**European Journal of Human Genetics (1998) 6,** 201–206

http://www.stockton-press.co.uk/ejhg

ORIGINAL PAPER

# MEHMO (mental retardation, epileptic seizures, hypogonadism and -genitalism, microcephaly, obesity), a novel syndrome: assignment of disease locus to Xp21.1–p22.13

Rolf Steinmüller, Daniela Steinberger and Ulrich Müller

*Institut für Humangenetik der Justus-Liebig-Universität Giessen, Germany*

**A previously unrecognised X-chromosomal mental retardation syndrome is described. Clinical hallmarks are mental retardation, epileptic seizures, hypogonadism, and -genitalism, microcephaly and obesity. Life expectancy of patients is less than two years. Based on the major clinical symptoms this condition is referred to by the acronym MEHMO. Haplotype and two-point linkage analyses in a large three-generation family assign the disease locus to Xp21.1–p22.13, to a region that is flanked by CYBB and DXS365.**

**Keywords: X-linked mental retardation; epilepsy; hypogonadism; microcephaly; obesity; X-chromosome**

## Introduction

X-linked mental retardation (XLMR) can be divided into three main categories, non-specific forms, variants associated with chromosomal characteristics, and types that occur within a well defined clinical syndrome. Gene loci in non-specific mental retardation (MR) are designated as *MRX*.[1] To date more than 30 *MRX* loci have been described based on linkage with lod scores of $>2.0$ in different families.[2,3,4] The second category includes the fragile X (fraXA)[5] and the fraXE[6] syndromes. The third group comprises syndromic XLMR, ie MR syndromes with additional clinical features such as neurological and metabolic symptoms, auditory problems, and dysmorphological stigmata. Frequently, the various combinations have been described in one or in a few families only. Gene loci mapped in such families are given temporary *MRXS* locus numbers until a syndrome name is assigned. Well established XLMR syndromes are MASA (mental retardation, adducted thumbs, shuffling gait and aphasia),[7,8] and the mental retardation α-thalassemia[9] syndrome.

Here we describe another form of XLMR that is associated with a previously not recognised combination of clinical symptoms including epileptic seizures, hypogonadism and -genitalism, microcephaly, and obesity. Linkage and haplotype analyses in this syndrome assign the disease locus to Xp21.1–p22.13.

Correspondence: U Müller, Institut für Humangenetik, Schlangenzahl 14, D35392 Giessen, Germany. Tel: +49 641 99 41600; Fax: +49 641 99 41609; E-mail: ulrich.mueller@humangenetik.med.uni-giessen.de
Received 18 August 1997; revised 25 November 1997; accepted 9 December 1997

## Materials and Methods

### *DNA Analysis*

DNA was extracted from peripheral blood of most family members in Figure 1 according to standard procedure. From patient IV/7 formalin-fixed and paraffin-embedded portions of brain tissue were used. DNA was extracted essentially as

