected. The possibility of germinal mosaicism in this grandfather was considered very unlikely a priori, especially taking into account that both mothers share most of their maternal chromosome. Thus, the closest informative markers exhibiting at least one recombinant event with the disease were DXS1068 on the short arm and DXS1196 on the long arm. These markers limit the interval possibly containing the MRX locus to Xp11.4-q21. The fact that an unaffected maternal cousin shares the haplotype for most markers in this interval, except for one microsatellite intragenic to the Menkes gene, points to Xq12-q21 as the most likely localisation of the locus, although a de novo mutation in grandmother II.5 cannot be ruled out.

The multipoint linkage analysis was also performed with the 15 informative markers. The resulting lod scores are shown in fig 2. Owing to the limitations of the program, it was not possible to analyse all 15 markers in a single run. However, several six point analyses (the disease locus plus five markers) were run in such a way that each interval analysed was flanked by fully informative markers. In this way, a maximum lod of 1.3 was reached for the pericentromeric markers, resulting in negative values for any other possible localisation.

All the females tested showed a non-random X inactivation pattern, with more than 95% methylation of one allele (fig 3), a pattern not found in 25 normal females previously analysed (data not shown). Moreover, the inactive allele was the same in the four females, and is

the allele associated with the disease since it was also shared by the three patients.

Discussion

The maximum lod score of 1.3 is low, but is the highest figure achievable with this family and, in addition, the lod scores corresponding to all the other chromosomal regions were as much as two units lower. If X linked inheritance is assumed for this condition, linkage to the pericentromeric markers of the X chromosome can be considered significant. The possibility that this condition is autosomal is very low given the absence of family antecedents with the disease and of consanguinity. In addition, taking into consideration that (1) the three patients are males, (2) all of them share the grandmaternal haplotype, and (3) there is an absence of affected patients in generations II and III, where females are predominant, an X linked condition is clearly most probable.

As estimated elsewhere,¹⁵ only 10% of women have skewing of X inactivation >90%. So, the probability of the extremely skewed X inactivation occurring by chance in the four females for the chromosome associated with the disease is $<10^{-5}$ ($0.1^4 \times 0.5^4$). Non-random X chromosome inactivation, once a normal karyotype has been determined, is usually detected under three circumstances: negative selection of X chromosomes bearing single gene mutations which affect cell survival or growth; positive selection; or primary skewed X inactivation.¹⁶ Positive selection for such skewing can be readily discarded since the common X chromosome in the three generations is inactive. In addition, it is important to note the physical proximity of the localised region of the syndrome to the X inactivation centre (XIC) in Xq13.2. The primary inactivation skewing appears to be rather uncommon and apparently not always linked to XIC,¹⁵ the only X chromosomal region shared by the four females in this family. We consider that the two possibilities which most easily explain the skewing pattern are: (1) a primary cis acting inactivation associated with the disease causing mutation; (2) a negative selection effect of the mutation, which affects the survival of cells bearing the mutation in the active X chromosome. In any case, the skewed inactivation would suggest additional evidence for X linked inheritance of the present syndrome.

Additional evidence in favour of linkage of the disease to the pericentromeric region is that the syndrome in this family is similar to that described by Sutherland *et al.*,¹⁷ localised to the same interval in Xp21.1-Xq22.¹⁸ The syndrome described by Sutherland *et al.*¹⁷ consists of mental retardation, microcephaly, short stature, small testes, spastic diplegia, and possible intrauterine growth retardation. The phenotype is clearly less severe and more variable than the one described here: the intellectual status ranges from normal with dysarthria to moderate, including one borderline case and another mildly retarded. The spastic diplegia

also varies from absent to moderate, being mild for most of the cases without affected upper limbs. It is worth noting, however, that the lower limbs are more severely affected in the family described here. Despite this variation in expression, clinical similarity is evident and there is no evidence for locus heterogeneity since the severe and mild forms apparently map to the same region. Variable expression between families can be accounted for by allelic heterogeneity. It is thus reasonable to assume that the same gene might be altered in both families to a different degree. This would be a similar situation to that found for the gene encoding the neuronal cell adhesion molecule L1, where different mutations are responsible for distinct clinical entities, with another form of complicated X linked spastic diplegia being one of them.¹⁹

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