LETTER TO JMG

A new MRXS locus maps to the X chromosome pericentromeric region: a new syndrome or narrow definition of Sutherland-Haan genetic locus?

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Xlinked mental retardation (XLMR) is classically divided into syndromal (MRXS) and non-specific (MRX) forms, depending on the presence or absence of specific distinguishing clinical, morphological, neurological, or metabolic anomalies. In the last XLMR update¹ (<http://xlmr.interfree.it>), genetic loci have been assigned for 117 of 202 XLMR by linkage studies, of which only 33 genes have been cloned. From these data, several forms of both syndromal (MRXS) and non-specific mental retardation (MRX) remain without a gene(s) associated disease.¹ Moreover, several allelic disorders have been described for some individual cloned genes, reflecting the heterogeneous functional role of a single XLMR associated gene. For instance, molecular defects on the zinc finger/helicase *XNP/ATR-X* gene,^{2,3} the proteolipid protein (PLP),⁴ and neuronal cell adhesion molecule L1⁵ (MIM 308840) have been associated with distinct clinical entities. Recently, the *MECP2* gene (MIM 300005) responsible for Rett syndrome (MIM 312750) has been shown to account for about 2% of MRX.⁶

In recent years, cloning and molecular characterisation of XLMR syndromes have provided an increase in the number of these disorders, also as a result of the “lumping together” of allelic syndromes. From this viewpoint, the delineation of specific clinical characteristics accompanying a syndromal form of XLMR would allow the description of a sufficient number of subjects, leading to more accurate genetic counselling, and consequently the identification of the disease associated gene.

We report here the clinical findings and linkage analysis of a family with five mentally retarded males. Examination showed the pedigree to be consistent with an X linked recessive mode of inheritance. Affected males were characterised by a mild form of MR associated with microcephaly and spastic diplegia. All obligate female carriers exhibited a totally skewed pattern of X chromosome inactivation (>90:10).

X chromosome linkage analysis maps the gene responsible for this form of MR to Xp11-q12, a location which overlaps the genetic locus of various MRXS,¹ including Sutherland-Haan syndrome⁷ (SHS, MIM 309470), but outside the *XNP/ATR-X* gene responsible for the 0.0016 SHS-ATR-X syndrome allelic

form (from *Online Mendelian Inheritance in Man*, OMIM, <http://www.ncbi.nlm.nih.gov/OMIM>).

MATERIALS AND METHODS

Clinical findings

The family referred for genetic counselling includes five affected males in two different generations, two cousins (IV.2 and IV.4), and three uncles (III.3, III.6, and III.8). The family pedigree is shown in fig 1. Genetic consultation was requested for subject IV.4, as a result of which complete clinical data and DNA samples were sought only for cases III.3, IV.2, and IV.4. No anomalies were found in these three subjects in a 500 band level karyotype, plasma and urine amino and organic acid studies, and *FMR1* gene analysis for FRAXA syndrome. For subjects III.6 and III.8, clinical information was obtained during genetic counselling.

Case III.3

A 45 year old male was born at term by normal delivery after an uneventful pregnancy (birth weight 3400 g). Psychomotor development was delayed. He was first examined by us at the age of 39 years and had, on WAIS, a mild degree of MR with a mental age of around 11 years (IQ=65). He often shows peevish behaviour, does not work, and attends daily occupational therapy in a specialised centre.

Clinical examination showed short stature (158.9 cm, <3rd centile) (fig 2A), microcephaly (OFC 51 cm, <2nd centile), triangular face, open mandibular angle, prognathism, and malocclusion (fig 2B). The nasal septum was deviated. The joints at the hands, wrists, and elbows have limited extension. Camptodactyly was confirmed in the patient by x ray of both hands, which showed subchondrial bone cysts (fig 2C). Testicular volume was normal (20 ml). Patellar and cubital hyperreflexia was present. The EEG showed frontal intermittent repetitive delta activity (FIRDA). Brain CT scan showed hypoplasia of the cerebellar vermis. Weight was 61.2 kg (between the 25th and 50th centiles).

