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Androgen Receptor Locus on the Human X Chromosome: Regional Localization to Xq11-12 and Description of a DNA Polymorphism

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Summary

The gene for the androgen receptor, mutations at which cause the X-linked androgen insensitivity syndrome, has been localized to the q11→q12 region of the human X chromosome by analysis, using a cloned cDNA for the androgen receptor, of somatic cell hybrid panels segregating portions of the X chromosome. A moderate-frequency *Hin*dIII RFLP has been found which should be useful in genetic linkage analysis of the various inherited forms of androgen insensitivity.

Introduction

A series of inherited disorders known as the androgen insensitivity syndromes is characterized by androgen unresponsiveness that is due to a variety of defects in the action of the androgen receptor. The androgen receptor is a high-affinity androgen-binding protein that mediates the effect of testosterone and dihydrotestosterone by functioning as a trans-acting inducer of gene expression. For proper male sexual development to occur during embryogenesis and puberty, both androgen and its receptor are required (Wilson et al. 1983; Pinsky and Kaufman 1987).

Both the complete and incomplete forms of androgen insensitivity, including the form known in animals as testicular feminization (Tfm), are inherited as X-linked traits (Lyon and Hawkes 1970; Meyer et al. 1975; Fichman et al. 1980; Wilson and Griffin 1985). Overall, the reported incidence of these disorders ranges from 1/20,000 to 1/60,000 male births. The genetic defects underlying the androgen insensitivity syndromes are believed, on the basis of both biochemical and genetic evidence, to involve the androgen receptor itself (Wilson et al. 1983; Wilson and Griffin 1985). Migeon et al. (1981) mapped the X-linked defect in Tfm to the pericentromeric region of the human X chromosome, on the basis of measurements of androgen-binding activity in rodent/human somatic cell hybrids. Consistent with this localization, Wieacker et al. (1987) have reported genetic linkage between a DNA polymorphism located in the proximal long arm of the X and androgen insensitivity in family studies.

Neither of these studies, however, provides direct evidence that the gene for the androgen receptor itself underlies Tfm or the other androgen insensitivity syndromes. Recently, several groups have reported isolation of X-linked genomic and cDNA clones for the androgen receptor (Chang et al. 1988; Lubahn et al. 1988; Trapman et al. 1988). Initial mapping studies indicated localization of the gene for the androgen receptor (designated *AR*) to the proximal long arm of the X chromosome, a result consistent with its presumed involvement in the X-linked androgen insensitivity syndromes (Lubahn et al. 1988).

In the present study, we have investigated further the localization of the X-linked *AR* gene by using two additional series of rodent/human somatic cell hybrids segregating different regions of the human X chromosome. These analyses indicate that the *AR* gene is located in the Xq11→Xq12 region of the X. In addition,

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we describe an *AR* RFLP (Botstein et al. 1980) which should facilitate genetic linkage analyses of this gene, both in normal families and in those segregating the androgen insensitivity syndrome. This *AR* RFLP should be of value in the potential prenatal diagnosis of androgen receptor defects.

Material and Methods

DNA Probes and DNA Analysis

The DNA probe used was the 0.7-kb *Hin*dIII-*Eco*RI fragment from ARHFL1, described by Lubahn et al. (1988). This fragment represents about one-quarter of the androgen receptor cDNA. Methods and conditions for DNA analysis and filter hybridization analysis have been described elsewhere (Willard et al. 1983). For the 0.7-kb probe, all filters were given a final wash in 0.1 × SSC/0.1% SDS at 68°C.

Rodent/Human Somatic Cell Hybrid Series

Two series of somatic cell hybrids have been used for X chromosome regional localization. The first involves a series of mouse/human or hamster/human hybrids segregating portions of various X-autosome translocations, with breakpoints ranging from Xp21 to Xq23 (Willard et al. 1985; Willard and Riordan 1985; Mahtani and Willard 1988). This panel has been characterized both cytogenetically and molecularly with a variety of X-linked DNA probes and potentially divides the Xp11→Xq13 pericentromeric region of the X into eight intervals.

