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# Basics of androgen synthesis and action

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Androgens are essential sex steroid hormones for both sexes. Testosterone (T) is the predominant androgen in males, while in adult females, T concentrations are about 15-fold lower and androgen precursors are converted to estrogens. T is produced primarily in testicular Leydig cells in men, while in women precursors are biosynthesised in the adrenal cortex and ovaries and converted into T in the periphery. The biosynthesis of T occurs via a series of enzymatic reactions in steroidogenic organs. Notably, the more potent androgen, dihydrotestosterone, may be synthesized from T in the classic pathway, however, alternate metabolic pathways also exist. The classic action of androgens on target organs is mediated through the androgen receptor, which regulates nuclear receptor gene transcription. However, the androgen—androgen receptor complex may also interact directly with membrane proteins or signaling molecules to exert more rapid effects. This review summarizes the current knowledge of androgen biosynthesis, mechanisms of action and endocrine effects in human biology, and relates these effects to respective human congenital and acquired disorders.



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**List of abbreviations**

| | |
|---|---|
| T | testosterone/4-androsten-17β-ol-3-one |
| DHT | dihydrotestosterone/5α-androstan-17β-ol-3-one |
| AR | androgen receptor |
| DHEA-S | dehydroepiandrosterone-sulfate/5-androsten-3β-ol-17-one-sulfate |
| DHEA | dehydroepiandrosterone/5-androsten-3β-ol-17-one |
| A4 | androstenedione/4-androsten-3,17-dione |
| HPG | hypothalamus-pituitary-gonadal |
| GnRH | gonadotropin-releasing-hormone |
| LH | luteinizing hormone |
| FSH | follicular-stimulating hormone |
| 11OHA4 | 11β-hydroxyandrostenedione/4-androsten-11β-ol-3,17-dione |
| ACTH | adrenocorticotropic hormone |
| MC | mineralocorticoids |
| GC | glucocorticoids |
| PREG | pregnenolone/5-pregnen-3β-ol-20-one |
| 17OHPREG | 17α-hydroxypregnenolone/5-pregnen-3β,17α-diol-20-one |
| androstenediol | 5-androsten-3β,17β-diol |
| PROG/P4 | progesterone/4-pregnen-3,20-dione |
| 17OHPROG/17OHP4 | 17α-hydroxyprogesterone/4-pregnen-17α-ol-3,20-dione |
| 17OH-ALLO | 17α-hydroxy-allopregnanolone/5α-pregnan-3α,17α-diol-20-one |
| androsterone | 5α-androstan-3α-ol-17-one |
| DHPROG | dihydroprogesterone/5α-pregnan-3,20-dione |
| 11OHT | 11β-hydroxy-T/4-androsten-11β,17β-diol-3-one |
| 11KT | 11-keto-T/4-androsten-17β-ol-3,11-dione |
| 11KDHT | 11-keto-DHT/5α-androstan-17β-ol-3,11-dione |
| 11KA4 | 11-keto-A4/4-androsten-3,11,17-trione |
| 17OH-DHP | 17α-hydroxy-dihydroprogesterone/5α-pregnan-17α-ol-3,20-dione |
| 11OHDHT | 11β-hydroxydihydrotestosterone/5α-androstan-11β,17β-diol-3-one |
| 11KAST | 11-ketoandrosterone/5α-androstan-3α-ol-11,17-dione |
| 11OHAST | 11β-hydroxyandrosterone/5α-androstan-3α,11β-diol-17-one |
| 11KP4 | 11-ketoprogesterone/4-pregnan-3,11,20-trione |
| 11KDHP4 | 5α-pregnanetrione/5α-pregnan-3,11,20-trione |
| 11OHDHP4 | 11OH-dihydroprogesterone/5α-pregnan-11β-ol-3,20-dione |
| Alfaxalone | 5α-pregnan-3α-ol-11,20-dione |
| 3α,11β-diOH-DHP4 | 3α, 11β-dihydroxy-dihydroprogesterone |
| 21dF | 21-deoxycortisol/11β,17α-dihydroxypregnan-4-ene-3,20-dione |
| 21dE | 21-deoxycortisone/17α-hydroxypregn-4-ene-3,11,20-trione |
| 11OHPdione | 5α-pregnan-11β, 17α-diol-3,20-dione |
| 11KPdione | 5α-pregnan-17α-ol-3,11,20-trione |
| zR | zona reticularis |
| StAR | steroidogenic acute regulatory protein |
| TSPO | translocator proteins |
| CYP11A1 | cytochrome P450 side chain cleavage P450scc |
| FDX1 | ferrodoxin |
| FDXR | ferrodoxin-reductase |
| CYP17A1 | cytochrome P450 17α-hydroxylase/17,20-lyase |
| POR | cytochrome P450 oxidoreductase |
| CYB5 | cytochrome $b_5$ |
| HSD3B2/3βHSDII | 3β-hydroxysteroid dehydrogenase type II |
| HSD17B3 | 17β-hydroxysteroid dehydrogenase type 3 |
| AKR1C3/HSD17B5 | 17β-hydroxysteroid dehydrogenase type 5 |
| SRD5A2/5αRed2 | steroid-5α-reductase type II |
| SRD5A1/5αRed1 | steroid-5α-reductase type I |

| | |
|---|---|
| HSD11B | 11β-hydroxysteroid dehydrogenase |
| AKR1C2/4 | aldo-keto reductase family 1 member C2/C4 |
| CAH | congenital adrenal hyperplasia |
| PCOS | polycystic ovary syndrome |
| CRPC | castration-resistant prostate cancer |
| CYP11B1 | 11β-hydroxylase |
| NR3C4 | nuclear receptor subfamily 3, group C, member 4 |
| NTD | N-terminal domain |
| AF1 | activation functional motif |
| DBD | DNA binding domain |
| LBD | ligand binding domain |
| AF2 | activation functional motif |
| AIS | androgen insensitivity syndrome |
| APOD | apolipoprotein D |
| HSPs | heat shock proteins |
| ARE | androgen response elements |
| MAPK | mitogen-activated protein kinases |
| ERK | extracellular signal-regulated kinases |
| PC | prostate cancer |
| PI3K | phosphoinositide 3-kinases |
| Akt | protein kinase B |
| ARKO | AR knockout mouse |
| LC-ARKO | Leydig cell-specific ARKO |
| GU-AKRO | gubernaculum-specific ARKO |
| DSD | disorders/differences of sex development |
| CYP21A2 | cytochrome P450 21-hydroxylase |
| CYP19A1 | cytochrome P450 aromatase |
| SF1/NR5A1 | steroidogenic factor 1/nuclear receptor subfamily 5 group A member 1 |
| CAIS | complete androgen insensitivity |
| PAIS | partial androgen insensitivity |

## Introduction

Androgens are steroid hormones that are essential for human sexual development and reproduction, but they also modulate other organs including bone, muscle, adipose tissue, skin, hair, the brain and the cardiovascular system, thereby effecting growth, body shape and human behavior.

Androgens are produced in the adult female ovaries and male testes and in the adrenal glands, from where they are secreted into circulation to exert their biological effects on target organs. In addition, secreted androgens also serve as substrates for peripheral organs for the intermediate steroid metabolism and its action. Androgens also serve as precursors for estrogen biosynthesis in the gonads and peripherally. Ultimately, the androgens biosynthesised in the adrenals and gonads are metabolized in the liver, and then excreted in urine.

