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Research Article

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Reduced Affinity of the Androgen Receptor for 5α -Dihydrotestosterone but not Methyltrienolone in a Form of Partial Androgen Resistance

Studies on Cultured Genital Skin Fibroblasts

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Abstract

We have studied a child with posterior labial fusion, clitoral phallus, female urethra, and a short, blind vagina born to a mother with decreased axillary and pubic hair. Her karyotype is 46,XY. At 2 yr of age, the child's basal level of plasma testosterone was <0.35 nM and after human chorionic gonadotropin stimulation, it rose to 2.6. Testis and epididymis histology were normal. Her cultured genital (labial) skin fibroblasts have normal testosterone 5α -reductase activity, and metabolize 5α -dihydrotestosterone (DHT) normally, but they do not augment (up-regulate) their basal androgen-receptor binding activity during prolonged incubation with DHT. With DHT, the androgen receptor in her genital skin fibroblasts has a normal binding capacity (maximum binding capacity = 25fmol/mg protein), but an increased rate constant of dissociation $(k = 11.6 \times 10^{-3} \text{ min}^{-1}; \text{ normal, } 6 \pm 1.2 (\pm \text{SD})), \text{ and a decreased}$ apparent equilibrium binding affinity ($K_d = 0.6$ nM; normal, 0.22±0.09) that is evident in the results of 2-h assays but not of those lasting 0.5 h. With the synthetic androgen, methyltrienolone, all three binding properties of the receptor are normal, and her receptor activity up-regulates normally. We interpret these results to mean that the subject has a ligandselective defect in the time-dependent transformation of initial, low-affinity androgen-receptor complexes to serial states of higher affinity, presumably as the result of a structural mutation at the X-linked locus that encodes the androgen receptor protein.

Introduction

Androgen resistance $(AR)^1$ can be classified in clinical and biochemical terms (1). Clinically, complete AR is synonymous with the syndrome, complete testicular feminization, in which XY subjects are born with female external genitalia and

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undergo a feminine puberty with breast development, but sparse or absent sexual hair, despite normal to elevated levels of plasma androgens. In partial forms of AR, there is frank ambiguity of the external genitalia or some degree of penile hypospadias at birth, usually accompanied by gynecomastia and poor virilization at puberty, and occasionally with highly variable intrafamilial expressivity (2). The mildest forms are characterized by male external genitalia that may include micropenis, and normal or near-normal virilization at puberty except for gynecomastia (3). In the extreme case, the latter class is exemplified by otherwise normally virilized men in whom AR is expressed solely by azospermia or severe oligospermia (4). Each of the clinical classes is heterogeneous in respect to the quantity and quality of the specific androgen receptor-binding activity in cultured genital skin fibroblasts. Thus, subjects with complete AR may have normal (receptorpositive), intermediate (receptor-deficient), or barely detectable (receptor-negative) concentrations of this activity. In the former two situations, qualitative defects of the androgen-receptor system have often been found (5-9). In this communication, we define a form of receptor-positive partial AR that is apparently due to a mutant androgen receptor that has a lower than normal affinity for 5α -dihydrotestosterone (DHT) as expressed both by an elevated apparent equilibrium dissociation constant (K_d) and an increased rate constant of dissociation (k). In contrast, both measures of affinity are normal when the mutant receptor interacts with the synthetic androgenic ligand, methyltrienolone (MT). This ligand-selective defect of the androgen receptor is reflected functionally by the fact that the subject's fibroblasts augment their basal androgen receptor activity during prolonged incubation with MT, but not with DHT.

Methods

Clinical summary. The child who is the subject of this report presented to the Saskatchewan University Hospital at 3 mo with "a normal clitoris and labial fusion." Behind the fused labia were a female urethra and a small, blind vagina as defined by cystography and endoscopy. Gonads were palpable in each inguinal canal. The peripheral lymphocytes had a normal 46,XY karyotype. At 4 mo, urinary 17-ketosteroids (0.3 mg/24 h [×2]) and the levels of testosterone (<0.3 nM), LH (10 mIU/ml), and FSH (8 mIU/ml) in the blood were all within normal limits. A labium majus skin biopsy was explanted to develop a strain of fibroblasts. At 2 yr, a dexamethasone (0.25 mg/d \times 2)-human chorionic gonadotropin (250 IU/d \times 4) stimulation test revealed a basal plasma testosterone (T) of <0.35 nM and a post-stimulation level of 2.6 nM. Thereafter, the gonads were histologically verified to contain immature seminiferous tubules, efferent ductules, and epididymes unremarkable for age.

