

Disorders linked to insufficient androgen action in male children

Charles Sultan^{1,2,3}, Françoise Paris^{1,2}, Béatrice Terouanne², Patrick Balaguer²,
Virginie Georget², Nicolas Poujol², Claire Jeandel¹, Serge Lumbroso² and
Jean-Claude Nicolas²

¹Unité d'Endocrinologie et Gynécologie Pédiatriques, Service de Pédiatrie I, Hôpital A. de Villeneuve, Montpellier, France and

²Hormonologie (Développement et Reproduction) and INSERM U-439, Pathologie Moléculaire des Récepteurs Nucléaires, Hôpital Lapeyronie, CHU Montpellier, France

³To whom correspondence should be addressed at: Unité d'Endocrinologie et Gynécologie Pédiatriques, Service de Pédiatrie I, Hôpital A. de Villeneuve, 34295 Montpellier Cedex 5, France. E-mail: chsultan@u439.montp.inserm.fr

Virilization of the external genitalia in the male fetus requires testosterone and dihydrotestosterone (DHT), which is formed from testosterone by the action of the enzyme, 5 α -reductase type 2 (5 α R-2). Mediation of the effects of both testosterone and DHT requires a functional androgen receptor (AR) located in the cytoplasmic compartment of target cells. DHT (or testosterone) binding induces a conformational change which facilitates AR nuclear transport, phosphorylation and dimerization, ultimately regulating of the rate of transcription of androgen-dependent genes. Any event which impairs DHT formation (mutation within the 5 α R-2 gene or 5 α R-2 inhibitors) or normal function of the AR (mutation in the AR gene, antiandrogens) may result in insufficient androgen action in the male fetus and in subsequent undervirilization in the newborn. Hypospadias may be due to a defect in androgen action due to mutation of the 5 α R-2 or of the AR gene. Mutation of unidentified genes is likely to underlie this displacement of the urethral meatus from the tip to the ventral side of the phallus. An aetiological role for environmental chemical products has been postulated, since ethnic as well as geographical differences in the incidence of hypospadias have been noted. Increasing evidence has been gathered indicating that widely used industrial and agricultural chemicals have deleterious effects on normal male sexual differentiation. Cryptorchidism and micropenis may represent an intersex phenotype, even if they are isolated. Aetiological factors include 5 α R-2 gene mutation, AR gene mutation or environmental hormonal disruptors. In conclusion, several phenotypes have been attributed to insufficient androgen action during fetal life. Whereas mutations in the 5 α R-2 gene and AR gene are natural, attention should be focused on environmental endocrine disruptors that are able to mimic steroid 5 α -reductase deficiency or partial androgen insensitivity syndrome.

Key words: androgens/cryptorchidism/endocrine disruptors/hypospadias/sexual differentiation

TABLE OF CONTENTS

Introduction
Role of androgens in fetal male sexual differentiation
Recombinant receptor-reporter gene assay
Disorders linked to insufficient androgen action
Conclusions
References

Introduction

Normal human male sexual differentiation begins with the sex determination (testis development), and is followed by sex

differentiation (genital development). The stimulatory events of male sex differentiation require the presence of androgens and a functional androgen receptor (AR) (Migeon *et al.*, 1994).

Development of the internal male genitalia is induced by the action of testosterone itself; masculinization of the external genital primordia requires dihydrotestosterone (DHT), a 5 α -reduced testosterone. Although defects in genes coding for the action of androgens in target cells have been identified that act to alter normal sex differentiation, disorders linked to insufficient androgen action can be also caused by any environmental chemical that impairs DHT synthesis or AR mediation in the male fetus (Sultan *et al.*, 2000).

Role of androgens in fetal male sexual differentiation

In the human male embryo, the testes begin to secrete androgens at 9 weeks of gestation. Testosterone, which peaks between 11 and 18 weeks of gestation, stimulates differentiation of the Wolffian duct system into the epididymis, vas deferens and seminal vesicles. Development of the prostate from the urogenital sinus and masculinization of the external genital primordia into the penis and scrotum require the more potent androgen, DHT, which is converted from testosterone by the action of the enzyme 5α -reductase type 2 that is expressed in these tissues (Figure 1).

Development of the Wolffian duct, as well as virilization of external genitalia, occurs between 9 and 15 weeks of gestation. In parallel, the Sertoli cells of the fetal testes secrete anti-Müllerian hormone (AMH), which induces the regression of the Müllerian duct. Mediation of the action of both testosterone and DHT requires the presence of a nuclear receptor within the internal as well as the external genitalia. In the human male fetus, AR is expressed as early as 8 weeks of gestation, before the onset of androgen secretion: androgen binding is higher in genital than in urogenital areas. This AR is a transcriptional factor that requires high-affinity androgen binding to initiate a series of molecular events leading to the specific gene activation required for male sex development.

AR is a member of the nuclear receptor (NR) family that includes receptors for steroid and thyroid hormones, vitamin D₃ and retinoic acids, and numerous orphan receptors for which no ligands are known (Mangelsdorf *et al.*, 1995). NRs are modular proteins that can be divided into separable domains with specific functions, such as ligand binding, dimerization, DNA binding and transactivation. In the absence of ligand, the AR resides in the cytoplasm (Georget *et al.*, 1997). Hormone binding induces a transconformation of the receptor and allows its translocation into the nucleus, where it initiates transcription through specific interactions with the transcription machinery (Figure 2).

To better understand the specific recognition of ligands by the human androgen receptor (hAR), we recently constructed a homology model of the ligand-binding domain (LBD) of AR, based on the progesterone receptor LBD crystal structure (Poujol *et al.*, 2000). This model revealed that: (i) the ligand-binding pocket is lined by 19 residues, mainly hydrophobic; (ii) R752 and Q711 participate in the binding of the 3-ceto group of the androgen ligand; and (iii) N705 is presumably the anchoring site of the 17β hydroxyl group. This has been further confirmed by in-vitro investigations.

