Eur J Pediatr (1993) 152 [Suppl 2]:S62–S69

European Journal of
Pediatrics

Molecular genetics of human androgen insensitivity*

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Abstract. Androgen insensitivity syndromes represent one cause of human male pseudohermaphroditism related to defects in the androgen receptor. The formation of a biologically active androgen receptor complex with testosterone and 5α-dihydrotestosterone is required for normal androgen action during fetal development and differentiation of the internal accessory sex glands and external genitalia. Cloning of the human androgen receptor complementary DNA and genetic screening of human subjects with the clinical and biochemical features of androgen insensitivity using the polymerase chain reaction, denaturing gradient gel electrophoresis and nucleotide sequencing techniques have led to the identification of molecular defects in the androgen receptor. The complexity of phenotypic presentation by affected subjects with the complete or partial forms of androgen insensitivity is represented by the heterogeneity of androgen receptor gene mutations which include deletions and point mutations, with the latter causing inappropriate splicing of RNA, premature termination of transcription and amino acid substitutions. The naturally occurring mutations in the androgen receptor of subjects with androgen insensitivity represent a base upon which we can increase our understanding of the structure and function of the androgen receptor in normal physiology and disease.

Key words: Androgen – Receptor – Genetics – Mutations – Human

Introduction

Male pseudohermaphroditism in humans is defined as a condition of incomplete masculinization of the fetal external genitalia in a karyotypically normal 46, XY individual. Among the multiple genetic disorders that result in male pseudohermaphroditism are a group of abnormalities of the androgen target cells, including 5α-reductase deficiency and androgen insensitivity syndromes (AIS) [10]. Although AIS is a relatively rare condition estimated to be present in ~ 1:60,000–100,000 male births, accounts of affected individuals with AIS outnumber those with other causes of male pseudohermaphroditism. The AIS are characterized by variable phenotypic expression, ranging from a complete female phenotype to male genitalia with mild hypospadias [10]. Evidence suggests also that the syndrome includes some rare cases of phenotypically normal males with azoospermia [1, 2, 27].

Wilkins originally proposed in 1950, that this syndrome was caused by target organ insensitivity to the actions of androgens. Multiple clinical and biochemical studies have since confirmed in affected individuals, the insensitivity of target tissues to testosterone and its more potent 5α-reduced metabolite, dihydrotestosterone (DHT) [10]. Testosterone is able to induce fetal differentiation and development of the Wolffian duct system in the fetus but DHT is required by the developing prostate gland and the male external genitalia. Androgenic effects during fetal development require not only the appropriate androgen, but also the formation of a biologically active androgen receptorsteroid complex. A defect in androgen receptor function can account for target organ insensitivity and evidence for such impairment has been reported for numerous subjects with AIS [10].

The heterogeneity in phenotypic expression of AIS is due to a variety of AR defects, some of which are detectable by biochemical methods [10]. For example, AR binding in cultured genital skin fibroblasts of subjects with a female phenotype due to complete AIS may be undetectable, in the so-called receptor negative (AR-) form [20], whereas others have quantitatively normal binding, termed the receptor positive (AR+) form [4, 9]. In subjects with sexual ambiguity due to the partial form of AIS, androgen receptor binding activity may be either deficient (AR±) or may also by quantitatively normal (AR+) [3]. Cloning of human cDNA encoding the AR has presented the opportunity to pursue molecular analyses of the AR gene in affected subjects with AIS.

*This work was supported by NIH Grants DK-43147 and DK-00180.

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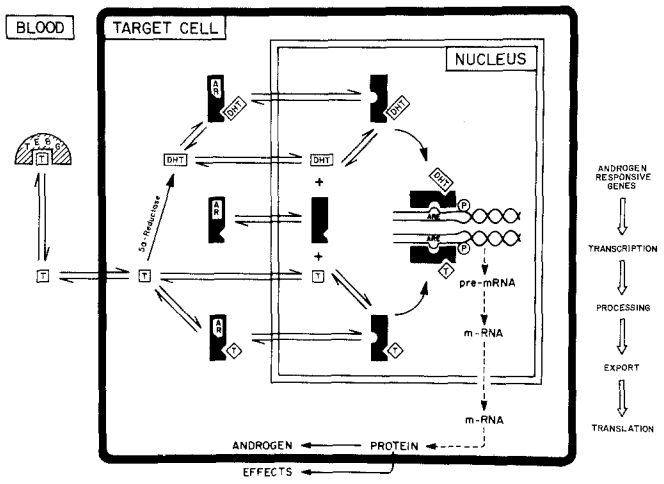

Fig. 1. Diagram of a target cell showing our present concept for the receptor-mediated action of androgens. Abbreviations: *TEBG,* testosterone-estradiol binding globulin; *T,* testosterone; *DHT,* 5α-dihydrotestosterone; *AR,* androgen receptor protein; *ARE,* androgen response element; *P, RNA* polymerase II

