Incomplete Androgen Insensitivity Associated with a Thermolabile Androgen Receptor

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Abstract. One infant and a cousin with incomplete androgen insensitivity syndrome were reported. The familial pedigree showed that the disorder was inherited in three generations in X-linked recessive fashion. An androgen binding study of cultured genital skin fibroblast from patients showed normal maximum binding capacity and a normal apparent dissociation constant. Heat stability assay showed binding decreased to less than 30% at 41°C compared with the amount at 30°C, indicating that the androgen receptor was thermolabile.

Key words: Incomplete androgen insensitivity syndrome, Thermolabile androgen receptor.

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INCOMPLETE androgen insensitivity syndrome (AIS) is one of the clinical variants of androgen receptor disorders which is inherited as an X-linked trait [1]. A genetic male patient has female external genitalia with partial virilization. The frequency of incomplete AIS is uncertain. We describe here an infant and a cousin with incomplete AIS. The familial pedigree showed patients with ambiguous genitalia in three generations in X-linked recessive fashion. Thermolabile androgen receptor was found in both patients' cultured genital skin fibroblasts.

Case Reports

The pedigree of this family is shown in Fig. 1.

The subjects with ambiguous genitalia were seen in three generations in X-linked recessive fashion.

Case 1

The proband (IV-14 in Fig. 1), a one-year and 9-month-old social girl was referred to our clinic because of ambiguous genitalia. She was the only child of unrelated parents. Her mother (III-11 in Fig. 1) achieved menarche at the age of 11 years and was cycling regularly. On examination, mild clitoromegaly was observed (Fig. 2). Masses presumed to be gonads were palpable in the bilateral labia majora. The labia minora were hypoplastic. The urethra was inside the vagina on the anterior wall. The vagina was 0.5 cm in depth. Other examination was unremarkable.

The patient's karyotype was found to be 46, XY.

Case 2

A cousin of the proband (IV-4 in Fig. 1), a three-year and 10-month-old social girl was also referred to our clinic because of ambiguous genitalia. There
were one sister and three brothers who were phenotypically normal. Her mother achieved menarche at the age of 12 years and was cycling regularly.

On examination (Fig. 3), the clitoris was enlarged and masses presumed to be gonads were palpable in the bilateral inguinal area. The degree of clitoromegaly was somewhat severer than that of Case 1. The labia minor were hypoplastic. The urethra was inside the vagina on the anterior wall. The vagina was 1.0 cm in depth.

The patient's karyotype was found to be 46, XY.

Exploratory laparotomy was performed with parental consent. In both cases, bilateral testes were found in the labia majora or in the inguinal canal and removed. Epididymides and vasa deferentia were present bilaterally. No uterus or other female structures were found in the pelvis. On histological examination, the testes were normal.

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Fig. 1. Family pedigrees. Circles denote social female; squares social male. □ and ★ indicate subjects with ambiguous genitalia.

Fig. 2. External genitalia in case 1.

Fig. 3. External genitalia in case 2.
Methods

Endocrinological studies were performed before laparotomy. Serum LH and FSH were measured by radioimmunometric assay (Dinabot). Serum testosterone and 5α-dihydrotestosterone (DHT) were measured by RIA (Diagnostic Product Cooperation and Medical System Service, respectively). After overnight fasting, GnRH (100 µg/m²) was administered intravenously, and serum samples were obtained at 30 min intervals for 2 h. Human chorionic gonadotropin (hCG) (4000 U/m², once a day for 3 days) were administered intramuscularly and serum samples were obtained before first injection and at 24, 48, 72 and 96 h after the last injection.

With parental consent a skin biopsy was obtained from the foreskin to assess androgen receptor in both patients. Fibroblasts were cultured from explants from the pubic skin, as previously described [2]. In whole cell assay, pubic skin fibroblasts from patients and control subjects were harvested with 0.01% trypsin-0.02% EDTA in phosphate-buffered saline (PBS) and suspended in 25 mM tricin in Dulbecco's Modified Eagle's Medium, pH 7.4. Fibroblasts (3 × 10⁵ cells/tube) were incubated with increasing concentrations (0.1–2.0 nM) of [3H]methyltrienolone ([3H]R1881, New England Nuclear Research Products) in the presence or absence of a 1000-fold concentration of R1881 at 30°C and 41°C for 1 h at room temperature. After the incubation, the cells were washed three times with 2 mL PBS and lysed with ethanol. Aliquots were transferred to scintillation vials containing 10 mL aquasol. The results were analyzed by the Scatchard method [3] and expressed as binding sites per cell by means of a computer.