In summary, virilization of the external genitalia in the male fetus is completed at 14 weeks of gestation. From 10 weeks gestation to birth, the penile length increases linearly. The hormonal control of the descent of the testis involves the presence of testosterone, DHT, AMH, insulin-like factor (INSL)-3 and functional AR (Figure 3).

Any event which could impair DHT formation (mutation within the 5α -R-2 gene or 5α R-2 inhibitors) or normal function of the AR (mutation in the AR gene, antiandrogens) may result in insufficient androgen action in a male fetus, and in subsequent undervirilization in the male newborn (Sultan and Savage, 1998).

Recombinant receptor-reporter gene assay

Recombinant receptor-reporter gene bioassays are required to evaluate the oestrogenic/antioestrogenic, androgenic/antiandrogenic activities of environmental chemicals and to identify new products present in food and water. Simple cell models that express a gene under the control of defined promoters responding to specific drugs and that produce a signal that is easy to quantitate are of great interest for rapid screening of the biological effects of artificial or natural compounds. In integrated systems, these cell models are very useful to study the synergy or antagonism of different substances and, in the field of environ-

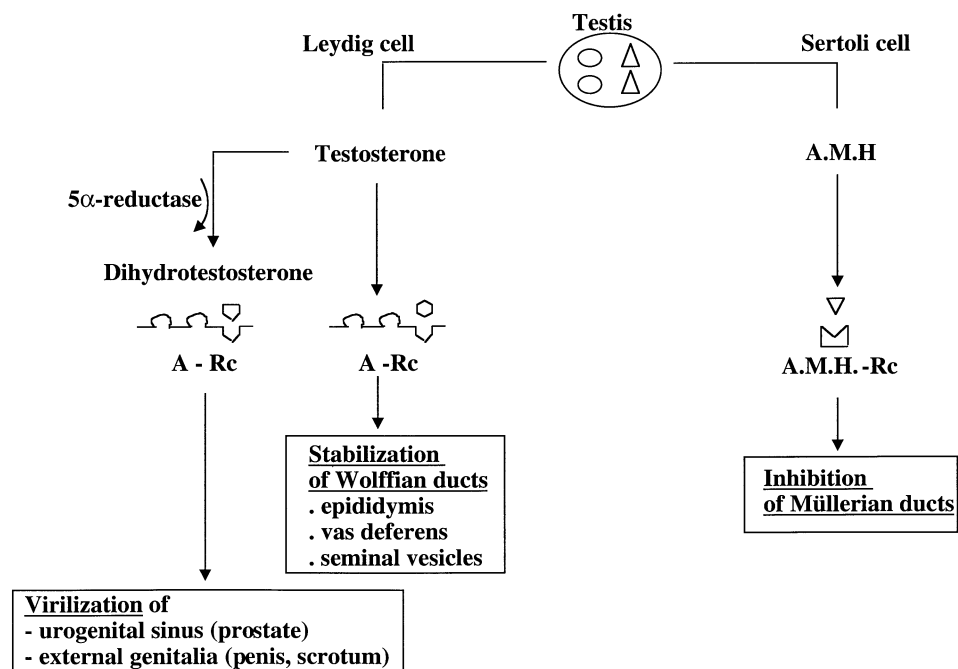


Figure 1. Sexual differentiation of the male fetus. A-Rc = androgen receptor; A.M.H. = anti-Müllerian hormone; A.M.H.Rc = AMH receptor.

mental research, they are excellent tools to identify compounds able to disrupt endocrine functions.

Numerous cell lines have been established using a technology based on the bioluminescent gene reporter (Balaguer *et al.*, 1999, 2000; Terouanne *et al.*, 2000). Analysis and selection of stable transfectants were simplified using a low-light imaging system. These systems have been used to evaluate the biological activity

of compounds found in the environment, and to identify new products present in wastewater effluents.

Stable bioluminescent cells responding to oestrogens

Several cell lines responding to oestrogen have been obtained, including cells with different enzymatic activity and cells expressing chimeric receptor or natural oestrogen receptor (ER)

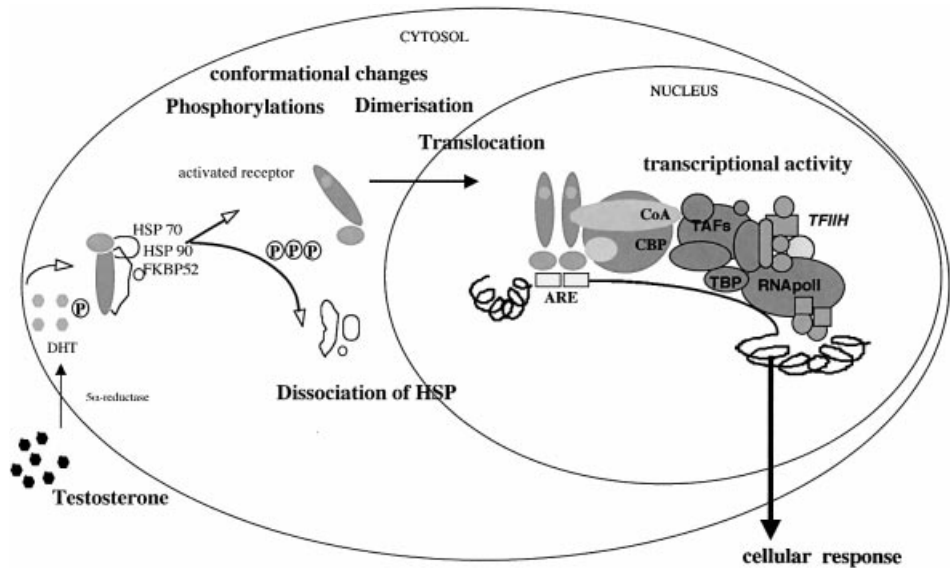


Figure 2. Molecular action of androgens within target cells. ARE=Androgen-Responsive element; HSP=heat shock protein; CBP=CREB-binding protein; DHT=dihydrotestosterone; TAFs=TBP-Associated Factors; TBP=TATA-binding protein; TFIIF=Transcriptional Factor IIF.

