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Androgen Receptor Alternative Splicing and Human Infertility

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Abstract

About 95% of multi-exonic genes express more than one mRNA and downstream proteins by alternative splicing (AS) through the inclusion or exclusion of specific exons. Although AS provides a significant advantage in human evolution by increasing proteomic diversity, deregulation of AS can result in various pathologic conditions. The androgen receptor (AR), encoded by AR gene, is a steroid receptor transcription factor which mediates the cellular functions of androgen. The AR-mediated androgen actions play important and dual roles in the human reproduction development and function. Dysregulation of AR will result in human infertility. Multiple AR alternative splicing variants have been identified in different pathologies conditions, including androgen insensitivity syndrome, which will cause male infertility. More recently, our group has identified two AR splice variants expressed in granulosa cells from patients with polycystic ovary syndrome, which is one of the most common causes of female infertility. All of the aforementioned indicate that androgen receptor alternative splicing may be an important pathogenic mechanism in human infertility. The purpose of this review is to summarize the various alternatively spliced AR variants that have been discovered, with a focus on their role and origin in the pathologies of the human infertility diseases, including polycystic ovary syndrome and androgen insensitivity syndrome.

Graphical Abstract

Figure 2

Keywords: Androgen receptor; Alternative splicing; Polycystic ovary syndrome; Androgen insensitivity syndrome; Human infertility

Abbreviations: AS: Alternative Splicing; AR: Androgen Receptor; PCOS: Polycystic Ovary Syndrome; AIS: Androgen Insensitivity Syndrome; NTD: N-Terminal Domain; DBD: DNA-Binding Domain; CTD: COOH-Terminal Domain; LBD: Ligand-Binding Domain; T: Testosterone; A: Androstenedione; DHT: Dihydrotestosterone; E_1: Estrone; E2: Estradiol; AF-1: Activation Function 1; ARE: Androgen-Response Element; Gcs: Granulosa cells; ARKO: *Ar* Knockout

Introduction

Alternative splicing (AS) is a process by which multiple different mRNAs and downstream proteins can be generated from one gene through the inclusion or exclusion of specific exons [1]. This process occurs in 95% of all multi-exonic genes [2] and provides a significant advantage in evolution by increasing proteomic diversity [3]. However, deregulation of this process may lead to inappropriate spliced mRNA, impaired proteins, and eventually to diseases. Indeed, disturbances of AS are frequently observed in different types of pathologic conditions, such as cancers [4], neurological disorders [5], and endocrine system diseases [6].

The androgen receptor (AR), encoded by AR gene, is a steroid receptor transcription factor that mediates the cellular functions of testosterone (T) and dihydrotestosterone (DHT). The AR gene is located at Xq12 and composed of 8 exons. As with other members of the nuclear receptor superfamily, the AR is characterized by a modular structure including four functional domains: an N-terminal domain (NTD), a DNA-binding domain (DBD), a hinge region, and a COOH-terminal domain (CTD) [7,8] (Figure 1). The N-terminal domain, encoded by AR exon 1, is relatively long and poorly conserved displaying the most sequence variability by virtue of polymorphic (CAG)n and (GGN)n repeat units encoding polyglutamine and polyglycine tracts, respectively [9-11]. The AR NTD contains the major transactivation function of the AR, termed activation function 1 (AF-1). AF-1 harbors two trans-activating regions, transcriptional activation unit-1 (TAU-1) and 5 (TAU-5), which is indispensable for AR activation [12]. The DNA-binding domain, the most conserved region in the nuclear receptor family, contains two zinc fingers encoded by exon 2 and exon 3, respectively. The first zinc finger in the AR DBD determines the specificity of DNA recognition, which makes contact with major groove residues in an androgen-response element (ARE) half-site [13]. The second zinc finger is a dimerization interface that mediates binding with a neighboring AR molecule engaged with an adjacent ARE half-site [13]. The short flexible hinge region, encoded by exon 4, regulates DNA binding, nuclear translocation, and transactivation of the androgen receptor [14]. The AR COOH-terminal domain (CTD), encoded by exon 4-8, harbors the AR ligand-binding domain (LBD) and transcriptional activation function 2 (AF2) co-regulator binding interface [15-17]. The ligand-free AR is mainly located in the cytoplasm. The molecular chaperone complex, including Hsp90, other molecular chaperones, and high-molecular-weight immunophilins, is critical to maintain the ligand-free AR protein in a stable, inactive, intermediate configuration that has a high affinity for androgenic ligands. Androgen binding induces a 3-dimension structure change, including the Hsp90 dissociate from AR, and the transformed AR undergoes dimerization, phosphorylation, and translocation to the nucleus, which is mediated by the nuclear localization (NLS) in the hinge region [18,19]. In the nuclear, the AR then recognized the tissue-specific AREs located in the promoter or enhancer region of AR target genes to enable the recruitment of an array of co-regulators and the general transcription machinery, thereby triggering the transcription of androgen-dependent genes.