The subject's mother has decreased pubic and axillary hair but had a normal age of menarche.

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^{1.} Abbreviations used in this paper: Δ^4 , androstenedione; AR, androgen resistance; B_{max} , maximum specific binding capacity; DHT, 5α -dihy-drotestosterone; GSF, genital skin fibroblast(s); k, rate constant of dissociation; K_d , apparent equilibrium dissociation constant; MT, methyltrienolone; s-f, serum-free; T, testosterone.

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The labium majus skin fibroblast strain of the subject was developed in Saskatoon and sent to Montreal for analysis. Those strains developed locally were derived, as described in detail previously (10), from small pieces of labium majus, scrotum, or prepuce skin obtained with informed consent according to a protocol approved by the Hospital Ethics Committee.

Androgen receptor assay. Fibroblast monolayers were grown to confluence in 5-cm plastic petri dishes with Eagle's modified essential medium supplemented by 10% (vol/vol) fetal calf serum, 2 mM glutamine, 1 mM pyruvate, $1 \times$ nonessential amino acids, and 60 mg/ liter each of penicillin G and streptomycin sulfate. Replicate dishes were incubated at 37°C for 30-120 min (as specified) with varying concentrations (0.1-3 nM) of [1,2,4,5,6,7-3H]DHT (120 Ci/mmol) or $[17\alpha$ -methyl-³H]MT (87 Ci/mmol) (New England Nuclear, Boston, MA) to measure "total" binding, or together with a 200-fold excess of the relevant radioinert androgen (to measure nonspecific binding) in 3 ml of serum-free (s-f) medium buffered to pH 7.4 by the addition of 15 mM Hepes. Thereafter, the monolayers were washed twice with 5 ml of ice-cold 20 mM Tris-HCl (pH 7.4) containing 0.15 M NaCl and 0.3% bovine serum albumin, and then twice more with the same solution minus bovine serum albumin. The monolayers were then treated for 5 min with 2 ml of 0.1% trypsin, the loosely adherent cells were scraped with a rubber policeman, and the suspension was centrifuged (4°C) at 200 g for 5 min. After resuspension in 5 ml Tris-HCl-NaCl and recentrifugation as above, the cell pellets were dissolved in 0.5 N NaOH and sampled for protein by the technique of Lowry et al. (11) and for radioactivity in 10 ml of a toluene solution containing Omnifluor (4 g/liter; New England Nuclear).

Specific (total minus nonspecific) binding activity was plotted as a function of free androgen concentration, and maximum specific binding capacity (B_{max}) and the apparent equilibrium dissociation constant (K_d) were derived from lines fitted to Scatchard plots (12) by linear regression. Only lines with an r = -0.89 or greater and a P < 0.01 were considered.

When normal genital skin fibroblast (GSF) monolayers are incubated with sufficient DHT in s-f medium at 37°C, they saturate their specific androgen-binding activity within 30 min (13–15), and they maintain that level for at least 2–3 h (5, 14). We have found, however, that saturation curves performed on replicate groups of GSF yield lower values of apparent K_d after incubations of 1 or 2 h than after those of 0.5 h (16). This phenomenon occurs despite the continuing catabolic consumption of DHT during the prolonged assays; indeed, it occurs, as well, with the synthetic, nonmetabolizable androgen, MT. Further data supporting this apparently paradoxical observation are presented in the Results section, and are interpreted in the Discussion.

