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Role of Aldo-Keto Reductase Family 1 (AKR1) Enzymes in Human Steroid Metabolism

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Abstract

Human aldo-keto reductases AKR1C1-AKR1C4 and AKR1D1 play essential roles in the metabolism of all steroid hormones, the biosynthesis of neurosteroids and bile acids, the metabolism of conjugated steroids, and synthetic therapeutic steroids. These enzymes catalyze NADPH dependent reductions at the C3, C5, C17 and C20 positions on the steroid nucleus and side-chain. AKR1C1-AKR1C4 act as 3-keto, 17-keto and 20-ketosteroid reductases to varying extents, while AKR1D1 acts as the sole Δ^4-3-ketosteroid-5β-reductase (steroid 5β-reductase) in humans. AKR1 enzymes control the concentrations of active ligands for nuclear receptors and control their ligand occupancy and *trans*-activation, they also regulate the amount of neurosteroids that can modulate the activity of $GABA_A$ and NMDA receptors. As such they are involved in the pre-receptor regulation of nuclear and membrane bound receptors. Altered expression of individual *AKR1C* genes is related to development of prostate, breast, and endometrial cancer. Mutations in *AKR1C1* and *AKR1C4* are responsible for sexual development dysgenesis and mutations in *AKR1D1* are causative in bile-acid deficiency.

Keywords

cancer; bile-acids; hydroxysteroid dehydrogenase; inherited mutations; neurosteroids; synthetic steroids

1. Enzymes of steroid metabolism

Imbalance in the biosynthesis and inactivation of steroids can lead to development of disease, including hormonally dependent cancer, of the breast, prostate, endometrium and ovary [1], and to diseases such as benign prostatic hyperplasia, endometriosis, cholestasis, neonatal liver failure, neurological disorders [2–6] and malformation or differentiation of the genitalia [7]. The metabolism of steroids involves phase I and phase II enzymes and has an important role in human health and disease. Phase I steroid biosynthetic enzymes include:

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monooxygenases from the cytochrome P450s superfamily (CYP), and oxidoreductases from the short-chain dehydrogenase reductase (SDR) and the aldo-keto reductase (AKR) superfamilies. An outlier are the 5α-reductases which belong to the polyprenol reductase family. The phase II conjugating enzymes include the sulfotransferases (SULTs), UDP glucuronosyl transferases (UGTs) and catechol-*O*-methyl transferases (COMT).

The SDR and AKR enzymes catalyze NAD(P)H dependent oxidoreductions and act at the C3, C5, C11, C17 and C20 positions of the steroid nucleus and side-chain and function as 3α/β-hydroxysteroid dehydrogenases (HSDs), 5β-reductases, 11β-HSDs, 17β-HSDs and 20α-HSDs, respectively [8]. HSDs from the SDR and AKR superfamilies differ in their protein folds, stereochemistry of hydride transfer and kinetic mechanism but share a common catalytic mechanism that has been evolutionary conserved [8,9]. Human enzymes from the AKR1 family catalyze either the biosynthesis of steroid hormones, bile acids and neurosteroids, or their inactivation and thus regulate activity and action of these mediators [8]. In addition, these enzymes act on conjugated steroids and synthetic steroids used as therapeutics [10–13].

2. Human AKR1 enzymes implicated in steroid metabolism

Human AKR1 enzymes implicated in steroid metabolism are members of the AKR1C and AKR1D subfamilies. AKR1C enzymes (AKR1C1-AKR1C4) function *in vivo* as 3-keto-, 17-keto- and 20-ketosteroid reductases to form 3α/β, 17β- and 20α-hydroxy-metabolites to varying extents and thus metabolize a broad spectrum of natural and synthetic therapeutic steroids [14], Figure 1. These enzymes are expressed in different tissues, while AKR1C4 is mainly liver specific [14]. AKR1C enzymes share a high percentage of amino-acid sequence identity that ranges from 84% to 98%. In particular AKR1C1 and AKR1C2, differ by only seven amino-acid residues. A large number of crystal structures of human AKR1C enzymes in complex with different ligands have been solved (37 as of May 5, 2013) and are deposited in the Protein Data Bank. AKR1C enzymes share the protein fold in common with other members of the superfamily, which is the triose phosphate isomerase (TIM) barrel characterized by an alternating arrangement of α-helix and β-strand to form a β-barrel. At the back of the barrel three large loops exist which define substrate specificity. In each of the AKR1 proteins the cofactor binds in a conserved manner across the lip of the β-barrel, while the steroid lies perpendicular to the cofactor. The conserved catalytic tetrad resides at the base of the barrel and consists of Asp50, Tyr55, Lys84 and His117, where Tyr55 acts as a general acid-base [8]. These cytosolic enzymes use nicotinamide adenine dinucleotides as cofactors, which are bound in an extended *anti*-conformation thus facilitating 4-pro-*R*-hydride transfer to the acceptor group [8]. These enzymes preferably act as reductases *in vivo* since they have K_d values for NADPH in the nanomolar range, there is a high NADPH/$NADP^+$ ratio within cells and NADPH inhibits the NAD^+ dependent oxidation reactions at low μM concentrations [15–17].

Extensive comparison of the steroid binding sites of the individual isoforms has elucidated the structural basis of substrate specificity. For example ketosteroid substrates can bind in reverse orientation so that the D-ring binds at the base of the pocket instead of the A-ring. In addition stereochemistry of the hydroxysteroid product can be inverted by either the ketosteroid being pushed across the steroid pocket by steric forces or the ketosteroid can be bound upside down so that a different face of the steroid is presented to the cofactor (Figure 2) [18, 19].

In contrast to AKR1C enzymes the only human member of the AKR1D subfamily, AKR1D1, catalyzes the NADPH dependent reduction of the C4-C5 double bond in Δ^4 ketosteroids to form A/B *cis*-ring junction (Figure 1). This configuration introduces a 90°

bend into the planar steroid and introduces amphipathic properties into the sterol which is essential for emulsification of fats and cholesterol [20]. The AKR1D1 enzyme has the typical $(\alpha/\beta)_8$ barrel structure of the AKR superfamily with cofactor and the steroid substrate bound at the C-terminal end of the β-sheets [21]. AKR1D1 has a conserved catalytic tetrad: Tyr58, Lys87 and Glu120 and Asp53 [22] which differs from catalytic tetrad of AKR1C members, as His is replaced by Glu. A mechanism of AKR1D1 catalyzed 5β-reduction has been proposed, in which Tyr58 acts as a general acid and Glu120 facilitates enolization of the Δ^4 double bond by acting as a superacid [23]. AKR1D1 has broad substrate specificity; it catalyzes the reduction of C18, C19, C21 and C27 Δ^4-ketosteroids and some synthetic drugs [23, 24]. Thus only one enzyme is required for the 5β-reduction of all Δ^4 ketosteroids. AKR1D1 is expressed mainly in liver as might be expected by its role in bile acid biosynthesis [25, 26].

All AKR1 enzymes catalyze a sequential ordered bi bi kinetic mechanism in which there is an obligatory requirement for the cofactor to bind before the steroid substrate can bind to form the central complex. Following the chemical step, steroid product then leaves followed by the oxidized cofactor. Different steps of the reaction can be rate-limiting depending upon the substrate. The chemical step or the release of either product may contribute to rate determination. However, with substrates with high turnover numbers the rate of release of the $NADP^+$ cofactor places an upper limit on the rate of reaction. The release of the $NADP^+$ cofactor is a two step process in which an isomerisation event occurs to convert a $E.NADP^+$ tight complex to form a $E.NADP^+$ loose complex [27–29].

2.1. AKR1 enzymes in androgen metabolism

In peripheral tissues, AKR1C enzymes act as 3-keto and 17-ketosteroid reductases to catalyze either the formation of the most potent androgen 5α-dihydrotestosterone (5α-DHT) or its inactivation to yield the inactive androgens 5α-androstane-3α,17β-diol (3α-Diol) or 5α-androstane-3β,17β-diol (3β-Diol) (Table 1, Figure 3). AKR1C3, also known as 17β-HSD type 5, acts as 17-ketosteroid reductase and has the highest catalytic efficiency of the AKR1C enzymes for the reduction of Δ^4-androstene-3,17-dione to form testosterone [30, 31]. LNCaP-cells stably transfected with AKR1C3 can recapitulate this reaction [32]. The conversion of Δ^4-androstene-3,17-dione to testosterone and its subsequent reduction to 5α-DHT by SRD5A1 and SDR5A2 is known as the “classical pathway” of 5α-DHT formation [33]. AKR1C3 also reduces 5α-androstane-3,17-dione to 5α-DHT which by-passes testosterone (this is known as the alternative pathway to DHT); and reduces androsterone to 3α-Diol (a backdoor precursor of 5α-DHT). Thus all pathways to the potent androgens testosterone and 5α-DHT proceed through AKR1C3. AKR1C2 and AKR1C4 also catalyze the reduction of 5α-pregnane-3,20-dione to yield 3α-hydroxy-5α-pregnane-20-one (allopregnanolone) which is the precursor of androsterone (Table 2) [34]. In this manner AKR1C2 and AKR1C4 enzymes play a critical role in the “backdoor pathway” of androgen formation in which 5α-reduction occurs at the levels of the pregnanes [33, 35].

The inactivation of 5α-DHT is also catalyzed by AKR1C enzymes. AKR1C2 has the highest catalytic efficiency as a 3-ketosteroid reductase and catalyzes reduction of 5α-DHT to 3α-Diol. COS-1, PC-3 and LNCaP cells transfected with AKR1C2 were able to recapitulate this reaction [15, 16]. On the other hand, AKR1C1 forms mainly 3β-Diol, a potent agonist of ERβ which exerts anti-proliferative effects. Liver specific AKR1C4 forms 3α-Diol with the highest k_{cat} among the AKR1C enzymes [16]. AKR1C3 is much less efficient as a 3-ketosteroid reductase and converts 5α-DHT mainly to 3α-Diol [16].