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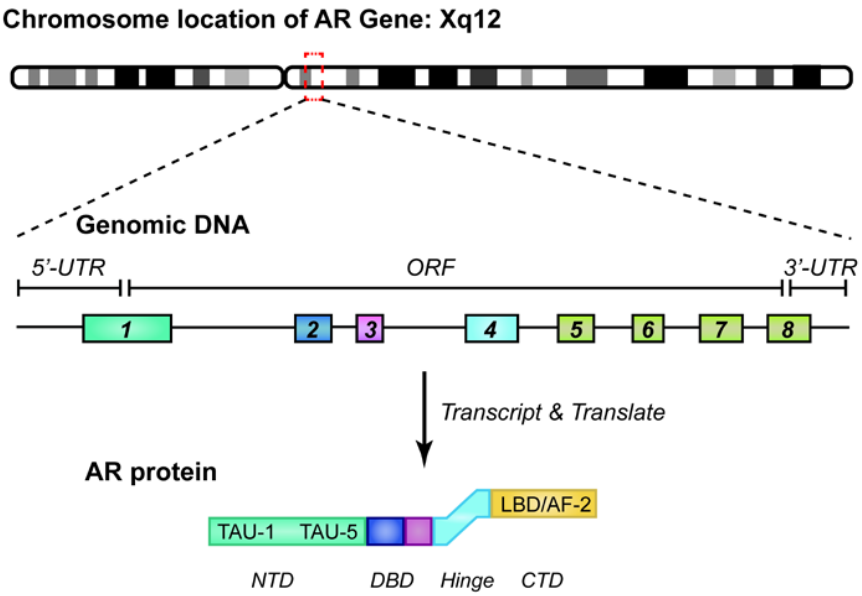

Figure 1: Structural organization of the AR gene and protein. The N-terminal transactivation domain (NTD), the first and second zinc fingers of DNA-binding domain (DBD), the hinge region and the COOH-terminal domain (CTD) of the AR protein were shown in green, dark blue, pink, light blue and orange, respectively. The exon numbers were shown in boxes and the colors of the exons were the same as the domain they encoded. TAU-1, Transcriptional Activation Unit-1; TAU-5, Transcriptional Activation Unit-1; LBD, Ligand-Binding Domain; AF2, Activation Function 2.

The AR-mediated androgen actions play key roles in the development and maintenance of male and female phenotype and reproduction function [20,21]. Given the complicated structure and the important function of each AR domain, it is not surprised that AR alternative splicing would affect the AR cellular signal and result in pathologic conditions. Numerous alternative spliced AR isoforms have been identified in different pathologic conditions including prostate cancer, Kennedy disease, androgen insensitivity syndrome (AIS) and so on [22-24]. AIS is characterized by a variety of phenotypes ranging from male infertility to completely normal female external genitalia [23,25]. More recently, our group have identified two AR splice variants expressed in granulosa cells (GCs) from patients with polycystic ovary syndrome (PCOS), which is one of the most common causes of female infertility [26,27] The altered AR splicing patterns are strongly associated with hyperandrogenism and abnormal folliculogenesis in PCOS [26]. All of the aforementioned indicate that androgen receptor alternative splicing may be an important pathogenic mechanism in human infertility. In this review, we will summarize the various alternatively spliced AR variants that have been discovered, with a focus on their role and origin in the pathologies of the human infertility diseases, including PCOS and AIS.

