Molecular Genetics and Pathophysiology of 17β-
Hydroxysteroid Dehydrogenase 3 Deficiency*

STEFFAN ANDERSSON, WAYNE M. GEISSLEK, LING WU, JAPHNE L. DAVIS,
MELVIN M. GRUMBACH, MARIA I. NEW, HANS P. SCHWARZ,
SANDRA L. BLETHEN, BERENICE B. MENDONCA, WALTER BLOISE,
SELMA F. WITCHEL, GORDON B. CUTLER, JR., JAMES E. GRIFFIN,
JEAN D. WILSON, AND DAVID W. RUSSELL

Department of Biochemistry, Merck Research Laboratories (S.A., W.M.G., L.W.), Rahway, New Jersey
07065; Department of Pediatrics, University of California (M.M.G.), San Francisco, California 94143;
the Department of Pediatrics, New York Hospital/Cornell University Medical Center (M.I.N.), New
York, New York 10021; the Department of Pediatrics, Ludwig-Maximilians-University of Munich
(H.F.S.), Munich, Germany; the Department of Pediatrics, State University of New York (S.L.B.), Stony
Brook, New York; the Department of Medicine, University of Sao Paulo (B.B.M., W.B.), Sao Paulo,
Brazil; the Department of Pediatrics, University of Pittsburgh (S.F.W.), Pittsburgh, Pennsylvania;
National Institute of Child Health and Human Development (G.B.C.), Bethesda, Maryland 20892; and
the Departments of Molecular Genetics (D.I.D., D.W.R.) and Internal Medicine (J.E.G., J.D.W.),
University of Texas Southwestern Medical Center, Dallas, Texas 75235

ABSTRACT

Autosomal recessive mutations in the 17β-hydroxysteroid dehydrogenase 3 gene impair the formation of testosterone in the fetal testes and give rise to genetic males with female external genitalia. Such individuals are usually raised as females, but virilize at the time of expected puberty as the result of increases in serum testosterone. Here we describe mutations in 12 additional subjects/families with this disorder. The 14 mutations characterized to date include 10 missense mutations, 3 splice junction abnormalities, and 1 small deletion that results in a frame shift. Three of these mutations have occurred in more than 1 family. Complementary DNAs incorporating

THE ESTABLISHMENT of the male phenotype during
embryogenesis is dependent on two hormones se-
creted by the fetal testes. The peptide hormone Mullerian
inhibiting hormone is responsible for regression of the
Mullerian ducts, and the steroid hormone testosterone regulates development of the male urogenital tract and external geni-
talia (reviewed in Ref. 1). Disorders that disrupt the syn-
thesis or action of testosterone impair male phenotypic de-
velopment and cause the condition of human intersex termed
male pseudohermaphroditism. Defects in androgen action are the most common of these disorders and include muta-
tions in the androgen receptor gene and mutations in the steroid 5α-reductase 2 gene, which encodes an enzyme re-
sponsible for the conversion of testosterone to dihydrotest-
tosterone in the urogenital tract (2). Autosomal recessive mutations have also been described that impair each of the

five enzymatic reactions involved in the conversion of cho-
lesterol to testosterone, including the side-chain cleavage reaction, 3β-hydroxysteroid dehydrogenase (3βHSD), 17α-
hydroxylase/17,20-lyase, and 17βHSD (3). We identified the
defect in the latter disorder as the consequence of mutations in the 17βHSD-3 gene and described six different mutations in five unrelated affected individuals (three homozygotes and two compound heterozygotes) (4).

17βHSD deficiency was originally described by Saez and his colleagues (5, 6); the characteristic phenotype is that of a
46,XY individual with testes and male Wolfian duct-derived urogenital structures (epididymides, vas deferentia, seminal vesicles, and ejaculatory ducts) but female external genitalia (7–9). Such individuals are usually classified as female at birth and raised as females, but at the time of expected puberty, plasma testosterone rises to levels that in some instances approximate the normal male range. As a conse-
quence, many such individuals undergo a marked virilization during the teenage years. Two explanations have been proposed to explain the fact that the impairment of testos-
terone synthesis is more severe during embryogenesis than in later life, namely that testosterone can be formed in ex-
traglandular tissues from the circulating precursor andro-
stenedione that accumulates in the disorder (10) and that the
impairment of the enzyme is incomplete, so the testis can secrete some testosterone (11).