In liver, circulating testosterone is metabolized either via 5α-reductase or 5β-reductase to form 5α-DHT and 5β-DHT, respectively. Subsequent 3-ketosteroid reduction by the AKR1C enzymes yields four stereoisomeric tetrahydrosteroids which can be further

conjugated by phase II enzymes. AKR1D1 has a high catalytic efficiency for the reduction of Δ^4-androstene-3,17-dione and testosterone to form 5β-androstane-3,17-dione and 5β-DHT, respectively (Table 1) [23]. It is noteworthy that some Δ^4 ketosteroids show distinct substrate inhibition with AKR1D1 e.g. testosterone which can be explained by two binding modes for the steroid; productive and non-productive. In the former instance testosterone binds in the steroid cavity so that it is bound perpendicular to the cofactor and the 3-ketone is tethered to the catalytic tyrosine. In the latter instance testosterone lies across the steroid cavity and lies parallel to the cofactor where it blocks access to the channel by the other steroids [21]. Further reduction of 5β-DHT to etiocholanone is catalyzed by the AKR1C enzymes, where AKR1C4 is the most efficient enzyme, followed by AKR1C2, AKR1C3 and AKR1C1 [36].

2.2. AKR1 enzymes in estrogen and progesterone metabolism

AKR1C enzymes also act as 17-ketosteroid reductases to reduce the weak estrogen, estrone to form the potent estrogen, 17β-estradiol in peripheral tissues (Figure 4) [14]. AKR1C3 is the most efficient enzyme for this reaction (K_M = 9 μM, k_{cat} = 0.068 mM^{-1}; k_{cat}/K_M = 76 min^{-1} mm^{-1}). In MCF-7 cells stably transfected with AKR1C3 it was found that the enzyme converts 0.1 μM estrone to 17β-estradiol faster than it catalyzes either the conversion of Δ^4-androstene-3,17-dione to testosterone or the conversion of progesterone to 20α-hydroxy-pregn-4-ene-3-one [37]. Other AKR1C enzymes have much lower activities for 17β-estradiol formation [14].

AKR1 enzymes also have important roles in metabolism of progesterone (Table 2, Figure 4). AKR1C1, but also AKR1C2 and AKR1C3 catalyze progesterone reduction to form the less potent progestagen 20α-hydroxy-pregn-4-ene-3-one [13, 14]. In liver and some peripheral tissues (e.g. placenta and myometrium) AKR1D1 catalyzes reduction of progesterone to 5β-dihydroprogesterone (5β-DHP) while the 5α-reductases SRD5A1 and SRD5A2 form 5α-dihydroprogesterone (5α-DHP) [23, 38, 39, 40]. Further metabolism of 5α/β pregnanes proceeds by AKR1C enzymes [34]. 5α-Pregnane metabolites stimulate proliferation of breast cancer cell lines [41] while 5β-pregnanes have been implicated in human parturition [40, 41].

2.3. AKR1 enzymes in neurosteroid metabolism

5α-Pregnane metabolites formed from progesterone by the action of SRD5A1 potentiate the action of γ aminobutyric acid receptor A ($GABA_A$) agonists [42]. Thus in the absence of GABA they are ineffectual but in the presence of GABA they can potentiate chloride ion channel opening. Allopregnanolone is a potent allosteric modulator of $GABA_A$, while 5α-pregnane-3α,20α-diol is less neuroactive [42]. All AKR1C enzymes act on 5α-pregnanes (Table 2) [34]. AKR1C1, AKR1C2 and AKR1C4 have high catalytic efficiencies for 5α-DHP reduction, where AKR1C1 preferentially forms 20α-hydroxy-5α-pregnane-3-one and AKR1C2 preferentially forms allopregnanolone. Additionally, AKR1C1 catalyzes the reduction and thus inactivation of allopregnanolone by forming 5α-pregnane-3α,20α-diol. AKR1C3 has low k_{cat} values for the reduction of 5α-DHP and 20α-hydroxy-5α-pregnane-3-one [34, 43] The kinetic data thus suggest that in brain AKR1C2 catalyzes formation of the potent neurosteroid allopregnanolone, while AKR1C1 is involved in its inactivation [43]. AKR1C1 can regulate the cellular concentrations of allopregnanolone by preventing its formation from progesterone and by catalyzing allopregnanolone inactivation. Recently it has been shown that allopregnanolone also acts as *N*-methyl-*D*-aspartate (NMDA) receptor antagonist in rat [44], modulates NMDA receptor action, and thus induces LHRH release [45].

2.4. AKR1 enzymes in bile acid biosynthesis

AKR1D1 and AKR1C4 are predominately expressed in liver [14, 25, 26] where they play essential and sequential roles in the biosynthesis of bile acids (Figure 5) [20]. These enzymes act in concert, where AKR1D1 catalyzes 5β-reduction of bile acid precursors 7α-hydroxy-4-cholestene-3-one and 7α,12α-dihydroxy-4-cholestene-3-one to yield 7α-hydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-5β-cholestan-3-one, respectively and AKR1C4 catalyzes the further reduction of 3-keto group [20, 23]. The later reactions proceed with a higher catalytic efficiency than double bond reduction (Jin and Penning, unpublished data). Subsequent steps in bile acid biosynthesis include side chain oxidation and truncation, and conjugation of the bile acid with taurine or glycine [20].

2.5. AKR1C enzymes in the metabolism of steroid conjugates

AKR1C enzymes catalyze the reduction of carbonyl groups to hydroxyl groups in the steroid molecule to enable further conjugation by sulfation or glucuronidation by phase II enzymes. It is generally accepted that phase I and phase II enzymes act sequentially. Recently AKR1C enzymes were shown to act on conjugated steroids (Table 3) [10], which suggested an alternative route where the steroid is conjugated first at the 17β-position and then reduced at the 3-position. Among AKR1C enzymes, AKR1C4 has by far the highest catalytic efficiency for reduction of 5α-DHT-17β-glucuronide or sulfate. AKR1C3 has a low k_{cat} for this reaction. By contrast, AKR1C1 and AKR1C2 prefer sulfate to bulky glucuronide conjugates where AKR1C2 is the more efficient enzyme [10]. AKR1C1, AKR1C2 and AKR1C3 form mainly 3β-hydroxy-5α-androstane-17β-glucuronide whereas AKR1C4 preferentially forms the corresponding 3α-stereoisomer. AKR1C1 and AKR1C4 thus show unchanged stereochemical preference when compared to the reduction of the unconjugated 5α-DHT, forming 3β- and 3α-metabolites, respectively. However, the stereospecificity of AKR1C2 and AKR1C3 is reversed with 5α-DHT-17β-glucuronide forming 3β-hydroxy-5α-androstane-17β-glucuronide instead of 3α-hydroxy-5α-androstane-17β-glucuronide [10]. As longer incubation times were required to measure the weak 3-ketosteroid reductase activity of AKR1C3 for the glucuronide conjugate, the observed change in stereoselectivity may be related to its epimerase activity [10].

2.6. AKR1 enzymes in metabolism of synthetic steroids

Many synthetic steroids contain the Δ^4 or 3-keto, 20-keto-steroid structural motif and are anticipated to be substrates for the AKR1 enzymes. In support of this contention AKR1 enzymes are involved in the metabolism of tibolone and the progestins, norethynodrel and dydrogesterone (Table 4, Figure 6), as well as the synthetic glucocorticoids budesonide and flunisolide [11–13, 18]

Tibolone is a prodrug used for the treatment of post-menopausal symptoms. Its tissue specific effects which make it a “selective tissue estrogen activity regulator” is likely mediated by AKR metabolism. This synthetic steroid maintains bone without the undesirable side effects on the endometrium and breast. AKR1C1 and AKR1C2 form 3β-hydroxytibolone and are responsible for pro-drug activation in peripheral tissues where the 3β-hydroxymetabolite activates ERα/β. The formation of the 3β-hydroxymetabolite is preferred over the 3α-hydroxymetabolite because the presence of the 5(10)-ene and 17α-ethinyl group enables the steroid to be flipped over at the active-site relative to 5α-DHT. By contrast the mainly liver specific AKR1C4 forms the 3α-hydroxy-metabolite which is the major metabolite found in the circulation [18]. The same outcome is observed for the norethynodrel suggesting that the presence of the 7α-methyl group in tibolone does not determine the stereochemcial outcome.

The progestin dydrogesterone is used for hormone-replacement therapy, for treatment of endometriosis, menstrual disorders, corpus luteum insufficiency and habitual or threatened abortions [46]. Dydrogesterone is metabolized to the active 20α-hydroxy-metabolite, 20α-hydroxy-9β,10α-pregna-4,6-diene-3-one by AKR1C1 and AKR1C2 enzymes and acts as μM inhibitor of AKR1C3 [11, 13].

The inhaled synthetic corticosteroids budesonide and flunisolide used in anti-inflammatory drug therapy in asthma patients can be metabolized by AKR1D1 and AKR1C enzymes. These steroids also act as inhibitors of AKR1 enzymes. Interestingly, budesonide was a more potent inhibitor of the AKR1D1 and AKR1C4 enzymes in the liver than flunisolide suggesting that this inhibition might contribute to the undesirable systemic effects of budesonide [24]. More extensive studies on the roles of AKR1 enzymes on the metabolism of synthetic steroids are still required. It is anticipated that single nucleotide polymorphisms (SNPs) in the *AKR1C1* and *AKRID1* genes are likely to influence the metabolism of different classes of therapeutic steroids (see section 5).

3. AKR1 enzymes control concentrations of receptor ligands

Human AKR1 enzymes catalyze the conversion of either weak ligands to form potent ligands for nuclear receptors or they are involved in the elimination of these ligands. In this manner AKR1 enzymes control the levels of potent ligands that can occupy and *trans*-activate nuclear receptors within endocrine target tissues. These receptors bind to DNA response elements as homo or heterodimers, recruit co-activators or co-repressors and thus regulate gene transcription [4, 14, 47]. Additionally, AKR1 enzymes also control biosynthesis of neurosteroids and modulate activity of GABA receptor A which act as a ligand-gated ion channel [14, 16, 42] and the ligand-gated and voltage dependent NMDA receptor [44, 45].

