Chapter 16
Androgen Receptors in the Pathology of Disease

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Abstract Androgen receptor (AR) belongs to the steroid hormone receptor group of ligand-activated transcription factors in the nuclear receptor superfamily. AR mediates the action of physiological and exogenous androgens to regulate the expression of a network of genes in target tissues that are essential for the development and maintenance of the male phenotype and reproductive function as well as the function of numerous other tissues in both males and females. AR is ubiquitously expressed throughout the body. AR is a modular protein that comprises an N-terminal domain (NTD) that contains all of its transcriptional activity, a DNA-binding domain, a flexible hinge region, and a C-terminal ligand-binding domain (LBD). All clinically approved hormonal therapies target the AR LBD, either directly with antiandrogens and selective AR modulators or indirectly by reducing levels of androgens. Pathological conditions related to AR dysfunction involve altered levels of androgens and structural alterations in the AR. These include mutations, polymorphisms in the polyglutamine tract of the NTD, and alternative splicing of AR to yield constitutively active receptors. From the extensive list of AR-related diseases, herein we describe prostate cancer, androgen-insensitivity syndrome, polycystic ovary syndrome, breast cancer, and a few more pathological conditions in more detail.

Keywords Androgen receptor · Androgen receptor mutation · Prostate cancer · Breast cancer · Androgen insensitivity syndrome · Polycystic ovary syndrome

Abbreviations

AF-1	activation function 1
AF-2	activation function 2
AR	androgen receptor

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ARKO	*AR* knockout
AR-Vs	androgen receptor splice variants
CAIS	complete androgen insensitivity syndrome
CRPC	castration-resistant prostate cancer
CTCs	circulating tumor cells
CTE	C-terminal extension
DBD	DNA-binding domain
DHEA	dehydroepiandrosterone
DHT	5α-dihydrotestosterone
E2	17β-estradiol
EMS	external masculinization score
ER	estrogen receptor
fl-AR	full-length AR
GR	glucocorticoid receptor
HSP	heat-shock protein
KLK3/PSA	prostate-specific antigen
LBD	ligand-binding domain
LH	luteinizing hormone
LH-RH	luteinizing hormone-releasing hormone
MAIS	mild androgen insensitivity syndrome
NTD	N-terminal domain
PAIS	partial androgen insensitivity syndrome
PCOS	polycystic ovary syndrome
PR	progesterone receptor
SARM	selective androgen receptor modulator
SBMA	spinal-bulbar muscular atrophy
SHBG	sex-hormone-binding globulin
TAU	transactivation unit
TNBC	triple-negative breast cancer

16.1 Androgens

Historically, androgens have been referred to as male sex hormones due to their importance in the control of normal development and reproductive function in males. The most abundant endogenous androgens are testosterone and its more active metabolite 5α-dihydrotestosterone (DHT). For the average adult male, 3–10 mg of testosterone is produced per day, and approximately 4% of it is converted to DHT by 5α-reductase and 0.2% to 17β-estradiol (E2) by aromatase. The Leydig cells in the testis synthesize >95% of the testosterone in circulation from cholesterol, through a pathway of enzymes in response to luteinizing hormone (LH) signaling. Peripheral tissues including the adrenal glands as well as the ovaries are also sources of weaker androgens, which include androstenedione and dehydroepiandrosterone (DHEA). The normal physiological range of testosterone in healthy

men is between 350 and 600 ng/dL (>12 nM), and levels below 300 ng/dL are considered low testosterone [1]. The upper range of testosterone levels in women is between 12 and 58 ng/dL (0.4–2 nM). Chronically elevated levels of testosterone in women can be associated with polycystic ovary syndrome (PCOS, 0.34–5.5 nM) or congenital adrenal hyperplasia (1.32–5.62 nM). Virilization is observed in women with three times above the normal concentrations of testosterone. In the circulation, only 2% of testosterone is free, whereas 50% is bound to albumin with low-to-moderate affinity, 44% tightly-bound to sex-hormone-binding globulin (SHBG), and 4% loosely-bound to corticotropin-binding globulin [2]. Testosterone bound to SHBG is not bioavailable, since it restricts cell permeability, and thereby SHBG is involved in regulating biological responses to androgens. SHBG levels are down-regulated by androgens and are decreased in pathological conditions, such as diabetes, obesity, hypothyroidism, and aging. Estrogens, hyperthyroidism, cirrhosis, and tamoxifen increase the levels of SHBG [3]. Tissue concentrations of androgens may therefore not reflect changes in the concentrations of circulating androgens [4]. The biological effects of androgens are mediated by the androgen receptor (AR). Pathologies associated with the androgen axis are carried out by AR and may involve altered levels of androgens and/or changes in the structure or function of AR.

16.2 Androgen Receptor Structure and Function

16.2.1 Expression of AR

AR is ubiquitously expressed throughout the body, with the possible exception of the spleen [5]. The AR has important roles in the reproductive tissues of men and women, and it influences cognition, hematopoiesis, coagulation, skin, hair, bone, muscle, and some brain malignancies [6–8] (Fig. 16.1). Tissue-specific expression of AR cofactors mediates the differential effects measured between different tissues [9]. The AR is encoded by a gene (AR; NR3C4) located on the X chromosome (locus: Xq11-Xq12). Males carry a single copy of the *AR* gene, whereas females have one functional copy due to X-chromosome inactivation (also known as Lyonization) [10]. The regulatory regions of the *AR* gene lack TATA and CCAAT elements and have binding sites for SP1, NF-kB, and c-MYC (for reviews, see [11, 12]). Androgen autoregulates AR expression to increase as well as decrease levels of AR mRNA (for a review, see [12]).

16.2.2 AR Structure

Full-length AR (fl-AR) is a 98.8 kDa protein encoded from eight canonical exons in the *AR* gene and at least seven other cryptic exons (Fig. 16.2). Generally, the wild-type full-length protein is described to be 910 to 919 amino acid residues, with

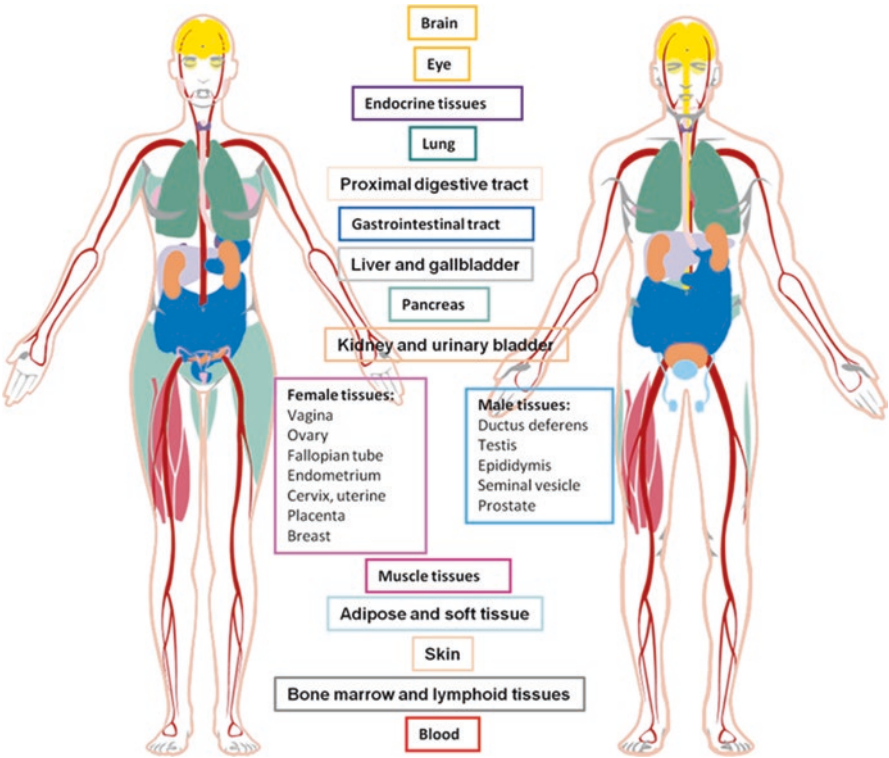

Fig. 16.1 AR expression in the human body. AR expression is detected in various organs in both males and females. Diagram of the human body showing the expression of AR in different organs was retrieved from the RNA and Protein Expression Summary in Human Protein Atlas (https://www.proteinatlas.org/ENSG00000169083-AR/tissue) [7]

deviations predominantly due to polymorphisms in the polyglutamine (CAG) and polyglycine (GGC) repeats in the amino-terminal domain (NTD). Posttranslational modifications of AR include phosphorylation, SUMOylation, methylation, and ubiquitination and can impact AR structure, protein-protein interactions, transcriptional activity, cellular localization, and stability. The amino acid sequence similarity between human AR and related steroid hormone receptors is crucial for understanding its specificity for ligands, DNA-binding sites to regulate gene expression, and drug development. For examples, the AR C-terminal ligand-binding domain (LBD) shares 54% sequence similarity with the LBD of progesterone receptor (PR), and antiandrogens can inhibit the transcriptional activity of PR [13, 14]; the AR DNA-binding domain (DBD) is 76% identical to that of the glucocorticoid receptor (GR), and they share common regulatory sequences within the same loci of chromatin [15, 16]. The specificity of steroid hormone receptors is generally believed to be achieved through receptor-specific residues in their ligand-binding pockets and tissue-specific expression (for a review, see [17]). Using the prostate as an example, benign prostate epithelial cells express AR but do not express GR,

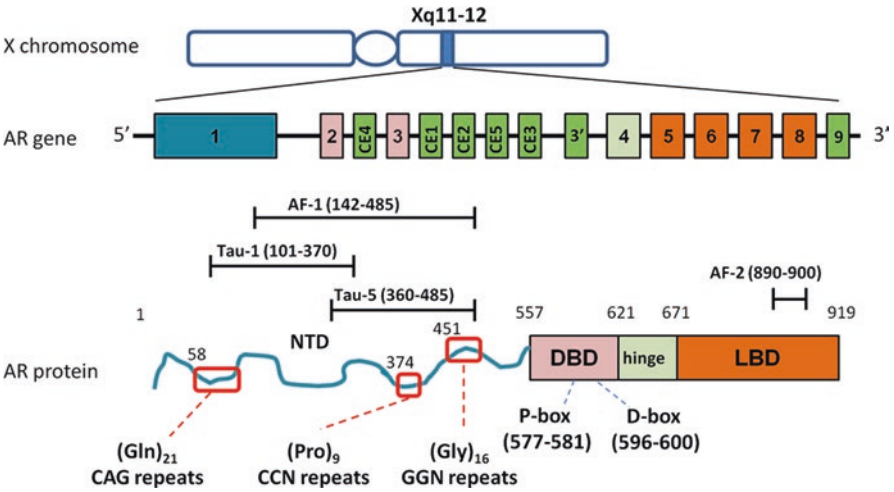

Fig. 16.2 Domains and functional regions of AR. AR gene is located on X chromosome and contains 8 exons that encode for full-length AR. Domains of AR are shown in the same color as respective exons. AF-1 is within NTD whereas AF-2 is in LBD. Tau-1 and tau-5 are located in AF-1. Locations of polyglutamine (CAG repeats), polyproline (CCN repeats), and polyglycine (GGN repeats) on AR NTD are indicated. P-box and D-box are located in the two zinc fingers within DBD. CE, cryptic exon

whereas in advanced prostate cancer, both AR and GR are coexpressed following castration [18]. Based upon these observations, the GR has been suggested as a mechanism of resistance to hormonal therapies for advanced prostate cancer [19].