In the present study, we characterized the mutations in 12 additional individuals/families with 17βHSD-3 deficiency and studied the functional consequences of some of the mutant enzymes expressed in reporter cells. Most of these mutations cause either single amino acid substitutions or disrupt splice acceptor sites, and all but 1 of the missense mutations characterized to date impair enzyme function completely. This latter finding is in agreement with studies in 2 of the subjects, in whom we show that negligible amounts of testosterone are secreted by the testes. We conclude that circulating testosterone in most individuals with this disorder is derived from extraglandular conversion of androstenedione to testosterone by 1 or more of the unaffected 17βHSD isoenzymes.

Subjects and Methods

Subjects

Four of the subjects/families shown in Table 1 were previously reported in detail, namely 17HSD-3-Gaza (11-14), 17HSD-3-Sao Paulo 3 (15), 17HSD-3-Syria (10), and 17HSD-3-New York (16). Two others (17HSD-3-Dallas and 17HSD-3-Sao Paulo 1) have been described in capsule form (4). The remaining 11 subjects/families have not previously been reported.

17HSD-3-San Francisco 1. This girl was evaluated at University of California-San Francisco at age 15 yr. The external genitalia had been considered normal at birth, but at 7 months of age she was noted to have a mass in the right labia majora. Beginning at age 13 yr, she noted breast enlargement, deepening of the voice, and enlargement of the clitoris. The family history was uninformative, and two siblings were normal. On physical examination she had facial hirsutism, 6 x 6-cm breast tissue, a 3 x 1.5-cm clitoris, scrotalization of the labia majora, and a blind-ending vagina. The karyotype was 46,XY. Endocrine findings are summarized in Table 2. Gonadectomy was performed; the testes measured 3 x 2 cm, and by microscopic examination the seminiferous tubules contained mostly Sertoli cells and a few germ cells. DNA was prepared from tissue blocks.

17HSD-3-San Francisco 2. This girl was thought to be normal at birth, but was evaluated at age 13 yr at University of California-San Francisco because of a deepening voice, hirsutism, male musculature, and an enlarged clitoris. The family history was uninformative, and a 15-yr-old sister was normal. Breasts were Tanner stage II, and pubic hair was Tanner stage IV. The vagina was blind-ending, and masses (3.5 x 2.5 cm) were palpable in the labia majora. The karyotype was 46,XY. Plasma androgen levels are summarized in Table 2. After extensive psychiatric and social evaluation, she underwent bilateral gonadectomy and partial resection of the clitoris; spermatic venous blood was obtained at the time of surgery, and the levels of testosterone and androstenedione were compared with those previously reported in normal men (17,18) (Table 2). The epididymides were hypoplastic. The seminiferous tubules contained Sertoli cells but no germ cells. DNA was prepared from tissue blocks.

17HSD-3-Munich. This female infant was thought to be normal until age 3 weeks, when she was found to have masses in the labia majora bilaterally; these masses were shown by biopsy to be testes. The karyotype was 46,XY. A hCG stimulation test was performed at 6 months of age; plasma testosterone rose from less than 20 to 26 ng/dL, and plasma androstenedione rose from 50 to 161 ng/dL. Bilateral gonadectomy was performed at age 8 months. DNA was prepared from peripheral blood.

17HSD-3-Stony Brook. This newborn infant with ambiguous genitalia was the product of a 35-week twin gestation; the other twin is 46,XX and has a normal female phenotype. The parents are first cousins of German descent; a 12-yr-old half-brother is normal. On physical examination the
infant had clitoromegaly (1.5 cm) and posterior fusion and scrotalization of the labia majora, which contained palpable masses bilaterally. The karyotype was 46,XY. At 9 h of life, serum testosterone was 86 ng/dL, dihydrotestosterone was 12 ng/dL, and androstenedione was 215 ng/dL. DNA was prepared from peripheral blood.