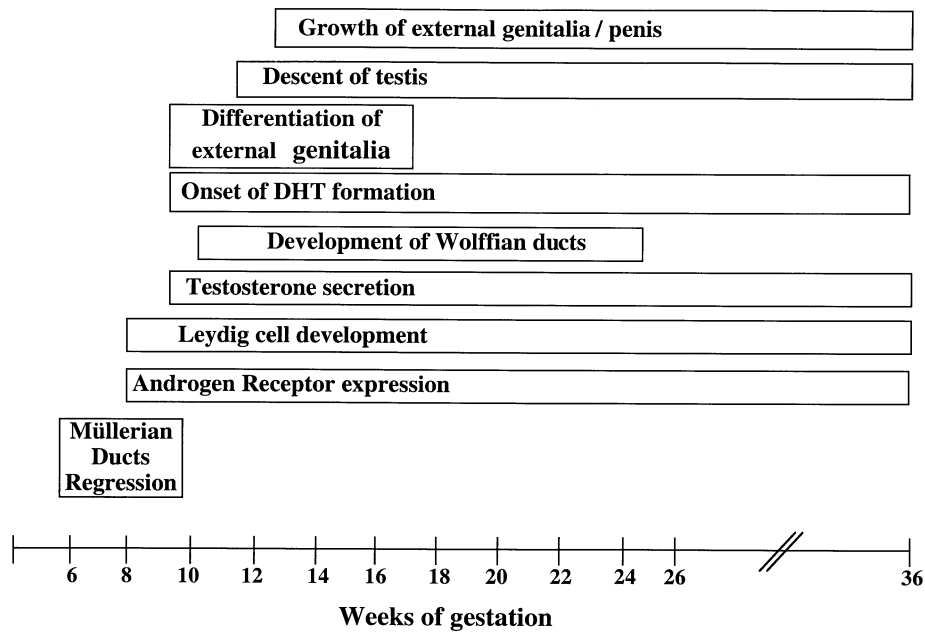


Figure 3. Schematic view of development of male sex differentiation. AR=androgen receptor.

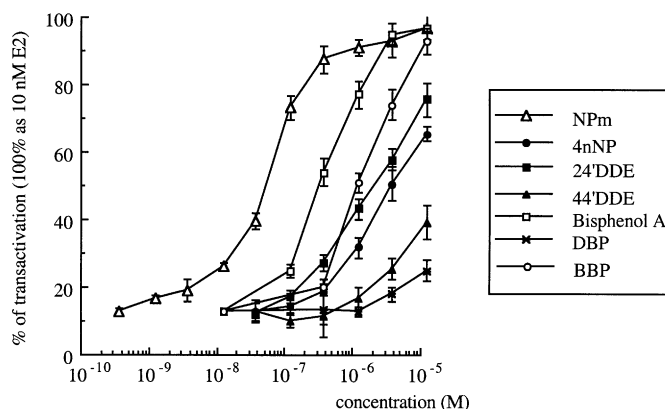


Figure 4. Induction of luciferase activity by xeno-oestrogens in the HeLa cell line, stably transfected with the reporter plasmid ERE-Luciferase and the expression vector ER α . Results are expressed as a percentage of luciferase activity measured per well. The 100% value represents the value obtained in presence of oestradiol 10^{-8} mol/l. NPm=nonylphenol mixture; 4nNP=4-n-nonylphenol; 2,4'DDE=2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene; 4,4'DDE=2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; BisA=2,2-bis(4-hydroxyphenyl)propane; DBP=dibutyl phthalate; BBP=benzyl butyl phthalate.

α or β . These cell lines could not only be used in pharmaceutical research, but they would also be helpful for monitoring the biological activity of pesticides, fungicides and chemicals found in plastics or discarded in the environment (Balaguer *et al.*, 1999, 2000).

The detection limit of these bioassays is lower than 10^{-12} mol/l oestradiol, and high-throughput screening could be performed using a 96-well microplate format.

The oestrogenic activity of plasticizers (nonylphenol), fungicides, pesticides (DDE products) and phthalates are presented in Figure 4. Using a HELN cell line (derived from a HeLa cell line), a luciferase activity was seen to be induced by these chemicals when tested at concentrations above 10^{-8} mol/l. Transcriptional activities of phyto-oestrogens were analysed in stable cell lines expressing either ER α or ER β .

Cells expressing the AR and a bioluminescent reporter gene

In order to generate a powerful tool for the investigation of androgen action and the rapid screening of novel agonists and antagonists, a new stable prostatic cell line was developed. A line of AR-deficient PC-3 cells was stably transfected with a human AR (hAR) expression vector and the reporter gene MMTV-luciferase (Terouanne *et al.*, 2000). One highly inducible clone was selected and named PALM, for PC-3-Androgen receptor-Luciferase-MMTV. It was characterized by its response to androgens and antiandrogens, as reflected by the expression of measured luciferase.