The second panel consists of a series of 21 hamster/human hybrids segregating portions of the human X created by gamma-irradiation of human chromosomes before fusion (Goss and Harris 1975). This panel has been extensively characterized (Goss and Harris 1975; Buck et al. 1976) and has been used by us previously to map both the gene for phosphoglycerate kinase (*PGK1*) and its X-linked pseudogene *PGK1P1* on the proximal long arm of the X (Willard et al. 1985).

DNA Polymorphism Analysis

To detect potential RFLPs with the androgen receptor cDNA, genomic DNA from at least four individuals (at least eight X chromosomes) was digested with the following restriction enzymes; *Eco*RI, *Bgl*I, *Kpn*I,

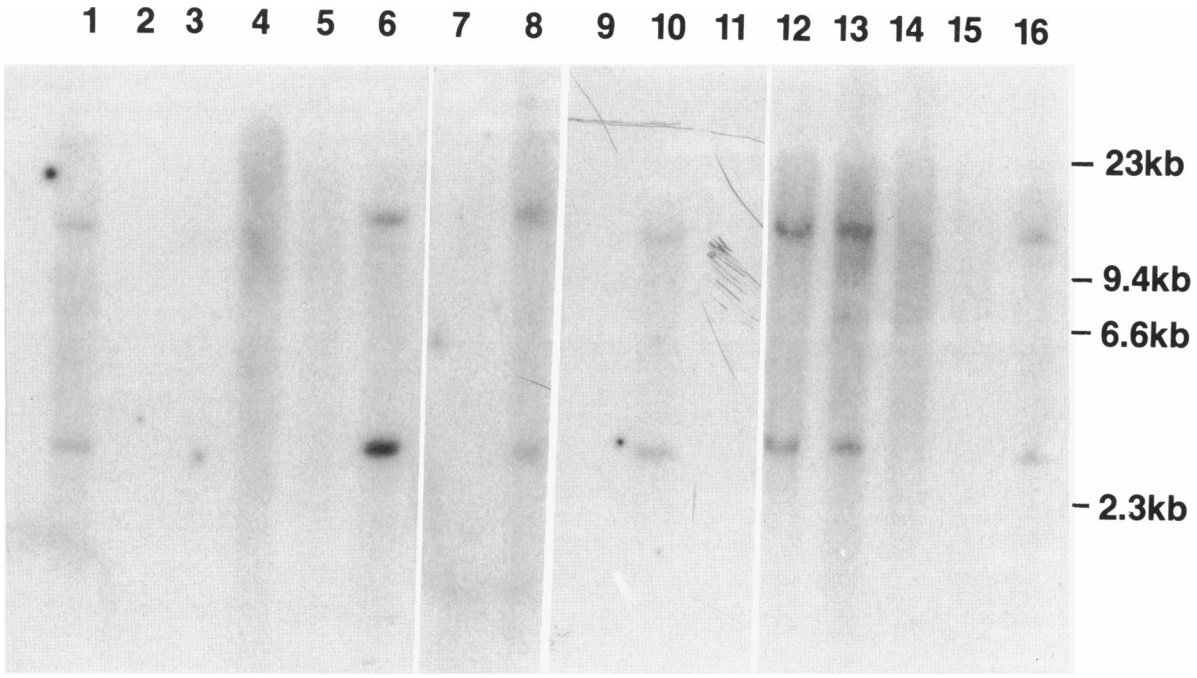

Figure 1 Regional localization of the human androgen receptor gene. Human genomic DNA (lane 1), mouse genomic DNA (lane 2), hamster genomic DNA (lane 3), and DNA from hamster/human (lanes 4–6) or mouse/human (lanes 7–16) somatic cell hybrids were digested to completion with *Eco*RI and a filter hybridization experiment was performed, using a 0.7-kb probe from the androgen receptor cDNA as probe. Hybrids in lanes 6, 8, 10, 12, 13, and 16 are positive for human androgen receptor sequences. For the X chromosome content of the hybrids, see table 1. Size markers are indicated to the right.

*Sma*I, *Xho*I, *Sac*I, *Sal*I, *Msp*I, *Pvu*II, *Bgl*II, *Pst*I, *Xba*I, *Bam*HI, *Bcl*I, and *Hin*dIII. Polymorphism was detected only with *Hin*dIII. To confirm this RFLP, Mendelian segregation was demonstrated in a series of large 2- and 3-generation pedigrees provided by the Centre d'Etude du Polymorphisme Humain (CEPH), as part of an international collaboration to map the human genome.