Dual Roles of Androgen Receptor in Human Fertility

AR plays important roles in male spermatogenesis and fertility and has been well reviewed previously [28]. While in female reproduction, it remains to be illustrated. For decades, it has been believed that androgens and AR in women are associated with poor reproductive health and are dispensable or harmful in follicular development and ovulation induction [29-31]. In hypophysectomized immature female rats, single androgen injection could result in decreased ovarian weight, as androgen induced the development of follicular atresia and reduced the number of healthy follicles of all types [32]. Androgens could also block the antiatretogenic effect induced by estrogen, enhance follicle apoptotic DNA fragmentation, and stimulated cell apoptosis [33], probably via repressing Ca^{2+} oscillations in oocytes [34]. Moreover, androgen excess could prevent the oocyte from resuming meiotic activity [35]. Tesarik et al. demonstrated that androstenedione could inhibit estradiol-induced Ca^{2+} response of oocytes, which posed detrimental effects on oocyte maturation and developmental potential [34,36]. After *in vitro* DHT treatment, more follicular cells were arrested at gap I (GI) phase and few proceeded to DNA synthesis phase by decreasing the cyclin D2 expression [37]. More importantly, follicular androgen levels were negatively associated with oocyte maturity in human samples [38]. In addition, excess androgen impairs oocyte developmental competence. Prenatally exposure to androgens reduced the percentage of zygotes developing into blastocysts [39]. The most typical evidence came from PCOS. Most PCOS women (60-80%) suffer from hyperandrogenism [27] and ovary is the main source of the excessive circulating androgens. PCOS patients always encountered the high rate of oocyte immaturity and poor fertilization rate in spite of more oocytes retrieved during IVF [40]. Pregnancy was associated with the higher follicular fluid estradiol/ testosterone (E2/T) ratio and follicles with the decreased follicular fluid E2/T ratio or high T levels tended to come across implantation failure or cleavage failure in *vitro* [38,40-42]. After anti-androgenic therapy, the ovarian morphology of polycystic ovary for PCOS women was significantly ameliorated and ovulation was restored [43].

However, with the introduction of the *Ar* knockout (ARKO, *Ar* $^{-/-}$) female mouse models and various *in vitro* studies, it is now widely accepted that androgens and AR also play irreplaceable roles in normal follicle development and female fertility. Androgen-AR signaling can affect follicular development by promoting pre-antral follicle growth and development into antral follicles while simultaneously preventing follicular atresia [44] .The ARKO mouse models, which mainly presented as loss of integrated AR protein or function, gave the most powerful evidence for the role of androgen and AR in normal ovarian physiology and function [45-47]. Although the ARKO female mice had normal ovarian and oviductal morphology, they showed considerable sub fertile with lower numbers of antral follicles, fewer corpora lutea,

and significantly higher rates of GCs apopotosis. And these mice finally presented as defective folliculogenesis and the development of premature ovarian failure. Further studies in GC-specific ARKO (GC-ARKO) mice and oocyte-specific ARKO (oocyte-ARKO) mice showed that the reproductive phenotypes of the GC-ARKO mice was the same as the global ARKO mice [48,49], whereas the oocyte-ARKO had normal reproductive phenotype [48], indicating that the local androgen signals within GCs were critical regulators to normal follicular development and fertility in female mice. Different androgens, including T, androstenedione (A), and DHT, could stimulate ovarian follicle development *in vitro* [50-52]. Since some androgens (T and A), could be aromatized into corresponding estrogens (estradiol, estrone), it causes confusion to determine the precise molecular mechanisms causing the effect. Ovarian follicles of mouse cultured by androgen antibodied or AR antagonists presented arrested follicular growth during pre-antral phase, decreased proficiency of pre-antral to pre-ovulatory follicles and reduced meiotic maturation rate [50,51]. These results sheded light on the direct stimulatory effect and essential role of AR in follicular maturation. Sen et al. [53] unveiled new molecular mechanisms of androgen-AR actions in follicular physiology. Androgens could enhance the expression of an antiapoptotic microRNA-125b (miR-125b), thereby contributing to AR-mediated follicular survival by pro-apoptotic protein expression suppression and follicular atresia inhibition. Meanwhile, androgens could up-regulate follicle-stimulating hormone receptor (FSHR) levels in a transcription-independent way, thus sensitizing pre-antral follicles towards FSH actions and involving androgen-mediated follicle growth [53].

In general, the balance exists between essentiality of androgens in follicular development and their negative effects in androgen excess conditions, regulates women's reproduction. Therefore, we strongly suggest the preventive strategy to rectify hyperandrogenism or deficiency of androgen/AR signaling to establish a beneficial environment for oocyte development and subsequent process.

