

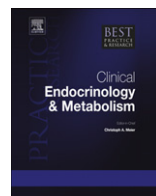


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46,XY disorders of sex development – the undermasculinised male with disorders of androgen action

Ralf Werner, PhD, Helga Grötsch, PhD, Olaf Hiort, MD, PhD *

Division of Paediatric Endocrinology and Diabetes, Department of Paediatrics, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

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Insensitivity to the action of androgens is a common cause of undermasculinisation in 46,XY individuals. These disorders are a result of the failure of major androgens to act via the intracellular androgen receptor and, thus, the genomic effects of androgen signalling are disrupted. The phenotype of affected individuals can vary considerably, depending on the dysfunction of the receptor. In childhood, the diagnosis is often complicated due to the lack of sensitive biochemical determinants, whilst during adolescence and in adults, the diagnosis can be readily made because of the striking clinical feminisation and a conclusive laboratory analysis. A variety of mutations in the androgen receptor have been analysed, providing insight into the complex pathways of intracellular processing and signal transduction via the androgen receptor. Endocrine therapy in androgen-insensitivity syndrome is controversial, because till date the special hormonal profiles in androgen insensitivity have not been acknowledged in replacement strategies.

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In clinical practice, paediatricians are confronted with children suffering from a 46,XY disorder of sex development (DSD), in whom the presence of histologically normal testicular tissue is confirmed and endocrine function of the gonads is perceived as normal at the time of investigation; therefore, androgen insensitivity is diagnosed clinically based on a presumed defect in androgen action. However,

Abbreviations: CAIS, complete androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome; MAIS, minimal androgen insensitivity syndrome; AR, androgen receptor.

* Corresponding author. Tel.: +49 451 500 2191; Fax: +49 451 500 6867.

E-mail address: hiort@paedia.ukl.mu-luebeck.de (O. Hiort).

thorough investigation of the biochemical and genetic pathways will elucidate the molecular mechanisms of decreased androgenic response of genital tissues only in a subset of patients.

The major androgens acting in the androgenic cascade are testosterone (T) and dihydrotestosterone (DHT). As DHT is synthesized mainly in extratesticular target tissues, the conversion defects due to 5 α -reductase type II deficiency have also been attributed as part of the ‘androgen insensitivity syndromes’ (AISs). However, at present, 5 α -reductase deficiency is described as a special form of androgen-biosynthesis defect.

A key player in the translation of androgen action is the androgen receptor (AR) – a nuclear transcription factor which can bind various androgenic steroids as ligands and then act via differential DNA targeting and genetic control. With the elucidation of the genetic structure of the AR 20 years ago,^{1a} it was believed that most 46,XY patients with presumed defects of androgen action would carry mutations in the coding regions of the AR gene. While this holds true for the majority of patients with complete AIS (CAIS), patients clinically assigned as partial AIS (PAIS) or minimal AIS (MAIS) to an increasing proportion do not carry relevant mutations in the AR.

This article focusses on our current understanding of the time-dependent physiology of androgen action to explain the clinical variability of ‘XY-undervirilisation’. We describe the features of androgen insensitivity with regard to clinical and laboratory findings and explain in detail the knowledge on cellular inhibition of androgen action, especially in PAIS. Finally, a brief explanation of the current strategy on therapeutic interventions in AIS is presented.

Developmental aspects of androgen action

In addition to genetic cascades of developmental regulatory genes, gene regulation controlled by sex hormones plays a fundamental role in male sexual differentiation and development. By the action of androgens, the bipotential anlagen of the internal and external sex organs develop irreversibly into the male phenotype.

Starting at nearly 9 weeks of gestation, testosterone, as a crucial factor for masculinisation, is secreted by the Leydig cells of the foetal gonad.^{1b} The AR is expressed in the Wolffian ducts and testosterone acts locally on these structures, preventing degeneration and promoting differentiation of these ducts into vas deferens, epididymis and the seminal vesicles.^{2,3}

Testosterone acts via the endocrine pathways following secretion into the blood. It induces differentiation of the genital tubercle, the anlage of the external genitalia, and promotes morphogenesis and differentiation of the prostate.⁴ As the concentration of testosterone in foetal circulation is relatively low, target cells of the external genitalia tissue and the prostate express 5 α -reductase type II – an enzyme that converts testosterone into DHT.⁵ DHT has a much higher affinity for the AR than testosterone and thus exhibits 10-fold stronger trans-activation potency.⁶

At 10–12 weeks of gestation, prostatic morphogenesis starts by outgrowth from the urogenital sinus⁴, and AR expression in the developing prostate has been detected in tissue specimens from 11.5 to 36 weeks.^{7,8} Androgens are necessary and sufficient to induce prostate morphogenesis and differentiation. This has been demonstrated by organ-culture experiments where androgens could promote prostate development from foetal urogenital sinuses of female rats.⁹ Although prostate development begins with the formation of an epithelial bud, during the first steps of organogenesis the AR is expressed only in the surrounding mesenchyme, indicating that androgen-dependent paracrine signals from the mesenchyme to the epithelium are required for prostatic bud formation.¹⁰

Similarly, as in the case of prostate organogenesis, mouse models and studies on patients with AIS have clearly shown that masculinisation of the external genitalia is mediated through the AR. The ambiguous stage of the external genitalia consists of the genital tubercle and the bilateral genital swellings, and both primordial structures express the AR.¹¹ Under the influence of androgens, the genital tubercle develops into the penis and the genital swellings fuse to form the scrotum.¹²

In addition to androgen-dependent virilisation of the internal and external genital structures, the descent of the testes from their origin at the urogenital ridge into the scrotum is another important step of male sexual development that depends on AR-dependent signalling. Testicular descent commences at 10 weeks in the human embryo and two different phases of the process can be distinguished.¹³ While the first trans-abdominal phase is mainly mediated by the peptide hormone insulin-like 3

hormone (InsI3) secreted by the Leydig cells of the foetal testis¹⁴, the second inguinoscrotal phase of testicular descent (from 26 to 35 weeks of gestation) is predominantly regulated through the AR. Owing to a defect in testicular descent in patients with AIS, the testes are usually localised in the abdominal or inguinal positions.^{15,16} It has been suggested that the gubernaculum is the primary site of androgen action during testicular descent, since normal migration of the gubernaculum does not occur in androgen-resistant mice and AIS patients.¹⁷ However, the expression of AR and the activity of 5 α -reductase in the gubernaculum were reported to be rather low^{18,19}, and more recent data suggest that androgens act via an indirect mechanism on the gubernaculum. In rats, androgens have been shown to be important for the sex-specific development of the genitofemoral nerve, which releases calcitonin gene-related peptide (CGRP) required for normal migration and development of the gubernaculum and thus for testis descent.^{20,21} So far, data from humans that clearly support a model for androgen action via the genitofemoral nerve are still missing.

The essential role of AR-dependent pathways for the masculinisation of the external genitalia and reproductive organs during embryonic development is well established. One of the future goals will be to elucidate the downstream effectors of the AR that transmit androgen signals into cellular proliferation and differentiation. Yamada and co-workers have recently identified β -catenin as an essential masculinisation factor for external genitalia development that acts downstream of the AR and suggested the involvement of Wnt/ β -catenin signalling in this process.²² The identification of further AR downstream factors and the elucidation of AR-dependent signalling pathways during embryonic organogenesis will not only lead to a deeper understanding of male development, but it might also help to find new causative factors for AIS phenotypes in patients where a mutation in the AR could not be identified.

Clinical and biochemical features of androgen insensitivity

In AIS, testicular function is apparently normal. Therefore, affected individuals will have a clinical phenotype that is consistent with a varying degree of presumable androgen deficiency, but testes may be of normal size and histology in childhood and Müllerian structures are absent due to normal anti-Müller hormone (AMH) synthesis and action. In this regard, at clinical investigation, AIS in childhood is indistinguishable from disorders of androgen biosynthesis action not affecting adrenal function, such as 5 α -reductase deficiency or 17 β -hydroxysteroid dehydrogenase deficiency. At the time of puberty, however, the clinical features of AIS become prominent with a varying degree of feminisation in conjunction with a lack of virilisation. These divergent findings do not occur so extensively in other forms of DSD. These features make clinical assessment of androgen insensitivity much easier during puberty and thereafter than during infancy and childhood.