3.1. Androgen receptor

AKR1 enzymes control concentrations of active androgens and regulate the occupancy and *trans*-activation of androgen receptor (AR) in the prostate. AKR1C3 catalyzes the reduction of Δ^4-androstene-3,17-dione to form the potent androgen testosterone ($K_d = 10^{-9}$ M for AR) and the reduction of 5α-androstane-3,17-dione to yield the most potent androgen 5α-DHT ($K_d = 10^{-10}$ M for the AR). On the other hand, AKR1C2 catalyzes inactivation of 5α-DHT to yield the less active 3α-Diol ($K_d = 10^{-6}$ M for the AR) [15, 16]. The reverse reaction, which involves oxidation of 3α-Diol to 5α-DHT is catalyzed by the SDR members, mainly HSD17B6 also designated as SDR9C6 [48]. These reactions thus represent molecular switches that can control ligand occupancy of the AR and likely influence prostate cancer progression.

3.2. Estrogen receptors

AKR1 enzymes control concentrations of estrogens and regulate occupancy and *trans*-activation of estrogen receptors α and β (ERα and ERβ). AKR1C3 catalyzes reduction of estrone (K_d = 0.3 nM for ERα and ERβ) to yield the more potent estrogen 17β-estradiol, (K_d = 0.1 nM for ERα and ERβ) and thus controls activation of ERα and ERβ [37]. The reverse reaction is catalyzed by HSD17B2 also designated as SDR9C2 [49, 50]. Thus these reactions represent molecular switches that can finely tune and control ligand occupancy of the ER. AKR1C1 also regulates occupancy and *trans*-activation of ERβ as it catalyzes reduction of 5α-DHT to yield 3β-androstanediol (3β-Diol) a potent agonist ($K_d = 10^{-9}$ M for ERβ) [16]. These reactions are important in estrogen sensitive tissues, such as breast and endometrium where occupancy of ERα is thought to be proproliferative and occupancy of ERβ is thought to be antiproliferative., The ERβ agonist 3β-Diol may also have important

role in androgen target tissues, e.g. prostate where it promotes cell apoptosis. ERβ is also thought to play a role in the etiology of lung cancer in women [51].

3.3. Progesterone receptors

AKR1 enzymes also control local concentrations of progesterone and thus regulate occupancy and *trans*-activation of progesterone receptors A and B (PR-A and PR-B), both encoded by the *PGR* gene. AKR1C1, as well as AKR1C2 and AKR1C3, catalyze inactivation of progesterone (K_d = nM for the PR) to the less potent 20α-hydroxyl-pregn-4-ene-3-one [13, 14] (Kd = μM for the PR), while the oxidation back to progesterone is catalyzed by HSD17B2 (SDR9C2) [52]. These reactions thus act as molecular switches to regulate the PR in progesterone target tissues e.g. endometrium and breast.

3.4. $GABA_A$ Receptor

AKR1C2 catalyzes reduction of 5α-DHP to yield the neurosteroid allopregnanolone, a potent allosteric modulator of $GABA_A$ (K_d =10^{-9}M), while AKR1C1 catalyzes its inactivation to form the less neuroactive 5α-pregnane-3α,20α-diol [34]. The reverse reactions are catalyzed by SDR enzymes RODH4, HSD17B6 and RDHL also designated as SDR9C8, SDR9C6 and SDR9C4, respectively [53].

3.5. Orphan Receptors

AKR1D1 and AKR1C4 have crucial roles in bile acid formation and thus regulate levels of bile acid ligands, which activate the orphan nuclear receptors the farnesoid X receptor (FXR) and the pregnane X receptor (PXR). Bile acids may also act as signaling molecules to the G protein coupled bile acid receptor (GPBAR1) [4]. In this manner they regulate their own homeostasis, and prevent the accumulation of toxic *allo*-bile acids in the liver which leads to liver injury and/or liver fibrosis [47]. Bile acids also stimulate the expression of inflammatory modulators (IL-8 and COX-2), and ensuing oxidative stress and DNA damage has been associated with gastro-intestinal cancers, esophagus adenocarcinoma and hepatocellular carcinoma [4]. Additionally 5β-DHP produced by the action of AKR1D1 is a ligand for the constitutive androstane receptor (CAR) and PXR, which regulate the expression of *CYP3A4*, which is one of the major hepatic enzymes involved in the metabolism of most drugs [54]. Activation of these receptors also affects bile acid homeostasis, but also glucose and energy metabolism [4, 47].

4. Expression of human *AKR1* genes in disease

As AKR1 enzymes play pivotal roles in regulating steroid levels changes in gene expression are anticipated to play roles in disease pathogenesis. The *AKR1C1-AKR1C4* genes are located on chromosome 10p15-p14, and the *AKR1D1* gene is located on chromosome 7q32-q33. *AKR1C1-AKR1C4* genes comprise 12 exons and AKR1D1 has 9 exons. Three alternatively spliced protein encoding mRNA variants were predicted for *AKR1C2* and *AKR1C3* and four for *AKR1D1* genes in the NCBI database (Table 5). *AKR1C2* transcript variants 1 and 2 translate the same full-length 323 amino acid protein, while variant 3 encodes a shorter, probably inactive protein of 139 amino acids that retains the conserved catalytic tetrad but has a truncated coenzyme binding site. Similarly, the *AKR1C3* transcript variants 1 and 2 encode the same full length 323 amino acid protein but variant 3 translates to a protein of only 138 amino acids. *AKR1D1* transcript variants encode four protein isoforms, variant 1 encodes the active protein of 326 amino acids, while variant 2 encodes a protein of 285 amino acids and has a compromised β-barrel structure. Variant 3 encodes a 290 amino acid protein which lacks the C-terminal flexible loop and the last helix [55]. Variant 4 encodes a short protein of 96 amino acids and consists only of exons 1 through 4. It is not known if all these truncated variants are transcribed into proteins and whether these

protein isoforms would have any activity. In many instances these isoforms are predicted to be inactive since they are devoid of functional components of the protein [55].

In diseased tissues the expression of *AKR1* genes and mRNA splicing is likely to be altered leading to different transcript variants, protein isoforms and thus changed protein levels and enzymatic activities [55]. Altered *AKR1* expression has been reported in hormone dependent diseases of the prostate, breast, as well as in endometrial cancers.

4.1. AKR1 enzymes and prostate cancer

AKR1C2 and *AKR1C3* genes are abundantly expressed in normal prostate [14] (Table 6). Early transcript measurements on a limited number of cases showed reduction in expression of *AKR1C1, AKR1C2* and *AKR1C3* in primary prostate cancer versus the associated normal tissue in 8/13, 11/13 and 6/13 cases, respectively) [56]. In another study *AKR1C2* transcript levels were elevated in prostate cancer epithelial cells when compared to normal epithelial cells [15]. In later studies, up-regulation of *AKR1C1, AKR1C2* and *AKR1C3* was seen in epithelial cells of prostate cancer as compared to normal epithelial cells [57]. Further studies revealed that AKR1C3 was the most up-regulated steroidogenic enzyme in castration-resistant prostate cancer (CRPC) and soft-tissue metastases [58–60]. While expression of *AKR1C1* and *AKR1C2* was increased in CRPC as compared to primary PC [58], this increase failed to reach statistical significance in all studies [59, 61]. Microarray analysis in 218 prostate adenocarcinoma samples [62, 63] showed that the *AKR1C1*, *AKR1C2*, *AKR1C3* and *AKR1C4* genes were up-regulated in 17.5%, 10.7%, 21.4% and 25% of 28 CRPC metastatic tumors cases, respectively [63]. This altered expression of *AKR1C* genes was associated with a marked decrease in overall survival of CRPC patients [63]. The recent microarray study by Mitsiades et al. [64] showed marked increased *AKR1C3* expression in metastatic PC (in 19 metastatic samples from 8 noncastrate and 11 castrate patients) as compared to normal prostate (29 samples) and primary PC (131 samples). Also *AKR1C1*, *AKR1C2* and *AKR1C4* were up-regulated in metastatic PC where expression of all *AKR1C* genes was co-regulated and negatively associated with expression of AR targeted genes [64].

Studies on prostate cancer cell lines (LNCaP, DuCaP, VCaP) and xeonografts of human CRPC revealed that in steroid-depleted serum both *AKR1C3* and AR are upregulated [60, 64, 65] and a similar response is seen with the potent anti-androgen enzalutamide. Down regulation of *AKR1C3* is seen in the presence of the potent synthetic androgen R1881 [64]. Collectively, these studies show that at the transcript level *AKR1C3* is consistently overexpressed in CRPC and that it is upregulated by androgen deprivation and this is part of an adaptive response of the tumor to make its own androgens in the presence of castrate levels of circulating androgens.

Immunohistochemical studies revealed positive AKR1C3 immunoreactivity in 77% to 82% of primary prostate adenocarcinoma [66–68] where staining correlated with clinical stage [66, 68]. AKR1C2 was observed in 89.5% to 90.3% of prostate cancer samples [69] and expression correlated with disease status, tumor grade, cigarette smoking and expression of AR [69].

The published data (Table 6) also suggest that in the transition from metastatic PC to CRPC the individual *AKR1C* genes may be induced by inflammatory and proliferative agents: such as interleukin IL-6 (*AKR1C2* [69]), activin A (*AKR1C3* [70]) and parathyroid hormone related protein (PTHrP) (*AKR1C1-AKR1C3* [71]). In this way induction of *AKR1C* genes may contribute to local androgen formation and prostate cancer relapse after androgen deprivation therapy. Measurement of intratumoral androgens by LC-MS/MS shows that in metastatic tumors the ratio of T: 5α-DHT increases 30-fold to show that the tumor is more

dependent upon T than 5α-DHT and that this may be driven by AKR1C3 [59]. Depending on the substrate availability AKR1C2-AKR1C4 enzymes also have roles in the so called "backdoor" pathway of 5α-DHT formation [33, 35, 72] where AKR1C2 and AKR1C4 catalyze reduction of 5α-DHP to allopregnenolone and AKR1C3 catalyzes reduction of androsterone to 3α-Diol, which is oxidized to 5α-DHT predominately by the SDR member, HSD17B6 (SDR9C6) [48, 72].

