**CURRENT DEVELOPMENT**

DOI: 10.34763/devperiodmed.20172101.0712

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# RECENT HIGHLIGHTS OF RESEARCH ON ANDROGEN RECEPTORS IN WOMEN

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**Abstract**

*In this brief review we present an outline of the current state of research on examples of hyperandrogenism that can be strongly associated with diverse modifications in the androgen signaling pathway. We discuss the most prominent clinical features of androgen excess and correlate them with studies on androgen receptor (AR) alterations. For the first time we summarize the confirmed localizations of all known AR receptors in women. The knowledge of ARs may be the basis for AR-targeted therapies of androgenic disorders in women, including malignancy, as it has recently been demonstrated for triple-negative breast cancer. Moreover, we summarize the structure and characterization of key AR splice variants, which could be involved in the androgenization in women.*

**Key word**s: androgen receptors; androgens; hyperandrogenism; protein splice variants; women

DEV PERIOD MED. 2017;XXI,1:7-12

## INTRODUCTION

Hyperandrogenism in women is a highly important clinical and social problem affecting 5-10% of the population of reproductive age [1, 2]. Often described as androgen excess, this disorder encompasses a wide array of manifestations such as: polycystic ovary syndrome (PCOS), idiopathic hirsutism, hirsutism and hyperandrogenemia, non-classical congenital adrenal hyperplasia, hyperandrogenism, insulin resistance, acanthosis nigricans (HAIR-AN), ovarian or adrenal androgen-secreting neoplasms, androgenic drug intake, Cushing's syndrome, and hyperprolactinemia [3]. Importantly, the above list may not be complete. Moreover, it includes disorders with normal and modified androgen production and metabolism, as well as those with altered androgen receptor (AR) or its defective signaling pathways. Overall, hyperandrogenism includes both clinical and biochemical androgen excess. In this paper we focus on the crucial role of ARs in the pathophysiological and clinical manifestations of hyperandrogenism in women.

In recent years the pace of research on ARs has been gaining a particular momentum since more and more scientific evidence supports their importance for tissue homeostasis in women. For example, roles of ARs have been studied in such complex processes as reproductive failure (on ovarian, oocytic, and uterine levels), endometrial hyperplasia, and cancer [4-7]. However, few studies analyzed specific truncated forms of the AR, called splice variants.

## FULL-LENGTH ANDROGEN RECEPTOR VERSUS ITS SPLICE VARIANTS

The expression of full-length AR (AR-FL) has been proven across most of the female reproductive tissues, which emphasizes its apparent functional importance, especially within the endometrium [8]. Tuckerman et al. established that androgens exert a direct effect on endometrial function by demonstrating the presence of ARs in cultured endometrial cells, and showing that this effect is nullified after AR inhibitor treatment [9]. The human AR is a transcription regulator belonging to the nuclear receptor family. AR gene structure, mature spliced mRNA, and protein domains bear a resemblance to other family members: estrogen receptors and progesterone receptors. *AR* includes 8 different exons forming the AR protein consisting of 4 domains, each of specialized function: 1) the N-terminal domain (NTD); 2) the DNA binding domain (DBD); 3) a hinge region (HR); and 4) the C-terminal ligand binding domain (LBD) [10]. When AR

is present in the cytosol, it remains in its transcriptionally inactive form stabilized by molecular chaperone heat shock protein 90 (HSP90). Once a ligand (such as testosterone or dihydrotestosterone) attaches to LBD, AR dissociates from HSP90. Subsequently, it dimerizes and translocates into the nucleus, where it binds to the androgen response element in the regulatory region of its target genes, thus modulating their expression [11].

Figure 1 presents the current understanding of the *AR* structure and the most prevalent AR transcripts compositions [12].

