

# Androgen receptor gene mutation, rearrangement, polymorphism

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**Abstract:** Genetic aberrations of the androgen receptor (AR) caused by mutations, rearrangements, and polymorphisms result in a mutant receptor that has varied functions compared to wild type AR. To date, over 1,000 mutations have been reported in the AR with most of these being associated with androgen insensitivity syndrome (AIS). While mutations of AR associated with prostate cancer occur less often in early stage localized disease, mutations in castration-resistant prostate cancer (CRPC) patients treated with anti-androgens occur more frequently with 10-30% of these patients having some form of mutation in the AR. Resistance to anti-androgen therapy usually results from gain-of-function mutations in the LBD such as is seen with bicalutamide and more recently with enzalutamide (MDV3100). Thus, it is crucial to investigate these new AR mutations arising from drug resistance to anti-androgens and other small molecule pharmacological agents.

**Keywords:** Androgen receptor (AR); mutations; rearrangements; polymorphisms; androgen insensitivity syndrome (AIS); castration-resistant prostate cancer (CRPC)



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## Androgen receptor (AR)

The human AR is an important steroid hormone receptor that plays a critical role in male sexual differentiation, development and maintenance of secondary male characteristics and the ignition and maintenance of spermatogenesis (1). AR, as a ligand-activated transcription factor, is activated by binding either of the androgenic hormones testosterone or dihydrotestosterone (2). In humans, AR is a 110 kD protein composed of 919 amino acids that is encoded by the AR gene located on the X chromosome at Xq11-12 (3). The AR contains four domains: (I) the amino terminal activation domain (NTD); (II) the DNA-binding domain (DBD); (III) the hinge region (HR) and (IV), the carboxyl ligand-binding domain (LBD) (4). AR exon 1 encodes the entire N-terminal domain (NTD) (a.a. 1-556) which comprises the bulk of the AR and is the least conserved of the four domains. This variability allows AR to differentially recruit co-regulators conferring androgen specific transactivation. The first 30 amino acids

of the NTD are essential for the amino-carboxyl terminal (N/C) interaction that is required for the appropriate activation of AR (5-10). The NTD region contains the activation function 1 (AF1) element through which AR transactivational activity is predominantly mediated. This distinguishes AR from the other steroid receptors that primarily utilize the AF2 region in the LBD (11). The NTD also contains polyglutamine and polyglycine repeats which are polymorphic. Polyglutamine repeat length has been correlated with PCa risk (11,12) and mutations in polyglutamine repeats have been shown to affect N/C interaction (13).

Exons 2 and 3 encode the two zinc fingers in the AR DNA-binding domain (DBD) which is responsive for binding to the androgen responsive element (ARE) sites (14). The DBD (a.a. 556-624) contains two zinc finger motifs and forms part of the hinge region (15). In addition to mediating binding to AREs, the second zinc finger stabilizes DNA bound AR and facilitates AR dimerization. Adjacent to the DBD is the hinge region (a.a. 625-668). A bipartite

nuclear localization signal (NL1) is present in the DBD and HR (16,17). In addition, the HR contains sites that are important in the phosphorylation, acetylation and degradation of AR. Exons 4-8 encode the short flexible hinge region and LBD which is capable of binding to the ligands and contains activation domain 2 (AF-2) that mediates N/C interaction and has also been shown to interact with a number of co-regulators (18). The LBD (a.a. 669-919) binds to ligands via its ligand binding pocket that is comprised of 12 alpha helices (19,20). In addition, the LBD contains a second nuclear localization signal (NL2) upon androgen binding and a nuclear export signal (NES), in the absence of androgens (17,21).

### Role of AR in prostate cancer

Both normal prostate and prostate cancer (PCa) depend on the presence of androgens for growth, and prostate development is dependent on a functional AR. Testosterone, the primary circulating androgen in men is mainly produced by the Leydig cells of the testis (22). In the prostate, testosterone is converted into 5 $\alpha$ -dihydrotestosterone (DHT) by the enzyme 5 $\alpha$ -reductase so it can bind to the AR and induce growth of the male urogenital structures. AR is the principle mediator of androgen action in the prostate. AR activation by DHT is critical for complete prostate development as men lacking a functional 5 $\alpha$ -reductase gene have only a small partial prostate or the prostate is completely undetectable (23).