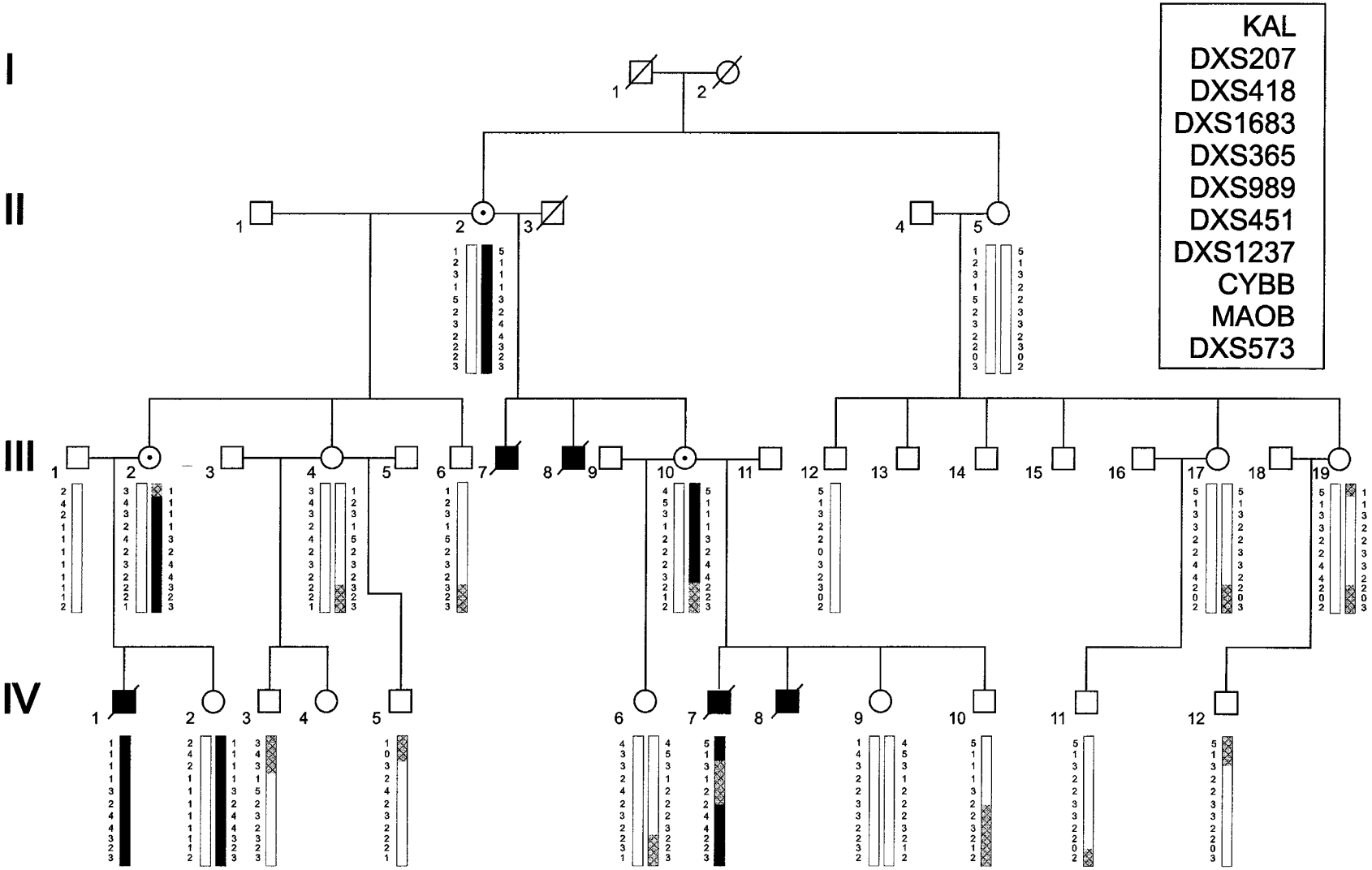


**Figure 1** *Haplotype analysis in the large family studied. Solid bars indicate the chromosomes segregating with the disease and hatched regions give recombinations. The haplotypes were derived by hand. Alternatively, the phase of alleles at CYBB in II/2 and II/5 could be inverted. This, however, does not change the conclusion regarding the critical interval of the disease locus.*

described.[10] In brief, paraffin-embedded tissue was scraped off with a sterile scalpel and paraffin was removed by xylene treatment. Subsequently, the tissue was washed in ethanol (twice in 100% and once in 70% ethanol) and air dried. The air-dried pellet was digested with proteinase K over night and DNA was purified by phenol and phenol/chloroform extraction.

DNA was amplified by the polymerase chain reaction (PCR) using primers flanking the various X-chromosomal short tandem repeat polymorphisms (STRPs) tested. STRPs at the following loci were analysed: DXS1283E, KAL, DXS207, DXS418, DXS365, DXS989, DXS451, DXS1683, DXS1237, DXS1238, 5'DMD, CYBB, MAOB, DXS337, DXS573, ALAS2, AR, DXS981, DXS135, DXS339, DXS106, DXS453, DXS7113, DXS7114, DXS7119, DXS227, DXS441, DXS3, DXS458, DXS454, DXS101, DXS424, CD40L, HPRTB, DXS102, DXS1113, DXS1108. These markers cover the X chromosome at intervals of less than 10 cM on average. The following markers were not informative in the family: DXS458, DXS454, DXS135 and DXS1108.

Each PCR reaction contained 30 ng genomic DNA (less when DNA extracted from paraffin-embedded tissue was used), 10 mM Tris, pH 8.3, 1.5 mM $MgCl_2$, 50 mKCl, 0.01% gelatin, 200 μM of each dGTP, dATP, dTTP, 2.5 μM dCTP, and 0.5 μCi $^{32}$P-dCTP, 10 ng of each primer and 0.15 U Taq polymerase in a final volume of 10 μl. All amplifications were done during 30 cycles (30 s denaturation at 94°C, 30 s annealing at the temperature described for each primer pair, 30 s elongation at 72°C), and were preceded by 3 min at 94°C and followed by a final extension at 72°C for 5 min. Amplification products were separated on a denaturing sequencing gel and autoradiographed.

