Steroid 5α-Reductase Deficiency in Man: An Inherited Form of Male Pseudohermaphroditism

Abstract. *In male pseudohermaphrodites born with ambiguity of the external genitalia but with marked virilization at puberty, biochemical evaluation reveals a marked decrease in plasma dihydrotestosterone secondary to a decrease in steroid 5α-reductase activity. In utero the decrease in dihydrotestosterone results in incomplete masculinization of the external genitalia. Inheritance is autosomal recessive.*

Significant progress has been made toward defining the role of androgens in sexual differentiation and development in the past 25 years. Jost in pioneer experiments on rabbit fetuses demonstrated that female organogenesis, that is, Mullerian stimulation and Wolffian inhibition, will occur in the absence of the gonads (*1*). Male sexual differentiation is imposed upon the natural tendency of the fetus toward femaleness. Normal male sexual differentiation requires the secretion of two factors by the testes. At a critical period of embryogenesis, testosterone, secreted by the Leydig cells, stimulates differentiation of the Wolffian anlage to the epididymis, vas deferens, and seminal vesicles, and differentiation of the urogenital sinus, urogenital tubercle, and urogenital swellings to form the male external genitalia and prostate. Mullerian inhibition, however, is not mediated by androgens, but results from the action of Mullerian inhibiting factor, probably secreted by the seminiferous tubules (*2*).

Within the last 10 years, investigators have shown that testosterone may act as a prehormone, that is, in specific androgen-dependent target areas, it is converted by the microsomal enzyme Δ^4-steroid 5α-reductase to form 5α-dihydrotestosterone, a more potent androgen (*3*). It has been demonstrated in human fetuses that, at the time of sexual differentiation in utero, dihydrotestosterone formation occurs in the urogenital sinus, urogenital tubercle, and urogenital swellings, but dihydrotestosterone formation does not occur in the Wolffian anlage until after differentiation has occurred (*4*).

The data suggest that there may be at least two androgens involved in sexual differentiation, with selective roles for testosterone and dihydrotestosterone during embryogenesis. The male pseudohermaphrodites described below define the necessity for dihydrotestosterone during embryogenesis and delineate the actions of testosterone and dihydrotestosterone in sexual differentiation and development.

To date we have found 13 families with 24 male pseudohermaphrodites, in the village of Salinas in the Dominican Republic (*5*). The affected males (46 XY) (*6*) are born with marked ambiguity of the external genitalia, and before the disorder became obvious to the community were raised as girls. At birth, they have bilateral testes presenting as inguinal or labial masses, a labial-like scrotum, a urogenital sinus with a blind vaginal pouch, and a clitoral-like phallus. No Mullerian structures are present.

At puberty, their voice deepens and they develop a typical male phenotype with a substantial increase in muscle mass; there is no breast enlargement. The phallus enlarges to become a functional penis, and the change is so striking that these individuals are referred to by the townspeople as "guevedoces" —penis at 12 (years of age). The scrotum becomes rugated and hyperpigmented, the testes descend from the inguinal canal, and there is an ejaculate. The prostate remains small, beard growth is scanty, and there is no temporal recession of the hairline or acne. Psychosexual orientation is unequivocally male. Testicular biopsy demonstrates complete spermatogenesis, with normal Leydig cells. There is a normal epididymis and vas deferens.

Thus, at birth the defect is limited to incomplete differentiation of the male extrenal genitalia; masculinization of the internal structures is normal. At puberty, virilization occurs with the exception of a scanty or absent beard, lack of temporal recession of hairline, and a small to absent prostate.

Because of the virilization at puberty, and despite marked ambiguity of the external genitalia at birth, we hypothesized that the affected individuals would not have a disorder of testosterone biosynthesis. The male puberty without breast development and with complete spermatogenesis also precludes a defect due to impaired androgen action. We proposed, therefore, that the abnormality was due most likely to a defect in the metabolism of testosterone at the target issue, that is, biotransformation of testosterone to 5α-dihydrotestosterone by the enzyme Δ^4-steroid 5α-reductase (*7*).