AR Alternative Splicing in Polycystic Ovarian Syndrome

As mentioned above, polycystic ovary syndrome (PCOS), manifested with hormonal imbalance, is one of the most common endocrine disorders in women of reproductive age. PCOS affects nearly 6-10% women worldwide in accordance with 1990 NIH criteria [54-57] and the occurrence will dual or perhaps triple when using the 2003 Rotterdam Consensus (ESHRE/ASRM criteria), which is the most prevalent diagnostic criteria all over the world [58,59]. According to Rotterdam Consensus, women with the presence of two of the three criteria: oligo- or anovulation, signs of clinical hyperandrogenism, and/or biochemical signs of hyperandrogenism and polycystic ovaries on ultrasonography after exclusion of specific identifiable disorders (congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome) could be diagnosed as PCOS.

There have been attempts over 40 years to try and work out the etiology of the condition. The high degree of familial aggregation of PCOS suggests that genetic factor plays an important role in its etiology [60,61]. Increasing evidences indicate that epigenetic alterations, including aberrant DNA methylation, and environmental factors could also contribute to the development of PCOS [62,63]. Underlying the condition of PCOS is the belief that hyperandrogenism is fundamental to the pathophysiology and presentation of the condition [62,64]. Using mice model with exon 3 deleted mutant AR, the classical genomic AR action is verified to be critical for normal ovarian function. Haploin sufficiency for an inactivated AR may contribute to a premature reduction in female fecundity [65]. In human studies, the association of shorter CAG repeats of AR with PCOS suggests that inherited alteration in androgen sensitivity may contribute to PCOS [66]. Chinese individuals carrying the rs6152A AR allele had significantly higher susceptibility to polycystic ovary syndrome than those that were GG homozygotes [67]. All of these indicate that alterations in the AR singling pathway play an important role in PCOS.

Recently, our group have identified two AR splice variants in GCs from patients with PCOS [68]. As shown in figure 2A, these AR alternative splicing variants comprised an insertion of a 69-bp fragment of intron 2 (ins) and exon skipping of exon 3 (del), both of which have been identified in individuals with AIS or prostate cancer previously [23]. The AR splice variants were identified in ~62% of the enrolled PCOS women, and none of the non-PCOS women expressed either of the variants. The numbers of nucleotides on both exon 3 and the insertion fragment of intron 2 were a multiple of 3, thus alteration in both ins and del were in-frame and significantly affected the second finger of DBD of AR. The prediction of protein 3D structure of AR variants implied that ins and del changed the length of alpha-helix in the zinc finger domain. Inspection of clinical background of above patients suggested that AR splice variants are strongly correlated with hyperandrogenism and abnormal folliculogenesis.

Further chromatin immunoprecipitation sequencing demonstrated that genomic-wide AR recruitment was remarkably reduced in GCs expressings or del. More interestingly, the binding sites of the two variants were not enriched for ARE motifs, and they had their characteristic genomic recruitment patterns and enriched motifs that the wild-type AR did not have. Consequently, the gene expression patterns were also significantly altered in GCs expressing ins or del. Combined with luciferase assays, the AR splice variants had attenuated nuclear shuttling and binding ability to the ARE of AR targeted genes. Specifically, we found that AR recruitment of the ins or del to the ARE site in the promoter of *CYP19A1* (encoding aromatase, the rate limiting enzyme for the conversion of androgens into estrogens) was reduced compared to the wild-type AR, resulting in less transcription of *CYP19A1*, deficient aromatase expression and impaired androgen conversion to estrogens, thus contributing to hyperandrogenism (Figure 2B). Meanwhile, the AR splice variants, especially the ins, appeared to promote the expression of *CYP17A1* (encoding 17 α-hydroxylase which catalyzes A biosynthesis) more than the wild-type AR, thus resulting elevated A levels (Figure 2B). This was in accordance with higher serum and follicular levels of androstenedione in patients expressing the mutant variants. We are trying to propose that these effects in the ovary might favor more androgen production and therefore contribute to the phenotype of patients with PCOS.

