Mutations in the Ligand-binding Domain of the Androgen Receptor Gene Cluster in Two Regions of the Gene

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Abstract

We have analyzed the nucleotide sequence of the androgen receptor from 22 unrelated subjects with substitution mutations of the hormone-binding domain. Eleven had the phenotype of complete testicular feminization, four had incomplete testicular feminization, and seven had Reifenstein syndrome. The underlying functional defect in cultured skin fibroblasts included individuals with absent, qualitative, or quantitative defects in ligand binding. 19 of the 21 substitution mutations (90%) cluster in two regions that account for ~ 35% of the hormone-binding domain, namely, between amino acids 726 and 772 and between amino acids 826 and 864. The fact that one of these regions is homologous to a region of the human thyroid hormone receptor (hTR-β) which is a known cluster site for mutations that cause thyroid hormone resistance implies that this localization of mutations is not a coincidence. These regions of the androgen receptor may be of particular importance for the formation and function of the hormone-receptor complex. (*J. Clin. Invest.* 1992. 90:2097–2101.) Key words: androgen • receptor • mutation • steroid • resistance

Introduction

Like other members of the steroid-thyroid hormone-retinoid class of receptors, the androgen receptor contains three distinct domains: a hormone-binding region, a DNA-binding region, and an amino-terminal region (1). Mutations of the androgen receptor in 46,XY individuals cause a spectrum of androgen-resistance syndromes, ranging from women with complete testicular feminization to men with infertility or minor degrees of undervirilization (2). In part because the gene that encodes the androgen receptor is X-linked, and hence mutations of the androgen receptor are expressed in hemizygous males, mutations of the human androgen receptor may be more common than mutations of all other receptors of this class combined. The mutant androgen receptors were initially characterized by studying the functional properties in genital skin fibroblasts cultured from patients (3, 4). The cloning of cDNAs encoding the receptor protein (5–8) and the elucidation of the androgen receptor gene structure (9, 10) then made it possible to characterize the underlying mutations in molecular terms.

Several categories of these mutations are now recognized. Deletion of the coding sequence for the gene (11, 12) or nucleotide substitutions that cause the insertion of premature termination codons (10, 13–15) result in the failure to form a functional protein, and hence cause profound androgen resistance associated with absent ligand binding in fibroblasts. In contrast, mutations that cause amino acid substitutions in the DNA-binding domain cause receptor positive androgen resistance in which ligand binding to the receptor is normal, but the hormone-receptor complex does not bind normally to DNA (16, 17). Mutations also occur in the androgen-binding domain of the receptor (18–23). In this study, we describe the localization of the mutation from 20 previously unreported subjects and from two previously studied subjects with mutations of the hormone-binding domain.

Methods

Clinical history and phenotype. The patients described in this study were referred by various physicians. Phenotype was established by the referring physicians or by the authors in selected instances (2). Two patients in this report have been reported separately, as noted in the legend to Table I.

Characterization of receptor binding in cultured genital skin fibroblasts. Monolayer binding assays (2) were performed, and qualitative abnormalities of receptor binding (e.g., thermolability and dissociation rate) were characterized. For quantification of immunoreactive androgen receptor in fibroblasts, genital skin fibroblasts were scraped in PBS, pelleted, and homogenized in SDS-polyacrylamide gel loading buffer (24). The samples were electrophoresed on 7.5% SDS-polyacrylamide gels, transferred to nitrocellulose, and detected with an antibody directed at the amino terminus of the receptor protein (24).

Androgen receptor gene analysis. Individual exons were amplified from 22 patients with various phenotypes (10). In 16 patients, the entire coding sequence of the androgen receptor gene was determined, with two exceptions. First, because of inaccuracies introduced during the amplification and cloning of the glutamine repeats, these analyses are only estimates of the size. In the case of the glycine repeats, sequencing has not been performed. In the remaining six patients, exons 2–8 were completely sequenced.

Results

Characterization of ligand binding in genital skin fibroblast cultures show limited agreement with clinical phenotype. Androgen resistance is commonly associated with qualitative or quantitative abnormalities of receptor binding in genital skin

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Table I. Phenotypic and Biochemical Characteristics of Subjects with Androgen Resistance Caused by Amino Acid Substitutions in the Ligand-binding Domain of the Androgen Receptor

