Molecular Defects in the Androgen Receptor Causing Androgen Resistance

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Patients with androgen resistance exhibit a spectrum of abnormalities of male sexual development ranging from 46,XY phenotypic women (complete testicular feminization) to undervirilized fertile men. The definition of the androgen receptor gene structure has permitted the identification of the defects causing androgen resistance in a number of patients. In some individuals androgen resistance is caused by large-scale structural alterations in the androgen receptor gene. In most patients, however, the androgen receptor mutation is the result of single nucleotide substitutions, which cause premature termination or amino acid replacement that result in the synthesis of defective androgen receptor proteins. These amino acid substitutions identify residues crucial to the normal function of the androgen receptor protein. J Invest Dermatol 98:975–998, 1992

The actions of androgens are mediated by a high-affinity intracellular receptor, termed the androgen receptor, which binds both testosterone and dihydrotestosterone in androgen target tissues. These hormone-receptor complexes modulate the transcription of androgen-responsive genes, controlling such important processes as prostate growth and male sexual differentiation. The importance of the androgen receptor in this pathway has been underscored by the recognition of a large number of mutations that impair the function of the X-linked androgen receptor. In affected 46,XY individuals, these mutations result in a spectrum of clinical phenotypes ranging from women affected by the syndrome of complete testicular feminization to undervirilized men with gynecomastia [1] (Fig 1).

These different phenotypes have been associated with four categories of androgen-receptor abnormalities defined on the basis of the dihydrotestosterone-binding properties assayed in cultures of genital skin fibroblasts. In receptor binding negative androgen resistance, no specific binding of dihydrotestosterone is detected. In other patients, androgen resistance is associated with a reduced quantity of an apparently normal androgen receptor detected in fibroblast cultures. In a third category, an abnormality of the receptor protein can be detected using one of several qualitative tests of the androgen receptor, such as assays of receptor upregulation, thermal stability, or ligand dissociation rate. In a number of patients, however, normal levels of dihydrotestosterone binding are present and no qualitative defects of the receptor are present, despite a family history, endocrine profile, and phenotype consistent with androgen resistance. This latter entity is termed receptor binding positive androgen resistance [1] (see Fig 1).

Androgen Receptor Structure  Following the initial reports of Chang et al [2] and Lubahn et al [3], the nucleotide sequence of cDNA encoding the human androgen receptor have been reported by six groups [4–9]. Analysis of the predicted amino acid sequence of the androgen receptor revealed that the androgen receptor contains regions that are highly conserved when compared to other members of the steroid receptor family, such as the mineralocorticoid, progesterone, and glucocorticoid receptors [10]. This conservation is most pronounced in the segments encoding the regions responsible for DNA and hormone binding (see Fig 2).

The gene encoding the androgen receptor is located near the centromere on the long arm of the X-chromosome [11] and spans at least 50 kilobases of genomic DNA. Like many other members of the steroid receptor family, it is divided into eight coding exons [12–14]. In humans, this gene gives rise to two principal species of androgen receptor mRNA, which are detected in tissues expressing the androgen receptor. These mRNAs are approximately 7 and 10 kilobases in length and appear to encode the same protein. The available evidence suggests that these mRNA are derived from the same promoter segment and differ in the size of 3' untranslated segments they contain [15,16].

Receptor Binding Negative Complete Testicular Feminization  Initial studies have focused on those patients affected by complete testicular feminization associated with a complete absence of specific androgen binding in assays of genital fibroblast cultures (receptor binding negative). These studies indicate that in most patients the organization of the androgen receptor gene is intact with no large-scale rearrangements or deletions detected using either Southern analysis or the polymerase chain reaction in an exonspecific fashion [17], although exceptions have been reported where segments of the androgen receptor gene have been deleted [18–20]. In most instances of receptor binding negative androgen resistance, the mutations that are identified are single nucleotide substitutions. In one group of patients, the causative mutations result in the introduction of premature termination codons in the androgen receptor coding segment [13,17,21]. These truncated receptor proteins are inactive in assays of ligand binding and are unable to activate androgen-responsive reporter genes in co-transfection assays [13].

A completely different mechanism is operative in a second group of patients with receptor binding negative complete testicular feminization. The androgen receptor genes of this second group also contain single nucleotide changes. In these patients, however, the nucleotide substitutions do not lead to truncation of the receptor.

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protein, but instead result in the replacement of single amino acids within the androgen receptor coding segment [22,23]. Biochemical characterization of these mutant receptors in transfection assays reveals that the receptor proteins are substantially impaired in assays of ligand binding. As a group, it appears that these replacement mutations identify amino acids that are crucial to the maintenance of the hormone-binding domain of the receptor protein in a conformation that is competent to bind hormone. These mutant androgen receptor proteins are also defective in assays of transcriptional activation. It appears that in some instances the defect in receptor function is compounded by a diminution in the steady-state level of androgen receptor mRNA [22] or protein expressed in genital skin fibroblasts (and presumably in intact tissues as well).

Receptor Positive Androgen Resistance In receptor positive androgen resistance normal levels of dihydrotestosterone binding can be assayed in genital skin fibroblasts. The androgen receptor in such patients is qualitatively normal by all tests applied, such as upregulation, thermal stability, and the rate of dissociation of ligand from the receptor. When the coding exons of the androgen genes are sequenced in these patients, single nucleotide substitutions are identified. In this group of patients, however, the substitutions are located within the DNA-binding domain of the receptor protein. When these mutations are inserted into expression vectors and transfected into eukaryotic cells, the level and kinetics of ligand binding are found to be normal, consistent with the binding kinetics observed in fibroblasts from these patients. Despite these normal hormone binding characteristics, when these mutant receptors are assayed in cotransfection assays using an androgen-responsive reporter gene, the mutant receptor proteins are unable to stimulate transcriptional activation [24]. These results suggest that the androgen receptors predicted for patients with receptor positive androgen resistance are defective in a step subsequent to hormone binding, such as DNA binding or dimerization.

The Biochemical and Clinical Spectrum of Androgen Resistance The principal focus of research up to this point has been to elucidate the genetic defects that underlie the androgen resistance in patients with androgen receptor gene defects. It appears that in most patients the causative mutations can be traced to single nucleotide substitutions within the coding segment of the androgen receptor gene. It is likely that the variations of the mechanisms operative in the receptor binding negative and receptor-positive categories will be responsible for the defects described for patients with qualitatively abnormal or reduced amounts of the receptor.

A major challenge will be to relate the genetic and biochemical abnormalities defined within patients with androgen resistance to the range of clinical phenotypes that are observed in the individual patients, as it is quite clear that a variety of different mutations can give rise to similar phenotypes. At this juncture it appears plausible that the observed phenotype is related both to the quantity of androgen receptor protein that is expressed in tissues and the amount of residual activity the defective androgen receptor can exert. Whereas the results obtained to date are consistent with this con-

Figure 1. Spectrum of clinical phenotypes resulting from androgen receptor mutations in 46,XY individuals.

Figure 2. Schematic structure of the androgen receptor.

cept, detailed analyses of many patients with differing degrees of androgen resistance will be required to confirm or refute this hypothesis.

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REFERENCES


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