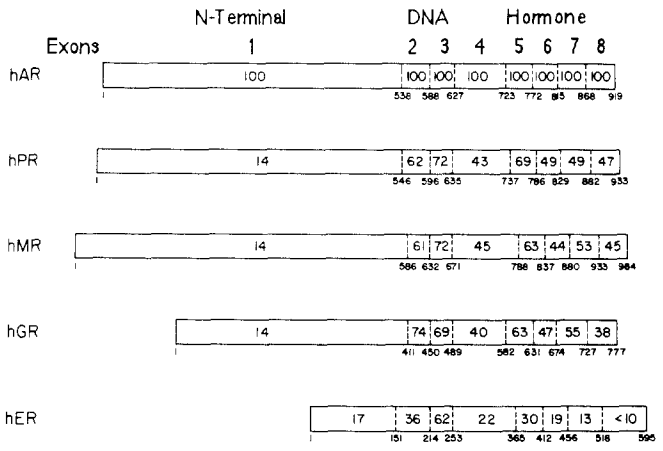

Fig. 2. Amino acid homology among human steroid receptors compared by exons. The percent homologies with *hAR* (boxed) and amino acid residue numbers (below boxes) are shown. Intron/exon splice sites are indicated by the vertical dashed lines. Abbreviations: *h,* human; *AR,* androgen receptor; *PR,* progesterone receptor; *MR,* mineralocorticoid receptor; *GR,* glucocorticoid receptor; *ER,* estrogen receptor. (From ref. [22])

Results and discussion

Structure and function of the androgen receptor

Androgen action within target cells occurs in a manner similar to that observed for all members of the steroid receptor family [5, 29] (Fig. 1). Testosterone from the blood enters cells by passive diffusion and may be converted to DHT in some tissues such as the prostate by the enzyme, steroid 5α-reductase [11]. Testosterone or DHT binds to a single class of intracellular androgen receptor protein, although the latter steroid has a demonstrably greater binding affinity which manifests itself as an enhanced biological potency. The human androgen receptor is expressed in androgen-sensitive target tissues of both men and women, with the concentration of available androgen being the primary factor determining tissue responsiveness. The receptor-steroid complex undergoes an "activation" process which confers high-affinity nuclear binding of the complex to DNA within target cells. Androgen receptors function as transcriptional regulatory proteins that recognize specific nucleotide base sequences, termed "androgen-response elements", within flanking regions of androgen-responsive genes [37]. A transcriptional complex comprised of RNA polymerase II, appropriate accessory protein factors and the androgen receptor-steroid complex activates expression of specific messenger RNAs. These RNA transcripts are processed and their translation leads to synthesis of androgen-dependent proteins. These proteins may function in the growth, structure, enzymatic or secretory activity of the target cell. Androgens may also act as negative effectors of gene transcription as occurs in the auto-regulation of androgen receptor gene expression [31]. A defect in any of the sequential steps outlined above, will lead to impaired masculinization of the internal and external genitalia in a male fetus.

The androgen receptor is a member of the ligand dependent superfamily of nuclear transcription factors, which includes receptors for progesterone, glucocorticoid, mineralocorticoid, estrogen, vitamin D, thyroid hormone and retinoic acid [12, 23]. Studies of human AR have revealed the presence of three conserved functional domains known to be common to each class of receptor [18, 35, 37] (Fig. 2). The amino terminus is the least conserved region with the greatest variation in length among these receptors, but is essential for transcriptional activation. In the human AR, this region includes amino acids 1–537 and is encoded by the first and largest exon of its gene [22]. In addition, this region of the human AR contains homopolymeric amino acid repeats of proline, glutamine and glycine, with the latter two polymeric regions being of variable length, and thus polymorphic, within the normal population. The most highly conserved regions is the central, cysteine-rich DNA-binding domain between amino acid residues 538 and 626, which forms two "zinc-fingers". Exons 2 and 3 of the AR gene encode the first and second zinc-fingers, respectively. The carboxyl terminus, amino acid residues 627–919, is encoded by exons 4–8 and specifies the steroid binding properties of the receptor protein. Additional amino acid sequences, concerned with other characteristics common to steroid receptors, such as interaction with the 90-kDa heat shock protein, receptor dimerization and nuclear translocation, are also conserved within the AR [18, 37]. The human AR gene encodes a protein of 919 amino acids with a molecular weight of ~110kDa [23].

The amino acid sequence of the DNA- and steroid-binding domains of AR share a particularly high degree of conservation with the progesterone, glucocorticoid and mineralocorticoid receptors [22]. The previously described X chromosome linkage of the AR gene [26] and the extraordinarily conserved region of the DNA-binding domain among these receptors [22] led to the synthesis of consensus oligonucleotides for hybridization screening of a human X-chromosome specific DNA library and the subsequent isolation and identification of an AR genomic

DNA clone [24]. The human AR gene locus has subsequently been localized just proximal to the centromere on the long arm of the X chromosome in the region, q11–12 [6]. Additonal cloning procedures led to the isolation and characterization of human AR complementary DNA (cDNA) encoding the entire protein [12, 23].

Complete androgen insensitivity

In the complete form of androgen insensitivity syndrome [10], affected 46,XY subjects present a female external genitalia but with a short vagina and no uterus. Mullerian ducts are absent due to the normal secretion of Mullerian inhibiting substance by testicular Sertoli cells. Gonads are testes located in the abdominal or inguinal area containing hyperplastic Leydig cells and seminiferous tubules lined by Sertoli cells but without germ cells. Wolffian derivatives are hypoplastic due to the insensitivity to testosterone. At puberty, normal female secondary sex characteristics and breast development occur in response to estrogen formation from testosterone, but pubic and axillary hair is sparse or absent. In adulthood, serum testosterone and LH are at or above the normal adult male range.