PCa is also initially dependent on the actions of androgens and functional AR expression and tumors will regress temporarily with castration. AR is expressed in both androgen-dependent (AD) and -independent (AI) PCa and is sustained throughout progression of the disease to hormone refractory PCa (24,25). PCa therapy is focused upon blocking androgen activity and androgen ablation therapy causes atrophy of the prostate epithelium. Treatment of PCa first involves androgen deprivation therapy (ADT) through blocking production of androgens by castration and/or by using anti-androgens such as bicalutamide or enzalutamide (MDV3100). When androgen ablation therapies fail, advanced PCa ultimately progresses to an AI late stage that is refractory to current therapies, also known as castrate resistant prostate cancer (CRPC), and this recurrence results from a reactivation of AR activity.

Decreasing levels of AR protein expression reduces both primary localized PCa and CRPC growth. While

ADT is initially successful in most patients (~80%) resulting in tumor regression and AR suppression, these therapies eventually fail and the cancer progresses to a stage where it is unresponsive to blockage of androgens and growth becomes androgen independent. Hormone suppression appears to induce an eventual overexpression or amplification of the AR in CRPC (26). Several mechanisms have been proposed to play a role in this reactivation of AR following ADT including: deregulation (causing overexpression of AR), mutation of AR (gain of function), alternative splicing (causing AR to be constitutively active), co-activator gain of function or loss of co-repressor function, and intracrine androgen synthesis [reviewed in (24)]. Restored AR activity in turn induces an increase in prostate specific antigen (PSA) levels signifying the development of CRPC. Therefore, AR signaling pathways must play critical roles in both AD and CRPC. Unlike AD signaling that depends on actions of androgens to bind AR and activate it, androgen independent pathways do not require androgens, but can be activated by growth factors acting through kinase pathways such as the mitogen-activated protein kinase (MAPK) pathway or the phosphatidylinositol 3-kinase (PI3K) pathway, which phosphorylate and activate AR in the absence of androgens (27). Thus determining how AR drives prostate tumor growth in the absence of androgens is critical for the development of effective therapy for CRPC.

### Overview of mutations of the AR

Mutations in the AR can result in defective AR function. Defective AR, including loss-of-function AR alterations and gain-of-function AR alterations, are associated with androgen insensitivity syndrome (AIS), spinal and bulbar muscular atrophy and PCa (28). In the AR gene, four different types of mutations have been detected to generate defective AR: (I) single point mutations resulting in amino acid substitutions or premature stop codons; (II) nucleotide insertions or deletions most often leading to a frame shift and premature termination; (III) complete or partial gene deletions; and (IV) intronic mutations causing alternative splicing (28,29).

Over 800 different AR mutations have been identified in patients with AIS according to the report from the Androgen Receptor Gene Mutations Database (29). AIS is a recessive genetic disorder of sex development characterized by androgen unresponsiveness which can impair the masculinization of male genitalia in the developing fetus (30,31). AIS consists of three classes based on phenotype:

complete androgen insensitivity syndrome (CAIS), partial androgen insensitivity syndrome (PAIS), and mild androgen insensitivity syndrome (MAIS) (31). The most common mutations of the AR gene in AIS are single point mutations that result in an amino acid substitution. However, insertions/deletions resulting in a reading frameshift, a complete/partial gene deletion, and mRNA alternative splicing have also been identified in patients with AIS syndrome (29,32). Loss-of-function mutations distribute unequally along the length of the exonic regions of the AR. Although exon 1 encodes more than half of the AR protein, the total of exon 1 mutations only represents 25% of all of the mutations in AIS patients (29). More than 70% (89 out of 124) of AIS mutations in exon 1 appear to cause CAIS, and about 18% (22 out of 124) of exon 1 mutations are related with MAIS which is due to single-base substitution (29). In the DBD region, 74 different mutations have been published of which most are single-base substitutions (29). The most common molecular defects of the AR gene associated with AIS are clustered primarily in the LBD region, and the single-base substitution mutation is predominant (29). Interestingly, in 33.3% (25 out of 75) patients with CAIS and 58.7% (37 out of 63) patients with PAIS, no AR mutations have been identified, which challenges the classical assumptions that the AIS phenotype is directly the result of a mutation in the AR (29).