16.2.3 AR Domains

AR is a modular protein with an intrinsically disordered polymorphic NTD (polymorphic, 547 to 556 amino acid residues), a folded DBD (65 amino acid residues), a flexible hinge region (49 amino acid residues), and a structured LBD (249 amino acid residues).

16.2.3.1 AR NTD

The AR NTD is essential for its transcriptional activity and acts as a hub for interactions with many other proteins. No crystal structure for the AR NTD has been resolved due to its limited stable secondary structure. The AR NTD contains all of its transcriptional activity with activation function-1 (AF-1) instead of AF-2 in the LBD like estrogen receptor (ER). At 547 to 556 amino acid residues, the AR NTD is approximately three times longer than the NTDs of ERα and ERβ. AR AF-1 has

approximately 13% helical secondary structure which is increased with binding to interacting proteins [20, 21]. There are two transactivation units (tau) within AF-1, tau-1 (amino acid residues 101–370) and tau-5 (amino acid residues 360–485), that interact with basal transcriptional machinery to mediate the transcriptional activity of the AR.

The polymorphic AR NTD contains multiple repeat regions that vary in length that include the polyproline tract (average 9 repeats), polyglycine tract (average 16 repeats), and polyglutamine tract (average 21 CAG repeats) (Fig. 16.2). Variable lengths of the polyglutamine tract are the most studied due to its association with diseases such as infertility [22], male pattern baldness [23], symptomatic benign prostatic hyperplasia [24], spinal-bulbar muscular atrophy (SBMA), PCOS, prostate cancer, breast cancer, and ovarian cancers [25–29]. The length of the polyglutamine tract impacts AR solubility and its transcriptional activity. A tract of 9 to 39 is considered in the "normal" range [30]. Short polyglutamine tracts have increased AR transcriptional activity, whereas a longer tract has less activity. Tracts longer than 37 CAG residues can form cytotoxic fibrillar aggregates that are associated with SBMA. The propensity for aggregate formation is increased with androgens due to the release of heat-shock protein (Hsp) 40 and Hsp70 chaperone proteins from the $_{23}$FQNLF$_{27}$ motif in the AR NTD. Shedding of Hsps allows AR NTD interaction between the $_{23}$FQNLF$_{27}$ and the AR C-terminal LBD (called N/C interaction) that is required to mediate transcriptional activity in response to androgen [31]. N/C interactions delay dissociation of androgen from the ligand-binding pocket, stabilize the AR protein, and most importantly provide the main site for binding of coregulators to mediate transcriptional activity through AF-1 rather than AF-2 unlike ER [31–33]. Low-resolution cryoelectron microscopy (cryo-EM) has revealed the structure of transcriptionally active fl-AR to be unique from ERα [34] (Fig. 16.3). Dimerization of AR is in a head-to-head and tail-to-tail manner which allows direct interactions at different sites in the AR NTD with a single molecule of the cofactors SRC-3 and p300 [34]. These data revealed that the AR dimer consists of two different conformations of NTD. One conformation directly interacts with SRC-3 close to its $_{23}$FQNLF$_{27}$ motif [34] that is consistent with coimmunoprecipitation studies from two decades ago that showed SRC interacts within 1–233 amino acid residues of the AR NTD [35]. The p300 molecule interacts with both conformations of NTD [34]. Presumably, interactions with CREB-binding protein (CBP), which is highly related to p300, may also behave similarly to p300 in its mechanism of interaction with the AR NTD. Such direct interactions and stoichiometry for SRC-3 and p300 are unique to AR compared to ERα, which has a strong AF-2 function and weak AF-1 function. The AR NTD is also highly modified by phosphorylation and SUMOylation and contains multiple sites for the peptidyl-prolyl *cis/trans* isomerase Pin1 [36–38]. These modifications can impact the conformation of a protein to potentially alter protein-protein interactions.

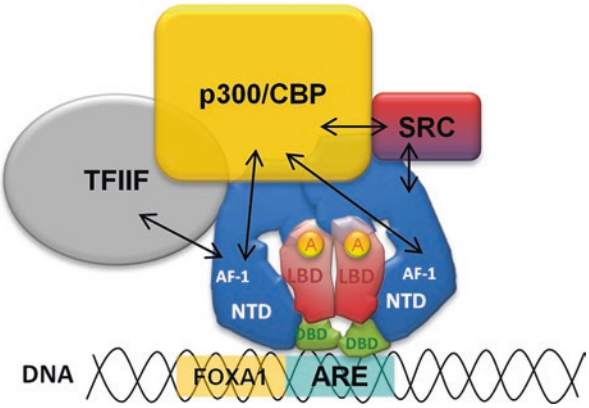

Fig. 16.3 Structure of transcriptionally active AR. The AR dimer forms when androgen binds to LBD. DNA-bound AR dimer interacts with one molecule of SRC-3 and p300 (CBP) through NTD. SRC-3 interacts with a region close to the $_{23}$FQNLF$_{27}$ motif on AR1-233 of one AR monomer. p300 interacts with AF-1 on two AR monomers [34, 35]. CBP is presumed to be similar to p300 in its interaction due to their structural similarities. CBP and the RAP74 subunit of TFIIF interacts with AR 423–448 [306]. Arrows indicate interactions between molecules. FOXA1 binding site is shown on DNA. A, androgen; ARE, androgen response element

16.2.3.2 AR DBD and Hinge Region

The AR interacts with DNA through its structured three-dimensional DBD that has a resolved crystal structure [39]. The AR DBD has three helices consisting of two zinc fingers with four cysteine residues that bind a zinc ion plus a C-terminal extension (CTE). Within AR DBD are the P-box and D-box that are essential for AR transcriptional activity. The first zinc finger is the recognition helix that binds AREs through the P-box [40]. The second zinc finger contains the D-box required for dimerization between monomers of AR [41]. The CTE provides specificity for AR to recognize AREs [42]. These AREs are found in enhancers and less so in promoter regions of target genes and are arranged as repeats of a hexamer separated by a spacer of three base pairs (for a review, see [17]). The hinge region is unstructured and links the AR DBD to its LBD. Nuclear translocation is a major function of the hinge region, but it has other functions and is regulated by acetylation, methylation, phosphorylation, and ubiquitination [43].

16.2.3.3 AR LBD

The effects of androgen are mediated through binding the folded C-terminus LBD. To date, there are only crystal structures resolved for the agonist conformation of AR, which reveals two antiparallel β-sheets and 11 α-helices that encompass a ligand-binding pocket [14]. The AR is missing helix 2, and this lack of helix 2 is seen in PR, GR, and mineralocorticoid receptor, but not in ER [44]. Androgens

cause a shift in conformation to reposition helix 12 over the ligand-binding pocket to create the AF-2 surface for N/C interactions [45]. The ligand-binding pocket consists of hydrophobic residues that interact with lipophilic testosterone and DHT. The AR LBD is the direct or indirect target for all currently FDA-approved drugs against the androgen axis. These drugs include those that reduce the levels of androgen that bind to the AR LBD such as luteinizing hormone-releasing hormone (LH-RH) analogs and CYP17 inhibitors that block steroidogenesis, selective androgen modulators (SARMs), as well steroidal and nonsteroidal antiandrogens. Antiandrogens compete with androgens for the AR LBD. Therefore, since DHT has a binding affinity in the low nM range for AR LBD, an effective antiandrogen must have a very strong affinity to be able to compete with DHT for the ligand-binding pocket in the AR LBD. Structural alterations in the AR LBD involved in disease include deletion or truncation of LBD that results in constitutively active AR that is independent of androgens [46]. Expressions of these constitutively active AR splice variants (AR-Vs) lacking the AR LBD have been detected in numerous tissues [47] and are a major mechanism of resistance to hormonal therapies for the treatment of prostate cancer [48]. Gain-of-function mutations in the AR LBD are also a major mechanism for the failure of current hormone therapies [49].

16.2.3.4 Transactivation of AR

Androgens enter into cells from the circulation by passive diffusion. Within the cell, testosterone can be converted by 5α-reductase to the more active androgen, DHT. Both testosterone and DHT bind with strong affinity within the ligand-binding pocket in the LBD of the cytosolic AR. DHT has approximately ten times improved affinity for the AR compared to testosterone predominantly due to the small difference in its chemical structure that impacts its interaction within the ligand-binding pocket of the LBD to result in a slower dissociation rate compared to testosterone [50]. Here, we focus on genomic signaling of AR and direct readers to a recent review on non-genomic signaling of the cytosolic AR [51]. Genomic signaling of AR is initiated upon androgen binding to the AR LBD to induce a conformational change that decreases AR interactions with chaperones which results in the reduction of its solubility that enhances its affinity for DNA. The nuclear localization signal in the hinge region becomes unmasked, thereby allowing the AR to form intramolecular N/C interactions and translocate into the nucleus. Within the nucleus, the AR forms an intermediate AR homodimer through intermolecular N/C interactions through their D-boxes [52]. Upon binding to androgen-response elements (AREs) within the regulatory regions of androgen-responsive genes, N/C interactions are lost to allow interaction with coactivators and recruitment of the basal transcriptional machinery. Over 300 coregulators have been described for nuclear receptors that function to stimulate or repress transcription without binding directly to DNA. These include proteins that regulate the structure of chromatin and bridge components of the basal transcriptional machinery to the site of transcription. Coregulators include ATPases and histone modifiers (for reviews, see [53, 54]). The

p160 steroid receptor coactivator-1 (SRC-1) and SRC-3 are examples of coactivators of AR that have histone acetyltransferase (HAT) activity. The bHLH/PAS, S/T, and HAT domains of SRC-3 directly interact with region 1–233 amino acid residues of the AR NTD [34, 35]. The AR is unique from other steroid hormone receptors in that p300, and presumably also CBP, directly interacts with the AR NTD rather than indirectly through recruitment to SRC [34]. The AR NTD is the site for interaction with the basal transcriptional machinery including recruitment of RNA polymerase II which is necessary for transcriptional activity. Other important coactivators of AR include the methyltransferases CARM1 and PRMT1 [55, 56]. The requirement of tissue-specific pioneer factors that co-localize with the AR on DNA-binding sites include FOXA1 with fl-AR as well as HOXB13 with AR-V7 in the prostate [57–60]. In response to androgens, the AR both induces and represses the expression of genes that are involved in development, metabolism, differentiation, proliferation, and DNA damage repair [61–65]. Thus, altered transcriptional activity of AR due to structural changes and/or variation in the levels of available androgens has a profound impact on human physiology and disease. An important recent discovery is the role for AR in modulating the expression of androgen-regulated genes such as *TMPRSS2* and *ACE2* that are required for the entry of the SARS-CoV-2 virus into cells to mediate COVID-19 disease [66, 67]. Due to space constraints, in the following sections, the roles of AR will be discussed in only a handful of these diseases, such as prostate cancer, androgen insensitivity syndrome (AIS), PCOS, breast cancer, and a few other AR-associated diseases.

