Androgen receptor disorder in three brothers with bifid prepenile scrotum and hypospadias

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Abstract Three brothers with congenital transposition of the penis, scrotal hypospadias, bifid scrotum, and bilateral undescended testes are described. Further signs of incomplete virilization, but no gynecomastia were seen. LH and FSH were elevated, whereas testosterone levels were reduced or in the normal range. Serum concentrations of 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione, 5α-dihydrotestosterone and estradiol measured in two affected brothers were in the normal range. Fibroblasts from scrotal skin biopsies performed in two patients showed normal 5α-reductase activity (419 and 214 pmol · (mg protein)⁻¹ · h⁻¹; normal >1), whereas androgen receptors had reduced maximal binding capacity (Bₘₐₓ 4 and 14 fmol · (mg protein)⁻¹; normal ≥18) and an increased equilibrium dissociation constant (0.7 and 1.26 nmol/l; normal 0.2±0.08) indicating a quantitative and qualitative androgen receptor defect. These patients represent a further variant of androgen insensitivity.

The term «male pseudohermaphroditism» covers a number of distinct clinical forms of intersexuality characterized by an XY genotypic individual with testes but without phenotypically normal male genitalia. Disorders of testosterone biosynthesis, resistance of androgen target organs to the action of circulating androgens or a disturbance in the action of Mullerian-inhibiting-hormone may be the molecular causes for male pseudohermaphroditism. Androgen resistance can be caused either by disturbed conversion of testosterone to 5α-dihydrotestosterone (5α-DHT; 5α-reductase defect), a qualitative or quantitative defect in androgen receptor binding or a postreceptor defect (1). We present another variant of incomplete androgen insensitivity which occurred spontaneously in 3 brothers. The syndrome is characterized by hypospadias, bifid and prepenile scrotum, maldescended testes, and a qualitatively and quantitatively abnormal androgen receptor.

Patients and Methods

The three patients were brothers and belonged to a family with eight siblings. Based on physical examination, hormone values and semen parameters the fourth brother was normal. The four sisters were normal females. Consanguinity and abnormal relatives were not reported from the family. The individual clinical and endocrine findings in the three affected brothers are listed in Table 1.

Patient No. 1 attended our clinic because of underdeveloped genitalia and erectile dysfunction. A first genital operation had been performed during childhood; however, medical records were no longer available. Further surgical treatments for hypospadias by the technique of Denis Browne (see 2) followed at the ages of 8 and 9. Physical examination at the age of 26 revealed sparse body hair, weak facial hair and a very high-pitched voice. The divided scrotum was positioned in front of the penis which had a ventral deviation and an urethral opening in the lower third (Fig. 1). Thus intercourse was not possible. The right testis had a volume of 6 ml, the left of 3 ml. Ultrasonography (Siemens Sonoline II, 75 MHz) revealed cystic structures and inhomogenus testicular tissue. The patient had a normal red blood count and bone density in the lower normal range (134.6 K₂HPO₄/cm³ H₂O) as mea-
sured by quantitative computer tomography (CT; Philips Tomoscan (3)).

Owing to subnormal testosterone values, this patient was first given substitution therapy with injections of 250 mg testosterone enanthate (Testoviron-Depot-250®) at 2 to 3 week intervals. After 4 injections he developed a painful gynecomastia on the right side and was switched to 19-nortestosterone (Anadur®), 100 mg every 3 weeks. However, no improvement in secondary sexual characteristics, libido or erectile function was noted after 2 months of treatment, so testosterone therapy was discontinued. The patient refused any further androgen supplementation.

Patient No. 2 was referred because of intersexual genitalia, erectile dysfunction, and disturbance of ejaculatory function. Congenital hypospadias was treated at an early age by surgery according to Edmunds (see 2). The exact date of operation and degree of hypospadias are unknown owing to loss of early medical reports. Because of a ventral flexion of the penis, further operations were necessary and performed with the technique of Denis Browne at the ages of 6 and 7. Bilateral orchidopexy was carried out at 7 years of age. Physical examination at the age of 24 showed a deviation of the penis with the opening of the urethra in the lower proximal third. The penis is positioned between the two halves of the divided scrotum. Testis volume was 6 ml on both sides. Intercourse was not possible. The patient was not able to produce an ejaculate for semen analysis. Ultrasonography revealed inhomogeneous testicular tissue and showed cystic structures in the left testis. The patient had sparse body hair, weak facial hair and a high-pitched voice. Bone density measured by quantitative CT of the spine was in the normal range, (176 K₂HPO₄/cm³ H₂O).