This new and original cell line model provides an easy and rapid bioluminescent test to characterize new agonists and antagonists. The antiandrogenic activities of pesticides, herbicides and fungicides are reported in Figure 5. As described by several authors in transient transfection (Kelce *et al.*, 1994, 1995; Vinggaard *et al.*, 1999), at concentrations higher than $1 \mu\text{mol/l}$,

Table I. Summarized results of oestrogenic and antiandrogenic activities of different chemicals on stable cell lines expressing oestrogen receptor (ER) α or androgen receptor (AR)

Chemical	Cell line	
	HELN (ER α)	PALM (AR)
4nNP	↑	0
DBP	↑	0
BBP	↑↑	0
NPm	↑↑	↓
2,4'-DDE	↑	↓
4,4'-DDE	↑	↓
Bisphenol A	↑	↓
PCB	0	↓
Arochlor	0	↓
M1	0	↓
M2	0	↓↓

↑ = agonist activity; ↓ = antagonist activity.

4nNP=4-n-nonylphenol; DBP=dibutyl phthalate; BBP=benzyl butyl phthalate; NPm=nonylphenol mixture; 2,4'DDE=2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene; 4,4'DDE=2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; Bisphenol A=2,2-bis(4-hydroxyphenyl)propane; PCB=3,3', 4,4'-tetrachlorobiphenyl; Arochlor=polychlorinated biphenyl mixture; M1=2-[(3,5-dichlorophenyl)-carbonyl]oxy-2-methyl-3-butenic acid; M2=3',5'-dichloro-2-hydroxy-2-methylbut-3-analide.

xenohormones are able to antagonize the activity mediated by R1881 methyltrienolone, a synthetic androgen at 0.1 nmol/l, while the vinclozoline metabolite M2 antagonizes this activity at 30 nmol/l.

A summary of oestrogen-like activity, as well as antiandrogenic activity, of the tested environmental disruptors is presented in Table I.

Cells expressing green fluorescent protein (GFP)-AR

The analysis of the subcellular localization of the steroid receptors has usually been performed by immunocytochemistry. It is generally acknowledged that the ER and the progesterone receptor (PR) are predominantly nuclear, with a continuous shuttle between the nucleus and cytoplasm. The intracellular localization of the mineralocorticoid receptor (MR), the glucocorticoid receptor (GR) and the AR are more controversial. Depending on the immunostaining protocol, these receptors have been described as being either in the cytoplasm or in the nucleus in absence of ligand, and exclusively in the nucleus after incubation with ligand. These techniques require the fixation and permeabilization of cells, which can lead to artefacts in the pattern of subcellular localization. Moreover, the AR can be in different states, i.e. associated with the heat shock proteins in an unliganded form or associated with DNA or transcription factors in the liganded form. The accessibility of the epitope to antibodies may vary for these different forms and this could induce artefactual results in the immunostaining. Having considered all the limits of immunocytochemistry, a model was developed using a chimera of AR fused to the green fluorescent protein (GFP) (Georget *et al.*, 1997). This fluorescent reporter permitted visualization of the AR in living transfected cells.

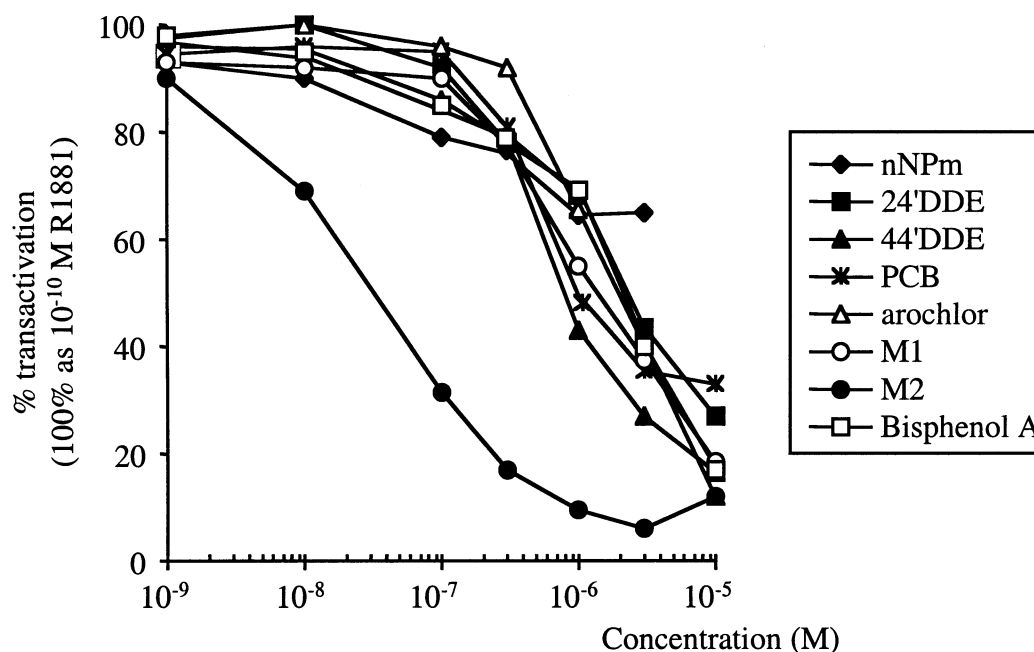


Figure 5. Detection of antiandrogenic activities of xeno-oestrogens with the PALM cell line. Cells were treated with various concentrations of chemicals (1 nmol/l to 10 μ mol/l) with 0.1 nmol/l R 1881. Luciferase activities were expressed relative to androgen receptor (AR) activities with 0.1 nmol/l R 1881, which was set at 100%. NPm = nonylphenol mixture; 4nNP = 4-n-nonylphenol; 2,4'DDE = 2(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethylene; 4,4'DDE = 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene; PCB = 3,3', 4,4'-tetrachlorobiphenyl; Arochlor = polychlorinated biphenyl mixture; M1 = 2-[[3,5-dichlorophenyl]-carbamoyl]oxy]-2-methyl-3-butenic acid; M2 = 3'5'-dichloro-2-hydroxy-2-methylbut-3-analide.