DHT metabolism. To evaluate the extent and quality of [³H]DHT catabolism during 30-120 min of incubation at 37°C, the medium from a 5-cm dish that contained confluent monolayers was supplemented with 4 \times 10⁴ dpm of [¹⁴C]DHT and 10 μ g each of T and androstenedione (Δ^4), and then extracted twice with diethyl ether. The 5α -catabolites of DHT [$3\alpha(3\beta)$ -androstanediol, androstanedione, and androsterone] were separated from DHT and each other by thin layer chromatography using two or three ascents in 99.5:0.5 chloroform/ methanol, T and Δ^4 were visualized under shortwave ultraviolet light; DHT was visualized by radioautography. [14C]DHT was used to monitor recovery and to estimate purity of the [3H]DHT spot by recrystallization to constant isotopic ratio. On all occasions, it was >90% pure. These assays were run in duplicate; the results, which seldom differed by more than 5%, are expressed as percent recovery of DHT or one of its catabolites relative to the 0-time concentration of [3H]DHT. The cells contained relatively insignificant amounts of radioactivity at any time.

GSF do not catabolize R1881 for at least 72 h at 37°C (9).

 5α -Reductase activity. This was determined according to the method of Wilson (17). The substrate was $[1,2,6,7,16,17-^{3}H]$ testosterone (138 Ci/mmol; New England Nuclear), and DHT, the only 5α -reduced metabolite generated to a significant extent, was identified, recovered, and measured as described above for the assessment of DHT catabolism. Dissociation rates of androgen-receptor complexes. To determine the rate constant of dissociation (k) of androgen-receptor complexes formed within GSF that had been incubated with 2–5 nM [³H]androgen for 30–120 min at 37°C, the assay medium in some dishes was replaced by a "chase" medium containing 0.6 μ M radioinert androgen, and the percent original complexes remaining after various times at 37°C was plotted semilogarithmically. Unchased replicate monolayers maintained their specific receptor activity, indicating no receptor degradation during the period of dissociation.

Prolonged incubation with DHT or MT. We assessed the ability of the subject's GSF to augment their specific androgen receptor activity in response to prolonged exposure to either androgen at 37°C as described in detail previously (9, 18, 19). Briefly, for DHT, replicate cultures were preincubated in s-f medium alone, or in s-f medium with 2-3 nM [³H]DHT plus or minus 200-fold radioinert DHT for 4 or 19 h before initiating a 1-h assay of specific DHT-receptor activity by replacement of spent media with appropriate fresh media. For MT, replicate cultures were incubated in s-f medium alone or with 2-3 nM [³H]MT plus or minus 200-fold radioinert MT for up to 72 h, and specific MT-receptor activity was assayed at intervals without replacement of spent media 1 h before assay. As pointed out previously (20), specific basal androgen-receptor activity in GSF is stable in s-f or androgen-free medium for up to 72 h; therefore, augmentation of specific androgen receptor activity after prolonged exposure to androgen is not simply a consequence of receptor stabilization.

Results

 B_{max} and K_d . The results of the 2-h determination of B_{max} and K_d with DHT and MT in the GSF of the mutant subject and normal controls are listed in Table I and exemplified, for DHT, in Fig. 1. It is apparent that the mutant androgen receptor has a normal B_{max} for DHT and MT and a reduced apparent binding affinity (increased apparent K_d) for DHT but not MT.

Relations among time of incubation, apparent K_d , and extent and quality of DHT catabolism in GSF of control and the mutant subjects. Fig. 1 shows that the apparent K_d for

Table I. Properties of the Specific Androgen-receptor Activity in GSF of the Mutant Subject (5555) and Controls

	B _{max}		K₄		
	DHT	МТ	DHT	MT	
	fmol/mg protein	fmol/mg protein	nM	nM	
5555	16	22	0.28	0.19	
	41	26	1.60	0.18	
	24	30	0.47	0.17	
	25		0.55		
	19		0.46		
	17		0.48		
	32		0.52		
	27		0.46		
Mean±SD	25±7.8	26	0.60±0.38	0.18	
Controls					
Mean±SD	28±8	31±12	0.22±0.09	0.16±0.08	
n	26	8	26	8	

Replicate confluent monolayers in 5-cm petri dishes were incubated for 2 h in s-f medium with 0.1-3.0 nM [³H]DHT or [³H]MT plus or minus 200-fold radioinert androgen. Specific androgen-binding activity was plotted as a function of free [³H]androgen concentration according to Scatchard (12). B_{max} and K_d were determined as explained in the legend to Fig. 1.