The origin of these AR alternative splicing variant (ASVs) were still needed to be studied. Sequencing of the intron-exon junctions and approximately 100 bp of the flanking introns was used to detect DNA changes in the patients with the AR variants, as well as more than 100 bp upstream sequence of the 69-bp insertion fragment. Unfortunately, no mutations were identified in the conserved regions. Nevertheless, one PCOS patient with ins was heterozygous for one single nucleotide in the 69-bp intron fragment of genomic DNA. The variant band was only produced from one of the two alleles of the patient, while the origin of the WT band is still unknown. SNP is reported to influences the presence or absence of splicing regulator, which are important in alternative splicing [69]. As rs6152G/A polymorphism in exon 1 is associated with susceptibility to PCOS in Chinese women [67], we screened this SNP in peripheral lymphocytes for all the patients. However, all the patients in both group carried the same G/G genotype. Epigenetic modifications, such as DNA differential methylation at

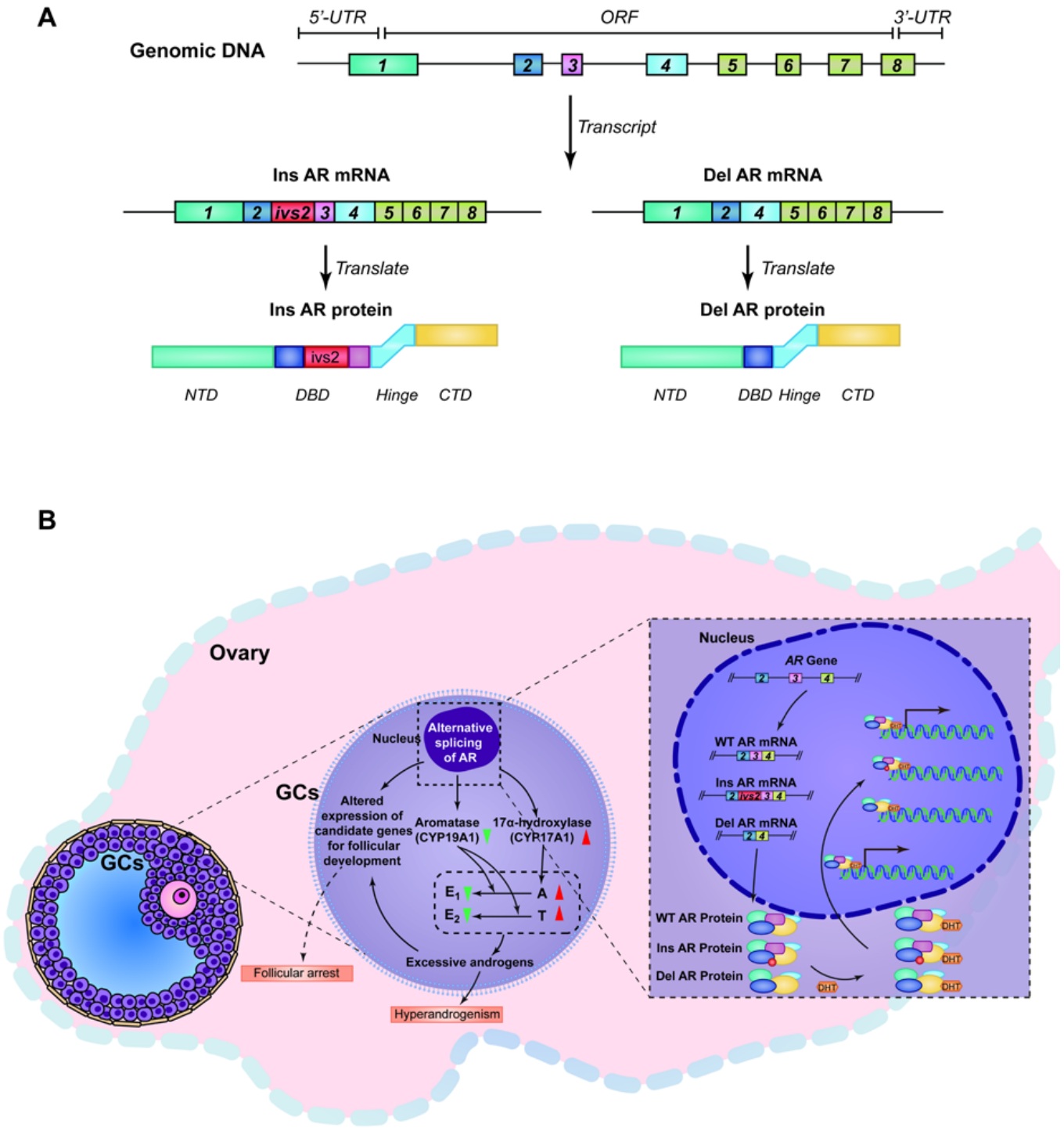

Figure 2: AR alternative splice variants (ASVs) and its roles in polycystic ovary syndrome. (A) Schematic diagram of AR ASVs and their predicted translation proteins. The ivs2 (red) represents the 69-bp insertion in intron 2 in the mRNA and its translation in the protein. (B) Schematic diagram of the roles of AR alternative splicing variants in granulosa cells (GCs) in the context of hyperandrogenism and abnormal follicular development. The AR ins or del reduced the *CYP19A1* expression resulting in impaired androgen (T, testosterone; A, androstenedione) conversion to estrogens (E1, estrone; E2, estradiol) and increased the *CYP17A1* expression resulting elevated A levels, thus contributing to hyperandrogenism. Green (downward) and Red (upward) triangles denote decreases and increases, respectively. The dihydrotestosterone (DHT) induced genome-wide recruitment pattern of AR ASVs (ins/del) were reduced comparing to the wild type (WT) AR. The color of AR domains shown in this schematic diagram was the same as in Figure1.

exon-intron boundaries, which altered the binding ability to splicing-regulatory proteins, also have a role in alternatively splicing regulation [70]. We analyzed the methylation status of individual DNA strands of exon 3 and its flanking region of AR including 11 CpG sites for individuals with different AR splicing patterns by bisulfite genomic sequencing PCR. Differential methylation of two CpG sites were observed at intron 2 and exon 3 junction in PCOS individuals with ins, suggesting the possibility that the ins isoform may be the result of altered DNA methylation. Nevertheless, it is still promising that epigenetic modifications in transacting factor machinery could result in AR alternative splicing in PCOS women.