Phenotype	Strain	Binding Bmax	Androgen receptor in genital skin fibroblasts				
			Thermolability	Dissociation rate[¶]	Immunoreactivity*	Mutation	Exon
		(fmol/mg protein)					
Receptor binding negative							
CTF	14	2.0	—	—	—	P902H	8
CTF	69	2.7	—	—	77%	W739R	5
CTF	105[‡]	0.2	—	—	86%	R772C	6
CTF	406[§]	3.5	—	—	—	R829Q	7
CTF	511[§]	0.3	—	—	—	R853C	7
CTF	571	4.0	—	—	49%	F762L	5
REIF	593	2.6	—	—	98%	R838H	7
CTF	895	0.7	—	—	6%	R829Q	7
CTF	909[§]	1.1	—	—	38%	Y832C	7
CTF	420[§]	0.8	—	—	—	V864E	7
Qualitative abnormality of receptor binding							
REIF	16[‖]	16.3	No[‖]	—	—	V901M	8
REIF	48	33.0	Yes	Abnormal	—	A746D	5
Inc TF	93	10.0	Yes	—	—	L726S	5
Inc TF	370	13.2	Yes	—	—	V864M	7
REIF	604[§]	22.5	Yes	—	—	R838C	7
Inc TF	450	13.6	No	Abnormal	104%	R838H	7
REIF	691	9.9	Yes	Normal	32%	R838C	7
Inc TF	762[§]	19.7	Yes	—	—	R852K	7
REIF	787**	24.0	Yes	Abnormal	70%	Y761C	5
CTF	851	13.0	No	Abnormal	100%	P764S	5
Quantitative abnormality of receptor binding							
CTF	855	11.0	No	Normal	100%	R853H	7
REIF	217	8.4	—	—	80%	R853H	7

* Expressed as percentage of immunoreactivity compared to a normal genital skin fibroblast strain (704) expressing an average of 34 fmol/mg protein specific 5α-dihydrotestosterone binding (24). ‡ Previously reported in Ref. 19. § Patients in which only exons 2–8 of the coding sequence have been sequenced. ‖ Transformation-labile as previously described (25). ¶ Determined as described in Ref. 26. ** Previously reported in Ref. 20. Patients were classified clinically as complete testicular feminization (CTF), incomplete testicular feminization (Inc TF), or Reifenstein (REIF) (1). Monolayer binding was performed in genital skin fibroblast cultures from each patient, and qualitative binding tests were performed as reviewed in Ref. 26. The location of the mutations are given using the single amino acid code and the numbering system of Tilley et al. (7). Dashes indicate that the tests have not been performed.

fibroblasts (2). The term "receptor binding" used throughout this manuscript denotes abnormalities in hormone binding by the androgen receptor, in keeping with the classification scheme that we have previously used (2). Despite the utility of this methodology as a marker of androgen resistance, ligand binding assays do not uniformly correlate with patient phenotype. The group of mutations of the ligand-binding domain of the androgen receptor in this study (Table I) caused both female (complete or incomplete testicular feminization) and male phenotypes (Reifenstein syndrome). Furthermore, in cultured skin fibroblasts, these mutations were associated with absent (< 4 fmol/mg protein), reduced, or qualitative abnormalities of ligand binding.

Mutations in the androgen receptor gene cluster in specific segments of the androgen receptor. The polymerase chain reaction was used to amplify the coding segment of the androgen receptor gene. This has permitted the nucleotide sequence analysis of the coding sequence in 22 subjects with mutations of the androgen-binding domain (Table I and Fig. 1). Amino acid substitutions causing receptor binding negative androgen resistance are present in the same regions of the hormone-binding domain responsible for qualitative abnormalities of the receptor protein. In both categories, most amino acid substitutions are localized in two specific regions of the hormone-binding domain: between amino acids 726 and 772 and between amino acids 829 and 853. This finding suggests that the distinction between qualitative and quantitative abnormalities of ligand binding is one of degree. That is, lesser perturbations of the structure of the hormone-binding domain cause qualitative abnormalities, whereas more marked disruption of the hormone-binding domain structure prevents ligand binding completely.

However, the differences in phenotypic expression between patients cannot be explained solely by the different amino acid substitutions. Two unrelated individuals in this study (subjects 450 and 593) each have the same amino acid substitution (R838H), and yet one (subject 450) had incomplete testicular feminization and the other (subject 593) had the Reifenstein phenotype. In like fashion, although patients 217 and 855 har-