Case IV.2

This man was born at term after an uneventful pregnancy (birth weight 3000 g). Psychomotor development was delayed (first steps at 16 months and first words after 2 years). Microcephaly was diagnosed at 2 months. He came to our notice at the age of 20 years, showing a mild degree of mental retardation (IQ=60) following CPM of RAVEN. He has attained adequate self-sufficiency in daily life. He does not work, but participates in the social life of the parish.

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Abbreviations: MR, mental retardation; XLMR, X linked mental retardation; MRXS, syndromal X linked mental retardation; MRX, non-specific X linked mental retardation; SHS, Sutherland-Haan syndrome; AR androgen receptor; RENS1, Renpenning syndrome

Figure 1 Pedigree of the family. The haplotype for markers in Xp11-q12 is boxed. X inactivation ratio is indicated for each female tested. Affected males are shown by a filled square.

He had a microbrachycephalic skull (OFC 51.4 cm, <2nd centile, cephalic index 81.92) with a triangular face, prognathism, malocclusion, open mandibular angle, and anteverted ears with thickened helix. Brain MRI or CT scan were not performed. Patellar hyperreflexia was evident.

Case IV.4

He was born at 42 weeks of gestation. Birth weight was 2550 g. Neonatal history was characterised by mild jaundice and poor feeding. Psychomotor development was delayed. At 4 months, he already showed some degree of spasticity, and neuromotor rehabilitation was started. At 8 months, a testicle was surgically removed because of teratoma. He came to our attention at 11 years, showing a mild degree of MR on the WISC-R (IQ=50), with a mental age corresponding to 5 years (fig 3A, B). He shows hyperactive behaviour and attends a school for the intellectually handicapped.

Height and weight were between the 50th and 75th centile (145.8 cm and 37 kg, respectively). Clinical examination

showed microcephaly (OFC 49.2 cm, <2nd centile), sparse hair, one café au lait spot, and strabismus. The facial features were similar to the other two probands: triangular face, prognathism, malocclusion, open mandibular angle, and deviated nasal septum (fig 3C, D). Brain MRI showed slight dilatation of the cerebral lateral ventricles. Spastic diplegia is a permanent clinical feature.

DNA studies

Consent for DNA analysis was obtained from only 12 family members. DNA was extracted from 5 ml of peripheral blood using standard methods.

Inactivation pattern

To establish the pattern of X inactivation, the methylation pattern was assayed at the human androgen receptor (*AR*)⁸ and Mβ27 (DXS225) loci.⁹ Methylation studies were performed on DNA from five females, three of whom are mothers of index

Figure 2 Case III.3 (45 years old). (A) Front view. (B) Side view, note open mandibular angle. (C) Right hand.

cases (III.3, IV.2, and IV.4). For *AR* containing a CAG polymorphic repeat, 2 μ g of DNA were digested with 20 U of *Hha*I and *Hpa*II restriction enzymes and subsequently amplified with forward and reverse Humara primers, as previously described.⁸ The DXS225 locus was analysed following the protocol suggested by Fraser *et al.*⁹

The inactivation ratio for both loci was determined by densitometry measurement of relevant bands on radiosensitive film.

DNA linkage studies

Linkage analysis of the X chromosome was performed using 38 markers spanning the X chromosome with ranges below 10 cM. After PCR amplification, the amplicons generated were subjected to electrophoresis on a 6% denaturant polyacrylamide gel, then blotted onto a nylon membrane, and hybridised with γ -³²P labelled oligonucleotides. Resulting alleles were scored and genotypes generated were entered into the pedigree file of the LINKAGE computer program.¹⁰

Two point and multipoint analyses were performed using MLINK and LINKMAP software. Lod scores were generated assuming X linked recessive inheritance with complete penetrance in males. Three mutually exclusive classes of phenotype were established: (1) affected males, (2) carrier females with skewed X inactivation, and (3) phenotypically normal males and females with random X inactivation. Among normal females, the frequency of skewed X inactivation¹¹ was taken to be 0.1.