In male adults, testosterone (T, 4-androsten-17β-ol-3-one) is the most abundant androgen produced in the testes that is present in circulation. Total T concentrations are about 10–30 nmol/L at age 30 years in men and decline at an average rate of 1–2% per year with aging (Fig. 1) [1–6]. T can be converted to the most potent endogenous androgen, dihydrotestosterone (DHT, 5α-androstan-17β-ol-3-one) [7,8], as DHT has about 5-10-fold greater affinity for the androgen receptor (AR) compared to T [8]. In a 30 year old woman, the most abundant androgens in circulation are dehydroepiandrosterone-sulfate (DHEA-S, 5-androsten-3β-ol-17-one-sulfate; 1.2–10 μmol/L), dehydroepiandrosterone (DHEA, 5-androsten-3β-ol-17-one; 0.1–23 nmol/L) and androstenedione (A4, 4-androsten-3,17-dione; 0.5–7.9 nmol/L) [9], which are all considered weak androgens according to their low affinity towards the AR [10]. However, these weak androgens can be metabolised to more potent androgens (such as T or DHT) in peripheral tissues through multiple pathways. In menstruating women, circulating androgens originate in part from the adrenal cortex (mainly DHEA/-S) and more so from the ovaries (A4)

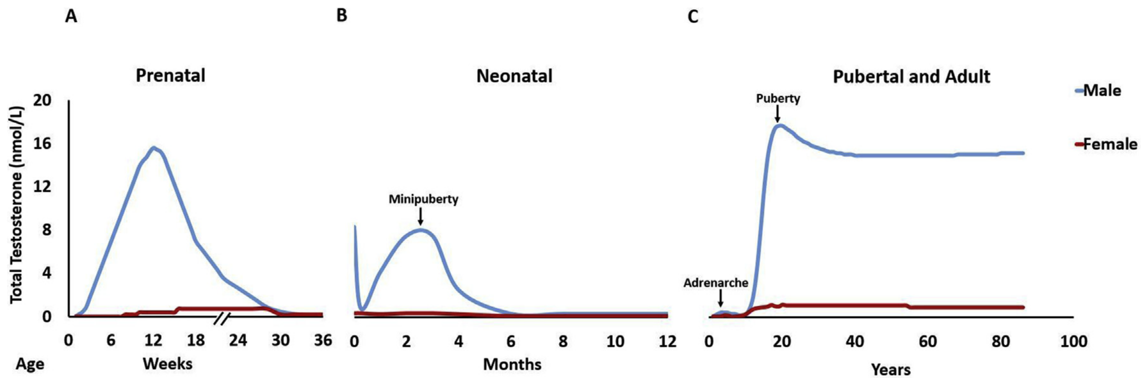


**Fig. 1. Lifetime curves of circulating testosterone (T) concentrations in males and females. A.** Prenatal period. Androgens are produced by the fetal testis and adrenals, while T is predominantly biosynthesised in the testes. In the fetal testis, the Leydig cells start producing T for sexual differentiation of male sex organs at 6 weeks of gestation. T levels keep rising until the end of the second trimester, peaking with values similar to levels measured in adult men; thereafter T's concentration decrease until the end of the third trimester. T levels in a female fetus remain low during pregnancy. **B**. Neonatal Period. At birth (0–3 days of life), T levels are increased in males due to a surge of luteinizing hormone (LH)/follicular-stimulating hormone (FSH), and again rises with minipuberty between 2 and 3 months of life stimulated once again by LH. **C**. Pubertal and adult period. During adrenarche around the age of 6–8 years, T levels increase slightly in both sexes. Finally, activation of the hypothalamic–pituitary–gonadal axis at the age of 12 years (puberty in boys) results in a significant increase in T levels peaking between the second and fourth decade of life in men. With aging, however, T production slightly decreases in men. Although T levels also rise during puberty in females, after about 10 years of age, T remains markedly lower in middle-aged women and decreases further with menopause.

[11]. Total plasma T levels of a 30-year-old woman are about 10 to 15-fold lower than in a same-aged male (0.4–2.1 nmol/L) (Fig. 1) [2,3]. After menopause, when ovarian steroidogenesis has ceased, circulating A4 levels are cut in half and total T levels decrease by about 25% [12].

Production of androgens in the adult gonads is controlled by the hypothalamus-pituitary-gonadal (HPG) axis, which involves the gonadotropin-releasing-hormone (GnRH), luteinizing hormone (LH) and follicular-stimulating hormone (FSH), and comprises of balanced feed forward and feedback loops (Fig. 2). This system is sex specific and thus characterized by the testis or ovary as target organs. Within the testis, Leydig cells produce and secrete T abundantly. By contrast in the ovary, theca cells produce A4 that is mostly transferred to granulosa cells as precursor for the production of estrogens, while only small amounts are secreted into circulation (Fig. 2). The adult adrenal cortex produces adrenal androgens in its zona reticularis (zR) (predominantly DHEA/-S, A4 and 11β-hydroxyandrostenedione [11OHA4, 4-androsten-11β-ol-3,17-dione]) [13]. However, unlike androgen production in the HPG axis, the exact regulation of adrenal androgen production is still not fully elucidated, although adrenocorticotropic hormone (ACTH) is an important co-regulator [11].

The human gonads and adrenals contribute to androgen production pre- and postnatally in a developmental specific fashion [14]. As both the adrenals and the gonads originate from a common embryologic anlage, the urogenital ridge, they share many genetic and steroidogenic characteristics. Prenatally, the fetal adrenal cortex consists of a large fetal zone that resembles the adult zR and produces predominantly DHEA-S which, together with the conversion to its hepatic 16α-hydroxylated metabolite, 16α-DHEA-S (5-androsten-3β,16α-diol-17-one-sulfate), contribute to the androgen pool of the fetal-placental unit [11,14]. These androgens are efficiently converted to estradiol and estriol in the placenta due to its aromatase activity, thereby regulating the androgen exposure to the fetus and the mother.

After birth the fetal adrenals involute and give rise to the adult adrenals which start to produce mineralocorticoids (MC) and glucocorticoids (GC) immediately after birth, while production of androgens in the zR occurs only around age 6–8 years at adrenarche. Similarly, the fetal testis is formed early and produces high amounts of T throughout the first and second trimester of pregnancy to masculinize the male fetus (Fig. 1) [7,15]. After birth T levels fall dramatically but raise again for a short time in the event of minipuberty (days 30–100 of life) predominantly in males [16,17]. After that T levels stay low until puberty (Fig. 1). In contrast to the testis, the fetal ovary seems steroidogenic inactive during the prenatal and neonatal period and starts its steroid production only with puberty (Fig. 2).

While the biochemistry of the classic androgen biosynthesis pathway is long known and the action of its conventional products on the AR well described, alternative pathways and novel active androgens have recently been reported (Fig. 3) [11]. In addition, newer studies on the regulation of the AR itself and its stimulated signaling have enhanced our knowledge on androgen action in health and disease [18]. The purpose of this review is to give an update on recent advances in androgen synthesis and action. In our literature search we therefore focused on the literature of the past decade whenever possible.

## Androgen synthesis and metabolism

### *Androgen biosynthesis by the classic pathway*

All steroid hormones are produced from cholesterol through a cascade of enzymes which are encoded by genes that are common to all steroid producing organs [11,19]. In most cases the organ specific gene expression determines the steroid profile of each specialized organ. For instance, the testis is determined to produce T from cholesterol while the theca cells in the ovary produce A4 (Figs. 2 and 3A). Cholesterol is transported to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). Its cooperation with translocator proteins (TSPO) for this transport has been suggested but remains controversial [20]. Inside the mitochondria, cholesterol is the substrate for the first step of steroidogenesis. In the mitochondria, cholesterol is converted to pregnenolone (PREG, 5-pregnen-3β-ol-20-one) by the side chain cleavage system comprised of CYP11A1 (cytochrome P450 side chain cleavage, P450scc), ferrodoxin (FDX1) and ferrodoxin-reductase (FDXR). In the classic