Receptor negative form of complete androgen insensitivity. Our initial search for mutations of the AR gene concentrated on subjects with the receptor negative (AR–) form of complete AIS. Six unrelated 46,XY phenotypic female subjects with undetectable androgen receptor binding were studied by Southern blotting of genomic DNA hybridized to human AR cDNA probes [8]. In one AIS patient, a deletion was identified by the failure of human AR cDNA probes, hAR1 and hAR2 but not hAR3, to hybridize with restriction fragments created by digestion of genomic DNA with the restriction endonucleases, Eco RI and Bam HI. These results indicated that the deletion was localized in the steroid-binding domain. Analysis of other members of the family confirmed the deleted region and its association with the AR- form of AIS (Fig. 3). Deletion of the entire steroid binding domain encoded by exons 4–8 was confirmed by amplification of genomic DNA using the polymerase chain reaction (described below). Furthermore, in poly A RNA extracted from genital skin fibroblasts of this AIS patient, neither the normal-sized 10kb AR mRNA nor smaller hybridizing fragments were detected on Northern blots [7], suggesting that the partial gene deletion resulted in a very unstable truncated transcript.

Complete deletion of the androgen receptor gene represents the null phenotype of AIS and has been reported in two subjects, studied independently by our group or in collaboration with others [15, 33, 41]. In addition, we have identified another subject with a partial AR gene deletion of exons 3–8 encoding the second zinc-finger of the DNA-binding domain and the entire carboxy-terminal steroid-binding domain [15]. Another deletion of only exon 3 which encodes the second zinc-finger of the DNA-binding domain has been reported in a subject with the AR+ form of complete AIS (see below) [32]. However, gross alterations in the AR gene structure resulting from

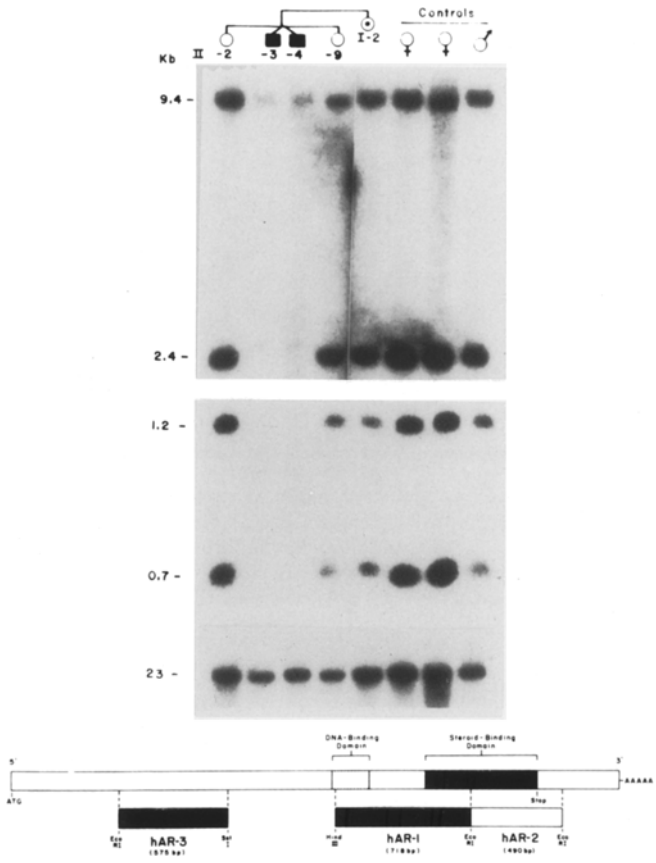

Fig. 3. Southern blot of DNA from five members of a pedigree with complete AIS, AR- and three control subjects and a diagram of the human androgen receptor cDNA denoting the three fragments used as hybridization probes on the Southern blot. DNA was digested with Eco RI and probed with hAR-1 *(top)*, hAR-2 *(middle)* and hAR-3 *(bottom)*. (From ref. [8])

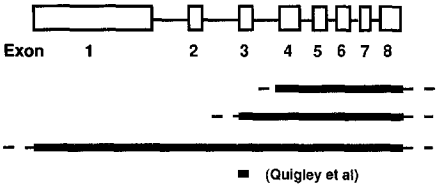

Fig. 4. Diagram of deletions in the human *AR* gene of subjects with complete *AIS*. The deleted regions are indicated by the solid black bars below the schematic of the human AR cDNA showing the eight exons. Three of these deletions were identified in our laboratory, the other was reported by Quigley et al. (ref. [32])

deletions, insertions or rearrangements are very rare events leading to androgen insensitivity, even among those with the receptor negative form (Fig. 4). This became evident from our studies using both Southern blotting to analyze the genomic organization of the AR gene and from Northern blots demonstrating the transcription of a normal-size AR mRNA in the vast majority of subjects with AIS.

Single nucleotide substitutions. We have applied a rapid genetic screening technique, termed denaturing gradient gel electrophoresis (DGGE) [28, 36, 39, 40], to the analy-

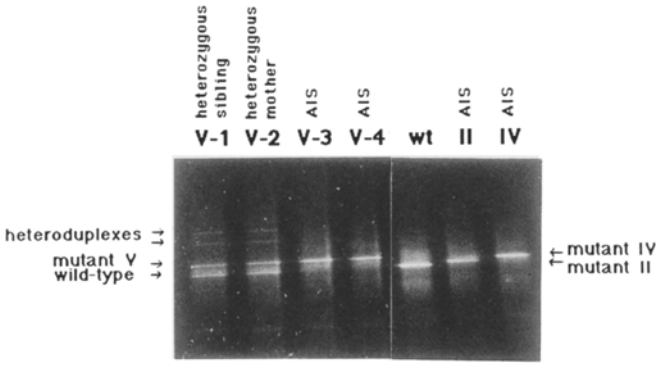

Fig. 5. Denaturing gradient polyacrylamide gel stained with ethidium bromide to show the relative mobilities of the PCR amplified DNA fragments representing exon 5 of the human AR gene and its adjoining intron regions. Putative mutations in three different families, represented by subjects V-3 and V-4, II, and IV with a 46,XY karyotype and AIS were detected by the altered mobility of the amplified DNA fragment relative to normal (wt). The mother, subject V-2, and a female sibling, subject V-1, have 46,XX karyotypes and are heterozygous carriers for the normal and mutant alleles of the AR gene as observed by the presence of four different bands on the gel, including the formation of heteroduplexes