RESULTS

Clinical features

X linked recessive inheritance was established in a four generation family with five affected males, of whom two were reported during the recording of the family history (III.6 and III.8, fig 1). In the probands, mild mental retardation, microcephaly, deep tendon hyperreflexia, or spastic diplegia were the main clinical features. Facial characteristics were constant in all affected males, suggesting that this syndrome is characterised by a typical facial gestalt. Neither clinical history nor examination of the affected subjects showed signs of progression of symptoms, confirming that intellectual and neuromuscular impairment was a persistent feature of the syndrome.

The syndrome segregates in the family with an extremely skewed X chromosome inactivation pattern in all obligate carriers (fig 1), who show no other clinical manifestations.

Linkage study

We considered 12 subjects, indicated in fig 1, for linkage investigation. Two point linkage analysis of the entire X chromosome showed that the disease was linked to nine markers (fig 1): DXS8015, DXS993, *MAOA*, DXS8035, DXS8054, DXS1003, DXS1039, DXS991, and *AR*. A maximum lod score of 2.1 at theta (θ)=0 was obtained with all markers spanning the interval DXS993-DXS991. All markers were fully informative. No other loci yielded positive lod scores at θ =0.

From these data, the candidate region extending from DXS8015 to the androgen receptor (*AR*) mapping in Xq12 encompasses a large region of about 29 cM, corresponding to the cytogenetic localisation Xp11.2-Xq12.

DISCUSSION

Disease segregation in the pedigree under study was consistent with an X linked disease with recessive mode of inheritance. Linkage analysis showed a significant lod score of 2.1 at θ =0 in the pericentromeric region Xp11-q12. Unfortunately, not all members of the family collaborated in the DNA studies, lowering the significance of the test.

The associated gene spans a genetic interval of 29 cM in which numerous XLMR syndromes have been mapped, including seven MRXS forms, some of which are potentially allelic.¹ In the Xp11-q12 region, allelic forms have recently been hypothesised for some mapped syndromes, such as Sutherland-Haan syndrome (SHS)⁷ and Renpenning syndrome (RENS1).¹² These syndromal XLMRs share similar phenotypic features (microcephaly, short stature, testicular anomalies). However, males with SHS presented spastic diplegia, while neuromuscular abnormalities were not reported in patients with RENS1 described by Stevenson *et al.*¹² Carrier females show no abnormalities in either syndrome.

The question is whether the family reported here represents an allelic form of one of the most phenotypically similar syndromes already mapped in the Xp11-q12 chromosome region or is a candidate for a new XLMR gene. On the basis of the clinical findings, we excluded Xp11-q12 mapped XLMR syndromes because of major clinical differences from those observed in our patients and focused on SHS syndrome.

Figure 3 Case IV.4 (11 years old). (A) Front view. (B) Side view. (C) Front view of face. (D) Side view of face.

In this family, affected males show microcephaly, hyper-reflexia or spastic diplegia, mild mental retardation, and a similar facial appearance, all features being characteristic of SHS. Small testes were not present in affected males and short stature, which may be a feature of SHS, appears only in one of our cases. However, these discrepancies may not be significant, since to date only two families have been considered, through linkage analysis, to be affected by this syndrome. The first was described by Sutherland *et al*⁷ in 1988, in which linkage analysis was refined by Gedeon *et al*¹³ in 1996, and the second was reported by Martinez *et al*¹⁴ in 1998 as a possible allelic form of SHS. Linkage results reduce, at least in the distal part, the genetic interval in which the SHS gene candidate is located by more than 3 cM, between the AR and DXS1125 loci, assigning it to the cytogenetic interval Xp11-Xq12 (fig 4).

