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Topic 1.10

Nuclear receptor action involved with sex differentiation*

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Abstract: Sex determination and differentiation in the male is an orderly sequence of events coordinated by genetic and hormonal factors operating in a time- and concentration-dependent manner. The constitutive sex in mammals is female. Disorders of fetal sex development have provided the means to identify testis-determining genes and the molecular mechanisms of hormone action. Thus, the androgen receptor, a nuclear hormone receptor critical for androgen-induced male sex differentiation, displays unique intra-receptor and protein–protein interactions which, when disturbed, can result in extreme forms of sex reversal. Polymorphic variants are associated with milder disorders of sex development. Against this genetic background, endocrine active substances may further contribute to the underlying causes of an increase in male reproductive tract disorders.

INTRODUCTION

The androgen receptor (AR) is a key mediator in the control of male sexual differentiation. It is expressed in fetal tissues, and activated in a ligand-dependent manner to coordinate expression of suitably responsive genes. The classic experiments of Jost [1], and observations in syndromes such as Turner, show that the ovary, and therefore estrogens, are not required for prenatal sex differentiation of the female. In contrast, male differentiation requires prior formation of the testis and subsequent testosterone production to masculinize the embryo [2]. This is mediated by the AR as a ligand-activated transcription factor, directing both internal and external male gonadal development.

Terminology must be clearly defined. Sex determination refers to formation of a testis or an ovary from the indifferent, bipotential gonad. Sex differentiation is the phenotypic expression of male or female development in the formation of internal and external genitalia. In the male, this requires the active intervention of androgens. The terms, determination and differentiation, refer to prenatal events. Sex development continues postnatally with the onset of secondary sexual characteristics appropriate for gender at puberty, and the acquisition of reproductive capacity.

SEX DETERMINATION

The process of sex determination in mammals represents a genetically regulated choice between divergent pathways of development. The result of this choice is the programming of the bi-potential gonad to either an ovarian or a testicular fate. A number of genes critical to these processes have been isolated,

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but mutation analysis in syndromes of sex reversal indicate that a large number of additional genes in the pathways that regulate sex determination and facilitate gonad differentiation remain to be identified. It has been assumed that the default pathway of gonad determination was female, suggesting a male dominant genetic control. By implication, mutation in key testis regulatory genes and abnormal regulation would redirect the pathway toward a female endpoint.

This original view is now being modified according to an understanding that the regulation of sex determination must also involve anti-testis genes or ovarian determinants, in addition to the currently known male determinants.

SRY (SEX-RELATED GENE ON THE Y CHROMOSOME)

For male sex determination, probably the most significant regulator is *SRY*, located on the short arm of the Y chromosome. Despite the fact that it is a small single exon gene, transgenic studies in female mice have clearly demonstrated that it is the only gene required to initiate development of the testis [3]. Studies in humans have confirmed the testis-determining role of *SRY* by the appearance of a complete sex reversal phenotype in XY individuals with mutant versions of the gene [4].

The critical region of the SRY protein is believed to be the HMG (high mobility group) box motif. This is highly conserved across species and within which the majority of sex-reversing mutations are located [5]. It is this motif that allows SRY to bind DNA and induce a sharp structural bend [6]. The kinetic stability of the bended HMG box-DNA complex appears to regulate the transcription of downstream genes [7].

SRY has a small window of male specific expression in the developing genital ridge which is programmed to form the testis or ovary [8]. This takes place in the mouse between 10.5 dpc (days post coitum), with *Sry* expression having ceased by 12.5 dpc. What regulates *Sry* remains unknown. *Sry* expression spreads through the genital ridge in a defined pattern, resulting in the programming of supporting cells with a bi-potential ability to develop as Sertoli cells. Subsequent cellular events include an increase in Sertoli cell mass, the attraction of cells from the mesonephros and the outer surface of the genital ridge, and the formation of testicular cords by 13.5 dpc. The cords comprise Sertoli cells surrounding germ cells. The somatic cell migrations are male specific and occur as a response to *Sry* expression [9]. Germ cells arise during early embryogenesis at the base of the allantois. They become incorporated into the hindgut during their migration to colonize the developing gonads in the genital ridge [10]. The newly programmed Sertoli cells express another HMG box-containing protein, SOX9 (Sry like HMG box), one of the earliest markers of Sertoli cell differentiation. *Sox9* has a significant role in the control of male sex determination, and is capable of programming the bi-potential genital ridge to a male fate in genetic females expressing *Sox9* as a transgene [11].

