LETTER TO JMG

A novel 2 bp deletion in the TM4SF2 gene is associated with MRX58

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X-linked mental retardation (XLMR) represents around 5% of all MR, with a prevalence of 1 in 600 males. Non-fragile X mental retardation was subdivided into syndromal and non-syndromal conditions by Neri et al. in 1991. The syndromal XLMR entities (MRXS) are those in which there is a specific pattern of physical, neurological, or metabolic abnormalities associated with the presence of mental retardation. Non-syndromic XLMR (MRX) are conditions in which a gene mutation causes mental retardation in the absence of other distinctive dysmorphic, metabolic, or neurological features. At present, XLMR conditions consist of 136 MRXS and 75 MRX entities. To date, 35 genes have been cloned. However, so far only nine non-syndromic XLMR genes have been identified: TM4SF2, FMR2, OPHN1, DGI1, PAK3, TM4SF2, RSK2, IL1RAPL1, ARHGEF6, and MECP2.

TM4SF2, a member of the transmembrane 4 superfamily, maps to Xp11.4 and is one of the genes associated with non-syndromic XLMR. Mutations in TM4SF2 have been previously described in two families (L28 and T15) with non-syndromic XLMR. As a part of our XLMR candidate gene testing, we have screened probands from 14 XLMR families (10 linked to Xp11 and four small families with no linkage data) for mutations in the TM4SF2 gene. Here we report a novel 2 bp deletion (564delGT), which segregates with mental retardation in the MRX58 family. The deletion causes a frameshift and a subsequent stop codon six amino acids downstream (stop codon 192). This finding supports the hypothesis that different XLMR conditions, especially non-syndromic XLMR, result from mutations in the same gene.

MATERIAL AND METHODS

Patients

Probands from 14 XLMR families (three MRXS and 11 MRX) were used in this study. The three linked MRXS families consisted of MRXS10 and two unpublished families. Of the seven linked MRX families, five families (MRX12, MRX18, MRX33, MRX56, MRX58) have been published previously, while two families are unpublished. The remaining four XLMR families were small and no linkage analysis had been done.

Incorporation PCR SSCP (IPS)

Genomic DNA was isolated from lymphocytes using a high salt procedure. IPS was performed using 50 ng of genomic DNA in a total volume of 10 μl containing 1 × PCR reaction buffer (10 mmol/l Tris, 50 mmol/l KCl, 1.5 mmol/l MgCl2, pH 8.3) with 1 μmol/l of each primer, 50 μmol/l of dNTPs, 0.05 μCi of α32P dCTP (3000 Ci/mmol, NEN Life Science), and 0.5 units of Taq DNA polymerase (Sigma). The information on the PCR primers and their optimal annealing conditions are listed in table 1. The primers were designed to be approximately 50 bp away from the exon/intron boundary to ensure detection of any alteration at the splice junction. Since the PCR products for exons 2 and 5 were greater than 310 bp, they were digested with HaeIII and PvuII respectively (table 1). Amplification was done in a PTC-200 thermocycler (MJ Research). Following PCR, 10 μl of the IPS loading dye (95% formamide, 10 mmol/l NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol) was added. The samples were denatured at 96°C for three minutes and loaded on a 0.5 × MDE gel (FMC) prepared in 0.6 × TBE. The gel was run at 8 W for 15–20 hours at room temperature. The gel was dried and the radioactive signal was visualised using the BIOMAX AM films (Eastman Kodak).

Sequence analysis

PCR products that showed an abnormally migrating pattern on IPS were sequenced directly using the fmol kit (Promega) according to the manufacturer’s protocol. Sequences were analysed using a 6% denaturing gel containing 6 mol/l urea. The gel was dried and the radioactive signal was visualised using the BIOMAX AM films (Eastman Kodak). The primers used for the PCR amplification were also used for sequencing.

Mutation analysis

The 564delGT in MRX58 created a BsrI site in the altered sequence. The forward and reverse primers for exon 5 were used to amplify the region spanning the deletion (table 1). The 371 bp of amplified fragment was then digested with BsrI (NEB) and the digestion products were run on an 8% acrylamide gel. Upon digestion, the normal sequence yielded fragments of 137 bp, 149 bp, and 85 bp, whereas the sequence carrying the deletion resulted in fragments of 137 bp, 95 bp, 52 bp, and 85 bp.