In the family described by Martinez *et al*,¹⁴ an ARG1742LYS missense mutation in the *XNP/ATR-X* gene mapping in Xq13

was later reported by Lossi *et al*.¹⁵ Molecular defects in this gene encoding for a zinc finger/helicase protein^{2,3} result in the ATR-X syndrome (MIM 301040). However, the gene is involved in a wide spectrum of MRXS (updated in OMIM database, MIM 300032). ATR-X and SHS syndrome share few clinical features. ATR-X is characterised by severe mental retardation, microcephaly, typical facial dysmorphism, an unusual form of α thalassaemia shown by the presence of Hb H inclusions in blood smears in a few cases, and generalised hypotonia in contrast to mild to moderate intellectual impairment and spastic paraplegia in SHS. However, female carriers in both syndromes show an extremely skewed X chromosome inactivation pattern.

We believe that SHS and ATR-X are not allelic syndromes as reported in the OMIM database. Actually, the probands of the family reported by Martinez *et al*¹⁴ have specific characteristics of the ATR-X syndrome, such as severe mental retardation and

Figure 4 Ideogram of Xp11-q12 showing the linkage limit for RENSI, SHS, and our XLMR family. The bold line on each genetic locus represents the positive lod score interval. X chromosome markers were assigned according to www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps.cgi?org=hvm&chrX.

the presence of Hb H inclusions. The presence of spastic paraplegia would then represent an expansion of the phenotypic spectrum of the *ATR-X* gene, probably the result of its "pleiotropic effect" on other genes involved in neurological development.

The *ATR-X* locus in our family was excluded in the first instance by the linkage data where the absence of a positive lod score excluded the *ATR-X* linked markers AR and DXS1275.¹⁵ However, in order to find any possible recombination events in the *ATR-X* gene, disease associated haplotype analysis, using five (CA)_n repeats,¹⁶ was conducted on all members of the family. The affected males segregate a different *ATR-X* gene haplotype, ruling out *ATR-X* gene anomalies as a possible molecular cause (data not shown). We suggest that SHS be considered as a specific syndrome whose molecular basis is as yet unknown.

In conclusion, the XLMR family reported here could represent the second true SHS pedigree described, allowing the redefinition of both the genetic locus and clinical features, including facial appearance. The assignment of a new pedigree to a rare syndrome is crucial for a more precise definition of the syndrome itself.

The biased X inactivation in carrier females could certainly be an important phenotypic clue for the identification of other similar XLMR families. However, because of the small number of normal females who agreed to DNA study, we are unable to confirm that the putative gene is directly involved in the X inactivation processes. However, the occurrence of an extremely skewed inactivation pattern and linked disease associated markers in all three obligate female carriers studied suggests that this feature is specific to the syndrome. Recently, Raynaud *et al*¹⁷ reported extremely skewed X inactivation in obligate carriers in three MRX families of the 19 XLMR families studied, concluding that the biased pattern of X inactivation in leucocytes may reflect the segregation of a MRX defect influencing cell survival or proliferation.

Linkage studies on other phenotypically similar XLMR families can help restrict the genetic locus and thus favour the cloning of the candidate gene locus. This is also true if our family represents a new XLMR syndrome, rather than an allelic form of SHS.

In silico analysis of the Xp12-q12 region shows that about 100 potential genes have been mapped (www.ncbi.nlm.nih.gov/genome/guida/HsChrX.shtml). The functional and phenotypic roles of only a small number of these are known. Even if some Xp12-q12 mapped genes could be involved in neurodevelopmental processes, a systematic search for mutations in these

genes would probably prove time consuming and unsuccessful. Chromosome region specific microarray technology, even with tissue specific limitations, surveying genome wide expression profiles in normal versus diseased states, may provide a useful tool for the study of mapped genes in large chromosomal regions, such as that mapped for SHS.

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