Clinical findings

In CAIS the external genitalia appear completely female; the gonads may be palpable in the labia majora or in the inguinal groin, and may also be intra-abdominal but inaccessible for clinical investigation. In infancy, a diagnosis is made on the absence of Müllerian structures (i.e., oviducts, uterus and the upper part of the vagina) as well as on the hypoplasia or absence of androgen-dependent Wolffian structures, mainly the lack of epididymis as well as the prostate on either ultrasound investigation or surgical exploration. In PAIS, the phenotype is very variable, ranging from only slight signs of virilisation as marked by an elongated ano-vaginal distance, overt signs of androgen action with rugation of labio-scrotal folds, urogenital sinus, phallic enlargement, to a predominantly male phenotype with apparently normal male phallic size, but with severe-to-moderate hypospadias. However, in most patients with PAIS, a combination of reduction of phallic size with severe, mostly penoscrotal to perineal, hypospadias is seen. Cases with MAIS during infancy have rarely been reported. The striking feature is the lack of a genital malformation, so there is a normal localisation of the urethral meatus and a normal-appearing prepubertal phallus and scrotum.²³

At the time of puberty, the specific clinical appearance of AIS is much more striking. A main feature of pubertal development is the feminisation that occurs in almost all patients with CAIS and PAIS with gonads *in situ*. Patients with CAIS show female puberty with normal onset of breast development.

Papadimitriou et al.²⁴ reported that breast enlargement started at age 11.1 and pubertal auxology followed female standards. An important feature of CAIS is the insufficiency of secondary sexual hair growth. In some patients with CAIS, pubic hair growth is reported, but this may appear slighter than normal and may not reach Tanner stage 5. In PAIS, a mixture of virilisation and feminisation may occur. This leads to phallic and testicular enlargement with sexual hair growth as well as breast development. Puberty may not fully develop despite laboratory values for hormones measured in the adult reference range. The voice may not deepen and acne is scarce. The body proportions tend to be female or intermediate. Gynaecomastia is present in almost all patients with PAIS as we reported in a recent survey of 15 individuals assigned to male sex.²⁵

In MAIS, a main feature is the lack of genital malformation. At the time of puberty, a significant proportion of patients may have gynaecomastia.^{25,26} Scant sexual hair may be a feature as well as a varying degree of infertility due to oligoasthenospermia, but fertility has also been reported.²⁷

Assessment of laboratory values in AIS

The determination of laboratory values relies on age-dependent reference values during infancy and childhood and is difficult to assess in androgen insensitivity. Bouvattier et al.²⁸ reported that, in children with CAIS, luteinising hormone (LH) levels were comparably low during mini-puberty (measurement at day 30), while in PAIS, they rose to a normal level, which corresponds to a reference range of Tanner 4–5. In addition, the testosterone levels were low in CAIS infants, but testosterone secretion appeared to be stimulated properly in PAIS. The authors concluded that, due to the impaired pituitary feedback mechanism in AIS, the postnatal surge of gonadotrophins and testosterone is absent in CAIS, but may be normal in PAIS. AMH measurements were reported as normal or even elevated, because the inhibited AR expression accounts for the absence of AMH repression during testis development.²⁹ Rey et al.³⁰ had reported AMH as a valuable marker of testicular differentiation. While AMH was low in testicular dysgenesis, values were within the normal range in disorders of androgen biosynthesis or action. Inhibin B as a Sertoli cell marker showed a similar response. It was measured at low levels in anorchia or gonadal dysgenesis and normal or elevated levels in disorders of androgen biosynthesis or AIS compared to normal 46,XY boys.³¹

Gonadotrophin levels normally increase at the time of puberty. In due course of puberty, follicle-stimulating hormone (FSH) levels may become elevated above the normal range because of secondary testicular atrophy. LH is in the normal male reference range or may even be elevated. Consecutively, a surge of testosterone is seen that does not correspond to external masculinisation, thus giving rise to the diagnosis of androgen insensitivity. An androgen sensitivity index has been postulated as the mathematical product of LH and testosterone serum values³² and is helpful in the discrimination of adult patients with MAIS as a cause of male infertility, although it does not differentiate AIS from other causes of hormonal imbalance such as oestrogen insufficiency in MAIS.²⁶ Thus, the higher the androgen sensitivity index, the more likely an abnormality of the AR gene.

Prior to the time of molecular characterisation of the AR gene, analysis of specific androgen binding in genital skin fibroblasts of patients had been a major diagnostic procedure. In the age of molecular genetics, this type of analysis has been generally avoided, because it entails an invasive method of genital skin biopsy to obtain cells for investigation.

An androgen sensitivity test was described on the basis of the response of serum levels of sex hormone-binding globulin (SHBG) following intake of stanozolol, a synthetic anabolic steroid. In normal probands, serum levels of SHBG decline to about 50% of the initial value 5–8 days following initiation of stanozolol treatment.³³ In AIS, this response is diminished, correlating somewhat with the phenotypic degree of undermasculinisation. While the SHBG test can be valuable as the only available *in vivo* test of androgen insensitivity, it has several pitfalls: Namely stanozolol has been taken off the regular market due to its high potential for being misused as a doping agent. Second, due to the endogenous testosterone surge during the first months of life, this test is not sensitive during this important period for diagnosis and management of the child with suspected AIS. Third, the test might fail in patients with somatic mosaicism of a mutation in the AR gene.^{33,34} Therefore, it

should only be proposed in patients older than 1 year of age with unclear disorders of androgen action.

Differential diagnosis of clinical disorders of androgen action

The diagnosis of AIS in infancy and childhood is difficult and nowadays depends on the identification of the underlying mutation of the AR. However, a mutation in the AR cannot be identified in all patients with presumed AIS. Ahmed et al.¹⁵ reported a case series of 278 patients with presumed AIS. While, in most patients with a clinical or biochemical diagnosis of CAIS, a relevant molecular genetic abnormality of the AR gene was demonstrated, it was noted that the less severe the phenotype, the more likely it was to encounter a normal sequence analysis of the AR gene. Deeb et al. pointed out that in PAIS patients there is no direct association between phenotype and the likelihood to bear a relevant mutation of the AR gene.³⁵ Holterhus et al.³⁶ studied children with presumed AIS, abnormalities of AR expression and the possibility of a mutation in the AR gene. Genital skin fibroblasts of affected patients were thoroughly analysed, including specific androgen-binding analysis, investigation of transcription and translation of the AR as well as complete sequencing of the coding region of the AR. In patients with CAIS the mutations were finally localised in the variable trinucleotide repeat regions of the gene; however, a patient with severe PAIS did not bear any molecular genetic abnormality of the AR gene despite severely diminished transcription and translation of the AR.³⁶ These findings point to defects of androgen action that might affect the AR expression due to promoter alterations, although this is yet to be proven.