16.3 Prostate Cancer

The prostate is part of the male reproductive system and is an androgen-dependent tissue that relies on functional androgen signaling for growth and survival. Castration leads to involution of the prostate in the mature male with apoptosis of prostate luminal epithelial cells. The androgen dependency of the prostate provided the rationale for Dr. Charles Huggins to test if a reduction of circulating levels of testosterone could induce tumor regression in prostate cancer patients [68, 69]. The success of those studies paved the way for the development of numerous approaches to block the androgen axis for the treatment of prostate cancer and other diseases driven by the AR. Today, androgen ablation therapy remains the standard of care for various stages of prostate cancer and can be combined with antiandrogens that target the AR LBD or other treatment modalities such as radiation therapy [70, 71]. Unfortunately, remissions to first-line androgen ablation for advanced prostate cancers are not durable and within 2–3 years the disease returns. These patients' disease will progress to lethal metastatic castration-resistant prostate cancer (CRPC). The transition to CRPC is characterized by a gradual rise in serum levels of prostate-specific antigen (PSA), the AR-regulated gene *KLK3*, which signifies a resurgence of transcriptionally active AR and biochemical recurrence. Mechanisms of resistance to androgen ablation therapies and antiandrogens implicated in the

progression to CRPC include synthesis of intratumoral androgens, amplification or overexpression of the AR gene, gain-of-function mutations in AR protein, ligand-independent activation by alternate signaling pathways, and expression of constitutively active truncated AR variants [72]. Regardless of androgen deprivation therapy and current AR-targeted therapies, genomic profiling shows a disproportional alteration of the AR signaling pathway compared to other pathways, which suggests that AR remains a key regulator of prostate cancer and an essential therapeutic target [73, 74]. Neuroendocrine prostate cancer is also considered to be a type of CRPC and represents about 20% of CRPC cases, but it does not rely on AR for growth and survival.

16.3.1 AR Mutations and Alterations in the Progression of Prostate Cancer

Amplification of the *AR* gene is the most common gene alteration and occurs in ~28% of CRPC tumors and 50% of CRPC metastases compared to less than 1% for primary prostate cancer tumors [73, 75, 76]. These frequencies support the notion that amplification of the *AR* gene is an adaptive response to androgen deprivation and that CRPC cells remain reliant on AR signaling. Increased sensitivity to a lower threshold of androgen is proposed to be a response to the elevated expression of AR. Castrate levels of androgen where serum testosterone is less than 50 ng/dL may be sufficient to transactivate the AR. Extragonadal sources of androgen including steroidogenesis from the tumor [77] or residual androgen biosynthesis from the adrenal glands [78] also contribute to AR signaling in spite of castrate serum levels of testosterone. Due to these discoveries of androgen still driving the disease, the nomenclature of this stage of the disease was changed from “hormone-refractory” or “androgen-independent” to “CRPC” [79].

AR mutations have been long suspected to drive the etiology and progression of prostate cancer and include the following: (i) point mutations that result in an amino acid substitution or premature stop codon, (ii) nucleotide insertions and deletions that cause a frameshift, (iii) complete or partial deletion of the *AR* gene, and (iv) intronic mutations that interrupt the processing of *AR* transcripts. Currently, there are more than 150 mutations reported in the Androgen Receptor Gene Mutations Database (http://androgendb.mcgill.ca) at the Lady Davis Institute for Medical Research [80]. These mutations predominantly occur in the LBD (48%) or NTD (39%) and are less commonly found in the DBD (7%) (Fig. 16.4a). Intronic mutations and large deletions that span multiple exons are considered to be rare and represent 3% and 2% of all detected mutations, respectively.

Missense mutations in the coding region of exon 8 that encodes the AR LBD are the most frequent and can confer ligand promiscuity and activation by antiandrogens or alternative steroids (Fig. 16.4b and Table 16.1). These AR LBD mutations primarily occur in “hot spots” that impact the structure of the ligand-binding pocket

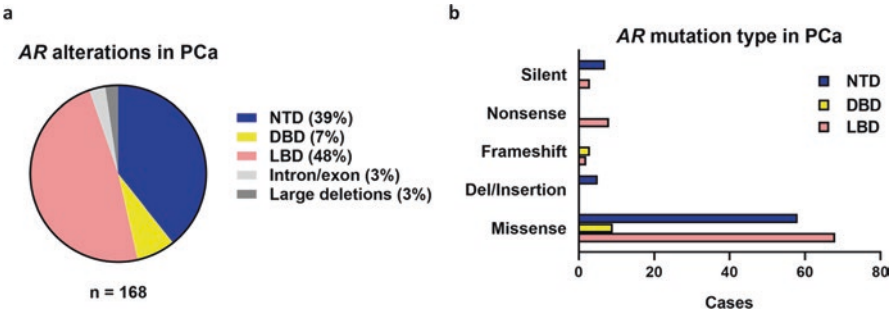

Fig. 16.4 Summary of *AR* gene alterations reported in prostate cancer patients. (**a**) Relative distribution of gene alterations in the AR N-terminal domain (NTD), DNA-binding domain (DBD), or ligand-binding domain (LBD), and intronic mutations or large deletions spanning multiple exons. (**b**) The number of cases reported for alterations occurring in the AR NTD, DBD, or LDB is shown, based on the type of mutation. Data shown were retrieved from the AR Gene Mutations Database (http://androgendb.mcgill.ca)

and are associated with therapies blocking the AR signaling axis, such as antiandrogens or CYP17 inhibitor abiraterone acetate. AR T877A was the first mutant identified to confer agonist activity to the antiandrogen flutamide. T877A reduces the specificity of the LBD for androgen such that the mutant AR can be activated by progesterone, E2, and various antiandrogens (hydroxyflutamide and bicalutamide) [81–83]. This mutation is present in the LNCaP human prostate cancer cell line, a widely used androgen-sensitive model of prostate cancer. Mutations associated with bicalutamide gain-of-function include W741C and W741L [84]. Another noteworthy mutation, AR F876L, was discovered in CRPC patients and confers agonist activity to second-generation antiandrogens enzalutamide and apalutamide [85, 86]. This AR F876L mutation remains sensitive to inhibition by bicalutamide, thereby indicating a difference in mechanism between these highly related compounds [87]. AR L701H was found in CRPC and is less sensitive to androgen but highly responsive to the glucocorticoids cortisol and cortisone [88]. Mutant AR harboring an L701H/T877A double mutation can be found in MDA-PCa cell lines, which were originally derived from a prostate cancer bone lesion.

Polymorphisms in the length of the AR NTD may influence the risk for men to develop prostate cancer. Most men have on average 21 repeats. Fewer CAG repeats, and therefore a shorter NTD, increases AR transcriptional activity in vitro, whereas increasing the length of the CAG region reduces transactivation [89, 90]. Several studies have shown an increased risk of developing prostate cancer for men with shorter (<21) CAG repeats [29, 30], but others have found no association between CAG repeat length and prostate cancer risk [91]. Thus, whether CAG repeat length of the AR NTD predisposes men to prostate cancer remains somewhat controversial.

Table 16.1 Recurring AR alterations from human prostate cancer

Domain	Exon	Mutation[a]	Findings	References
NTD	1	E43G/V	No endocrine treatment	Steinkamp et al. (2009) [281]
NTD	1	Q58L	Treated and untreated	Robins (2012) [282]
NTD	1	Q84del	Treated and untreated	Steinkamp et al. (2009)
NTD	1	S119S	Synonymous mutation, bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	L192Q	Bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	E211E	Synonymous mutation; bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	T227A	Treated and untreated patients	Steinkamp et al. (2009)
NTD	1	T227C	Bicalutamide- and flutamide-treated	Robins (2012)
NTD	1	E250V	Adjacent to conserved CHIP E3 ligase interaction site, bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	A251V	Bicalutamide- and flutamide-treated	Robins (2012)
NTD	1	E253K	Adjacent to conserved CHIP E3 ligase interaction site, prolonged protein half-life and nuclear localization without hormone, bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	A356V/T	Flutamide-treated	Steinkamp et al. (2009)
NTD	1	R360H	Treated and untreated	Robins (2012)
NTD	1	G414S/D	Treated and untreated	Steinkamp et al. (2009)
NTD	1	W433C	Treated and untreated	Steinkamp et al. (2009)
NTD	1	W433L	Impact on WxxLF motif, increased transactivation function and N/C interaction, bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	T438P	Bicalutamide- and flutamide-treated	Robins (2012)
NTD	1	G454S	Bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
NTD	1	G455D	Bicalutamide-treated	Robins (2012)
NTD	1	R484C	Treated and untreated	Robins (2012)
NTD	1	T497I	Treated and untreated	Robins (2012)
NTD	1	V508L	Bicalutamide-treated	Robins (2012)
NTD	1	V508L/G	Bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
DBD	3	C619Y	Cannot bind DNA and is transcriptionally inactive	Nazareth et al. (1999) [283], Marcelli et al. (2000) [284]
H	4	E665D	Bicalutamide- and flutamide-treated	Robins (2012)
LBD	4	L701H	Less responsive to androgens, responsive to glucocorticoids, partial agonist activity with flutamide and bicalutamide	van de Wijngaart et al. (2010) [88], Lallous et al. (2016) [285]
LBD	4	V715M	Responsive to progesterone, partial agonist activity with flutamide and bicalutamide	Culig et al. (1993) [286], Lallous et al. (2016)

(continued)

Table 16.1 (continued)

Domain	Exon	Mutation[a]	Findings	References
LBD	5	R726L	Activated by estradiol, germline mutation	Elo et al. (1995) [287], Mononen et al. (2000) [288]
LBD	5	V730M	Partial agonist activity with flutamide and bicalutamide	Lallous et al. (2016)
LBD	5	W741L	Confers agonist activity to bicalutamide	Bohl et al. (2005) [289]
LBD	5	W741C	Confers agonist activity to bicalutamide	Yoshida et al. (2005) [82]
LBD	5	R752Q	Reduced ligand-binding and N/C interaction, differential gene expression, reported as a germline mutation in some cases of AIS	Robins (2012)
LBD	5	R760R	Bicalutamide- and flutamide-treated	Steinkamp et al. (2009)
LBD	5	R760K	Bicalutamide-treated	Robins (2012)
LBD	6	T786*	Treated and untreated	Steinkamp et al. (2009)
LBD	6	L797P	Flutamide-treated	Robins (2012)
LBD	7	Q867*	Treated and untreated	Steinkamp et al. (2009)
LBD	8	L873P	Flutamide-treated	Robins (2012)
LBD	8	H874Y	Responsive to progesterone and estrogen; partial agonist activity with bicalutamide, enzalutamide, and apalutamide	Taplin et al. (1995) [81], Lallous et al. (2016)
LBD	8	H874Q	Partial agonist activity with flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	F876L	Partial agonist activity with flutamide, enzalutamide, and apalutamide	Korpal et al. (2013) [86], Joseph et al. (2013) [85]
LBD	8	T877A	Responsive to progesterone and estrogen, confers agonist activity to flutamide and bicalutamide, present in LNCaP cells	Wilding et al. (1989) [290], Veldscholte et al. (1992) [291]
LBD	8	T877S	Responsive to progesterone and estrogen, confers agonist activity to bicalutamide	Taplin et al. (1995), Lallous et al. (2016)
LBD	8	D879E	Partial agonist with flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	L881I	Partial agonist with flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	S888G	Responsive to progesterone and estrogen, confers agonist activity to flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	D890H	Confers agonist activity to flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	E893K	Partial agonist with flutamide and bicalutamide	Lallous et al. (2016)
LBD	8	M895V	Confers agonist activity to bicalutamide, partial agonist activity with flutamide	Lallous et al. (2016)