SOX9

Targets of SOX9 have been identified. It has a role in bone morphogenesis, whereby the syndrome of skeletal campomelic dysplasia and associated sex reversal results from mutations in human *SOX9* [12]. The gene is expressed in developing chondrocytes, and regulates the control of anti-Müllerian hormone (AMH) expression in Sertoli cells [13]. This peptide causes regression of the female internal genital structures (Müllerian ducts) in a time-dependent fashion. *SOX9* is located on chromosome 17q24 and is initially expressed in both the male and female genital ridge. Recent studies have suggested how specific regulation ensures that inappropriate expression of *SOX9* does not occur in the female pathway. Long-range repressor elements have been detected by fortuitous deletion upstream of *Sox9*, located on mouse chromosome 11 [14]. This deletion removed repressors of *Sox9* expression so that in XX mice, *Sox9* remained active resulting in the development of testes and complete sex reversal. Regulation of this repressor function is believed to involve both *Sry* and *Dax1*, an orphan nuclear receptor with a known role in the antagonism of *Sry* [15].

DAX1 (DOSAGE-SENSITIVE SEX REVERSAL–AHC CRITICAL REGION ON THE X, GENE 1)

DAX1 is an unusual member of the nuclear receptor superfamily. The C-terminus is similar to the ligand-binding domain (LBD) of nuclear hormone receptors, but the N-terminal region bears no similarity to known proteins. There is no central DNA-binding domain. Powerful transcriptional repression activity is present in the putative LBD, although no specific ligand has been identified [16,17].

Human *DAX1* is located on chromosome Xp21. Mutations of this gene in affected males causes congenital X-linked adrenal hypoplasia congenita, which is also associated with hypogonadotropic hypogonadism. Most are nonsense mutations affecting the N-terminus, whereas missense mutations cluster within the putative LBD [18]. All severely impair the function of DAX1 as a transcriptional repressor. Affected males are not sex reversed. Duplication of *DAX1* in males, however, does lead to sex reversal, confirming *Dax1* as a dose-dependent sex reversal locus [19]. Further characterization suggests that *Dax1* acts to antagonize *Sry*, as a putative anti-testis gene [15]. In the mouse genital ridge, *Dax1* is expressed in both the male and female genital ridges at about 11.5 dpc; thereafter expression is downregulated in the male after the start of testis determination (12.5 dpc) but expression persists in the female genital ridge [20].

SF1 (STEROIDOGENIC FACTOR 1)

SF1 is also an orphan nuclear receptor that is expressed in the genital ridge, adrenals, and the hypothalamo-pituitary region. Targeted mutagenesis in mice and rare natural mutations in humans indicate the role this receptor plays in the control of a myriad of processes involving male sex differentiation, steroidogenesis, and regression of Müllerian structures via AMH [21]. Typically, loss of function *SF1* mutations in humans causes XY sex reversal and adrenal insufficiency.

Recent studies in mice identified a highly conserved *SF1* consensus binding site upstream of *Dax1* [22]. *Sf1*-deficient mice display much reduced expression of *Dax1* in the developing gonad, evidence of the role of *Sf1* in regulating *Dax1* gonadal expression. Two unique, novel orphan nuclear receptors appear to interact critically in the early events of gonadogenesis.

WNT4 (WINGLESS-TYPE MOUSE MAMMARY TUMOR VIRUS INTEGRATION SITE)

WNT4 is a member of a large family of signaling glycoprotein molecules which is expressed in the developing mesonephros. *Wnt4* is downregulated in the testis at 11.5 dpc but persists in the developing ovary [23]. Targeted deletion in mice results in the masculinization of XX pups, with stabilization of male internal genitalia (the Wolffian ducts) and degeneration of the female equivalent, Müllerian ducts [23]. Duplication of *WNT4* results in XY sex reversal and in vitro analysis suggests that *Dax1* is upregulated by *Wnt-4* expression [24]. These observations imply that *Dax1* and *Wnt-4* may represent anti-testis genes, contributing to ovarian development by preventing testis formation [25,24].

Several other genes with roles in the control of mammalian gonad development have been identified, primarily from targeted gene disruption experiments. None are members of the nuclear receptor family. They can be broadly grouped according to their function in the early stages of differentiation of the urogenital system. A prime example is *WT1* (Wilm's tumor suppressor gene). This is characterized by a proline and glutamine-rich transregulatory region in the N-terminus of the protein and four zinc fingers in a C-terminal DNA-binding domain [26]. There is thus similarity with the nuclear receptor family as far as the zinc finger moieties, but there is no LBD. Mutations in *WT1* in humans gives rise to syndromes such as the Denys-Drash and Frasier syndromes, characterized by gonadal dysgenesis, nephropathy, and Wilm's tumor of the kidney [27,28]. A number of useful reviews may be referred to for a description of other genes that may play key roles in the development of the genital ridge and testis determination [29,30].