It was first verified that the fusion protein (GFP-AR) conserved the functional characteristics of AR. The advantages of this GFP-AR tool versus immunodetection were demonstrated. The intracellular dynamics of AR were evaluated and quantified in living cells, which suggested some applications of the GFP-AR model, such as antiandrogen screening and androgen insensitivity study (Georget *et al.*, 1998).

An example of inhibition of AR nuclear trafficking by pure antiandrogen is shown in Figure 6. Using GFP-AR protein, a correlation was established between the rate of AR nuclear transfer and the antagonist/agonist activity. Antiandrogens which affect stabilization of active conformation of AR alter the dynamics of nuclear translocation.

Using this method, it was possible to select new compounds able to bind to the AR but unable to trigger the translocation to the nucleus. Unlike classical antiandrogens, these compounds do not exhibit low agonist activity, even at high concentration.

Besides the classical *in-vivo* tests to identify chemicals with endocrine-disrupting activity, such as the uterine weight bioassay, the sex accessory gland weight, or the induction of developmental malformations in offspring, we plan to implant bioluminescent cell lines in the nude mouse in order to evaluate *in vivo* the biological consequences of environmental oestrogens and anti-oestrogens.

Disorders linked to insufficient androgen action

Steroid 5 α -reductase-2 deficiency

Patients with steroid 5 α -reductase-2 deficiency (5 α R-2D), with low synthesis of DHT, are characterized at birth by an

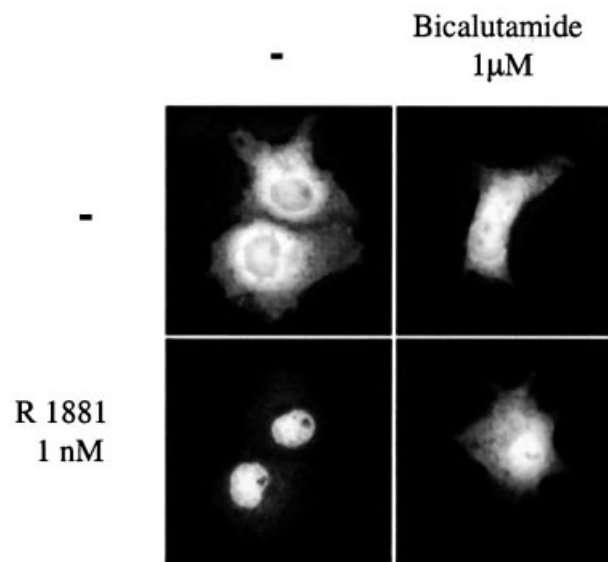


Figure 6. COS-7 cells were transiently transfected with the plasmid coding the fusion protein between the green fluorescent protein (GFP) and the androgen receptor (GFP-AR). After 48 h, cells were incubated for 3 h in presence of R1881 (1 nmol/l), bicalutamide (1 μ mol/l) alone, or in competition with R1881. Cells were observed and recorded directly with an epifluorescence microscopy coupled to a CCD camera. The AR nuclear localization induced by R1881 (bottom left) is inhibited by bicalutamide (bottom right).

undervirilized phenotype: affected newborns exhibit ambiguous genitalia with a hypospadiac phallus resembling a clitoris, a bifid scrotum which is labia-like, and a urogenital sinus opening on the

perineum. It is worth noting that the testes have been found in the inguinal canal, labia majora or scrotum (Wilson *et al.*, 1993).

The clinical presentation can actually range from almost normal female phenotype to a clear-cut male phenotype with isolated hypospadias, but in all cases Wolffian ducts have been differentiated normally into vas deferens, epididymis and seminal vesicles.

The main characteristic of patients with 5 α R-2D is virilization of the external genitalia that occurs at puberty, along with the acquisition of male genetic identity in these patients usually raised as females.

Diagnosis should be based on physical examination, pedigree analysis, analysis of basal and post-human chorionic gonadotrophin (HCG) stimulation, plasma testosterone and DHT concentrations, 5 β /5 α urinary steroid metabolite ratio, measurement of 5 α -reductase activity in cultured genital skin fibroblasts, and finally by analysis of the 5 α -R2 gene.

The characteristic endocrine features of 5 α R-2D are as follows: normal male to high concentrations of plasma testosterone and low concentrations of plasma DHT; an elevated ratio of the concentration of plasma testosterone to DHT in adulthood and after stimulation with HCG in childhood; and elevated ratios of urinary 5 β - to 5 α -metabolites of androgen and C21 steroids. The diagnosis of steroid 5 α -reductase deficiency is mainly supported by an increased testosterone/DHT ratio.

From a biochemical point of view, the decrease in 5 α R-2D activity in the intact genital skin fibroblasts supports the diagnosis of 5 α R-2D, but enzymatic activity is sometimes in the normal range. The decreased activity in sonicated cell extracts at acidic pH provides strong evidence that the mutation resulted in a loss of type 2 enzyme activity (Russell and Wilson, 1994).

Isolation and sequencing of the cDNA encoding 5 α -reductase type 2 (Andersson *et al.*, 1989) provides the molecular tools required for definition of the gene abnormalities responsible for steroid 5 α -reductase deficiency. To date, only three gene deletions have been described (Russell *et al.*, 1994). Indeed, when a sequence alteration is identified it is usually a point mutation (Boudon *et al.*, 1995). These mutations vary greatly and are found throughout the gene. To date, the standard methods of molecular genetic analysis have revealed three nonsense mutations, two splicing defects, and 33 missense mutations. About half of the missense mutations result in a protein with no detectable enzyme activity, whereas the remaining half give rise to proteins with severely decreased but measurable enzyme activity. The last group of mutations can be divided into two classes: those that affect the ability of the enzyme to bind testosterone substrate; and those that decrease the affinity for the NADPH cofactor.