### *Data Analysis*

Map distances between loci were estimated based on the data published in the Proceedings of the 6$^{th}$ International Workshop on X chromosome mapping and on previously published maps.[11, 12] Two-point linkage analysis was carried out using the programs MLINK and LINKAGE package version 5.2.[13] Penetrance was assumed to be complete but lod scores with the linked markers were also calculated at < 100% penetrance. The frequency of the mutant allele was set to $10^{-6}$.

## Results

### *Clinical Description*

The pedigree of the large family studied is given in Figure 1. It is consistent with X-linked recessive inheritance. Only males are affected and male to male transmission does not occur. Minor symptoms were not detected in unaffected males and there was no evidence of greatly variable expression (see below). Given that far more than 50% of the sons of carrier females are affected, penetrance appears to be complete.

Of the five affected males, only one was alive (IV/1) at the time of investigation. Patients III/7, III/8, and IV/7 had died at 7, 2, and 10 months old, respectively. IV/8 was diagnosed prenatally with microcephaly and the pregnancy was terminated at week 24. IV/1 was also diagnosed prenatally with microcephaly but at a later stage (week 31). Due to oligohydramnion and an abnormal stress test he was delivered by Caesarean section during the 32nd week. At birth his weight was 1420 g, length 40.5 cm, head circumference 26.5 (10th percentile). Postnatal development of the infant was greatly delayed. At 2 years (Figure 2) he was small for his age (74 cm, ie < third percentile), obese (10.5 kg, ie > 70th percentile) and non-ambulatory. He was severely mentally retarded and microcephalic (head circumference 37.7 cm, ie < third percentile) with a narrow forehead. His ears were poorly formed, the auricular lobuli were large. He had full cheeks, facial telangiectasias and downturned corners of the mouth. Both hands and feet were edematous, fingers tapered, there was a simian crease of the left hand, and he had bilateral talipes. He had cryptorchidism and a micropenis. Epileptic seizures were first noted at week 7. Both frequency and severity increased over time. Towards the end of his life at 2 he had permanent seizures refractory to treatment. Diabetes was diagnosed at age 6 months due to a ketoacidotic crisis. Abnormal laboratory results included increased levels of acylcarnithine and of saturated and unsaturated fatty acids but absolute values are not available.

Examination records of III/7 at age 5 months are available. He was described as short for his age, obese and microcephalic. He had several epileptic seizures, yet milder than those observed in IV/1. In addition, there was hypogonadism and hypogenitalism. The patient died at 7 months. The examination record of III/8 describes epileptic seizures, microcephaly, slight obesity, and hypogonadism and -genitalism. IV/7, at age 4 months, also had epileptic seizures, hypogenitalism, and dramatic developmental delay. The autopsy record at 10 months describes pronounced obesity, microcephaly ( < second percentile), a fatty liver, and a small thyroid. The cause of death could not be determined. No autopsy record is available of IV/8.

### *Linkage Mapping*

In order to delineate the disease locus on the X chromosome, linkage analysis was performed using short tandem repeat polymorphic (STRP) markers spanning the entire chromosome at intervals of 10 cM. Recombinations were observed among affecteds and between affecteds and unaffecteds with all informative STRP loci of Xq tested, and no evidence of linkage was obtained with any Xq locus. Lod scores were < –2 with all sufficiently informative Xq markers (not shown), and several recombinations were observed between