SEX DIFFERENTIATION

Fetal sex differentiation proceeds in an orderly sequence in the male following testis determination (Fig. 1). The first event is regression of the Müllerian ducts mediated by anti Müllerian hormone (AMH) secreted from the Sertoli cells. AMH is a member of the transforming growth factor β family and acts through an AMH Type II receptor expressed in the mesenchyme of the Müllerian duct [31]. This results in regression of the Müllerian ducts by 10 weeks of fetal age. Mutations in either the *AMH* gene or the AMH Type II receptor gene lead to persistence of uterus and Fallopian tube development in otherwise normally differentiated males [32]. The most frequent mutation is a 27 bp deletion in the *AMH Type II* receptor gene [33]. During this period of regression, Leydig cell activity is initiated resulting in fetal testosterone synthesis. Androgen production is initially gonadotrophin-independent before becoming placental hCG-dependent and then later in gestation, under the control of fetal pituitary LH secretion. Fetal serum testosterone concentrations increase to 10 nmol/L or more, levels comparable to adult males. Testosterone stabilizes and differentiates the Wolffian ducts to form the male internal genitalia. The central role of androgens in stabilization of the Wolffian ducts to form the vas deferens, epididymis, and seminal vesicles was originally proposed based on the results of the classic fetal rabbit castration experiments of Jost [1]. However, the observation of Wolffian duct stabilization in males with testosterone biosynthetic effects (17β-hydroxysteroid dehydrogenase deficiency) and resistance to the action of androgens suggests non-androgen dependent effects may have a role in Wolffian duct stabilization [34]. The external male genitalia are also masculinized by androgens although this is primarily mediated by dihydrotestosterone (DHT), a metabolite of testosterone which is more potent. Development of the prostate is also DHT-dependent.

The final step in the sequence of male fetal development is the descent of the testes into the scrotum. The testes migrate from the lower pole of the kidney to the scrotum in a two-stage process of transabdominal migration and inguinoscrotal descent [35,36]. The testis is initially attached to the lower pole of the kidney by the cranial suspensory ligament. This persists in the female to maintain the ovary on the pelvic wall. In the male, the suspensory ligament degenerates by apoptosis in a part-androgen dependent manner. The gubernaculum is also critical for testicular descent. It develops from the caudal suspensory ligament; contraction and thickening produces a bulbar outgrowth to guide the migrating testis towards the internal inguinal ring. The gubernaculums persists as a thin cord in the female. Gubernacular development may be partly androgen dependent, but also appears to be influenced by Insl3 (insulin-like 3) and its receptor [37–39]. Failure of the testes to descend by birth in boys is a com-

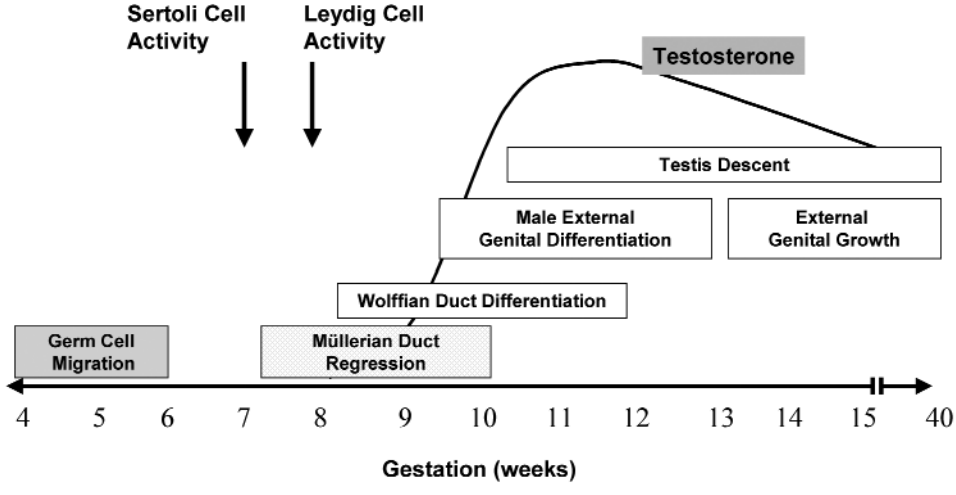

Fig. 1 A schematic of the embryology fetal male development.

mon problem. However, mutations of *INSL3* in boys with bilateral cryptorchidism are rare [40]. The final phase of inguinoscrotal descent of the testes is androgen-dependent, as shown by the gonadal positioning in disorders of androgen insensitivity and hypogonadotrophic hypogonadism [41].

The ontogeny of fetal male sex development involving differentiation of the internal and external genitalia is under the trophic control of androgens. This process is time- and concentration-dependent. Critical to stabilization of the Wolffian ducts and differentiation and growth of the external genitalia is ligand-activation of the nuclear AR. The receptor is ubiquitously expressed, but particularly in sex-dependent tissues. The role of the AR as a transcription factor and its importance in male development can be observed by the study of patients with the androgen insensitivity syndrome (AIS).