Alternatively, other genetic abnormalities have to be taken into the differential diagnosis. Coutant et al.³⁷ investigated two related 46,XY children with severe undermasculinisation, without Müllerian structures as well as good Leydig cell response following stimulation with human chorionic gonadotrophin (hCG). While the AR gene did not reveal any mutation, a steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) mutation was demonstrated although the patients had no sign of adrenal insufficiency. A study by Lee et al.³⁸ investigated undermasculinised 46,XY children with 17 β -hydroxysteroid dehydrogenase (17 β -HSD) type III deficiency and demonstrated that the ratio of testosterone to androstenedione is not always discriminative. In addition, they concluded that the phenotype of these children might be indistinguishable from those with PAIS. Therefore, 17 β -HSD type III deficiency should always be taken into account in the differential diagnosis of AIS. The same holds true for 5 α -reductase type II deficiency, where the ratio of testosterone to DHT is difficult to determine and the clinical findings may mimic AIS in childhood.³⁹

All these reports and investigations indicate the fact that the clinical and laboratory diagnosis of AIS in childhood is difficult and non-specific. Further research aiming at stringent and sensitive methodology and reference values for the discrimination of the various causes of 46,XY undermasculinisation and its differential diagnosis need to be undertaken. Moreover, investigations should consider abnormalities in the regulation of AR expression as well as defects in other cellular components involved in the facilitation of androgen action during sexual development. At present, this is under investigation in the European research collaborative study EuroDSD – a project financed by the 7th European framework programme of the European Commission (for further information, see www.eurodsd.eu)

Gene structure and functional domains of the AR

The AR is located on the X chromosome at q11–12. Due to the hemizygous status of the AR gene, mutations in the gene directly affect male sexual development and, in most cases, also lead to infertility, while heterozygous women are unaffected and can pass the mutation on to their children. The AR is a nuclear receptor that acts as a transcription factor and is composed of four distinct domains: (1) a large N-terminal domain (NTD) containing a strong ligand-independent activation function 1 (AF1), (2) a DNA-binding domain (DBD) mediating the binding of the receptor to androgen-responsive elements (AREs) in the promoters and enhancers of target genes, (3) a small hinge region containing most of the bipartite nuclear localisation signal and (4) the ligand-binding domain (LBD), which undergoes structural changes following hormone binding and forms a ligand-dependent activation

function 2 (AF2). The large NTD contains two polymorphic repeats – a repeat of 9–36 glutamine (Q) residues and a repeat of 10–27 glycine (G) residues (Fig. 1). Expansion of the polyglutamine repeat over 40 residues leads to spinal and bulbar muscular atrophy (SBMA or Kennedy disease) – a late-onset neurodegenerative disorder associated with mild symptoms of androgen insensitivity.⁴⁰ The NTD is thought to exist in a molten-globule or pre-molten-globule-like structure that adopts a higher structure by induced folding when activated by transcriptional regulators, such as transcription factor TFIIIF⁴¹; however, crystal structures of this domain could not be obtained. In contrast to the NTD, the DBD and the LBD are highly structured and conserved amongst the steroid receptor family. Crystal structures of the DBD, bound to AREs, as well as of ligand-activated LBDs, with peptides bound to AF2, have been obtained.^{42–44} The DBD consists of two zinc fingers followed by a C-terminal extension. The first zinc finger containing the so-called P-Box interacts with the DNA major groove of the ARE and determines the specificity of DNA recognition. The second zinc finger is involved in AR dimerisation.⁴⁵ The LBD consists of 12 α -helices and four small β -strands folded into a three-layer sandwich-like structure.⁴⁴ Following hormone binding, helix 12 folds back into the core of the LBD and seals the ligand-binding pocket in a manner similar to a mouse trap.⁴⁶ Thereby, a hydrophobic groove at the surface of the LBD is formed that interacts with LxxLL-like and FxxLF-like motifs of co-regulators as well as with the ²³FQNLF²⁷ motif in the NTD of the AR.

Genomic action of the AR

Testosterone and the more potent DHT, which is converted from testosterone *in situ* by 5 α -reductase type II, are the two main androgens involved in the genomic action of AR. In the absence of ligand, the AR is cytoplasmic and forms a large protein complex with the molecular chaperone heatshock protein 90 (Hsp90) and p23 – a co-chaperone that stabilises the binding of HSP90 to the receptor together with various co-chaperones, including cyclophilin 40 (Cyp40) and FK506-binding protein 52 (FKbp52).⁴⁷ Following ligand binding, alterations in the composition of the HSP complex occur and the AR undergoes a conformational change. The NTD interacts intramolecularly with the LBD⁴⁸ and the AR dissociates from the chaperone complex, unmasking the bipartite nuclear localisation signal in the hinge region and DBD composed of two clusters of basic amino acids.⁴⁹ The AR translocates rapidly to the nucleus, homodimerises and binds in a head-to-head conformation to its cognate hormone-response elements (HREs).⁴³ The AR can bind to the so-called classical AREs, which are recognised by all class I nuclear receptors (glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR) and AR) as well as to selective AREs – which have been shown not to be recognised by the GR. Natural androgen-responsive promoters and enhancers contain monomeric and dimeric AR-binding sites and are also enriched for binding sites of other transcription factors that may prime the chromatin for AR binding.⁵⁰

Following binding to AREs, the AR can directly recruit components of the transcription pre-initiation complex, interact with other transcription factors within the target promoter or recruit a variety of co-regulator proteins, which either promote or suppress transcription (co-activators and co-repressors, respectively). Nearly 200 co-regulator proteins of the AR have been described so far.⁵¹ Depending on the cell type and the state of the cell, different subsets of co-regulator proteins

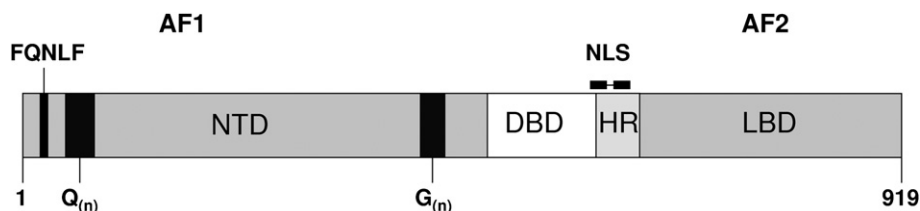


Fig. 1. Domains of the AR. AF1: activation function 1, AF2: activation function 2, NTD: N-terminal domain, DBD: DNA-binding domain, HR: hinge region, LBD: Ligand binding domain. NLS: bipartite nuclear localization signal. Q(n): polymorphic glutamine repeat, G(n): polymorphic glycine repeat. FQNLF: peptide interacting with AF2.

associate with the AR, possessing a diverse array of functions. AR co-regulators differ from general and specific transcription factors in that they typically do not bind to DNA and do not affect the basal rate of transcription, but their enzymatic activities lead to enhanced or repressed expression of target genes. AR co-regulators act on chromatin modification or as bridging factors between the AR and the basal transcriptional machinery. They exhibit enzymatic activities on the AR itself, on other co-regulator proteins or on the chromatin. By their enzymatic activities, AR co-regulators act on the state of post-translational modifications, such as phosphorylation, ubiquitination, sumoylation, acetylation, methylation, ADP-ribosylation and others, causing a constant remodelling of the chromatin and the transcriptional complex. Large chromatin-remodelling complexes, such as the ATP-dependent SWI/SNF complex or related multi-protein complexes^{52,53}, as well as steroid receptor coactivator (SRC) proteins that can recruit methyltransferases, such as coactivator-associated arginine methyltransferase 1 (CARM1)⁵⁴, or histone acetylases, such as CBP/p300, assist in inducing transcriptional activation. They are necessary to generate an accessible open chromatin environment for subsequent transcriptional activation by recruitment of the RNA polymerase II complex⁵⁵ (Fig. 2). Concerted post-translational modifications of co-factors on the AR and other transcription factors or co-factors determine protein stability, protein–protein recognition and activity, degradation by the 26S proteasome and turnover or compartmentalisation by nuclear–cytoplasmic shuttling of components of the transcriptional complexes and ensure the highly regulated and dynamic gene expression of target genes.