(continued)

Table 16.1 (continued)

Domain	Exon	Mutation[a]	Findings	References
LBD	8	M895T	Confers agonist activity to bicalutamide, partial agonist activity with flutamide	Lallous et al. (2016)
LBD	8	E897G	Partial agonist activity with flutamide	Lallous et al. (2016)
LBD	8	T918S	Partial agonist activity with flutamide and bicalutamide	Lallous et al. (2016)

Note. [a]del, deletion; *, stop codon

16.3.2 Roles of AR Splice Variants in Prostate Cancer

The expression of some truncated AR splice variants that lack the LBD is now established as a major resistance mechanism for CRPC. To date, 22 AR splice variants have been reported in the literature with available transcript sequences [92]. AR-V7 (also known as AR3) is the most extensively studied and the most common splice variant expressed in CRPC. AR-V7 is comprised of the NTD, DBD, and a unique C-terminus with 16 amino acids encoded by cryptic exon 3 [93, 94]. AR-V7 is constitutively active and does not encode the LBD, which is the therapeutic target all of currently approved therapies for CRPC that target AR. Thus, AR-V7 is considered a major resistance mechanism for all therapeutic approaches that target the AR LBD, including next-generation antiandrogens (enzalutamide, apalutamide, and darolutamide) and abiraterone acetate. Clinical evidence for the importance of AR-V7 in CRPC has been drawn from studies showing AR-V7 expression is associated with shorter survival and limited responses to approved AR-targeted therapies for CRPC patients [95–97]. Approximately 10%–30% of patients with metastatic CRPC have detectable AR-V7 expression, based on clinically validated assays that detect nuclear protein or mRNA in circulating tumor cells [98]. Alternative splicing of AR-V7 transcripts is induced by androgen deprivation and antiandrogens. Both the rate of *AR* gene transcription and recruitment of RNA splicing factors and enhancers (U2AF65 and ASF/SF2) that generate the AR-V7 transcript are upregulated when fl-AR transcriptional activity is suppressed [99, 100]. AR-V7 is almost always coexpressed with fl-AR, but V7 transcript levels are usually lower (5%–30%) than fl-AR. AR-V7 is commonly detected in samples that also have *AR* gene amplification.

Proliferation of prostate cancer cells that express mixed populations of fl-AR and AR-Vs tends to be androgen-independent and resistant to antiandrogens. This is observed in clinical findings, where AR-V7-positive CRPC patients treated with enzalutamide or abiraterone had poor responses and lower overall survival than patients without detectable AR-V7 [96]. AR-V7-positive patients are associated with better PSA responses with taxane chemotherapy compared to treatment with enzalutamide or abiraterone, whereas for AR-V7-negative patients, there were no obvious differences in efficacy between taxanes and these hormonal therapies [101]. Serial analysis of AR-V7 expression in CRPC patients further revealed that

inhibition of AR signaling by androgen-deprivation, enzalutamide, or abiraterone may exert a selective pressure for promoting the expression AR-V7 [102], confirming in vitro observations [96, 103]. Reversion to AR-V7-negative status is observed in some taxane-treated AR-V7-positive patients; however, this did not occur with all taxane-treated patients, and thereby further investigation is warranted to understand the mechanism of this phenomenon.

Protein-protein interactions between fl-AR and AR-Vs remain an important area of investigation. A study by Xu et al. in 2015 [104] using bimolecular fluorescence fusion constructs reported that truncated splice variants, AR-V7 and AR^{v567es}, can interact with fl-AR by N/C interactions mediated by AF-2 of fl-AR or by DBD-DBD interactions mediated by the D-box motif. These data suggest that constitutively active AR-Vs may promote transactivation of fl-AR in the absence of androgen or transactivate target genes without fl-AR by using their D-box to form variant homodimers or heterodimers [104]. Analysis of an AR-V gene expression signature in CRPC cell lines suggested that AR-V7 and AR^{v567es} can activate some canonical fl-AR target genes, in addition to a subset of variant-specific genes that include *AKT1* and cell cycle genes, such as *UBE2C*, *CDC25B*, and *CCNA2* [93, 105]. Ectopic expression of AR-V7 can increase the expression of *ETS2* and *EDN2,* which are otherwise co-repressed by fl-AR and the pioneer factor FOXA1 [106]. Cofactors and interacting proteins that uniquely interact with AR-Vs but not fl-AR have also been reported. An analysis of AR-V7 cistromes in CRPC cell lines and patient specimens suggested that homeobox protein HOXB13 may interact with AR-V7 as an essential coactivator and pioneer factor to open the chromatin for access to DNA-binding sites [107]. Genomic profiling of AR-V7 and fl-AR binding sites in 22Rv1 human prostate cancer cells showed a proportion of sites (2221 out of 17,409) were specific to AR-V7 binding [108]. In contrast to the binding sites shared by fl-AR and AR-V7, which were enriched in ARE and FOXA1 motifs at enhancer regions, these AR-V7-specific binding sites were associated with zinc finger X-chromosomal protein (ZFX) and located primarily at promoter regions of MYC-bound genes or genes regulating cell cycle progression (*SKP2*), autophagy (*ZNF32*), and WNT signaling (*FZD6*) [108]. ChIP-sequencing analysis supports the notion that fl-AR and AR-V7 can heterodimerize to mostly the same genomic foci, but AR-V7 preferentially interacted with transcriptional corepressors (NCOR1 and NCOR2), whereas fl-AR was associated with both coactivators and corepressors [109]. These findings suggest that AR-V7 may have a significant repressor function in CRPC, which may contribute to prostate cancer progression by preventing the expression of tumor suppressor genes [109].

16.3.3 Treatments Targeting AR

The two main therapeutic approaches to inhibit AR signaling are surgical or pharmaceutical reduction of androgens and the direct inhibition of binding of androgen to the AR LBD with competitive antagonists called antiandrogens. Castration by

orchiectomy or analogs of LH-RH quickly reduce circulating levels of androgen by >90%. Abiraterone acetate is a CYP17 inhibitor that blocks steroid synthesis to reduce de novo androgen synthesis. The development of antiandrogens as antagonists of the AR commenced approximately 60 years ago first with the development of steroidal progestogens such as cyproterone acetate and then later with the development of flutamide as a first-in-class nonsteroidal pure antagonist that lacked partial agonist activity (for a review, see [110–112]). Steroidal antiandrogens are used today for numerous indications mediated by AR, including prostate cancer, PCOS, congenital adrenal hyperplasia, benign prostatic hyperplasia, acne, hirsutism, and androgenic alopecia. All nonsteroidal antiandrogens, including flutamide, nilutamide, bicalutamide, enzalutamide, apalutamide, and darolutamide, are competitive AR LBD inhibitors with chemical structures based upon flutamide and bicalutamide with the exception of darolutamide. The crystal structure of the folded AR LBD has only been resolved for the agonist conformation bound to ligand with no antagonist conformation reported. The mechanism of how antiandrogens antagonize AR involves blocking N/C interactions required for agonist activity and preventing essential protein-protein interactions with AF-2 in the AR LBD. Differences in AR-binding affinity to the chromatin and reduction of AR nuclear localization have also been reported for the various nonsteroidal antiandrogens [100, 113].

Enzalutamide is a second-generation antiandrogen developed for CRPC using LNCaP human prostate cancer cells engineered to express elevated levels of wild-type AR in the background of the LNCaP AR mutation T877A [114]. Enzalutamide binds to the AR LBD, with about an eightfold improved affinity compared to bicalutamide, and impairs AR nuclear translocation and chromatin binding [114]. Enzalutamide was FDA-approved in 2012 as second-line therapy for metastatic CRPC following results of the AFFIRM trial that showed an improvement for overall survival by 4.8 months [115]. Enzalutamide was subsequently approved for first-line therapy for metastatic CRPC following the PREVAIL study [116] and was later approved for nonmetastatic CRPC after results from the PROSPER trial showed a 71% reduction for the risk of progression for nonmetastatic CRPC patients on androgen deprivation therapy [117].

Apalutamide is a second-generation antiandrogen with high chemical similarity to enzalutamide. It was discovered using the same screen as used for enzalutamide [118]. Apalutamide is the first drug to be approved for the treatment of nonmetastatic CRPC. The SPARTAN trial for nonmetastatic CRPC patients reported a significant improvement to metastasis-free survival by 23.3 months with apalutamide compared to a placebo [119]. Apalutamide has comparable properties to enzalutamide including its binding affinity for the AR LBD and reducing AR nuclear translocation or DNA binding [118]. Preclinical evaluation of apalutamide demonstrated that it has a greater in vivo efficacy on human CRPC xenografts compared to enzalutamide, such that 30 mg/kg/d of apalutamide had a maximum response that was equivalent to 100 mg/kg/d of enzalutamide [118].

Darolutamide is the most recent FDA-approved second-generation antiandrogen for nonmetastatic CRPC. Darolutamide and its active metabolite have an eight to tenfold improved the binding affinity for AR compared to enzalutamide and

apalutamide in ligand competition assays as well as having activity against the AR F876L mutant, which is resistant to enzalutamide and apalutamide [120]. Darolutamide has also been shown to inhibit other clinically relevant AR LBD point mutations, including F876L, H874Y/T877A, F876L/T877A, and T877G [121]. In contrast to enzalutamide and apalutamide, darolutamide does not share structural similarity to first-generation antiandrogens and has negligible brain penetrance [120, 122]. The ARAMIS trial of darolutamide for men with nonmetastatic CRPC reported a metastasis-free survival of 40.4 months compared to 18.4 months for the placebo group [123]. These results are consistent with the PROSPER and SPARTAN studies, where metastasis-free survival was 36.6 and 40.5 months for enzalutamide and apalutamide, respectively [117, 119]. Since these second-generation antiandrogens all target the AR LBD and appear to provide a similar clinical benefit, their differences in cost and improvement on the quality of life are important factors to consider.

Abiraterone acetate is a selective inhibitor of CYP17A1 that blocks androgen biosynthesis from steroid precursors in the testes, adrenal glands, or any sources from the tumor itself [124]. Cytochrome P450 enzymes (CYP11A1 and CYP17A1) synthesize the weak adrenal androgens DHEA and androstenedione, which can be converted by some prostate cancer cells to testosterone and DHT. Castrate levels of serum testosterone following surgical or chemical castration are typically within the 20–50 ng/dL range. The addition of abiraterone can further reduce serum testosterone to a "super-castrate" level of 1–2 ng/dL [125]. Tumor biopsies from CRPC patients following abiraterone therapy showed an upregulation of CYP17A1 expression, which suggests that CRPC cells may remain steroid-dependent [126].