It should be noted that AR-FL coexists in tissues with its truncated isoforms lacking LBD that are referred to as AR splice variants (AR-Vs), and with variant 2 of the AR named AR45. The truncated variants are products of alternative splicing of the human *AR* gene [13, 14]. As of now, 17 different AR splice variants have been characterized and labeled [15]. Overall, AR-Vs fall into two categories depending on their structure: the truncated variants, and the exon skipping variants [16]. It has been established that AR-Vs differ significantly in function both from AR-FL and among themselves depending on alterations in their structure. First, the most frequent alteration is the loss of LBD, which results in ligand-independent constitutive actions [17]. Secondly, the actions of AR-Vs can be mediated through heterodimerization with the wild-type receptor, as well as homodimerization, therefore a potential interplay between the two types has been discussed in depth [18, 19]. Finally, AR-V7 (also known as AR3) and $AR^{V567es}$ (also known as AR-V12) have been commonly identified in cancer tissue and ascribed an important role in cancer pathogenesis [15]. For example, strong correlations between AR-V7 and prostate cancer staging and prognosis have been established [20, 21]. Moreover, in prostate cancer cells, AR-V7-targeted specific protein immunoreactivity has been found to be greater than that of AR-FL. This observation was somewhat unexpected, because the expression of *AR-V7* gene in these cells is relatively less pronounced than that of the *AR-FL* gene. However, such a high yield protein transcription can be explained by excessive constitutive activity of AR-Vs, especially in the absence of any ligand, compared to AR-FL ligand-activated transcription [17, 22]. Both AR-V7 and AR-FL share impact on expression of at least 71 genes [23]. Nonetheless, recent research in