17HSD-3-San Francisco 1. This 16-yr-old Iranian girl was evaluated at Wilfred Hall U.S. Air Force Hospital because of hirsutism, clitoromegaly, and failure to menstruate. The karyotype was 46,XY. A diagnosis of 17βHSD-3 deficiency was made on the basis of a male level of testosterone and an elevated level of androstenedione (exact values not available). DNA was prepared from cultured skin fibroblasts.

17HSD-3-Bethesda. This girl was thought to be normal until age 10 yr, when a testis was discovered in a right herniorrhaphy sac. Breast enlargement was noted by age 13 yr, but because of failure to menstruate she was evaluated at age 15 yr in Portland, ME, where a diagnosis of 17βHSD-3 deficiency was made. This diagnosis was confirmed at the NIH, where she was found to have a serum testosterone level of 221 ng/dL and a serum androstenedione level of 1031 ng/dL. The parents are first cousins, and she has two brothers with ambiguous genitalia. On physical examination she had a deep voice; coarse hair over the lip, chin, and abdomen; clitoromegaly (8 X 2 cm); a blind-ending vagina (6 cm in depth); and no palpable breast tissue. A 4 X 2.5-cm mass was palpable in the left inguinal canal. Serum testosterone was 620 ng/dL, and serum androstenedione was 980 ng/dL; these values increased to 810 and 1810 ng/dL, respectively after HCG administration and did not change significantly after 4 days of dexamethasone administration. Serum LH was 38 U/L, and serum FSH was 51 U/L. The decision was made to remove the testes. At surgery, the testes measured 3 X 1.8 cm with separate epididymis and vas deferens. A diagnosis of Gartner’s duct cyst was made. DNA was prepared from cultured skin fibroblasts.

17HSD-3-Sao Paulo 2. This woman was evaluated at age 21 yr because of failure of breast development and deepening of the voice. No breast tissue was palpable. The clitoris measured 4.5 X 1 cm, and 3 X 1.4-cm masses were present in the inguinal areas. The vagina was blind-ending and shallow. The karyotype was 46,XY. Serum testosterone was 330 ng/dL, and androstenedione was 1010 ng/dL. Serum LH was 27 U/L, and serum FSH was 29 U/L. After psychological and psychiatric examination, bilateral orchidectomy and clitoral resection were performed. Histological examination of the testes revealed abundant Leydig cells and seminiferous tubules that contained Sertoli cells but no germ cells. She is currently receiving estrogen replacement therapy. DNA was prepared from peripheral blood.

17HSD-3-Sao Paulo 4. This subject was born with ambiguous genitalia and was registered and raised as a female until age 10 yr, at which time he began to function as a male. By age 16 yr, increased body hair, male pattern muscle development, and penile growth were prominent. At age 21 yr, psychological testing confirmed that he had a male gender identity. He is the offspring of a first cousin marriage and is one of seven siblings, including a similarly affected younger brother. Physical examination revealed a male habitus, no palpable breast tissue, and male pattern facial and chest hair. The penis measured 6.8 X 2.5 cm with perineoscrotal hypospadias; the scrotum was bifid, and a blind-ending vagina was present. Testes (5 X 3 cm) were palpable in the inguinal canals. Endocrine findings included serum testosterone of 285 ng/dL and serum androstenedione of 480 ng/dL; these values increased to 525 and 1800 ng/dL, respectively after HCG administration and did not change significantly after 4 days of dexamethasone administration. Serum LH was 38 U/L, and serum FSH was 64 U/L. He subsequently underwent surgical repair of the hypospadias.