All current FDA-approved hormonal therapies for CRPC target the AR LBD and will inevitably fail from de novo or acquired resistance. Targeting solely the AR LBD is inadequate to completely block all AR signaling. The AR NTD contains the AF-1 region which is required for transcriptional activity, including the activity of truncated AR-Vs lacking the LBD. Thus, targeting the AR NTD would potentially inhibit fl-AR and all transcriptionally active AR-Vs. Ralaniten acetate is a prodrug of ralaniten, which is a first-in-class AR NTD antagonist that specifically binds to AF-1. Ralaniten inhibits the growth of prostate cancer in vitro and in vivo and maintains AR inhibition despite overexpression of AR coactivators, gain-of-function mutations in the AR LBD, or expression of AR-V7 [127–129]. Nuclear magnetic resonance studies revealed that ralaniten and its stereoisomers bind to a pocket formed by amino acids of 345–448 of tau-5 in AF-1 [130]. Proof of concept for the chemical scaffold and efficacy of ralaniten was provided in a phase I clinical trial with heavily pretreated CRPC patients who had previously failed enzalutamide or abiraterone [131]. Due to the rapid metabolism of ralaniten acetate, there was an excessive pill burden that stopped the trial. A second-generation analog, EPI-7386, is more potent with an improved pharmacokinetic profile compared to ralaniten, and it commenced clinical trials in mid-2020 (NCT04421222).

On-target complications associated with blocking the AR axis are associated with anemia, bone and muscle loss, gynecomastia, cognitive impairment, depression, diabetes, coronary heart disease, and cardiovascular disease [132–134].

Cycling of androgen levels by application of intermittent androgen suppression has been proposed as an approach to reduce the incidence of adverse side effects from decreased levels of androgen (NCCN Guideline 2020). High levels of androgen may also be beneficial in blocking the progression of some prostate cancers [135]. Phase 2 clinical trials of bipolar androgen therapy that cycles high and low levels of androgen were well-tolerated but did not improve the outcomes for AR-V7-positive disease [136]. Thus, stimulating AR activity such as with SARMs or exogenous androgen may have beneficial effects for some prostate cancers in addition to other diseases, such as for some breast cancer, sarcopenia or cachexia, osteoporosis, hypogonadism, Duchenne muscular dystrophy, and AIS [137].

16.4 Androgen Insensitivity Syndrome

Testosterone and DHT both play roles in virilization during embryogenesis, with testosterone for the Wolffian structures and DHT for the virilization of the Anlagen, which forms the prostate and external genitals (for a review, see [138]). In the absence of androgens or functional AR, male sexual differentiation fails to occur. Inactivating mutations of the *AR* gene that cause a partial or complete inability of androgen-sensitive cells to respond to androgen is associated with AIS, which is a disorder of sex development [139]. The first detailed report of AIS (formerly known as testicular feminization) was described by John Morris in 1953, who recognized it to be an inherited disorder affecting male sexual differentiation. In general, individuals affected by complete AIS (CAIS) have developed testes and physiological production of testosterone plus its conversion to DHT, but they appear phenotypically female [140, 141]. Over the decades with the identification of the *AR* gene and an increased understanding of the structure and function of AR and the androgen axis, it is now appreciated that this overall feminizing effect arises predominantly from the lack of androgen action and an abundance of E2 resulting from the aromatization of testosterone.

Insensitivity to androgen during development of the male fetus prevents the masculinization of external genitalia. Instead, partial female external genitals are formed from the urogenital sinus, which in most cases results in a blind-ended vagina. Phenotypic variation of the external genitalia in AIS is directly attributed to the binding affinity of androgen to a mutant AR and its residual function. Table 16.2 provides a list of AR mutations. Most individuals impacted by AIS have undescended testes that can be located anywhere along the path of embryonic testicular descent, for instance, in the abdomen, inguinal canal, or labia, since androgen signaling regulates testicular descent to the scrotum [142]. Secondary sexual characteristics that are regulated by androgen actions include the development of axillary and pubic hair, and deepening of the voice at puberty, which can be absent or minimal in AIS. Breast development occurs at the onset of puberty, which is supported by the aromatization of testosterone to E2. AIS is the most common disorder of sex development reported in genetic (46,XY) males. The prevalence of CAIS is estimated to

Table 16.2 Select AR alterations identified from individuals with AIS

Pathology	Domain	Exon	Mutation[a]	Sex of rearing	External genitalia	Findings	References
CAIS	NTD	1	V30M	F	Normal		Topcu et al. (2015) [155]
CAIS/ PAIS	NTD	1	E211E	M or F	Normal to ambiguous	Synonymous mutation at the end of 3′ acceptor splice site	Topcu et al. (2015), Gottlieb et al. (1999) [292]
PAIS/ MAIS	NTD	1	P390S	M	Ambiguous to normal		Hiort et al. (2000) [170], Audi et al. (2010) [169]
MAIS	NTD	1	R405S	M	Ambiguous	Creates a phosphorylation site that inhibits interaction with MAGE-11 and p300	Lagarde et al. (2012) [171]
MAIS	NTD	1	A474V	M	Normal	Male infertility	Zuccarello et al. (2008) [168]
MAIS	NTD	1	L547F	M	Normal	Male infertility, azoospermia	Zuccarello et al. (2008)
CAIS	DBD	3	R607*	F	Normal		Cheikhelard et al. (2008) [293], Boehmer et al. (2001) [143], Giwercman et al. (2000) [294]
PAIS	DBD	3	R607Q	M or F	Ambiguous	Partial AR transactivation	Deeb et al. (2005) [161]
CAIS	DBD	3	R615C	F	Normal		Zhou and Wang (2013) [159], Hughes et al. (2012) [141]
CAIS	DBD	3	R615H	F	Normal	Absence of epididymis and vas deferens	Boehmer et al. (2001), Hiort et al. (1998) [151]
CAIS	DBD	3	R615P	F	Normal		Ahmed et al. (2000) [146], Marcelli et al. (1991) [156]
CAIS	DBD	3	R615S	F	Normal		Sharma et al. (2011) [158]
CAIS	LBD	4	N705S	F	Normal		Hughes et al. (2012), Audi et al. (2010), Pinksy et al. (1992) [295]
PAIS	LBD	4	Q711E	F	Ambiguous		Méndez et al. (2001) [296], Topcu et al. (2015)

(continued)

Table 16.2 (continued)

PAIS	LBD	4	A645D	M	Ambiguous	2 cases with long polyQ (30/28) and short polyG (10)	Hughes et al. (2012), Hiort et al. (1996) [297]
CAIS	LDB	5	D732Y/N	F	Normal		Hannema et al. (2004) [298], Pinksy et al. (1992)
PAIS	LBD	5	M742V/I	F or M	Ambiguous		Audi et al. (2010), Boehmer et al. (2001)
CAIS	LBD	5	M749V	F	Normal		Bouvattier et al. (2002) [299]
CAIS	LBD	5	P752*	F	Normal		Ledig et al. (2005) [300], Hughes et al. (2012), Pinksy et al. (1992)
CAIS	LBD	5	P752Q	F	Normal		Hughes et al. (2012)
PAIS	LBD	5	Y763C	M	Ambiguous		Ahmed et al. (2000)
CAIS	LBD	5	A765T	F	Normal		Ledig et al. (2005), Hannema et al. (2004), Hiort et al. (2000)
CAIS	LBD	5	P767fs	F	Normal	1 nucleotide frameshift and stop codon at 807	Hannema et al. (2004)
CAIS	LBD	6	R774C	F	Normal		Hiort et al. (2000)
CAIS	LBD	6	R774H	F	Normal		Ledig et al. (2005), Ahmed et al. (2000)
CAIS	LBD	6	R779W	F	Normal		Ledig et al. (2005), Ahmed et al. (2000)
PAIS	LBD	6	M780I	F	Ambiguous		Boehmer et al. (2001)
PAIS/ MAIS	LBD	6	Q798E	F or M	Ambiguous to normal	Male infertility and azoospermia, defective AR transactivation	Hiort et al. (2000), Wang et al. (1998) [167], Bevan et al. (1996) [301]
PAIS	LBD	7	I816I	M	Ambiguous	Synonymous mutation, additional E211E synonymous mutation	Topcu et al. (2015)
PAIS	LBD	7	Q824L	M	Ambiguous	EMS = 12	Hellmann et al. (2012) [302]
CAIS	LBD	7	R831*	F	Normal		Giwercman et al. (2000)
CAIS	LBD	7	R831Q	F	Normal		Audi et al. (2010), Ahmed et al. (2000)

PAIS	LBD	7	R840C	M or F	Ambiguous	AR is transcriptionally active only at high concentrations of androgen (EC50 ~300x Wt)	Georget et al. (1998) [303], Bevan et al. (1996)
PAIS	LBD	7	R840H	F	Ambiguous		Beitel et al. (1994) [304]
CAIS	LBD	7	R855C	F	Normal		Ledig et al. (2005), Ahmed et al. (2000)
PAIS	LBD	7	R855H	M or F	Ambiguous		Audi et al. (2010), Deeb et al. (2005)
PAIS	LBD	7	V866L/M	M or F	Ambiguous		Ledig et al. (2005)
CAIS	LBD	8	H874R	F	Normal		Cheikhelard et al. (2008)
CAIS	LBD	8	V889M	F	Normal		Audi et al. (2010), Ledig et al. (2005)

Note. Notable mutations and alterations reported five or more times for CAIS and PAIS, or three or more times for MAIS were retrieved from the Androgen Receptor Gene Mutation Database [80]. The numbering of residues is based on the 919 amino acid reference sequences for the human androgen receptor, NCBI Accession No. AAA51729.1 [305]

[a]*, stop codon; fs, frameshift

vary between 1:20,000 and 1:99,000 in 46,XY live births [80, 143, 144] and is identified in 0.8% to 2.4% of phenotypic females with inguinal hernia [145].

16.4.1 Clinical Presentation of AIS

Androgen resistance in AIS may be suspected when serum androgen levels are physiological or elevated but clinical effect is lacking or suboptimal. Individuals affected by AIS are classified by their clinical phenotype as either CAIS, partial (PAIS), or mild (MAIS) [141]. The external masculinization score (EMS) was devised as a tool for the initial assessment of ambiguous genitalia in infants (ranging from 0 to 12); however, it should be noted that gender assignment does not necessarily depend on the appearance of the external genitalia and gender identity may change during or after puberty [146, 147]. For CAIS, there are no clinical indications of androgen action, and individuals are born with female-appearing external genitalia, but structures, such as the clitoris, labia minora, and labia majors, are typically underdeveloped. CAIS individuals are almost always raised as females, and the condition is rarely diagnosed in childhood unless a family history is known. CAIS can be suspected prenatally when the karyotype (46,XY) of the fetus is not consistent with the developing female phenotype [139]. It is also not uncommon for CAIS to be diagnosed during puberty when breast development occurs but pubic and axillary hair is lacking and menarche does not occur.