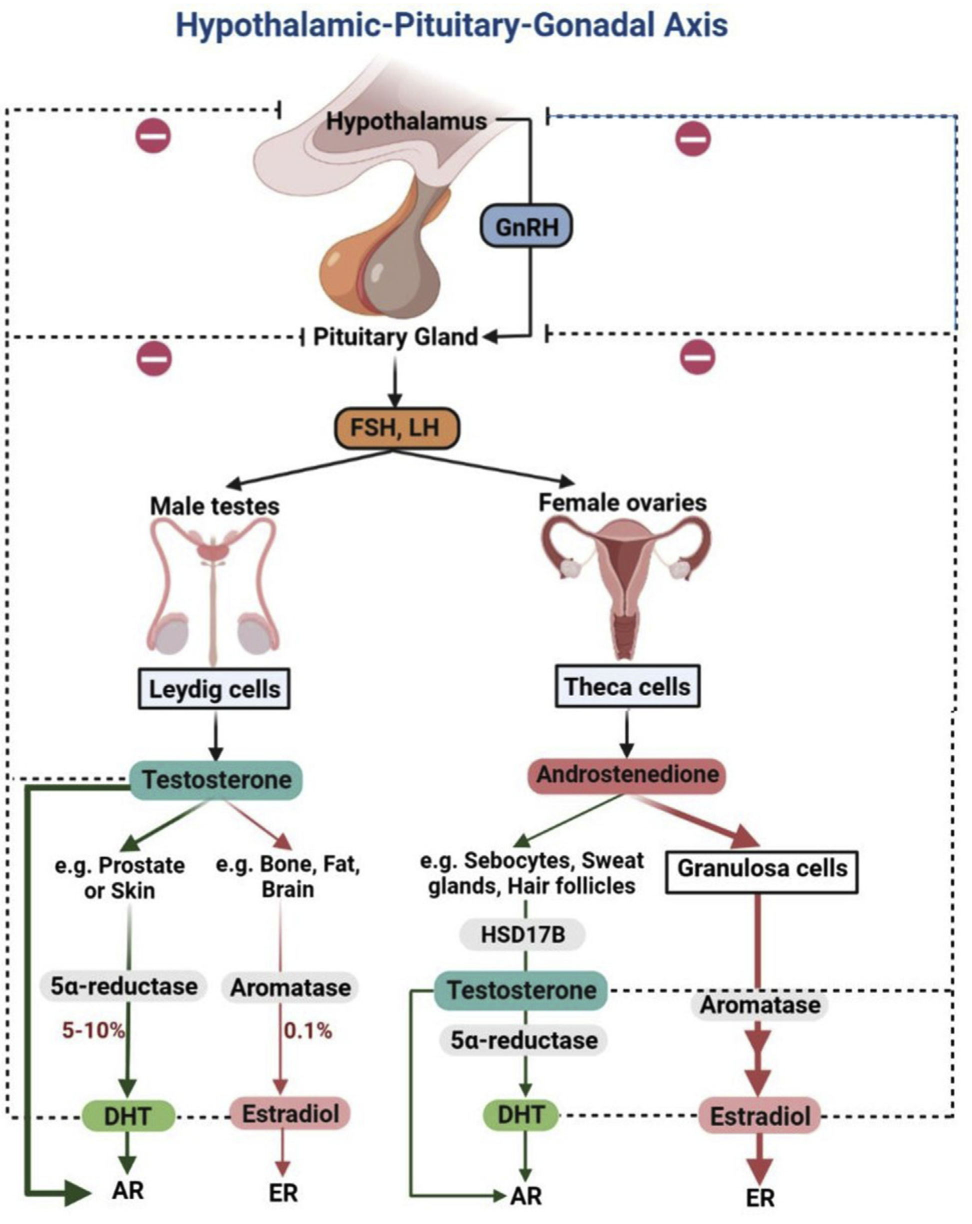


**Fig. 2. Schematic diagram of the hypothalamic-pituitary-gonadal (HPG) axis, resulting in androgen biosynthesis and their biological actions.** Androgens are produced upon central gonadotropin-releasing-hormone (GnRH)-luteinizing hormone (LH)/follicular-stimulating hormone (FSH) stimulation of the gonads. In the testicular Leydig cells, the most abundant androgen testosterone (T) is produced at a daily rate of 5−7 mg. About 90−95% of the circulating T reaches the target organs and exerts its effect via the androgen receptor (AR) (left thick green arrow), while 5−10% of T is converted to dihydrotestosterone (DHT) by 5α-reductase activity in the prostate or skin. Only a small amount of T (0.1%) is converted to estrogens by aromatase (CYP19A1) activity in peripheral tissues (e.g., bone, fat or the brain), where these hormones exert their effect via the estrogen receptor (ER). In the female ovarian theca cells, androstenedione (A4) is produced, where most of it is converted to estradiol by aromatase in the ovarian granulosa cells. Only a small amount of A4 is converted peripherally to T and DHT in hair follicles or in sweat glands. Eventually all steroids, including T, DHT and estrogens, exert negative feedback on the HPG axis.

pathway, PREG is then converted through the delta-5 pathway by cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1, P450c17) to 17α-hydroxypregnenolone (17OHPREG, 5-pregnen-3β,17α-diol-20-one) and DHEA with the first reaction catalyzed by its 17α−hydroxylation activity supported by cytochrome P450 oxidoreductase (POR) and the second reaction by the 17,20-lyase activity supported by POR and additionally also cytochrome $b_5$ (CYB5). DHEA is then turned over to A4 or androstenediol (5-androsten-3β,17β-diol) by the enzymes 3β-hydroxysteroid dehydrogenase type II (HSD3B2/3βHSDII) and 17β-hydroxysteroid dehydrogenase type 3 or type 5 (HSD17B3 or AKR1C3 also known as HSD17B5) (Fig. 3A), or to T through the action of both enzymes. Thereafter, T is converted to DHT by steroid-5α-reductase activities; either type II (SRD5A2/5αRed2), which is specifically expressed in skin and the prostate, or type I (SRD5A1/5αRed1), which is expressed more abundantly in the liver, cerebellum and hypothalamus [21]. In humans, only little conversion to A4 occurs through the delta-4 pathway following the conversion of PREG to progesterone (PROG, 4-pregnen-3,20-dione) to 17α-hydroxyprogesterone (17OHPROG, 4-pregnen-17α-ol-3,20-dione) and then to A4, as 17OHP4 is a poor substrate of CYP17A1 compared to 17OHPREG [22].

In the ovary, androgen production leads to A4 in theca cells (Fig. 2), which is either further converted to estrogens in the granulosa cells or secreted into the periphery where it serves as a substrate for the intermediate androgen metabolism. Likewise, adrenal androgens such as DHEA, DHEA-S and 11OHA4 are secreted into the peripheral androgen pool and further converted to active and inactive (androgen) products by different pathways that have been explored in greater detail recently, but are still not fully understood [23–25].

*Novel androgens and biosynthesis pathways*

In addition to the well-known classic pathway of T and DHT biosynthesis, alternative biochemical pathways and novel bioactive androgens have been revealed more recently (Fig. 3B-D) [26,27]. It has been recognized that the intermediate metabolism and peripheral androgen pool play an important role for the formation of active androgen metabolites that has thus far mostly been regarded as ‘inactive’ products on their way out of the body. This has been illustrated for several disorders which divert normal steroidogenesis and produce alternative steroids, and these disorders therefore serve as models to fully understand the role of novel steroids and metabolic pathways.

Androgen production by the so-called **backdoor, alternative pathway** (Fig. 3B) was first described while studying sexual development of the tammar wallaby pouch young [28,29]. The same pathway has also been described in mice [30], before its metabolites were identified in the human steroid disorders, POR deficiency [31,32] and 21-hydroxylase deficiency [33]. Soon after, the first human mutations in genes (aldo-keto reductase family member C2/C4, *AKR1C2/4*) specific to this pathway were reported, thereby establishing a role for this pathway in humans [34]. In this pathway, mainly 17OHPROG is 5α-reduced (by SRD5A1) and then 3α-reduced (by AKR1C2/4), producing 17α-hydroxy-allopregnanolone (5α-pregnan-3α,17α-diol-20-one), a perfect substrate for CYP17A1, which is then converted to androsterone (5α-androstan-3α-ol-17-one) (Fig. 3B). Androsterone is thereafter converted to androstanediol (5α-androstane-3α,17β-diol) and finally to DHT. Alternatively, the backdoor pathway may start with the 5α-reduction of PROG, producing dihydroprogesterone (5α-pregnan-3,20-dione) (Fig. 3B). Of note, androsterone and androstanediol are androgenic, however, androsterone has only an AR affinity of one-seventh compared to T and androstanediol has only very low affinity and thus weak androgenic activity [35].

The existence of 11-oxy androgens has long been known, but that the human adrenal cortex produces abundant precursors (predominantly 11OHA4, less 11β-hydroxy-T [11OHT, 4-androsten-11β,17β-diol-3-one]) [10] to feed this **C11-oxy pathway** and provide an important source for the production of active androgens in the periphery (11-keto-T [11KT, 4-androsten-17β-ol-3,11-dione],11-keto-DHT [11KDHT, 5α-androstan-17β-ol-3,11-dione]), has only recently been recognized (Fig. 3C) [13,36]. In this C11-oxy pathway, the 11β-hydroxylation (by 11β-hydroxylase, CYP11B1) of A4 in the adrenals leads to 11OHA4 [37], which can be converted to 11-keto-A4 (11KA4, 4-androsten-3,11,17-trione) (by 11β-hydroxy steroid dehydrogenase type II, HSD11B2), then 11KT (by HSD17B3/5) and finally 11KDHT (by SRD5A1/2) in peripheral organs [38]. Other parallel metabolic paths may also be used, but they do not lead to other active androgens (Fig. 3C) [27,35]. Overall, 11-oxy androgens are associated with androgen