Although AR mutations occur very rarely in the early stages of PCa, approximately 10-30% of patients with CRPC carry AR mutations, especially in PCa patients treated with ADT (33,34). To date, over 150 AR mutations have been identified in PCa tissue, and most of them consist of single-base substitutions due to somatic rather than germline mutations (29). About 45% of the mutations identified in PCa patients occur in the LBD, while 30% occur in exon 1 (29). These gain-of-function mutations allow prostatic epithelial cells to grow in an androgen-refractory manner, suggesting that these mutations in the AR may allow it to bind and be activated by ligands that are normally present in the body (e.g., adrenal androgens) but that do not normally cause substantial activation of the AR (35,36). The presence of multiple mutations within tissues from single PCa patients has been found, which leads to the hypothesis that cancers are based on tissues accumulating mutations in a number of genes in advanced PCa. Interestingly, AR mutations have been identified in PCa patients that are associated with both a loss-of-function and a gain-of-function (i.e., p.R753Q) (29), which further complicates the relationship between genotype and phenotype and suggests the important role

of post-translational events in protein generation and carcinogenesis. New mutations in the AR can also lead to resistance to current forms of treatment. For example, mutation in the LBD of AR can lead to resistance of MDV3100 causing AR to become active even in the presence of anti-androgens (37). Thus, it is crucial to investigate these new AR mutations arising from drug resistance to anti-androgens and other small molecule pharmacological agents.

### Mutations in the NTD of the AR

The NTD is located in the first exon of the AR and spans amino acids 1-558. It is known to contain the transcriptional regulatory regions or transcription activation units, the ligand-dependent TAU-1 (amino acids 101-370) and the ligand-independent constitutively active TAU-5 (amino acids 360-528) which together are also referred to as AF-1 (8). Mutations in the NTD of the AR account for nearly a third of all mutations in the receptor. In the AR database the NTD is listed as having 42 single-base substitutions in PCa (29). Most of these mutations are thought to occur following androgen ablation therapy (38,39).

Two mutations at amino acids 142 and 221 of the AR which are located in the TAU-1 region of the NTD were discovered from patients who had CRPC (40). The 142 mutation was characterized as a substitution of glycine to valine (G142V), while the 221 mutation was characterized as a substitution of aspartic acid to histidine (D221H). Both of these mutations had an increased response to DHT. In addition, these two mutations had a shorter than normal CAG repeat length than the median of this polymorphic variation in the Chinese population (40,41). Since a reduction of the CAG repeat is associated with increased risk of PCa, it is possible that in addition to the mutations in these two patients, the shortening of the CAG repeat might have also contributed to tumor development and PCa progression.

Another mutation discovered in a primary PCa biopsy is known to affect the N/C interaction of the AR. The L179R somatic substitution mutation results in a receptor that is highly activated and more potent than wild type AR (42,43). Callewaert *et al.* (43) also discovered that the L179R mutation disrupts the N/C interaction and indirectly alters NTD interaction with the coactivator SRC-1. Further characterization of this mutation revealed that this residue is important for TAU-1 function. The 179 lysine is part of the LKDIL motif that is involved in hydrophobicity and the

helical structure of TAU-1. Two alpha helices that surround the lysine at position 179 make up the core of TAU-1, which can act independently of p160 coactivators as an autonomous activation function. These two alpha helices are necessary for full activity of wild type AR and thus the L179R mutation may increase this activity acting as a gain of function mutation (43).