PAIS includes a broad range of external genitalia phenotypes, which may vary from female-like to male-appearing depending on the level of residual AR function. The management of PAIS is highly complex, since sexual identity and gender assignment may be unclear at birth. In milder presentations of PAIS, the external genitalia appear morphologically male, but there may be an underdeveloped penis, severe hypospadias, and bifid scrotum with or without undescended testes [145]. PAIS is thought to be as commonly occurring as CAIS. In the case of MAIS, individuals have unambiguous male external genitalia, but there may be evidence of mild impairment of masculinization, such as decreased terminal body hair or perhaps isolated micropenis. Impotence is commonly reported as a concern in MAIS, and spermatogenesis may be impaired but may be sufficient to preserve fertility [148]. MAIS is the least understood type of AIS, since it has the mildest phenotype and may not be actively investigated unless there are issues regarding fertility. In many cases, male infertility is the only reported symptom in patients with MAIS [148]. Other phenotypic characteristics of MAIS include minor gynecomastia, sparse terminal body hair, and lack of vocal deepening at puberty [149]. The prevalence of MAIS is not known, but it is reported at a lower frequency than CAIS and PAIS [141]. Expansion of the AR polyglutamine tract to more than 38 CAG repeats is related to a progressive onset of MAIS in the form of gynecomastia and reduced fertility in adulthood [150].

16.4.2 AR Mutations in Patients with AIS

More than 500 unique mutations in the *AR* gene have been identified from over 900 AIS patients [80] (Fig. 16.5a). The majority of mutations that cause AIS are inherited with about 30% identified as de novo mutations [151]. Defects in the *AR* gene that result in a loss of AR function are sufficient to be a single causative factor for AIS; however, polymorphisms in AR coactivator genes or genes related to steroid biosynthesis and metabolism are also important factors that may contribute to the AIS phenotype [143, 152]. Mutations in the *AR* gene are detected in the majority (90%–95%) of CAIS cases [153]. According to the AR mutation database (http://androgendb.mcgill.ca), the majority of *AR* mutations from CAIS patients affect the AR LBD (66%) and are predominantly missense single base-pair substitutions, resulting in an amino acid change (Fig. 16.5b–d). AR LBD mutations that cause CAIS are clustered in the amino acid regions 688–712, 738–784, and 827–870 [80, 141]. These mutations are predicted to primarily alter the AR LBD protein structure and disrupt the ligand-binding pocket and ligand specificity or render the mutated AR to be functionally inactive [14, 141]. Mutations associated with AIS have also been found in the AR LBD dimerization interface mediating AR LBD-LBD interactions and may disrupt allosteric regulation and impair AR transcriptional activity [154]. Mutations of the AR NTD and DBD have also been reported for CAIS but are less common than the LBD, representing 17% and 13% of detected mutations, respectively (Fig. 16.5e). Notably, a missense mutation resulting in a substitution of valine to methionine (V30M) in the AR NTD was identified from a patient with CAIS [155]. Mutations of arginine 615 (R615C, R615H, R615P, R615S) of the second zinc finger of AR DBD has been documented in several CAIS cases [156–159]. Other *AR* gene alterations identified in CAIS include mutations that impact the intron or exon splice sites and large deletions spanning multiple exons, which are less frequent and cover less than 5% of all detected *AR* mutations (Fig. 16.5e).

In contrast to CAIS, *AR* gene mutations are only identified in 20%–40% of PAIS patients [80, 160]. Mutations associated with PAIS are more frequently detected in the AR LBD (71%) than in the DBD (19%), NTD (8%), or at intron-exon junctions (2%) (Fig. 16.5f). Whether a PAIS patient carries an *AR* gene mutation is phenotypically indistinguishable from the external genitalia and the EMS criteria; however, birth weight was reported to be significantly lower for the gestational age of PAIS infants without an *AR* mutation [160]. It is noteworthy to mention that identical mutations have been associated with different conditions of PAIS [161, 162], such that related affected individuals with the same *AR* mutation may have a different phenotype and sex assignment [143, 163]. These cases imply that additional factors are accountable for the extent of virilization in PAIS. Other identified genetic causes that may promote PAIS in the form of underdeveloped male external genitalia include defects in LH receptor and deficiencies in androgen biosynthesis enzymes (i.e., 17,20-lyase, P450 oxidoreductase, 17β-hydroxysteroid dehydrogenase, and 5α-reductase) [164, 165]. Fewer AR mutations are reported in patients with MAIS, with 40% identified in the NTD and 47% in the LBD (Fig. 16.5g).

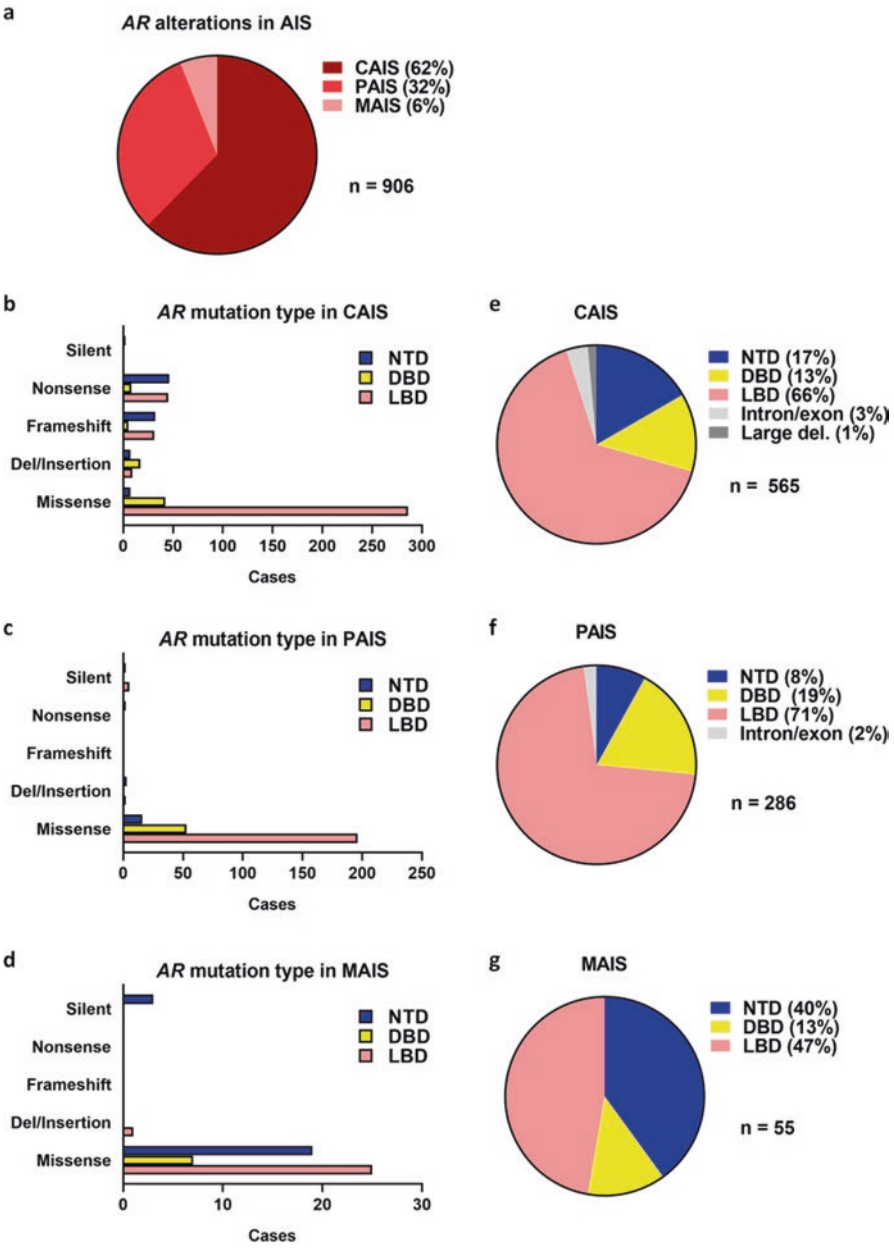

Fig. 16.5 Summary of *AR* gene alterations reported from patients with androgen insensitivity syndrome. (**a**) Relative distribution of AIS phenotypes (complete, partial, or mild) associated with a mutation in the *AR* gene. (**b-d**) The number of cases with a mutation impacting the AR NTD, DBD, or LBD is shown, based on the type of mutation. (**e-g**) Relative distribution of the gene alterations occurring in the AR NTD, DBD, or LBD, or introducing a large deletion or intronic mutation

The AR NTD has a flexible disordered structure; therefore, mutations in this domain have a milder effect on protein structure and are less likely to be detrimental to AR function. The pathogenicity of AR NTD mutations may also be difficult to prove since transactivation of AR in vitro can vary depending on the promoter of the reporter gene or cell line used. Such studies suggest that mutations of the AR NTD associated with a MAIS phenotype might impact coactivator binding or impair the structural flexibility of the AR NTD rather than cause a significant structural change as those observed with mutations in the folded AR LBD [166]. Missense mutations located in the AR NTD have been documented in mild to partial states of AIS (Table 16.2). G216R is associated with reduced AR transactivation and has been reported in multiple patients with PAIS [161, 167]. A474V has been detected in several cases of infertile men with MAIS [168] and P390S in mild to partial AIS cases [169, 170], but neither the A474V and P390S mutations are associated with a significant difference in transcriptional activity in vitro compared to the wild-type AR. Interestingly, an R405S mutation in AR from a PAIS patient creates a phosphorylation site that inhibits interaction with essential transcriptional coactivators such as p300 [171]. Although hyperexpansion of the AR polyglutamine tract is associated with SBMA and progressive onset of androgen deficiency in the form of MAIS, CAG polymorphisms within the normal range of CAG repeats are not single causative factors for AIS [170, 172–174].

16.4.3 Clinical Management of AIS

There is currently no standardized treatment for patients impacted by AIS. Individualized care with a multidisciplinary approach is strongly recommended for the management of a disorder of sex development, such as AIS, from pursuing a diagnosis and providing information about the condition appropriately to monitoring puberty and considering the need and optimal timing for gonadectomy [139]. Gonadectomy is recommended for CAIS due to the increased risk of testicular malignancy that increases with age which is estimated to be about 3.6% at 25 years and 33% at 50 years [175]. Continued support for the adult patient is especially important to promote adequate sexual function and quality of life.

The management of PAIS is far more complex than for CAIS since it encompasses a range of ambiguous phenotypes and patient sexual identity may not be clear. Gender assignment for PAIS should not only consider the external genitalia but also the virilization potential, complexity of genioplasty, likelihood of gaining fertility, and projected gender identity in a case-by-case manner. The majority of PAIS infants are raised as males and would require surgery to repair hypospadias, orchiopexy for the undescended testes, and corrective mammoplasty after puberty. Several studies have demonstrated that some PAIS patients respond to high pharmacologic doses of androgens to improve virilization and masculine self-identity [149, 162, 176, 177]. Patients with shorter CAG repeats appear more likely to respond to testosterone supplementation, but further investigation is required to completely

assess the value of CAG length as a selection marker [178]. PAIS assigned as females would require gonadectomy to prevent further virilization and the risk of developing gonadal tumors later in life, and they may elect for vaginal reconstruction procedures to improve sexual function. Most patients affected by AIS are infertile, but since AIS is an X-linked recessive heritable disorder with significant consequences, genetic counseling is recommended to affected families.