DHT BOUND (fmol/mg protein)

Figure 1. Representative Scatchard plots on a control strain (2036) and that of subject 5555 from incubations on replicate groups of monolayers incubated for 0.5 or 2 h. K_d is computed from the slope of the Scatchard line and B_{max} is extrapolated from its intercept on the abscissa.

DHT decreases (apparent binding affinity increases) with increasing time of incubation, both for a control strain and that of subject 5555. Table II reveals that this occurs despite concurrent catabolism of DHT, and without any relation between percent recovery of DHT and apparent K_d for DHT after either 0.5- or 2-h incubations. Indeed, the mutant cells do not catabolize DHT to a greater extent than control cells, yet they have an apparent K_d for DHT that is in the normal range at 0.5 h but above the normal range (0.1–0.3 nM) at 2 h. Furthermore, the effect of prolonged incubation on apparent

 K_d is also seen for the nonmetabolizable and rogen, MT, but with this ligand the mutant cells behave normally.

The GSF of subject 5555 not only catabolize DHT to a normal extent, but they also produce DHT catabolites in proportions that do not differ from those of control strains as shown in Table III.

 5α -Reductase activity. The subject's GSF converted testosterone to DHT at a rate of 30 pmol/mg protein per hour. A control strain (2036) assessed simultaneously had 20 U of activity. These are normal values (17).

Rates of dissociation of androgen-receptor complexes. The rates of dissociation of DHT- and MT-receptor complexes are given in Table IV. It is apparent that DHT dissociates from the subject's androgen receptor at a rate twice normal, while MT does so at a normal rate. A representative experiment is depicted in Fig. 2. The rates for DHT in the controls and the subject correspond to half-lives of 90 and \sim 45 min, respectively.

Prolonged incubation with DHT or MT. The response of the specific androgen-receptor activity to prolonged incubation with either ligand is shown in Fig. 3. With MT, the GSF of subject 5555 augmented their activity with a time-course and to an extent (more than double their basal [1-h] value) typical of control strains (19). The response of the subject's GSF to 4 h of exposure to DHT was not sustained during the next 15 h. In contrast, the control strain assessed simultaneously exhibited a progressive augmentation of specific DHT-receptor activity through 19 h of exposure to DHT, and thus behaved in the typical fashion (9).

Discussion

The subject described in this report was born with ambiguous external genitalia including a clitoral phallus, labial fusion, female urethra, short blind vagina, and inguinal gonads bilaterally. The gonads were verified as normal testes histologically, and were shown to augment their secretion of testosterone adequately in response to submaximal human chorionic gonadotropin stimulation.

These clinical observations suggest that the child has male pseudohermaphroditism due to a form of partial androgen resistance, and that the reduced axillary and pubic hair in her

Table II. K_d (nM) and B_{max} (fmol/mg protein) of Specific Androgen Binding within Replicate Groups of GSF of Subject 5555 and Controls in Relation to Length of Incubation and Recovery of $[^3H]5\alpha$ -Dihydrotestosterone

		0.5-h Incubation			2-h Incubation		
Strain	Androgen	Kd	B _{max}	Recovery	Kd	B _{max}	Recovery
				%			%
2036 (experiment 1)	DHT	1.0	29	83	0.23	27	40
2036 (experiment 2)		0.4	28	87	0.10	27	54
82612		0.51	25	62	0.15	21	29
5555		1.1	23	72	0.46	27	64
141141	MT*	0.3	17	_	0.13	18	_
82612		0.2	25		0.07	29	_
5555		0.5	28	_	0.17	30	

Confluent monolayers were incubated in s-f medium with 4 nM [3 H]DHT. The catabolites of DHT in the medium were separated by thin-layer chromatography and localized in relation to the mobilities of T, Δ^4 , and [14 C]DHT. The latter also served to monitor overall recovery and estimate the purity of the [3 H]DHT recovered. * MT is not catabolized.