Results

Regional Localization of the X-linked AR Gene

Elsewhere, we used a small group of five hybrids to map the human *AR* gene to the centromere→Xq13 region of the human X chromosome (Lubahn et al. 1988). Here, we have used hybrids which localize the *AR* gene with respect to eight additional breakpoints in the Xp11→Xq23 region. As shown in figure 1 and summarized in table 1, the androgen receptor cDNA hybridizes to two prominent *Eco*RI fragments in human genomic DNA, but not in mouse or hamster DNA. Hybrids containing the q11→qter portion of the X retain human *AR* sequences, but those containing the q12→qter, the q13→qter, or the q21–23→qter portion of the X revealed no *AR* sequences. Thus, when these results are considered in conjunction with data reported elsewhere (Lubahn et al. 1988), the human *AR* gene can be localized to the Xq11→q12 region of the long arm (table 1).

Radiation-induced Segregation Analysis of the AR Gene

The Goss and Harris (1975) panel of hybrids potentially divides the long arm of the X chromosome into at least 18 intervals; in previous studies, at least eight patterns of X-linked marker retention/segregation have been detected with this panel (Willard et al. 1985). When DNAs from this panel were hybridized with the human androgen receptor cDNA, 9 of the 21 hybrids showed retention of the human *AR* sequences (table 2). By comparison with previous data obtained with this panel, the human *AR* gene could be assigned proximal to the *PGK1* locus in Xq13 and coincident with the *PGK1P1* pseudogene, which has previously been assigned to Xq11→Xq13, proximal to the *PGK1* gene (Willard et al. 1985). In none of the 21 hybrids did we observe discordant segregation between *PGK1P1* and *AR* sequences.

HindIII Polymorphism in the AR Gene

To search for RFLPs at the *AR* locus, genomic DNA samples from at least four normal individuals were digested with 15 restriction enzymes (see Material and Methods), and filter hybridization analysis was carried out with the androgen receptor cDNA. Polymorphism was detected only with the enzyme *Hin*dIII. Constant bands were detected at ~8.0, 4.5, and 2.4 kb. The polymorphic band was observed either at 7.0 kb (A1 allele) or at 3.5 kb (A2 allele). The A2 allele was detected on 10 of 98 X chromosomes screened for this polymorphism. The frequency of the predominant A1 allele is, therefore, ~.90. Mendelian inheritance of the polymorphism was demonstrated in several 3-generation families, one of which is shown in figure 2.

Table 1

Regional Localization of the Human Androgen Receptor Gene

Hybrid	Lane (fig. 1)	Portion of X Present	Human *AR* Gene
c1 2D	6	Entire X	+
A62-9A	16	p11.2–qter	+
L62-4A	15	pter–p11.2	–
A48-1Fa	10[a]	cen–qter	+
L48-2A	11	pter–cen	–
A63-1A	12	q11–qter	+
A67-5A	9	q12–qter	–
A68-1A	13	pter–q13,q13–qter	+
A68-2A	14	q13–qter	–
W4-1A	4[a]	q13–qter	–
A50-1A	. . .[a]	q21.1–qter	–
A50-2A	8	pter–q21.1,q21.1–qter	+
A49-5A	7	q22–qter	–
W51-6A	5	q23–qter	–

[a] Data also reported elsewhere (Lubahn et al. 1988).

Table 2

Radiation-induced Segregation Analysis of the Androgen Receptor Gene

Class	X-linked Markers						
	G6PD	HPRT	PRPS	GLA	PGK1	PGK1P1	AR
Controls:							
c12D	+	+	+	+	+	+	+
Hamster	−	−	−	−	−	−	−
Hybrids:							
A	+	+	+	+	+	+	3/3
B1	+	+	+	+	−	+	2/2
B2	+	+	+	+	−	−	0/3
C	+	+	+	−	−	−	0/2
D	+	+	−	−	−	−	0/5
E,F1	−	+	+	+	+	+	4/4
F2	−	+	+	+	+	−	0/2

Note. — The A–F classes have been defined by Willard et al. (1985). The B and F classes are subdivided by the presence or absence of *PGK1P1* sequences. The F class is distinguished from the E class by the presence or absence of the S10 surface antigen in Xq27 (Buck et al. 1976). For the purposes of the present study, these classes were pooled.

