STEROID 5α-REDUCTASE 2 DEFICIENCY

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OUTLINE

INTRODUCTION

Between 1968 and 1973 a large body of evidence accumulated in our laboratory and by others demonstrated that the conversion of testosterone to dihydrotestosterone plays a critical role in androgen action, including virilization of the external genitalia in male embryos (reviewed in 1). We had postulated that mutations that impair dihydrotestosterone formation might be the cause of some cases of human male pseudohermaphroditism (2), and in the course of reviewing the hereditary disorders of human sexual development Joe Goldstein had suggested that a candidate disorder for 5α-reductase deficiency was the rare autosomal recessive disorder termed pseudovaginal perineoscrotal hyposapdias (3, 4). On November 8, 1973, I gave these Grand Rounds on hereditary male pseudohermaphroditism, and afterwards Zaven Chakmakjian told me that his brother Souren Chakmakjian had seen two sisters with an unusual form of male pseudohermaphroditism that he thought would be of interest to us. The girls were subsequently hospitalized by James Marks at the Children's Medical Center at Dallas, and as the result of endocrine, phenotypic, and enzymatic studies it was established that these sisters do have a defect in the 5α-reductase enzyme (5). At almost the same time an extensive family from the Dominican Republic was described in whom male pseudohermaphroditism was due to impairment of the conversion of testosterone to dihydrotestosterone (6, 7). In the intervening years we and others have spent a great deal of effort in investigating the pathogenesis of this disorder, culminating in the cloning of the genes that encode steroid 5α-reductases in David Russell's laboratory (8-10). 5α-reductase deficiency is of interest not because of its frequency (it is rare) but because of its importance for understanding the

mechanism of androgen action, the process of sexual differentiation, and the factors that influence normal sexual behavior.

This review is designed to describe the train of events that led to the deduction of the cause of the disorder, to summarize our own studies and those of others in more than 40 families or family groups from various countries with 5α-reductase deficiency, to review the management, and to consider some of the still unresolved problems in dihydrotestosterone physiology and 5α-reductase deficiency.

ANDROGEN ACTION

The current concepts of androgen action (Fig. 1) were formulated as the result of studies of androgen metabolism and physiology in normal animals and normal embryos (1). Testosterone, the major androgen secreted by the testis and the major circulating androgen in men, enters target tissues down an activity gradient by a passive diffusion mechanism. Inside the cell testosterone can be 5α-reduced to dihydrotestosterone or aromatized to estradiol. Dihydrotestosterone and testosterone bind to the same high-affinity receptor protein in cell nuclei. The androgen receptor is a member of the steroid-thyroid-retinoid family of transcription regulatory factors (Fig. 2) and acts to control the transcription of certain genes by binding to regulatory sites in or adjacent to the genes (11). Although dihydrotestosterone and testosterone bind to the same receptor, the two hormones perform different physiological roles. The testosterone-receptor complex is responsible for the regulation of the secretion of luteinizing hormone by the hypothalamic-pituitary system, for stimulation of the wolffian ducts during sexual differentiation, and (possibly) for the control of spermatogenesis (12). The dihydrotestosterone-receptor complex is responsible for external virilization (development of the male external genitalia and prostate) during embryogenesis and for most androgen-mediated events of male sexual maturation at puberty (growth of facial and body hair, temporal regression of scalp hair, maturation of the external genitalia). The reason that two different hormones bind to the same receptor but perform different functions is only partially understood; testosterone binds less avidly to the receptor than does dihydrotestosterone (13), primarily as the consequence of a slower dissociation rate (14). The dihydrotestosterone-receptor complex is more readily transformed to the DNA-binding state (15), and the dihydrotestosterone-receptor complex activates a reporter gene system more efficiently (16). The net consequence is that dihydrotestosterone formation amplifies a rather weak signal. However, the dihydrotestosterone-receptor complex may also regulate specific genes that do not respond to testosterone (17). Consequently, dihydrotestosterone formation probably plays two roles - a general amplification and a specific role in the regulation of some genes.

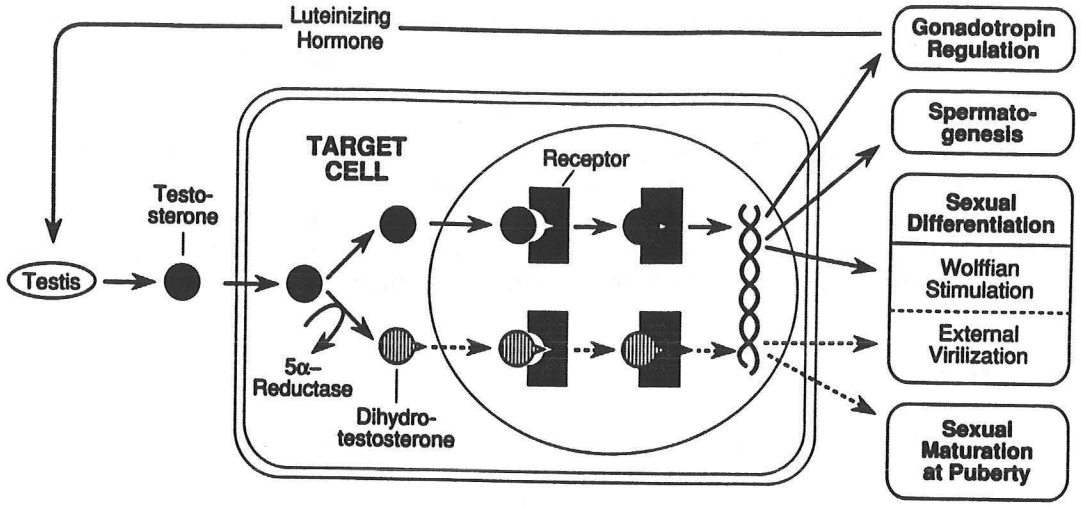

Fig. 1. Schematic diagram of the mechanism of androgen action

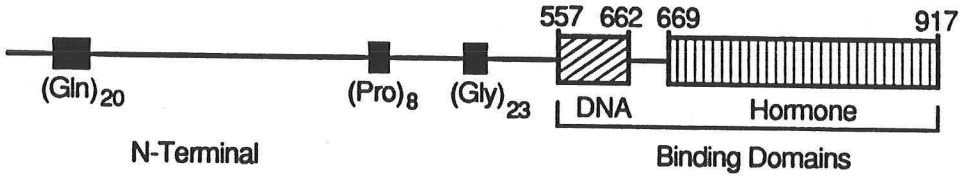

Fig. 2. Schematic diagram of the normal androgen receptor.

Whether estrogen plays a physiological role in normal men is not known, but in some species the hormone is essential for normal male sexual drive. Excess estrogen - either relative or absolute - causes feminization in men, including the induction of gynecomastia (18). As a consequence, estrogen can influence the phenotype in disorders of androgen action, acting via its own receptor.

ROLE OF DIHYDROTESTOSTERONE IN DEVELOPMENT OF THE MALE PHENOTYPE

Normal sexual development during embryogenesis consists of three sequential, ordered, and interrelated processes (19). The first involves the establishment of chromosomal sex at the time of fertilization. In the mammal the heterogametic sex (XY) is male, and the homogametic sex (XX) is female. In the second phase, chromosomal sex is translated into gonadal sex. The exact mechanisms by which the genetic information determines that an indifferent gonad differentiates into a testis or an ovary and secretes the hormones characteristic of the testis or ovary are not understood entirely, but genetic determinants that induce the indifferent gonad to develop into a testis are present on the Y

chromosome and are termed the SDY gene(s) (20). The third phase, the translation of gonadal sex into phenotypic sex, is the direct consequence of the type of gonad formed and the endocrine secretions of the fetal testis. In the formation of phenotypic sex, indifferent internal and external anlagen are converted to male or female forms, and the sexual, behavioral, and functional characteristics are ultimately determined.

The embryologic processes involved in the development of phenotypic sex are summarized in Fig. 3. The internal ducts arise from the wolffian and mullerian ducts, both of which are present in early embryos of both sexes. In the male, the wolffian ducts give rise to the epididymides, vasa deferentia, and seminal vesicles, and the mullerian ducts disappear. In the female, the mullerian ducts give rise to the fallopian tubes, uterus, and upper vagina, and the wolffian ducts either disappear or persist in vestigial form as Gartner's ducts. Thus, the internal genital tracts in males and females arise from different anlagen. In contrast, the external genitalia and lower urogenital tracts of both sexes develop from common precursors, the genital tubercle, genital folds, and genital swellings. In the female the system elongates but changes very little; the genital tubercle becomes the clitoris, the genital swellings become the labia majora, and the genital folds become the labia minora. In the male, fusion and elongation of the urethral folds cause formation of the urethra and shaft of the penis and ultimately bring the urethral orifice to the genital tubercle (glans penis). The fused genital swellings become the scrotum, and a prostate forms in the wall of the urogenital sinus.

In the absence of the testes, as in the normal female or in male embryos castrated prior to the onset of phenotypic differentiation, the development of phenotypic sex proceeds along female lines (19). Thus, masculinization of the fetus is the positive result of action by testicular hormones, whereas development of the female phenotype does not require hormone from the fetal ovary. Under ordinary conditions, development of the sexual phenotype conforms faithfully to the chromosomal sex, i.e., chromosomal sex determines gonadal sex, and gonadal sex in turn determines phenotypic sex.

Three hormones act to control the development of the male phenotype (Table 1). Two, antimullerian hormone and testosterone, are secretory products of the fetal testes. Antimullerian hormone is a glycoprotein formed by the Sertoli cells of the fetal and newborn testis; it causes regression of the mullerian ducts and hence prevents development of the uterus and fallopian tubes in the male (21, 22).