Table III. DHT Catabolism in GSF of Subject 5555 and a Control Incubated with 4 nM [${}^{3}H$]DHT for 0.5 and 2 h at 37°C

Strain	h	Total recovery	Diols	Andro	DHT	Ane
		%		%		
82612	0.5 2	98 91	7 10	14	62 29	29 38
5555	0.5 2	100 101	5 8	4 9	76 53	15 31

DHT catabolism was assessed as described in the legend to Table II. Andro, androsterone; Ane, androstanedione; Diols, androstanediols.

mother is the heterozygous manifestation of a mutation at the X-linked locus (21) for the androgen-receptor protein that is known to undergo random inactivation (22). Indeed, there is no other genetic cause of male pseudohermaphroditism in which carrier females are known to express their genotype clinically (23).

We have used GSF to show that the subject's androgen receptor has a normal B_{max} , but an increased apparent K_d and k for the natural androgen, DHT. In contrast, all three binding properties of the mutant receptor are normal with the synthetic,

Table IV. Rates of Dissociation of [³H]Androgen-receptor

Complexes Within GSF of Subject 5555 and Controls

100 80 60 $= 5.8 \times 10^{-3} \text{ min}^{-1}$ -0.98 40 $= 7.4 \times 10^{-3} \text{ min}^{-1}$ = -0.94 $= 13.3 \times 10^{-3} \text{ min}^{-1}$ 20 r = -0.99 30 60 90 120 MIN AT 37°C

DHT-RECEPTOR COMPLEXES

2

Figure 2. A representative experiment in which the rate of dissociation of DHT-receptor complexes at 37° C was assessed in GSF of subject 5555 (•) and those of two controls (•, •) after 0.5 h of incubation at 37° C with 5 nM [³H]DHT.

nonmetabolizable, androgenic ligand, MT. While making these observations, we have confirmed a previous report (13) that MT binds to the androgen receptor in normal GSF with a

	Rate constant of dissociation (k)		
	DHT	МТ	
	10 ⁻³ min ⁻¹	10 ⁻³ min ⁻¹	
5555	8*	16	
	10	15	
	13		
	13	_	
	12	_	
	11	_	
	15‡		
Mean±SD	11.6±2.8	15.5	
Controls			
Mean±SD	6.0±1.2§	12.1±3§	
(<i>n</i>)	(15)	(26)	

* The majority of values in this column were obtained when GSF of the subject and a control were assayed simultaneously. ‡ In this experiment (see also Fig. 2) the same result was obtained from replicates that were chased after a 0.5-h incubation. § These results are unchanged after incubation for 0.5 or 2 h. Confluent monolayers in 5-cm petri dishes were incubated at 37°C for 2 h (or as specified) with 2 or more nM [³H]DHT or [³H]MT plus or minus 200-fold radioinert androgen. At 2 h, the assay medium in some dishes was replaced by a "chase" medium containing 0.6 μ M of the radioinert androgen, and the percent original androgen-receptor complexes remaining after various times at 37°C was plotted semilogarithmically. To insure that this rate of disappearance was a measure of androgen-receptor complex dissociation, we ascertained that unchased replicates maintained their specific androgen-receptor activity over the same time period.

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INCUBATION (hours)

Figure 3. Experiments revealing the response of androgen-receptor activity to prolonged incubation with DHT or MT in the GSF of subject 5555, one normal control, and one strain (61479) from a subject with primary 5α -reductase deficiency (36). "4+1" and "19+1" mean that fresh s-f medium containing 2 nM [³H]DHT plus or minus 0.4 μ M DHT was given after 4 and 19 h of incubation to initiate a 1-h assay of specific DHT-binding activity.

somewhat higher equilibrium binding constant (lower K_d) than DHT, yet it dissociates from it about twice as fast as DHT.

It is easy to imagine that primary hypercatabolism of DHT could generate a form of androgen resistance that might be expressed by an artificially high K_d for DHT. However, we have eliminated this possibility in our subject directly by showing that her GSF do not catabolize DHT excessively, or in a qualitatively abnormal way. In addition, we have demonstrated that the mutant DHT-receptor complexes dissociate abnormally quickly, using a chase protocol that nullifies the factor of DHT catabolism entirely. Therefore, we conclude that the subject has a primary, qualitative, ligand-selective, androgen-receptor defect that is presumably due to a structural gene mutation.

