Identification by STS PCR screening of a microdeletion in Xp21.3-22.1 associated with non-specific mental retardation

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X-linked non-specific mental retardation (MRX) is a heterogeneous condition in which mental retardation (MR) appears to be the only consistent manifestation. The genetic and phenotypic heterogeneity exclude any possibility of pooling families and, therefore, of fine-mapping the related disease genes. In order to identify genomic critical regions involved in the MRX condition assigned to Xp21.3-22.1 region, we have implemented the PCR screening of non fragile X MR patients for the presence of deletions in this region. The amplification by PCR of 12 markers located between POLA and DXS704 using genomic DNA from 192 MR males led to the identification, in a 9 year old mentally retarded boy, of a microdeletion which extends from DXS1202 to DXS1065. None of the known genes, POLA, MAGE genes cluster, DAX1, GK and DMD, that map in the Xp21.3-22.1 region is affected by this deletion. This approach, which could easily be applied to several other MRX loci, allowed not only a confirmation of the presence of a potential locus in Xp21.3-22.1 involved in non-specific mental retardation, but also a better definition of the genomic critical region corresponding to this locus.

INTRODUCTION

X-linked mental retardation (XLMR) is a common and vastly heterogeneous group of disorders which can be roughly categorized as syndromic or non-specific (MRX) (1,2). Families with syndromic disorders usually have a quite distinct phenotypic presentation whereas families with non-specific disorders present no distinctive somatic features. Despite recent advances in identifying genes such as FMR1, L1CAM, FGD1 and XH2, involved in XLMR conditions, progress in unravelling nonspecific X-linked mental retardation has been slow partly because of the clinical and genetic heterogeneity which preclude any possibility of pooling families and, therefore, to fine map the related disease genes. Although mapping data showed at least 28 nonspecific loci with an apparent clustering around the pericentric region of the X chromosome (1), it is almost impossible to evaluate how many MR genes there are in reality, partly because of the broad localisation and the presence of several overlaps between intervals of assignment. Thus, fine mapping and identification of genes implicated in nonspecific X-linked mental retardation will essentially depend on thorough investigation of molecular abnormalities and systematic screening of many probands for mutations in many candidate genes.

Given these difficulties, we thought that the screening of genomic DNA from MR patients for deletions could allow the identification of genomic MRX critical regions. This screening could be carried out by PCR using very dense set of STS markers previously mapped to specific regions of the X chromosome. We have implemented the PCR screening of 192 MR patients for the presence of deletions in Xp21.3-22.1 region in which a non-specific mental retardation locus has been assigned on the basis of (i) deletions associated with contiguous gene syndromes (3), (ii) linkage analysis (4), (iii) recent data reported by Fryn’s group describing the identification of an inherited deletion encompassing the DXS1218 CA-repeat (5). In this report, we describe the identification of a microdeletion in Xp21.3-22.1 associated with a severe non specific mental retardation. Also, we demonstrate the value of screening for microdeletion to define MRX loci using an STS PCR-based approach.

RESULTS

PCR screening and molecular investigation of the deletion

DNA samples from 192 MRX patients have been tested with a panel of 12 X-linked microsatellites or STS markers located between POLA and DXS1020 (Fig. 1A) (6). PCR amplification of these 12 markers using genomic DNA from patients led to the identification of one microdeletion which extends from DXS1202 to DXS1065 (Fig. 1A and B). None of the known genes, POLA, the MAGE genes family, DAX-1, GK and DMD, that map in Xp21.3-22.1 region is affected by this deletion. Deletion of the MAGE genes was ruled out by Southern blot analysis using HindIII, PstI and EcoRI digested DNA hybridized with a probe corresponding to exon 4 of the MAGE-Xp gene, known to be present in a minimum of five copies in a 30 kb interval of Xp23.1 region (7). Analysis of the patient’s DNA showed qualitatively...
Figure 1. PCR amplification and Southern blot analysis of genomic DNA from the MRX patient (P) and a control male (Ct). (A) Schematic representation of the STS map (6) between POLA and DMD genes. PCR amplification results are indicated as follows: (+) presence of PCR product, (–) absence of PCR product. (B) Gel electrophoresis of PCR products stained with ethidium bromide. Sizes of PCR product are compatible with published data; for the DXS6764 PCR product, the expected size is 138 bp. PCR products corresponding to POLA and DXS1218 markers are not shown on this figure. (C) Southern blot analysis of EcoRI/EagI double digested DNA using C7 probe (upper) or StB12-3 probe (lower). This figure (lane MRX patient) confirms the deletion using C7 probe and the presence of a normal pattern with FRAXA probe. Controls with premutated alleles and full mutated allele of FRAXA gene are indicated by asterisk and bracket respectively.

and quantitatively patterns similar to those of a non-deleted male used as a control (data not shown). In addition, owing to the fact that DXS1074 and DXS319 which map within the 30 kb interval of the MAGE genes family are proximal and at least 300 kb far from DXS7188 (the non deleted proximal marker) (6), we can exclude the involvement of these genes in the deletion.