A specific role of certain co-regulators in the pathophysiology of AIS is not established yet, although some mouse knockout models of co-activators displayed various degrees of androgen resistance. Male SRC2 knockout mice showed defects in spermiogenesis and age-dependent testicular degeneration⁵⁶ and FKBP52 knockout mice developed penile hypospadias, prostate dysgenesis and compromised

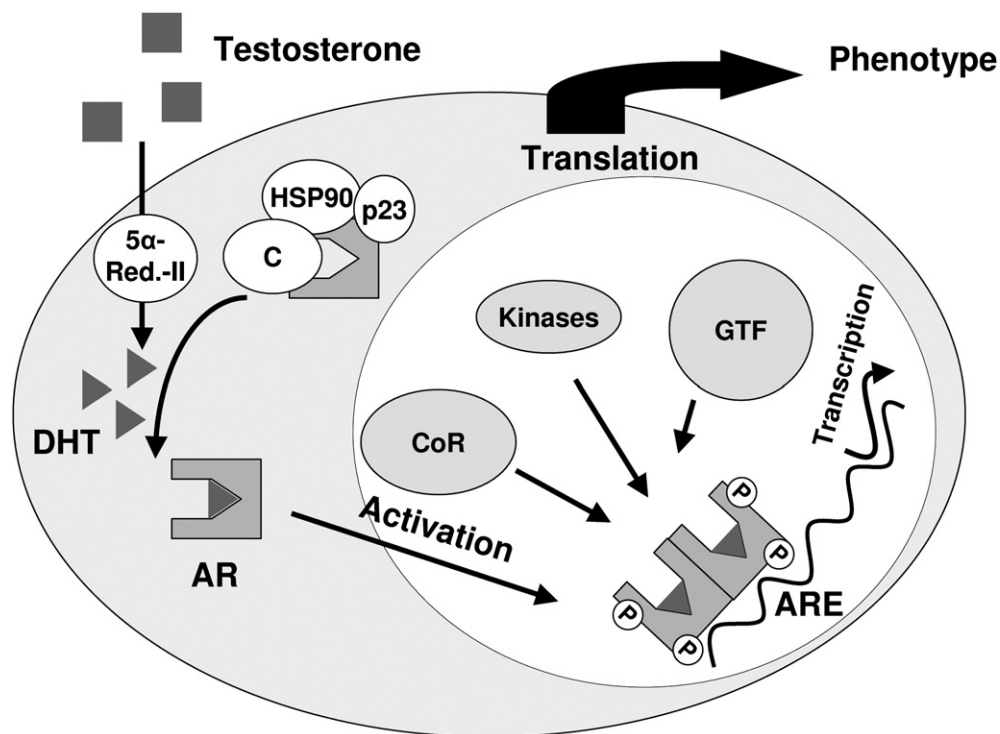


Fig. 2. Scheme of the genomic action of the AR: 5α-Red.-II: 5α-reductase II, HSP90: heat shock protein 90, C: co-chaperones, CoR: co-regulators, GTF: general transcription factors.

fertilising capacity of sperm.^{57,58} This observation led to the screening for mutants in FKBP52 as a candidate gene for hypospadias in men, but no sequence variation could be associated with isolated hypospadias.⁵⁹

AR mutations in PAIS

At present, >600 mutations within the AR gene have been identified.⁶⁰ Most of them are inactivating mutations and are linked to AIS. Activating mutations or mutations leading to a more promiscuous receptor have been identified in prostate cancer. Furthermore, extensions of the glutamine repeat have been linked to spinal and bulbar muscular atrophy – a neurodegenerative disease.⁴⁰

Spectrum of mutations in AIS

In general, a broad array of AR-inactivating mutations, such as nonsense and missense mutations, small insertions and deletions, splice site and mosaic mutations, have been detected in AIS. Nonsense mutations as well as most cases of small deletions or insertions lead to a disruption of the reading frame, consequently to a premature stop codon and, thus, to a non-functional receptor. Even the loss of the last four amino acid residues of the AR leads to the complete loss of ligand-binding capacity and CAIS.^{61,62} In-frame deletions have been rarely detected. A nine-nucleotide deletion in the NTD has been observed in a patient with PAIS and an insertion of three nucleotides in the NTD has been found in another patient with male infertility; however, functional impairment by this deletion or insertion could not be demonstrated so far.^{63,64}

By contrast, most in-frame insertions or deletions in the DBD or LBD disrupt these highly structured domains and lead to CAIS.^{65–68} Because of the highly structured nature of these two domains, not surprisingly, most missense mutations lead to a more-or-less severe disturbance of their structure and thus to all forms of AIS.⁶⁰ Proof of the impact of these mutations on AR function has been obtained in various *in vitro* assays described below. Compared with the DBD or LBD, within the flexible NTD only a few missense mutations associated with MAIS or PAIS have been detected and, so far, a proof of their impact on the impairment of AR function was barely successful.^{63,64} Splice-site mutations are difficult to detect and have been associated with CAIS⁶⁹ as well as with PAIS. In a PAIS patient, a silent mutation in exon 8 of the AR has been detected that leads to a partial aberrant splicing.⁷⁰ Thus, the residual expression of wild-type AR might explain the PAIS phenotype. In addition, post-zygotic mutations are sometimes hard to detect. Post-zygotic mutations in the AR leading to somatic mosaicism have been identified in rare cases of AIS.^{71–74} In somatic mosaicism, different ratios of cells expressing mutant or wild-type protein are present in various tissues of the same individual. Hence, depending on the distribution of the mutant cells, the same mutation might lead to a very variable phenotype. All types of mutations – from missense to nonsense as well as frame-shift and splice-site mutations – have been identified in AIS patients with somatic mosaicism and are associated with both, PAIS and CAIS.

The polymorphic glutamine (Q) and glycine (G) repeats: modulators of AR activity

The polymorphic glutamine and glycine repeats vary from 9 to 36 glutamine residues and from 10 to 27 glycine residues in the normal population (Fig. 1). The length of the polyQ and polyG repeat has an inverse influence on AR transactivity *in vitro*. A long polyQ repeat reduces AR transactivity⁷⁵, while a long polyG repeat increases AR activity.⁷⁶ A combination of a long polyQ repeat (Q_{28–30}) and a short polyG repeat (G₁₀) in combination with or without an A645D mutation was identified not only in several PAIS patients⁷⁶, but also in normal men.^{77,78} Because the polymorphic repeats modulate AR activity, one would also expect this combination of repeats in apparently normal men, but it might predispose for virilisation disorders or infertility – depending on the androgen levels in the given individual. Additional studies have analysed the impact of the polymorphic repeats on impaired sperm production and male infertility, but the results are contradictory. Elongation of the CAG repeat within

the normal range has been correlated with decreased virilisation, male infertility or impaired sperm morphology^{79–83}; however, contradictory studies have also been reported.^{84–86}

Functional characterisation of mutants in AIS

AR function in genital skin fibroblasts

Until cloning of the AR in 1989⁸⁷, analysis of specific androgen binding in fibroblasts obtained from a genital skin biopsy was an important tool to confirm the diagnosis of AIS. Genital skin fibroblasts were grown from a labio-scrotal biopsy and used to calculate the androgen-binding capacity in saturation-binding curves and Scatchard plots using radioactively labelled ligands such as the non-metabolisable androgen methyltrienolone (R1881). Low maximum binding (B_{max}) or an elevated dissociation constant (K_d) indicate low AR expression or a compromised ligand binding; however, normal androgen binding cannot exclude other defects, such as a dysfunctional DNA binding. In case of somatic mosaicism of mutations that affect the ligand binding, both AR proteins might influence the ligand-binding assay and generate a hyperbola instead of a linear graph in the Scatchard plot.⁷⁴

At present, the diagnosis of AIS is confirmed, in most cases, by abnormal results of molecular genetic analysis of the AR gene. Nevertheless, in some cases, patients with clinically presumed AIS do not seem to have mutations in the AR gene and, in these cases, biochemical data from skin biopsies may assist as a diagnostic tool. Recently, in a search for molecular biomarkers for AIS, genital skin fibroblasts of labio-scrotal origin from 46,XY males and 46,XY CAIS females were compared concerning their genome-wide gene-expression pattern⁸⁸ and their responsiveness to DHT by microarray analysis.⁸⁹ While inactivating mutations appear to induce lasting changes in gene expression in cultured fibroblasts, only the mRNA of apolipoprotein D (APOD) and two other mRNAs were identified to be up-regulated in response to DHT treatment in normal scrotal fibroblasts but not in labia-majora-derived fibroblasts from CAIS patients. This observation makes APOD a promising biomarker for AIS in cases without proven AR mutation; however, further studies are required to confirm its usefulness.