Overall, we identified AR splice variants from women with PCOS for the first time, and found the mutant AR also had gained functions that different from the wild-type AR. The findings considerably change our understanding of the role of mRNA splicing, a major component of epigenetic modifications, in the pathogenesis of PCOS.

AR Alternative Splicing in Androgen Insensitivity Syndrome

Androgen insensitivity syndrome (AIS, OMIM 300068/312300) is an X-linked recessive mendelian disease caused by mutations in the androgen receptor (AR) gene, though also mutations in the AR interaction genes have been suggested [71,72]. Affected patients with AIS usually have a male 46, XY karyotype and undescended testes which produce age-related androgen levels but present with female external genitalia due to defects of androgen action in end-organ [73]. The phenotypic spectrum ranges from phenotypic females to an infertile males. Depending on the type and localization of *AR* mutations, AIS is clinically sub-classified into three groups: complete AIS (CAIS), partial AIS (PAIS), and mild type (MAIS) [73-75].

CAIS is the typical form of AIS characterized by a completely female appearance of the external genitalia but internal female genitalia are absent. The CAIS patients present primary amenorrhea in an adolescent female, and inguinal hernia or labial swelling containing testes in infancy or childhood [71]. The estimated incidence of CAIS is between 1/20,400 and 1/99,100 genetic males based upon proven molecular diagnosis [76]. Distinct from CAIS, PAIS is characterized by varying degrees of masculinisation of the external genitalia with normal testis development and partial responsiveness to age-appropriate levels of androgens [73]; while the MAIS usually have typical male genitalia but present gynaecomastia at puberty or male factor infertility at adult [73].

The Androgen Receptor Gene Mutations Database (ARDB) (http://androgendb.mcgill.ca) now contains over 500 different *AR* mutations causing AIS [77]. The ligand binding domain (LBD) is the mutation hotspot containing about 20% of all mutations, although the N-terminal transactivation domain is the largest AR domain [78,79]. About two thirds of these mutations are inherited in an X-linked pattern; the remainders are either germline or somatic *de novo* mutations [72]. The most frequent AR pathogenic mutation are point mutations leading to an amino acid substitutions in the protein structure. Loss-of-function mutations of the AR gene due to exons deletions or premature termination codon (PTC) generating mutations usually lead to CAIS, while splicing mutations can result in diverse phenotypes that are generally hard to predict. Notably, most splice-junction mutations identified to date are associated with CAIS/PAIS phenotype, although mutations in AR can lead to other diseases [80]. To date, 20 splice mutations accounting for AIS have been documented in the AR gene (Table 1) [77,81].