Testosterone is the androgen secreted by both the fetal and the adult testes. The onset of testosterone secretion occurs just

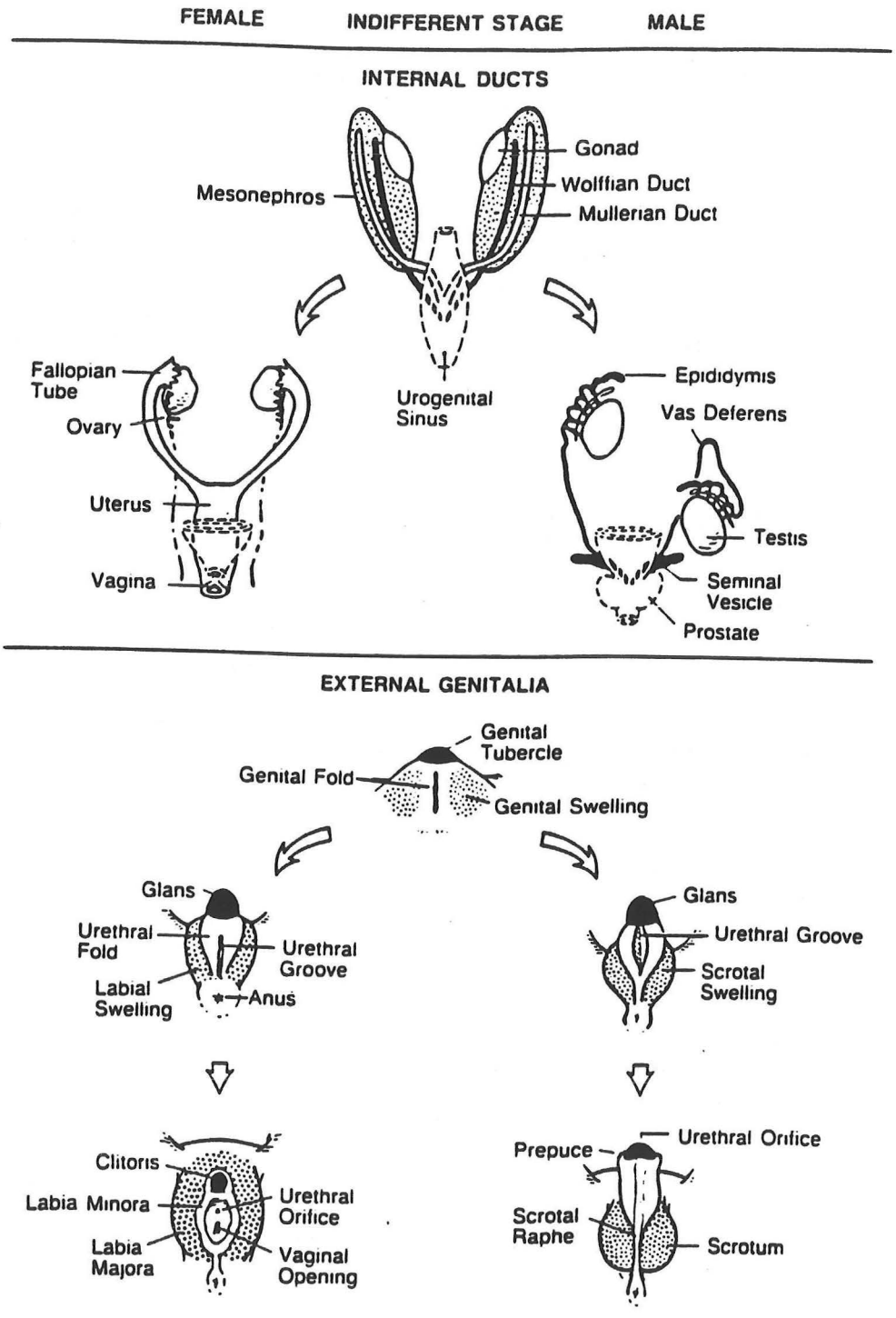

Fig. 3. Phenotypic differentiation of internal ducts and external genitalia in male and female embryos (from Ref. 12).

Table 1

HORMONES INVOLVED IN VIRILIZATION OF THE MALE UROGENITAL TRACT

Hormone	Major Function
Antimullerian Hormone	Regression of the mullerian Ducts
Testosterone	Virilization of the wolffian Ducts
Dihydrotestosterone	Virilization of the urethra and development of the penis and scrotum

prior to the onset of virilization of the male embryo (at about the eighth week of human development) (23). The factors that regulate the initial secretion of testosterone have not been defined completely. However, the initiation and early maintenance of Leydig cell function appear to be autonomous, and gonadotropin control of testosterone formation is probably not acquired until the later phases of embryogenesis (24).

Testosterone promotes virilization of the urogenital tract in two ways. Testosterone acts directly to stimulate the wolffian ducts and to induce development of the epididymides, vasa deferentia, and seminal vesicles (23, 25). Differentiation of the wolffian ducts into seminal vesicles and epididymis is completed in the human male embryo at about 13 weeks of development, before the capacity to form dihydrotestosterone is acquired by these tissues (Fig. 4). In contrast, in the early urogenital sinus and external genitalia testosterone acts as a prohormone for dihydrotestosterone, the third hormone of fetal virilization (Fig. 4). Dihydrotestosterone acts in the urogenital sinus to induce development of the male urethra and prostate and in the urogenital tubercle, swelling, and folds to cause the midline fusion, elongation, and enlargement that eventuate in the penis and scrotum.

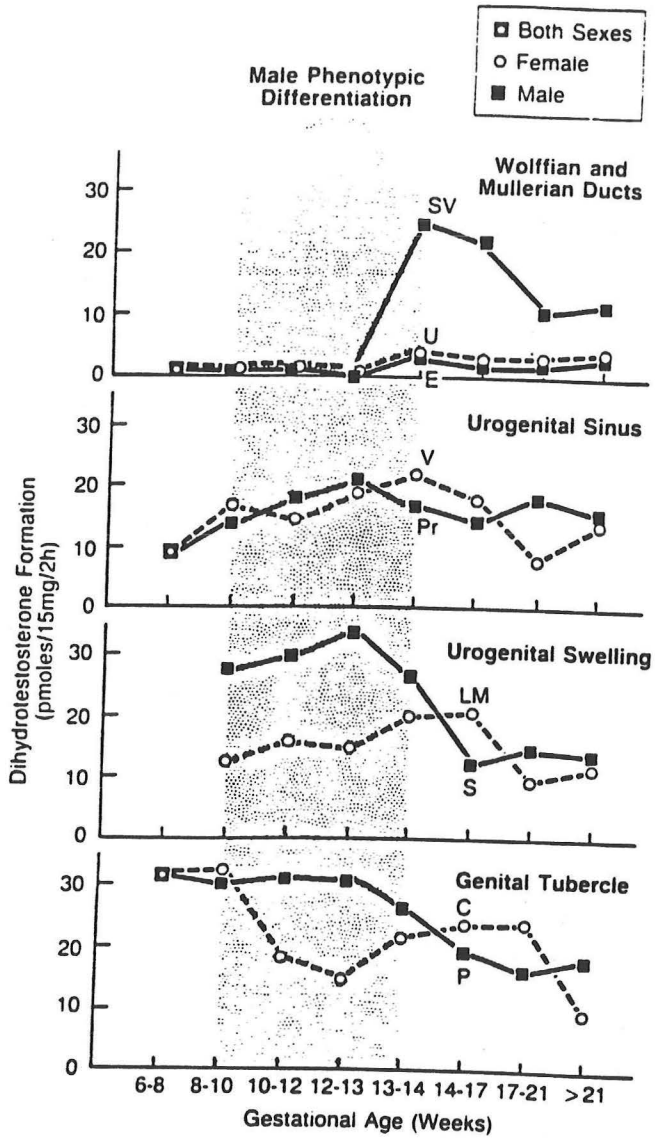

Fig. 4. Dihydrotestosterone formation by the urogenital tract during phenotypic differentiation of the human embryo. SV, seminal vesicles; U, uterus; E, epididymis; V, vagina; Pr, prostate; LM, labia majora; S, scrotum; C, clitoris; P, penis (from Ref. 23).

The deduction that testosterone and dihydrotestosterone perform separate roles in male embryogenesis has been substantiated by studies of the effects of specific inhibitors of the 5α-reductase enzyme in rat embryos (26, 27). More importantly, the recognition that the two hormones play separate roles during male embryogenesis led us to the prediction that a specific form of hereditary male pseudohermaphroditism might be due to impairment of the conversion of testosterone to dihydrotestosterone.

5α-REDUCTASE DEFICIENCY IS A CAUSE OF MALE PSEUDOHERMAPHRODITISM

A form of hereditary male pseudohermaphroditism termed pseudovaginal perineoscrotal hypospadias was defined on clinical and genetic grounds in 1961 by Nowakowski and Lenz (28-30). Additional affected subjects were described subsequently by Simpson et al (31) and Opitz and coworkers (32). Affected persons are 46,XY males who have an autosomal recessive disorder characterized by an external female phenotype at birth, bilateral testes, and normally virilized wolffian structures that terminate in the vagina. This entity (also described under the term familial incomplete male pseudohermaphroditism type 2) constitutes a distinct disorder on genetic, phenotypic, and endocrine grounds. While this eponym encompassed more than one disorder, the fact that the phenotype is commonly the result of deficient production of dihydrotestosterone was established by studies of two families with the disorder in 1974, one in Dallas (5) and the other in the Dominican Republic (6, 7). The disorder is now termed steroid 5α-reductase 2 deficiency.