To define a defect in 5α-reductase activity, plasma testosterone and 5α-dihydrotestosterone were measured in four affected males by a double isotope derivative technique (*8*). In the affected males the plasma testosterone

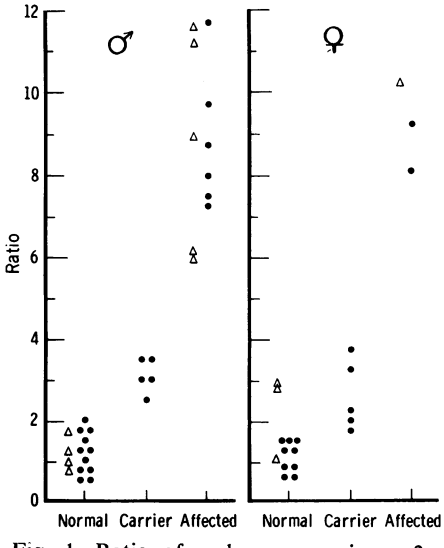

Fig. 1. Ratio of endogenous urinary 3α, 5β-etiocholanolone to 3α,5α-androsterone (●) and 3α,5β-etiocholanediol to 3α,5α-androstanediol (△) in males and females —that is, normals, carriers, and affected.

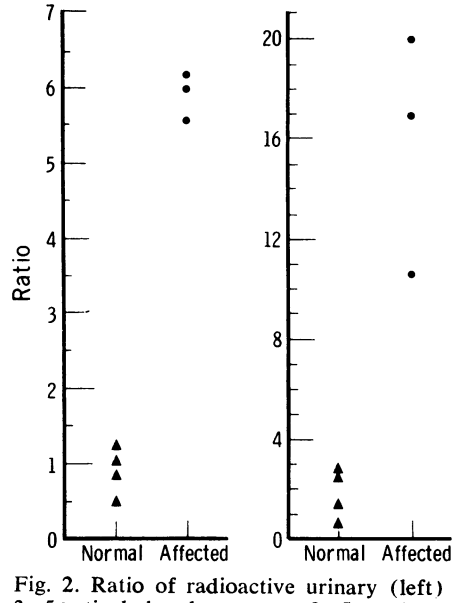

Fig. 2. Ratio of radioactive urinary (left) 3α,5β-etiocholanolone to 3α,5α-androsterone and (right) 3α,5β-etiocholanediol to 3α,5α-androstanediol after [^{3}H]testosterone infusion in normal (▲) and affected (●) males.

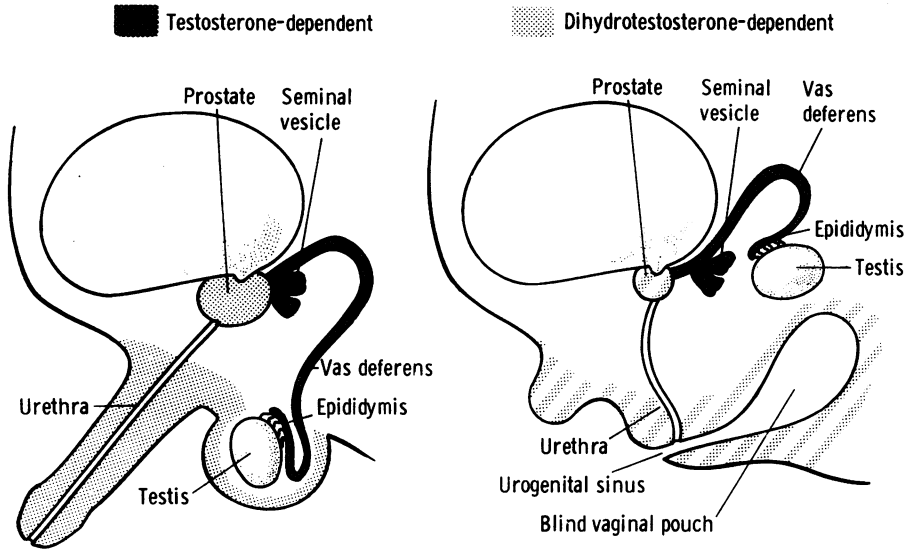

Fig. 3. Illustration of the hypothesis for the role of testosterone and dihydrotestosterone in male sexual differentiation in utero.

concentration ranged from 470 to 960 ng per 100 ml, which was within the normal male range of 300 to 1200 ng per 100 ml. However, dihydrotestosterone concentrations were 16, 17, 21, and 29 ng per 100 ml, which were below the normal male range of 40 to 80 ng per 100 ml. The ratio of plasma testosterone to dihydrotestosterone in normal males was approximately 14/1, and in the affected males it was approximately 40/1.

In two affected males, the percentage conversion of testosterone to 5α-dihydrotestosterone was measured during continuous infusion of radioactive testosterone (*9*). The percentage conversion was 0.48 and 0.85, and was approximately one-sixth of the reported normal range of 3.5 to 7.0.

Reduction of the double bond between rings A and B of neutral steroids, such as testosterone, is catalyzed in the liver by Δ^4-steroid 5β-reductase (or reductases) localized to the cytosol, and the Δ^4-steroid 5α-reductase (or reductases) of the membranes of the endoplasmic reticulum. However, a substantial fraction of testosterone is metabolized in extrahepatic tissue (*10*), and most, if not all, proceeds to the *trans* or 5α configuration (*11*).