Further investigation of this deletion using supplementary sets of STS markers showed that the proximal breakpoint lies between DXS1065 and DXS7188, and the distal breakpoint lies between DXS1202 and DXS6764. Although we have not yet accurately defined the deletion breakpoints, the size could be estimated at 1 Mb according to Ballabio’s physical map (6) and the Genethon genetic map (8).

The presence of the deletion was confirmed by Southern blot analysis using C7 probe at DXS28 locus (9). As shown in Figure 1C, no signal is observed in the lane corresponding to the MRX patient. This deletion was found in a 9 year old boy, with severe mental retardation; at 7 years his IQ was less than 45. He had neither abnormal biological findings nor dysmorphic features. This patient was born of healthy non consanguinous parents. Pregnancy and neonatal periods were normal. Cytogenetic analysis of the patient’s chromosomes did not detect any abnormality and FRAXA was excluded by molecular investigations (Fig. 1C).

A frequent deletional polymorphism in the population can be ruled out since 100 male control samples, from ongoing investigations into families with DMD, showed normal signals on Southern blot using C7 probe. Also, to exclude a rare deletional polymorphism, we tested an additional set of 470 unrelated males with DXS1218-CA-repeat, which is deleted in the mentally retarded patient reported here and in the Belgian family described by Fryn’s group. As no deletion was found in 570 tested males a rare deletional polymorphism can be confidently ruled out.

DISCUSSION

PCR amplification of STSs in the Xp21-3 locus using genomic DNA from 192 non fragile X MR patients led to the identification of one microdeletion which extends from DXS6764 to DXS7188. These results are in line with the inherited microdeletion associated with nonspecific mental retardation reported by Fryn’s group during the 7th International Workshop “Fragile X and X-linked Mental Retardation” (5). They also confirm the presence of a potential locus in Xp21.3-22.1 involved in nonspecific mental retardation. This specific-MR deletion will enable focus on the genomic critical region involved in this MRX condition and further investigations aiming to identify expressed sequences.

This very simple PCR-based approach could also be applied to: (i) several other regions of the X chromosome exhibiting deletions of contiguous genes with mental retardation, such as, Xp22.3 (10), Xq21.1 (11) and Xq28 (12); (ii) MR-intervals of assignment based on linkage analysis carried out on individual large families; (iii) genomic regions flanking balanced X-autosome translocation breakpoints associated with mental retardation. The screening for deletions which will be enhanced by the increasing number of mapped STS and EST might allow to pinpoint small specific-MR critical regions both in large genetic-
intervals of assignment and in contiguous genes syndromes. In MR cases exhibiting balanced X;autosome translocations, the screening for deletion could allow to favour the involvement of X-linked genes in the phenotype rather than autosomal ones. J. L. Mandel reported a proposal (13) based on systematic screening of many probands for mutations in many candidate genes. He proposed a scheme that associates standardized reporting of XLMR families including small ones and deposit of a lymphoblastoid cell line for one proband of each family to an accessible repository. The STS and EST PCR-based screening for deletions is a complementary approach to Mandel’s proposal and to Flint’s strategy (14) based on the screening for subtelomeric DNA rearrangements in patients with idiopathic MR. Ultimately these systematic approaches could speed up identification of critical regions and genes involved in XLMR.

MATERIALS AND METHODS

MR patients

In this study, patients with idiopathic MR were identified by examining the fragile X case record of J. C. Kaplan’s molecular diagnosis unit at Cochin Institute. Male patients were selected on the basis of (i) a low IQ or, if too young for psychological assessment, the presence of significant developmental delay; (ii) no recognizable syndrome whether chromosomal, Mendelian, acquired or environmental. Fragile X syndrome was excluded by Southern blot analysis using DNA digested with EcoRI/I, endonucleases and StB12-3 probe corresponding to FRAXA locus (15).

STS amplification and Southern blot analysis

PCR were carried out in microtitre plates using 50 ng of genomic DNA in a final volume of 50 µl. PCR primers and sizes of fragments were previously reported (8,10). Amplification conditions were: (i) 5 min denaturation step at 94°C, (ii) 35 cycles of 30 s at 94°C, 30 s at 52°C, 1 min at 72°C, (iii) a final 10 min extension step at 72°C. PCR products were analysed by electrophoresis using 2% Nusieve™ agarose gel and ethidium bromide staining. PCR products corresponding to microsatellite markers were also analysed by Southern blot probed with a (CA)10 oligonucleotide labelled by kination.

REFERENCES

5. 7th International Workshop on the Fragile X and X-linked Mental Retardation. Tromso, Norway, 2–5 August 1995.