The mutations identified by our group are reported in Figure 7.

The management of steroid 5 α -reductase deficiency is primarily dependent upon the phenotypic findings and gender at the time of diagnosis. Given the severe defect of the external genitalia, most newborns are raised as female! Gonadectomy should be performed early to prevent masculinization, along with vaginoplasty and clitoral reduction. If the diagnosis is made in puberty, one can consider raising such a child as male.

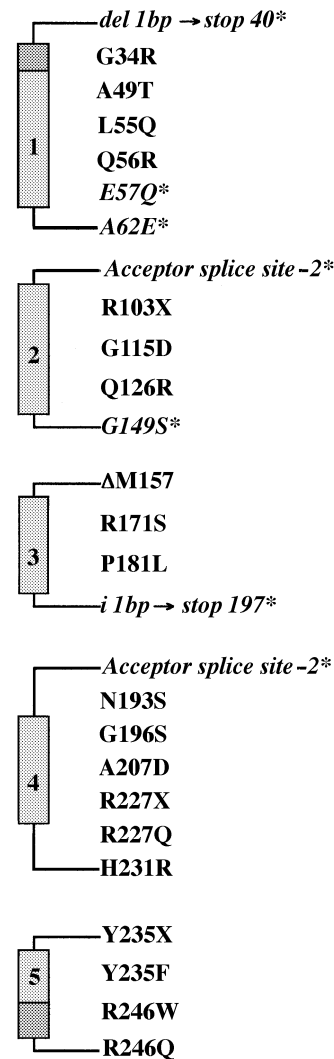


Figure 7. Mutations of 5 α -reductase type 2 gene identified by the authors' group. Location of the mutations is shown on the five exons of the gene. Mutations also described by other groups are shown in bold; new mutations not yet reported in the literature are shown in italics.

Partial androgen insensitivity syndrome (AIS)

Partial AIS covers a wide spectrum of clinical phenotypes, from patients with predominantly female phenotype (i.e. mild clitor-omegaly) to an undervirilized male phenotype (McPhaul, 1999). In addition, the Wolffian duct may develop to a variable extent. Simple hypospadias or micropenis in children, or undervirilization and gynaecomastia in adolescent boys, should also come to medical attention. It would be helpful to refer to a more detailed system by which to categorize patients with partial AIS (Quigley *et al.*, 1995).

The classification of partial AIS (according to Quigley *et al.*, 1995) is as follows:

Grade 1 (male phenotype): individuals with normal male external genitalia such as infertile males with azoospermia and

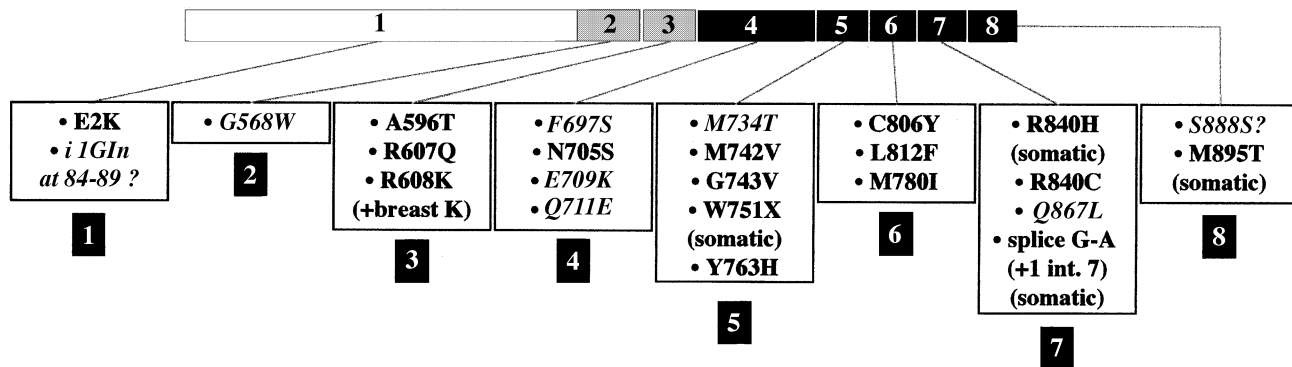


Figure 8. The figure displays the mutations of the androgen receptor gene identified by the authors' group in patients with partial androgen insensitivity. Mutations are located on the different exons of the gene. Mutations depicted in bold are already reported in the androgen receptor database; mutations in italics are new ones. Two mutations, annotated with a '?', are not proved to be responsible for the phenotype. However, the silent mutation S888S was found in a large kindred, segregated perfectly with the disease and, furthermore, was reported by other groups (personal communication). In brackets are added some specific features of some mutations. In particular, four cases are shown of patients presenting somatic mutations of the androgen receptor (AR). In these instances, mosaicism of normal and mutant AR is responsible for a less severe phenotype than for patient with germline mutations.

hormonal features of androgen resistance; those with reduced virilization at puberty (so-called 'minimal' androgen resistance).

Grade 2 (partial AIS with male phenotype): individuals who have a univocally male phenotype, but who have mildly defective fetal masculinization, manifested by defects such as isolated hypospadias and/or micropenis.

Grade 3 (partial AIS with male phenotype): individuals with predominantly male phenotype but with more severely defective masculinization *in utero*, as evidenced by perineal hypospadias, small penis and cryptorchidism and/or bifid scrotum.