4.2. AKR1 enzymes and breast cancer

In human breast, *AKR1C1* and *AKR1C3* are the main isoforms expressed [14] (Table 7). Decreased expression of *AKR1C1*, *AKR1C2* and *AKR1C3* in breast cancer tissue versus adjacent normal control tissue has been reported by semiquantitative RT-PCR in 11 samples and by Affymetrix gene chip analysis in 43 samples [73–75]. Also Ji et al. [73] reported decreased expression of *AKR1C1* and *AKR1C2* in 24 paired breast cancer samples at the mRNA level and unchanged expression of *AKR1C3*. Recent microarray analysis in 463 invasive breast carcinoma samples [63, 76] showed that genes *AKR1C1* and *AKR1C3* are up-regulated in 12.3% and 11.1% of 81 PAM50 basal cases of breast cancer (the PAM50, minimal gene set is used for classification of breast cancer subtypes, [77]) while their expression was not affected in luminal or $HER2^+$ groups. This data suggests that AKR1C1 and AKR1C3 have roles in more aggressive, triple negative (ER^-, PR^-, $HER2^-$) basal-like subtype of breast cancer, where the changes in these genes decrease overall survival of these patients [63].

AKR1C3 protein was detected in 56% of breast cancer specimens and 92% of nonmalignant adjacent tissues of 50 patients by immunohistochemistry [78]. On the other hand, Oduwole et al. [79] found increased *AKR1C3* expression in breast tumor specimens as compared to adjacent normal tissue by *in situ* hybridization of 669 paraffin sections originating from pre- and post-menopausal women, where *AKR1C3* over-expression correlated with poor survival. In another study high *AKR1C3* expression in ER^+ patients correlated with significantly increased risk of relapse later than 5 years after diagnosis [80]. These studies included patients with invasive ductal carcinoma and invasive lobular carcinoma..

The alteration in expression of *AKR1C1* and *AKR1C3* genes in breast cancer may also affect progesterone, estrogen and androgen metabolism and activation of the corresponding nuclear receptors. Increased expression of *AKR1C1* and *AKR1C3* in triple negative cancers may be related to enhanced metabolism of progesterone to 20α-hydroxy-preg-4-ene-3-one, which may prevent activation of membrane bound progesterone receptors mPR which are expressed in PR negative basal-like type of breast cancer [81]. Increased levels of AKR1C3 may affect local production of pro-proliferative estradiol, which may drive proliferation of cancerous cells via membrane-bound estrogen receptor GPER [82, 83]. AKR1C3 may also have a role in the production of testosterone, and both anti-proliferative as well as proliferative effects of androgens have been observed in breast cancer [84, 85]. Triple negative patients are currently treated with chemotherapy only, thus it is critical to find new drug targets for this molecular subtype of breast cancer [76]. Being involved in estrogen and androgen formation AKR1C3 may represent appropriate target for a subgroup of these patients.

4.3. AKR1 enzymes and endometrial disease

In human uterus the expression of *AKR1C* genes is relatively low, where *AKR1C2* and *AKR1C3* represent the major *AKR1* genes expressed [14]. *AKR1C* genes are also expressed in endometrial cancer (EC) and ectopic endometrium of endometriosis patients (Table 8). In endometrial cancer increased *AKR1C3* mRNA levels have been seen in some patients, but overall no significant difference in *AKR1C3* expression in cancer versus the adjacent control

endometrium was observed [86-89]. There was, however, a trend for a higher expression of AKR1C3 in G2/G3 cancers [88]. Also *AKR1C1* and *AKR1C2* were not significantly differentially expressed in cancer of the endometrium versus adjacent control tissue and both higher and lower levels were seen [89]. Recent microarray analysis in 373 endometrial adenocarcinoma samples [63, 90] showed that the *AKR1C1* and *AKR1C2* genes were up-regulated in only 3.6% of cases while *AKR1C3* and *AKR1C4* were up-regulated in 6.3% and 4.5% of grade 3 cases, respectively. Altered expression and activity of AKR1C1-AKR1C4 enzymes thus seems to have roles only in individual patients with more aggressive grade 3 endometrial cancer where these enzymes may modulate progesterone, estrogen and androgen metabolism. AKR1C1-AKR1C3 may decrease levels of protective progesterone and AKR1C3 may increase levels of mitogenic estradiol and more probably testosterone. The unpublished data suggests that AKR1C3 does not play an important role in estrogen biosynthesis in endometrial cancer as estrogens are mainly derived from estrone-sulfate. By contrast AKR1C3 probably has a role in androgen biosynthesis (Hevir and Lanišnik Rižner, unpublished data). Androgens have been shown to stimulate endometrial stromal migration and survival [91] and AR is up-regulated in 9.8% of endometrial adenocarcinoma grade 3 samples [63, 90].

At the cellular level, Ito et al. [92] reported higher AKR1C3 staining in endometrial hyperplasia and EC, but showed no experimental data for the stained sections. Later weaker AKR1C3 staining in hyperplastic endometrium and cancerous endometrium *versus* the normal proliferative endometrium was reported [93], but this data may be biased by different menopausal status of the case and control group.

AKR1C genes are also expressed in ectopic endometrium of endometriosis patients. Significantly higher mRNA levels of *AKR1C1* and *AKR1C2* were seen in ovarian endometriosis *versus* normal endometrium of healthy women [94]. The increased expression of *AKR1C3* was borderline significant in ovarian endometriosis [94]. Immunohistochemical staining showed no significant differences in AKR1C3 in ovarian endometriosis *versus* control endometrium, but a significant increase was seen in AKR1C2 total scores and AKR1C2 scores in stromal cells [94]. Higher levels of AKR1C1 and AKR1C2 in endometriotic tissue may contribute to enhanced metabolism of the protective progesterone to 20α-hydroxy-pregn-4-ene-3-one, and the concurrent action of SRD5A1 may lead to the formation of the pro-proliferative 5α-pregnanes [39] (N. Beranič and T. Lanišnik Rižner, unpublished data). This suggests that the AKR1C enzymes, and especially AKR1C1 and AKR1C2, may be associated with pathogenesis of ovarian endometriosis. The expression of AKR1 genes in peritoneal and deep infiltrating endometriosis has not yet been studied.

5. Genetics of human AKR1 enzymes

Inherited or SNP allelic variants in *AKR1* genes may affect the metabolism of exogenous and endogenous steroids and may also contribute to the development of pathophysiological processes.

5.1. Inherited Mutations

Inherited mutations in *AKR1D1* have been studied in patients with bile-acid deficiency that develops in infants and is characterized by reduced formation of bile acids and accumulation of Δ^4-3-keto and 5α-reduced (*allo*-bile acids) which are hepatotoxic. This deficiency is exacerbated by the inability to from sufficient bile acids to feed-back and inhibit the rate determining step in the pathway, 7α-hydroxylase (*CYP7A1*), and thus the formation of *allo*-bile acids will be further elevated [95–99]. Defects in bile acid synthesis are rare but can lead to cholestasis and neo-natal liver failure and can be lethal if untreated. In addition to missense mutations at Arg50* and 511 T deletion leading to truncated inactive proteins,

seven point mutations have been identified in patients with 5β-reductase deficiency: Leu106Phe, Pro133Arg, Gly223Glu, Pro198Leu, Asp241Val, Arg261Cys and Arg266Glu [95–97, 99, 100]. Except Pro133Arg, these mutations reside in domains highly conserved across AKR1D1 homologues and are not involved in catalysis, coenzyme or substrate binding [21, 101]. Mutant Pro133Arg was prepared in a recombinant form and showed a 12-fold decreased catalytic efficiency for testosterone reduction [101]. Expression of the mutated enzymes in HEK293 cells revealed lower expression levels of Leu106Phe, and Arg261Cys probably due to decreased stability, and lower enzymatic activity and expression levels of both Pro198Leu and Gly223Glu. The estimated K_m value for Pro133Arg was 10-fold higher and V_{max} about 2-fold lower as compared to the wild type enzyme for the bile acid precursor, 7α-hydroxy-cholesten-3-one [101]. The mutants thus showed lower 5β-reductase activity as compared to wild type which is in agreement with clinical observations of bile acid deficiency [98, 101].

Recently several inherited mutations in the *AKR1C2* and *AKR1C4* genes have been identified in two Swiss families which were associated with disordered sexual dysgenesis (DSD). Proper sexual development in male fetus requires synthesis of 5α-DHT which is crucial for differentiation of male external genitalia. Genetic males with incompletely developed (ambiguous) genitalia usually have disorders in the classical pathway of androgen biosynthesis which proceeds from cholesterol via pregnenolone to form dehydroepiandrosterone and Δ^4-androstene-3,17-dione [33, 102]. In this pathway AKR1C3 converts Δ^4-androstene-3,17-dione to testosterone, and in the alternative pathway it converts 5α-androstane-3,17-dione to 5α-DHT [102]. However, mutations in *AKR1C2* and *AKR1C4* in these patients suggest that in fetal development the backdoor pathway [33, 102], which involves the conversion of progesterone into 5α-DHT plays a crucial role in androgen biosynthesis. In this pathway, AKR1C2 and AKR1C4 catalyze the reduction of 5α-pregnane-3,20-dione to yield allopregnanolone which is the precursor of androsterone. Androsterone is converted to 3α-Diol by AKR1C3 which is the immediate precursor of 5α-DHT. In the first family identified with DSD, missense *AKR1C2* muations: Ile79Val, His906Glu, and Asn300Thr were identified in the affected individuals. In addition to these *AKR1C2* mutations these individuals also carried a mutation causing aberrant splicing of the *AKR1C4* gene (Val29_Lys84del). In the second family recombination between *AKR1C1* and *AKR1C3* was detected and a missense *AKR1C2* mutation His222Gln was identified. The mutants Ile79Val, His90Gln, Asn300Thr and His222Gln have been prepared in recombinant forms or over-expressed in COS-1 cells and showed reduced enzymatic activities [103]. The authors observed *AKR1C2* and *AKR1C4* expression in fetal testes thus they concluded that these enzymes are needed for fetal 5α-DHT synthesis via the backdoor pathway and normal development of the genitalia [103].

5.2. Population Genetics

A large number of synonymous and nonsynonymous single nucleotide polymorphisms (SNPs) have been identified in *AKR1* genes (Table 5). However, SNP databases are not always complete and accurate. Based on a bioinformatics approach it has been predicted that 8.3% of biallelic, coding SNPs in the dbSNP database represent single nucleotide differences (SNDs) [104]. Among all AKR1 SNPs included in the SNP database there are currently only 5 SNPs with a minor allelic frequency (MAF) greater than 0.05. These correspond to Phe46Tyr in AKR1C2 with a MAF = 0.051; His5Gln and Lys104Asn in AKR1C3 with MAF of 0.428 and 0.132, respectfully; and Ser145Cys and Leu311Val in AKR1C4, both with MAF of 0.104. So far SNPs in *AKR1C1*, *AKR1C2* and *AKR1C4* have been studied at the protein level and Phe46Tyr has been associated with disease [95–97, 99, 103, 104].