AR Alternative Splicing at the Splice Donor Site

Ris-Stalpers et al. [82] firstly reported the *AR* c.2173+1G>T

Phenotype	AR splicing mutation1,2	Exon / Intron	Reference
AR mutation in splicing donor site			
CAIS	c.1616+2_1616+3insT	exon 1 / intron 1	Trifiro et al. [95]
CAIS	c.1616+5G>C	exon 1 / intron 1	Philibert et al. [96]
CAIS	c.1768+1G>A	exon 2 / intron 2	Hellwinkel et al. [97]; Hiort et al. [98]
CAIS	c.1885+1G>A	exon 3 / intron 3	Ahmed et al. [70]
CAIS	c.2173+1G>T	exon 4 / intron 4	Ris-Stalpers et al. [81]
CAIS	c.2318+1G>A	exon 5 / intron 5	Vasu et al. [77]
CAIS	c.2449+3A>T	exon 6 / intron 6	Trifiro et al. [95]; Pinsky et al. [99]
CAIS	c.2449+5G>C	exon 6 / intron 6	Chávez et al. [82]
PAIS	c.2449+5G>T	exon 6 / intron 6	Sammarco et al. [83]
CAIS	c.2607+1G>A	exon 7 / intron 7	Lim et al. [100]; Kohler et al. [101]; Yong et al. [102]
CAIS	c.2607+1G>T	exon 7 / intron 7	Choi et al. [103]
AR mutation in splicing acceptor site			
CAIS	c.1769-1G>A	intron 2 / exon 3	Jaaskelainen et al. [78]
CAIS	c.1769-2A>C	intron 2 / exon 3	Audi et al. [104]
CAIS	c.1769-3C>G	intron 2 / exon 3	Audi et al. [104]; Gannage-Yared et al. [105]
PAIS	c.1769-11T>A	intron 2 / exon 3	Bruggenwirth et al. [86]
CAIS	c.1886-1G>T	intron 3 / exon 4	Avila et al. [106]
PAIS	c.1886-60G>A	intron 3 / exon 4	Melo et al. [107]
CAIS	c.2319-1G>T	intron 5 / exon 6	Zhang et al. [108]
CAIS	c.2450-44G>A	intron 6 / exon 7	Audi et al. [104]
AR splicing mutation in coding region			
PAIS	c.2667C>T	exon 8	Hellwinkel et al. [90]; Chávez et al. [82]

1 The nucleotide numbering system is based on AR reference sequence NM_000044.2.
2 Mutation descriptions are using the HGVS standard nomenclature.

Table 1: AR splicing mutations related to AIS phenotype include in the Androgen Receptor Gene Mutations Database (ARDB) and the Human Gene Mutation Database (HGMD).

mutation in the splice donor site of intron 4 in a CAIS patient and describe the consequences in detail. This mutation was indicated to induce aberrant RNA splicing at the exon 4 / intron 4 boundary by use of a cryptic splice donor site in exon 4, which lead to an in-frame deletion of 123 nucleotides from the mRNA and deletion of 41 amino acid (p.684_724del) in the steroid-binding domain of the AR protein. The mutated receptor was unable to bind androgens and did not activate transcription of an androgen-regulated reporter gene construct [82].

Similarly, Chávez et al. [83] described a *de novo AR* splicing donor site mutation (c.2449+5G>C) at exon 6 / intron 6 boundary associated with a CAIS phenotype with total absence of androgen binding. The c.2449+5G>C mutation could prevent the normal splicing of intron 6 in the mature transcript. As a consequence, a PTC within the unsliced intron 6 could give rise to a truncated protein lacking part of the AR LBD. Interestingly, a different c.2449+5G>T mutation at the same position had also been reported in a PAIS XY girl [84]. The c.2449+5G>T mutation lead to intron 6 retention in most of the AR mRNAs; as a result, most of the expressed protein lacks part of the C-terminal LBD, and a low level of full-length AR is also observed. The presence of full-length AR suggests that the primary transcript can be partially spliced correctly even in the presence of the c.2449+5G>T mutation, probably explaining the PAIS phenotype of the patient.