Patient No. 3. The youngest of the 3 affected brothers suffers from severe infantile cerebral paralysis. He is not

Table 1.
Clinical and endocrine findings in three brothers with the prepenile scrotum syndrome.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>188</td>
<td>183.5</td>
<td>183.5</td>
</tr>
<tr>
<td>Span (cm)</td>
<td>196.5</td>
<td>195.5</td>
<td>190</td>
</tr>
<tr>
<td>Body hair</td>
<td>sparse</td>
<td>sparse</td>
<td>sparse</td>
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<tr>
<td>Facial hair</td>
<td>weak</td>
<td>weak</td>
<td>beginning</td>
</tr>
<tr>
<td>Voice</td>
<td>very high-pitched</td>
<td>high-pitched</td>
<td>n/a</td>
</tr>
<tr>
<td>Gynecomastia</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Penis length (cm)</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Testes (ml; right/left)</td>
<td>6/3</td>
<td>6/6</td>
<td>not palpable</td>
</tr>
<tr>
<td>Karyotype</td>
<td>46,XY</td>
<td>46,XY</td>
<td>n/a</td>
</tr>
<tr>
<td>Ejaculate</td>
<td>azoospermia</td>
<td>not possible</td>
<td>n/a</td>
</tr>
<tr>
<td>LH (IU/l) (normal&lt;10)</td>
<td>26.5</td>
<td>17.6</td>
<td>47</td>
</tr>
<tr>
<td>LH post GnRH (+25 min/+ 45 min)</td>
<td>46.8/47.3</td>
<td>70.8/68.8</td>
<td>109/112</td>
</tr>
<tr>
<td>FSH (IU/l) (normal &lt;7)</td>
<td>48.2</td>
<td>25.6</td>
<td>101</td>
</tr>
<tr>
<td>FSH post GnRH (+25 min/+45 min)</td>
<td>66.5/64.8</td>
<td>35.5/32.4</td>
<td>108.4/112.9</td>
</tr>
<tr>
<td>Testosterone (nmol/l) (normal &gt;12)</td>
<td>7.4</td>
<td>19.5</td>
<td>15.1</td>
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<td>Testosterone post hCG</td>
<td>14.5</td>
<td>38.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Stimulation coefficient</td>
<td>2.0</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>5α-dihydrotestosterone (nmol/l) (normal 0.3-3.0)</td>
<td>1.8</td>
<td>1.6</td>
<td>n/a</td>
</tr>
<tr>
<td>5α-reductase activity (pmol/(mg protein)⁻¹·h⁻¹) (normal &gt;1)</td>
<td>418</td>
<td>279</td>
<td>n/a</td>
</tr>
<tr>
<td>Androgen receptor binding B_max (fmol/mg protein) (normal 30±12; mean ± sd)</td>
<td>4</td>
<td>14</td>
<td>n/a</td>
</tr>
<tr>
<td>Dissociation constant, K_d (nmol/l) (normal 0.2±0.08)</td>
<td>0.7</td>
<td>1.26</td>
<td>n/a</td>
</tr>
<tr>
<td>Thermostability of receptor at 40°C, percent</td>
<td>91</td>
<td>88</td>
<td>n/a</td>
</tr>
</tbody>
</table>

n/a = not assessable
Hormone assays

Serum testosterone was measured by radioimmunoassay as previously described by Schürmeyer et al. (5). All other steroids in serum were measured by commercially available radioimmunoassay reagents: 5α-DHT (after chromatography on celite columns), dehydroepiandrosterone (DHEA), and androstenedione (DRG Instruments, Marburg, FRG); 17-hydroxy progesterone (Mérieux, Charbonnières-les-Bains, France); estradiol (Serono, Freiburg, FRG). Serum LH was measured using reagents provided by the WHO Matched Reagents Programme (6) and serum FSH by fluoroimmunoassay (Pharmacia, Freiburg, FRG).