The initial studies in Dallas were performed in a 13-year-old 46,XY phenotypic girl with primary amenorrhea. She was partially virilized (Fig. 5, left), and plasma testosterone values were in the adult male range. Because she was undergoing virilization the decision was made to remove the testes and to repair the external genitalia. Documentation at surgery of normal male wolffian duct structures - epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts - that terminated in a blind-ending vagina established the phenotype as that of pseudovaginal perineoscrotal hypospadias (Fig. 5, right). Dihydrotestosterone formation was examined in tissue slices of foreskin, epididymis, and labia majora obtained from the subject at the time of surgery and compared with rates of formation in tissue slices from control subjects (Table 2). Dihydrotestosterone formation was virtually undetectable in tissues from the subject but was high in tissues from control groups, establishing that deficiency in dihydrotestosterone formation is the cause of this disorder. This interpretation was further supported by studies of the 5α-reductase enzyme in fibroblasts cultured from the skin of the Dallas family (33-35).

A similar conclusion as to the etiology of the disorder was reached by Imperato-McGinley and colleagues (6, 7) as the result of analyses of plasma and urinary steroids in a large family with an autosomal recessive form of male pseudohermaphroditism in the Dominican Republic (6, 7). Namely, the urinary excretion of 5α-androstanediol and androsterone (the end-products of dihydrotestosterone metabolism) was low, as would be predicted if dihydrotestosterone formation were deficient.

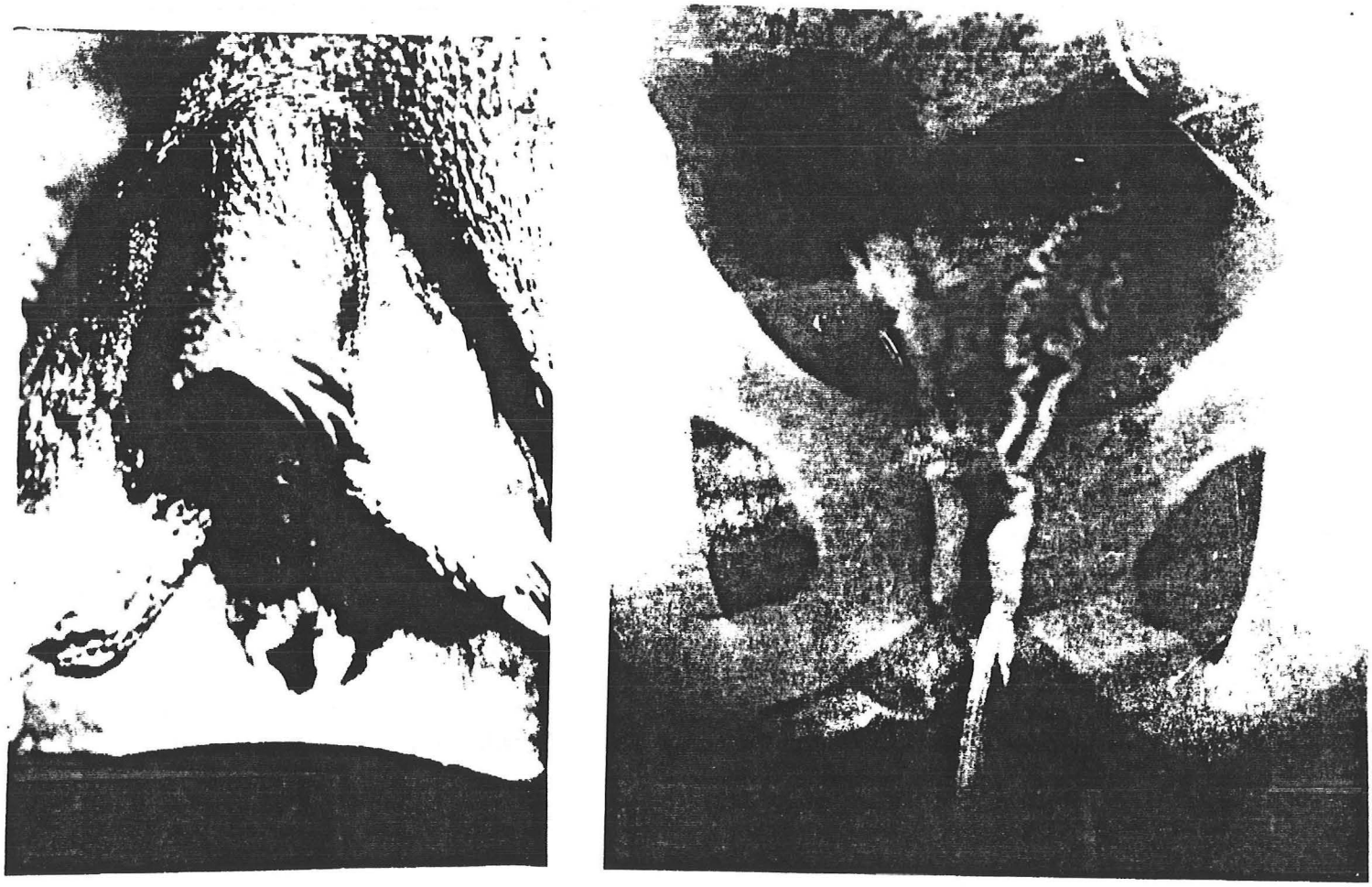

Fig. 5. External genitalia and internal ducts of a patient with steroid 5α-reductase 2 deficiency (Ref. 5). Left: photograph of external genitalia showing clitoromegaly and the opening of a pseudovagina. Right: X-ray of the abdomen after injection of diatrizoate sodium into the vasa differentia at the time of abdominal surgery. vd, vas deferens; sv, seminal vesicles; ed, ejaculatory ducts. The dye emptied into the vagina.

Table 2

Dihydrotestosterone Formation by Tissue Slices from Normal Subjects and from a Patient with 5α-Reductase Deficiency

Group	Age range, years	Dihydrotestosterone formation, pmol/(h•100 tissue) ± SEM		
		Foreskin	Epididymis	Labia majora
Miscellaneous control subjects	6-85	211 ± 26	142 ± 32	183 ± 25
5α-Reductase deficiency	13	8	3	0

NOTE: Tissues slices (40 to 100 mg) were incubated with 0.5 μM [^{3}H]testosterone, 10 mM glucose, and Krebs-Ringer phosphate buffer, at pH 7.4 in a total volume of 2.5 ml. After incubation for 1 h (genital tissue) or 2 h (miscellaneous body sites) the steroids were extacted and analyzed. Samples from 5 to 20 subjects were pooled for each of the control tissues analyzed, and 6 patients with complete testicular feminization were studied. The other groups represent 1 subject each. (Ref. 5).

ENZYMATIC CHARACTERISTICS

The molecular features of 5α-reductase deficiency were characterized in fibroblasts cultured from the skin of persons with the disorder (33-35). 5α-reductase activity is high on average in normal genital skin, where a single enzyme catalyzes the 5α-reduction of many C19 and C21 steroids including cortisol. The pH optimum of enzyme activity in homogenates of normal genital skin fibroblasts is 5.5, with a broad shoulder of activity extending over a more alkaline range (Fig. 6). When this pH curve was obtained, the meaning was not clear, but after the cDNAs for the enzymes were eventually cloned it became apparent that the two activities are in fact encoded by separate genes (8-10). The more alkaline pH enzyme is termed steroid 5α-reductase 1, and the acidic pH enzyme is steroid 5α-reductase 2. At any rate, it was established that measurement of the activity in fibroblast homogenates at pH 5.5 is the most sensitive means to detect the enzyme deficiency, and over the years we characterized the enzyme deficiency in fibroblasts grown from more than 20 families from various parts of the world that fulfil the endocrine, genetic, and phenotypic criteria for the diagnosis (12). Low rates of enzyme activity were observed in cells grown from skin biopsies from subjects from the original Dallas family (data points labelled "deficient enzyme" in Fig. 7). Families with deficient enzyme activity include the Dominican Republic family studied by Imperato-McGinley and coworkers, additional families reported in the literature, and some families that have not been reported in detail (12). Thus, deficiency of enzyme activity is the most common cause of the syndrome.

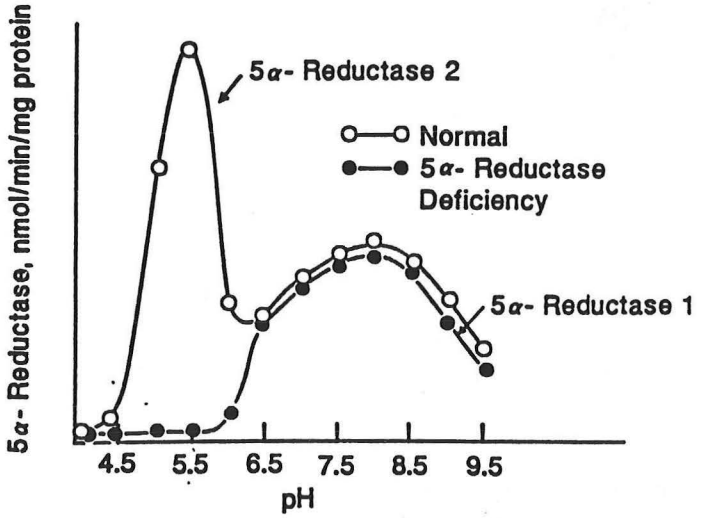

Fig. 6. Schematic diagram of the relation between pH and 5α-reductase activity in extracts of genital skin fibroblasts. (Redrawn from Ref. 35)

In a second category of patients (Fig. 7), pH 5.5 enzyme is synthesized but is abnormal. The first family of this category was from Los Angeles and was studied in collaboration with Dr. Maurice Kogut and his colleagues (36, 37). The two affected individuals have typical endocrine findings of 5α-reductase deficiency, a phenotype identical to that of previously characterized families, and deficient 5α-reductase in direct biopsy material. However, activity of the enzyme in fibroblasts cultured from the genital skin of these subjects was measurable and in some within the normal range (labelled "abnormal enzyme" in Fig. 7). Furthermore, in contrast to the situation in the previous cases, the enzyme from these patients had normal apparent Km for testosterone, but affinity of the enzyme for reduced nicotinamide adenine dinucleotide phosphate (NADPH), the cofactor for the reaction, was decreased, and as a consequence the enzyme was unstable and exhibited a rapid turnover. The enzymes in cells from subjects from three additional families exhibited similar evidence of a qualitatively defective enzyme. In these four families 5α-reductase appears to be synthesized at a normal rate but is degraded more rapidly than normal so that the steady state activity in tissues is profoundly decreased. Consequently, the phenotypic expression is identical to that in families with deficient enzyme.