The genetically engineered TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse model of prostate cancer (44) has revealed that hormone ablation selects for AR mutations and these mutations can cause the development of aggressive and metastatic disease (38,45). Most of the mutations (seven out of nine, 78%) identified in this study were localized to the NTD, while the other two were found in the LBD. Two mutations were identified in the most highly conserved region of the NTD that is critical for its structure and recruitment of transcription factors. A229T and E231G exhibit increased basal activity independent of ligand and E231G has a higher responsiveness to coactivators ARA160 and ARA70. Additionally, these two mutations significantly reduced the interaction of AR with the Hsp70-interacting protein (CHIP) which functions as a negative regulator of AR transcriptional activity (38,45). These findings suggest that this evolutionarily conserved AR NTD signature motif plays a role in modulating AR action and support a direct causal relationship between AR-E231G expression and malignant prostate cancer.

More recently, Steinkamp *et al.* (39) found 26 recurring missense mutations, (mostly in the NTD) that occurred in multiple PCa tumors that had been treated with anti-androgens. Fourteen of the 19 mutations in the NTD localized to four regions: the polyQ tract, the COOH-terminus of Hsp70 interacting Protein (CHIP) interaction domain, the WxxLF motif, and the end of AF5 which is involved in interactions with coactivators (39). Two novel mutations, E255K and W435L were characterized further. E255K (which was found to be mutated in a domain of the AR that interacts with E3 ubiquitin ligase) increased protein stability and nuclear localization in the absence of ligands, while W435L (located within the WxxLF motif) enhanced N-C interaction 50% greater than wild-type AR indicating once again that treatment with anti-androgens selects for gain-of-function AR mutations (39).

### Mutations in the DBD and hinge region of the AR

The DBD and hinge region (H) of the AR (located between

the NTD and LBD from amino acids 559-670) contain fewer reported mutations associated with PCa than the NTD and LBD regions. As of 2012, there were 10 reported single-base substitutions in the DBDH (7 in the DBD and 3 in the hinge region) (29).

One double mutation in the DBD and LBD of AR has been thoroughly studied for functional relevance (46). Substitutions of two threonines for two alanines (T->A) were found on the same AR transcripts of a mutant AR isolated from CRPC samples. The 575 threonine in the first zinc finger of the DBD of AR and the 877 threonine in the LBD of AR were both replaced by alanine. The authors describe this double mutant as being both “promiscuous” and “unfaithful” as the T877A mutation allows activation by abnormal ligands and the T575A mutation modifies AR transcriptional activity by strengthening AR binding to AR nonspecific promoters (46). Compared with wild-type AR, T575A AR binds preferentially to nonspecific palindromic androgen response elements (AREs) suggesting that the T575A mutation could enrich the binding of AR to non-canonical AR binding elements.

Another mutation in the DBD of AR is a rare inactivating mutation reported in the receptor. C619Y is a somatic substitution (tyrosine for a cysteine at amino acid 619) mutation causing loss of transcriptional activity of AR that was identified in a Caucasian man with stage D1 metastatic PCa (47). This mutation was shown to be transcriptionally inactive and unable to bind DNA in androgen-responsive reporter assays. Treatment of ligand causes C619Y AR to localize abnormally in nuclear aggregates located in both the nucleus and cytoplasm. Additionally, C619Y colocalizes with the coactivator SRC-1 in these aggregates thus demonstrating that interactions between steroid receptors and coactivators may occur in the absence of DNA binding and transcriptional activity (47). While the significance of all known mutations in the DBD has not been fully elucidated, mutations in this region of the AR likely interfere with DNA binding, nuclear export, and coregulator recruitment which would affect AR transactivation (48,49).

Interestingly, a study by Hu *et al.* (50) identified a novel germline mutation in the DBD of the AR in African American males with familial PCa. This study used genomic DNA from 30 high-risk African American and Caucasian families and identified a germline AR (T559S) substitution in the DBD in three members of an African American family who had a history of early-onset familial PCa (50). This mutation may contribute to disease progression by altering AR-DNA binding affinity and affect AR signaling

in response to androgens or anti-androgen therapy. Since African American men have a higher incidence (70% higher than Caucasian men) and mortality rate (double that of Caucasian men) of PCa than Caucasians and other ethnic groups (51,52), further investigation into the relevance of such mutations in the AR may provide useful information needed to treat these high risk individuals in a personalized manner.