ANDROGEN INSENSITIVITY SYNDROME

The clinical manifestation of AIS varies in severity from completely female external genitalia (CAIS) to varying degrees of partial masculinization (PAIS). Testis determination and function is normal during fetal life in AIS. Thus, Sertoli cell production of AMH causes Müllerian duct regression and Leydig cells produce an abundant amount of testosterone with normal conversion to DHT. However, there is resistance to the action of androgens. In postnatal life, CAIS can present in infancy with bilateral inguinal swellings due to herniated testes. Otherwise, presentation is delayed until adolescence with primary amenorrhoea being the stimulus for investigation. This would include a karyotype that reveals the XY chromosomes. Breast development is normal, as androgens are readily aromatized to estrogens. Pubic and axillary hair is absent or scanty because of the androgen resistance. Internal female genitalia are absent, but there is a shortened, blind-ending vagina. In PAIS, there is some responsiveness to androgens which typically is manifest with the appearance of micropenis, bifid scrotum, and a perineoscrotal hypospadias. In some cases, the resistance to androgens is very minimal with a mild, isolated hypospadias or even oligospermia in an otherwise normally masculinized male [42]. Serum concentrations of LH are generally elevated in AIS, despite the increased testosterone levels. Histology of the testes shows absence of spermatogenesis, but often interstitial cell hyperplasia. There is an increased risk of gonadoblastoma formation.

ANDROGEN RECEPTOR

The AR is encoded within a 90 kb region on Xq11-12. It consists of 8 exons and is translated from a 10.5 kb mRNA comprising 919 amino acids [43]. The AR protein comprises three functional domains, in common with other nuclear receptors. The N-terminal domain is the least conserved amongst members of the nuclear receptor family. The AR contains an activation function domain AF-1 comprised of two subdomains, AF-1a and AF-1b. It has a transcriptional activation role, regulating ligand-dependent activation of responsive genes. This regulation is attenuated by polymorphic regions in the N-terminus, with a variable number of glutamine repeats in the 5′ terminus and glycine repeats in the 3′ end. A link between variation in transactivational capacity, and the number of glutamine repeats has been confirmed [44].

The central region of the AR protein comprises the DNA-binding domain (DBD) and represents the region of highest homology amongst the nuclear receptors. It contains two zinc fingers which each employ two pairs of cysteine residues to bind the zinc ions. The DBD encompasses 68 amino acids encoded by exons B and C. The C-terminal domain of the AR comprises the LBD containing a second activation function domain AF-2. A number of additional functions map to this domain and include heat shock protein interactions, dimerization, and nuclear localization signals. The role of the AF regions appears to entail interaction with coregulator proteins to regulate specific genes via the general transcriptional machinery [45]. This interaction is ligand-dependent and results in either induction or repression of transcription depending on whether coactivators or corepressors are complexed with the AR [46,47]. AF-1 functions in a ligand-independent manner, as shown by deletion mutants of the AR lacking the

LBD, with constitutive activity in vitro [48,49]. The AF-2 domain functions in a ligand-dependent manner and is located within helix 12 of the LBD. The AR, in common with several other nuclear receptors, has 12 alpha helices arranged in an anti-parallel fashion in three layers in the form of a sandwich fold. Helix 12 is the most C-terminal helix and forms a hydrophobic cleft in the presence of ligand, allowing coregulator binding via L*XX*LL (L, leucine; *X*, any amino acid) motifs. A number of coregulators are known to interact with the nuclear hormone receptors, a few being more AR specific. Examples include ARA70, which interacts with the AR in a ligand-dependent manner, even though it appears to act only as a weak activator in vitro [50]. Mutation analysis in XY patients with incomplete masculinization, however, has not identified mutations in ARA70 that may affect AR-dependent function [51].

N/C INTERACTION

N-terminal/C-terminal interaction has been demonstrated for the AR protein, as well as the estrogen and progesterone receptors [52]. The AF-2 region of the AR is required for N/C interaction, a function believed to be important for stabilization of the receptor by reducing ligand dissociation rate and degradation kinetics of the LBD/ligand complex [53]. The result is enhanced DNA-binding affinity and subsequent transactivation ability of the AR. A number of LBD mutants have been identified that disrupt N/C interaction potential but retain ligand-binding ability; these mutations can result in a phenotype consistent with AIS [54].