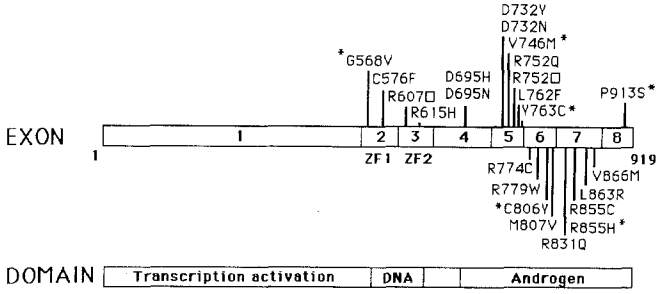

Fig. 6. Diagram of point mutations in the human AR gene of subjects with AIS. The numerical position of the amino acid residues affected is flanked on the left by the normal amino acid and on the right by the amino acid encoded by the nucleotide substitution. The single letter amino acid code is used and the open boxes represent termination codons. The presence of an asterisk denotes partial AIS in an affected subject, all other mutations occur in subjects with complete AIS

sis of genomic DNA for detection of human AR gene mutations at the level of sensitivity involving a single nucleotide. We have combined the amplification of each of the exons of the human AR gene from DNA by the polymerase chain reaction (PCR) with analysis by DGGE [7]. Oligonucleotide primers were synthesized to anneal to intron regions adjoining the 5' and 3' splice junctions of exons 2 through 8, whereas the much larger exon 1 was divided into several overlapping regions for PCR amplification [22]. In our laboratory, the 5' oligonucleotide primer was synthesized to contain an additional 40-bp GC-clamp sequence to improve the sensitivity of the DGGE method [36, 39]. This method is based upon the melting property of DNA under denaturing conditions [14]. As DNA fragments move through polyacrylamide gels containing an ascending gradient of denaturant of formamide and urea at a constant but elevated temperature, regions termed "melting domains" undergo a strand separation to produce partially denatured molecules with decreased electrophoretic mobility. As these DNA molecules continue to move slowly into higher concentrations of denaturant, additional melting domains undergo strand separation. Single nucleotide base changes in any of these domains will alter the melting temperature and lead to differences in the pattern of electrophoretic mobility of a given DNA fragment in the denaturing gel. However, when the final, or most stable, domain melts, the fragment undergoes complete strand separation and the resolving power of the gel is lost. Therefore, base substitutions in the highest temperature melting domain of the DNA molecule cannot be detected by DGGE. The addition of a GC-clamp, because of its resistance to melting, holds the DNA strands together through a higher concentration of denaturant, thus allowing a more complete comparison of melting domains and hence, of the nucleotide sequence within the amplified DNA fragment. While this method may not detect all mutations, its technical simplicity and the absence of required incorporation of radioactive nucleotide into the PCR amplified DNA fragment, makes it attractive as an initial genetic screening procedure. DNA fragments exhibiting altered mobility relative to control samples are subjected to nucleotide sequence analysis [42]. The resolving power of DGGE can be further increased by forming DNA heteroduplexes from the mixing of normal and mutant amplified AR exon DNA fragments, thus creating a nucleotide base mismatch when the DNA mixture is denatured and allowed to reanneal prior to electrophoresis [39]. The DGGE method has led us to detect a multitude of single nucleotide base mutations in the human AR gene open reading frame (Fig. 5).

The majority of subjects with receptor negative complete androgen insensitivity have single nucleotide substitutions within the coding region of the human AR gene that result in alternative splicing of AR mRNA, premature termination of AR gene transcription or missense mutations. In one subject with all coding exons present and without sequence alterations within the AR open reading frame, a single nucleotide substitution was detected at the consensus splice donor site located at the 3' terminus of exon 4 [34]. Inactivation of the consensus splice site resulted in alternative use of a cryptic splice donor site within exon 4 and synthesis of a truncated AR mRNA that encodes a protein with a 41 amino acid deletion (residues 682–722). The truncated AR protein did not bind steroid and was unable to stimulate reporter gene transcription.

Mutations which create premature termination codons for transcription of AR mRNA represent a mechanism by which truncated mRNAs or proteins are produced leading to unstable macromolecules or proteins lacking necessary functional domains. These so-called nonsense mutations have been identified in exons 1, 3, 4, 5, 6, 7, and 8 of the human AR gene [25, 30] (Fig. 6). Cultured fibroblasts from these subjects are reported to lack androgen binding and for the few cases in which the mutated AR cDNA has been expressed in transient transfection assays, both androgen binding and transcriptional activation of reporter genes have been undetectable [25]. These results confirm data from deletion mapping studies performed with the human AR, as well as other steroid receptors, and demon-