### Mutations in the LBD of the AR

To date, of the more than 150 mutations identified in PCa tissue, most occur in the LBD, and a substantial minority occurs in exon 1 (29). The majority of AR mutations identified in the LBD in clinical PCa cluster to four discrete regions: (I) amino acids 670-678, which is located at the boundary of the HR and the LBD; (II) amino acids 701-730, a region which covers the helix 3 which contributes to form the ligand-binding pocket surface, and also the “signature sequence”, the highly conserved loop between helices 3 and 4 of nuclear receptors, directly involved in coactivator recruitment; (III) amino acids 741-763, a part of the ligand-binding pocket; (IV) amino acids 872-910, a region which spans both helix 11 and the core domain of AF-2 (53).

Somatic missense mutations in the LBD usually result in decreased specificity of AR to other hormones such as progesterone, estrogens and adrenal steroids, and affect both ligand affinity and coregulator recruitment (54). Importantly, many mutated AR can be activated by anti-androgens, which may be partly responsible for progression to CRPC. Several independent studies revealed that mutations in the LBD affect the ligand pocket and modify the conformational structure of the receptor, which in turn reduces ligand discrimination but does not affect the agonist-induced coactivator recruitment (55-58). Due to the different location and the nature of the substitution, the mutations in the LBD will alter the ligand-induced conformational change of AR, which results in altered ligand binding affinity, N/C-terminal interactions, as well as interactions with coactivators and chaperones (53). However, not all mutations in the LBD reduce ligand specificity by altering the dimensions of the pocket. For example, the H874Y mutant AR is also activated by hydroxyflutamide, oestradiol, and progesterone besides androgens, but the side chain of the residue points away from the pocket and is buried in a cavity between helices 11 and 12, which is formed by ligand induced activation (57).

Different agonists activating the LBD mutant AR may

result in different coactivators binding and regulating different subsets of genes (59). Brooke *et al.* (60) studied the preference of several of the most commonly identified LBD mutants (H874Y, T877A and T877S) for the motifs of LxxLL and phenylalanine-rich motifs like FxxLY and found striking differences in motif utilization dependent upon which ligand was activating the receptor. In the presence of cyproterone acetate, LBD mutants interact with the LxxLL motif while in the presence of hydroxyflutamide the receptors interact with the FxxLY motif. Moreover, the authors demonstrated the mutant AR induced different “patterns” of regulation of a subset of androgen-regulated genes. In the LNCaP cell line which endogenously expresses the well-known T877A mutant AR, the expression of the prostate differentiation factor *KLK2* and cell cycle regulator *CDK2* were induced most strongly by androgen, then hydroxyflutamide and then cyproterone acetate, while another cell cycle progression associated gene *CDK4* was not regulated by androgen, but by the two anti-androgens. As for the differentiation associated gene *DRG-1*, its expression was highly up-regulated by androgen, and only weakly by hydroxyflutamide (60).

The T877A mutation was the first identified AR mutation in prostate cancer and was initially described in the LNCaP human PCa cell line early in 1990 and was frequently found in flutamide-treated PCa patients (~31%) (61,62). This mutant not only responds to androgens but also to oestrogens, progestins and even the anti-androgens cyproterone acetate and hydroxyflutamide (63). The threonine 877 residue in AR LBD comprises a large portion of the ligand-binding pocket surface, and forms hydrogen bonds with 17 $\beta$ -hydroxyl group of androgen, and the substitutions to the smaller alanine affects the size and conformation of the receptor such that the other ligands can fit into the pocket and activate the receptor (21,38). The AR T877A may drive tumor growth through aberrant activation by the anti-androgen used for treatment.