16.5 Polycystic Ovary Syndrome

An excess of androgen in women can be associated with a hormone disorder known as PCOS. The first description of PCOS was reported by Stein and Leventhal in 1935 [179] from a series of women with enlarged bilateral polycystic ovaries, hirsutism, and infrequent or absence menstrual periods. PCOS is a heterogeneous disorder that affects 6% to 20% of women of reproductive age [180]. It is the most common endocrine condition for childbearing age. According to the Rotterdam criteria, a patient diagnosed with PCOS will have two of the following features: clinical or biochemical androgen excess, infrequent or lack of ovulation, and a characteristic polycystic ovarian morphology as observed by ultrasound [181, 182]. Approximately 60% of PCOS patients have high levels of circulating androgen (hyperandrogenism) in the form of testosterone, androstenedione, and DHEA, and possibly also elevated levels of 3β-hydroxysteroid dehydrogenase [183, 184]. In PCOS patients, a greater number of follicles are recruited to the preantral and antral stage; however, the follicles fail to progress to ovulation. This leads to follicular atresia, giving rise to ovaries with the characteristic polycystic appearance. Moreover, increased LH pulse frequency by the anterior pituitary stimulates testosterone production by theca cells of the follicle to further exacerbate the hyperandrogenic state and PCOS condition. There is a wide variety of comorbidities with PCOS comprising of endocrine, reproductive, and metabolic symptoms [185]. The primary endocrine and reproductive features of PCOS include LH excess and hyperandrogenism, ovulatory perturbations, aberrant follicle development, and reduced fertility. Women with PCOS who achieve pregnancy also have an increased risk of miscarriage and for developing complications including gestational diabetes, hypertensive disorders, and premature delivery [186]. A metabolic component of PCOS is associated with hyperinsulinemia and insulin resistance, increased intra-abdominal fat, fatty liver disease, and dyslipidemia, all of which amplify the risk of cardiovascular disease and type 2 diabetes [187].

16.5.1 AR-Mediated Actions in the Ovary and Brain

The phenotype of *AR* knockout (ARKO) mice has been critical for our understanding of androgen action and how AR maintains ovarian function, primarily in regulating the early stages of folliculogenesis. Although AR is not essential for the survival and reproduction of female mice, ARKO females have reduced fertility and show a progressive decline in reproductive potential with age. ARKO female mice produce fewer offspring and smaller numbers of litters, where fecundity is reduced by about 70% compared to wild-type littermates [188, 189]. Ovaries of ARKO female mice appear relatively normal at 4 weeks of age, but by 8 weeks, there are fewer corpora lutea and more atretic follicles compared to wild-type littermates, and the follicles are completed depleted by 40 weeks [189]. Analysis of ARKO mouse ovaries suggests that several genes that are involved in folliculogenesis are regulated by AR signaling, including *Kitl*, *Bmp15*, *Gdf9*, and *Hgf* [189]. Chronic exposure to exogenous androgens is sufficient to induce PCOS-like traits in rodents, including disruption of the estrous cycle, the appearance of polycystic atretic follicles, and metabolic symptoms such as increased body fat and glucose intolerance [190, 191]. Global ARKO female mice supplemented with excess androgen do not develop PCOS, which supports the hypothesis that functional AR is required for the development of PCOS phenotypes [192]. Neuron-specific ARKO prevented the development of most reproductive and metabolic symptoms induced by androgen excess but still had cycle irregularity and partial polycystic ovary morphology [193]. A more recent mouse model with double ARKO in the brain and adipocytes showed further protection against developing irregular cycles, polycystic ovary morphology, and hepatic steatosis in response to androgen excess [194]. Collectively, these findings support that AR-driven neuroendocrine actions from the brain are major drivers to the onset of reproductive and metabolic PCOS traits induced by hyperandrogenism. Other potential tissue targets include adipocytes, liver, and muscle cells, which are believed to be involved in the pathogenesis of PCOS and could also involve AR.

16.5.2 Regulation of AR Signaling in PCOS

Ovulatory women with an AR that has more than 23 CAG repeats in AR NTD are associated with higher aromatase levels and lower intrafollicular testosterone than in patients with fewer than 20 CAG repeats [195]. This suggests that CAG length may influence hormone levels in the follicular milieu. Studies examining the association between CAG length and hyperandrogenism in PCOS have yielded conflicting results. In cohorts of Australian and Chinese women, longer CAG repeats were more frequent in PCOS women [196, 197]. CAG length in a Croatian population was reported to be associated with total testosterone in PCOS women, but it was not a significant predictor of PCOS or PCOS traits like hirsutism or acne [198]. Although

polymorphism in CAG length of AR does not appear to be a major determinant of PCOS, there may be an association between CAG length and the variations in androgen levels among women with PCOS.

The presence of alternatively spliced *AR* transcripts has been identified in granulosa cells of some patients with PCOS. A study by Wang et al. in 2015 identified two AR-Vs, which were found in the granulosa cells of most (62%, 42/68) women with PCOS but not from non-PCOS control subjects [199]. One of these variants has a 69-base-pair insertion in intron 2 in the *AR* gene, whereas the other has a deletion skipping exon 3 [199]. Both of the splice variants are in-frame alterations that only affect the second zinc finger of the AR DBD. The expression of either of these AR-Vs was more common in PCOS women that had severe hyperandrogenism [199]. Notably, these AR-Vs were shown to attenuate AR nuclear translocation in response to androgen and reduce the overall number of DNA sites of AR in ChIP-sequencing analyses [199, 200]. Since these AR-Vs appear to primarily suppress the transcriptional activity of AR, these findings imply that nongenomic AR functions could be involved in hyperandrogenism and PCOS at the ovarian cell level. Furthermore, an analysis of AR phosphorylation from marmoset ovaries by immunostaining showed that phosphorylation of AR can occur at serine resides 81, 309, and 650 in granulosa and theca cells [201]. Phosphorylation of these serine residues was not impacted by hormone manipulation with testosterone or LH-RH antagonist. The biological significance of AR phosphorylation in ovarian cells remains to be fully elucidated, but posttranslational regulation of AR could potentially have a distinct function in ovarian cells.

16.5.3 Targeting AR for the Treatment of PCOS

Currently, there is no cure for PCOS, and the management of PCOS relies primarily on alleviating symptoms to improve quality of life. There are no therapies approved for PCOS specifically, and the majority of treatments for PCOS are used in an off-label fashion. Thus, there is a significant need for continuing research to improve our understanding of the etiology of PCOS and to develop mechanism-specific drugs that are more effective. Therapies targeting the AR signaling axis, including antiandrogens (spironolactone, cyproterone acetate, and flutamide) or the 5α-reductase inhibitor finasteride, have been able to provide some clinical benefit in alleviating PCOS symptoms for women [202–207].

16.6 Breast Cancer

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer mortality among women worldwide [208]. Its incidence is approximately 100 times more common in women than in men [209]. Breast cancer is highly

heterogeneous and may be categorized into four major molecular subtypes based on the expression of ER, PR, and human epidermal growth factor receptor 2 (HER2). The major subtypes include luminal A (ER^+, PR^+, and $HER2^-$), luminal B (ER^+, PR^+, and $HER2^+$), HER2-expressing (ER^- and $HER2^+$), and basal-like, which are mostly triple-negative (ER^-, PR^- and $HER2^-$). Approximately, 75% to 80% of the basal-like subgroup is triple-negative breast cancer (TNBC) [210, 211]. TNBC is an aggressive disease that is usually associated with higher grade, poor prognosis, and an increased rate of mortality [212, 213]. Although TNBC patients respond to chemotherapy, they commonly develop distant recurrence and metastasis [214]. Lack of molecular targets for therapies is a challenge for the treatment of TNBC. In recent years, targeting AR for the treatment of TNBC has been a growing interest in translational research and clinical trials. In addition to TNBC patients, other patients with AR-expressing breast cancers may also benefit from AR-targeted therapies. AR expression is detected in all stages of breast cancer: ductal carcinoma in situ, primary breast cancer, and metastatic disease [215]. From different studies employing various methodologies that vary in their sensitivity of detection and antibody of choice, AR can be detected in 70% to 90% of all breast cancers and 20% to 40% of TNBC patients [213, 216, 217].

16.6.1 AR Roles in Different Types of Breast Cancer

AR signaling plays a role in regulating normal breast development as demonstrated by ARKO mice [218]. Although female ARKO mice appear healthy in general, they display abnormal phenotypes that include decreased ductal branching during prepuberty and decreased lobuloalveolar development with fewer milk-producing alveoli in the mammary gland in adulthood. Most research has been focused on ERα due to its proliferative effects on breast cancer cells; however, AR is more abundantly expressed than ERα and PR in mammary epithelial cells [219]. The main active androgen in females is testosterone, which is produced by the ovaries. In mammary tissues, testosterone and DHT can transactivate the AR, and testosterone can be converted to E2 [213, 220]. The levels of circulating androgens are not consistently correlated with the risk of developing breast cancer [216, 221]. Some studies have shown an increased risk with elevated circulating androgens; however, others have shown that increased levels of circulating estrogens, but not androgens, are linked to the increased risk. Since testosterone can be converted to E2 by aromatase, both androgen and estrogen might be indirectly associated with breast cancer risk [221]. Indeed, both AR and ERα signaling pathways appear involved in the development and progression of breast cancer.

AR is expressed in approximately 75% of ER-positive breast cancers [222]. Depending on the disease stage and ER expression level, AR signaling may have a proliferative or antiproliferative effect, depending on the subtype of the breast cancer cells. In ARKO mice, abnormal development of the mammary gland is associated with impaired ERα and MAPK signaling [218]. There are similarities between

the AR and ERα in regard to their genomic and non-genomic actions. Studies demonstrating cross talk between AR and ERα signaling have been discussed in many reviews [209, 213, 215, 219, 221]. In clinical studies, higher levels of AR are generally associated with improved outcomes and better survival in patients with ERα-positive breast cancer [223, 224]. In these patients, AR behaves as an antiproliferative factor to mitigate estrogen-driven proliferation; therefore, AR expression may have a prognostic value for predicting patient outcomes [213, 215]. In addition to using AR as an independent prognostic biomarker, the ratio of AR to ERα is used as an indicator to predict treatment outcomes, although controversial results have been reported in different studies [225–227]. These discrepancies may be explained by the subtype of breast cancer and the threshold criteria for ratio cutoffs. A more standardized evaluation of these receptors will be required to have a reliable and consistent outcome prediction. The AR has also been reported to support ER signaling in breast cancer growth. D'Amato and colleagues demonstrated that inhibition of AR nuclear translocation with enzalutamide could reduce estrogen-mediated growth driven by ER in breast cancer cell lines and patient-derived xenografts [228]. Furthermore, gene expression analysis of AR-positive circulating tumor cells (CTCs) isolated from patients with metastatic breast cancer identified 18 genes associated with AR. Six of these 18 genes – *XBP1*, *ERBB2*, *CELSR2*, *ESR1*, *TFF1,* and *CA12* – are also regulated by ERα, which further supports the notion that the ERα and AR signaling pathways are connected for certain breast cancers [229]. Interestingly, a correlation between the duration of treatment with aromatase inhibitor and AR expression was determined from CTCs derived from breast cancer patients with bone metastases [230]. These findings support that increased AR expression might enhance tumor cell survival in response to long-term endocrine treatment in some breast cancers.