AR Alternative Splicing at the Splice Acceptor Site

Even though most of the AR splicing mutations occurred in the splicing donor sites [78], mutations in splicing acceptor sites that lead to CAIS/PAIS had also been reported. Jaaskelainen et al. [79] performed AR mutation analysis and cDNA sequencing in CAIS patients and identified a mutation occurred in the intron 2 acceptor splice site (c.1769-1G>A). The mutation was shown to lead to an insertion of 69 nucleotides (c.1768_1769ins1769-69_1769-1) between exon 2 and exon 3, exactly the same as the AR insert variant identified in PCOS [68] and prostatic cancer [85,86]. This phenomenon can be explained by the activation of cryptic splice site located 71/70-bp upstream of exon 3 [87]. The 69 nucleotides insertion in mRNA was predicted to in-frame and would lead to a substitution p.Gly590Glu combined with an insertion of 23 amino acids. Further androgen binding assay suggested the C-terminal LBD of the AR protein maintains ligand binding ability. However, the 23 amino acids insertion located between the two zinc fingers of the AR DNA binding domain (DBD) can impair the binding of the mutated AR with the androgen response element of target genes [87,88].

The 69 nucleotides insert transcript had also been reported in an unusual type of intronic mutation (c.1769-11T>A) in a PAIS pedigree comprised 3 patients [87]. The c.1769-11T>A mutation located in the pyrimidine-rich consensus sequence upstream of the splicing acceptor site at the intron 2/exon 3 boundary. cDNA analysis revealed aberrant splicing involved the 69 nucleotides insert (c.1768_1769ins1769-69_1769-1) between exon 2 and exon 3. In addition, a low level of the other aberrant transcript with exon 3 skipping and a very small amount of wild-type transcript were detected. These different types of transcripts might contribute to the patients PAIS phenotype.

AR Alternative Splicing Caused By Coding Region Mutation

Point mutations in the coding regions are commonly studied for their pathological functions through altered amino acid sequences. However, approximately 50% of all point mutations responsible for genetic diseases result in aberrant pre-mRNA splicing [89,90]. An presumably synonymous mutation (c.2667C>T; p.Ser889Ser) in exon 8 of the *AR* gene had been reported in PAIS patients [83,91]. The mutation led to a shorten transcript by intraexonic splicing, which merges a downstream 3'-UTR-region to codon 887, removing the internal sequence [91]. Consequently, the translation product consists of 8 missense amino acids from codon 888 followed by a PTC (p.Val888Ilefs*9). As expected, the androgen-binding property and the androgen-induced transcriptional activity were defective.

Conclusion and Perspective

In the condition that the *AR* gene includes some cryptic exons and long intron regions, it can be anticipated that more AR alteration splicing variants will be identified in different pathologic conditions as well as in different cells and tissues. A key question related to the AR alternative splicing variants is the mechanisms by which they control this process and how these splicing variants lead to diseases. Up to date, the origins of these AR alternative splicing variants were quite controversial. At least four potential mechanisms underlying generation of the AR alternative splicing forms were proposed, including alternative translation start codons, proteolysis cleavage, premature stop codon resulted from mutation, and alternative transcription start site [92,93]. However, the naturally occurred AR alternative splicing isoform, AR45, which expressed in various kinds of normal tissues including heart, muscle, uterus, prostate, and breast, indicates that there may be normal regulation systems attribute to AR alternative splicing. This hypothesis is also supported by recent studies that truncated AR isoform, AR-V7 which consisted of AR exons 1/2/3/CE3, can also been found in normal prostate tissues, demonstrating the splicing can occur naturally and non-pathologically. The alternative splicing variants identified in AIS indicated that the changes in the DNA sequence may be an important mechanisms related to AR alternative splicing. Indeed, some studies found that the copy numbers of the cryptic exon regions in the AR gene were increased in various prostate cancer cell lines [94,95]. However, no genomic mutation in the AR gene were identified in PCOS individuals harboring AS ins or del variants, although the same splicing variants were also found in AIS caused by a T/A mutation 11 bp upstream of exon 3 [87]. Moreover, our result suggested that altered DNA methylation may play an important role in splicing regulation [26,70]. The rapid development of molecular genetic testing technology, such as next generation sequencing, methylation sequencing and copy number variation detection in the whole genome level, will greatly facilitate the mechanism study of alternative splicing and new diagnostic and therapeutic approaches may be proposed based on the detailed characterization of this process.

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