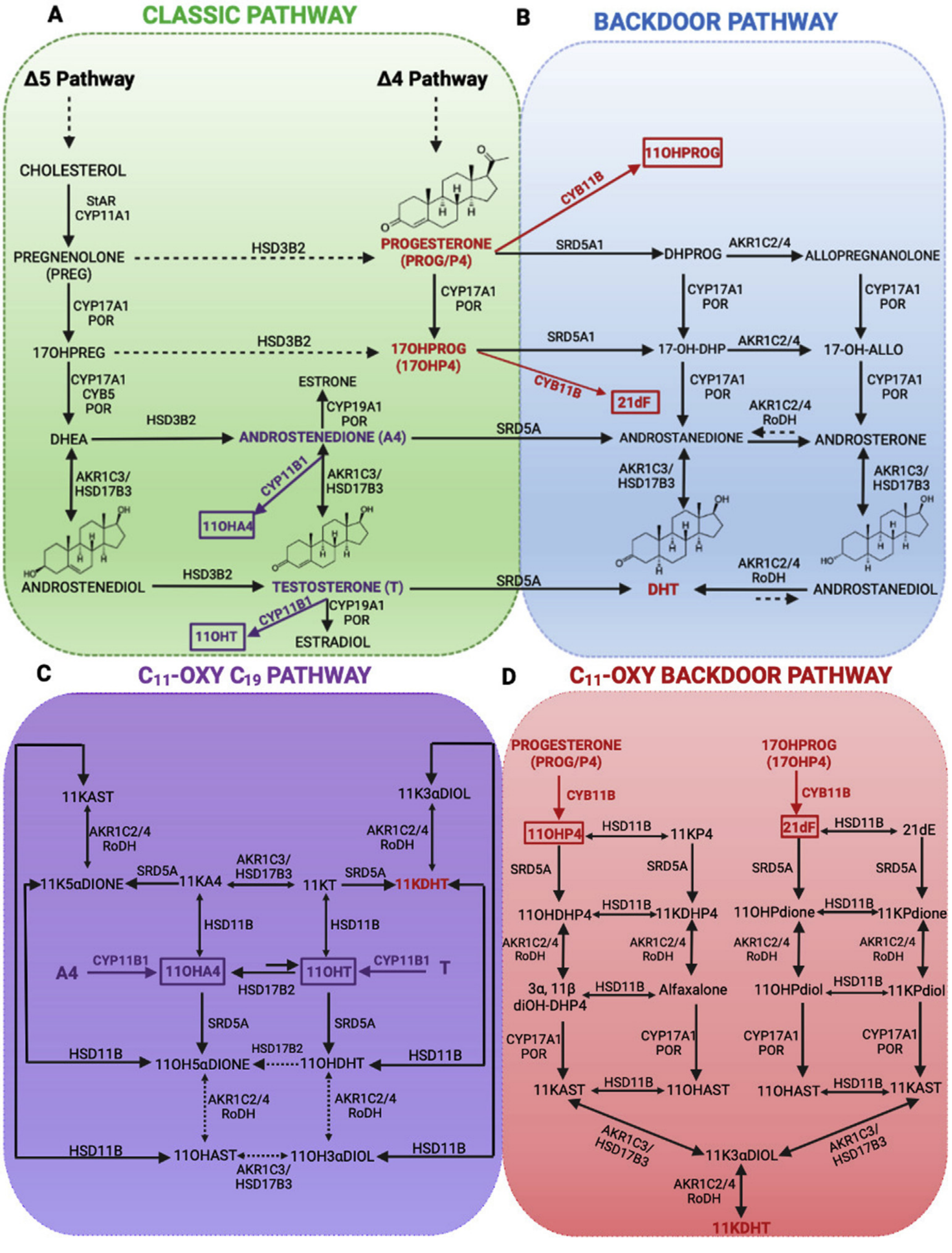


**Fig. 3. Classic and alternative androgen biosynthesis pathways. A. The classic pathway** of testosterone (T) biosynthesis, metabolically active in endocrine organs such as the human testis Leydig cells. Cholesterol is transported to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR). At the inner mitochondrial membrane cholesterol is then converted to pregnenolone (PREG) by the side-chain cleavage catalytic unit consisting of the enzyme cytochrome P450 side chain cleavage (CYP11A1), ferrodoxin (FDX1) and ferrodoxin-reductase (FDXR). PREG is then sequentially converted to 17α-hydroxypregnenolone (17OHPREG) and dehydroepiandrosterone (DHEA) by cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1) supported by cytochrome P450 oxidoreductase (POR) in the first hydroxylase step and cytochrome $b_5$ (CYB5) in the second lyase step. Following the biosynthesis of DHEA, both androstenediol and androstenedione (A4) are biosynthesized by the enzyme 17β-hydroxysteroid dehydrogenase (AKR1C3/HSD17B3) and 3β-hydroxysteroid dehydrogenase (HSD3B2), respectively. Thereafter, both metabolites are

excess disorders such as congenital adrenal hyperplasia (CAH), premature adrenarche and polycystic ovary syndrome (PCOS) [13,24,39–46], and diseases such as castration-resistant prostate cancer (CRPC) [47–50] and (most recently) breast cancer [51].

Most recently, a possible connection between the C11-oxy and the backdoor pathways has been suggested through *in vitro* studies (Fig. 3D) [52–55]. The entry point to this **C11-oxy backdoor pathway** could be either through PROG or 17OHPROG, which are 11β-hydroxylated by CYP11B in the first step, and then turned over to 11KDHT (a very potent androgen) by multiple conversions involving enzymes common to the backdoor pathway (Fig. 3B-D). Whether this pathway plays a role in human biology, to what degree and under which circumstances remains to be studied.

## Androgen action and the androgen receptor

Androgens exert their effect typically through the AR and modulate a vast range of different biological processes (Table 1) [56–58]. Accordingly, the *AR* is differentially expressed in many tissues including female and male reproductive organs, bones, muscles, the brain, the cardiovascular system, neural tissues, as well as the immune and haematopoietic systems (Fig. 4). Once androgens are bound to the AR, their effect may be at the genomic or at the non-genomic level (Fig. 4) [7,57,59].

### *Characteristics and regulation of the androgen receptor*

The AR is a member of the class I nuclear receptor transcription factor family, also known as *NR3C4* (nuclear receptor subfamily 3, group C, member 4). It is a ligand-activated transcription factor. In humans, the *AR* gene is located on the X chromosome at position $Xq_{11-12}$. Hence, 46,XX females have two copies of the *AR* and 46,XY males have only one copy. The *AR* consists of eight exons which are separated by relatively large introns (Fig. 4A). The *AR* expressed region encodes a 110-kDa protein of