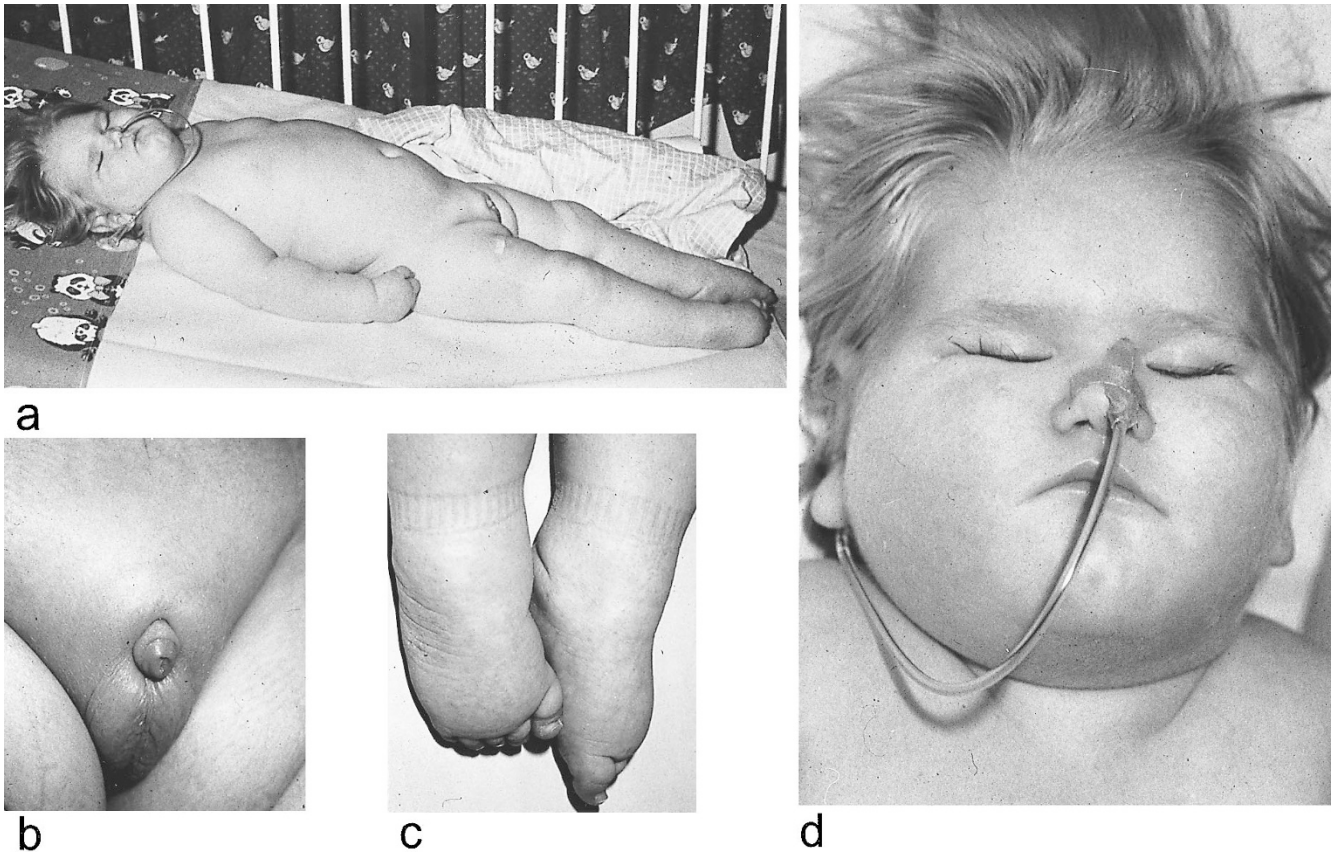


**Figure 2** *Patient IV/I at 2 years of age (***a***). Note hypogenitalism (***b***), talipes (***c***), fatty face, down-turned corners of mouth and microcephaly (***d***).*

these markers and the disease locus. Of the Xp markers studied, several were closely linked to the MEHMO locus. Maximum Lod scores ($Z_{max}$) of 2.3 at $\theta = 0$ were obtained with DXS451 and DXS1237. Locus DXS989 was less informative in the family and a $Z_{max}$ of 0.29 was found at $\theta = 0$. Recombinations with the disease locus were detected with the flanking loci DXS365, DXS1683, DXS418, DXS207, KAL (distal) and CYBB (proximal). The two-point lod scores obtained on the family with Xp markers are given in Table 1. Even