In *AKR1C2* Takahashi et al. functionally characterized 11 nonsynonymous SNPs known at the time but later bioinformatics studies showed that only 5 of these represent real SNPs: Phe46Tyr, Vall11Ala, Lys179Gln, Lys185Gln and Arg258Cys [104]. Phe46Tyr had a 2.5-fold reduced enzymatic activity versus wild-type enzyme (V_{max}) and two SNPs, Lys185Glu and Arg258Cys had significantly lower apparent K_m values, when the reduction of 5α-DHT to 3α-Diol was followed [105]. The authors observed that the frequency of Phe46Tyr SNP parallelled the risk for prostate cancer in different populations (individuals of African descent 15%, Europeans 5.9%, and undetected in the Asian population). Lower AKR1C2 activity in the Phe46Tyr carriers may increase 5α-DHT levels and may thus stimulate prostate cell proliferation. Homology modeling explained the decreased activity in the Phe46Tyr variant since this changed the interaction between the enzyme and coenzyme and could lead to weak cofactor binding [106]. For the other *AKR1C1/2* SNPs (Val111Ala, Lys179Gln, Lys185Gln and Arg285Cys) it was rationalized that they are unlikely to affect enzyme activities as they are located on the periphery, and distant from the active site.

6. Future Directions

AKR1 enzymes play important roles in metabolism of androgens, estrogens, progesterone, glucococorticoids, neurosteroids, conjugated steroids, and in the biosynthesis of bile acids. Although AKR1 activities towards representative substrates from these steroid classes have been studied the list is far from comprehensive. A more complete understanding of substrate specificities of AKR1 enzymes and the products they generate may reveal additional physiological and/or pathophysiological roles for these enzymes.

Synthetic steroids found in oral contraceptives, hormone replacement therapies and steroid anti-inflammatory drugs are metabolized by AKR1C enzymes. In this manner AKR1C enzymes may affect treatment of a particular disease or condition and susceptibility to therapy. For instance; the prodrug tibolone is metabolized by AKR1C enzymes to active compounds and inhaled corticosteroids inhibit AKR1D1 and AKR1C4, which may disrupt endogenous steroid metabolism in liver. Other synthetic steroids, e.g. dydrogesterone, and the glucocorticoids budesonide inhibit AKR1 enzymes and may thus interfere with the normal physiological roles of these enzymes. The ability of AKR1C enzymes to metabolize other steroid based drugs needs to be examined.

Steroid preferences of AKR1 enzymes are characterized by determination of their kinetic properties *in vitro*, where catalytic efficiencies (k_{cat}/K_M values) are compared, and products of the reactions are identified, preferably by liquid chromatography mass spectrometry. However, in human tissues cellular levels of particular isoforms should be taken into consideration, as they determine the physiological significance of a particular enzyme in that context. It is thus of paramount importance to determine the cellular levels of AKR1 enzymes in diseased versus normal tissue and how these levels are regulated. It is likely that different ratios of AKR1 enzyme expression may tune ligand availability for nuclear and membrane bound receptors.

The physiological and pathophysiological roles of AKR1 enzymes are currently not known in every instance. A functional genomics approach would contribute enormously. After studying enzymatic activities of recombinant AKR1 enzymes (substrate specificity, catalytic efficiency, product profiling), their activities in model or transfected cell lines, and their ability to alter *trans*-activation of nuclear receptors should be examined. Proof-of-principle experiments in human cell lines using siRNA or sh-RNA approaches are needed to clarify the pathophysiological roles of AKR1 enzymes.

Altered regulation of *AKR1* gene transcription, including epigenetic modifications, transcriptional regulation (induction, repression, alternative splicing) should be further investigated. *AKR1C* genes are up-regulated by androgen deprivation in prostate cancer but it is not known how AKR1C genes are regulated by hormone deprivation and whether this would be seen in other malignancies.

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Abbreviations

AKR	Aldo-keto reductase
AR	androgen receptor
CAR	constitutive androstane receptor
CRPC	castrate resistant prostate cancer
COMT	catechol-*O*-methyl transferase
CYP	cytochrome P450 superfamily
3α/β-Diol	5α-androstane-3α/β,17β-diol
5α/β-DHT	5α/β-Dihydrotestosterone
DSD	disordered sexual dysgenesis
ER	estrogen receptor
FXR	farnesoid X receptor
GABA	γ-amninobutyric acid
GPBAR1	G protein coupled bile acid receptor
HSD17B6	17β-hydroxysteroid dehydrogenase type 6
MAF	minor allelic frequency
NMDA	*N*-methyl-*D*-aspartate
PR	progesterone receptor
PXR	pregnane X receptor
SDR	short-chain dehydrogenase/reductase
SND	single nucleotide difference
SNP	single nucleotide polymorphism
SRD5A1/A2	5α-reductase type 1 or 2
SULT	sulfotransferase
UGT	UDP glucuronosyl transferase

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Highlights

- The aldo-keto reductase superfamily contains five human steroid-metabolizing enzymes
- AKR1C isoforms act as 3-, 17- and 20-ketosteroid reductases
- AKR1D1 is the sole steroid 5β-reductase in humans
- AKR enzymes control ligand access to nuclear and membrane bound receptors
- Expression profiles, inherited mutations and SNP support their roles in human disease

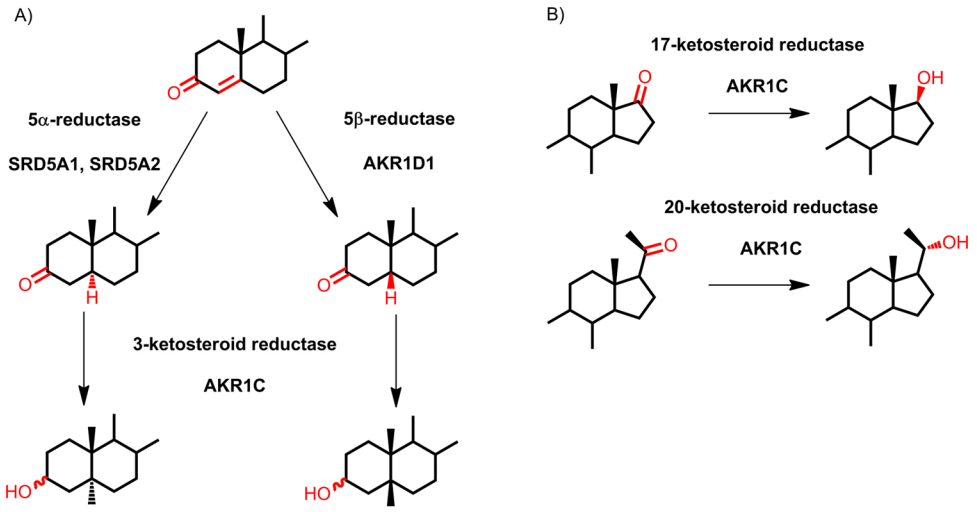

Figure 1. Reactions catalyzed by human AKR1 enzymes

Reduction of Δ^4-3-ketosteroids are catalyzed by AKR1D1 to form 5β-reduced steroids or by SRD5A1/SRD5A2 to form 5α-reduced steroids. 5α/β-Reduced steroids are further reduced by AKR1C enzymes to form tetrahydrosteroids of defined stereochemistry (A). 17-Ketosteroid reduction is catalyzed mainly by AKR1C3 to form 17β-hydroxysteroids and 20-ketosteroid reduction is catalyzed mainly by AKR1C1 to form 20α-hydroxysteroids (B).

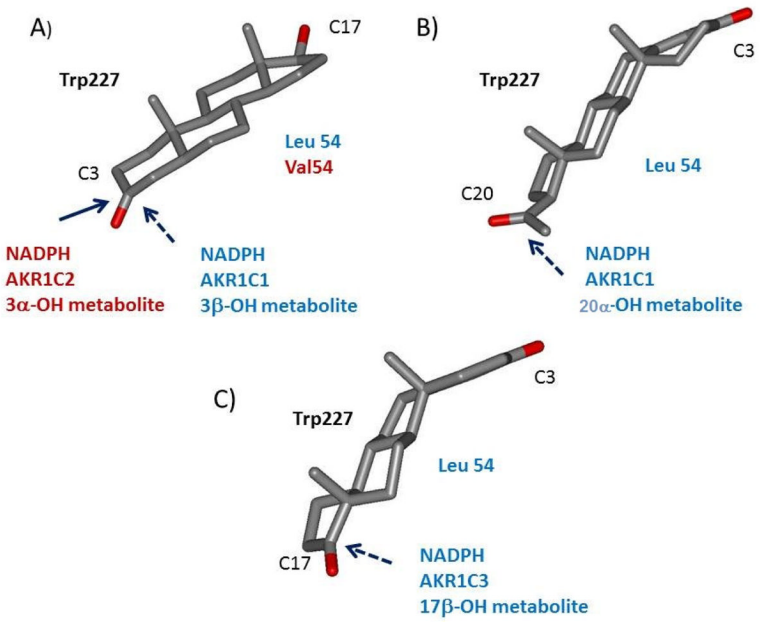

Figure 2. Binding orientations of ketosteroids within the active sites of human AKR1 enzymes

The A-ring of 3-ketosteroid substrates can orient towards the nicotinamide ring of the cofactor to form 3α and 3β-hydroxy metabolites (A); they can bind in reverse orientation so that the D-ring binds at the base of the pocket and thus form 20α- (B); and 17β-hydroxy (C) metabolites. Different stereochemistry of the hydroxysteroid product is achieved by the ketosteroid being pushed across the steroid pocket by steric forces (Leu54 versus Val54).