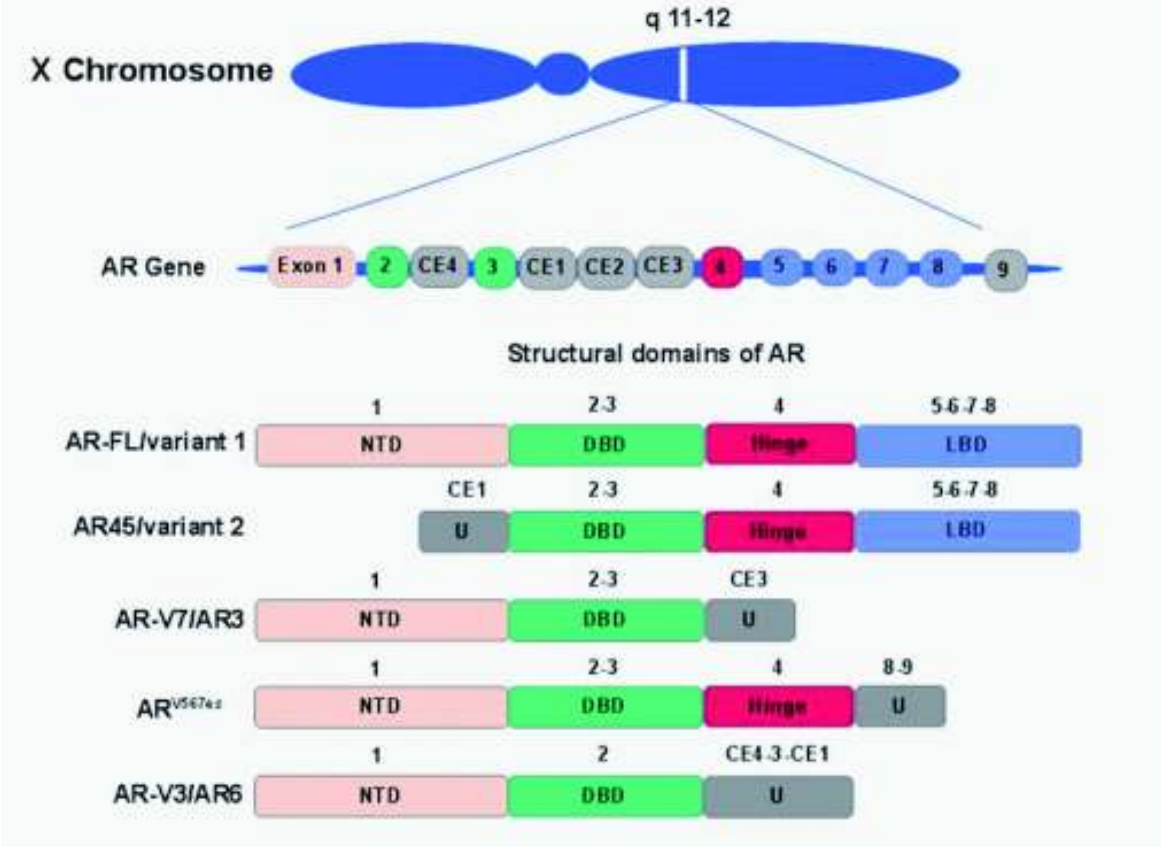


Fig. 1. The *AR* gene and its key protein transcripts. The *AR* is located on the X chromosome (*locus*: Xq11-Xq12) and includes 8 exons encoding 4 distinct domains: N-terminal domain (NTD), DNA-binding domain (DBD), hinge region, and ligand-binding domain (LBD). The above simplified scheme distinguishes: exons 1 to 8 of full-length AR (AR-FL), cryptic exons (i.e., CE1-4) of AR splice variants (AR-Vs) and AR45, and exon 9 of $Ar^{V567es}$. U - unique sequences not found in AR-FL. Adapted from [12].

Table I. The localization of ARs found in various tissues in women, and characterization of known AR-Vs [15 ,24-27]. NTD – N-terminal domain; DBD – DNA-binding domain; HR – hinge region; LBD – ligand-binding domain; CE – cryptic exon; ZF – zinc finger; U – unique N-terminal extension.

| Name | Aliases | Splice junction in AR-Vs and other structural changes in AR45 | Domains excluded | Confirmed tissue localization in women | Activity |
|---|---|---|---|---|---|
| AR-FL | AR wild-type | Reference structure | None | Ovary, uterus, endometrium, breast, cervix, placenta, vagina, vulva, skin | Ligand-stimulated |
| AR45 | AR transcript variant 2 | NTD replaced by U which is linked to the DBD, HR, and LBD of the AR | NTD | ovary, cervix, breast, placenta | Conditional |
| AR-V1 | AR4 | 3/CE | HR to LBD | ovary, cervix, breast, placenta | Conditional |
| AR-V2 | AR1/2/2B | 3/3/CE1 | HR to LBD | | Constitutive |
| AR-V3 | - | 2/CE4 | ZF2 to LBD | ovary, cervix, breast, placenta | Constitutive |
| AR-V4 | AR5, AR1/2/3/2B | 3/CE4 | HR to LBD | | Constitutive |
| AR-V5 | - | 3/CE2 | HR to LBD | | Constitutive |
| AR-V6 | - | 3/CE2 | HR to LBD | | Constitutive |
| AR-V7 | AR3 | 3/CE3 | HR to LBD | ovary, cervix, breast, placenta | Constitutive |
| AR-V8 | - | 3/intron 3 | HR to LBD | | Constitutive |
| AR-V9 | - | 3/CE5 | HR to LBD | ovary, cervix, breast, placenta | Conditional |
| AR-V10 | - | 3/intron 3 | HR to LBD | | Constitutive |
| AR-V11 | - | 3/intron 3 | HR to LBD | | Unknown |
| AR-V12 | $AR^{V567es}$ | 4/8/9 | LBD (disrupted) | | Constitutive |
| AR-V13 | - | 6/9 | LBD (disrupted) | | Inactive |
| AR-V14 | - | 7/9 | | | Unknown |
| AR-V15 | - | 6/9 | LBD (disrupted) | | Unknown |
| AR-V16 | - | 8/9 | | | Unknown |
| AR-V17 | - | 8/9 | | | Unknown |
| AR-V18 | - | 6/9 | | | Unknown |

mice highlighted a paramount functional difference: AR-V7 regulates its own unique set of genes that boost cell proliferation and division, whereas unique genes regulated by AR-FL are responsible mainly for cell differentiation and maturation [23].