RESULTS

We have screened the seven exons of the TM4SF2 gene, using IPS combined with sequencing of the PCR product in 14 XLMR families. Two populations of IPS shifts were observed in exon 5, with the patient from MRX58 having a different shift from the other samples (indicated by arrow 1 in fig 1A). Sequencing of exon 5 from the MRX58 patient showed that he was carrying a 2 bp (GT) deletion at position 564 (564delGT, fig 1B). This deletion results in a frameshift giving rise to a stop codon six amino acids downstream (stop codon 192). The deletion falls in the second extracellular domain and results in a truncated protein missing the fourth transmembrane as well as the carboxy end of the gene. The deletion created a BsrI restriction enzyme site in the abnormal sequence, making it possible to test for cosegregation in the family. This analysis determined that the deletion segregated with the XLMR in MRX58 (fig 2).

Abbreviations: MR, mental retardation; XLMR, X linked mental retardation; MRXS, syndromal XLMR; MRX, non-syndromal XLMR; IPS, incorporation PCR SSCP
Sequencing of the second IPS shift (indicated by arrow 2 in fig 1A) observed in exon 5 showed a dA→dG change at position +60 in intron 5 (data not shown). The alteration was found in MRX12, MRX18, MRX33, MRX56, MRX58, and two unpublished families linked to this interval, along with one small family (K8951) with no linkage data. Twenty normal X chromosomes were tested for the dA→dG change and 13 of them carried the alteration. Thus, the second alteration is a polymorphism and occurs at a frequency estimated to be 65%.

Testing of the dA→dG polymorphism was attempted in some of the XLMR families to see if the existing linkage intervals could be narrowed. Unfortunately, the polymorphism was

<table>
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<th>Product size (bp)</th>
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Figure 1  [A] An IPS gel of exon 5 of the TM4SF2 gene showing the two abnormally migrating bands as indicated by arrows 1 and 2. Lane 1: proband from MRX33. Lane 2: proband from MRX56. Lane 3: male CMS1934 (from a small unlinked family). Lane 4: proband from MRXS10. Lane 5: proband from MRX58. Lane 6: proband from MRX12. Lane 7: normal female showing the normal and altered pattern. Lane 8: normal male. [B] DNA sequence analysis of exon 5 of the TM4SF2 gene from a control and the proband from MRX58 showing the 2 bp (GT) deletion. The 5′→3′ sequence is read from the bottom to the top. Small arrows in the control lane indicate the deleted G and T in the patient. Arrow on the left indicates the point of deletion.
The number of genes on the X chromosome responsible for non-syndromic XLMR was first estimated by Herbst and Miller as seven to 19 in 1980. Their estimate was predicted on a prevalence estimate of 1.83 cases of non-syndromic XLMR/1000 males. Their estimate appeared to be reasonable based on the ability of later researchers to group the localisations of XLMR conditions into either eight or 10-12 non-overlapping loci depending on the criteria used to determine limits of the published localisations. However, it is now evident that the number of genes responsible for the 75 presently known XLMR conditions will probably exceed the upper limit of 19 proposed by Herbst and Miller. This conclusion is based on results recently generated in various laboratories. For example, in Xq28, which contains at least nine XLMR conditions (MRX3, MRX6, MRX16, MRX25, MRX28, MRX41, MRX46, MRX48, MRX72), two genes have been found that account for only three of these conditions, GDI1 (MRX41, MRX48), and MECP2 (MRX16). This would mean at least one more gene must exist in Xq28. A similar situation exists for the Xq22-q23 region where PKD3 is found to be mutated in MRX30 and MRX47, but not in another seven XLMR entities whose localisations span this region. Thus, the minimum number of XLMR genes is likely to be three times the number of non-overlapping loci defined by Lubs et al.

However, even this rationale for estimating the number of XLMR genes is complicated by the finding that genes associated with syndromic XLMR conditions may also be involved in XLMR. RSK2, the gene associated with Coffin-Lowry syndrome, is mutated in MRX2. MECP2, the Rett syndrome gene, is mutated in MRX16, and FGD1, the Aarskog gene, has been found to have a mutation in a family with non-syndromic XLMR. Therefore, to determine the number of genes on the X chromosome responsible for XLMR conditions it will be necessary to screen every known candidate XLMR gene in all of the known linked XLMR families, MRX and MRXS, within the localisation interval.

ACKNOWLEDGMENTS

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