The AR is expressed in approximately 50% of ERα-negative breast cancers and may replace ERα as an oncogenic driver [215]. Cells that express the AR but not ERα tend to differentiate into apocrine-like cells (molecular apocrine cells). In clinical samples of ERα-negative breast cancer, there is a correlation between AR and HER2 expression [209, 213, 215]. Cross talk between AR and HER2 regulates cell proliferation and apoptosis in molecular apocrine cell lines. Activation of HER2 leads to increased AR binding to target genes (such as *FOXA1*, *XBP1*, *TFF3,* and *KLK3*), and AR reciprocally upregulates the expression of the HER2 gene (*ERBB2*) [231, 232]. Despite cross-regulation between AR and HER2 signaling pathways, the AR in breast cancer with amplified HER2 has no clear association with overall survival [209, 213]. Among ERα-negative breast cancer patients, TNBC patients have the worst prognosis. TNBC can be further stratified into molecular subtypes based on gene expression profiling: basal-like 1, basal-like 2, mesenchymal, and luminal AR (LAR) [233]. Approximately 22% of TNBC is the LAR subtype [234], which is associated with a worse clinical outcome [235]. Clinical LAR tumors can express high levels of AR and coactivators or downstream targets, such as *FKBP5*, *APOD*, *PIP*, *DHCR24*, *ALCAM*, *FASN*, *SPDEF,* and *CLDN8* [214]. The AR has proliferative effects in TNBC based upon studies showing that modulation of AR

can reduce the growth of some subtypes of TNBC. EGFR and PI3K signaling pathways were shown to be involved in AR-mediated proliferation [236–238].

Since AR signaling is involved in breast cancer progression, there has been a considerable effort to investigate alterations in AR structure and function. One such alteration is the length of the polyglutamine tract in the AR-NTD. Some reports reveal no association for patients younger than 40 years of age or in patients that are carriers for BRCA1 and BRCA2 mutations [239, 240]. However, an earlier study reported that a short polyglutamine track with less than 20 CAG repeats may protect against breast cancer [241]. This is contrary to a meta-analysis that revealed a longer polyglutamine track of more than 22 CAG repeats might be protective [242]. Another important AR alteration to consider in breast cancer patients is the expression of AR-Vs. Transcripts and protein of AR-Vs are detectable in breast cancer cell lines as well as in some primary breast cancer specimens from patients without prior antiandrogen treatments [243, 244]. High levels of AR-V7 protein were detected in a subset of ERα-negative/HER2-enriched breast cancer cells, which were likely to be molecular apocrine cells [243]. Moreover, the expression of AR-V7 was detected in CTCs from patients with metastatic breast cancer and was associated with bone metastasis [230] similar to findings from patients with metastatic CRPC [95]. AR-V7 was upregulated in ex vivo primary breast cancer cells treated with enzalutamide [243]. In advanced prostate cancer, AR-V7 upregulates the expression of *UBE2C*, which is involved in cell cycle progression and enhances malignancy [105]. AR-V7-regulated genes in the breast cancer cell line MDA-MB-453 were found to be involved in immune function and cell movement [243]. The exact roles of AR-Vs in breast cancer continues to be an active area of investigation.

16.6.2 Treatments Targeting AR

Historically, androgens were used systemically to treat breast cancer patients and provided tumor regression in 15%–30% of patients. Following the advent of antiestrogen therapies, systemic androgen treatment is no longer used due to the undesirable side effects of virilization and the conversion of testosterone to E2 [209, 215, 220]. With more research on the roles of AR in breast cancer, specific therapeutic strategies for targeting AR are actively being tested and developed. The finding of LAR subtype in TNBC [214] was embraced with numerous preclinical studies and clinical trials to test if existing therapies that target the AR signaling axis by antagonizing the AR LBD or by inhibiting steroidogenesis would be effective. Antiandrogens (bicalutamide and enzalutamide) and ablation of androgen biosynthesis with abiraterone acetate are being evaluated in clinical trials for breast cancer patients. Preclinical studies have shown that bicalutamide could inhibit androgen-induced tumor growth in vivo in mice bearing MDA-MB-453 human breast cancer xenografts [245]. Moreover, bicalutamide has also been shown to inhibit the growth of TNBC xenografts with different subtypes and variable sensitivities [214]. Phase 2 clinical trial was conducted to evaluate the safety and efficacy of enzalutamide in

patients with ≥10% nuclear AR in locally advanced or metastatic TNBC [246]. Patients were dosed daily with 160 mg of enzalutamide until disease progression. Results from the trial indicated that enzalutamide was well-tolerated and led to a median overall survival of 17.6 months for the evaluable subgroup who met the criteria (78 patients) compared to 12.7 months for all 118 enrolled patients [246].

Clinical trials evaluating antiandrogens or an androgen biosynthesis inhibitor have shown promising results for AR-positive TNBC patients especially [246–249], but these therapeutics rely on targeting the AR LBD and would be expected to have limited to no response on the transcriptional activity of truncated AR-Vs. AR-Vs are expressed in breast cancer [243, 244]. Clinical resistance to drugs that only target the fl-AR may also develop from these agents as already seen for prostate cancer. Targeting both fl-AR and AR-Vs by an AR NTD inhibitor, such as ralaniten, may yield therapeutic responses in some breast cancer patients. Next-generation and more potent ralaniten analogs have been developed and undergone preclinical testing for their activity against AR-Vs [61, 250]. A more potent and metabolically stable second-generation ralaniten analog EPI-7386 is in clinical trials for metastatic CRPC patients (NCT04421222).

16.7 AR in Other Diseases

Discoveries from global ARKO murine models and cell-type-specific and tissue-specific ARKO models have vastly expanded our understanding of the pathophysiological roles of AR that were not previously possible by castration and AIS experiments in mice [251]. The unique roles for AR in the function of immune cells, bone mineralization, muscle, brain, liver, wound healing, metabolism, regulating insulin sensitivity, and glucose homeostasis have been described from such murine models [251]. In the following, we highlight some key findings on the role of AR in hypertension and atherosclerosis in humans as well as some other malignancies.

16.7.1 Role of AR in the Progression of Hypertension and Atherosclerosis

The AR is involved in cardiovascular diseases, where its role in hypertension and atherosclerosis is the most established. Men with cardiovascular diseases are observed to have lower levels of serum testosterone [252, 253]. Notably, men with total testosterone levels lower than 241 ng/dL were 40% more likely to die from cardiovascular disease compared to those with higher testosterone levels [254]. Androgen deprivation therapy for prostate cancer patients is also associated with an increased risk for peripheral artery disease [255]. In general, men have a higher blood pressure than women, where the difference is gradually diminished after

women have gone through menopause and men have decreased testosterone levels from the age of 70 years old. Thus, androgen appears to be involved in modulating blood pressure. In preclinical models, castration or flutamide could reduce mean arterial pressure in spontaneously hypertensive male rats to levels that were comparable females [256]. Interestingly, blocking the conversion of testosterone to DHT with a 5α-reductase inhibitor was not able to decrease blood pressure. It is noteworthy to mention that having low endogenous testosterone levels is also associated with higher blood pressure in male populations [257, 258]. Overall, androgens and AR signaling have a role in modulating arterial pressure and may exacerbate the progression of hypertension.

Atherosclerosis is a cardiovascular disease that is associated with the chronic expansion of arterial intima by a gradual accumulation of lipids, cells, and extracellular matrix, which may lead to occlusion and thrombosis, myocardial infarction, sudden cardiac death, or stroke. Males in general have a thicker intima-media during early carotid atherosclerosis relative to females. Total testosterone and SHBG levels are inversely correlated with atherosclerosis [259], where low androgen levels are strongly linked to the production of triglycerides, total cholesterol, and low-density lipoprotein cholesterol [260]. Androgen deprivation therapy for prostate cancer can increase the metabolic burden, which may accelerate the progression of atherosclerosis [261]. In castrated rabbits, DHT supplement was sufficient to inhibit the accumulation of foam cells from oxidized low-density lipoprotein [262]. These findings suggest that physiological levels of testosterone could help to prevent the formation of atherosclerosis.

16.7.2 AR in Other Types of Cancers

AR signaling is also implicated in the development of other cancer types and may be a therapeutic target to influence patient survival such as in salivary duct carcinoma (reviewed in [263]), glioblastomas [8], bladder (reviewed in [264]), kidney [265], endometrial [266], pancreatic [267], and liver cancer [267, 268]. While the bladder is not generally considered to be an androgen-responsive organ, AR expression has been described in the urothelium, submucosa, smooth muscle cells, and neurons of the bladder in primates and humans [264]. Males innately have a three to four times increase in the risk of developing urinary bladder cancer than females, even after accounting for lifestyle and environmental factors that include cigarette smoking and occupational exposure to carcinogens [269]. Notably, the oncogenic action of *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine, a known carcinogen for bladder cancer, was identified to act through AR signaling [270]. Miyamoto et al. identified that the incidence of urothelial carcinoma was much greater in male mice treated with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine compared to female mice (92% vs. 42%, respectively), where tumors did not develop in ARKO mice. In rodents, androgen deficiency induces a decrease in bladder capacity, smooth muscle bladder mass, and autonomic nerve function, whereas testosterone supplementation can reverse

these effects [271, 272]. AR signaling may also promote migration, invasion, and metastasis of bladder cancer cells by interaction with β-catenin/Wnt signaling and its downstream targets c-myc and cyclin D1 [273–275].

In renal cell carcinoma, elevated *AR* expression is generally associated with better patient outcomes [276]. AR expression is negatively correlated with tumor stage, tumor grade, and tumor status [277, 278]. In the case of hepatocellular carcinoma, the AR was reported to be overexpressed in the nuclei of hepatocellular carcinoma cells in approximately one-third of tumors and was associated with advanced disease and poor survival [279]. It was proposed that co-targeting the AR and the mTOR pathway may be a necessary therapeutic approach for hepatocellular carcinoma, since feedback activation of AKT-mTOR from inhibiting the AR could promote AR expression and nuclear localization [279]. Others have also demonstrated that the AR may have a protective role in suppressing hepatocellular carcinoma metastasis, supporting cell adhesion, and increasing tumor cell death by anoikis mechanisms [280]. Thus, the AR may have distinct and opposing roles in hepatocellular carcinoma cells, by promoting tumor initiation and inhibiting metastasis.

16.8 Summary

Some common trends in the AR-associated diseases discussed in this chapter include mutations in AR, polymorphic variants of AR, and the expression of AR-Vs. Although most diseases caused by an imbalance of androgen, deviation from the physiological levels of androgen, or alteration of AR transcriptional activity are clinically manageable, most are not curable and have severe consequences on quality of life and may lead to mortality. These tend to be genetic diseases, and therefore examining the genetic alterations in AR from patients may be beneficial for selecting optimal and effective therapeutic options for personalized medicine. Furthermore, standardizing methodologies to detect and to define AR positivity and status, either at a genetic or protein level, will be required to identify patients who might benefit from AR-targeted therapies. Continued research remains paramount to facilitate drug discovery and to develop more specific, efficacious, and cost-effective therapeutic strategies to target AR and the androgen axis.

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