17HSD-3-Dallas. This 17-yr-old girl with known hemoglobinopathy (SC disease) was evaluated because of clitoromegaly (4 cm) and failure to menstruate. The family history was uninformative. On physical examination a blind-ending vagina was present, testes were palpable in the inguinal canals bilaterally, no breast tissue was palpable, and there was no hirsutism. No uterus was identified by ultrasonography. The karyotype was 46,XY. Serum testosterone was 740 ng/dL, serum androstenedione was 880 ng/dL, and serum dihydrotestosterone averaged 35 ng/dL. Serum LH was 27 U/L, and FSH was 51 U/L. The decision was made to remove the testes. At surgery, the testes measured 3 X 1.8 cm with separate epididymis and vas deferens. A diagnosis of Gartner’s duct cyst was made. DNA was prepared from cultured skin fibroblasts. Subject 2 was evaluated at age 12 yr. an enlarged clitoris in an otherwise normal female infant had been noted at birth, but she was not evaluated until the sister was diagnosed. No breast tissue was palpable. The clitoris measured 4 X 1 cm, and the blind-ending vagina was 2.5 cm in depth. Tests-like masses (4 X 2 cm) were palpable in the inguinal regions. The karyotype was 46,XY. The endocrine findings are shown in Table 2. Bilateral orchidectomy was performed; the seminiferous tubules contained Sertoli cells and rare germ cells. She was treated with conjugated estrogens and medroxyprogesterone. DNA was prepared from cultured skin fibroblasts. Subject 2 was evaluated at age 12 yr. an enlarged clitoris in an otherwise normal female infant had been noted at birth, but she was not evaluated until the sister was diagnosed. No breast tissue was palpable. The clitoris measured 4 X 1 cm, and the blind-ending vagina was 2.5 cm in depth. Tests-like masses (4 X 2 cm) were palpable in the inguinal regions. The karyotype was 46,XY. The endocrine findings are shown in Table 2. Bilateral orchidectomy was performed; the seminiferous tubules contained Sertoli cells and rare germ cells. She was treated with conjugated estrogens and medroxyprogesterone. DNA was prepared from cultured skin fibroblasts.
ng/dL after hCG administration and changed very little after dexamethasone administration. LH was 29 U/L, and FSH was 30 U/L. After careful psychological testing she underwent bilateral orchidectomy (the right testis was intraabdominal) and clitoral resection. Histological examination of the testes revealed abundant Leydig cells and seminiferous tubules that contained predominantly Sertoli cells. She now receives estrogen replacement. DNA was prepared from peripheral blood.

**17HSD-3-Pittsburgh.** This 13-year-old girl was referred to the Children’s Hospital of Pittsburgh because of clitoromegaly and consennting of the voice. The family history was incomplete, but she has four normal brothers. Physical examination revealed no palpable breast tissue, clitoromegaly (4 × 3 cm), scrotalization of the labia majora, and bilateral masses in the inguinal regions. Karyotype was 46,XY. Serum androstenedione was 512 ng/dL, and serum testosterone was 212 ng/dL. After KCl stimulation, the androstenedione level was 817 ng/dL, and testosterone was 344 ng/dL. After LH-releasing hormone administration, the androstenedione level was 675 ng/dL and testosterone was 377 ng/dL. After careful evaluation, the decision was made to remove the gonads and administer estrogen. The right undescended testis exhibited Leydig cell hyperplasia and seminiferous tubules lined with Sertoli cells, but no germ cells, and the left partially descended testis had tubules with germ cell hyperplasia and a maturation arrest. DNA was prepared from peripheral blood.

**17HSD-3-Indianapolis.** This infant was noted at birth to have perineal hypospadias with a single orifice on the perineum and a bifid scrotum with rugated labioscrotal folds. The karyotype was 46,XX. After a hCG stimulation test at 4 months of age, the plasma testosterone level was 430 ng/dL, and the plasma dihydrotestosterone level was 12 ng/dL. After hCG administration the androstenedione level was 512 ng/dL, and serum testosterone was 212 ng/dL. After LH-releasing hormone administration, the androstenedione level was 675 ng/dL and testosterone was 377 ng/dL. After careful evaluation, she underwent bilateral orchidectomy (the right testis was intraabdominal) and clitoral resection. Histological examination of the testes revealed abundant Leydig cells and seminiferous tubules with germ cell hyperplasia and a maturation arrest. DNA was prepared from peripheral blood.