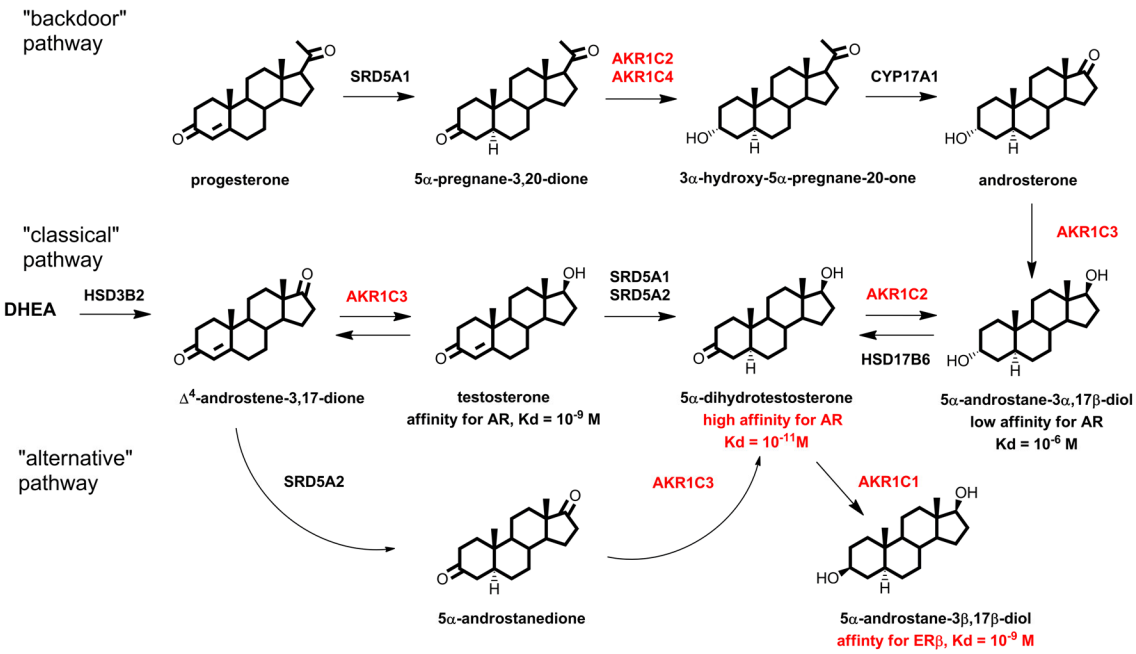

Figure 3. Human AKR1 enzymes involved in androgen biosynthesis via the classical, the alternative and the backdoor pathways

In prostate androgens can be synthetized from DHEA or *de novo* from cholesterol, where AKR1C3 catalyzes activation of androstenedione to testosterone, the reverse reaction is catalyzed by HSD17B2. Testosterone is further activated by SRD5A1 and/or SDR5A2 to form the most potent androgen 5α-dihydrotestosterone (5α-DHT), which stimulates proliferation via AR. This is the classical pathway. 5α-DHT can be formed also by an alternative pathway from 5α-androstane-3,17-dione by the action of AKR1C3. Enymes AKR1C1 and AKR1C2 catalyze inactivation of 5α-DHT to form the less potent 3α-Diol and 3β-Diol with high affinity for proapoptotic ERβ. Recent data suggest that 5α-DHT may also be formed from cholesterol via pregnenolone, progesterone, 5α-DHP, allopregnanolone and androsterone by the so called «backdoor« pathway». AKR1C2 and AKR1C4 catalyze reduction of 5α-DHP and AKR1C3 catalyzes the reduction of androsterone to from 3α-Diol. 3α-Diol is oxidized back to 5α-DHT by the action of several oxidases from the SDR superfamily including HSD17B6.

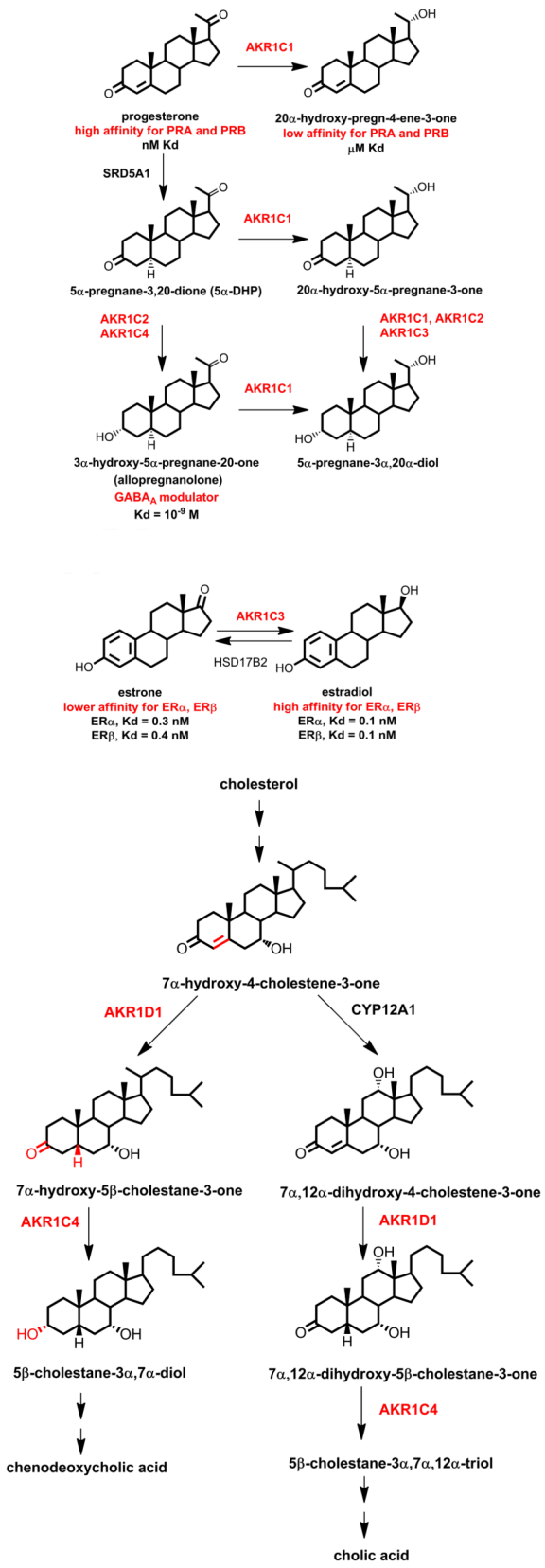

Figure 4. Human AKR1 enzymes implicated in progesterone and estrogen metabolism

A) In peripheral tissues including breast and endometrium AKR1C enzymes catalyze reduction of progesterone which has high affinity for the progesterone receptor to from the the less potent progestagen 20α-hydroxy-pregn-4-ene-3-one. AKR1C enzymes also reduce 5α-DHP to yield allopregnanolone, 20α-hydroxy-5α-pregnane-3-one, and 5α-pregnane-3α, 20α-diol. In the CNS allopregnanolone acts as a positive allosteric modulator of $GABA_A$ receptor, while in breast cancer 5α-preganes stimulate cell proliferation. B) Among AKR1 enzymes AKR1C3 is involved in biosynthesis of the most potent estrogen, estradiol from estrone, the reverse reaction is catalyzed by HSD17B2.

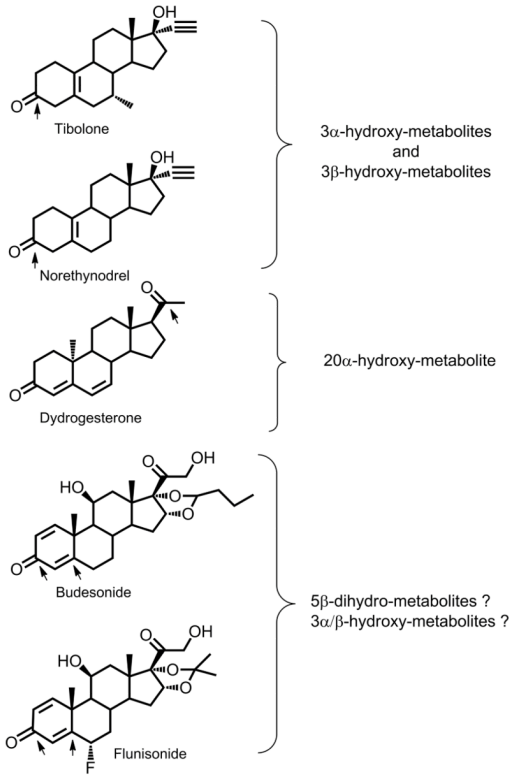

Figure 5. A sequential action of AKR1D1 and AKR1C4 in bile acid metabolism

In liver AKR1D1 and AKR1C4 catalyze sequential reactions in biosynthesis of chenodeoxycholic and cholic acids from precursors 7α-hydroxy-4-cholestene-3-one and 7α, 12α-dihydroxy-4-cholestene-3-one. AKR1D1 catalyzes the first step, formation of 7α-hydroxy-5β-cholestan-3-one and 7α,12α-dihydroxy-5β-cholestan-3-one, while AKR1C4 catalyzes reduction of 3-keto group to 3α-hydroxy-group.