In vitro transactivation assays

If a new mutation is detected, the impact of that mutation on protein activity has to be proven. With respect to the AR, mutations are often recreated in an expression vector and the AR is co-transfected into an AR-negative mammalian cell line, together with a reporter gene preceding an androgen-responsive promoter. These assays are sensitive for several functions of the AR, such as hormone binding, nuclear transport, DNA binding and transactivation. Impaired AR function is measured as reduced expression of the reporter gene compared with a wild-type construct. Various androgen-responsive promoters have been used – minimal promoters containing only two androgen responsive elements in front of a TATA-Box and natural promoters, for example, the mouse mammary tumour virus (MMTV) long terminal repeat (LTR)^{90,91}, the sex-limited protein (slp) promoter of the mouse, the probasin (PB) promoter of the rat⁹² or the human prostate-specific antigen (PSA) promoter. In complete and severe forms of AIS, these functional studies often demonstrate a good genotype–phenotype correlation, but, for mutations associated with MAIS or less severe forms of PAIS, experimental evidence is often lacking or scarce. In some cases of MAIS, an impairment of AR transactivity could be demonstrated on the PEM promoter of the mouse⁹³, suggesting that this promoter might be more informative to test mildly dysfunctional mutations.

AR mutations can cause distinct patterns of reduced activation of androgen-responsive promoter constructs, depending on the kind of mutation and the ligand or promoter used in the assay.⁹⁴ The transactivation of androgen-responsive reporter genes depends on folding by chaperones, binding of the hormone and subsequent conformational changes, post-translational modifications, nuclear transport, intra- and intermolecular interactions such as N/C-interaction and dimerisation, binding to the various HRE motifs as well as on the recruitment of cofactors and the subsequent recruitment of the RNA-Pol II complex. Since the set of expressed AR co-regulators differs between cell types and

tissues⁹⁵, especially mild mutations may lead to different reporter gene expression levels, depending on the promoter, cell type, mutation or ligand used in the assay.

N/C-interaction assays

Following hormone binding, helix 12 in the LBD undergoes conformational changes and seals the ligand-binding pocket in a manner similar to a mouse trap.⁴⁶ Thereby, a binding groove on the surface of the LBD is formed, called AF2, which is a binding site for coactivators harbouring LxxLL or FxxLF motifs.⁹⁶ This AF2 also shows a strong inter- and intramolecular interaction with the ²³FQNLF²⁷ motif of the NTD.^{48,97} The N/C interaction appears to further stabilise helix 12 and bound androgen.⁹⁸ Mutations in the AF2 can cause AIS without altering equilibrium androgen binding affinity of the receptor.⁹⁹ The strong N/C-interaction can be analysed by reporter gene activation in a mammalian two-hybrid assay. Missense mutations found in the AR-LBD that revealed that near-normal androgen-binding kinetics may display disrupted N/C-interaction and the decreased N/C-interaction was shown to mirror the degree of AIS.^{92,100} However, in rare cases, contrasting results have also been obtained. Recently, a new F826L mutation found in a boy with severe penoscrotal hypospadias was described. This mutation revealed normal androgen binding, but, surprisingly, N/C-interaction was increased twofold compared to the wild type, when tested on different promoters as well as with different androgens.¹⁰¹

DNA-binding assays

Missense mutations in the DBD may lead to a complete disruption of AR binding to AREs associated with CAIS or only to an altered affinity and selectivity of AR–ARE interactions¹⁰² associated with PAIS or MAIS. The reduced or absent DNA binding could be demonstrated by electrophoretic mobility-shift assays (EMSAs)¹⁰³ or by fluorescence recovery following photobleaching (FRAP) experiments, comparing cells transfected with GFP-tagged mutant and wild-type AR.^{104,105}

Phenotypic variability of AIS mutations

Since AIS is a rare disease, only a few patients bearing the same AR mutation and displaying a PAIS phenotype have been identified, making it difficult to estimate the range of virilisation associated with a particular mutation. Nevertheless, estimating the anticipated grade of virilisation during puberty and the pubertal outcome of the affected patient is a prerequisite for adequate counselling and patient management. For instance, the mutation Q798E was found in several patients with PAIS who have been raised in the female sex.^{106,107} In other patients with the same mutation, it was associated with infertility and MAIS.^{26,108} For the mutations L712F, I737 T and F725L, even in the same family, a high variability of PAIS phenotypes was observed.^{23,109} Interestingly, these three mutations do not affect the ligand binding but disrupt AF2 and N/C-terminal interaction. So far, it is not clear if a strong phenotypic variability of PAIS mutations is a feature of only a small set of mutations affecting key residues of AR function or whether this applies to all PAIS mutations. The analysis of a larger patient cohort and their mutations is one of the aims of the ongoing EuroDSD project (www.eurodsd.eu).

Therapy

Endocrine therapy of androgen insensitivity at the time of puberty may follow two pathways: Either patients assigned to the female sex receive an oestrogen replacement therapy because they have been gonadectomised, or patients with male sex assignment receive additional androgen treatment to overcome virilisation deficits. In addition, sometimes, infants assigned to the male sex receive androgen treatment to induce phallic growth prior to surgical reconstruction. Any endocrine therapy in AIS, however, has not been validated or supported by surveys of larger patient groups.

The present consensus¹¹⁰ states that sex steroid supplementation at the time of puberty should attempt to achieve gender-appropriate pubertal maturation with induction of sexual characteristics, a pubertal growth spurt and optimal bone mineral accumulation as well as gender-appropriate

psychosexual maturation. In the present standard concepts, patients with CAIS following gonadectomy receive non-cyclic oestrogen therapy, because, at present, there is no evidence that progesterone as well as cyclic replacement is beneficial to women without a uterus. This is in contrast to patients with other forms of DSD – especially women with complete gonadal dysgenesis, who receive cyclic administration of oestrogens and gestagens. At this time, the ‘female substitution theory’ is challenged by some patients with CAIS as well as their support groups, because they argue that the normal endocrine profile of post-pubertal non-gonadectomised women with CAIS does not mirror a normal 46,XX sex steroid profile, namely that the administration of testosterone as a precursor to oestrogens is mandatory in CAIS. In self-studies, some gonadectomised patients with CAIS have reported a restoration of libido, better muscle power and better quality of life following supplementation of testosterone rather than oestradiol. This new therapeutic option has to be evaluated in controlled studies before any further conclusion can be made.

In males with PAIS, high-dose androgen therapy in addition to the elevated endogenous testosterone levels has been tried to achieve better masculinisation. However, reports on the outcome of such therapies are scarce. Weidemann et al.¹¹¹ report on a single patient, who received 250 mg of testosterone enanthate intramuscularly per week and responded with marked progress of virilisation despite a mutation in the DBD of the AR. Any such therapy has to be individualised in light of the type and localisation of the underlying aberration of the AR. Patients treated in this fashion should be followed up closely and the outcome should be documented. Again, this is currently undertaken in the *EuroDSD* project mentioned earlier.

Practice points

- Androgen insensitivity has a very variable phenotypic appearance and may be clinically undistinguishable from defects of androgen biosynthesis during childhood.
- During childhood, laboratory analysis is often difficult and lacks specificity and sensitivity.
- Molecular genetic analysis of the androgen receptor gene can detect a relevant mutation only in a subset of patients. The less severe the phenotype, the less likely is the chance of demonstrating a mutation.
- In adolescence and adulthood, the diagnosis of androgen insensitivity can be made on the grounds of undermasculinisation and/or feminisation, albeit with high measurable androgen levels.

Research agenda

- Highly specific tools for early and specific detection of androgen receptor abnormalities must be developed.
- Future research should evolve model systems to elucidate better individualised genotype–phenotype correlations.
- The characterisation of other factors of androgen-controlled genital development is mandatory to get further insight into the cellular and time-dependent events of androgen action and to define further mechanisms for androgen insensitivity.