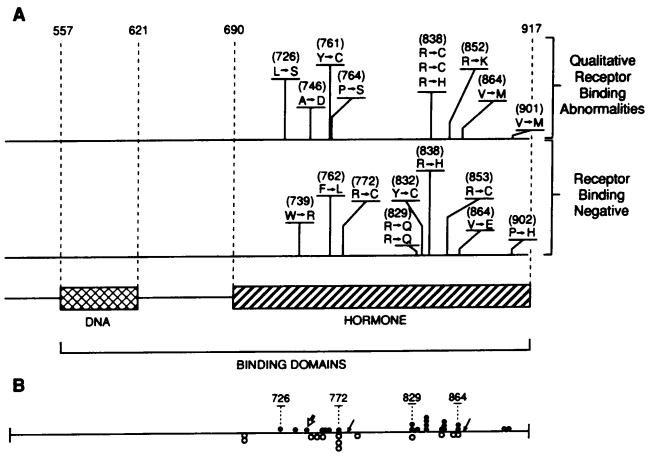

Figure 1. Localization of amino acid substitutions in the hormone-binding domain of the androgen receptor that cause androgen resistance. (*A*) A schematic of the DNA- and hormone-binding domains of the normal androgen receptor gene is shown. The location of mutations in the ligand-binding domain for 10 subjects with qualitative receptor binding abnormalities and 10 subjects with receptor binding negative (androgen resistance; Table I) are shown. Two mutations (R853H) associated with a quantitative abnormality in receptor binding (Table I) are not shown on this schematic. (*B*) A histogram of amino acid substitution mutations in the hormone-binding domain of the androgen receptor described in this report (*filled circles*) and in other laboratories (*open circles*) (18, 21–23, 27). For the purposes of this diagram and discussion, all of the mutations have been assigned using the numbering system of Tilley et al (7). The two solid arrows indicate the relative position of amino acid substitutions in the glucocorticoid receptor that are reported to cause glucocorticoid resistance (28, 29). The open arrow indicates the relative positions of an amino acid substitution reported to cause vitamin D resistance (30).

bor the same mutation, one is classified as complete testicular feminization and the other as having the Reifenstein phenotype. These findings imply that genetic determinants outside the coding sequence of the androgen receptor can influence the function and/or stability of the receptor protein.

A group of mutations in the hormone-binding domain of the androgen receptor is centered on a region affected by mutations in the thyroid hormone receptor-beta. Amino acid substitutions in the hormone-binding domain of the thyroid receptor-β that cause generalized thyroid hormone resistance also cluster in two regions of the thyroid receptor hormone-binding domain (31, 32). When the predicted amino acid sequence of the ligand-binding domains of the androgen and thyroid hormone receptors are aligned, an interesting pattern emerges (Fig. 2). First, several mutations in the thyroid hormone receptor-β have no correlates in the androgen receptor. Likewise, many androgen receptor mutations are not paralleled by thyroid receptor mutations; this is particularly true for the cluster of androgen receptor mutations located between residues 829 and 864. By contrast, the segment between residues 726 and 772 of the androgen receptor is homologous to an area of the thyroid hormone receptor (residues 310–342) in which several amino acid substitutions that cause thyroid hormone resistance are localized. The androgen receptor amino acid substitution at position 764 involves a residue that corresponds to a residue in the hTR-β that is mutated in two different families with thyroid hormone resistance. The amino acid substitution at amino acid 772 in the androgen receptor is a mutation that has been identified in several different pedigrees and is situated five amino acids from an amino acid residue that is mutated in the thyroid receptor-β in three families with thyroid hormone resistance. In addition, androgen receptor mutations at residues 761, 762, and 746 are located in close proximity to mutations in the thyroid receptor-β causing thyroid hormone resistance at amino acid residues 305, 312, 317, and 327 in the thyroid receptor.

Discussion

Single amino acid substitution mutations of the ligand-binding domain of the androgen receptor can cause either absent (< 4 fmol/mg protein), decreased, or qualitatively abnormal ligand binding in genital skin fibroblasts from patients with androgen resistance. Furthermore, these mutations can cause a spectrum of phenotypes, from women with complete or incomplete testicular feminization to men with Reifenstein syndrome (perineoscrotal hypospadias and gynecomastia). These variable phenotypes and ligand-binding characteristics appear to reflect the degree to which ligand binding and receptor function are disrupted by the various substitutions.

The finding that 20 of 22 such mutations examined cluster in two relatively discrete regions of the hormone-binding domain is intriguing. Although the amino terminal boundary of the ligand-binding domain of the androgen receptor has not been determined, studies of the glucocorticoid receptor hormone-binding domain (33) and the definition of amino acid substitutions in the androgen receptor at residue 693 (22) that cause abnormalities of hormone binding in two patients with androgen resistance suggest that it extends at least to the area of residue 690. Using amino acid residue 690 as the amino terminal boundary of the hormone-binding domain would localize 90% of the mutations in our patients to two segments that account for ~ 20 and 15% of the coding sequences of the androgen-binding domain. This suggests that these regions are of particular importance for receptor function and for the stable association of receptor with ligand.