Grade 4 (partial AIS with ambiguous phenotype): individuals with ambiguous phenotype and severely limited masculinization, as evidenced by a phallic structure that is intermediate between a clitoris and a penis, generally accompanied by a urogenital sinus with perineal orifice and labioscrotal folds.

Grade 5 (partial AIS with female phenotype): individuals with essentially female phenotype (i.e. minimal fetal androgen action), including separate urethral and vaginal orifices, with minimal androgenization as evidenced by mild clitoromegaly or a small degree of posterior labial fusion.

Grade 6 (partial AIS with female phenotype): individuals with a normal female genital phenotype (i.e. no fetal androgen action) who develop androgen-dependent pubic and/or axillary hair at puberty.

Grade 7 (complete AIS): individuals with female phenotype and absence of pubic or axillary sexual hair after puberty.

Newborns with partial AIS have increased LH and testosterone secretion, while oestrogen production is higher: serum sex hormone-binding globulin (SHBG) concentrations are intermediate between those of normal male and normal female. The SHBG response to the increase in serum testosterone induced by an HCG stimulation test has been used as an aid in the differential diagnosis between partial AIS and other forms of male pseudohermaphroditism: substantial SHBG suppression during stanozolone administration correlates with changes in penile size (Sinnecker *et al.*, 1997).

Androgen binding in genital skin fibroblasts has revealed that defects are heterogeneous, since they range between reduced

capacity, reduced affinity, thermolability, increased ligand dissociation rate and altered ligand specificity. As has been the case for other investigators, we were unable to report any consistent correlation between the concentration of AR and the degree of undervirilization.

Since the AR gene was cloned, the tools of molecular biology have made it possible to identify mutations within the gene from patients with different phenotypes of partial AIS (Figure 8). Screening procedures with sequencing of the AR gene allow identification of the subtle changes responsible for missense or nonsense mutations. Measurements of AR mRNA have been useful in identifying mutations that cause partial AIS by altering the steady state levels or the size of the mRNA. Transfection of constructs expressing the mutant AR in mammalian cells is the main approach for demonstrating the causative role of the mutation in the development of the androgen insensitivity (Lumbroso *et al.*, 1996).

AR gene alterations may be classified into two groups according to the DNA and mRNA alterations: (i) loss or gain of genomic information, such as macro- and microdeletions and basepair insertions; and (ii) point mutations, responsible for nonsense, missense and splice mutations. The molecular genetics of the AR gene has revealed that there is a wide variety of molecular defects underlying the clinical and biochemical heterogeneity of androgen insensitivity syndrome (Gottlieb *et al.*, 1999). All AR defects appear to disturb its ability to regulate transcription of its target genes, although AR gene sequencing and in-vitro studies of receptor function do not explain every aspect of androgen insensitivity. Moreover, the same mutation can be associated with different phenotypes from different—but even within the same—families (Rodien *et al.*, 1996). Markedly different molecular defects (major deletion, premature termination, or single amino acid substitution in the protein) can produce the same phenotype. Thus, it seems that other genetic determinants may influence androgen action within its target cells.

The management of patients with partial androgen insensitivity syndrome must be individualized depending on the degree

of genital ambiguity, the growth response of the penis to supraphysiological doses of testosterone, and the type of AR mutation. Although certain AR defects may be amenable to androgen therapy, multiple reconstruction of external genitalia and azoospermia are good arguments to prefer rearing as a female sex.

Hypospadias

Hypospadias is defined by a displacement of the urethral meatus from the tip to the ventral side of the phallus. Hypospadias can be found as an isolated defect of fetal virilization or accompanied by micropenis and/or cryptorchidism.

According to the location of the urethral meatus, hypospadias is termed either glandular (the ventral surface of the glans penis) or coronal (at the junction of the glans and the shaft). It is correct to use the terms distal, median or proximal hypospadias according to the severity of this defect. Hypospadias can also be associated with chordee, a ventral curvature of the penis.

Mutation within the steroid 5 α -reductase gene or the AR gene has been reported rarely in patients with isolated hypospadias (Russell *et al.*, 1994).

Micropenis

Micropenis refers to an anatomically correct penis that is, nevertheless, extremely small in size. A micropenis has a stretched length of less than 2.5 SD below the mean for age.

Any defect of the hypothalamo-pituitary-gonadal axis can result in a micropenis. Briefly, it may be due to inadequate testosterone production or action.

Mutations within the steroid 5 α -reductase gene or the AR gene are exceptional.

Cryptorchidism

Cryptorchidism corresponds to an incompletely descended testis, and can be uni- or bilateral. Cryptorchidism is the most common disorder of sex differentiation in man, the incidence being 1–1.6% in the newborn. Cryptorchidism is commonly found in patients with defects of gonadotrophin or androgen and AMH production and action. Insulin-like factor-3 (Insl-3) is a possible candidate gene for cryptorchidism (Nef and Parada, 2000).

Secular trends in the incidence of disorders of male sexual differentiation, the occurrence of genital abnormalities in the sons of women exposed to diethylstilboestrol during pregnancy, and the adverse effects of prenatal oestrogen/antiandrogen treatment in experimental animals, have persuaded several authors to advance the hypothesis that fetal exposure to xeno-oestrogens and/or antiandrogens may account for the reported chronological changes in the incidence of disorders of male sexual differentiation (Toppari and Skakkebaek, 1998).

Conclusions

In conclusion, the systematic screening of environmental chemicals and the chemicals present in human foods and water is needed to identify putative causal agents and to assess their ability to disrupt the endocrine system. Prospective epidemiological studies of genital malformation need to be conducted in birth cohorts from Europe, Japan and the United States, as these

countries are expected to have significant differences in the prevalence of urogenital malformations. In a simultaneous case-control study, diet, drug usage, exposure to chemicals during pregnancy and lifestyle parameters need to be evaluated as possible causal factors in neonatal genital malformations.