**Table 1** Two-point lod score analysis of MEHMO locus with DNA sequences from Xp

| | | | Z *values at* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Locus* | *Localisation* | *Primer pair* | *0.0* | *0.1* | *0.2* | *0.3* | *0.4* | $Z_{max}$ | $\theta_{max}$ |
| KAL | Xp22.32 | pHX82-2A/B | – | –0.76 | –0.25 | –0.04 | 0.026 | 0.028 | 0.42 |
| DXS207 | Xp22.2 | DXS207-A/B | – | 0.20 | 0.345 | 0.29 | 0.14 | 0.347 | 0.21 |
| DXS418 | Xp22.2 | DXS4218-A/B | – | 0.70 | 0.68 | 0.52 | 0.28 | 0.716 | 0.14 |
| DXS1683 | Xp22.13 | A0563ME5-5/6 | – | –1.09 | –0.48 | –0.19 | –0.04 | 0 | 0.5 |
| DXS365 | Xp22.13 | RX314-a/b | – | 0 | 0.29 | 0.31 | 0.2 | 0.32 | 0.26 |
| DXS989 | Xp22.13 | AFM135xe7-A/B | 0.29 | 0.20 | 0.13 | 0.06 | 0.02 | 0.29 | 0 |
| DXS451 | Xp22.13 | kQST80-A/B | 2.3 | 1.9 | 1.48 | 1.03 | 0.54 | 2.3 | 0 |
| DXS1237 | Xp21.3-p21.2 | DMD-45A/B | 2.3 | 1.9 | 1.48 | 1.03 | 0.54 | 2.3 | 0 |
| CYBB | Xp21.1 | CYBB A/B | – | –0.44 | –0.16 | –0.05 | –0.01 | 0 | 0.5 |
| MAOB | Xp11.4-p11.3 | MAOB1/2 | 0.31 | 0.34 | 0.32 | 0.25 | 0.15 | 0.34 | 0.11 |
| DXS573 | Xp11.3-p11.23 | pHX82-2A/B | 1.09 | 0.91 | 0.70 | 0.48 | 0.25 | 1.09 | 0 |
| DXS1199 | Xp11.22-p11.21 | AFM248wf9A/B | – | 0.40 | 0.40 | 0.29 | 0.14 | 0.43 | 0.15 |
| ALAS2 | Xp11.21 | AI7 A/B | – | –1.16 | –0.57 | –0.28 | –0.11 | 0 | 0.5 |

under the assumption of a reduced penetrance of 0.8 of the trait, significant lod scores of greater than $2^{14}$ were obtained with DXS451 and DXS1237.

Identical alleles were detected at loci DXS451 and DXS1237 in affecteds and in carriers only. Furthermore, different alleles were found in IV/1 and in IV/7 at DXS365 and identical alleles were observed in patient IV/1 and in the unaffected male IV/10. This marks DXS365 as the distal flanking marker of the MEHMO locus. Similarly, identical alleles in patient IV/1 and in his unaffected uncle III/6 identify CYBB as the proximal flanking marker.

## Discussion

The X-linked mental retardation syndrome with epileptic seizures, hypogonadism and -genitalism, microcephaly and obesity described here has not been recognised before. It is being referred to by the acronym MEHMO.

Although several XLMR syndromes have been assigned to the X chromosome short arm, they are clinically distinct from the present syndrome. Thus Partington syndrome (PRTS),[15, 16] MRXS1, has been assigned to a region of Xp (Xpter-p21) that overlaps with the MEHMO locus critical region. Similar to MEHMO, MR and seizures are characteristic of PRTS. However, PRTS differs dramatically from MEHMO by the concurrence of dystonia and ataxia and by a much longer life span of patients (up to 57 years, range 4–57). MRXS2, now referred to as Prieto syndrome, was also assigned to Xp (p11.22–p21.1). It is characterised by MR, dysmorphism and cerebral atrophy.[17] This syndrome differs from MEHMO by the nature of dysmorphic stigmata (abnormal growth of teeth, ear malformation, clinodactyly) and the much longer lifespan of patients. Additional X-linked mental retardation syndromes have been assigned to the pericentromeric region of the X chromosome thus placing the respective disease loci proximal to MEHMO. These syndromes include MRXS6, now Wilson-Turner syndrome[18] in Xp21.1–q22,[19] MRXS3, now Sutherland-Haan syndrome[20] in Xp21–q21.3,[21] and MRXA in Xp11.[22] Wilson-Turner syndrome is characterised by MR, obesity and gynecomastia, Sutherland-Haan syndrome by MR and spastic diplegia, and MRXA by concurring aphasia. Several symptoms of MEHMO such as epileptic seizures were not described in either of these syndromes. Furthermore, patients with Wilson-Turner syndrome, Sutherland-Haan syndrome, and MRXA live much longer than those suffering from MEHMO. They also display features not found in this syndrome such as spastic diplegia.

The underlying defect of MEHMO is at present unknown. It may be that it is a contiguous gene syndrome and caused by a submicroscopic deletion within the critical interval in Xp11.3–p22.13. Alternatively, MEHMO might be caused by a mutation in a single gene. Good candidate genes, however, have not yet been assigned to the critical region. In order to clarify the molecular basis of the MEHMO syndrome, STSs densely spaced within the critical interval need to be tested in patients. If these studies do not reveal evidence of a deletion and thus of a contiguous gene syndrome, ESTs in the region need to be analysed. The elucidation of the gene represented by a given EST will then facilitate mutation analyses in patients.