It is possible, of course, that this clustering could be the result of coincidence or some type of selection bias. Two observations make this unlikely. First, when the location of reported hormone-binding domain mutations from other patient collections (18, 21–23) are compared with our own, there is predominance of mutations in these regions as well (see Fig. 1 *B*). Even the mutation causing androgen resistance in the *Tfm* rat (27) falls into the first of these segments. Second, comparison of the locations of mutations of the androgen receptor with mutations in the thyroid hormone receptor-β that have been reported to cause thyroid hormone resistance also suggests that the clustering is not a coincidence (31, 32). In both the thyroid and androgen receptors, two clusters of mutations are recognized. The locations of the carboxy-terminal mutations show little agreement, but the locations of the amino-terminal clusters (726-772 in the androgen receptor and 310-342 in the thyroid hormone receptor hTR-β) are homologous, suggesting that this segment plays a critical role in the function of both receptor proteins. Although fewer in number, amino acid substitutions associated with glucocorticoid resistance (28, 29) and vitamin D resistance (30) are positioned at near or within the clusters of mutations evident for the androgen receptor (see Fig. 1 *B*).

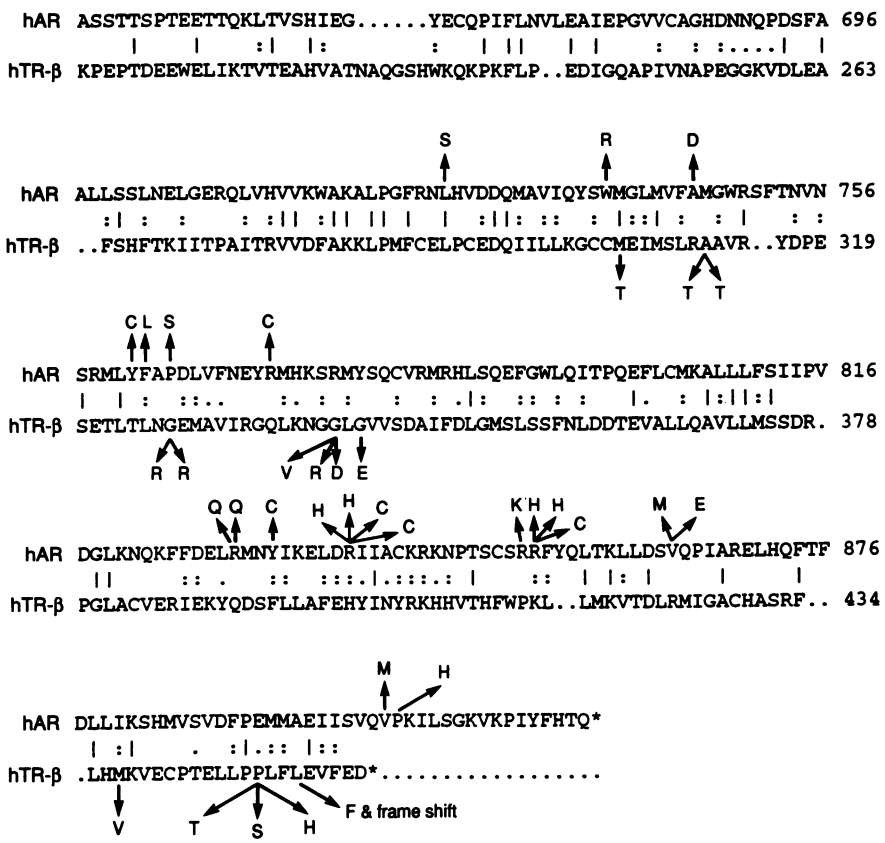

Figure 2. Alignment of ligand-binding domain mutations causing generalized thyroid hormone and androgen resistance. The predicted amino acid sequence of the human androgen receptor (*hAR*) is shown above, and that for the human thyroid receptor beta (*hTR-β*) is shown below. The single amino acid code is used. The arrows and letters indicate the positions of amino acid substitutions. Vertical lines show identical amino acids, and two dots indicate conservative amino acid changes.

The significance of this clustering of mutations in two segments of the androgen receptor hormone-binding domain is not clear. The fact that all of these mutations are associated with abnormal receptor binding suggests that the two regions may define physical surfaces (e.g., a cleft) important for the interaction of the receptor with ligand.

The observation that identical mutations in the androgen receptor gene are associated with different receptor binding defects or different clinical phenotypes is provocative. These differences are not caused by differences in the level of immunoreactive receptor detected in fibroblast cultures, suggesting that genetic determinants outside the androgen receptor coding segment are able to influence the function of the receptor.

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