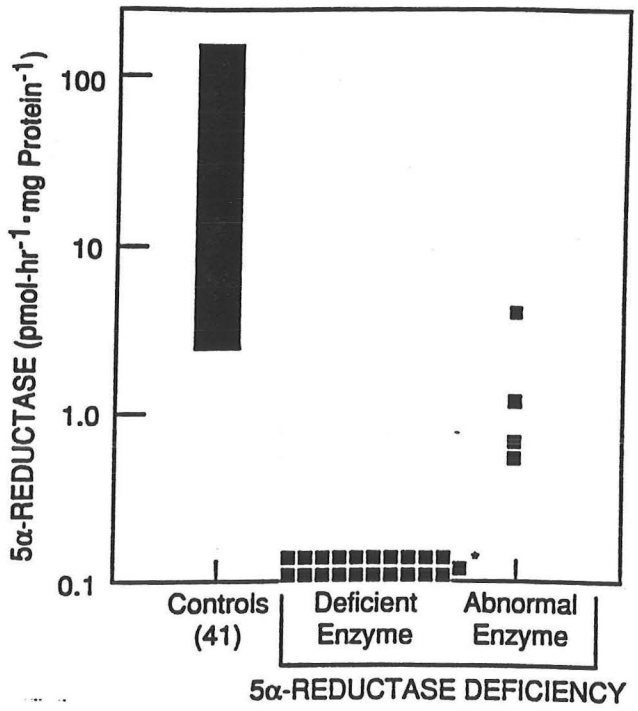

Fig. 7. 5α-reductase activity at pH 5.5 in genital skin fibroblasts cultured from skin biopsies of 25 unrelated subjects with steroid 5α-reductase deficiency (black squares) and 41 control subjects (black bar). The asterisk denotes the family in which the gene for steroid 5α-reductase 2 has been shown to be defective (Ref. 9).

In summary, as the result of these functional studies, it was established that 5α-reductase deficiency is the result of an abnormal pH 5.5 enzyme, and two categories of 5α-reductase deficiency were ascertained - one associated with an abnormal enzyme and the other associated with such low activity that we were uncertain whether it was due to missense mutations that block enzyme function completely or cause a decrease in enzyme protein. The real meaning of these functional studies only became apparent when the cDNAs for the enzymes were cloned.

MOLECULAR BIOLOGY OF THE STEROID 5α-REDUCTASES

Despite major attempts in this laboratory (38-40) and by others, 5α-reductase was never successfully solubilized or purified. Consequently, this problem remained dormant while we accumulated case material. (As of 1992 the disorder has been described in at least 46 families or family groups from different parts of the world.) The problem was broken, when Andersson and Russell utilized the technique of expression cloning to characterize the cDNA for the rat steroid 5α-reductase 1 and applied homology cloning to clone the analogous human 5α-reductase 1 (8). This enzyme has a broadly alkaline pH optimum and was shown by formal genetic testing to be normal in subjects with 5α-reductase deficiency (10). Recognizing that a second enzyme must be present, Andersson and colleagues again utilized expression cloning to identify the cDNA for steroid 5α-reductase 2, an enzyme with an acidic pH optimum, and demonstrated that this gene is deleted in a New Guinea cluster of 5α-reductase deficiency (9).

The characteristics of the two enzymes are summarized in Table 3. Enzyme 1 is encoded on Chromosome 5, and Enzyme 2 is encoded on Chromosome 2. The tissue distribution of the enzymes is still under investigation; on the basis of the initial studies it appears that 5α-reductase 2 is primarily localized in androgen target tissues whereas 5α-reductase 1 has a more ubiquitous distribution. Enzyme 2 has a lower Km for testosterone and is more sensitive to the 5α-reductase inhibitor finasteride. The two enzymes have about a 50% homology. Both are low abundance proteins. Both are hydrophobic (explaining the failure to solubilize), and they have similar gene structures with five coding exons and four introns each. The androgen-binding domain is encoded in the amino terminal end of the molecule (41).

Table 3

COMPARISON OF HUMAN STEROID 5α-REDUCTASE 1 AND 2

	5α-Reductase 1	5α-Reductase 2
pH Optimum	7.5	5.0
Location of gene	Chromosome 5	Chromosome 2
Tissue Distribution	Ubiquitous	Male Urogenital Tract
Km for Testosterone	4μM	1μM
Ki for Finasteride	300 nM	3-5 nM
Activity for 5α-reductase Deficiency	Normal	Impaired
Sequence Homology	50%	

Many unresolved issues about these two enzymes are now under investigation, for example, compartmentalization studies within tissues (epithelial vs. all stromal localization), studies of the mechanism of the enzymatic reaction itself and in particular whether NADPH acts as a direct hydrogen donor, analysis of whether the localization of the enzyme to the nuclear membrane is important in hormone action, and, most importantly, analysis of the embryologic relation of the two enzymes and specifically whether 5α-reductase 2 controls the expression of 5α-reductase 1. The cloning of these enzymes makes it possible to investigate many fundamental issues in androgen action and has stimulated a burst of activity in androgen physiology.

GENETIC STUDIES OF STEROID 5α-REDUCTASE 2 DEFICIENCY

More directly to today's discussion, the cloning of the 5α-reductase 2 gene has made it possible to define the mutations responsible for steroid 5α-reductase 2 deficiency. To this end, Thigpen et al utilized a variety of techniques including PCR amplification, conformation-dependent DNA polymorphism analysis, and DNA sequencing for analysis of the mutations in 32 families with 5α-reductase deficiency (42) (Fig. 8 and Table 4) (5-7, 9, 10, 12, 42-54). This patient population includes 25 families who have been diagnosed in various parts of the world and whose enzyme function has been studied in our laboratory. Four of the families were ascertained by Dr. Bernice Mendonca from the University of Sao Paulo, four families (including the Dominican Republic family) were ascertained by Dr. Julianne Imperato-McGinley at Cornell University Medical Center, New York, two families were ascertained by Drs. Paul Saenger and Maria New and their colleagues at Cornell University Medical Center, New York, and one family was studied by Dr. Moustafa El Awady, University of Cairo.

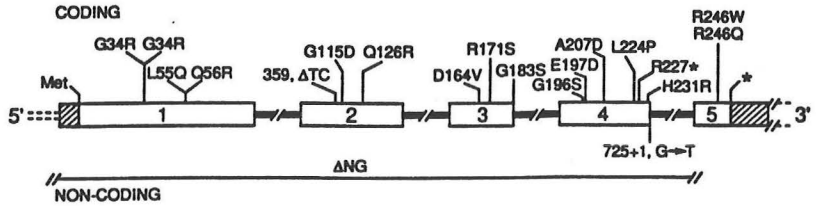

Fig. 8. Mutations in 5α-reductase type 2 gene and protein. A schematic diagram of the 5α-reductase 2 gene is shown together with the locations of 19 different mutations. The single letter amino acid code is used. All substitution mutations occur in amino acids that are conserved amongst the sequenced 5α-reductase proteins (359.ΔTC) portion of the gene alter conserved splice junction signals (725 + 1. G-T) or cause premature termination (R227*). Amino acid numbers refer to those of the normal 5α-reductase type 2 protein. Other numbers refer to the normal cDNA and indicate the position at which a given mutation occurred (e.g.,359.ΔTC indicates a deletion of a TC dinucleotide at position 359 of the cDNA). Two different mutations (GGG-AGG and GGG-CGG) result in the G34R substitution. (from Ref. 42)

Table 4

Steroid 5α-Reductase 2 Mutations in 32 Subjects and/or Families with 5α-Reductase Deficiency