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References

- *1a. Brinkmann AO, Faber PW, van Rooij HC et al. The human androgen receptor: domain structure, genomic organisation and regulation of expression. *J Steroid Biochem* 1989; **34**: 307–310;
- b. Siiteri PK & Wilson JD. Testosterone formation and metabolism during male sexual differentiation in the human embryo. *The Journal of Clinical Endocrinology and Metabolism* 1974; **38**(1): 113–125.
2. Sajjad Y, Quenby SM, Nickson P et al. Expression of androgen receptors in upper human fetal reproductive tract. *Human Reproduction (Oxford, England)* 2004; **19**(7): 1659–1665.
3. Rey R & Picard JY. Embryology and endocrinology of genital development. *Baillière's Clinical Endocrinology and Metabolism* 1998; **12**(1): 17–33.
4. Marker PC, Donjacour AA, Dahiya R et al. Hormonal, cellular, and molecular control of prostatic development. *Developmental Biology* 2003; **253**(2): 165–174.
5. Ellsworth K & Harris G. Expression of the type 1 and 2 steroid 5 alpha-reductases in human fetal tissues. *Biochemical and Biophysical Research Communications* 1995; **215**(2): 774–780.
6. Deslypere JP, Young M, Wilson JD et al. Testosterone and 5 alpha-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. *Molecular and Cellular Endocrinology* 1992; **88**(1–3): 15–22.
7. Adams JY, Leav I, Lau KM et al. Expression of estrogen receptor beta in the fetal, neonatal, and prepubertal human prostate. *The Prostate* 2002; **52**(1): 69–81.
8. Aumuller G, Holterhus PM, Konrad L et al. Immunohistochemistry and in situ hybridization of the androgen receptor in the developing human prostate. *Anatomy and Embryology* 1998; **197**(3): 199–208.
9. Takeda H, Lasnitzki I & Mizuno T. Analysis of prostatic bud induction by brief androgen treatment in the fetal rat urogenital sinus. *The Journal of Endocrinology* 1986; **110**(3): 467–470.
10. Takeda H, Mizuno T & Lasnitzki I. Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and postnatal prostate. *The Journal of Endocrinology* 1985; **104**(1): 87–92.
11. Sajjad Y, Quenby S, Nickson P et al. Immunohistochemical localization of androgen receptors in the urogenital tracts of human embryos. *Reproduction (Cambridge, England)* 2004; **128**(3): 331–339.
- *12. Yamada G, Satoh Y, Baskin LS et al. Cellular and molecular mechanisms of development of the external genitalia. *Differentiation; Research in Biological Diversity* 2003; **71**(8): 445–460.
13. Nef S & Parada LF. Hormones in male sexual development. *Genes & Development* 2000; **14**(24): 3075–3086.
14. Nef S & Parada LF. Cryptorchidism in mice mutant for *Insl3*. *Nature Genetics* 1999; **22**(3): 295–299.
15. Ahmed SF, Cheng A, Dovey L et al. Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. *The Journal of Clinical Endocrinology and Metabolism* 2000; **85**(2): 658–665.
16. Hughes IA & Acerini CL. Factors controlling testis descent. *European Journal of Endocrinology* 2008; **159**(Suppl. 1): S75–S82.
17. Hutson JM. Testicular feminization: a model for testicular descent in mice and men. *Journal of Pediatric Surgery* 1986; **21**(3): 195–198.
18. Heyns CF & Pape VC. Presence of a low capacity androgen receptor in the gubernaculum of the pig fetus. *The Journal of Urology* 1991; **145**(1): 161–167.
19. Heyns CF, Tate R, Sargent NS et al. Absence of 5 alpha-reductase activity in the gubernaculum during descent of the fetal pig testis. *The Journal of Urology* 1993; **150**(2 Pt 1): 510–513.
20. Ng SL, Bidarkar SS, Sourial M et al. Gubernacular cell division in different rodent models of cryptorchidism supports indirect androgenic action via the genitofemoral nerve. *Journal of Pediatric Surgery* 2005; **40**(2): 434–441.
21. Yong EX, Huynh J, Farmer P et al. Calcitonin gene-related peptide stimulates mitosis in the tip of the rat gubernaculum in vitro and provides the chemotactic signals to control gubernacular migration during testicular descent. *Journal of Pediatric Surgery* 2008; **43**(8): 1533–1539.
22. Miyagawa S, Satoh Y, Haraguchi R et al. Genetic interactions of the androgen and Wnt/{beta}-catenin pathways for the masculinization of external genitalia. *Molecular Endocrinology* 2009; **23**(6): 871–880.
23. Holterhus PM, Sinnecker GH & Hiort O. Phenotypic diversity and testosterone-induced normalization of mutant L712F androgen receptor function in a kindred with androgen insensitivity. *The Journal of Clinical Endocrinology and Metabolism* 2000; **85**(9): 3245–3250.
24. Papadimitriou DT, Linglart A, Morel Y et al. Puberty in subjects with complete androgen insensitivity syndrome. *Hormone Research* 2006; **65**(3): 126–131.
25. Steltenkamp S & Hiort O. Pubertal development of 46, XY DSD patients with partial androgen insensitivity due to mutation of the androgen receptor assigned to male sex. *Hormone Research* 2007; **68**(Suppl. 1): 205.
- *26. Hiort O, Holterhus PM, Hörter T et al. Significance of mutations in the androgen receptor gene in males with idiopathic infertility. *The Journal of Clinical Endocrinology and Metabolism* 2000; **85**(8): 2810–2815.
27. Galli-Tsinopoulou A, Hiort O, Schuster T et al. A novel point mutation in the hormone binding domain of the androgen receptor associated with partial and minimal androgen insensitivity syndrome. *Journal of Pediatric Endocrinology & Metabolism* 2003; **16**(2): 149–154.
28. Bouvattier C, Carel JC, Lecoindre C et al. Postnatal changes of T, LH, and FSH in 46, XY infants with mutations in the AR gene. *The Journal of Clinical Endocrinology and Metabolism* 2002; **87**(1): 29–32.
29. Boukari K, Meduri G, Brailly-Tabard S et al. Lack of androgen receptor expression in Sertoli cells accounts for the absence of anti-Müllerian hormone repression during early human testis development. *The Journal of Clinical Endocrinology and Metabolism* 2009; **94**(5): 1818–1825.
30. Rey RA, Belleville C, Nihoul-Fekete C et al. Evaluation of gonadal function in 107 intersex patients by means of serum antimüllerian hormone measurement. *The Journal of Clinical Endocrinology and Metabolism* 1999; **84**(2): 627–631.
31. Kubini K, Zachmann M, Albers N et al. Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. *The Journal of Clinical Endocrinology and Metabolism* 2000; **85**(1): 134–138.