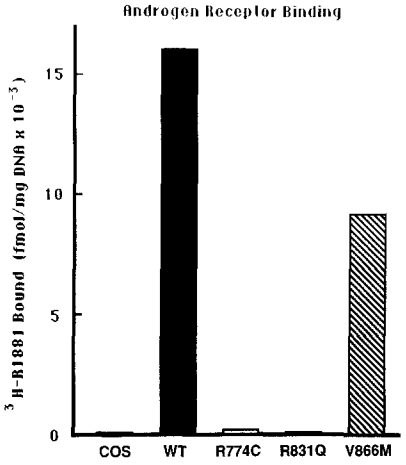

Fig. 7. Androgen binding in COS monkey kidney cells transfected with an AR cDNA expression plasmid. Cells were transfected with plasmid DNA lacking the AR cDNA insert *(COS)* or containing the normal *(WT)* or mutant *(R774C, R831Q,* or *V866M)* AR cDNA insert. Specific androgen receptor was quantitated in total cell extracts following incubation with a saturating concentration (5 n*M*) of the synthetic androgen, methyltrienolone (^{3}H-R1881). Abbreviations: *R,* arginine; *C,* cysteine; *Q,* glutamine; *V,* valine; *M,* methionine. (From ref. [7])

strate the essential contribution of steroid binding towards the biological activity of each receptor [18, 35, 37]. Furthermore, the occurrence of a premature termination codon in exon 8 at amino acid residue 883 [30], suggests that the entire carboxy-terminal steroid binding domain must be intact for ligand binding to occur. That complete androgen insensitivity with female phenotype occurs in these subjects, even when the premature termination codon is located 3' of the DNA-binding domain, indicates that constitutive transcriptional activity of a putative truncated receptor protein does not occur in vivo, contrary to results from in vitro deletion mapping studies with the various steroid receptors, including AR [18, 37].

The majority of subjects with the receptor negative form of complete AIS have missense mutations causing amino acid substitutions within the steroid binding domain of the human AR [25, 30]. The results from our laboratory exemplify the heterogeneity of such mutations (Fig. 6). Missense mutations in exons 4, 5, 6, and 7 are the molecular basis for undetectable ligand binding among numerous affected subjects with AIS. We reported, in detail, two subjects with undetectable AR binding in assays with cultured genital skin fibroblasts [7]. We identified missense mutations in exon 6 resulting from a C to T nucleotide substitution in one subject and in exon 7 resulting from a G to A substitution in the other subject. Hence the codons for the amino acid arginine (R) at residue numbers 774 and 831 were changed to cysteine (R774C) and glutamine (R831Q), respectively. Mutagenesis of human AR cDNA at the respective nucleotide positions within an expression vector and transfection of this plasmid into a mammalian cell line resulted in expression of an androgen receptor protein detectable by immunoblotting but which failed to bind either DHT or the synthetic androgen, methyltrienolone (R1881) (Fig. 7). Cotransfection of the mutant R774C or R831Q AR expression plasmid with a second plasmid containing an androgen-responsive element in the mouse mammary tumor virus (MMTV) promoter driving the chloramphenicol acetyltransferase (CAT) bacterial gene, in the presence or absence of androgen, failed to induce androgen-regulated gene transcription as evidenced by the lack of CAT activity in extracts from transfected cells.

We have identified other missense mutations in exon 4 (D695N), exon 5 (D732Y, D732N, R752Q and Y762F), exon 6 (M807V), and exon 7 (R855C and L863R) (Fig. 6). Each of these subjects has a complete female phenotype and the identified mutations are the only ones detected within the AR open reading frame of each subject. AR expression following transfection of plasmids containing the mutant cDNA and assays of androgen binding and transcriptional activation are currently in progress to correlate each mutation with impaired AR biological function. In summary, a heterogeneous group of human AR missense mutations in exons 4, 5, 6 and 7, encoding much of the steroid binding domain of the human AR, are related to the receptor negative form of complete AIS. Although missense mutations have been identified within exon 8 which encodes the 3' region of the steroid binding domain (Fig. 6), only partial forms of androgen insensitivity are related to these amino acid substitutions.

Receptor positive form of complete androgen insensitivity. AR binding in cultured genital skin fibroblasts of some subjects with complete androgen insensitivity syndrome may be quantitatively normal, thus adding to the heterogeneity of the abnormality [4, 9]. This property suggests the normality of the steroid binding domain of the receptor and that the defect might lie within a separate functional domain, such as for DNA binding. In fact, several mutations within the DNA binding domain of the human AR have been identified [13, 25, 30]. As mentioned earlier, one such mutation represents a rather surprising finding, the deletion of exon 3 encoding the second zinc-finger required for receptor binding to DNA [32]. Despite deletion of the entire exon, the AR mRNA is correctly spliced and a receptor protein lacking 39 amino acids is synthesized. The AR protein binds androgen with normal affinity and is localized intracellularly to the nucleus, but fails to bind to a hormone response element-MMTV DNA promoter sequence and does not activate androgen-sensitive reporter gene transcription.

Other subjects with AR(+) complete AIS have single nucleotide substitutions in exons 2 and 3 [13] (Fig. 6). Within the first zinc-finger encoded by exon 2, a missense mutation occurs at the base of the loop (C576F) where one of the cysteine residues essential for coordinate binding of zinc is mutated so as to disrupt the DNA-binding finger motif. We have recently demonstrated that this mutation results in the inability of an expressed AR fusion protein containing the mutated DNA-binding domain to bind to an androgen response element DNA sequence as analyzed by an electrophoretic mobility shift assay (Fig. 8). Another missense mutation (R615H) occurs immediately 3' to the second zinc finger coding region within exon 3.