Family Designation	Cell Strain	Ethnic Group	Consanguinity/ Positive Family History	5α-Reductase Activity in Genital Skin Fibroblasts N=1-100 pmol/h/mg protein[a]	Molecular Lesion Type	Location	Mutation	Comment	Reference
Class 1: Homozygotes:									
5R2-New Guinea[b]	848,849,850	New Guinean	Yes/Yes	<0.2	Deletion (>20 kb)	5'FR-3'FR	Deletion of all exons	Deletion not sequenced	9,10,42,43, 72,81,82
5R2-Dominican Republic[b]	40,41,42,338,506	Dominican Republic	Yes/Yes	<0.2	Missense	Exon 5	C→T,R246W	CG Dinucleotide, altered NADPH K_m	6,7,10,42,44, 53,54,75,79
5R2-São Paulo-1	-	White Brazilian	Yes/No	ND[c]	Missense	Exon 5	C→T,R246W	CG Dinucleotide, altered NADPH K_m	42
5R2-São Paulo-2	-	Creole Brazilian	Yes/Yes	ND	Nonsense	Exon 4	C→T,R227[d]	CG Dinucleotide	42
5R2-Pakistan	904	Pakistani	Yes/No	1.8; unstable[e]	Missense	Exon 5	G→A,R246Q	CG Dinucleotide	10,42,47
5R2-Chicago-3	537,538	Pakistani	No/Yes	0.2	Missense	Exon 5	G→A,R246Q	CG Dinucleotide	42
5R2-New Haven	728,729,759,760, 828,829	Greek American	No/No	variable	Missense	Exon 4	G→A,G196S	CG Dinucleotide, altered NADPH K_m	42,46
5R2-Louisiana	196	Creole American	Yes/Yes	<0.2	Missense	Exon 2	A→G,Q126R		42
5R2-Los Angeles-2	632	Vietnamese	No/Yes	<0.2	Missense	Exon 1	G→A,G34R	CG Dinucleotide, altered NADPH K_m	42,47 (Subject 5)
5R2-London-1	215,216,272,379, 380,381	Pakistani	Yes/Yes	<0.2	Splice Junction	Exon 4/ Intron 4	G→T		42,49 (Subject AA)
5R2-Chicago-1	26,739	Mexican American	No/Yes	<0.2	Nonsense	Exon 4	C→T,R227*	CG Dinucleotide	42,47 (Subject 1)
5R2-Phoenix	426,427	Native American	No/No	<0.2	Missense	Exon 4	T→C,L224P	No SSCP	42,47 (Subject 2)
5R2-São Paulo-3	-	Black Brazilian	Yes/Yes	ND	Missense	Exon 3	G→A,G183S		42
5R2-New York-4	-	Italian American	No/No	-	Missense	Exon 2	Q126R		48 (Subject A)
5R2-Sao Paulo-5									
5R2-Cairo-1	-	Egyptian	Yes/No	-	Missense	Exon 5	C→T,R246W		This report
5R2-Ghent	-	Portuguese	No/Yes	-	Missense	Exon 2	Q126R		50
5R2-Malta	-	Maltese	No/No	-	Deletion 2BB	Exon 2	Deletion of TC at nucleotide 359		This report
5R2-Bristol	-	Anglo-Saxon	No/No	-	-	-	-		51
Class 2A: Compound Heterozygotes:									
5R2-Austria	667	Austrian	No/Yes	1.6; unstable	Missense Missense	Exon 4 Exon 5	C→A,A207D G→A,R246Q	CG Dinucleotide	10,42,47,52 (Subject 3)
5R2-São Paulo-4	-	Creole Brazilian	No/Yes	ND	Missense Missense	Exon 2 Exon 3	A→G,Q126R A→T,D164V		42
5R2-Irvine	231,232	Mexican American	No/No	<0.2	Missense Missense	Exon 1 Exon 2	G→A,G34R G→A,G115D	CG Dinucleotide, altered testosterone K_m	42
5R2-New York-2	445	Jordanian	No/No	<0.2	Missense Missense	Exon 1 Exon 1	T→A,L55Q A→G,Q56R		42,53 (Subject 29)
5R2-New York-1	106,163	Sicilian	No/No	0.6; unstable	Missense Missense	Exon 1 Exon 3	G→C,G34R G→C,R171S		10,42,54
5R2-London-2	490	Maltese	No/No	0.6; unstable	Deletion 2 bp Missense	Exon 2 Exon 3	Deletion of TC at nucleotide 359 G→C,R171S		10,42,49 (Subject MM)
Class 2B: Heterozygotes:									
5R2-Los Angeles-1	70,71,73,74	Black American	No/Yes	3.0; unstable	Missense	Exon 5	G→A,R246Q	CG Dinucleotide	10,36,37,42
5R2-Dallas	65,66,121,129,139	Black American	No/Yes	<0.2	Missense	Exon 4	A→G,H231R		5,10,33-35, 42
5R2-Chicago-2	318,394,395,418	White American	No/No	0.38	Missense	Exon 4	A→G,H231R		42
5R2-New York-3	352	Russian American	No/No	0.2	Missense	Exon 4	G→C,E197D		42
Class 3: No Abnormality Identified:									
5R2-Los Angeles-3	526	Latvian American	Yes/No	<0.2	?[f]	?	?	Exon sequences normal	10,42
5R2-London-3	325,326	Cypriot	No/Yes	<0.2	?	?	?	Exon sequences 1B normal	42,49 (Subject CP)
5R2-New York-5	-	Italian American	No/No	-	-	-	-		49 (Subject 13)

[a]Normal range = 1-100 pmol/h/mg protein as described in Ref. 36

[b]Previously described, included for comparison

[c]ND = not done

[d]* = termination codon

[e]unstable = enzyme activity unstable at elevated temperatures

[f]? = no mutation found

These 32 families represent 21 ethnic groups; the diagnosis was based on clinical findings, family studies, endocrine criteria, and in most instances on analysis of 5α-reductase in cultured skin fibroblasts. Coding sequence abnormalities were discovered in 29 of the 32 families, and in all except one (5R2-New Guinea) the abnormalities identified were point mutations, consisting of 16 amino acid substitutions, a splice-junction alteration, a nonsense codon, and a small deletion. Nineteen of these families have homozygous mutations, and six are compound heterozygotes. Mutations of only one allele were present in four individuals (Los Angeles-1, Dallas, Chicago-2, and New York-3). No mutations were detected in two subjects (Los Angeles-3 and London-3). We believe that the latter two groups are compound heterozygotes and homozygotes respectively for mutations that map outside the exons and the immediate flanking intron sequences.

The locations of the mutations in the gene and their consequences are summarized in Fig. 8. The mutations are distributed throughout the coding sequence. Each of the substitution mutations alters an amino acid that is conserved among the sequenced 5α-reductases (9). No affected individual had more than two of the mutations shown in Fig. 8, and no mutations have been detected in the normal individuals screened to date. The meaning of our original caterorization of 5α-reductase deficiency as due to "deficient" or "abnormal" enzyme became clear as a result of this analysis, namely many subjects classified as "deficient" (such as the Dominican Republic family) actually synthesize an abnormal enzyme that is so unstable that it is difficult to assay except when overexpressed in reporter cells; in general, the mutations that cause abnormalities of NADPH binding and instability involve the carboxy terminal region of the gene. Moreover, there are several causes of deficient enzyme activity, including mutations that impair binding of testosterone to the enzyme, mutations outside the coding sequence that impair translation of the gene product (crm negative).

Identical mutations have been discovered in different ethnic groups (Fig. 9). In some instances, this recurrence is almost certainly due to a founder effect. For example, 5R2-Malta is the result of a homozygous deletion of two base pairs at nucleotide 359 in exon 2. Another subject from Malta (5R2-London-2) is a compound heterozygote, bearing this same mutation on one allele and a missense mutation on the other allele (R171S). Similarly, a subject from Sicily (5R2-New York-1) bears the R171S missense mutation on one allele and as second missense allele (G34R) on the other. In this instance it is likely that a carrier from Malta or Sicily was responsible for the spread of the gene. In other instances, for example, the sharing of the R246W mutation among Egypt (5R2-Cairo-1), Brazil (5R2-Sao Paulo-1) and the Dominican Republic may be due to recurring mutations. The formal genetic testing as to the frequency of recurring mutations has not been performed.

It is of interest that three large clusters of 5α-reductase deficiency have been described in different parts of the world - the Dominican Republic family involving some 38 members (6, 7), the Turkish cluster of 12 subjects (63), and 13 affected members of the Sambia tribe in New Guinea Highlands (43, 71). In the case of the Dominican Republic and Turkish clusters, the recurrence of the disorder is almost certainly the consequence of a founder effect in a geographic isolate of people with a high coefficient of inbreeding. It is of interest in this regard that the Turkish kindred also has a high incidence of another rare autosomal recessive trait, 17β-hydroxysteroid oxidoreductase deficiency (72). Whether the New Guinea cluster is the result of a founder effect is less clear; 5α-reductase deficiency also occurs in areas of New Guinea remote from the Sambia (73), and it is possible that the high frequency of the mutation there may be due to some other cause.

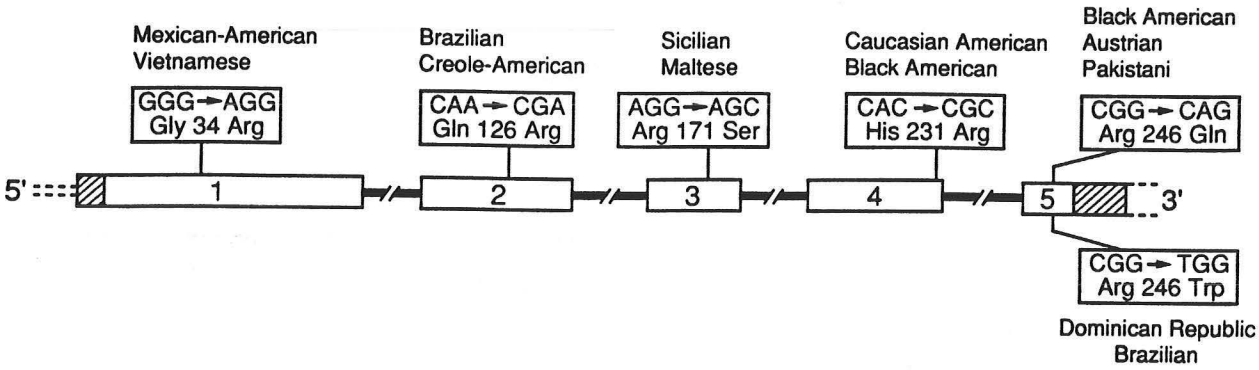

Fig. 9. Apparent recurrent 5α-reductase type 2 mutations. A schematic diagram of the 5α-reductase type 2 gene is shown. The location of six mutations that occur in individuals of different ethnic backgrounds is indicated above and below the gene structure. These mutations are designated apparent recurrent as DNA polymorphisms have not yet been identified that would allow detailed haplotype analysis and subsequent determination of whether a given lesion occurred on two different genetic backgrounds, or represents two descendants from a common ancestor. (From Ref. 42)

CLINICAL FEATURES

In addition to the subjects summarized in Table 4, at least 15 additional families have been reported by others (Table 5) (56-71). In the 46 families summarized in Tables 4 and 5, sufficient description is available to make it possible to make certain generalizations about the clinical manifestations and genetic characteristics (Table 6). Consanguinity has been documented in 16/40, and homozygosity was present in 19/31; this slight discrepancy is almost certainly due to the fact that several of the families in whom consanguinity was not documented came from areas of the world with known high coefficients of inbreeding. About 30% are compound heterozygotes or presumed compound heterozygotes.