We have found that appreciable DHT catabolism (for instance, up to 38% of 4 nM DHT in 0.5 h) is still compatible with normal 2-h K_d values for DHT. Furthermore, K_d values are always lower (and as a group less variable) after 2 h of incubation than after 0.5 h, despite progressive DHT depletion during the extended assays (Table II; and [16]). Indeed, in the latter respects, the receptor behaves similarly even with the nonmetabolizable androgen, MT.

The simplest interpretation of these facts is that androgenreceptor complexes are formed in an initially low-affinity state and are transformed ("activated") to one or more sequential higher-affinity states by a process that is time-dependent. Such transformation has been demonstrated in rabbit uterine cytosol with the k of progesterone-receptor complexes (24), in calf uterine cytosol with the k of estradiol-receptor complexes (25), and in whole circulating mononuclear leukocytes of the goat with the K_d of dexamethasone-receptor complexes (26). In the latter situation, the K_d decreased from 1.6 to 0.24 nM between 2 and 15 h of incubation at 20°C. In contrast, as judged by altered mobility on DEAE-cellulose columns, cytosolic dexamethasone-receptor complexes underwent transformation within 15 min when rat thymus cells were incubated at 37°C (27). These differences in the time apparently required for transformation of dexamethasone-receptor complexes are not interpretable in view of the different protocols employed; nor are they relatable to the time frame we have observed for transformation of androgen-receptor complexes in human GSF.

One deduction from our data is that the rate of transformation of androgen-receptor complexes in human GSF is subject to the initial concentration of androgen that is available for complex formation. Thus, increased apparent equilibrium binding affinity (decreased K_d) with time would merely reflect greater transformation of the complexes to the higher-affinity state(s) at the lower concentrations of ligand. It would also explain why normal GSF incubated with 2 or more nM [³H]androgen, whether for 0.5 or 2 h, form a single pool of high-affinity androgen-receptor complexes that dissociate at the same slow rate.

We have reasoned previously (9) that a primary defect in the transformation of initial, low-affinity complexes, rather than the possibility of secondarily accelerated DHT catabolism, might be the proximate cause for failure of prolonged incubation of mutant GSF with DHT to provoke augmentation of their DHT-receptor activity ("up-regulation"). This idea has been substantiated by our recent characterization of two different receptor-positive, androgen-receptor mutations that cause familial partial androgen resistance and that have equally abnormal K_d for both DHT and MT (16, 28). The mutant GSF from either family cannot up-regulate with DHT or MT, despite the fact that they catabolize DHT at a normal rate and MT not at all. On the background of this information, the ability of the GSF of subject 5555 to up-regulate normally in response to prolonged incubation with MT, but not with DHT, (despite the fact that they do not hypercatabolize DHT) affirms our conclusion that they have a ligand-selective defect in transformation of their androgen-receptor complexes that is expressed with DHT but not with MT.

One important corollary of this conclusion is that the structural attributes of the synthetic androgen enable it to complement those of the mutant receptor protein so as to generate an androgen-receptor complex with normal properties. The subtleties that underlie such a complementary interaction are obscure, but they are highly reminiscent of the faulty transformation behavior that is expressed by complexes formed from certain glucocorticoid antagonists with the glucocorticoid receptor (29), and from certain estrogen antagonists with the estrogen receptor (30). Indeed, full understanding of the physiological consequences of steroid-receptor interactions may have to await much more knowledge about the way normal steroid-receptor complexes regulate gene transcription (31).

Meanwhile, the ligand-selective dysfunction imposed on the androgen receptor by the mutation in the present subject makes it tempting to consider the therapeutic implications of our data. Oral MT is 50 times more potent than 17α -methyltestosterone in maintaining prostate weight of prepubertal castrated rats (32), and, given parenterally for 5 to 10 d in the neonatal period, can inhibit the capacity of ovariectomized rats to show female mating behavior when given ovarian hormones in adulthood (33). Oral MT has also been shown to have superior anabolic potency in man (34) presumably, in part, because it does not bind to the sex steroid-binding globulin, and is metabolized slowly, if at all (35). Unfortunately, it is equally potent in causing intrahepatic cholestasis in man (34). A mode of parenteral administration that avoids the firstpass effect of MT on the liver, or a search for other synthetic androgens that share the normalizing effects of MT in vitro, but not its hepatotoxicity when administered orally, may be fruitful for various clinical purposes in androgen-resistant subjects with receptor mutations like the one described here.