**Genomic DNA isolation**

Genomic DNA was extracted from cultured fibroblasts and white blood cells as described previously (19) using an Applied Biosystems model 340A Nucleic Acids Extractor. In some cases genomic DNA was extracted from embedded and formalin-fixed tissue specimens. Briefly, small sections of the tissue block were deparaffinized with xylene and then rehydrated by rinsing in a graded series of ethanol solutions. The tissue was treated twice with proteinase K (250 µg/mL in 10 mmol/L Tris-Cl (pH 8.0) and 1 mmol/L ethylene-diamine-tetraacetic acid-1% (wt/vol) SDS; 55 C; 1-16 h), and DNA was isolated using a kit (IsoQuick Nucleic Acid Extractor Kit, MicroProbe Corp., Garden Grove, CA). The purified DNA was then used as a template in PCR, as described below.

**TABLE 3.** Sequence and locations of oligonucleotides in 17βHSD3 gene used for PCRs

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Location</th>
<th>Amplification target</th>
<th>Sequence 5’ → 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5’-Untranslated, Exon 1</td>
<td>Exon 1</td>
<td>ACGGCCAGGGCTGAAACAGTGCCTGTT</td>
</tr>
<tr>
<td>2</td>
<td>Intron 1</td>
<td>Exon 2</td>
<td>TGAATTCTGCTTTTTAAAAGCA</td>
</tr>
<tr>
<td>3</td>
<td>Intron 1</td>
<td>Exon 3</td>
<td>GCTCATCATTCTTGGTCTTGGTT</td>
</tr>
<tr>
<td>4</td>
<td>Intron 2</td>
<td>Exon 4</td>
<td>GAGGGTCCACACACATCCTCCCTTA</td>
</tr>
<tr>
<td>5</td>
<td>Intron 2</td>
<td>Exon 5</td>
<td>TGATCCTGCTTCACTTGACACATTTTGG</td>
</tr>
<tr>
<td>6</td>
<td>Intron 3</td>
<td>Exon 6</td>
<td>AGCCAGGCGGGACGACACCACCCGG</td>
</tr>
<tr>
<td>7</td>
<td>Intron 3</td>
<td>Exon 7</td>
<td>AGTTCTCTGCTGCGCTTACCTTTGG</td>
</tr>
<tr>
<td>8</td>
<td>Intron 4</td>
<td>Exon 8</td>
<td>AGGGCGAGGGCCATGCTGCTCA</td>
</tr>
<tr>
<td>9</td>
<td>Intron 4</td>
<td>Exon 9</td>
<td>AGCTCATCTGCTTGGAACAGCTGAC</td>
</tr>
<tr>
<td>10</td>
<td>Intron 5</td>
<td>Exon 10</td>
<td>GATGATTGCTTCCTGTCCAGACAGATT</td>
</tr>
<tr>
<td>11</td>
<td>Intron 5</td>
<td>Exon 11</td>
<td>TTCAAGGAAAGGAGGAGTT</td>
</tr>
<tr>
<td>12</td>
<td>Intron 6</td>
<td></td>
<td>GAGAACTCTGACGCTTACCTTTG</td>
</tr>
<tr>
<td>13</td>
<td>Intron 6</td>
<td></td>
<td>GAGGAAGGAGGAGGAGGTGCTT</td>
</tr>
<tr>
<td>14</td>
<td>Intron 7</td>
<td></td>
<td>ACGGCCAGGGCTGAAACAGTGCCTGTT</td>
</tr>
<tr>
<td>15</td>
<td>Intron 7</td>
<td></td>
<td>TGAATTCTGCTTTTTAAAAGCA</td>
</tr>
<tr>
<td>16</td>
<td>Intron 8</td>
<td></td>
<td>GCTCATCATTCTTGGTCTTGGTT</td>
</tr>
<tr>
<td>17</td>
<td>Intron 8</td>
<td></td>
<td>GAGGGTCCACACACATCCTCCCTTA</td>
</tr>
<tr>
<td>18</td>
<td>Intron 9</td>
<td></td>
<td>TGATCCTGCTTCACTTGACACATTTTGG</td>
</tr>
<tr>
<td>19</td>
<td>Intron 9</td>
<td></td>
<td>AGCCAGGCGGGACGACACCACCCGG</td>
</tr>
<tr>
<td>20</td>
<td>Intron 10</td>
<td></td>
<td>AGTTCTCTGCTGCGCTTACCTTTGG</td>
</tr>
<tr>
<td>21</td>
<td>Intron 10</td>
<td></td>
<td>AGGGCGAGGGCCATGCTGCTCA</td>
</tr>
<tr>
<td>22</td>
<td>3’-Untranslated, exon 11</td>
<td>Exon 11</td>
<td>TTCAAGGAAAGGAGGAGTT</td>
</tr>
</tbody>
</table>