In normal, affected, and obligate carriers, we measured the C_{19},11-deoxysteroids—that is, the 17-ketosteroid metabolites 3α,5β-etiocholanolone and 3α,5α-androsterone, and the 17β-hydroxy metabolites 3α,5β-etiocholanediol and 3α,5α-androstanediol.

In both postpubertal normal and affected subjects, the urinary 17-ketosteroids 3α,5α-androsterone and 3α,5β-etiocholanolone were fractionated by an isotope dilution technique with the use of β-glucuronidase hydrolysis. Tritium-labeled androsterone and etiocholanolone were added to the urine samples (30 ml) before hydrolysis to correct for procedural losses. The steroids were purified by paper chromatography, and quantitated by the Zimmerman reaction.

After glucuronidase hydrolysis of the urines of 11 normal males, the 5β/5α ratios of the urinary 17-ketosteroids etiocholanolone and androsterone, were 0.5 to 2.0 with a mean of 1.2 (Fig. 1). In six affected males, the mean ratio was 8.5, with a range of 7.3 to 11.8.

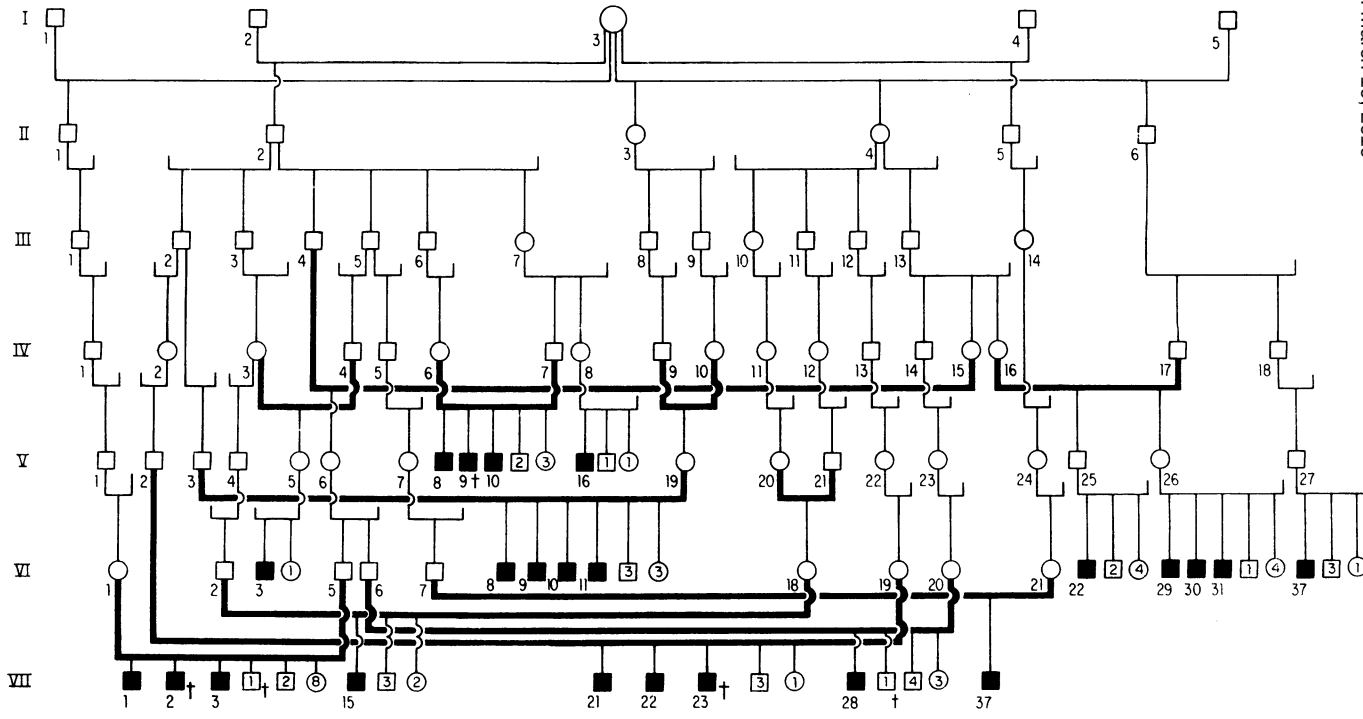

Fig. 4. Pedigree illustrating common ancestry in 12 of the 13 families, and transmission of the defect for male pseudohermaphroditism through seven generations.