32. Aiman J, Griffin JE, Gazak JM et al. Androgen insensitivity as a cause of infertility in otherwise normal men. *The New England Journal of Medicine* 1979; **300**(5): 223–227.
33. Sinnecker GH, Hiort O, Nitsche EM et al. Functional assessment and clinical classification of androgen sensitivity in patients with mutations of the androgen receptor gene. German Collaborative Intersex Study Group. *European Journal of Pediatrics* 1997; **156**(1): 7–14.
- *34. Holterhus PM, Wiebel J, Sinnecker GH et al. Clinical and molecular spectrum of somatic mosaicism in androgen insensitivity syndrome. *Pediatric Research* 1999; **46**(6): 684–690.
35. Deeb A, Mason C, Lee YS et al. Correlation between genotype, phenotype and sex of rearing in 111 patients with partial androgen insensitivity syndrome. *Clinical Endocrinology* 2005; **63**(1): 56–62.
36. Holterhus PM, Werner R, Hoppe U et al. Molecular features and clinical phenotypes in androgen insensitivity syndrome in the absence and presence of androgen receptor gene mutations. *Journal of Molecular Medicine* 2005.
37. Coutant R, Mallet D, Lahlou N et al. Heterozygous mutation of steroidogenic factor-1 in 46, XY subjects may mimic partial androgen insensitivity syndrome. *The Journal of Clinical Endocrinology and Metabolism* 2007; **92**(8): 2868–2873.
38. Lee YS, Kirk JM, Stanhope RG et al. Phenotypic variability in 17beta-hydroxysteroid dehydrogenase-3 deficiency and diagnostic pitfalls. *Clinical Endocrinology* 2007; **67**(1): 20–28.
39. Hiort O, Willenbring H, Albers N et al. Molecular genetic analysis and human chorionic gonadotropin stimulation tests in the diagnosis of prepubertal patients with partial 5 alpha-reductase deficiency. *European Journal of Pediatrics* 1996; **155**(6): 445–451.
40. La Spada AR, Wilson EM, Lubahn DB et al. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991; **352**(6330): 77–79.
41. Lavery DN & McEwan IJ. The human androgen receptor AF1 transactivation domain: interactions with transcription factor IIF and molten-globule-like structural characteristics. *Biochemical Society Transactions* 2006; **34**(Pt 6): 1054–1057.
42. Matias PM, Donner P, Coelho R et al. Structural evidence for ligand specificity in the binding domain of the human androgen receptor. Implications for pathogenic gene mutations. *The Journal of Biological Chemistry* 2000; **275**(34): 26164–26171.
43. Shaffer PL, Jivan A, Dollins DE et al. Structural basis of androgen receptor binding to selective androgen response elements. *Proceedings of the National Academy of Sciences of the United States of America* 2004; **101**(14): 4758–4763.
44. He B, Gampe Jr. RT, Kole AJ et al. Structural basis for androgen receptor interdomain and coactivator interactions suggests a transition in nuclear receptor activation function dominance. *Molecular Cell* 2004; **16**(3): 425–438.
45. Verrijdt G, Tanner T, Moehren U et al. The androgen receptor DNA-binding domain determines androgen selectivity of transcriptional response. *Biochemical Society Transactions* 2006; **34**(Pt 6): 1089–1094.
- *46. Moras D & Gronemeyer H. The nuclear receptor ligand-binding domain: structure and function. *Current Opinion in Cell Biology* 1998; **10**(3): 384–391.
47. Smith DF & Toft DO. Minireview: the intersection of steroid receptors with molecular chaperones: observations and questions. *Molecular Endocrinology* 2008; **22**(10): 2229–2240.
48. Schaufele F, Carbonell X, Guerbodot M et al. The structural basis of androgen receptor activation: intramolecular and intermolecular amino-carboxy interactions. *Proceedings of the National Academy of Sciences of the United States of America* 2005; **102**(28): 9802–9807.
49. Jenster G, Trapman J & Brinkmann AO. Nuclear import of the human androgen receptor. *The Biochemical Journal* 1993; **293**(Pt 3): 761–768.
- *50. Claessens F, Denayer S, Van Tilborgh N et al. Diverse roles of androgen receptor (AR) domains in AR-mediated signaling. *Nuclear Receptor Signaling* 2008; **6**: e008.
- *51. Heemers HV & Tindall DJ. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocrine Reviews* 2007; **28**(7): 778–808.
52. Link KA, Burd CJ, Williams E et al. BAF57 governs androgen receptor action and androgen-dependent proliferation through SWI/SNF. *Molecular and Cellular Biology* 2005; **25**(6): 2200–2215.
53. Chen J, Kinyamu HK & Archer TK. Changes in attitude, changes in latitude: nuclear receptors remodeling chromatin to regulate transcription. *Molecular Endocrinology* 2006; **20**(1): 1–13.
54. Chen D, Ma H, Hong H et al. Regulation of transcription by a protein methyltransferase. *Science* 1999; **284**(5423): 2174–2177.
55. Kang Z, Janne OA & Palvimo JJ. Coregulator recruitment and histone modifications in transcriptional regulation by the androgen receptor. *Molecular Endocrinology* 2004.
56. Gehin M, Mark M, Dennefeld C et al. The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and pCIP. *Molecular and Cellular Biology* 2002; **22**(16): 5923–5937.
57. Yong W, Yang Z, Periyasamy S et al. Essential role for Co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology. *The Journal of Biological Chemistry* 2007; **282**(7): 5026–5036.
58. Hong J, Kim ST, Tranguch S et al. Deficiency of co-chaperone immunophilin FKBP52 compromises sperm fertilizing capacity. *Reproduction (Cambridge, England)* 2007; **133**(2): 395–403.
59. Beleza-Meireles A, Barbaro M, Wedell A et al. Studies of a co-chaperone of the androgen receptor, FKBP52, as candidate for hypospadias. *Reproductive Biology and Endocrinology* 2007; **5**: 8.
- *60. Gottlieb B, Beitel LK, Wu JH et al. The androgen receptor gene mutations database (ARDB): 2004 update. *Human Mutation* 2004; **23**(6): 527–533.
61. Tahiri B, Auzou G, Nicolas JC et al. Participation of critical residues from the extreme C-terminal end of the human androgen receptor in the ligand binding function. *Biochemistry* 2001; **40**(29): 8431–8437.
62. Werner R, Zhan J, Gesing J et al. In-vitro characterization of androgen receptor mutations associated with complete androgen insensitivity syndrome reveals distinct functional deficits. *Sexual Development* 2008; **2**(2): 73–83.
63. Holterhus PM, Werner R, Struve D et al. Mutations in the amino-terminal domain of the human androgen receptor may be associated with partial androgen insensitivity and impaired transactivation in vitro. *Experimental and Clinical Endocrinology & Diabetes* 2005; **113**(8): 457–463.