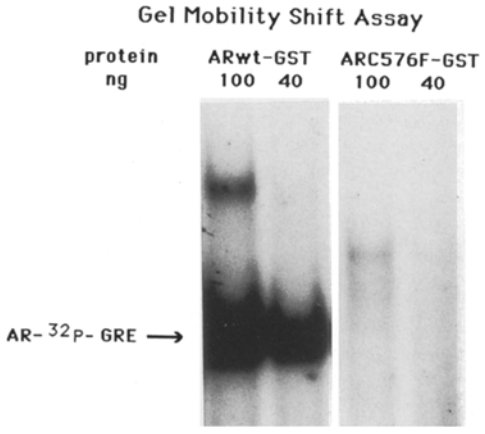

Fig. 8. Polyacrylamide gel electrophoretic mobility shift assay of a consensus glucocorticoid/androgen DNA response element binding to a fusion protein containing the normal or mutated DNA binding domain of the AR. A double-stranded DNA fragment representing a GRE/ARE response element *(bottom)* was radiolabeled with ^{32}P-ATP and incubated with a purified glutathione S-transferase/androgen receptor fusion protein (40 or 100 ng) purified from E. coli following expression in the pGEX-2T plasmid vector. The androgen receptor portion of the fusion protein included amino acid residues 559–645 from the normal *(wt)* or mutant *(C576F)* AR. The formation of a protein-DNA complex is indicated by the upward shift of the radiolabeled DNA appearing as a band on the autoradiogram.

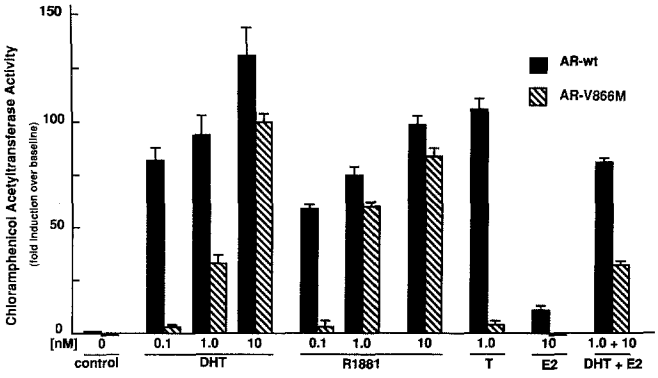

Fig. 9. Concentration-dependent transcriptional activation of chloramphenicol acetyltransferase (CAT) by androgens for normal and mutant AR. CV-1 monkey kidney cells were co-transfected with the normal (wt, solid bars) and mutant (V866M, stippled bars) AR expression vectors and the reporter gene plasmid vector containing the mouse mammary tumor virus promoter-CAT construct. Acetylation of chloramphenicol was quantitated in extracts from cells incubated in the absence or presence of different steroids at various concentrations. Abbreviations: *DHT,* 5α-dihydrotestosterone; *R*1881, methyltrienolone; *T,* testosterone; E_2, estradiol. (From Ref. [7])

By contrast, other subjects with complete AIS may have quantitatively normal androgen receptor binding but are distinguished by functional abnormalities in the AR. Qualitative defects in the AR, such as increased thermal lability, accelerated ligand dissociation and impaired up-regulation, have been demonstrated [9, 16, 17, 19]. We have extensively studied one pedigree with three affected 46,XY subjects with AR(+) complete AIS [9]. Assays of AR binding in cultured genital skin fibroblasts showed a decreased binding affinity for DHT, thermolability of the receptor, and an accelerated rate of dissociation for DHT. Sequence analysis of the entire coding region of the AR gene revealed a single nucleotide substitution (G→A) within exon 7 [22]. This same mutation was present in each of the three affected siblings and the mother was heterozygous for G and A nucleotides at the same position. Transfection of the mutant AR cDNA with methionine replacement of valine (V866M) revealed the expression of a receptor protein with decreased affinity for DHT (normal = 0.38 n*M* vs. V866M = 2.46 n*M*) and significantly reduced ability to activate reporter gene transcription in co-transfection studies [7]. The mutant receptor was almost completely inactive in transcriptional activity assays in the physiologic range of androgen concentrations (0.1 n*M*) when compared to the normal receptor which was near maximally active (Fig. 9).

Partial forms of androgen insensitivity syndrome

Varying degrees of sexual ambiguity are present in other subjects with an endocrine profile consistent with the diagnosis of AIS [10]. Most likely, those subjects described earlier by Reifenstein, Gilbert-Dreyfus, Lubs and Rosewater and associates, form part of the continuum related to insensitivity to androgen. In the partial form of androgen insensitivity, AR binding may be either quantitatively normal (AR+) or partially deficient (AR±) [3].

In our laboratory, we have identified missense mutations within both the DNA binding and steroid-binding domains that are associated with the phenotype of partial AIS. Single nucleotide substitutions within exon 2 (G568V), exon 5 (V746M and Y763C), exon 6 (C806Y), exon 7 (R855H) and exon 8 (P913S) have been detected (Fig. 6).

An interesting subject with a predominantly male phenotype associated with microphallus and perineoscotal hypospadias and a qualitatively abnormal receptor protein related to two separate mutations in the AR gene has been reported [25]. In assays of androgen binding in cultured fibroblasts, the quantity, up-regulation and apparent k_d of the AR were normal but an increased rate of ligand dissociation and thermolability were noted. Two alterations in the androgen receptor were identified; one was a missense mutation in exon 5 that substituted cysteine for tyrosine (Y763C) and the other was a truncation of the glutamine homopolymeric region encoded by exon 1. Analysis of the independent, as well as combined, effects of these alterations in the expressed AR demonstrated an enhanced ligand dissociation rate caused by the amino acid substitution at residue 763, an effect that was further extenuated by the shortened glutamine repeat region. In co-transfection assays with mutant AR and a reporter gene, the combination of Y763C and the truncated glutamine stretch, caused a greater decrease in reporter gene activity than with either mutation alone. These findings suggest the importance for interaction between the N-terminal transcriptional activation and the C-terminal steroid binding domains in achieving optimal transactivation.