## Acknowledgements

We thank Niki Katsarou for technical assistance with the amplification of several STRPs. This work was supported by the Deutsche Forschungsgemeinschaft (Mu668/5-1).

## References

1 Mulley J, Kerr B, Stevenson R, Lubs HA: Nomenclature guidelines for X-linked mental retardation. *Am J Med Genet* 1992; **57**: 383–391.
2 Gedeon AK, Donnelly AJ, Mulley JC, Kerr B, Turner G: How many X-linked genes for non-specific mental retardation (MRX) are there? *Am J Med Genet* 1996a; **64**: 158–162.
3 Lubs HA, Schwartz RE, Stevenson RE, Arena JF: Study of X-linked mental retardation (XLMR): Summary of 61 families in the Miami/Greenwood study. *Am J Med Genet* 1996; **64**: 169–175.
4 OMIM 1997 http://www3.ncbi.nlm.nih.gov/omim.
5 Verkerk AJMH, Pieretti M, Sutcliffe JS *et al*: Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; **65**: 905–914.
6 Knight SJL, Flannery AV, Hirst MC *et al*: Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell* 1993; **74**: 127–134.
7 Jouet M, Rosenthal A, Armstrong G *et al*: X-linked spastic paraplegia (SPG1), MASA syndrome and X-linked hydrocephalus result from mutations in the L1 gene. *Nat Genet* 1994; **7**: 402–407.
8 Vits L, van Camp G, Coucke P *et al*: MASA syndrome is due to mutations in the neural cell adhesion gene L1CAM. *Nat Genet* 1994; **7**: 408–413.

9 Gibbons RJ, Picketts DJ, Villard L, Higgs DR: Mutations in a putative global transcriptional regulator cause X-linkel mental retardation with α-thalassemia (ATR-X syndrome). *Cell* 1995; **80**: 837–845.
10 Kösel S, Graeber MB: Use of neuropathological tissue for molecular genetic studies: Parameters affecting DNA extraction and polymerase chain reaction. *Acta Neuropathol* 1994; **88**: 19–25.
11 Dib C, Fauré S, Fizames C *et al*: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152–154.
12 Fain PR, Kort EN, Chance PF *et al*: A 2D crossover-based map of the human X chromosome as a model for map integration. *Nat Genet* 1995; **9**: 261–266.
13 Lathrop GM, Lalouel JM: Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 1984; **36**: 460–465.
14 Ott J: Analysis of Human Genetic Linkage. Johns Hopkins University Press, Baltimore and London, 1991.
15 Partington MW, Mulley JC, Sutherland GR, Hockey A, Thode A, Turner G: X-linked mental retardation with dystonic movements of the hands. *Am J Med Genet* 1988; **30**: 251–262.
16 Gedeon A, Partington M, Mulley J: X-linked mental retardation with dystonic movements of the hands (PRTS): Revisited. *Am J Med Genet* 1994; **51**: 565–568.
17 Watty A, Prieto F, Beneyto M, Neugebauer M, Gal A: Gene localization in a family with X-linked syndromal mental retardation (Prieto syndrome). *Am J Med Genet* 1991; **38**: 234–239.
18 Gedeon A, Mulley J, Turner G: Gene localisation for Wilson-Turner syndrome (WTS:MIM 309585). *Am J Med Genet* 1996c; **64**: 80–81.
19 Wilson M, Mulley J, Gedeon A, Robinson H, Turner G: New X-linked syndrome of mental retardation, gynecomastia, and obesity is linked to DXS255. *Am J Med Genet* 1991; **40**: 406–413.
20 Gedeon A, Mulley J, Haan E: Gene localisation for Sutherland-Haan syndrome (SHS:MIM 309470). *Am J Med Genet* 1996b; **64**: 78–79.
21 Sutherland GR, Gedeon AK, Haan EA, Woodroffe P, Mulley JC: Linkage studies with the gene for an X-linked syndrome of mental retardation, microcephaly and spastic paraplegia. *Am J Med Genet* 1988; **30**: 493–508.
22 Wilson GN, Richards CS, Katz K, Brookshire GS: Nonspecific X linked mental retardation with aphasia exhibiting genetic linkage to chromosomal region Xp11. *J Med Genet* 1992; **29**: 629–634.