64. Ferlin A, Vinanzi C, Garolla A et al. Male infertility and androgen receptor gene mutations: clinical features and identification of seven novel mutations. *Clinical Endocrinology* 2006; **65**(5): 606–610.
65. Jeske YW, McGown IN, Cowley DM et al. Androgen receptor genotyping in a large Australasian cohort with androgen insensitivity syndrome; identification of four novel mutations. *Journal of Pediatric Endocrinology & Metabolism* 2007; **20**(8): 893–908.
66. Ledig S, Jakubiczka S, Neulen J et al. Novel and recurrent mutations in patients with androgen insensitivity syndromes. *Hormone Research* 2005; **63**(6): 263–269.
67. Avila DM, Wilson CM, Nandi N et al. Immunoreactive AR and genetic alterations in subjects with androgen resistance and undetectable AR levels in genital skin fibroblast ligand-binding assays. *The Journal of Clinical Endocrinology and Metabolism* 2002; **87**(1): 182–188.
68. Beitel LK, Prior L, Vasiliou DM et al. Complete androgen insensitivity due to mutations in the probable alpha-helical segments of the DNA-binding domain in the human androgen receptor. *Human Molecular Genetics* 1994; **3**(1): 21–27.
69. Ris-Stalpers C, Turberg A, Verleun-Mooyman MC et al. Expression of an aberrantly spliced androgen receptor mRNA in a family with complete androgen insensitivity. *Annals of the New York Academy of Sciences* 1993; **684**: 239–242.
70. Hellwinkel OJ, Holterhus PM, Struve D et al. A unique exonic splicing mutation in the human androgen receptor gene indicates a physiologic relevance of regular androgen receptor transcript variants. *The Journal of Clinical Endocrinology and Metabolism* 2001; **86**(6): 2569–2575.
71. Holterhus PM, Bruggenwirth HT, Hiort O et al. Mosaicism due to a somatic mutation of the androgen receptor gene determines phenotype in androgen insensitivity syndrome. *The Journal of Clinical Endocrinology and Metabolism* 1997; **82**(11): 3584–3589.
72. Hiort O, Sinnecker GH, Holterhus PM et al. Inherited and de novo androgen receptor gene mutations: investigation of single-case families. *The Journal of Pediatrics* 1998; **132**(6): 939–943.
73. Kohler B, Lumbroso S, Leger J et al. Androgen insensitivity syndrome: somatic mosaicism of the androgen receptor in seven families and consequences for sex assignment and genetic counseling. *The Journal of Clinical Endocrinology and Metabolism* 2005; **90**(1): 106–111.
74. Holterhus PM, Sinnecker GH, Wollmann HA et al. Expression of two functionally different androgen receptors in a patient with androgen insensitivity. *European Journal of Pediatrics* 1999; **158**(9): 702–706.
75. Mhatre AN, Trifiro MA, Kaufman M et al. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. *Nature Genetics* 1993; **5**(2): 184–188.
76. Werner R, Holterhus PM, Binder G et al. The A645D mutation in the hinge region of the human androgen receptor (AR) gene modulates AR activity, depending on the context of the polymorphic glutamine and glycine repeats. *The Journal of Clinical Endocrinology and Metabolism* 2006; **91**(9): 3515–3520.
77. Lundin KB, Giwercman A, Richthoff J et al. No association between mutations in the human androgen receptor GGN repeat and inter-sex conditions. *Molecular Human Reproduction* 2003; **9**(7): 375–379.
78. Lundin KB, Nordenskjöld A, Giwercman A et al. Frequent finding of the androgen receptor A645D variant in normal population. *The Journal of Clinical Endocrinology and Metabolism* 2006; **91**(8): 3228–3231.
79. Tut TG, Ghadessy FJ, Trifiro MA et al. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *The Journal of Clinical Endocrinology and Metabolism* 1997; **82**(11): 3777–3782.
80. Davis-Dao CA, Tuazon ED, Sokol RZ et al. Male infertility and variation in CAG repeat length in the androgen receptor gene: a meta-analysis. *The Journal of Clinical Endocrinology and Metabolism* 2007; **92**(11): 4319–4326.
81. Milatiner D, Halle D, Huerta M et al. Associations between androgen receptor CAG repeat length and sperm morphology. *Human Reproduction (Oxford, England)* 2004; **19**(6): 1426–1430.
82. Lim HN, Chen H, McBride S et al. Longer polyglutamine tracts in the androgen receptor are associated with moderate to severe undermasculinized genitalia in XY males. *Human Molecular Genetics* 2000; **9**(5): 829–834.
83. Dowsing AT, Yong EL, Clark M et al. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet* 1999; **354**(9179): 640–643.
84. Lund A, Tapanainen JS, Lahdetie J et al. Long CAG repeats in the AR gene are not associated with infertility in Finnish males. *Acta obstetrica et gynecologica Scandinavica* 2003; **82**(2): 162–166.
85. Hadjicacem L, Hadj-Kacem H, Boulila A et al. Androgen receptor gene CAG repeats length in fertile and infertile Tunisian men. *Annales de génétique* 2004; **47**(3): 217–224.
86. Lavery R, Houghton JA, Nolan A et al. CAG repeat length in an infertile male population of Irish origin. *Genetica* 2005; **123**(3): 295–302.
87. Lubahn DB, Brown TR, Simental JA et al. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. *Proceedings of the National Academy of Sciences of the United States of America* 1989; **86**(23): 9534–9538.
88. Holterhus PM, Deppe U, Werner R et al. Intrinsic androgen-dependent gene expression patterns revealed by comparison of genital fibroblasts from normal males and individuals with complete and partial androgen insensitivity syndrome. *BMC Genomics* 2007; **8**: 376.
89. Appari M, Werner R, Wunsch L et al. Apolipoprotein D (APOD) is a putative biomarker of androgen receptor function in androgen insensitivity syndrome. *Journal of Molecular Medicine* 2009; **87**: 623–632.
90. Cato AC, Henderson D & Ponta H. The hormone response element of the mouse mammary tumour virus DNA mediates the progestin and androgen induction of transcription in the proviral long terminal repeat region. *The EMBO Journal* 1987; **6**(2): 363–368.
91. Govindan MV. Specific region in hormone binding domain is essential for hormone binding and trans-activation by human androgen receptor. *Molecular Endocrinology* 1990; **4**(3): 417–427.
92. Thompson J, Saatcioglu F, Janne OA et al. Disrupted amino- and carboxyl-terminal interactions of the androgen receptor are linked to androgen insensitivity. *Molecular Endocrinology* 2001; **15**(6): 923–935.

93. Zuccarello D, Ferlin A, Vinanzi C et al. Detailed functional studies on androgen receptor mild mutations demonstrate their association with male infertility. *Clinical Endocrinology* 2007.
94. Werner R, Schutt J, Hannema S et al. Androgen receptor gene mutations in androgen insensitivity syndrome cause distinct patterns of reduced activation of androgen-responsive promoter constructs. *The Journal of Steroid Biochemistry and Molecular Biology* 2006; **101**(1): 1–10.
95. Bebermeier JH, Brooks JD, Deprimo SE et al. Cell-line and tissue-specific signatures of androgen receptor-coregulator transcription. *Journal of Molecular Medicine* 2006; **84**(11): 919–931.
96. Dubbink HJ, Hersmus R, Verma CS et al. Distinct recognition modes of FXXLF and LXXLL motifs by the androgen receptor. *Molecular Endocrinology* 2004; **18**(9): 2132–2150.
97. He B, Kemppainen JA & Wilson EM. FXXLF and WXXLF sequences mediate the NH₂-terminal interaction with the ligand binding domain of the androgen receptor. *The Journal of Biological Chemistry* 2000; **275**(30): 22986–22994.
98. He B, Gampe Jr. RT, Hnat AT et al. Probing the functional link between androgen receptor coactivator and ligand-binding sites in prostate cancer and androgen insensitivity. *The Journal of Biological Chemistry* 2006; **281**(10): 6648–6663.
99. He B, Kemppainen JA, Voegel JJ et al. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH₂-terminal domain. *The Journal of Biological Chemistry* 1999; **274**(52): 37219–37225.
100. Ghali SA, Gottlieb B, Lumbroso R et al. The use of androgen receptor amino/carboxyl-terminal interaction assays to investigate androgen receptor gene mutations in subjects with varying degrees of androgen insensitivity. *The Journal of Clinical Endocrinology and Metabolism* 2003; **88**(5): 2185–2193.
101. Wong HY, Hoogerbrugge JW, Pang KL et al. A novel mutation F826L in the human androgen receptor in partial androgen insensitivity syndrome; increased NH₂-COOH-terminal domain interaction and TIF2 co-activation. *Molecular and Cellular Endocrinology* 2008; **292**(1–2): 69–78.
102. Nguyen D, Steinberg SV, Rouault E et al. A G577R mutation in the human AR P box results in selective decreases in DNA binding and in partial androgen insensitivity syndrome. *Molecular Endocrinology* 2001; **15**(10): 1790–1802.
103. Bruggenwirth HT, Boehmer AL, Lobaccaro JM et al. Substitution of Ala564 in the first zinc cluster of the deoxyribonucleic acid (DNA)-binding domain of the androgen receptor by Asp, Asn, or Leu exerts differential effects on DNA binding. *Endocrinology* 1998; **139**(1): 103–110.
104. Farla P, Hersmus R, Geverts B et al. The androgen receptor ligand-binding domain stabilizes DNA binding in living cells. *Journal of Structural Biology* 2004; **147**(1): 50–61.
- *105. van Royen ME, Cunha SM, Brink MC et al. Compartmentalization of androgen receptor protein-protein interactions in living cells. *The Journal of Cell Biology* 2007; **177**(1): 63–72.
106. Jaaskelainen J, Deeb A, Schwabe JW et al. Human androgen receptor gene ligand-binding-domain mutations leading to disrupted interaction between the N- and C-terminal domains. *Journal of Molecular Endocrinology* 2006; **36**(2): 361–368.
107. Bevan CL, Brown BB, Davies HR et al. Functional analysis of six androgen receptor mutations identified in patients with partial androgen insensitivity syndrome. *Human Molecular Genetics* 1996; **5**(2): 265–273.
108. Wang Q, Ghadessy FJ, Trounson A et al. Azoospermia associated with a mutation in the ligand-binding domain of an androgen receptor displaying normal ligand binding, but defective trans-activation. *The Journal of Clinical Endocrinology and Metabolism* 1998; **83**(12): 4303–4309.
109. Quigley CA, Tan JA, He B et al. Partial androgen insensitivity with phenotypic variation caused by androgen receptor mutations that disrupt activation function 2 and the NH₂- and carboxyl-terminal interaction. *Mechanisms of Ageing and Development* 2004; **125**(10–11): 683–695.
- *110. Hughes IA, Houk C, Ahmed SF et al. Consensus statement on management of intersex disorders. *Archives of Disease in Childhood* 2006; **91**(7): 554–563.
111. Weidemann W, Peters B, Romalo G et al. Response to androgen treatment in a patient with partial androgen insensitivity and a mutation in the deoxyribonucleic acid-binding domain of the androgen receptor. *The Journal of Clinical Endocrinology and Metabolism* 1998; **83**(4): 1173–1176.