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converted to T via the same enzymes. Small amounts of T or A4 may also be converted to estrogens (estradiol and estrone, respectively) in the testis by aromatase (CYP19A1). Secreted T is then partially converted to dihydrotestosterone (DHT) in peripheral tissues by steroid-5α-reductases (SRD5A1/2). **B. The alternative backdoor pathway.** In this pathway 17α-hydroxyprogesterone (17OHPROG/17OHP4) or progesterone (PROG/P4) serve as substrate for 5α-reduction (by SRD5A1) to enter the backdoor pathway to DHT synthesis. SRD5A1 converts 17OHPROG to 17α-hydroxy-dihydroprogesterone (17OH-DHP, 5α-pregnan-17α-ol-3,20-dione) and the 3α-hydroxysteroid dehydrogenase activity of AKR1C2/4 will yield 17α-hydroxy-allopregnanolone (17OH-ALLO); this metabolite is then converted to androsterone by CYP17A1. Further conversion by AKR1C3/HSD17B3 leads to androstanediol and by AKR1C2/4 or retinol-like dehydrogenase (RoDH) to DHT. **C. The alternative $C_{11}$-oxy pathway.** The starting point of this pathway is the 11β-hydroxylation of A4 and T (by 11β-hydroxylase (CYP11B1)) yielding 11β-hydroxyandrostenedione (11OHA4) and 11β-hydroxytestosterone (11OHT), which are then metabolized by SRD5As to 11β-hydroxy-5α-androstane-3,17-dione (11OH5αDIONE) and 11β-hydroxydihydrotestosterone (11OHDHT, 5α-androstan-11β,17β-diol-3-one), respectively. Alternatively, 11OHA4 can also be metabolized to 11-ketoandrostenedione (11KA4) by 11β-hydroxysteroid dehydrogenase (HSD11B). 11KA4 is reversibly metabolized to 11-ketotestosterone (11KT) by HSD17Bs, and the latter to the 11-ketoandrostanolone (11KDHT) by SRD5A. Furthermore, 11OH5αDIONE can be converted to 11K5αDIONE, and 11OHDHT to 11KDHT by HSD11B. 11K5αDIONE and 11KDHT are both reversibly metabolized to 11-ketoandrosterone (11KAST, 5α-androstan-3α-ol-11,17-dione) and 11keto-5α-androstane-3α,17β-diol (11K3αDIOL) by AKR1C2/4, while 11OH5αDIONE is also reversibly metabolized to 11β-hydroxyandrosterone (11OHAST, 5α-androstan-3α,11β-diol-17-one), and 11OHDHT to 11OH-3α-androstanediol (11OH3αDIOL) by AKR1C2/4. Finally, 11OHAST and 11OH3αDIOL are metabolized to 11KAST and 11K3αDIOL by HSD11B. **D. The alternative $C_{11}$-oxy backdoor pathway.** In this pathway the 11β-hydroxylation (CYP11B) of PROG/P4 and 17OHPROG/17OHP4 is the starting point. From P4, 11OHP4 is reversibly metabolized to 11-ketoprogesterone (11KP4, 4-pregnen-3,11,20-trione) by HSD11B, whereafter both 11KP4 and 11OHP4 are metabolized by SRD5A to either 5α-Pregnanetrione (11KDHP4, 5α-pregnan-3,11,20-trione) or 11OH-dihydroprogesterone (11OHDHP4, 5α-pregnan-11β-ol-3,20-dione), respectively, with these metabolites also interconverted by HSD11B. Next, 11KDHP4 and 11OHDHP4 are reversibly metabolized to alfaxalone (5α-pregnan-3α-ol-11,20-dione) and 3α,11β-dihydroxy-dihydroprogesterone (3α,11β-diOH-DHP4), respectively, by AKR1C2/4, and these metabolites may be converted to 11OHAST and 11KAST by CYP17A1. On the other hand, from 17OHP4, 21-deoxycortisol (21dF, 11β,17α-dihydroxypregn-4-ene-3,20-dione) is reversibly metabolized to 21-deoxycortisone (21dE, 17α-hydroxypregn-4-ene-3,11,20-trione) by HSD11B. Then, 21dF and 21dE are converted by SRD5A to 5α-pregnan-11β,17α-diol-3,20-dione (11OHPdione, 5α-pregnan-11β,17α-diol-3,20-dione) and 5α-pregnan-17α-ol-3,11,20-trione (11KPdione, 5α-pregnan-17α-ol-3,11,20-trione), respectively, with these metabolites also interconverted by HSD11B. Thereafter, 11OHPdione and 11KPdione can be metabolized by AKR1C2/4 to 5α-pregnan-3α,11β,17α-triol-20-one (11OHPdiol) and 5α-pregnan-3α,17α-diol-11,20-dione (11KPdiol), respectively (with these metabolites also metabolized by HSD11B). Finally, 11OHAST and 11KAST is biosynthesised by CYP17A1 from the latter metabolites, and in the final steps of this pathway, 11KAST is converted to 11K3αDIOL (by HSD17Bs), followed by the production of 11KDHT (by AKR1C2/4 or RoDH).

919 amino acids [56,57,60,61]. The AR protein is comprised of three functional domains: the N-terminal domain (NTD) which harbours the first activation functional motif (AF1), the DNA binding domain (DBD) which is responsible for the AR interactions and dimerization with specific DNA sequences, and the ligand binding domain (LBD) which contains the second activation functional motif (AF2) which is responsible for most AR activities [56,57,60,61].

Activity of the AR is regulated at different levels. At the genomic level, the *AR* can comprise inactivating variants itself, or its expression can be epigenetically regulated by methylation or cofactors. Studies in patients with a profile of androgen resistance/insensitivity syndrome (AIS type I) revealed many pathogenic variants in the *AR* gene and are listed in the *AR* mutation database [62–65]. In many other patients without an *AR* gene mutation, low expression of the *AR* and low activity on DHT stimulated AR-dependent apolipoprotein D (APOD) expression in genital skin fibroblasts was found and consequently classified as AIS type II [18,66]. In some of these patients, further studies showed a direct correlation between the *AR* mRNA expression and the methylation of *CpG* regions within the proximal *AR* promoter [18,67]. In addition, a highly conserved region at the 3′-*UTR* of the *AR* gene has been identified through which RNA stability can be regulated by RNA binding proteins in multiple cell lines and tissues [68–71].

At the protein level, the expressed AR can be regulated by posttranslational modifications, which may affect its transcription activity, cellular localization, and stability (Fig. 4A) [72]. Posttranslational modifications that have thus far been identified include phosphorylation, methylation, acetylation, ubiquitination and SUMOylation. These modifications were discovered by performing overexpression experiments in androgen dependent cell lines [72]. Identified phosphorylation modifications were mostly mapped to the NTD, and several studies indicated that differentially phosphorylated residues regulate AR stability, cellular localization, and transcription [72,73]. Moreover, acetylation or methylation of residues in the AR hinge region regulate the ligand-dependent activation and nuclear localization, respectively [72,73]. Ubiquitination of residues in the AR LBD region and SUMOlyation in the NTD region also alter AR transcriptional activity and stability and provide another level of regulation of the AR protein [72,73].

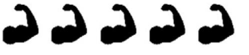
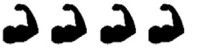
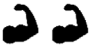
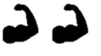
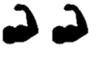
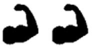
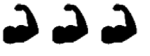
*Androgen receptor signaling*

Androgens are typically secreted from the female and male reproductive systems (ovaries and testes) and the cortex of the adrenal glands to exert their effect on AR in various tissues [57]. After reaching the targeted AR expressing cells through the blood stream (Fig. 4B), androgens bind to the AR. ARs are located in the cytoplasm bound to a complex of different heat shock proteins (HSPs), chaperones and co-chaperones (such as HSP40, HSP70, HSP90 and p23) in a conformation that is competent for ligand-binding [59,74]. Androgen-AR (A-AR) binding leads to signaling activation through a change in AR conformation and dissociation of the chaperones from the AR-complex [59,74]. Once the AR is activated, it can act either at the genomic level or at the non-genomic level.

In the **classical genomic action pathway,** the A-AR complex is transited through the nuclear pore complex into the nucleus. In the nucleus, the A-AR binds as a dimer through its DBD to androgen response elements (ARE) comprised in promoters of target genes [56]. Upon binding of an AR dimer, several co-regulators join the A-AR complex to promote the transcription of target genes [75]. Co-regulators may affect the complex assembly, formation and stability. Moreover, they can affect the chromatin occupancy, looping and remodelling, which can all modulate AR action [75].

Apart from the genomic action, AR signaling in the cell can also occur through a rapid **non-genomic action**. This signaling path of the AR may be activated within seconds or minutes and usually targets plasma membrane proteins or receptors, which activate intracellular kinase cascades that enhance cell proliferation and survival (Fig. 4B) [76,77]. One of the well-studied non-genomic pathways activated by the AR is mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK) signaling. In this signaling pathway, the activated A-AR binds to the tyrosine kinase Src, which then becomes unfolded and auto-phosphorylated resulting in modulation of the MAPK/ERK cascade (Fig. 4B) [76,77]. MAPK/ERK signaling then targets AR-independent transcription, regulating immediate early genes in the cell nucleus through activated phospho-ERK [76]. Interestingly, this modulation seems dose-dependent in prostate cancer (PC) cell lines where low concentrations of androgens

**Table 1**
Circulatory levels (reference ranges) of bioavailable androgens in females and males (group age: ± 18–49 [F; follicular phase or entire cycle]/18–62 [M] years) and their potency towards the mammalian AR in comparison to T. (Not available, N/a; these steroids are not commercially available for bioassays; not established, reference ranges for these hormones have not been confirmed *in vivo*). Abbreviations can be found in the list of abbreviations. *Only in tissue [10,35,115–122].