For heat stability, cultured pubic skin fibroblasts were preincubated at 41°C or 30°C for 30 min. The cells were incubated with 1.0 nM [3H]R1881 in the presence or absence of a 1000-fold concentration of R1881 at 41°C or 30°C for 1 h. After the incubation, the cells were treated as described above.

Results

In both patients, endocrinological studies showed that the serum LH response to the GnRH test was greater than in normal children. There were responses of testosterone and 5α-DHT to hCG stimulations (Table 1).

An androgen binding study showed that the maximum binding capacity (Bmax) (sites/cells) and apparent dissociation constant (Kd) (nM) were 4,245 and 0.43 in case 1 and 4,378 and 0.23 in case 2, respectively, indicating normal capacity and affinity (Fig. 4).

When assay was performed after preincubation of cells in 41°C or 30°C for 30 min, [3H]R1881 binding decreased to less than 30% at 41°C of that seen at 30°C, indicating thermolabile androgen receptor (Fig. 5).

Discussion

At least four phenotypic variants of the androgen receptor disorders are known - complete AIS, incomplete AIS, the Reifenstein syndrome, and the infertile male syndrome [1]. Incomplete AIS might be suspected in an infant with 46, XY karyotype, ambiguous genitalia, increased response of LH to GnRH test and normal response of testosterone and 5α-DHT to hCG stimulation. The diagnosis is made on the basis of studies of androgen receptor function in cultured skin fibroblasts. The two patients reported were consistent with incomplete

Table 1. Endocrinological study of cases 1 and 2

<table>
<thead>
<tr>
<th></th>
<th>Case 1 Basal</th>
<th>Case 2 Basal</th>
<th>Case 2 Peak</th>
</tr>
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<tbody>
<tr>
<td>GnRH test</td>
<td></td>
<td></td>
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<tr>
<td>LH (mIU/ml)</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>6.6 ± 3.4</td>
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<tr>
<td>PSH (mIU/ml)</td>
<td>1.1</td>
<td>1.3</td>
<td>8.2 ± 4.6</td>
</tr>
<tr>
<td>hCG test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.3</td>
<td>0.2</td>
<td>7.0 ± 3.0</td>
</tr>
<tr>
<td>5α-DHT (ng/ml)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.83 ± 0.25</td>
</tr>
</tbody>
</table>

* age, 1 year and 6 months - 3 years and 11 months. N=6 (GnRH test), N=5 (hCG test).
+ data, mean±SD; ( ), range.
AIS phenotypically and endocrinologically. The presence of thermolabile androgen receptor (qualitatively abnormal androgen receptor) made the diagnosis convincing.

Thermolabile androgen receptors have been observed in complete AIS [4, 5], incomplete AIS [6], the Reifenstein syndrome [7] and the infertile male syndrome [8], indicating that patients with thermolabile androgen receptors span a wide spectrum. Further study is required to elucidate these forms of phenotypic heterogeneity in patients with thermolabile androgen receptors.

It should be noted that the degree of clitoromegaly differed in Case 1 from that in Case 2 and two in this family (II-14 and III-5) have been reared as social males. Maes et al. [9] reported that in a family with incomplete AIS, phenotypic variation existed as in the family presented. These data suggested that factors other than androgen might contribute to the masculinization of the external genitalia.

Recently, the gene for the androgen receptor has been cloned and localized to Xq11-Xq12 [10-12]. Molecular genetic defects in the androgen receptor gene have already been reported in complete AIS, incomplete AIS, and the Reifenstein syndrome [12-14].

As far as thermolabile androgen receptor is concerned, McPhaul et al. [15] reported that the androgen receptor gene contained two alterations in the Reifenstein syndrome with a normal level of ligand binding but with both receptor thermolability and accelerated ligand dissociation. First, a nucleotide substitution in exon G results in a tyrosine to cysteine at amino acid 761. Second, the glutamine homopolymeric segment in the amino terminus of the receptor was short. Site-directed mutagenesis demonstrated the cooperative effects of these two mutations.

In the family presented, molecular study will elucidate the genetic abnormality of androgen receptor gene.
INCOMPLETE AIS WITH THERMOLABILE RECEPTOR

References