**Mutation detection**

Mutations in the 17HSD 3 gene were detected by amplification of individual exons using the PCR and single strand DNA conformation polymorphism analysis, as previously described (4). The DNA sequences of the oligonucleotide primers used to amplify the 11 exons of the gene are shown in Table 3. Exons 1, 3, 4, 5, 7, 9, and 11 were amplified with a thermocycler program consisting of 30 cycles of 94 C for 30 s, 65 C for 15 s, and 72 C for 30 s. Exons 2, 6, 8, and 10 were amplified using a thermocycler program consisting of 30 cycles of 94 C for 30 s, 62 C for 15 s, and 72 C for 30 s. The nucleotide sequence of exons suspected of harboring mutations on the basis of single strand DNA conformation polymorphism analysis were determined by cycle sequencing using a thermostable DNA polymerase (20).

**Expression analysis in transfected 293 cells**

Site-directed mutagenesis reactions in which individual missense mutations were introduced into an expressible complementary DNA (cDNA) vector were performed with a Mutagen Kit (Bio-Rad Laboratories, Richmond, CA). The mutagenic oligonucleotide primers were 21 bases in length. Manipulated DNA fragments were subjected to DNA sequence analysis and then transfected into cultured human embryonic kidney 293 cells (CRL 1573, American Type Culture Collection, Rockville, MD) using a calcium phosphate precipitation protocol (21). Forty-eight hours after transfection, cells were analyzed for 17βHSD activity as previously described (4, 22).

Immunoblotting of transfected cell extracts was carried out using an antipeptide antibody directed against residues 303–310 of the 17βHSD 3 isoenzyme. A polyclonal antibody was raised in rabbits by standard methods (23) after coupling the synthetic peptide to bovine thyroglobulin. Peptide-specific antibodies were purified by chromatography on antigen-agarose beads and used directly in the immunoblotting reactions. Aliquots of transfected cell extracts (5 µg protein) were separated by electrophoresis through 17% (wt/vol) polyacrylamide gels containing 0.1% (wt/vol) SDS. Separated proteins were transferred to nitrocellulose filters (Novex Corp., San Diego, CA) by electroblotting and subjected to a standard immunoblotting protocol (23). Enhanced chemi...
Results

Subjects

The geographical, clinical, and genetic characteristics of the subjects studied are summarized in Table 1; these individuals comprise about 45% of all subjects reported to date with 17βHSD deficiency. The affected individuals are from 17 families and represent diverse ethnic groups. In all instances, except 1, the diagnosis of 17βHSD deficiency was based on characteristic clinical features, namely 46,XY males with pseudohermaphroditism, measurable or male serum testosterone levels, and elevated androstenedione levels. In 1 instance (17HSD-3-Indianapolis), an infant with ambiguous genitalia was misdiagnosed as having 5α-reductase 2 deficiency, and the correct diagnosis was only made after molecular genetic analysis. Interestingly, only a single mutant allele was identified in the 17βHSD-3 gene in this individual; we assume that the other allele probably contains a mutation outside the coding sequence of the gene, as heterozygous males with other mutations of the gene are phenotypically and endocrinologically normal. Alternatively, the individual might be a heterozygous carrier of the mutation and have some unidentified cause of ambiguous genitalia. Of the subjects in Table 1, all have been raised as female except for 17HSD-3-Stony Brook, who is being raised as a male, and 17HSD-3-Gaza, some of whom are raised as males and some as females (11–14).