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References

1. Brown, T. R., and C. J. Migeon. 1981. Cultured human skin fibroblasts: a model for the study of androgen action. *Mol. Cell. Biochem.* 36:3-22.

2. Wilson, J. D., M. J. Harrod, J. L. Goldstein, J. L. Hemsell, and P. C. MacDonald. 1974. Familial incomplete male pseudohermaphroditism, type 1: evidence for androgen resistance and variable clinical manifestations in a family with the Reifenstein syndrome. N. Engl. J. Med. 290:1097-1103.

3. Larrea, F., G. Benavides, H. Scaglia, S. Kofman-Alfaro, E. Ferrusca, M. Medina, and G. Perez-Palacios. 1978. Gynecomastia as a familial incomplete male pseudohermaphroditism type 1: a limited androgen resistance syndrome. J. Clin. Endocrinol. Metab. 46:961–970.

4. Aiman, J., and J. E. Griffin. 1982. The frequency of androgen receptor deficiency in infertile men. J. Clin. Endocrinol. Metab. 54: 725-732.

5. Pinsky, L., M. Kaufman, and R. L. Summitt. 1981. Congenital androgen insensitivity due to a qualitatively abnormal androgen receptor. *Am. J. Med. Genet.* 10:91–99.

6. Kaufman, M., L. Pinsky, L. Simard, and S. C. Wong. 1982. Defective activation of androgen-receptor complexes: a marker of androgen insensitivity. *Mol. Cell. Endocr.* 25:151-162.

7. Brown, T. R., M. Maes, S. W. Rothwell, and C. J. Migeon. 1982. Human complete androgen insensitivity with normal dihydrotestosterone receptor binding capacity in cultured genital skin fibroblasts: evidence for a qualitative abnormality of the receptor. J. Clin. Endocrinol. Metab. 55:61-69.

8. Griffin, J. E., and J. L. Durrant. 1982. Qualitative receptor defects in families with androgen resistance: failure of stabilization of the fibroblast cytosol androgen receptor. J. Clin. Endocrinol. Metab. 55:465–474.

9. Kaufman, M., L. Pinsky, R. Hollander, and J. D. Bailey. 1983. Regulation of the androgen receptor by androgen in normal and androgen-resistant genital skin fibroblasts. *J. Steroid Biochem.* 18:383– 390.

10. Pinsky, L., J. Miller, B. Shanfield, G. Watters, and L. S. Wolfe. 1974. G_{m1}-gangliosidosis in skin fibroblast culture. Am. J. Hum. Genet. 26:563-577.

11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein determination with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.

12. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. Ann. NY Acad. Sci. 51:660-672.

13. Brown, T. R., S. W. Rothwell, and C. J. Migeon. 1981. Comparison of methyltrienolone and dihydrotestosterone binding and metabolism in human genital skin fibroblasts. *J. Steroid Biochem.* 14: 1013–1022.

14. Hodgins, M. B. 1982. Binding of androgens in 5α -reductasedeficient human genital skin fibroblasts and its inhibition by progesterone. J. Endocrinol. 94:415-432.

15. Eil, C. 1983. Familial incomplete male pseudohermaphroditism associated with impaired nuclear androgen retention. Studies in cultured skin fibroblasts. J. Clin. Invest. 71:850-858.

16. Kaufman, M., L. Pinsky, A. Bowin, and M. W. S. Au. 1984. Familial external genital ambiguity due to a transformation defect of androgen-receptor complexes that is expressed with 5α -dihydrotestosterone and the synthetic androgen methyltrienolone. Am. J. Med. Genet. 18:493-507.

17. Wilson, J. D. 1975. Dihydrotestosterone formation in cultured human fibroblasts. J. Biol. Chem. 250:3498-3504.

18. Kaufman, M., L. Pinsky, and R. Feder-Hollander. 1981. Defective up-regulation of the androgen receptor in human androgen insensitivity. *Nature (Lond.).* 293:735-737.