| Androgen metabolites | Circulatory levels (ng/dL) | | Potency toward the AR |
|---|---|---|---|
| | F | M | |
| **T** | 1.1–14.3 | 95–430 |  |
| **11K3αDIOL** | *Not established* | | N/a |
| **11K5αDIONE** | *Not established* | |  |
| **11KA4** | 17.4–21 | 8.4–9.5 |  |
| **11KAST** | <1 | <1 |  |
| **11KDHT** | <1 | <1 |  |
| **11 KT** | 5.0–60.6 | 9.5–70.8 |  |
| **11OH3αDIOL** | *Not established* | | N/a |
| **11OH5αDIONE** | *Not established* | | Inactive |
| **11OHA4** | 19.2–233 | 36.4–313 | Inactive |
| **11OHAST** | 3.9–4.7 | 4.4–5.3 | <  |
| **11OHDHT** | *Not established* | |  |
| **11OHT** | 8.2–10.8 | 8.5–9 |  |
| **A4** | 28–230 | 44–186 |  |
| **Androstanediol-glucoronide** | 11–249 | 112–1046 | Inactive |
| **Androstanediol*** | *Not established* | |  |
| **Androstanedione** | *Not established* | 5.8–20.2 |  |

(*continued on next page*)

**Table 1** (*continued*)

| Androgen metabolites | Circulatory levels (ng/dL) | | Potency toward the AR |
|---|---|---|---|
| | F | M | |
| **Androstenediol** | 38–45 | 116–125.5 |  |
| **Androsterone** | 31.3–35.2 | 17.1–18.6 |  |
| **DHEA** | 31–701 | 31–701 | Inactive |
| **DHEA-S** | 17,000–372,000 | 16,000–523,000 | Inactive |
| **DHT** | 4–22 | 30–85 |  |

activate the cascade, while high concentrations inhibit it [76,77]. As shown in non-tumoral cells and PC cell lines, activated A-AR may also directly interact with other signaling molecules like phosphoinositide 3-kinases (PI3K), thereby activating the protein kinase B (Akt) kinase pathway [76].

At the systemic level, much of the current knowledge on the role of the AR comes from mouse knockout models [78,79]. The global male *AR* knockout mouse (ARKO) revealed its important role for male fertility and the development of the testes, while the global female ARKO mouse showed an important role in normal breast and ovary development [78,79]. To study the exact role of the AR for specific cells, tissues and developmental processes, cell-specific ARKO mice were investigated [78,79]. Thus Leydig cell-specific ARKO (LC-AKRO) mice present with infertility, absence of sperm, arrested spermatogenesis and low serum T levels, indicating that the AR is critical for maintaining normal T production and spermatogenesis [78]. In gubernaculum-specific ARKO (GU-ARKO) mice, the crucial role of the AR for regulating testicular descent was illustrated [78]. In ovarian cell-specific ARKO mice, AR-mediated action for normal folliculogenesis and fertility was shown, for example its role to optimize follicular development, maximize ovulation rates, and maintain follicle health. Likewise, its role for cellular proliferation and growth of the uterus has also been shown [78,79].

## Implications of lack or excess of androgen action

Males and females need androgens and its action to develop normally and stay healthy, but the dosage of androgens is highly sex specific (Fig. 1). Both androgen deficiency and excess may lead to endocrine disorders that may manifest with a phenotype of disorders/differences of sex development (DSD) at birth, disturb pubertal development, and sexual functioning and fertility. Both lack and excess of androgen action may also manifest at any time of life with adverse effects on other organ systems including overall metabolism, the cardiovascular system, muscles, bones, brain and psychological system. Non-endocrine and non-reproductive effects are described in more details elsewhere [44,80,81].

The effects of androgen deficiency and excess on sexual development and function are best studied in patients carrying rare disease-causing monogenetic variants in genes involved in androgen biosynthesis and action. By contrast, acquired disorders of androgen deficiency and excess will not cause a DSD, but may affect pubertal development, fertility and overall health, nevertheless. Thus, androgen excess in adults is a common problem in women suffering from PCOS and in men with prostate hyperplasia/tumors [82–88]. Furthermore, androgen decline/deficiency is a controversial topic in healthy aging males and females [89,90].

To understand the clinical manifestations of genetic disorders of gonadal and adrenal steroidogenesis at birth it is important to keep in mind that adrenal and gonadal androgens are part of a concerted steroid network *in utero*. During fetal development, the liver and the placenta play major

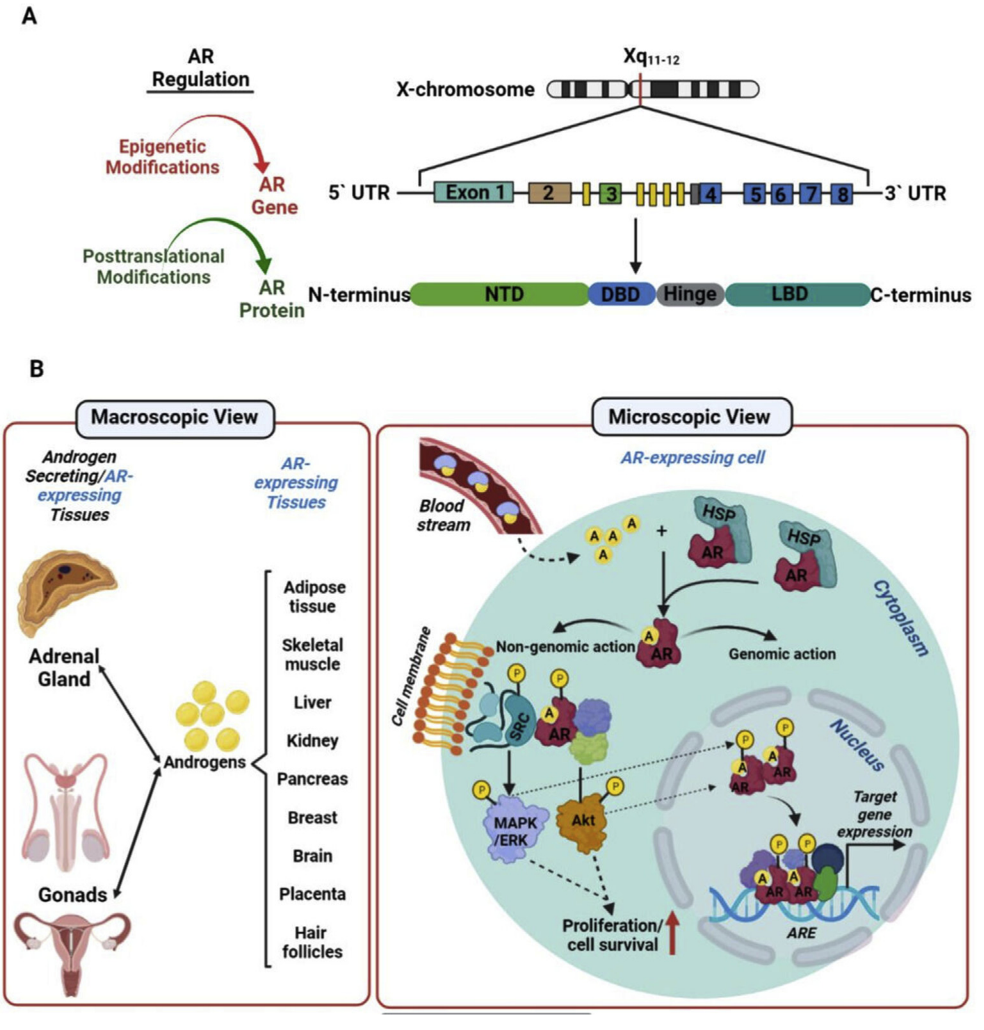



**Fig. 4. Androgen action through the androgen receptor (AR). A.** The human *AR* gene is located on the *X* chromosome at position *Xq11–12*. The *AR* consists of eight exons which are separated by long introns and the *AR* gene can be epigenetically regulated by methylation. Notably, the AR protein is comprised of three functional domains, the N-terminal domain (NTD), the DNA binding domain (DBD) and the ligand binding domain (LBD) and can be regulated by posttranslational modifications. **B. The macroscopic view:** androgens are secreted from the adrenal gland and the gonads. The secreted androgens exert their effect in *AR*-expressing tissues, including the kidney, liver, adrenal gland and gonads. **The microscopic view:** once the androgens (A) are released into the blood stream, they enter the AR-expressing cell and activate the AR. The A-AR complex can have a genomic action where it binds androgen receptor elements (ARE) in the target gene and initiates gene expression. While in the non-genomic action, the A-AR can bind membrane-bound receptors and initiate signaling cascades, which increases the cell proliferation and survival.

roles in this network [91]. The fetal liver functions to metabolise and inactivate androgens, and while the placenta is not considered an androgen producing organ, it does express all the necessary steroidogenic enzymes to convert maternal PROG to androgens, including those that can biosynthesise T [91–96]. Additionally, a role of the placenta in the fetal backdoor pathway in the production of androgens has also been suggested [91]. Moreover, predominant androgen levels have recently been measured in placental tissue [97], highlighting the production of placental androgens and that androgens are exchanged in the feto-placental unit. Notably, placental aromatase would convert A4 and T to estrogens, while other androgens might not be biotransformed to estrogen derivatives *in vivo* [98,99]. As the development of the typical female and male external genitalia relies largely on the absence or presence of T and DHT, any disturbance in androgen production may result in apparent virilization of a 46,XX fetus or undermasculinization of a 46,XY fetus. After birth and minipuberty, gonadal steroidogenesis is quiescent until puberty (Fig. 1), when activation of the HPG axis commands to resume sex steroid production for normal sexual maturation, fertility and reproduction (Fig. 2). Also, in postnatal/adult life, steroids secreted by the adrenals and gonads are converted to active and inactive metabolites by peripheral organs, and this complex peripheral steroid metabolism may then be responsible for the formation of unusual steroids through alternate pathways in genetic disorders of steroidogenesis (Fig. 3).