In two subjects studied at the University of California-San Francisco, androstenedione and testosterone were measured in spermatic venous blood obtained by cannulation at the time of orchidectomy, and the levels were compared with normal spermatic vein measurements from the same laboratory (Table 2). Androstenedione levels in spermatic venous blood were elevated 15- to 20-fold, and testosterone levels were decreased 15- to 70-fold. The testosterone to androstenedione ratio, on the other hand, was decreased 15- to 70-fold. The testosterone to androstenedione ratio, on the other hand, was decreased 15- to 70-fold.

Mutations

The mutations in this cohort are summarized in Table 1 and Fig. 1. Fourteen different mutations have been identified in 12 homozygotes, 4 compound heterozygotes, and 1 presumed compound heterozygote. These mutations occur in or affect the splicing of 7 exons and consist of 1 small deletion, 3 splice site mutations, and 10 missense mutations that cause single amino acid substitutions in the protein. Three of these mutations were identified in more than 1 family. The R80Q mutation, originally identified in homozygous form in 17HSD-3-Gaza and in heterozygous form in 17HSD-3-Sao Paulo 1 (4), was found in homozygous form in 17HSD-3-Sao Paulo 3. Likewise, the 655-1, G—A splice acceptor site mutation previously described in 17HSD-3-Syria (4) is present in homozygous form in 17HSD-New York, and the 325+4, A—T splice acceptor site mutation was present in homozygous form in 3 families and in heterozygous form in 2 others (Table 1).

Expression analysis of missense mutations

To confirm that the coding sequence mutations are deleterious, five of the missense mutations that cause single amino acid substitutions (S65L, V205E, F208I, E215D, and P282L) were introduced into expressible cDNA vectors by site-directed mutagenesis, and the enzyme activity was assessed after transfection into cultured cells by measuring the conversion of radioactive androstenedione to testosterone (Fig. 2A). The fact that the mutant cDNAs were expressed in the transfected cells was shown by immunoblotting using an antibody directed to a specific epitope of 17βHSD-3 (Fig. 2B). In each of the mutations studied, the substitution mutation appeared to inactivate enzyme activity almost completely (Fig. 2A). Thus, of the nine substitution mutations analyzed by this technique to date (4), only the R80Q mutation appears to possess residual enzyme activity when studied in this manner, namely approximately 20% of the normal activity.

DISCUSSION

17βHSD-3 deficiency is a rare autosomal recessive cause of male pseudohermaphroditism. The typical subject with this disorder is a 46,XY male who is born with a female external phenotype (but with testes located in the inguinal canals or labia majora) and who undergoes considerable virilization at the time of expected puberty associated with elevated levels of blood androstenedione and either low or normal male...
levels of testosterone (7–9). [Occasional individuals have ambiguous genitalia at birth (24), and two late-onset variants of uncertain pathophysiology have been described, one manifest by gynecomastia in boys (25, 26) and the other as poly cystic disease in women (27).]

It has been recognized for approximately 25 yr that this disorder is the result of a defect in the final step in testosterone synthesis in the testes, namely the conversion of androstenedione to testosterone (5, 6). We have shown that the molecular basis of this disorder is any of several mutations in the 17βHSD-3 gene, a gene that appears to be expressed predominately or exclusively in testes (4). In the present report we describe 9 additional mutations in this gene to make a total of 14 different mutations in 17 subjects/families. Twelve are present in homozygous form, 4 are compound heterozygotes, and 1 is a presumed compound heterozygote.

These mutations consist of a 7-nucleotide deletion that causes a frame shift, 3 splice site abnormalities, and 10 missense mutations that cause amino acid substitutions. Three of these mutations (R80Q, 655–1, G→A, and 325+4,A→G) have been identified in more than 1 family; we do not know at present whether this recurrence is due to a founder effect or to recurrent new mutations.

Two characteristic features of the disorder are particularly puzzling: 1) the defect in virilization and the deficiency in testosterone synthesis are usually more complete during embryogenesis than in later life, implying that an alternate route for testosterone synthesis must be activated at the time of expected puberty; and 2) the external genitalia at birth are usually female in character, but the Wolffian duct structures (epididymides, vasa deferentia, and seminal vesicles) virilize normally, indicating that androgen must act by an alternate mechanism in these tissues in utero.