19. Pinsky, L., M. Kaufman, C. Gil-Esteban, and D. Sumbulian. 1983. Regulation of the androgen receptor in human genital skin fibroblasts, with a review of sex steroid receptor regulation by homologous and heterologous steroids. *Can. J. Biochem. Cell Biol.* 61:770–778.

20. Kaufman, M., C. Straisfeld, and L. Pinsky. 1977. Expression of androgen-responsive properties in human skin fibroblasts of genital and nongenital origin. *Somatic Cell Genet.* 3:17-35.

21. Migeon, B. R., T. R. Brown, J. Axelman, and C. J. Migeon. 1981. Studies of the locus for androgen receptor: localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse. *Proc. Natl. Acad. Sci. USA*. 78:6339-6343.

22. Elawady, M. K., D. R. Allman, J. E. Griffin, and J. D. Wilson. 1983. Expression of a mutant androgen receptor in cloned fibroblasts derived from a heterozygous carrier for the syndrome of testicular feminization. *Am. J. Hum. Genet.* 35:376-384.

23. Imperato-McGinley, J. 1983. Sexual differentiation: normal and abnormal. Curr. Top. Exp. Endocrinol. 5:231-307.

24. Seeley, D. H., and P. D. Costas. 1983. Transformation of a rabbit progesterone receptor from an 8S form to 5.5S and 4S forms. *Mol. Cell. Endocr.* 30:161–178.

25. Weichman, B. M., and A. C. Notides. 1977. Estradiol-binding kinetics of the activated and nonactivated estrogen receptor. J. Biol. Chem. 252:8856-8862.

26. Murakami, T., D. Brandon, D. Rodbard, D. L. Loriaux, and M. B. Lipsett. 1979. Glucocorticoid receptor in circulating mononuclear leukocytes. *Endocrinology*. 104:500-505.

27. Munck, A., and R. Foley. 1979. Activation of steroid hormonereceptor complexes in intact target cells in physiological solutions. *Nature (Lond.).* 278:752-754.

28. Jukier, L., M. Kaufman, L. Pinsky, and R. E. Peterson. 1984. Partial androgen resistance associated with secondary 5α -reductase deficiency: identification of a novel qualitative androgen receptor defect and clinical implications. J. Clin. Endocrinol. Metab. 59:679–688.

29. Moguilewsky, M., and D. Philibert. 1984. RU 38486: Potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. J. Steroid Biochem. 20:271-276.

30. Rochefort, H., and J.-L. Borgna. 1981. Differences between oestrogen receptor activation by oestrogen and antioestrogen. *Nature* (Lond.). 292:257-259.

31. Groner, B., H. Ponta, V. Rahmsdorf, P. Herrlich, M. Pfahl, and N. E. Hynes. 1984. Glucocorticoid hormone interactions with cloned proviral DNA of mouse mammary tumor virus. J. Steroid Biochem. 20:95–98.

32. Feyel-Cabanes, T. 1963. Etude biologique d'un analogue triénique de la méthyltestostérone. C. R. Soc. Biol. (Paris). 157:1428-1429.

33. Olsen, K. L. 1983. Effects of 17β -hydroxy- 17α -methyl-estra-4,9,11-triene-3-one (R1881): evidence for direct involvement of androgens in the defeminization of behaviour in rats. *J. Endocrinol.* 98:431– 438.

34. Krüskemper, H. L., and G. Noell. 1966. Liver toxicity of a new anabolic agent: methyltrienolone $(17\alpha$ -methyl-4,9,11-estratriene-17 β -ol-3-one). Steroids. 8:13-24.

35. Bonne, C., and J.-P. Raynaud. 1975. Methyltrienolone, a specific ligand for cellular androgen receptors. *Steroids*. 26:227-232.

36. Peterson, R. E., J. Imperato-McGinley, T. Gautier, and E. Sturla. 1977. Male pseudohermaphroditism due to steroid 5α -reductase deficiency. *Am. J. Med.* 62:170-191.