The L701H mutation, a second AR mutational hot spot, was first identified in a hormone-refractory PCa patient (64). This mutation, in combination with T877A, is also identified in the PCa cell line MDA-PCa 2a, which was established from a bone metastasis of a castrated PCa patient (65,66). Both Leu701 and Thr877 residues are part of the ligand-binding pocket and interact with bound ligand. L701H and the double mutant L701H/T877A are highly responsive to circulating steroids such as glucocorticoids, cortisol and cortisone (67,68). However, the L701H mutation was not well characterized like the T877A until

most recently. Van de Wijngaert *et al.* (69) found that the presence of a hydroxyl group at position 17 $\alpha$  is critical for activation of AR L701H. Modeling of the various mutations in the AR LBD structure (such as L701H, L701M, and L701Q) revealed that a unique H-bonding network involving His701 or Gln701, the steroidal 17 $\alpha$ -OH group, and the backbone oxygen of Ser778 plays an important role in the cortisol response. Interestingly, the L701H mutants hardly respond to anti-androgens (69). These findings suggest that the L701H mutation does not drive prostate tumor growth upon binding of an anti-androgen used for treatment, which indicates in these cases, tumor growth is dependent on endogenously circulating ligands such as cortisol, a different mechanism of tumor growth observed in T877A mutants.

MDV3100 (enzalutamide) is a novel anti-androgen that was recently approved by the FDA for the treatment of CRPC (70-72). Although MDV3100 has shown significant efficacy in clinical trials, many patients who initially responded favorably develop resistance to this drug, however, the mechanisms driving resistance remain largely unknown. A most recent study by Korpál *et al.* (37) demonstrated that a mutation in the AR LBD, F876L, spontaneously emerges in the majority of MDV3100 resistant clones of LNCaP cell line, which strongly suggests that the emergence of AR F876L mutants may represent a dominant tumor-autonomous mechanism of resistance to MDV3100. Additional studies revealed that the benzamide motif of MDV3100 can extend into the access channel created by the smaller leucine residue, which could potentially prevent the compound from clashing with helix-12 of the AR LBD in the agonistic mode (37). Thus, the F876L mutation may abolish the antagonistic activity of MDV3100 and could potentially allow agonist activity. However, since the clinical relevance has not been identified, further research needs to be done to determine the effect(s) of this mutation. Additionally, since this study was performed in LNCaP cells, it remains to be elucidated whether or not this type of AR mutation is sufficient to convey drug resistance in patients.

### AR rearrangements

The recent identification of constitutively active forms of AR, known as AR variants (ARVs), has revealed another important mechanism underlying persistent AR signaling in CRPC (73,74). More than a dozen ARVs containing variable structures have been isolated, but each lacks all or a portion

of the ligand binding domain (LBD) (32). The lacking of LBD allows ARVs to be constitutively active in driving AR regulated gene expression and promoting tumor progression even without the presence of androgens (75,76), and leads to their resistance to the current LBD-targeting AR antagonists or other agents that repress androgen biosynthesis. Expression of ARVs is increased in CRPC compared to hormone-naïve metastases and associated with PCa progression and resistance to AR-targeted therapy (75-77).

Recently it was discovered that 22Rv1 cells as well as prostate tissue of some patients with CRPC contained rearrangements of the AR (78). 22Rv1 cells were shown to have increased copy number of AR exons 2b, 3, and CE3 compared with the androgen dependent CWR22Pc cell line which suggested rearrangement of this genomic segment. Further characterization of 22Rv1 cells determined that they contain truncated AR isoforms associated with an intragenic rearrangement of a 35-kb AR genomic segment which contains a cluster of alternative AR exons. Analysis of Genome-Wide Human SNP Array 6.0 (SNP6.0) data from primary PCa patients and metastatic CRPC patients (79,80) revealed that only patients with CRPC had high incidences of rearrangements associated with AR amplification. Additionally, increases in focal copy number between AR exons 2/3 and 3/4 were also observed in patients with CRPC but not in patients with primary androgen-dependent PCa (6/14 in CRPC patients *vs.* 0/44 primary PCa patients). Although the generation of ARVs is due to the aberrant AR splicing or gene rearrangements of the AR gene, it still remains unknown how such aberrant splicing is regulated.