ANDROGEN-BINDING ASSAY

Androgen receptor binding ability is readily measured in cultured genital skin fibroblasts. A normal range for binding parameters can be derived by using circumcised foreskin explants, which give consistently reproducible results. There is considerable variation in receptor binding activity when using skin derived from other sites, such as suprapubic and scrotal skin (personal observations). A typical analysis of an androgen-binding assay is shown in Fig. 2. Primary cell cultures are established from 2–4 mm genital skin explants obtained at the time of surgery. Isolated cells are grown to confluency and starved of serum for 24 h prior to assay. For the binding assay, cells are seeded at a known density and incubated with increasing concentrations of ^{3}H-DHT, with or without a 200-fold excess of unlabeled ligand. An aliquot of cells is retained to quantify DNA content. Binding capacity (Bmax) and dissociation constant (Kd) are derived from Scatchard analysis using linear regression analysis to provide a

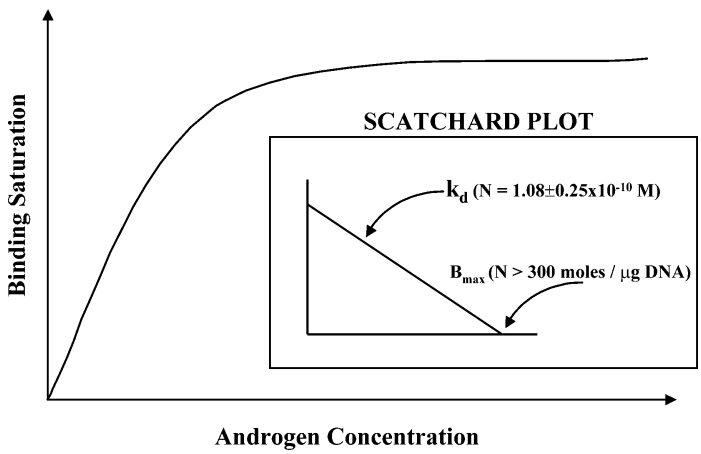

Fig. 2 Saturation curve of androgen binding in genital skin fibroblasts. The Scatchard analysis is based on bound/free hormone vs. bound.

best fit for the binding data [55]. AR expression in genital skin fibroblasts remains relatively constant, in contrast to 5 alpha reductase activity, which declines with age [56].

AR MUTATIONS

Numerous mutations have been identified throughout the AR and are detailed on an international database <http://www.mcgill.ca/androgendb>. Mutations associated with AIS cluster within the LBD, but are also located throughout the whole of the protein coding region, and less frequently within the intron/exon boundaries. A number of AR mutations associated with breast and prostate cancer are also detailed on this database.

The Cambridge Intersex Database contains a similar spread of mutations identified in a large number of patients with AIS (Fig. 3). The results are complemented by detailed clinical, biochemical, and histological information. Also included on the database is information on other sex reversal disorders whose phenotypes may be similar to CAIS and PAIS. Examples include complete and partial gonadal dysgenesis, androgen biosynthetic defects, sex chromosome abnormalities, hermaphroditism, and male factor infertility. This unique resource is now derived from information on more than 1000 cases.

Mutations of the AR in cases of CAIS are routinely identified in over 80 % of those analyzed, the majority of mutations located in the LBD. In contrast, only about 20 % of cases with findings consistent with PAIS have an identifiable *AR* gene mutation [57].

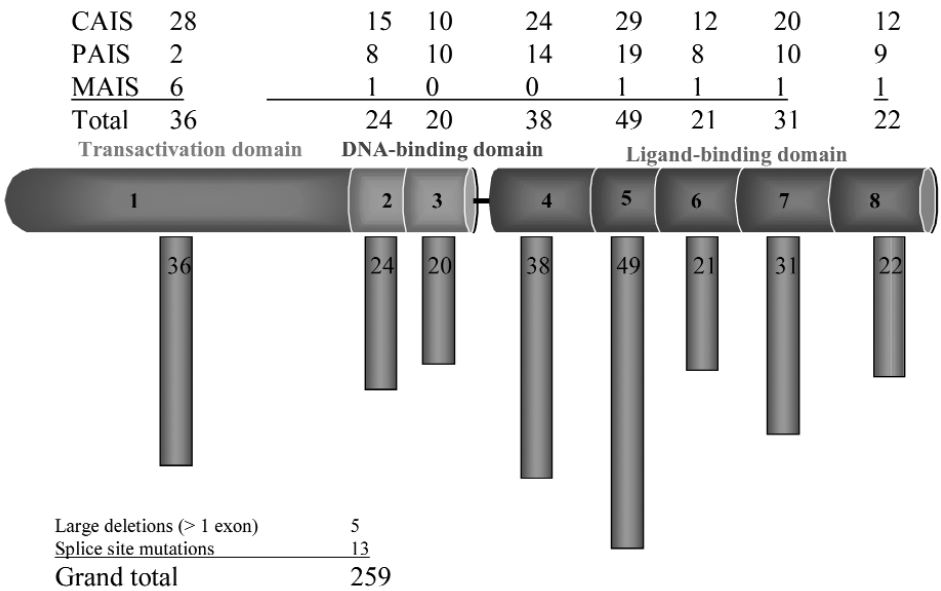

Fig. 3 The Cambridge AR mutation database. The number of mutations located in each exon (1–8) is shown. MAIS, mild androgen insensitivity syndrome.