Fibroblast studies

The fibroblast strains were derived from scrotal biopsy and were grown, stored and assayed as described previously (7,8). 5α-reductase activity was determined under standardized conditions in fibroblast cell sonicates at pH 5.5 with 100 nmol [3H]testosterone as described by Leshin et al. (9) but with these modifications: cell sonicates were incubated for 60 min at 37°C in the presence of 1 nmol NADPH and approximately 20 µg fibroblast protein.

Androgen receptor binding was assessed in confluent fibroblast monolayers utilizing [3H]methyltrienolone as the binding ligand. To determine thermolability of the receptor, identical incubation conditions were chosen except that the incubation temperature was raised to 42°C.

Results

Patient No. 1 showed increased serum gonadotropin levels. Basal serum LH was 3-fold above the upper normal range whereas basal serum FSH was 7-fold increased. In the GnRH test there was a further 1.7 fold rise in serum LH and a further 1.3-fold rise in serum FSH. Serum testosterone was subnormal and stimulation with hCG resulted in a 2-fold rise. Basal serum 5α-DHT, 17-hydroxyprogesterone, DHEA, androstenedione, 5α-reductase-activity, and the thermostability of the androgen receptor were in the normal range. However, the equilibrium dissociation constant (Kd) was increased and the androgen receptor binding capacity (Bmax) was reduced (Fig. 2).

Patient No. 2 also had increased gonadotropin serum levels. Serum LH was 2-fold higher and serum FSH 3-fold higher than the normal range. Stimulation with GnRH resulted in a 4-fold increase in LH and a 1.5-fold increase in FSH. Serum testosterone was in the normal range for men and the hCG injection was followed by a 2-fold rise in testosterone. Basal serum values for all other ste-

Fig. 1.

Genitalia of patient No. 1 (26 years): The prepenile position and the division of the scrotum are clearly visible. The hypospadias cannot be seen.

able to walk alone nor to speak. Orchidopexy and surgical treatments of scrotal hypospadias by the technique of Denis Browne were performed at 5 and 6 years of age. At the age of 23 the patient was examined by us. The habitus was eunuchoid and virilization was incomplete. Testes were not palpable and could not be identified by ultrasonography. The urethra ends in the lower third of the penis which is embedded between the halves of the divided scrotum.

GnRH test

Following a basal blood sample 100 µg GnRH (Relefact LHFR®, 0.1 mg, Hoechst AG, Frankfurt, FRG) was given iv. Further blood samples were taken 25 and 45 min later as standardized in our clinic (4).

hCG test

After a basal blood sample, 5000 IU human chorionic gonadotropin (hCG) (Pregnesin®, Serono, Freiburg, FRG) was injected im and 72 h later a second blood sample was taken for testosterone determination (4).
Fig. 2.
Left panel: Specific androgen binding in cultured fibroblasts from genital skin of a male with normal genital development (○-○) and patients No. 1 (●-●) and 2 (△-△). Fibroblasts were incubated with increasing concentrations of [3H]methyltrienolone. Methyltrienolone is a synthetic steroid that binds to androgen receptors such as 5α-dihydrotestosterone, but is not metabolized.
Right panel: Scatchard transformation of the binding data.

Androgens were in the normal range. 5α-reductase activity and the thermostability of the androgen receptor were normal. As in patient 1, Ka was increased and the Bmax reduced (Fig. 2).

The youngest brother (patient No. 3) had extremely high gonadotropin serum levels. Serum LH was 5-fold increased, whereas serum FSH was increased even 14-fold above the normal range. Stimulation with GnRH resulted in a further 2-fold increase in LH but in no further increase in FSH. Although this patient’s testes were not palpable, serum testosterone was in the lower normal range. In the hCG test for stimulation for testosterone was subnormal, indicating the presence of testes with primary failure. All other steroid serum levels were in the normal range.