### *Specific monogenetic defects of androgen biosynthesis*

Pathogenic variants in all genes involved in human androgen biosynthesis and metabolism may cause androgen deficiency and/or excess. Table 2 gives an overview of all genes, for which variants have been reported with a human phenotype associated with abnormal androgen production. For details we refer the reader to [11,57,100]. In the following section we provide a summary of possible genetic defects underlying specific phenotypes.

Genetic defects affecting early steps of steroid biosynthesis and cortisol production in particular are known as **CAH** [11,100]. These disorders may be grouped in defects causing 46,XY DSD CAH, 46,XX DSD CAH or DSD CAH in both chromosomal sexes according to the current DSD classification [101].

To the **first group of 46,XY DSD CAH** belong mutations in the genes for *StAR*, *CYP11A1*, *CYP17A1* and lack of androgens. Patients with autosomal recessive variants in these genes with loss of function are not able to synthesize cortisol and androgens. Thus, affected chromosomal male fetuses are born with typical female external genitalia and raised as girls. However, genetic mutations in these genes with partial loss of function may not affect prenatal sex development and only manifest with primary adrenal insufficiency in early life, while sex hormone failure may develop later. Affected 46,XX individuals with severe mutations in the *StAR*, *CYP11A1*, *CYP17A1* genes manifest at birth with adrenal insufficiency only and show typically lack of pubertal development later.

The **second group of 46,XX DSD CAH** with androgen excess comprises autosomal recessive defects of 21-hydroxylase (*CYP21A2)* and *CYP11B1* necessary for GC and MC synthesis. With these defects that affect GC and MC synthesis in the adrenal cortex, the lack of cortisol leads to negative feedback stimulation of the HPA axis, elevated ACTH and thus increased adrenal androgen production. Affected girls show variable degrees of external genital virilization at birth as the androgen excess already occurs very early *in utero,* when the female fetus needs to be safeguarded from high androgens to develop normally [102]. Affected 46,XY boys have no DSD phenotype but still suffer from neonatal onset adrenal insufficiency with severe variants.

Genetic mutations in *HSD3B2* and *POR* belong to the **third group** that can manifest with cortisol deficiency and variable severity of a **DSD at birth in both chromosomal sexes** depending on the specific variants.

**Genetic defects that only affect sex hormone biosynthesis** also display some sex specific characteristics. 46,XY DSD individuals with an isolated moderate to severe undervirilization at birth may harbour a defect in the *CYP17A1* (isolated 17,20-lyase deficiency), *CYB5A1*, *POR, AKR1C2/4, HSD17B3* or *SRD5A2* genes (Fig. 3). Regarding these genetic variants, only 46,XX females with variants in *CYP17A1* and *POR* have been found with a DSD phenotype (*POR*), with primary or secondary failure of pubertal development (*POR, CYP17A1*), or with a PCOS-like phenotype (*POR*). Moreover, aromatase (*CYP19A1)* mutations inhibiting the aromatase activity and the conversion of androgens to estrogens, cause fetal and maternal androgen excess during pregnancy and beyond. Affected girls are virilized at birth and show abnormal pubertal development and fertility. Affected boys are asymptomatic during childhood years, but then come to medical attention for tall stature and failure to stop growing. Bone age is typically delayed because estrogens are crucial for epiphyseal maturation and closure, and decreased bone mineral density can be observed later in life. A negative impact on glucose homeostasis and lipid profile has also been described in both adult males and females with aromatase deficiency.

Finally, genetic variants in the steroidogenic factor 1/nuclear receptor subfamily 5 group A member 1 (*SF1/NR5A1*) may cause a broad phenotypical range of 46,XY DSD, 46,XX DSD and failure of pubertal development and fertility [103,104]. In rare cases, *NR5A1* mutations also cause adrenal insufficiency. SF1 is an essential transcription regulator for many genes involved in sex determination, sex differentiation as well as (sex) steroid biosynthesis [103–107].

*Genetic defects of androgen action – AIS*

Complete or partial androgen insensitivity syndromes (CAIS/PAIS) are caused by genetic mutations affecting AR function [18,108]. In these syndromes of 46,XY phenotypic undermasculinization, androgen concentrations are typically elevated [62]. AIS incidence is reported internationally in 1 in 20,400 live born 46,XY infants, with CAIS occurring at a higher rate than PAIS [109]. This high incidence might be explained by low mortality and morbidity of AIS. So far about five hundred *AR* mutations have been described in individuals with AIS, classified as **AIS type I**. In CAIS, severe hemizygous *AR* mutations cause the loss of AR signaling in more than 95% of cases. Affected 46,XY individuals show male typical inner genital organs and undescended gonads with the prostate, vas deferens and seminal vesicles missing. External genitalia are typical female with a vaginal pouch. Female carriers are phenotypically normal. In 46,XY PAIS, the phenotypic variability in undermasculinization is large and depends on the residual activity of the AR. In the mildest form of PAIS, gynecomastia and male infertility may be the only clinical signs. Unlike in CAIS, the underlying genetic defect in PAIS is only found in the *AR* gene in about 40% of cases [64].

By contrast, individuals with AIS without *AR* mutations are classified as **AIS type II**. In these individuals largely unidentified regulators or cofactors of the AR are responsible for the impaired AR signaling as revealed by an AR-dependent bioassay using genital skin fibroblasts and the targeted APOD as a biomarker [66]. As AR activity can be regulated at various levels, the possible mechanisms of AIS type II are manifold. Thus far, altered DNA methylation of the *AR* promoter has been found in some individuals with PAIS [67].

*Common acquired disorders of androgen excess*

Androgen levels and AR activity control development, proliferation and growth of a variety of cells in many organs and regulate metabolic processes in both males and females (Table 1) [57].

Unbalanced levels of androgens are involved in the development of several types of cancers including PC and CRPC [57,82–84]. In men, PC is the second leading cause of cancer death worldwide. PC growth and development critically dependent on androgens and AR signaling [60,82,83]. Androgens promote prostate cell proliferation resulting in continuous growth of malignant cells. But it is important to note that there is no evidence of an association between the lifetime T exposure and the appearance of PC [110]. Therefore, local prostate tumors are cared for by watchful waiting or treated with surgery or radiation therapy, while high-grade and metastatic PCs are treated with androgen-deprivation therapies, lowering T levels. However, over time many PCs escape this treatment and progress to CRPC [83]. CRPC might be driven by potent androgens synthesized from alternate, non-gonadal steroid metabolites (for example metabolites originating from the adrenals) [47,49,50] that stimulate AR signaling [83]. However, the specific mechanisms associated are still unclear [82–84].

In women, PCOS is the most common endocrine condition affecting about 10% of reproductive-age women [85]. PCOS is characterized by hyperandrogenism, menstrual disturbances and polycystic ovaries [86,111]. Studies performed in animals and humans support the hypothesis that excess androgen and AR action are key players of PCOS pathomechanism as they are clearly associated with adverse reproductive and metabolic outcome [86], but the exact interplay remains a conundrum. Numerous studies have shown that androgens of the classic pathway (such as T and A4; Fig. 3A) are elevated in circulation of most women with PCOS when compared to healthy controls [85,87]. In line with these findings are *in vitro* studies showing ovarian theca cells isolated from PCOS women with steroidogenic hyperfunction resulting in androgen excess [88].