Five obligate carriers (fathers) showed an intermediate range of 2.5 to 4.3, with a mean ratio of 3.5. Nine normal females had a ratio of 0.7 to 1.6, with a mean ratio of 1.1. Two phenotypically normal females, from a family with three affected males, had ratios of 8.3 and 9.6, and are homozygous for the condition. In five obligate carriers (mothers), the ratio was 1.8 to 3.7, with a mean of 2.5.

The urinary 17β-hydroxysteroid glucuronides 3α,5α-androstanediol and 3α,5β-etiocholanediol were determined by a double isotope derivative procedure on 10-ml samples of urine, with the use of ^{14}C-labeled steroids to correct for procedural losses and tritium-labeled acetic anhydride (25 mc/mmole) to measure mass.

The 5β/5α ratio of the urinary glucuronides of etiocholanediol and androstanediol in four normal males and three normal females ranged from 0.8 to 3.0. In five affected males, the ratio was 6.0 to 11.8; in one phenotypically normal female with an abnormal 5β/5α ratio of urinary 17-ketosteroids, the ratio was 10.2.

[^{3}H]Testosterone was infused into four normal and three affected males (Fig. 2). After the urine was treated with glucuronidase and hydrolyzed with hot acid, the 5β/5α ratios of etiocholanolone to androsterone in the normal males ranged from 0.5 to 1.13 with a mean of 0.87 and from 5.5 to 6.4 in the affected males. In normals, the urinary 5β/5α ratio of etiocholanediol to androstanediol was 0.46 to 2.7; and in the affected males, 11.0 to 20.

Thus, analysis of the 5β- and 5α-11-deoxy C_{19}-steroid metabolites (both endogenous and radioactive) revealed a marked decrease in 5α reduced metabolites in affected males (*12*), and an intermediate decrease in obligate carriers.

The above studies demonstrate a defect in 5α reduction resulting in the decreased conversion of testosterone to dihydrotestosterone. Whether the biochemical error involves the synthesis, structure, or metabolism of the enzyme Δ^4-steroid 5α-reductase is not known.

From the clinical presentation of ambiguous external genitalia with normal male internal structures and the biochemical data demonstrating Δ^4-steroid 5α-reductase deficiency with decreased dihydrotestosterone formation, we hypothesize that during embryogenesis and again at puberty, both testosterone and dihydrotestosterone are necessary for complete male external differentiation and development (Fig. 3). Testosterone secreted in utero by the testes acts directly on the Wolffian ducts to cause differentiation to the vas deferens, epididymis, and seminal vesicles; but in the urogenital sinus and urogenital tubercle, testosterone functions as a prehormone, where its conversion to dihydrotestosterone results in differentiation of the external genitalia and prostate.

The anabolic events at puberty, in particular the increase in muscle mass, the growth of the phallus and scrotum, and the voice change, appear to be mediated by testosterone and occur in the affected males (*11, 13*). Prostate growth, facial hair, temporal recession of the hairline, and acne do not occur and appear to be mediated by dihydrotestosterone (*14*).

Psychosexual orientation (postpubertal) is male, and this is of considerable interest, since the sex of rearing in 18 of the affected males was female. Despite the sex of rearing, the affected were able to change gender identity at the time of puberty. They consider themselves as males and have a libido directed toward the opposite sex. Thus, male sex drive appears to be testosterone related and not dihydrotestosterone related (*15*), and the sex of rearing as female, appears to have a lesser role in the presence of two masculinizing events—testosterone exposure in utero and again at puberty with the development of a male phenotype.

Salinas (population 4300), is a geographic isolate 150 miles (1 mile = 1.6 km) west of Santo Domingo, Dominican Republic. Within the village, the frequency of normal to affected males is aproximately 90/1. The affected males range in age from 1½ to 60 years. Figure 4 is a pedigree illustrating the transmission of the defect through seven generations. In 12 of the 13 families, one line of descent can be traced back to Altagracia Carrasco I-3, and in seven of the families both lines can be traced to the same woman.

The isolation of the town, together with the pedigree demonstrating common ancestry, suggests that the increase in gene frequency is a consequence of genetic drift—a founder effect. However, the heterozygotes may have a selective advantage which contributes to gene frequency. The increased incidence of consanguinity, the presence of the biochemical defect in both sexes, and phenotypically normal carriers of both sexes with an intermediate biochemical abnormality, support autosomal recessive inheritance.

In summary, we have described an inherited form of male pseudohermaphroditism secondary to Δ^4-steroid 5α-reductase deficiency, elucidating the action of dihydrotestosterone in the development of the male external genitalia in utero. This entity also demonstrates for the first time an inherited disorder of steroid metabolism.

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