Mutant AR proteins

Functional analysis of AR mutations can provide further understanding on which regions of the receptor are critical for activity. The crystal structure of the AR-LBD has now been elucidated [58]. This has enabled a number of residues with roles in ligand selectivity and stability of the AR protein to be identified. Mutations located within the most C-terminal alpha-helix 12 display ligand selectivity in functional studies. Thus, mibolerone, which is a potent and nonmetabolizable androgen, is transactivational in a reporter gene assay, whereas that is not the case with DHT (personal observations).

We have recently reported molecular studies on a pair of identical twins with CAIS in whom two novel *AR* gene mutations were identified [59]. In functional assays using HeLa cells, mutation Phe856Leu transactivated more than the wild-type, whereas mutation Ser865Pro completely ablated androgen-dependent transactivation (Fig. 4). Serine865 is located in helix10/11 within a region important for the positioning of helix 12. The latter is critical for coactivator binding. Thus, the functional consequences of mutation Ser865Pro are related to ligand binding, dimerization, and receptor activation; features manifest phenotypically as complete sex reversal in XY twins.

A number of mutations residing outside the LBD have been identified with relevance to N/C interaction and AIS. Specific mutations associated with the hinge region of the AR (between amino acids 621 and 671) have been shown to regulate N/C interaction ability and may have a repressive role on transactivation as shown by deletion mutants in this region [60].

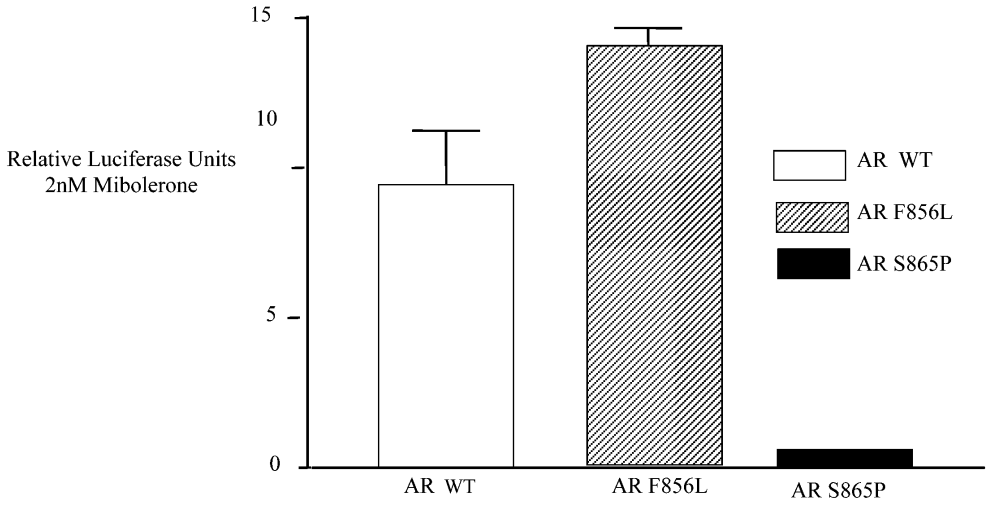

Fig. 4 Androgen-induced transactivation of wild-type AR and 2 mutant ARs transiently expressed in HeLa cells. A glucocorticoid response element-luciferase reporter system was used to measure transactivation capacity. Transfected cells were incubated for 48 h with 1 nM mibolerone.

AR polymorphisms

Located within the N-terminus of the AR is a CAG repeat trinucleotide producing a variable number of glutamine residues in the protein. This polyglutamine variation is an important modulator of receptor function, which may have a contributory role in disorders affecting the male reproductive tract. The neurodegenerative disorder, spinal and bulbar muscular atrophy (Kennedy's disease), is characterized by a hyper-expanded number of CAG repeats in the AR. Affected patients also show evidence of reduced AR function as mild androgen insensitivity and decreased spermatogenesis [61]. A longer (CAG)n tract, but within the normal population range (11–31 repeats in a Cambridge population) has been reported to be associated with reduced spermatogenesis in otherwise normal males in some Australian, North American, Japanese, and Singaporean populations [62,63]. However, this is not universally found, particularly in Scandinavian studies [64]. Shorter repeats are associated with an earlier age of onset of prostate cancer and the response to androgen ablation therapy [65,66]. A similar association was observed in men with androgenetic alopecia [67] and in older adults with indices of central obesity [68].