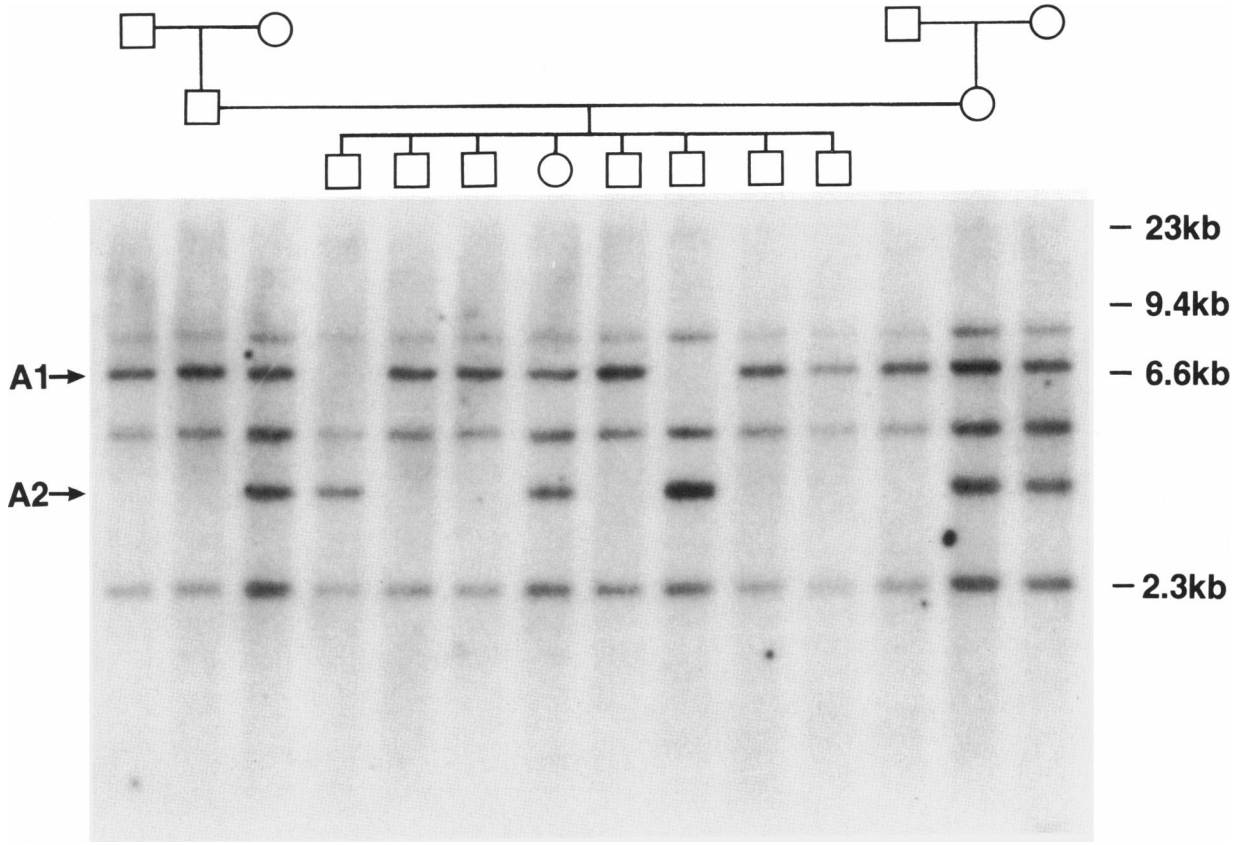

Figure 2 Mendelian inheritance of the *Hin*dIII RFLP in the human androgen receptor gene. Genomic DNAs from a 3-generation family were digested to completion with *Hin*dIII and a filter hybridization experiment was performed, as in fig. 1. Bands corresponding to alleles A1 and A2 are indicted by arrows at the left. Size markers are indicated to the right.