The findings in the present study provide insight into the source of circulating testosterone after the time of expected puberty in this disorder. Two theories have been proposed to explain this phenomenon. First, Akesode and his colleagues (10) concluded on the basis of studies of the metabolism of radioactive precursors in a single affected subject (17HSD-3-Syria) that testosterone is formed from circulating androstenedione by extraglandular conversion. Second, on the basis of studies of the hormone concentrations in testicular venous blood from several related subjects from the Gaza strip (17HSD-3-Gaza), Eckstein et al. (11) demonstrated that considerable amounts of testosterone can be secreted by the testes of affected members of that kindred. Using site-directed mutagenesis and transient transfection assays, we demonstrated that eight of the nine missense mutations inactivate enzyme activity almost completely. Furthermore, in two of the individuals with inactive missense mutations, assessment of testosterone and androstenedione levels in testicular venous blood indicated that these individuals secrete only 5% as much testosterone from the testes as normal controls. This finding is in keeping with the fact that the expressed enzyme from the subject described by Akesode et al. (10) (17HSD-3-Syria) was also inactive when transiently expressed in reporter cells (4). As the other mutations characterized to date, the frame-shift mutation and the splice acceptor site abnormalities, are predicted to preclude the formation of functional protein, we conclude that the common mechanism for testosterone synthesis (and for the resulting virilization) after the time of expected puberty in this disorder is by extraglandular formation. It is of interest in this regard that in the one exception to this generalization (subjects from the Gaza family who carry the R80Q mutation), not only was testosterone secreted by the testes (11), but in addition, the expression of the reconstructed DNA containing this mutation caused the formation of an enzyme with about 15–20% of normal enzyme activity at high substrate concentrations (4).

Whether the enzyme responsible for extraglandular conversion of androstenedione to testosterone is isoenzyme 1 or 2 or some other 17βHSD enzyme is, at present, unclear, nor is it understood why this process seems to be more efficient in later life than during embryogenesis. The enhancement of testosterone synthesis at the time of expected puberty may be the consequence of the increased output of androstenedione by the testes after the pubertal surge of LH secretion and and...
hence, the availability of more substrate for testosterone synthesis in extraglandular sites by other isoenzymes.

The present studies also leave unexplained the virilization of the Wolffian ducts in these subjects during fetal life. At the simplistic level, virilization in the absence of circulating testosterone could be due to the presence in the tissue of an embryonic receptor mechanism that lacks the specificity of the mature androgen receptor or to the presence in the tissue of an alternate mechanism for the conversion of circulating androstenedione. So far as can be ascertained, the embryonic Wolffian duct androgen receptor is identical to the receptor in other tissues (28), and we, therefore, conclude that this tissue must contain an alternate pathway for testosterone formation, possibly the same 17βHSD responsible for extraglandular testosterone formation in later life. The mechanism by which the Wolffian ducts virilize in this disorder is now under scrutiny.

Extraglandular testosterone formation may also be responsible for one other unexpected finding in the present study. Namely, 1 of the 16 homozygotes/compound heterozygotes in the present study (17HSD-3-Stony Brook) had clear-cut ambiguous genitalia at the time of birth, implying considerable testosterone formation during embryogenesis. It was, therefore, unexpected that when the reconstructed DNA containing this mutation (F208D) was expressed in reporter cells, the mutant 17βHSD was totally inactive. It is possible that there is considerable genetic variability among individuals in the capacity for extraglandular testosterone formation.

References

5. Saez JM, de Peretti E, Moreira AM, David M, Bertrand J. 1979 Familial male pseudohyperplasmidism with gynecomastia due to a testicular 17-ketosteroid reductase defect. J Clin Endocrinol Metab. 32:604-610.
6. Saez JM, Moreira AM, de Peretti E, Bertrand J. 1972 Further in vivo studies in male pseudohyperplasmidism with gynecomastia due to a testicular 17-ketosteroid reductase defect (compared to a case of testicular feminization). J Clin Endocrinol Metab. 34:598-600.