Table I presents the known localization and characterization of ARs, including AR-Vs, in tissues of women [15, 24-27].

## ANDROGEN RECEPTORS AND CLINICAL FEATURES OF HYPERANDROGENISM

In the light of ARs abundance in women (Tab. I), their pivotal role in the pathogenesis of hyperandrogenism should be thoroughly analyzed. Hence, it is important to distinguish the most common clinical manifestations facilitating the diagnosis of this disorder and select appropriate patients for further in-depth hormonal and receptor validation. One of such manifestations is hirsutism which affects up to 15% of all women and 70-80% of women with hyperandrogenism [1, 2]. Visual assessment methods of hair type growth, such as Ferriman-Gallwey score [28], are efficient in determining the severity of hirsutism and differentiating it from hypertrichosis (excess growth of androgen independent hair) [29]. Virilization represents a rapid process in which severe clinical features of marked androgen excess occur. As a part of a broader spectrum it can include: hirsutism, acne, androgenic alopecia, enlargement of the clitoris, deepening of the voice, increased muscle mass, decreased breast size, and frequently amenorrhea [3]. Furthermore, hyperandrogenism correlates with numerous metabolic alterations, for example: hyperinsulinemia, insulin resistance, hyperglycemia, dyslipidemia, and increased risk of atherosclerosis [30-32]. These features represent the core components of metabolic syndrome. Importantly, changes of AR in hyperandrogenism must be mediated via ARs-controlled pathway(s). For example, Apparao et al. established that women with PCOS exhibited elevated endometrial AR expression compared to controls. Further, AR in endometrial cell lines was upregulated not only by androgens but also by estrogens [32]. Another example of AR significance is the confirmed increased risk for breast cancer in premenopausal women with elevated serum concentrations of testosterone, androstenedione and dehydroepiandrosterone sulfate [33]. Therefore, novel breast cancer therapies are targeting the AR for its observed expression in the three main breast cancer subtypes [34-36]. Importantly, AR has already been demonstrated as a promising target for antiandrogen therapies in the most aggressive triple-negative breast cancer [37].

## OTHER CORRELATES OF ANDROGEN RECEPTOR INVOLVEMENT

To date, AR-Vs have been studied to some extent in human breast cancer cell lines and ovarian granulosa cells (GCs) [27, 38]. Recent research by Wang et al. identified two alternative splice variants (exon 3 deletion isoform, and 69 bp insertion into intron 2 isoform) in GCs from PCOS women. Moreover, there was no coexpression of both these isoforms in any of the patient studied. This investigation also described up-regulation of total AR mRNA in GCs of PCOS patients compared with controls; no expression of AR-Vs was observed in the control group. Since the levels of AR-FL were found reduced, the authors ascribed this up-regulation primarily to the increased mRNA levels of AR-Vs [38]. In this study AR-Vs were strongly associated with marked hyperandrogenism and abnormalities in folliculogenesis which, on the other hand, were absent in all control subjects. Therefore, Wang et al. claim that alternative splicing of the AR in GCs could be a major causative mechanism in PCOS (38). In contrast, in the opinion of Walters et al., increased AR-Vs expression in PCOS is considered rather the effect of the aberrant hyperandrogenic follicular milieu, rather than its cause [39].

All in all, we are currently experiencing an exciting era of new discoveries in the field of ARs and their possible multiple roles in women. Our brief review highlights that the knowledge of AR signaling and receptor structure alterations is crucial for the understanding of the pathogenesis of many androgenic disorders. Therefore, future scientific efforts targeted to further decipher the AR significance in women's health and disease will be of utmost importance.

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**Conflicts of interest**
The Authors declare no conflict of interest.

**Received:** February 20, 2017
**Accepted:** March 06, 2017

**Published online**

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