Approximately 45% of subjects had other affected family members. These findings - particularly the high rate of documented consanguinity - all reinforce the original concept of Lenz (28-30) that this autosomal recessive trait is rare. The fact that the disorder is genetically heterogeneous - at least 16 mutations responsible for the defect in 32 families - is in keeping with findings in other rare autosomal recessive mutations. This feature unfortunately complicates the development of rapid screening procedures for making the diagnosis.

Table 5

Fifteen Subjects with 5α-Reductase Deficiency Reported by Others

#	Family Designation	Age at Diagnosis	Ethnic Background	Consanguinity/ Positive Family History	Change in Gender Role	How Diagnosis Established	Reference	Unusual Features
1.	Guadalajara	18	?	Yes/No	No	T:D Ratio >35	56, 57 (Cantu)	Female psychosexual orientation, age 21; testes of 12 and 17 ml; 2 cm vagina
2.	Montpelier	25	?	?/No	?	T:D Ratio 38	58 (Jaffiol)	No vaginal orifice
3.	London-4	2½	Pakistani	No/No	Too young	Absence of 5α-reductase in cultured skin fibroblasts	59 (Greene)	"Small" vaginal pouch
4.	Paris-1	25	Algerian	?/No	Yes	T:D Ratio 42; low 5α-reductase in scrotal skin biopsy	60 (Kuttenn)	Urogenital sinus and small vaginal pouch; spermatogenic arrest
5.	Paris-2	20	?	?/No	No	T:D Ratio 26; low 5α-reductase in skin homogenates	61 (Mauvais-Jarvis)	Spermatogenic arrest; small vaginal pouch with urogenital sinus
6.	Jerusalem	13	Jordanian	Yes/Yes	Yes	Deficient 5α-reductase in cultured skin fibroblasts	62 (Okon)	Urogenital sinus; absent spermatogenesis
7.	Turkey	5-55	Turkish	Yes/Yes	Raised as male	T:D Ratios >36 abnormal urinary ratios of etiocholanolone to androsterone	63 (Akgun)	
8.	São Paulo-5	13-21	?	No/Yes	Yes	T:D Ratios >36	64 (Mendonca)	Vaginal pouch in 2 of 3
9.	Cairo	2	Egyptian	Yes/No	Too young	T:D Ratio 50; deficiency of 5α-reductase in cultured skin fibroblasts	65 (El Awady)	
10.	Naples	17	Italian	Yes/No	Yes	T:D Ratio 38	66 (Iudice)	5 cm vaginal pouch
11.	Bombay	4	Indian	No/No	Too young	Abnormal ratio of 5α:5β metabolites in urine	67 (Patel)	No vaginal pouch
12.	Zurich	NB	?	?/?	Too young	Abnormal ratio of etiocholonalone to androsterone	68 (Greene)	Urogenital sinus
13.	London-5	5 mo	United Arab Emirates	Yes/No	Too young	T:D Ratio 60 after hCG	69 (Stanhope)	Urogenital sinus
14.	Malmo	16	Swedish	No/Yes	Raised as male	T:D ratio 46; abnormal ratios of etiocholonalone:androsterone (3.16)	70 (Ivarsson)	Hypospadias repair in all 3
15.	Glasgow	6	Pakistani	No/Yes	Raised as male	Abnormal ratios of urinary tetrahydrocortisol to 5α-tetrahydrocortisol	71 (Odame)	Hypospadias repair

Table 6

CLINICAL FEATURES IN 45 FAMILIES WITH STEROID 5α-REDUCTASE 2 DEFICIENCY

Feature	Number Affected	Percent
Documented Consanguinity	16/40	40
Positive Family History	19/42	45
Homozygotes	19/31	61
Compound Heterozygotes or Presumed Compound Heterozygotes	10/31	32
Anatomical Features		
Pseudovagina	18/33	55
Urogenital Sinus	12/33	36
Hypospadias	5/38	9
Testes in inguinal canals, labia, or scrotum	43/43	100
Spermatogenesis, absent or profoundly impaired	9/9	100
Gynecomastia	1/45	2
How Diagnosis Established		
Ratios of 5β/5α metabolites in urine	12/45	27
Ratio of T/DHT in plasma (before or after hCG)	44/45	98
5α-reductase measurements in biopsy tissue or fibroblasts	29/45	64
Gonadotropin Values		
Elevated Luteinizing Hormone	10/23	43
Elevated Follicle Stimulating Hormone	12/23	52
Gender Role Behavior		
Raised as male	5/38	13
Changed from female to male	19/37	51
Female	8/37	22
Too young to ascertain	6/37	16

The anatomical features are also summarized in Table 6. Approximately 55% of patients have a blind-ending vagina (pseudovagina) as originally described by Lenz (28-30). However, it was recognized in the first two families that we worked up (5, 36, 37) that in one affected sister in each family had a pseudovagina, whereas the other was more severely virilized and had only a single perineal orifice, a urethra that on closer inspection provided the outlet for a urogenital sinus, namely a vaginal pouch or dimple being demonstrable on the side of the urethra (Fig. 10). In the larger series 12 of 33 individuals have a urogenital sinus. Of greater interest, perhaps, in five families the phallus was so large at birth that the children were identified as males with hypospadias and raised from the first as males. In summary, the anatomical features range from partial to profound impairment of virilization of the urogenital tubercle. Indeed, the most consistent features in the disorder are hypospadias and absence of the prostate. The nature of the variability in expression (even within families) is not clear, but one clear-cut implication of this analysis is that this disorder must now be added to the differential diagnosis of hypospadias (at least familial hypospadias).

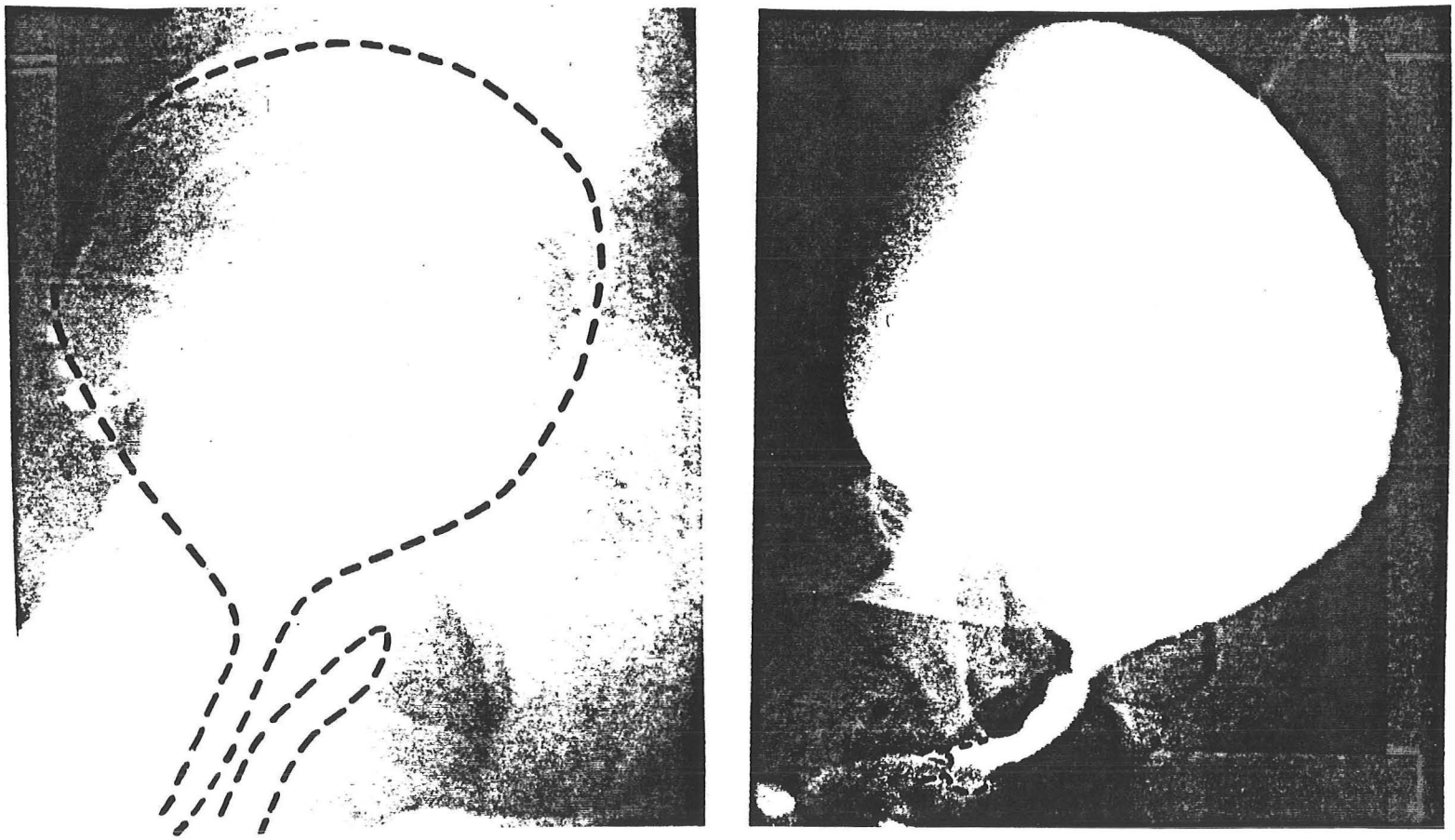

Fig. 10. Vagino-urethrograms on the index Dallas patient (A) and her younger sister (B) (Ref. 5).