Discussion

In the present study, we have refined the map location, on the X chromosome, of the *AR* gene. Analysis of somatic cell hybrids segregating X-autosome translocations allows localization of *AR* gene sequences to Xq11→Xq12. This assignment is consistent with and extends previously analyses by ourselves and others (Migeon et al. 1981; Lubahn et al. 1988). The region to which the *AR* gene can be localized probably contains less than 5–10 million bp of DNA and also contains the *PGK1P1* pseudogene and DXS1 sequences detected by the probe p8. It is worth noting that the X;9 translocation used in our hybrid A67-5A (table 1) was derived from a female patient with X-linked hypohidrotic ectodermal dysplasia, the gene for which is believed, therefore, to map to the position of the breakpoint in Xq12 (see discussion in Zonana et al. 1988). Since the *AR* gene maps above this breakpoint and *PGK1* maps below, the data suggest a gene order of *AR*–HED–*PGK1*. This order is identical with the order of the presumed homologous murine loci (*Tfm–Tabby–Pgk-1*) on the mouse X chromosome, as determined by linkage analysis (Davisson 1987).

Migeon et al. (1981) measured androgen binding activity in Tfm mouse/human somatic cell hybrids and reported localization of the *AR* gene to Xp11→q13, consistent with the results presented here. In one hybrid, abnormal binding kinetics were detected, prompting the suggestion that the X breakpoint in that hybrid interfered with normal *AR* gene expression. This breakpoint, in an X;11 translocation, was originally reported to be in Xq11 (Migeon et al. 1981) but has now been shown to be within the large alpha-satellite DNA array found at the centromere of the X (Mahtani and Willard 1988). This same X;11 translocation has been examined in the present study (hybrids L48-2A and A48-1Fa; see table 1), and the structure of the *AR* sequences appears normal by Southern blotting. Thus, it is clear that the breakpoint in this translocation does not directly affect the *AR* locus. However, as a way of reconciling our findings with those of Migeon et al. (1981), it is conceivable that disturbances of the X centromeric heterochromatin might alter the level of expression of the nearby *AR* gene. Additional work will be required to establish precisely how close the *AR* gene is to the centromeric alpha-satellite array.

Since accurate cytogenetic determination of breakpoints within this small region is likely to be limited, more precise localization of the gene will require physical mapping techniques of considerably greater resolution, such as pulsed-field gel electrophoresis (Barlow and Lehrach 1987). Alternatively, it may be possible to use approaches that do not depend on the availability of a finite collection of naturally occurring chromosome abnormalities. Physical mapping techniques that rely on the creation of a potentially unlimited number of de novo chromosome breaks or deletions in vitro, such as the radiation-induced segregation analysis approach of Goss and Harris (1975), may be useful for fine mapping. In the current application of this approach, we have demonstrated complete concordant segregation between *AR* and *PGK1P1*. Whether this cosegregation implies a close physical relationship on the chromosome or whether it reflects the relatively small size of the hybrid panel used is unclear. The current panel was selected on the basis of prior results with other X-linked DNA markers and gene products. A larger group of hybrids, unselected for coretention of specific traits, would permit statistical measures to be used to address this point, thus greatly increasing the power of the Goss-Harris technique.

The presence of a *Hin*dIII polymorphism in the *AR* gene (fig. 2), albeit one of only moderate frequency, should permit genetic linkage analysis of this gene, in conjunction with other genetic markers mapped to this region (Arveiler et al. 1987; Mahtani and Willard 1988), including the DXS1 marker used by Wieacker et al. (1987) in their family study of androgen insensitivity. For fine mapping, it will be necessary to uncover additional RFLPs in the *AR* gene, using either other portions of the cDNA (Lubahn et al. 1988) or genomic probes for the gene.

The availability of a cDNA probe for the androgen receptor has permitted direct confirmation of the role of androgen receptor defects in the X-linked androgen insensitivity syndrome by the demonstration of gene deletions in at least one family (Brown et al. 1988). Notwithstanding the modest information content of the *AR Hin*dIII polymorphism described here, it should allow immediate evaluation of the role of the *AR* gene in the nondeletion forms of androgen insensitivity and may permit prenatal diagnosis of affected fetuses in the ~20% of cases in which a carrier female should be informative for the polymorphism.

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