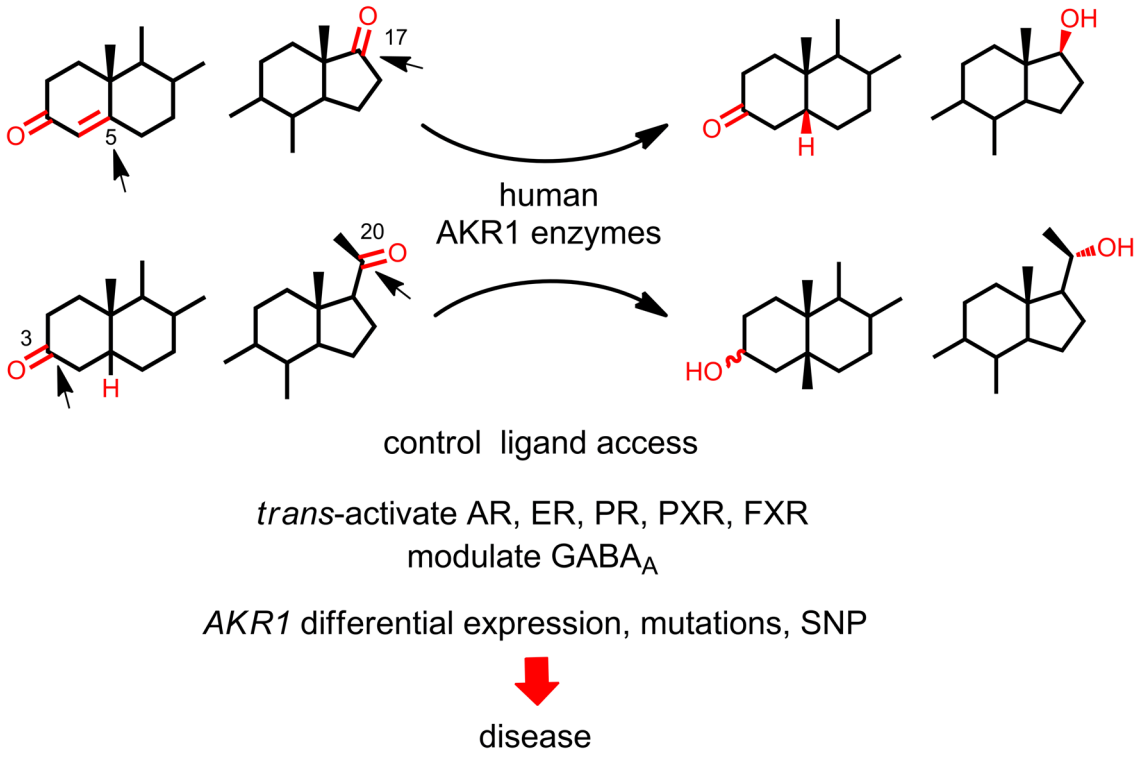

Figure 6. Synthetic steroids metabolized by AKR1 enzymes

AKR1C enzymes are involved in the metabolism of tibolone, the progestins dydrogesterone and norethynodrel and synthetic glucocorticoids budesonide and flunisolide. Tibolone and norethynodrel are metabolized to 3β-hydroxy-metabolites by AKR1C1 and AKR1C2 and to 3α-hydroxy-metabolites by AKR1C4. Dydrogesterone is metabolized by AKR1C1 and AKR1C2 to form 20α-hydroxy-metabolite. AKR1D1 and AKR1C4 metabolize glucocorticoids budesonide and flunisonide to 5β-dihydro and 3α/β-hydroxy-metabolites.

Table 1

Kinetic characteristics of the AKR1C enzymes for metabolism of androgens

Enzyme	Substrate	Product	* K_M (μM)	* k_{cat} (min^{-1})	* k_{cat}/K_M (min^{-1} mM^{-1})	Reference
AKR1C1	5α-DHT (R)	3α-Diol		0.2		[16]
	5α-DHT (R)	3β-Diol		0.6		[16]
	Androsterone (S)		21.0	0.2	9	[14]
	5α-androstane-3,17-dione (S)		6.8	0.5	70	[14]
AKR1C2	5α-DHT (R)	3α-Diol	6.6	3.0	458	[16]
	5α-DHT (R)	3β-Diol	11.0	0.2	19	[16]
	5α-DHT (R)	3α-Diol?	4.6	3.8	820	[31]
	5α-DHT (S)		4.3	3.1	714	[16]
	5α-androstane-3,17-dione (S)		6.3	1.4	222	[14]
AKR1C3	5α-DHT (R)	3α-Diol		0.1		[16]
	5α-DHT (R)	3β-Diol		0.1		[16]
	5α-DHT (R)	?	20	0.3	13	[17]
	Δ^4-Androstene-3,17-dione (R)	Testosterone	13.4	0.9	65	[30]
	Δ^4-Androstene-3,17-dione (R)	Testosterone	6.6	0.2	24	[31]
	Androsterone (S)		9.0	0.4	42	[14]
	5α-androstane-3,17-dione (S)		5.0	0.3	56	[14]
AKR1C4	5α-DHT (R)	3α-Diol		4.4		[16]
	5α-DHT (R)	3β-Diol		1.2		[16]
	5α-androstane-3,17-dione (S)		1.4	1.8	1243	[14]
AKR1D1	Androstenedione (F)		0.9	6.0	6720	[23]
	Testosterone (F)		2.7	8.4	3120	[23]

* The numbers have been rounded out to keep the information clear. R, radiometric assay, S, spectrophotometric assay, F, fluorimetric assay. Products identified by radiometric assay are shown.

Steckelbroeck et al., 2004 [16]; radiometric assay: 100 mM phosphate buffer pH 7.0; 2.3 mM NADPH, 4% ACN, 37°C, 41.25 μM concentration of 5α-DHT;

Penning et al., 2000 [14]; spectrophotometric assay: 100 mM phosphate buffer, pH 7.0; 200 μM NADPH, 4% ACN, 25 °C;

Byrns et al., 2008 [31]; radiometric assay: 100 mM phosphate buffer pH 7.0, 200 μM NADPH, 4% EtOH, 37 °C;

Sharma et al., 2006 [30]; radiometric assay: 100 mM phosphate buffer pH 7.0, 2.5 mM NADPH, 2 % EtOH, 37°C;

Penning and Bryns 2009 [17]; radiometric determination

Chen et al., 2011 [23]; fluorimetric assay: 100 mM phosphate buffer, pH 6.0; 15 μM NADPH, 4% acetonitrile, 37°C.

Table 2

Kinetic characteristics of the AKR1C enzymes for metabolism of progesterone and 5α-pregnanes

Enzyme	Substrate	Product	* K_M (μM)	* k_{cat} (min^{-1})	* k_{cat}/K_M (min^{-1} mM^{-1})	References
AKR1C1	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	5.7	0.9	210	[30]
	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	1.9	0.6	300	[11]
	Progesterone (S)		18	18	980	[107]
	5α-DHP (S)		1.1	2.6	2 400	[34]
	20α-hydroxy-5α-pregnane-3-one (S)		0.7	0.6	860	[34]
	3α-hydroxy-5α-pregnane-20-one (S)		2.0	4.4	2 200	[34]
AKR1C2	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	7.7	0.2	30	[13]
	5α-DHP (S)		0.6	0.5	700	[34]
	5α-DHP (R)	3α-hydroxy-5α-pregnane-20-one	1.7	0.2	135	[108]
	5α-DHP (R)	20α-hydroxy-5α-pregnane-3-one	3.4	0.2	72	[108]
	20α-hydroxy-5α-pregnane-3-one (S)		0.5	0.5	960	[34]
	3α-hydroxy-5α-pregnane-20-one (S)		3.1	0.3	80	[34]
AKR1C3	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	2.8	1.0	370	[30]
	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	20	0.6	30	[17]
	Progesterone (R)	20α-hydroxy-4-pregnen-3-one	5.6	0.2	30	[11]
	5α-DHP (S)		-	0.06	-	[34]
	20α-hydroxy-5α-pregnane-3-one (S)		2.1	0.52	250	[34]
	3α-hydroxy-5α-pregnane-20-one (S)		?	na	?	[34]
AKR1C4	5α-DHP (S)		0.6	1.2	2 000	[34]
	20α-hydroxy-5α-pregnane-3-one (S)		1.1	1.3	1200	[34]
	3α-hydroxy-5α-pregnane-20-one (S)		0.9	0.6	670	[34]

* The numbers have been rounded out to keep the information clear. R, radiometric assay, S, spectrophotometric assay, F, fluorimetric assay. Products identified by radiometric assay are shown.

Sharma et al., 2006 [30]; radiometric assay: 100 mM phosphate buffer pH 7.0, 2.5 mM NADPH, 2% EtOH, 37 °C;

Beranič et al., 2011 [11]; radiometric assay: 100 mM phosphate buffer pH 6.5; 2.3 mM NADPH; 4% ACN, 37 °C;

Beranič et al., 2012 [13]; radiometric assay: 100 mM phosphate buffer pH 6.5; 100 μM NADPH; 4% ACN, 37 °C;

Nishizawa et al., 2000 [106]; spectrophotometric assay: 0.1 M phosphate buffer, pH 6.5, 80 μM NADPH, 37 °C;

Higaki et al., 2003 [34]; spectrophotometric assay: 100 mM phosphate buffer, pH 7.0; 0.1 mM NADPH, 25 °C

Tranger et al., 2002 [107]; radiometric assay: 100 mM phosphate buffer pH 7.0; 2 mM NADPH; 5% DMSO, 0.5 mg/ml BSA, 37 °C; Penning and Bryns 2009 [17]; radiometric determination.

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Table 3

Kinetic characteristics of the AKR1C enzymes for metabolism of 5α-DHT glucuronides and sulfates

Enzyme	Substrate	Product 3α: 3β alcohol*	* K_M (μM)	* k_{cat} (min^{-1})	* k_{cat}/K_M (min^{-1} mM^{-1})
AKR1C1	5α-DHT	1: 3	4.2	0.7	180
	5α-DHT-G	1: 7	16.1	0.5	33
	5α-DHT-S	1: 3	5.2	0.8	140
AKR1C2	5α-DHT	20:1	2.9	2.0	680
	5α-DHT-G	1: 5	4.1	0.2	59
	5α-DHT-S	6: 1	4.2	1.0	240
AKR1C3	5α-DHT	1.6: 1		< 0.2	
	5α-DHT-G	1: 2.3		< 0.2	
	5α-DHT-S	1: 3		< 0.2	
AKR1C4	5α-DHT	3.6: 1	< 0.2	3.1	>15000
	5α-DHT-G	8: 1	< 0.2	3.3	>16000
	5α-DHT-S	1: 1	< 0.2	3.1	>15000

* The numbers have been rounded out to keep the information clear.

Jin et al. 2009 [10]; fluorimetric assay: 100 mM phosphate buffer, pH 6.0; 15 μM NADPH, 4% acetonitrile, 37 °C. Products were identified by liquid chromatography mass spectrometry.

