Short reports

Androgen insensitivity with mental retardation: a contiguous gene syndrome?

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Abstract

We present data to suggest the existence of a mental retardation (MR) locus at Xq11.2-q12 between DXS1 and DXS905, identified in two subjects with complete androgen insensitivity syndrome (CAIS) and MR. Androgen insensitivity syndrome is a disorder of male sexual differentiation caused by a defect in the androgen receptor (AR) gene (Xq11-q12). Two subjects with CAIS resulting from a complete deletion of the AR gene have previously been reported, one of whom also has MR. We have identified another mentally retarded person with a complete deletion of the AR gene. The deletion in the two patients with CAIS and MR extends past the AR gene and includes several marker loci both proximal and distal to the AR gene, the limits of the deletions being DXS1 and DXS905. The deletions in the CAIS patients who do not have MR do not include any of the markers outside the AR gene itself. These data suggest that located close to the AR gene is a gene which is implicated in non-specific MR.

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There are believed to be at least eight distinct loci for non-specific mental retardation (MR) on the X chromosome.^{1 2} As there are no other physical characteristics associated with this type of MR it is not possible to combine the results of linkage analysis in different affected families. For this reason it has been difficult to map the genes involved. We present data to suggest the existence of a MR locus close to the gene for the androgen receptor (AR) on Xq11.2-12, between DXS1 and DXS905. The data derive from an analysis of four patients with complete androgen insensitivity syndrome (CAIS), two of whom also have MR.

Androgen insensitivity syndrome (AIS) is a disorder of male sexual differentiation caused by an inability to respond appropriately to the male sex hormones, androgens.³ Affected subjects have a 46,XY karyotype, testes, and either normal female external genitalia (complete

AIS) or a range of degrees of undervirilisation (partial AIS). The disorder is caused by a defect in the AR gene which lies on Xq11-12. Over 150 different mutations in the AR gene responsible for AIS have been reported to date,^{4 5} the vast majority being point mutations resulting in missense mutations. There have been four previous reports of large deletions of the AR gene, all of which result in the CAIS. We have had the opportunity to study three of these four patients. In two cases the entire coding region of the gene is deleted.^{6 7} One of these patients (ARΔ1) is intellectually normal⁷; however, the other (ARΔ2) also has MR.⁶ A third patient (ARΔD-H) has a deletion of the five 3' exons of the gene, exons D to H, which code for the entire hormone binding domain of the receptor; this patient is intellectually normal.⁸ All of these deletions are submicroscopic. Limited deletion analysis was performed by Southern blotting in the original studies of both complete deletion patients, using cDNA probes to X chromosome loci in the region of the AR gene. The closest markers to the AR gene tested were DXS1 and PGK1P1 in ARΔ1 and DXS1 and DXS159 in ARΔ2. In both cases all the markers outside the AR gene were present.

We have identified a third patient (ARΔ3) with CAIS resulting from a complete deletion of the coding region of the AR gene. The AR gene was examined by genomic PCR using conditions specific for the eight exons of the gene.⁹ Although PCR of control DNA produced the expected products, all exons of the AR gene failed to amplify from the patient's DNA. This patient also has MR and mild developmental abnormalities. She attends a special school, has impaired vision and developmental delay, mild hypertelorism, large hands and feet, high arched palate, a small chin, and downward slanting eyes. This phenotype is similar to ARΔ2 although slightly less severe.

A deletion analysis of all four of the above patients has shown that the X chromosome deletion in the two patients with MR extends past the AR gene in both directions, whereas in the patients with no MR the deletion is limited to the AR gene itself. The markers tested were: DXS1161,¹⁰ PGK1P1,¹¹ DXS1160,¹⁰

Figure 1 (A) Examples of AR gene PCRs: lanes 1, 6, and 11 = normal male control, lanes 2, 7, and 12 = ARΔD-H, lanes 3, 8, and 13 = ARΔ1, lanes 4, 9, and 14 = ARΔ2, lanes 5, 10, and 15 = ARΔ3. (B) Southern blot analysis of DXS1 using probe p8 on genomic DNA from ARΔ3 and a normal male control, digested with EcoRI. (C) ³²P labelled PCR products for DXS1161. This marker includes a CA repeat and therefore a ladder pattern is produced by Taq polymerase stuttering. (D) Results of deletion analysis of the four CAIS patients using PCR based markers distal to the AR gene.

DXS908,¹² DXS897,¹² DXS905,¹² DXS159,¹³ and DXS981.¹⁴ All markers except for DXS1161 were analysed by genomic PCR and the products visualised by agarose gel electrophoresis. The sense primer used for DXS1161 has sequence similarity to L1 repeats and, as recommended in the original publication, the antisense oligo was end labelled with ³²P and the products analysed on a standard polyacrylamide sequencing gel.¹⁰

Fig 1A shows examples of the PCR reactions for the AR gene showing that ARΔ1, ARΔ2, and ARΔ3 are all deleted for the entire AR gene, while ARΔD-H is only deleted for the 3' portion of the gene. Southern blot analysis of genomic DNA from patient ARΔ3 using probe p8 (obtained from ATCC) for DXS1 confirmed that, as in the other patients, this marker is present (fig 1B). The deletion in ARΔ3 is slightly greater than that in ARΔ2, extending from DXS1161 to DXS905. In ARΔ2 the deletion extends from DXS1161 but only as far as DXS897. The results of the PCR analysis are shown in figs 1C and D and in schematic form in fig 2.

These results suggest that there is a locus for MR between DXS1 and DXS905, which is deleted in the two patients with CAIS and MR but not in the patients without MR. Several reports of linkage analysis in families with non-specific X linked MR have mapped a locus for

MR to this region.¹ This study provides a more precise estimate for the position of a locus for X linked MR near to the AR gene. Until candidate genes are characterised it is not possible to say whether the locus we have identified corresponds to any loci for non-specific X linked MR mapped by linkage analysis.

A possible candidate gene which has been mapped to this region is EPLG2, a potential ligand for ELK, a member of the EPH family of receptor tyrosine kinases. ELK is expressed exclusively in brain and testis and has been suggested to play a role in the development or maintenance of the nervous system.^{17,18} The gene for EPLG2 itself has been mapped to a 200 kb interval on Xq12, distal to PGK1P1 and on the same YAC as PGK1P1, DXS159, and DXS133.¹⁹ Although EPLG2 has not been detected in adult human brain by northern blot analysis, it does appear to be expressed in developing rat brain.¹⁸ Further characterisation of this ligand, together with the identification of other genes in this region of the X chromosome, will help to determine the gene (or genes) responsible for MR in these patients.

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Figure 2 A schematic representation of the extent of the X chromosome deletions in each of the four patients studied. The hatched areas represent the regions deleted in each case. Only the AR gene markers are deleted in ARΔD-H and ARΔ1. The deletion in ARΔ2 extends from DXS1161 to DXS897, while the deletion in ARΔ3 is slightly larger, extending from DXS1161 to DXS905. The order of the markers is taken from the report of the Sixth International Workshop on Human X-Chromosome Mapping.¹⁵ The estimated size of the deletion between DXS1 and DXS905 is 1.5 megabases.¹⁵ Note that the orientation of the partial AR gene deletion in ARΔD-H has been illustrated to take account of recent evidence that the AR gene is transcribed away from the centromere.¹⁶

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Note added in proof

As this paper was in press, another complete deletion of the AR gene was reported by Hiort *et al*²⁰ in a CAIS patient. The extent of the deletion is not indicated nor whether the patient is mentally retarded.

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