Another group of male subjects, those with spinal and bulbar muscular atrophy and associated gynecomastia and infertility, are characterized by a significant elongation of the glutamine polymeric region encoded within exon 1 of the AR gene [21]. This region is polymorphic within the normal population and varies in length from approximately 17 to 26 glutamine residues. However, in male subjects with spinal and bulbar muscular atrophy, the number of glutamine residues is increased between 40 and 52. The exact role of the homopolymeric region in determining the biologic function of the human AR is unknown at present, but may represent target cell specific alterations of AR activity in neuronal cells.

Summary. Cloning of the human AR cDNA has made it possible to establish the causal relationship of mutations in the AR gene with the androgen insensitivity syndromes. Upon inspection, one is immediately impressed by the diverse nature of the mutations involved in the complete and partial forms of androgen insensitivity and the heterogeneous distribution of these mutations throughout the coding region of the AR gene. Because of the large number and diverse array of these naturally occurring mutations and their associated clinical phentoypes, there is great potential for understanding the structure/function relationships of the AR from the in vitro expression of the mutant receptors in various cell lines. Whereas studies to date have focused upon the identification of mutations and their correlation with steroid binding and transactivation of reporter genes in heterologous cell lines, future studies will be directed toward understanding the interaction of functional domains within the AR, AR bindings to specific androgen response elements, AR dimerization, AR phosphorylation and AR interaction with heat shock protein(s), the nuclear localization of AR and the role of accessory factors that direct cell and temporal specific regulation of gene transactivation by AR. All represent future areas for intensive investigation using the naturally occurring mutations of AR found in subjects with AIS as a basis for increased understanding.

References

1. Aiman J, Griffin JE (1982) The frequency of androgen receptor deficiency in infertile men. J Clin Endocrinol Metab 54: 725–732
2. Aiman J, Griffin JE, Gazak JM, Wilson JD, MacDonald PC (1979) Androgen insensitivity as a cause of infertility in otherwise normal men. N Engl J Med 300:223–227
3. Amrhein JA, Klingensmith G, Walsh PC, McKusick VA, Migeon CJ (1977) Partial androgen insensitivity: The Reifenstein syndrome revisited. N Engl J Med 297:350–356
4. Amrhein JA, Meyer III WJ, Jones Jr HW, Migeon CJ (1976) Androgen insensitivity in man: evidence for genetic heterogeneity. Proc Natl Acad Sci USA 73:891–894
5. Beato M (1989) Gene regulation by steroid hormones. Cell 56:335–344
6. Brown CJ, Gross SJ, Lubahn DB, Joseph DR, Wilson EM, French FS, Willard HF (1989) Androgen receptor locus on the human X-chromosome: regional localization of Xq11–12 and description of a DNA polymorphism. Am J Hum Genet 44: 264–269
7. Brown TR, Lubahn DB, Wilson EM, French FS, Migeon CJ, Corden JL (1990) Functional characterization of naturally occurring mutant androgen receptors from patients with complete androgen insensitivity. Mol Endocrinol 4:1759–1772
8. Brown TR, Lubahn DB, Wilson EM, Joseph DR, French FS, Migeon CJ (1988) Deletion of the steroid-binding domain of the human androgen receptor gene in one family with complete androgen insensitivity syndrome: Evidence for further genetic heterogeneity in this syndrome. Proc Natl Acad Sci USA 85: 8151–8155
9. Brown TR, Maes M, Rothwell SW, Migeon CJ (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
10. Brown TR, Migeon CJ (1987) Androgen insensitivity syndromes: Paradox of phenotypic feminization with male genotype and normal testicular androgen secretion. In: Hormone Resistance and Other Endocrine Paradoxes, Cohen MP, Foa PP (eds), Springer-Verlag, New York, pp 157–203
11. Bruchovsky N, Wilson JD (1968) The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243:2012–2021
12. Chang C, Kokontis J, Liao S (1988) Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci USA 85:7211–7215
13. Chang YT, Migeon CJ, Brown TR (1991) Human androgen insensitivity syndrome due to androgen receptor gene point mutations in subjects with normal androgen receptor levels but impaired biological activity. 73rd Annual Meeting, The Endocrine Society, Abstract 28.
14. Fischer S, Lerman L (1983) DNA fragments differing by single-base-pair substitutions are separated in denaturing gradient gel: correspondence with melting theory. Proc Natl Acad Sci USA 80:1579–1583
15. French FS, Lubahn DB, Brown TR, Simental JA, Quigley CA, Yarbrough WG, Tan J, Jar M, Joseph DR, Evans BAJ, Hughes IA, Migeon CJ, Wilson EM (1990) Molecular basis of androgen insensitivity. Rec Prog Horm Res 46:1–42
16. Griffin JE (1979) Testicular feminization associated with a thermolabile androgen receptor in cultured human fibroblasts. J Clin Invest 64:1624–1631
17. Griffin JE, Durrant JL (1982) Qualitative defects in families with androgen resistance: Failure of stabilization of the fibroblast cytosol androgen receptor. J Clin Endocrinol Metab 55: 465–474
18. Jenster G, van der Korput HAGM, van Vroonhoven C, van der Kwast TH, Trapman J, Brinkman AO (1991) Domains of the human androgen receptor involved in steroid binding, transcriptional activation and subcellular localization. Mol Endocrinol 5:1396–1404
19. Kaufman M, Pinsky L, Feder-Hollander R (1981) Defective up-regulation of the androgen receptor in human androgen insensitivity. Nature (London) 293:735–737
20. Keenan BS, Meyer III WJ, Hadjian AJ, Jones HW, Migeon CJ (1974) Syndrome of androgen insensitivity in man: absence of 5α-dihydrotestosterone binding protein in skin fibroblasts. J Clin Endocrinol Metab 38:1143–1146
21. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77–79
22. Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM, French FS (1989) Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. Proc Natl Acad Sci USA 86: 9534–9538
23. Lubahn DB, Joseph DR, Sar M, Tan J, Higgs HN, Larson RE, French FS, Wilson EM (1988) The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis, and gene expression in prostate. Mol Endocrinol 2: 1265–1275

24. Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM (1988) Cloning of human androgen receptor complementary DNA and localization of the X-chromosome. Science 240:327–330
25. McPhaul MJ, Marcelli M, Tilley WD, Griffin JE, Wilson JD (1991) Androgen resistance caused by mutations in the androgen receptor gene. FASEB J 5:2910–2915
26. Migeon BR, Brown TR, Axelman J, Rothwell SW, Migeon CJ (1981) Studies on 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
27. Migeon CJ, Brown TR, Lanes R, Palacios A, Amrhein JA, Schoen EJ (1984) A clinical syndrome of mild androgen insensitivity. J Clin Endocrinol Metab 59:672–678
28. Myers RM, Lerman LS, Fischer SG, Maniatis T (1985) Modification of the melting properties of duplex DNA by denaturing gradient gel electrophoresis. Nucl Acids Res 13:3111–3129
29. O'Malley B (1990) The steroid receptor superfamily: more excitement predicted for the future. Mol Endocrinol 4:363–369
30. Pinsky L, Trifiro M, Kaufman M, Beitel LK, Mhatre A, Kazemi-Esfarjani P, Sabbaghian N, Lumbroso R, Alvarado C, Vasiliou M, Gottlieb B (1992) Androgen resistance due to mutation of the androgen receptor. Clin Invest Med 15:65–81
31. Quarmby VE, Yarbrough WG, Lubahn DB, French FS, Wilson EM (1990) Autologous down-regulation of androgen receptor messenger ribonucleic acid. Mol Endocrinol 4:22–28
32. Quigley CA, Evans BA, Simental JA, Marschke KB, Sar M, Lubahn DB, Davies P, Hughes IA, Wilson EM, French FS (1992) Complete androgen insensitivity done to deletion of exon C of the androgen receptor gene highlights the functional importance of the second zinc finger of the androgen receptor *in vivo*. Mol Endocrinol 6:1103–1112
33. Quigley CA, Friedman KJ, Johnson A, Lafrenier RG, Silverman LM, Lubahn DB, Brown TR, Wilson EM, Willard HF, French FS (1992) Complete deletion of the androgen receptor gene: definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. J Clin Endocrinol Metab 74:927–933
34. Ris-Stalpers C, Kuiper GGJM, Faber PW, Schweikert H-U, van Rooij HCJ, Zegers ND, Hodgins MB, deGenhart HJ, Trapman J, Brinkman AO (1990) Aberrant splicing of androgen receptor mRNA results in the synthesis of a nonfunctional receptor protein in a patient with androgen insensitivity. Proc Natl Acad Sci USA 87:7866–7870
35. Rundlett SE, Wu S-P, Miesfield RL (1990) Functional characterization of the androgen receptor confirm that the molecular basis of androgen action is transcriptional regulation. Mol Endocrinol 4:708–714
36. Sheffield V, Cox DR, Lerman LS, Myers RM (1989) Attachment of a GC-clamp to genomic DNA fragments by the polymerase chain reaction results in improved detection of single base changes. Proc Natl Acad Sci USA 86:232–236
37. Simental JA, Sar M, Lane MV, French FS, Wilson EM (1991) Transcriptional activation and nuclear targeting signals of the human receptor. J Biol Chem 266:510–518
38. Tan JA, Marschke KB, Ho KC, Perry JT, Wilson EM, French FS (1992) Response elements of the androgen-regulated C_3 gene. J Biol Chem 267:4456–4466
39. Theophilius BDM, Latham T, Gabrowski GA, Smith FI (1989) Comparison of Rnase A, a chemical cleavage and GC-clamped denaturing gradient gel electrophoresis for the detection of mutations in exon 9 of the human acid β-glucosidase gene. Nucleic Acids Res 19:7707–7722
40. Traystman MD, Higuchi M, Kasper CK, Antonarakis SE, Kazazian HH Jr (1990) Use of denaturing gradient gel electrophoresis to detect point mutations in the factor VIII gene. Genomics 6:293–301
41. Trifiro M, Gottleib B, Pinsky L, Kaufman M, Prior L, Belsham DD, Wrogemann K, Brown CJ, Willard HF, Trapman J, Brinkmann AO, Chang C, Liao S, Sergovich F, Jung J (1991) The 56/58 kDa androgen-binding protein in male genital skin fibroblasts with a deleted androgen receptor gene. Mol Cell Endocrinol 75:37–47
42. Wong C, Dowling CD, Saiki RK, Higuchi RG, Erlich HA, Kazazian HH Jr (1987) Characterization of β-thalassemia mutations using direct genomic sequencing of amplified single copy DNA. Nature 330:384–386