Table 4

Kinetic characteristics of the AKR1 enzymes for reduction of synthetic steroids

Enzyme	Substrate	Product	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (min^{-1} mM^{-1})	* K_I (μM)	References
AKR1C1	Tibolone (F)	3β-hydroxy tibolone	0.8	0.9	1 200		[12]
	Norethynodrel (F)	3β-hydroxynor ethynodrel	2.3	1.0	450		[12]
	Dydrogesterone (R)	20α-OHD*	2.4	2.88	1200	1.9	[11]
AKR1C2	Tibolone (F)	3β-hydroxy tibolone	0.9	12.7	14 600	0.7	[12]
	Norethynodrel (F)	3β-hydroxynor ethynodrel	1.5	8.2	5 600	2.3	[12]
	Dydrogesterone (R)	20α-OHD*	1.6	7.0	4 480	1.3/4.4*	[13]
AKR1C3	Tibolone (F)	≫3β-hydroxy tibolone					
	Norethynodrel (F)	3β-hydroxynor ethynodrel		< 0.2			[12]
	Dydrogesterone (R)	20α-OHD*	la	la	la	0.5	[11]
AKR1C4	Tibolone (F)	≫ 3α-hydroxy tibolone	1.0	1.8	1800		[12]
	Norethynodrel (F)	≫ 3β-hydroxynor ethynodrel	0.4	2.7	6400		[12]

*20α-OHD, 20α-hydroxy, 9β,10α-pregna-4,6-diene-3-one

Jin et al., 2012 [12]; fluorimetric assay: 100 mM phosphate buffer pH 7.0, 12 mM NADPH, 0.4% MetOH, 37 °C;

Beranic et al. 2011 [11]; radiometric assay: 100 mM phosphate buffer pH 6.5, 100 μM NADPH, 4% ACN, 37 °C

*Ki values for oxidation of 1-acenaphthenol and reduction of progesterone, la, low activity. Products identified by either radiometric assay or liquid chromatography mass spectrometry are shown.

Table 5

Single nucleotide polymorphisms within the *AKR1* genes

Gene	Gene	Transcript Info	Transcript	gSNP	cSNP			
	ID		Size (bp)		total	SYN	NSYN	MAF
			Protein (aa)				M/N/F	>0.01
AKR1C1	1645	NM_001353.5	1384 323	406	52	17	50/5/0	none
AKR1C2	1646	NM_001354.5	V1, 3621 323	884	52	19	33/0/0	Phe46Tyr, 0.051
		NM_205845.2	V2, 3521 323	884	52	19	33/0/0	Phe46Tyr, 0.051
		NM_001135241.2	V3 1098 139	207	37	14	23/0/0	Phe46Tyr, 0.051
AKR1C3	8644	NM_003739.5	V1. 1251 323	505	67	18	47/2/0	His5Gln, 0.428 Arg66Gln, 0.024 Glu77Gly, 0.047 Lys104Asn, 0.132 Pro180Ser, 0.012 Arg258Cys, 0.037
		NM_001253908.1	V2, 1228 323	1515	65	16	47/2/0	Arg66Gln, 0.024 Glu77Gly, 0.047 Lys104Asn, 0.132 Pro180Ser, 0.012 Arg258Cys, 0.037
		NM_001253909.1	V3 1064 138	148	27	9	17/2/0	His5Gln, 0.428 Arg66Gln, 0.024 Glu77Gly, 0.047 Lys104Asn, 0.132
AKR1C4	1109	NM_001818.3	1192 323	586	39	8	30/1/0	Gly135Glu, 0.027 Ser145Cys, 0.104 Leu311Val, 0.104
AKR1D1	6718	NM_005989.3	V1 2710 326	855	38		27/0/0	none
		NM_001190906.1	V2 2587 285	855	36		26/0/0	none
		NM_001190907.1	V3 2627 290	855	34		24/0/1	none

gSNP, SNP in gene region; cSNP, SNP within coding region; SYN, synonimous; NSY, nonsynonimous; M, missense; N, nonsense; F, frame shift; MAF, minor allele frequency; V1, V2, V3= protein variants

Table 6

Differential expression of AKR1 genes in prostate cancer

Gene	Method	Samples/Stage of disease	Regulation	Reference
AKR1C1				
	RT-PCR	Primary PC versus normal prostate (13 samples)	Decreased in 8 of 13	Ji et al. [56]
	RT-PCR	Epithelial cells of primary PC versus epithelial cells of normal prostate	Increased	Bauman et al. [57]
	Real-time PCR	CRPC versus primary PC	Unchanged Unchanged	Mongomery et al. [59] Locke et al. [61]
	Microarray analysis	CRPC versus primary PC	Increased	Stanbrough et al. [58]
	Microarray analysis	CRPC (28 samples)	Up-regulated in 18%	Taylor et al. [62]
	Microarray analysis	Metastatic PC	Increased	Mitsiades et al. [64]
AKR1C2				
	RT-PCR	Primary PC versus normal prostate (13 samples)	Decreased in 11 of 13	Ji et al. [56]
		PC epithelial cells versus normal prostate epithelial cells	Increased	Lanisnik Rizner et al. [15]
	RT-PCR	Epithelial cells of primary PC versus epithelial cells of normal prostate	Increased	Bauman et al. [57]
	Real-time PCR	CRPC versus primary PC	Unchanged Unchanged	Mongomery et al. [59] Locke et al. [61]
	Microarray analysis	CRPC versus primary PC	Increased	Stanbrough et al. [58]
	Microarray analysis	CRPC (28 samples)	Up-regulated in 11%	Taylor et al. [62]
	Microarray analysis	Metastatic PC	Increased	Mitsiades et al. [64]
	IHC	PC paraffin sections	90 % of sections; 77/86 Chinese patients, 28/31 American patients	Huang et al. [69]
AKR1C3	RT-PCR	Primary PC versus normal prostate (13 samples)	Decreased in 6 of 13	Ji et al. [56]
	RT-PCR	Epithelial cells of primary PC versus epithelial cells of normal prostate	Increased	Bauman et al. [57]
	Real-time PCR	CRPC versus primary PC	Upregulated	Mongomery et al. [59] Locke et al. [61]
	Microarray analysis	CRPC versus primary PC	Upregulated	Stanbrough et al. [58]
	Microarray analysis	CRPC (28 samples)	Up-regulated in 21 %	Taylor et al. [62]
	Microarray analysis	Metastatic PC	Increased	Mitsiades et al. [64]
	IHC	Primary PC paraffin sections	77% to 82%	Nakamura et al. [66] Fung et al. [67] Wako et al. [68]
AKR1C4	Microarray analysis	CRPC (28 samples)	Up-regulated in 25 %	Taylor et al. [62]
	Microarray analysis	Metastatic PC	Increased	Mitsiades et al. [64]

Table 7

Differential expression of AKR1 genes in breast cancer

Gene	Method	Samples/Stage of disease	Regulation	Reference
AKR1C1				
	RT-PCR	BC versus adjacent normal prostate (11 samples)	Decreased	Lewis et al. [73]
	Microarray analysis	BC versus adjacent normal prostate (43 samples)	Decreased	Ni et al. [74]
	Real-time PCR	BC versus adjacent control tissue (24 samples)	Decreased	Ji et al. [75]
	Microarray analysis	Invasive breast cancer (463 samples, 81 basal cancer)	Up-regulated in 12% of basal cases	The Cancer Genome Atlas, 2012 [76]
AKR1C2				
	RT-PCR	BC versus adjacent normal prostate (11 samples)	Decreased	Lewis et al. [73]
	Microarray analysis	BC versus adjacent normal prostate (43 samples)	Decreased	Ni et al. [74]
	Real-time PCR	BC versus adjacent control tissue (24 samples)	Decreased	Ji et al. [75]
AKR1C3				
	RT-PCR	BC versus adjacent normal prostate (11 samples)	Decreased	Lewis et al. [73]
	Microarray analysis	BC versus adjacent normal prostate (43 samples)	Decreased	Ni et al. [74]
	Real-time PCR	BC versus adjacent control tissue (24 samples)	Unchanged	Ji et al. [75]
	Microarray analysis	Invasive breast cancer (463 samples, 81 basal cancer)	Up-regulated in 11% of Basal cases (81)	The Cancer Genome Atlas, 2012 [76]
	IHC	BC versus adjacent control tissue (50 samples)	56% Breast cancer 92% Adjacent control tissue	Han et al. [78]
	In situ hybridization	BC versus adjacent control tissue (669 paraffin sections)	Increased expression	Oduwole et al. [79]

Table 8

Differential expression of *AKR1* genes in endometrial cancer and endometriosis

Gene	Method	Samples/Stage of disease	Regulation	Reference
AKR1C1	Real time PCR	EC and adjacent control tissue (25 samples)	No difference	Šmuc and Lanišnik Rižner [87]
	Real time PCR	EC and adjacent control tissue (47 samples)	No difference	Sinreih et al. [89]
	Microarray analysis	EC (373 samples)	Increased in 4% of G3 samples	The Cancer Genome Atlas, 2013 [90]
AKR1C2	Real time PCR	EC and adjacent control tissue (47 samples)	No difference	Sinreih et al. [89]
	Microarray analysis	EC (373 samples)	Increased in 4% of G3 samples	The Cancer Genome Atlas, 2013 [90]
AKR1C3	Real time PCR	EC and adjacent control tissue (16 samples)	No difference	Lanišnik Rižner et al. [86]
		(25 samples)		Šmuc and Lanišnik Rižner [87]
		(47 samples)		Sinreih et al. [89]
	Real time PCR		Trend for higher expression G2/G3	Cornell et al. [88]
	Microarray analysis	EC (373 samples)	Increased in 6% of G3 samples	The Cancer Genome Atlas, 2013 [90]
	IHC	EC and hyperplastic endometrium	Increased	Ito et al. [92]
	IHC	EC, hyperplastic endometrium	Decreased	Zakharov et al. [93]
AKR1C1	Real-time PCR	Ovarian endometriosis (24 samples) versus contol endometrium (10 samples)	Increased	Šmuc and Lanišnik Rižner [87]
	Real-time PCR	Ovarian endometriosis (31 samples) versus normal endometrium (28 samples)	Increased	Hevir et al. [94]
AKR1C2	Real-time PCR	Ovarian endometriosis (31 samples) versus normal endometrium (28 samples)	Increased	Hevir et al. [94]
	IHC	Ovarian endometriosis (18 sections) Control endometrium (9 sections)	Increased in stromal cells	Hevir et al. [94]
AKR1C3	Real-time PCR	Ovarian endometriosis (24 samples) versus contol endometrium (10 samples)	Increased	Šmuc and Lanišnik Rižner [87]
	Real-time PCR	Ovarian endometriosis (31 samples) versus normal endometrium (28 samples)	Increased	Hevir et al. [94]
	IHC	Ovarian endometriosis (18 sections) Control endometrium (9 sections)	Unchanged	Hevir et al. [94]