Interestingly, comprehensive steroid profiling in more recent studies showed that C11-oxy androgens of alternate pathways (such as 11OHA4, 11KA4, 11OHT and 11KT; Fig. 3C and D) are even more

**Table 2**
Characteristics of monogenetic disorders of steroidogenesis affecting androgen production and the gonads in 46,XX and 46,XY individuals [11,57,100].

| Disorder | Gene/ OMIM | 46,XY Gonadal Phenotype (T Deficiency) | 46,XX Gonadal Phenotype (E2 Deficiency) | Fertility | Adrenal Insufficiency | Other Features |
|---|---|---|---|---|---|---|
| **Lipoid congenital adrenal hyperplasia (LCAH)** | *StAR* 201710 | *Classic form*: 46,XY DSD, gonadal insufficiency *Non-classic form*: normal or NK | *Classic*: primary or secondary ovarian insufficiency (POI) *Non-classic*: NK or normal | *Classic:* Absent in 46,XY; variable in 46,XX | YES | |
| **P450 side chain cleavage syndrome (CAH)** | *CYP11A1* 118485 | *Classic form*: 46,XY DSD, gonadal insufficiency *Non-classic form*: normal | *Classic*: primary or secondary ovarian insufficiency (POI) *Non-classic*: NK or normal | Reported in 46,XX | YES | |
| **3β-Hydroxysteroid dehydrogenase II deficiency (CAH)** | *HSD3B2* 201810 | 46,XY DSD, gonadal insufficiency *Non-classic form*: normal, but premature adrenarche | 46,XX DSD with atypical genital development; gonadal insufficiency *Non-classic form*: normal, but premature adrenarche | Absent in 46,XY; reported in 46,XX | YES | |
| **21-Hydroxylase deficiency (CAH)** | *CYP21A2* 201910 | *Classic form*: normal *Non-classic form*: normal | 46,XX DSD with atypical genital development through androgen excess; *Non-classic form*: premature adrenarche, virilization, PCO | Normal in both 46,XX and 46,XY, if treated | YES | Cave: Testicular adrenal rest tumor (m >> f) CAH-X (when combined with Ehlers-Danlos syndrome with contiguous gene variants) |
| **11β-hydroxylase deficiency (CAH)** | *CYP11B1* 202010 | *Classic form*: normal *Non-classic form*: normal | 46,XX DSD with atypical genital development through androgen excess; *Non-classic form*: premature adrenarche, virilization, PCO | Normal in both 46,XX and 46,XY, if treated | YES | Hypertension |
| **Combined 17α-hydroxylase, 17,20 lyase deficiency (CAH)** | *CYP17A1* 202110 | 46,XY DSD, gonadal insufficiency | Lack of pubertal development, POI | Possible in 46,XX with assisted fertility measures | Rare | Hypertension and hypokalemic alkalosis (not seen with isolated lyase deficiency) |
| **P450 oxidoreductase deficiency (CAH)** | *POR* 124015 201750 | Mild to severe 46,XY DSD, gonadal insufficiency | 46,XX DSD with atypical genital development or premature adrenarche (androgen excess), virilisation, POI, PCO | Reported | Variable | Maternal virilization during pregnancy; Antley-Bixler skeletal malformation syndrome; changes in drug metabolism |
| **Cytochrome $b_5$ deficiency** | *CYB5A* 613218 | 46,XY DSD | NK | NK | NO | Methemoglobinemia |

**Table 2** (*continued*)

| Disorder | Gene/ OMIM | 46,XY Gonadal Phenotype (T Deficiency) | 46,XX Gonadal Phenotype (E2 Deficiency) | Fertility | Adrenal Insufficiency | Other Features |
|---|---|---|---|---|---|---|
| **17β-Hydroxysteroid dehydrogenase III deficiency/17-ketosteroid reductase deficiency** | *HSD17B3* 264300 | 46,XY DSD; progressive virilisation and gynecomastia at puberty | Normal | Decreased or absent in 46,XY | NO | |
| **5α-Reductase II deficiency** | *SRD5A2* 607306 | 46,XY DSD; progressive virilisation and gynecomastia at puberty | Normal | Impaired in 46,XY | NO | |
| **3α-Hydroxysteroid dehydrogenase deficiency** | *AKR1C2/4* 600450 600451 | 46,XY DSD; gonadal insufficiency | Normal | NK | NO | |
| **Aromatase deficiency** | *CYP19A1* 107910 | Normal | 46,XX DSD with variable degree of virilisation at birth, gonadal insufficiency, POI | Impaired in 46,XX | NO | Overgrowth and metabolic anomalies in males |
| **Steroidogenic factor 1** | *NR5A1/SF1* 184757 | Mild to severe 46,XY DSD; gonadal insufficiency – very variable | Mostly POI or normal 46,XX ovotesticular DSD | Mostly impaired in 46,XY; variable in 46,XX | Rare | |

Abbr.: DSD – Disorder/Difference of Sexual Development; E2 – estradiol; NK – not known; PCO – polycystic ovaries; POI – primary ovarian insufficiency; T – testosterone.

elevated than classic androgens in PCOS patients [24,112]. This indicates that androgen excess in PCOS originates from both the ovaries and non-gonadal tissues including the adrenals and peripheral tissues as well as the intermediary metabolism. This also suggests that the underlying defect of PCOS leads to a dysregulation of androgen production through different steroid producing pathways. In addition, hyperandrogenism in PCOS is very often associated with insulin resistance [43,86,111], and both may lead to metabolic dysfunction in the reproductive tissues and beyond [88]. However, although the metabolic consequences of PCOS have been well described, the underlying cause and detailed molecular mechanisms are still unsolved.

Paradoxically, there are no clear pathophysiological conditions in men where T is too high, and no such conditions in women where it is too low [113]. However, there are some discussions about a male PCOS phenotype with androgen excess and a female lack of libido with deficiency.

### *Acquired disorders of androgen deficiency*

In principle, the loss of circulating androgens in adults is caused by gonadal failure. This might be due to any disorders affecting the HPG axis (Fig. 2). In men, hypogonadism causes impaired sexual functioning, infertility, alterations in body composition and osteoporosis [89]. Moreover, it has adverse effects on metabolic and cardiovascular health as well as a negative impact on brain health [90]. When in women low androgen levels are due to hypogonadism, estrogen levels are also decreased, and the consequences of low estrogens are the same as with hypogonadism in men [89,113]. By contrast, no adverse consequences are seen when androgen levels are decreased because of a loss of adrenal androgens with primary or secondary adrenal disorders [6]; although this has been challenged in some studies for women without convincing evidence [114].

## Summary

Clinical, genetic and functional characterization of human mutations in genes involved in androgen biosynthesis and action have taught us plenty about the crucial role of androgens for sexual development, reproduction and beyond. All the same, many patients manifesting with rare congenital or common acquired disorders of androgen deficiency or excess remain unexplained indicating that androgen biology is still not fully understood. Only in the last two decades, two novel alternate pathways of androgen biosynthesis have been recognized to play important roles in normal biology and disease states. But the exact interplay and specific contribution of the different biosynthesis pathways to the active androgen pool need further investigation. Likewise, the regulation of the AR is more complex than initially assumed. In theory, multiple regulators and co-regulators may modulate AR signaling at the transcriptional and posttranslational level. Studies of patients with PAIS found differential *AR* promoter methylation in some, but other regulatory factors have yet to be identified.

Understanding the complex network regulating androgen biosynthesis, metabolism and action is essential for finding new targets for better diagnostic and therapeutic opportunities.

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## Declaration of competing interest

The authors declare no competing interest.

### Practice points

- Androgens are essential sex steroid hormones for both sexes.
- Testosterone is the predominant circulating androgen in males, while in females the major circulating classic androgens are DHEA-S, DHEA and androstenedione.
- Androgens exert their effect through binding to the androgen receptor to modulate many different biological processes.
- Androgen receptor signaling occurs at the genomic and non-genomic level.
- Rare human genetic disorders have been described for all genes involved in androgen biosynthesis and for the androgen receptor.
- Common disorders associated with androgen imbalance are castration resistant prostate cancer in men and polycystic ovary syndrome in women.

### Research agenda

- To better understand the role of newly discovered, alternate androgen biosynthesis pathways in health and disease.
- To study the impact of alternate' androgens on the androgen receptor in various tissues.
- To identify regulators and co-regulators of the androgen receptor affecting its expression, stability and activity.
- To understand the role of androgens' action in common disorders like prostate hyperplasia and cancers, and in polycystic ovary syndrome in order to identify targets for better treatments.
- To investigate the treatment potential of candidate drugs for disorders and diseases marked by androgen imbalances, such as selective androgen receptor modulators (SARMs).

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