We have demonstrated that longer CAG repeats are associated with undermasculinization to varying degrees of unknown cause [69]. When the cause of a developmental disorder is multifactorial, the significance of a polymorphism such as the AR (CAG)n repeat may be more than just an association,

but actually plays a causative role in the phenotype [70]. These observations in a heterogeneous group of pathophysiological situations (see Fig. 5) may be explained by the clear association found in vitro between the length of the CAG repeat and the activity of the AR as a transcription factor [71]. A number of control points operate in fetal male sex differentiation, several of the genes involved exhibiting polymorphisms. It is expected, therefore, that associations may be additive. This was the case when the cohort we studied with male undermasculinization of unknown cause was analyzed for the AR (CAG)n and a LH receptor polymorphism combined [72]. It would be anticipated that the effects of endocrine disruptors on fetal and early postnatal development may be more profound, depending on a particular genetic background.

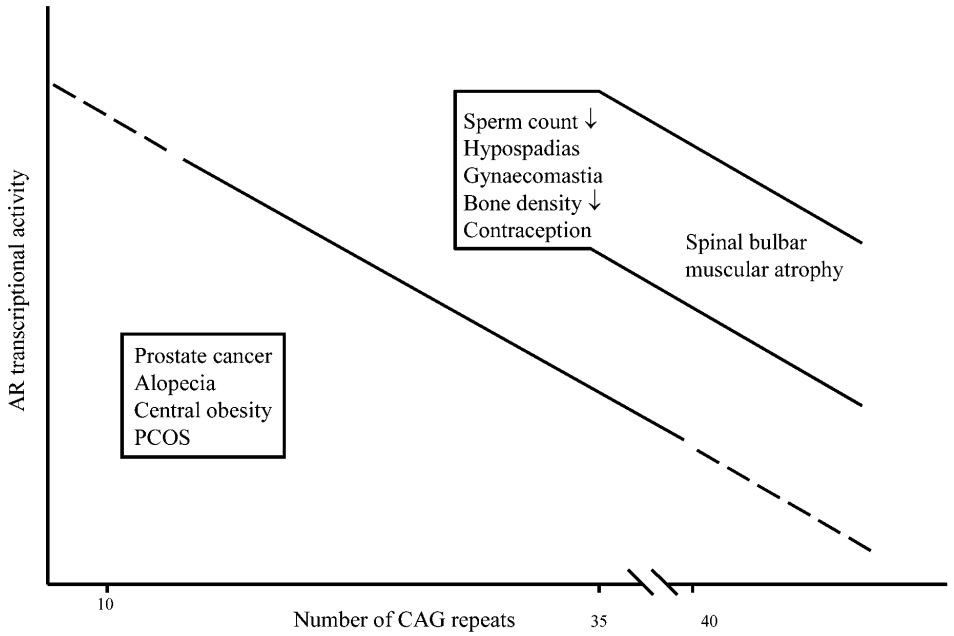

Fig. 5 Theoretical relationship between length of (CAG)n and AR transcriptional activity: relevance to a heterogeneous group of conditions. PCOS, polycystic ovarian syndrome.

AR cofactors

ARA70 has been identified as a coactivator specific to the AR in human prostate cells [73]. It is possible that this cofactor enhances transcription by weak androgens. Another cofactor, ARA24, is believed to interact with the AR polyglutamine repeat [74]. In our study of undermasculinized males, we found no evidence of mutations or polymorphisms in ARA24 associated with the patient phenotype [75]. In contrast, we did observe an association between a shorter number of glutamine repeats in SRC3, an AR coactivator [76,77]. It is quite plausible that these polymorphisms may account for the anti-androgen effects of some endocrine disruptors. AR transcription is regulated in an inhibitory fashion by a corepressor, SMRT (silencing mediator for retinoid and thyroid hormone receptors). Over-expression of SMRT inhibits androgen-dependent AR-mediated transactivation and enhances the effects of anti-androgens such as flutamide [78]. The mechanism of action probably involves inhibition of N/C terminal interaction.

Endocrine active substances

In the context of an apparent increase in reproductive disorders, AR function is relevant to the study of chemicals that may act as anti-androgens or directly as androgens [79,80]. To that end, we have developed an adenovirus-based assay using genital skin fibroblasts to assess AR function in response to test chemicals that may have androgenic or anti-androgenic properties (Fig. 6). The cell line derived from cultured foreskin explants, is telomerase immortalized (Clontech). It expresses the wild-type AR, binds androgens as a high affinity association, and displays a normally regulated AR signaling pathway (personal observations).