Discussion
Variations of male pseudohermaphroditism were described by Reifenstein (10), Lubs et al. (11) and Rosewater et al. (12). Later an androgen receptor deficiency was suggested as the cause of this intersexual development. Investigation of family history revealed that affected individuals within one family may show different symptoms such as gynecomastia, azoospermia, cryptorchism and hypospadias. Male pseudohermaphroditism characterized by infertility, ambiguous genitalia, gynecomastia and elevated testosterone, estradiol and LH values is known as the Reifenstein syndrome (1-13). It is a syndrome with variable mutations of the gene, which is possibly transmitted by the X-chromosome (14). Linkage studies with genes known to be X-linked, such as colour blindness and blood group antigen Xgα, provide one possible resolution of this question. Although no close correlation could be established between the blood group antigen Xgα as proof for the X-chromosomal heritage of the Reifenstein syndrome (15), there are indications that gene loci for colour blindness and development of Reifenstein syndrome are connected (16). Results from recent studies with cloned DNA support the hypothesis of the X-chromosomal heritage of the androgen resistance syndrome (17). In the family reported here, 3 of 4 sons are affected, whereas all 4 daughters are healthy. The cause of the androgen receptor defect in these patients is probably due to a spontaneous gene mutation in the maternal X-chromosome, because no further affected members are known in the family history. Possibly some of the daughters are healthy carriers of this gene mutation.

In patient No. 1 a quantitative and qualitative androgen receptor defect was demonstrated, with decreased receptor binding and an increased dissociation constant. This is most likely the cause for the development of the intersexual genitalia in this patient. None of the measured testosterone precursors or metabolites were elevated, making additional enzyme defects unlikely. Because of subnormal testosterone, underdevelopment of secondary male characteristics and erectile dysfunction, a testosterone substitution therapy was initiated. No response to this therapy was observed and because the patient developed unilateral gynecomastia, androgen substitution therapy was discontinued. Price et al. (18) reported success with testosterone therapy in some patients with androgen receptor deficiency with doses 3- to 6-fold higher than regular testosterone substitution therapy. Because this patient had no further clinical signs of hypoandrogenism such as anemia or osteoporosis, testosterone treatment did not appear necessary for medical reasons. Although basal serum testosterone was subnormal in this patient, testosterone could be stimulated by hCG. It is known that patients with male pseudohermaphroditism not due to enzyme defects respond to hCG stimulation (19). This patient is married, wanted to start a family and supplied an ejaculate for analysis. Azoospermia was diagnosed. This is also a common finding in patients with androgen resistance (15).
same androgen receptor defect as in patient No. 1 was found in patient No. 2. However, hormone analysis showed normal serum values for testosterone.

Although androgen receptor studies were not performed in patient No. 3, it can be assumed from the history of hypospadias, physical examination and endocrine data that an androgen receptor deficiency is also the cause of the intersexual genitalia, as in his 2 affected brothers. The extremely high FSH level in this patient which cannot be further stimulated by GnRH suggests severely impaired exocrine function of the testes. Neither testis was palpable. However, a positive hCG test showed the presence of tissue. Because of the increased risk of cancer associated with cryptorchid testes (20), exploratory surgery and resection of the testicular tissue with subsequent testosterone substitution is recommended for this patient.

A study of a family with members affected by Reifenstein syndrome showed fertility disorders with increased LH and testosterone values and an androgen receptor defect in cultured fibroblasts in phenotypically male family members (13). However, the fourth, phenotypically normal brother in the present study showed normal values for gonadotropins, testosterone, estradiol and seminal parameters so that an androgen receptor dysfunction is unlikely in this individual.

Because neither gynecomastia nor increased serum testosterone and estradiol values were observed in the three affected brothers, this variation of male pseudohermaphroditism owing to androgen resistance cannot be classified under the term Reifenstein's syndrome. A review of the literature revealed several urological papers reporting a prepenile scrotum syndrome. Shima et al. (21) even observed that the incidence of each anomaly, i.e. prepenile scrotum, undescended testis, male vagina or urticulus masculinus, and hypoplasia of the testis, increases in proportion to the severity of hypospadias. Although these authors suggest the necessity of investigating Leydig cell function in patients with severe hypospadias, no endocrinological data are presented in their study.

The known syndromes of androgen resistance are clinically, endocrinologically and genetically well characterized. The patients presented here show a spontaneous androgen resistance in one family, marked by a quantitative and qualitative androgen receptor deficiency and similar phenotype of incomplete virilization. This molecular defect was demonstrated in 2 of 3 affected brothers. Although this phenotypical form of incomplete virilization has been described before, the current investigation is the first to reveal an androgen receptor disorder as the cause of this type of pseudohermaphroditism.

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References