It is also worth noting that the testes have been extra-abdominal - present in the inguinal canals, labia majora, or scrotum in all subjects. Likewise, spermatogenesis was absent or profoundly impaired in all nine subjects in whom it has been examined; whether this is a direct effect of the mutation or the secondary consequence of incomplete testicular descent is uncertain.

ENDOCRINOLOGY

Simpson et al observed that normal male levels of testosterone in one affected individual rose even higher after administration of human chorionic gonadotropin (hCG) and fell to the castrate range after removal of the testes (31). They concluded that testosterone secretion in 5α-reductase deficiency is normal and is under normal feedback control. Subsequent studies have supported this interpretation.

The characteristic endocrine features are as follows: 1) normal male to high levels of plasma testosterone and low levels of plasma dihydrotestosterone (5-7), 2) elevation in the ratio of the concentration of plasma testosterone to dihydrotestosterone in adulthood and after stimulation with hCG in childhood (7, 55, 60, 68, 74, 75), 3) elevated ratios of urinary 5β- to 5α-metabolites of androgen (7, 53, 55, 60, 68, 74, 75), 4) diminished conversion of testosterone to dihydrotestosterone in tissues of affected subjects (5), 5) elevated ratios of urinary 5β- to 5α-metabolites of C21 steroids (53, 63, 75), and 6) increased ratio of plasma testosterone to dihydrotestosterone after the administration of testosterone (49, 68). Levels of plasma LH are either normal (5, 48, 49, 55) or slightly elevated (although never as high as in men with primary testicular failure or in subjects with male pseudohermaphroditism due to abnormalities of the androgen receptor (6, 7, 54, 62, 63, 74). It is of interest in this regard that elevated LH was present in 10/23 and elevated FSH in 12/23 subjects summarized in Table 6. This finding implies that dihydrotestosterone plays a role, probably minor, in the regulation of gonadotropin secretion.

Detailed studies of the origins and rates of production of androgen and estrogen have been conducted in one person (Fig. 11) (5); plasma levels of androstenedione (1.1 ng/ml) and testosterone (6.9 ng/ml) and the plasma production rates of androstenedione (2.7 mg/day) and testosterone (5.2 mg/day) were in the range of normal men. Estradiol production was also in the range for normal men in regard to the total production rate (45 μg/day). These quantitative studies demonstrating normal male androgen and estrogen production explain the failure of patients to undergo female breast development at the time of puberty and are in contrast to the situation in male pseudo- hermaphroditism caused by disorders of the androgen receptor; in the latter disorders the variable degree of feminization at the expected time of puberty is

associated with increased production of estrogen by the testes. In the one subject described in the literature with 5α-reductase deficiency and gynecomastia (Table 6) the gynecomastia was probably due to testicular failure (64).

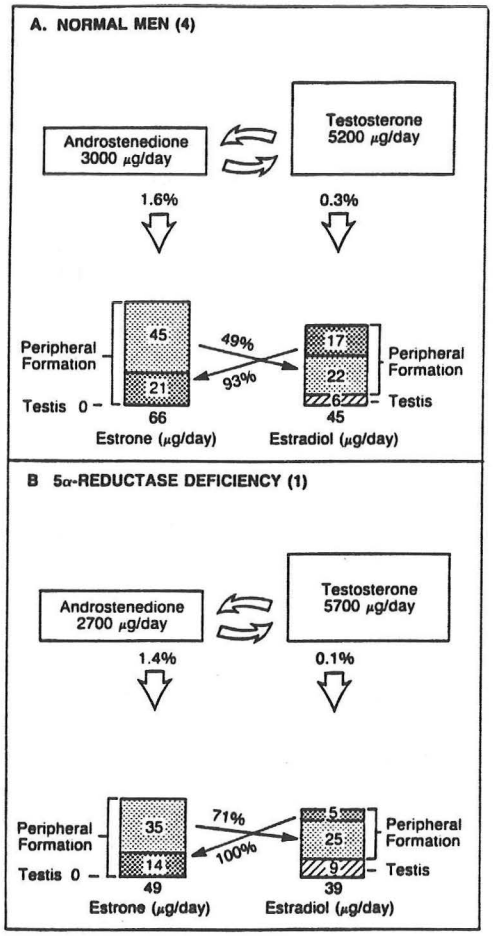

Fig. 11. Dynamics of androgen and estrogen production in normal men and in 46,XY subjects with androgen resistance. A. Four normal men. B. One subject with 5α-reductase deficiency. Average production rates of androgen are indicated in the upper boxes, and the production rates of estrogen are shown below the vertical bar. Extent of conversion of plasma testosterone and androstenedione to estradiol and estrone is indicated by the vertical arrows, and interconversion of estrone and estradiol and of testosterone and androstenedione are indicated by the horizontal arrows. The sources of estradiol and estrone are indicated in the vertical bars. Thus, estradiol arises from plasma testosterone, from estrone, and from direct secretion by the testis, and estrone arises from plasma androstenedione, from estradiol, and in some instances from direct secretion by the testis. (From Ref. 5)

Virilization at Puberty

In addition to the variable impairment of virilization at birth, other features of are puzzling. One, although plasma dihydrotestosterone levels are low (5) they are never undetectable and indeed in many subjects fall within the low normal range. The circulating dihydrotestosterone in this disorder could have two origins - it could be synthesized by the residual activity of the mutant enzyme, or it could be derived from 5α-reductase 1. Studies of two groups of affected individuals clearly indicate that the latter source predominates. Indeed, New Guinean subjects with a total deletion of the coding sequence for 5α-reductase 2 (9) and who, as a consequence, can make no functional enzyme nevertheless have measurable plasma dihydrotestosterone (43). Likewise, subject NA from a family with a splice junction abnormality (5R2-London-1) (49) does not synthesize a functional steroid 5α-reductase 2; nevertheless, basal levels of plasma dihydrotestosterone in this subject were in the low normal range and rose to supraphysiological levels (7.8 ± 1.1 nmol/L) when he was given large amounts of testosterone propionate by injection for three days (49). These findings demonstrate that steroid 5α-reductase 1 does contribute to plasma dihydrotestosterone, recognizing that in some patients dihydrotestosterone may also be formed in small amounts from the residual activity of mutated steroid 5α-reductase 2 (42). Elucidation of the role of 5α-reductase 1 in androgen physiology is a major imperative. Indeed, the fact that 5α-reductase activity is normal in hair follicles from the scalps of patients with 5α-reductase deficiency (76) suggests that androgen action in some tissues may be mediated by steroid 5α-reductase 1.

Second, the degree of virilization at the time of puberty can be striking, and in some subjects the habitus becomes truly masculine, although all affected subjects appear to be less virilized than their unaffected brothers. This masculinization at the time of puberty could either be mediated by the small amounts of circulating dihydrotestosterone present in all subjects or could be due to the effects of testosterone itself over a long period of time; studies attempting to resolve among these possibilities have yielded inconclusive results (49). Namely, in four patients, the parenteral administration of sufficient amounts of testosterone esters to elevate testosterone levels above normal for several months did promote virilization but simultaneously raised plasma dihydrotestosterone levels to the normal range. Consequently, it was not possible to deduce whether the improved virilization at puberty is mediated by testosterone or dihydrotestosterone. There is no question but that testosterone can enhance transcription of an androgen-responsive reporter gene (16), but the fact that dihydrotestosterone binds more tightly to the androgen receptor (14) favors the argument that dihydrotestosterone itself is the universal mediator of virilization at puberty.

Homozygous State in Women

On the basis of studies of the urinary excretion patterns of 5α-androstan-3α,17β-diol and 5β-androstan-3α,17β-diol in the Dominican Republic families Peterson et al (7, 74) identified seven apparently normal women (two obligate carriers and five sisters of affected men) who were presumed to be homozygous for the condition. In addition a homozygous sister of an affected male (5R2-Sao Paulo-2) who is homozygous for insertion of a premature termination at amino acid 227 has been ascertained in a Brazilian family; the latter individual has three children and no known endocrine abnormalities (B. Mendonca, unpublished). On the basis of these studies 5α-reductase 2 is presumed to play no essential role in endocrine physiology in women. Since 5α-reduced progesterone is a major metabolite in the blood of women during the luteal phase and during pregnancy (77, 78), it is possible either that this metabolite is formed by 5α-reductase 1 or that the pathway is of minor significance in women.