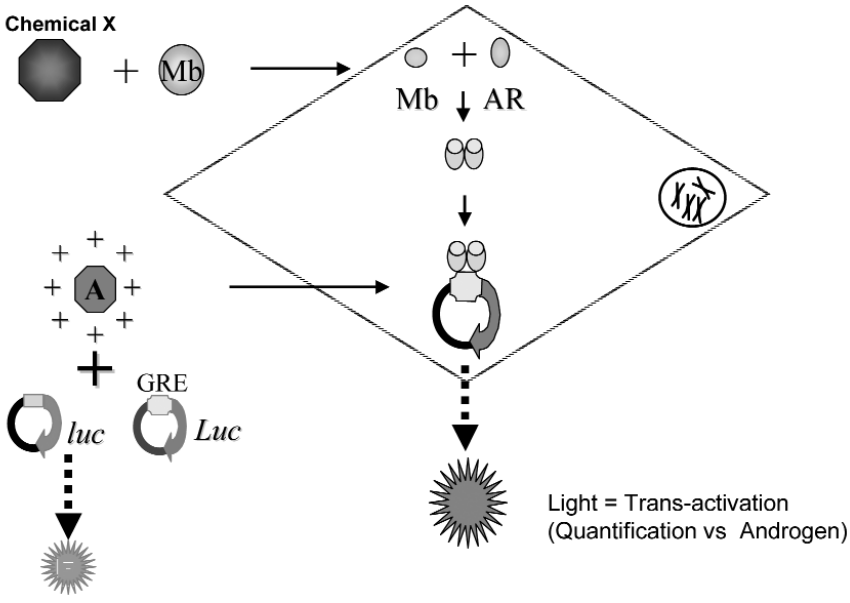

Fig. 6 Adenovirus based reporter gene assay to measure anti-androgenic activity of test chemicals. Genital skin fibroblast cells are transiently transfected with an androgen responsive—firefly luciferase reporter (GRE-*Luc*) and a control renilla luciferase plasmid (*luc*). Cells are exposed to test chemical either in isolation or in combination with 0.1 nM mibolerone. Antiandrogenic response is measured as a ratio of firefly to renilla activity, and compared to the AR transactivation ratio with 0.1 nM mibolerone. Androgenic activity is assessed by chemical-induced transactivation in the absence of mibolerone.

Figure 7 illustrates the effect of one chemical, bisphenol A, acting as an antiandrogen based inhibition of mibolerone-induced AR transactivation of luciferase. The methodology has now been validated for the study of a range of environmental chemicals, which may affect development of the reproductive system in fetal and early postnatal life.

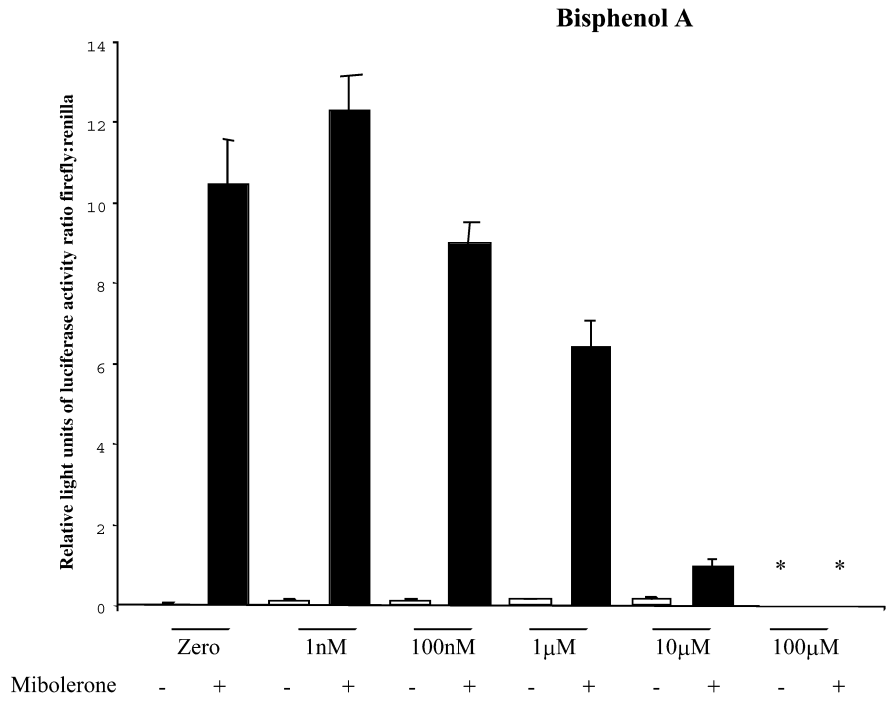

Fig. 7 Effect of bisphenol A as an antiandrogen. Increasing concentrations of bisphenol A demonstrate antiandrogenic activity by inhibition of mibolerone-induced AR transactivation. Lack of androgenic activity is confirmed by absence of bisphenol A-induced transactivation.

CONCLUSION

Two orphan nuclear receptors are inter-linked in contributing to the formation of the testis in the developing male. The nuclear AR, however, is the key molecular transducer of events in fetal male sex differentiation. The genetic and hormonal control of this process is time- and threshold-dependent. A number of single gene defects cause profound effects manifested sometimes as complete sex reversal. The exquisite sensitivity of the developing reproductive tract to hormones suggests that endocrine active substances may play a role in some disorders of human sex differentiation.

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