### AR polymorphisms

The human AR gene contains two polymorphic (CAG)<sub>n</sub> (polyGln/polyQ) and (GGC)<sub>n</sub> (polyGly/polyG) repeat sequences with different number in exon 1. The number of polyQ and polyG repeats is 21.6 $\pm$ 3.3 (range, 9-31) and 17.4 $\pm$ 1.4 (range, 8-21) respectively, in normal men (81). Abnormal length of the polyQ tract has been found to be associated with the pathology in Kennedy's disease (Spinal and bulbar muscular atrophy, SBMA) where the polyQ tract is expanded and varies between 38 and 75 repeat units (82,83). SBMA, as one of the classic trinucleotide repeat expansion diseases, results from a combination of a gain-of-function mechanism in motor neurons and a loss-of-function mechanism in androgen target cells, causing partial loss of AR function in androgen target tissues (28).

Abnormal polyQ lengths have also been associated with race. The average polyQ repeat number differs significantly among African-American (mean: 20.1), Caucasian (22.0), and Asian-American (22.4) populations in the USA (84).

PolyQ tract length has been reported to affect AR activity. Shorter polyQ tract can enhance the critical intramolecular N-C terminal interaction of AR, allowing response to lower androgen concentrations associated with higher levels of specific p160 coactivators (13,85). The relationships between AR polyQ tract and risk of getting certain diseases (including cancers, male infertility, bone and mineral density, Alzheimer's disease, hypertension, muscle and adipose tissue change and personality traits) have been investigated by several different groups (86-90). However the association between polyQ tract length with PCa remains controversial. AR with shorter polyQ tract is associated with increased PCa risk and has been found in the high-risk African-American population (91) whose average polyQ numbers are less than Caucasian's and Asian-American (84). Furthermore, somatic mosaicism of the AR polyQ tract has been found in PCa tumors, which may subsequently lead to the development of PCa (92,93). However, controversial results have been reported by recent publications with larger sample sizes (94,95). In those studies, no association of AR polyQ tract length with PCa risk was found, and the knowledge of AR polyQ tract length provides no clinically useful information to predict PCa risk. Despite failure as a predictor of PCa risk, polyQ tract has been reported to affect progression or treatment response of PCa. Both estradiol and testosterone levels were significantly elevated in men with greater polyQ tract length (94,96).

In order to use experimental tools to test the role of AR polyQ tract length on PCa, Dr. Robins' group developed knock-in mouse strains with human AR alleles containing 12, 21 or 48 CAG repeats (referred to as AR12Q, AR21Q, and AR48Q) (97). All three mouse lines were grossly normal in growth, behavior, fertility, and reproductive tract morphology, with no neurological problems evident in AR48Q, although transactivational differences due to polyQ tract length were detected in expression of AR downstream targets (97). Also, the hAR Q-tract polymorphism mediates *in vivo* tissue androgen sensitivity by impacting negative hypothalamic feedback and trophic androgen effects on target organs (98).

To further investigate the effect of polyQ tract length in oncogenesis, the three mouse strains (AR12Q, AR21Q, and AR48Q) were crossed with a transgenic model of prostate adenocarcinoma (TRAMP) (97). Striking

genotype-dependent differences in PCa initiation and progression were revealed due to the different length of polyQ. Although cancer in the mice with an average human polyQ tract length AR progressed similarly as in wild-type mice, the short polyQ tract AR resulted in significantly earlier tumor development, whereas the long polyQ tract appeared to be protective (97,99). Taken together, those mouse models demonstrate that a functional difference in AR activity within the normal range of polymorphic variation could affect PCa biology. The association between PCa and AR polymorphisms remains unclear, and further investigations with suitable models will provide us more information in the future.

### Summary/future perspectives

Currently there are over 1,000 known mutations in the AR and 159 have been reported in PCa tissues (29). While the number of mutations reported continues to rise, the relevance of these mutations in CRPC remains unclear. Mutations in patients with CRPC treated with anti-androgens, such as MDV3100 may provide vital information as to why therapies targeting the LBD of AR eventually relapse or fail. Further investigation is needed to determine the function of mutations derived from prolonged treatment with anti-androgens.

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### Footnote

*Conflicts of Interest:* D.W. is an AUA Research Scholar. L.E.P. is a Tippins Scholar. The other authors have no conflicts of interest to declare.

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