References

- Andersson, S., Bishop, R.W. and Russell, D.W. (1989) Expression, cloning and regulation of steroid 5 α -reductase, an enzyme essential for male sexual differentiation. *J. Biol. Chem.*, **264**, 16249–16255.
- Balaguer, P., Francois, F., Comunale, F. *et al.* (1999) Reporter cell lines to study the estrogenic effects of xenoestrogens. *Sci. Total Environ.*, **233**, 47–56.
- Balaguer, P., Fenet, H., Georget, V. *et al.* (2000) Reporter cell lines to monitor (anti)steroid activity in environmental samples. *Ecotoxicology*, **9**, 105–114.
- Boudon, C., Lumbroso, S., Lobaccaro, J.M. *et al.* (1995) Molecular study of the 5 alpha-reductase type 2 gene in three European families with 5 alpha-reductase deficiency. *J. Clin. Endocrinol. Metab.*, **80**, 2149–2153.
- Georget, V., Lobaccaro, J.M., Terouanne, B. *et al.* (1997) Trafficking of the androgen receptor in living cells with fused green fluorescent protein-androgen receptor. *Mol. Cell. Endocrinol.*, **129**, 17–26.
- Georget, V., Terouanne, B., Lumbroso, S. *et al.* (1998) Trafficking of androgen receptor mutants fused to green fluorescent protein: a new investigation of partial androgen insensitivity syndrome. *J. Clin. Endocrinol. Metab.*, **83**, 3597–3603.
- Gottlieb, B., Beitel, L.K., Lumbroso, R. *et al.* (1999) Update of the androgen receptor gene mutations database. *Hum. Mutat.*, **14**, 103–114.
- Kelce, W.R., Monosson, E., Gamcsik, M.P. *et al.* (1994) Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol. Appl. Pharmacol.*, **126**, 276–285.
- Kelce, W.R., Stone, C.R., Laws, S.C. *et al.* (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist [see comments]. *Nature*, **375**, 581–585.
- Lumbroso, S., Lobaccaro, J.M., Georget, V. *et al.* (1996) A novel substitution (Leu707Arg) in exon 4 of the androgen receptor gene causes complete androgen resistance. *J. Clin. Endocrinol. Metab.*, **81**, 1984–1988.
- Mangelsdorf, D.J., Thummel, C., Beato, M. *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835–839.
- McPhaul, M.J. (1999) Molecular defects of the androgen receptor. *J. Steroid Biochem. Mol. Biol.*, **69**, 315–322.
- Migeon, C.J., Berkovitz, G.D. and Brown, T.R. (1994) Sexual differentiation and ambiguity. In Kappy, M.S., Blizzard, R.M. and Migeon, C.J. (eds), *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. Charles C. Thomas, Springfield, pp. 573–681.
- Nef, S. and Parada, L.F. (2000) Hormones in male sexual development. *Genes Dev.*, **14**, 3075–3086.
- Poujol, N., Wurtz, J.M., Tahiri, B. *et al.* (2000) Specific recognition of androgens by their nuclear receptor. A structure-function study. *J. Biol. Chem.*, **275**, 24022–24031.
- Quigley, C.A., Debellis, A., Marschke, K.B. *et al.* (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocr. Rev.*, **16**, 271–321.
- Rodien, P., Mebarki, F., Mowszowicz, I. *et al.* (1996) Different phenotypes in a family with androgen insensitivity caused by the same M780I point mutation in the androgen receptor gene. *J. Clin. Endocrinol. Metab.*, **81**, 2994–2998.
- Russell, D.W. and Wilson, J.D. (1994) Steroid 5 alpha-reductase: two genes/two enzymes. *Annu. Rev. Biochem.*, **63**, 25–61.
- Russell, D.W., Berman, D.M., Bryant, J.T. *et al.* (1994) The molecular genetics of steroid 5 alpha-reductases. *Rec. Prog. Horm. Res.*, **49**, 275–284.
- Sinnecker, G.H., Hiort, O., Nitsche, E.M. *et al.* (1997) Functional assessment and clinical classification of androgen sensitivity in patients with mutations of the androgen receptor gene. German Collaborative Intersex Study Group. *Eur. J. Pediatr.*, **156**, 7–14.
- Sultan, C. and Savage, M.O. (1998) Intersex states. In Grossman, A. (ed.), *Clinical Endocrinology*, 2nd edition. Blackwell Science, Oxford, pp. 795–809.
- Sultan, C., Térouanne, B., Balaguer, P. *et al.* (2000) Interrupteurs hormonaux

- et anomalies de la différenciation sexuelle. Séminaire pédiatrique, Institut Pasteur, Paris, 24–25th January, pp. 136–139.
- Térouanne, B., Tahiri, B., Georget, V. *et al.* (2000) A stable prostatic bioluminescent cell line to investigate androgen and antiandrogen effects. *Mol. Cell. Endocrinol.*, **160**, 39–49.
- Toppari, J. and Skakkeback, N.E. (1998) Sexual differentiation and environmental endocrine disrupters. In *Baillière's Clinical Endocrinology and Metabolism*. Baillière Tindall, London, pp. 143–156.
- Vinggaard, A.M., Joergensen, E.C. and Larsen, J.C. (1999) Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic effects of environmental chemicals. *Toxicol. Appl. Pharmacol.*, **155**, 150–160.
- Wilson, J.D., Griffin, J.E. and Russell, D.W. (1993) Steroid 5 α -reductase 2 deficiency. *Endocr. Rev.*, **14**, 577–593.

Received on October 2, 2000; accepted on February 23, 2001