REVERSAL OF GENDER ROLE BEHAVIOR

Imperato-McGinley et al reported that 18 of 19 individuals studied by them from the Dominican Republic cluster were initially raised as females but subsequently changed their gender role behavior to male at the time of expected puberty (44, 79). A similar phenomenon has been described in additional families in other parts of the world, indeed in 19 of 37 families summarized in Table 6. This phenomenon has served to reinvigorate the argument as to the relative roles of biological determinants and psychological factors in the development of sexual identity. A similar phenomenon has been described in people with 17β-hydroxysteroid oxidoreductase deficiency, in subjects with 46,X/46,XY gonadal dysgenesis, and in other subjects with male pseudohermaphroditism in which the diagnosis is not clear (reviewed in 80). It is of interest that such behavioral change is not characteristic of mutations of the androgen receptor in which gender behavior conforms to gender assignment. In analyzing these various reports two facts seem clear: (1) the majority of individuals in whom gender identity does not conform with gender assignment have ambiguous genitalia at birth, i.e., they are genetic males in whom male development is incomplete; and (2) the decision to change gender role is nearly always from female to male. In other words, the shift is from a misassigned gender in biological terms to the correct one; the reverse sequence has been reported but is exceedingly rare.

The reports of gender role reversal in 5α-reductase deficiency are particularly striking because of the fact that the phenomenon occurs in different ethnic groups and in different social settings. Indeed the suggestion has been made that androgen action in the brain (in utero, during the neonatal period, and/or at puberty) has an impact on the determination of male gender identity that is so

pervasive that it can override female sex assignment and female rearing (44, 79). Interesting as this line of thought may be, the suggestion that these individuals undergo a true change of gender identity as contrasted to a change in gender role behavior is open to many problems of interpretation:

(1) No prospective studies have been performed so that it is impossible to ascertain whether the gender identity prior to puberty was in fact unambiguously female. Apparent gender reversal is a prominent feature only of those forms of abnormal sexual development in which genital ambiguity is prominent. Many individuals with ambiguous genitalia are aware of their abnormalities from an early age and consequently unclear as to their exact gender role prior to puberty (49).

(2) Many patients in whom gender role has changed from female to male at puberty have been raised in cultures in which the sexes have fairly rigid stereotypes as to sexual role and in which the traditional female roles centering around home and family life are difficult, if not impossible, for women with phallic enlargements and shallow vaginas. Consequently, in such an environment cultural forces serve to reinforce any biological forces involved in a change from a female to a male role. Furthermore, it is difficult to perform prospective studies of sexual behavior in these communities (80).

(3) Even if it were established that the changes in gender role in such patients were due to an actual change in gender identity and that gender identity and role were determined solely, or significantly, by biological forces rather than psychosocial factors, it would still be unclear whether the changes are due to effects on the central nervous system or the peripheral target tissues. The development of a functional penis might influence behavioral patterns independent of the central nervous system. It is plausible to argue that any effects of gonadal steroids on human behavior could be mediated largely, if not exclusively, by their effects on peripheral tissues. These could include such diverse actions as tomboyish behavior which might result from androgen actions on muscle growth as well as adoption of a male gender role due to virilization of the genitalia.

(4) If it is true that androgens can override female gender identity - by whatever mechanism - it is interesting that those disorders in which reversal apparently occurs (5α-reductase deficiency, defects in testosterone synthesis, and mixed gonadal dysgenesis) are those in which the androgen receptor mechanisms are intact and in which considerable virilization is still possible, despite the fact that initial testosterone levels during embryogenesis are not normal. If the behavioral consequences are androgen-mediated, it would still have to be ascertained whether such effects are mediated by testosterone itself, by dihydrotestosterone (formed via steroid 5α-reductase 1) or by other

androgen metabolites (such as estradiol formed within the central nervous system). Furthermore, it seems safe to assume that psychosocial forces interact with hormonal factors in determining the sexual behavior of humans, and definitive studies into this problem are difficult because of a variety of limitations in methods available for studying human behavior.

Two side aspects of this phenomena deserve special comment. First, the New Guinean cluster of subjects with 5α-reductase deficiency originally identified by Gadjusek (81) and subsequently studied by Herdt and Davidson (72, 82) and by Imperato-McGinley et al (43) includes some people raised initially as females and some raised from the first as males. In this tribe the disorder is common enough that affected individuals are frequently assigned initially to a third sex, but they eventually have the same type of difficulties fitting into adult life as do subjects with intersex in other parts of the world, most neither being accepted as women or as functioning men within society. Second, the first case of gender role reversal and indeed of steroid 5α-reductase 2 deficiency may well have been Herculine Barbin, a nineteenth century woman who changed her legal sex from female to male and whose phenotype, including evidence from autopsy, is compatible with the diagnosis (83). Indeed, Barbin's description of the psychological problems inherent in intersexuality is a masterpiece and a sad story indeed (83). Of all the unresolved problems of 5α-reductase deficiency, the mechanism by which androgens, including dihydrotestosterone, influence sexual behavior is certainly the most interesting.

DIAGNOSIS

The diagnosis of 5α-reductase deficiency is commonly made either at the time of expected puberty or in infancy. In the adolescent or young adult, diagnosis is usually straightforward, namely a 46,XY male pseudohermaphrodite with the characteristic phenotype, male plasma testosterone levels, and abnormal ratios of plasma testosterone to dihydrotestosterone or abnormal ratios of urinary 5α- to 5β-steroid metabolites (Table 6). In this group it is necessary to distinguish 5α-reductase deficiency from defects in testosterone biosynthesis on the one hand and partial defects of the androgen receptor on the other (12). In all three disorders, virilization of the wolffian ducts can be more complete than that of the external genitalia. Defects in testosterone biosynthesis are usually associated with low plasma testosterone for men, but in men with partial enzyme deficiency, testosterone can be normal at the expense of high LH values (12). The most common hereditary defect in testosterone biosynthesis, 17β-hydroxysteroid oxido-reductase deficiency, can be recognized on the basis of elevated androstenedione levels, and it is our practice to measure androstenedione routinely in suspected cases of 5α-reductase deficiency. The recognition of partial defects in the androgen receptor can be more perplexing, since such defects can impair the

development of tissues that are major sites of dihydrotestosterone biosynthesis and hence cause secondary forms of apparent 5α-reductase deficiency with abnormally high ratios of plasma testosterone to dihydrotestosterone (84, 85). In the latter cases, detailed family histories indicating the pattern of inheritance, careful phenotypic characterization to determine the presence or absence of gynecomastia, and measurements of the ratios of 5β- to 5α-urinary glucocorticosteroid metabolites (which reflect hepatic metabolism, predominantly) may provide insight into the true diagnosis.

Recognition of 5α-reductase in infancy presents special problems, particularly when the family history is uninformative. In this situation, as in all prepubertal subjects, determination of the ratios of plasma testosterone to dihydrotestosterone before and after administration of hCG generally serves to establish the diagnosis (86). In those situations in which the testes have previously been removed, the diagnosis can be established either by determining the ratio of urinary 5β- to 5α-glucocorticosteroid metabolites (73, 86) or by determining the ratio of plasma testosterone to dihydrotestosterone after the administration of testosterone esters by injection (51).

MANAGEMENT

For those individuals raised as males or who elect to function as males, several procedures are appropriate. First, urological consultation should be obtained regarding appropriate corrective surgery to repair chordee, correct hypospadias, and bring cryptorchid testes as low as possible into the labioscrotal folds. Second, in view of the fact that the degree of virilization is generally unsatisfactory, supplemental androgen therapy is indicated in such patients. The ideal agent would be one that replaces the missing dihydrotestosterone; in experimental studies the administration of dihydrotestosterone enanthate by injection at 4- to 6-week intervals results in a sustained elevation of plasma dihydrotestosterone levels (87), but at present the agent is not available for general use. A second technique has been to administer testosterone esters in quantities sufficient to elevate plasma testosterone to supraphysiological levels; when this has been done in patients with 5α-reductase deficiency, it is possible to bring dihydrotestosterone levels to the normal male range and to promote virilization in a satisfactory manner (49). Unfortunately, it is not known whether the supraphysiological levels of testosterone may produce deleterious side effects over the long term. A third approach would be to administer androgen in a form that does not require 5α-reductase to be active. For example, 19-nortestosterone is active in the absence of 5α-reduction (88) and can be given by injection in an esterified form such as nandrolone decanoate. A fourth approach in the administration of a dihydrotestosterone cream by inunction (46); this regimen raises plasma dihydrotestosterone levels and has resulted in considerable phallic

growth in infants (46). Although this means of androgen administration appears to be safe over the short term its long-term efficacy and safety have not been established (89).

In those subjects who elect to lead life as women, the management should be similar to that in women with testicular feminization and allied syndromes (12) but should be undertaken only after careful psychiatric and psychological evaluation. That is, the testes should be removed to preclude (or stop) the partial virilization at expected puberty, estrogen/progestogen therapy should be instituted at an appropriate age to promote feminization, and, when appropriate, vaginoplasty should be undertaken by either surgical or medical means (90).

SUMMARY

In the twenty years since it was established that impairment of dihydrotestosterone formation is the cause of a rare form of human intersex, a wealth of information has accumulated about the genetics, the endocrinology, and the variable phenotypic manifestations, culminating in the cloning of the cDNAs for the 5α-reductase genes and the analysis of the mutant genes responsible for steroid 5α-reductase deficiency in David Russell's laboratory. We are still left with perplexing and difficult unresolved problems, for example, whether the variability in manifestations is due to variable expressions of steroid 5α-reductase 1 or to variable effects of testosterone itself. It is also imperative to establish whether defects in steroid 5α-reductase 2, perhaps in the heterozygous state, are responsible for a significant fraction of sporadic hypospadias, to determine whether 5α-reductase plays a role in the physiology of progesterone in women, and to elucidate the relation between androgen action and gender role behavior.

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