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Human Aldo-Keto Reductases: Function, Gene Regulation, and Single Nucleotide Polymorphisms

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Abstract

Aldo-Keto Reductases (AKRs) are a superfamily of NAD(P)H linked oxidoreductases that are generally monomeric 34- 37 kDa proteins present in all phyla. The superfamily consists of 15 families, which contains 151 members (www.med.upenn.edu/akr). Thirteen human AKRs exist that use endogenous substrates (sugar and lipid aldehydes, prostaglandins, retinals and steroid hormones), and in many instances they regulate nuclear receptor signaling. Exogenous substrates include metabolites implicated in chemical carcinogenesis: NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), polycyclic aromatic hydrocarbon *trans*-dihydrodiols, and aflatoxin dialdehyde. Promoter analysis of the human genes identifies common elements involved in their regulation which include osmotic response elements, antioxidant response elements, xenobiotic response elements, AP-1 sites and steroid response elements. The human AKRs are highly polymorphic, and in some instances single nucleotide polymorphisms (SNPs) of high penetrance exist. This suggests that there will be inter-individual variation in endogenous and xenobiotic metabolism which in turn affect susceptibility to nuclear receptor signaling and chemical carcinogenesis.

Keywords

lipid aldehydes; prostaglandins; retinals; steroid hormones; polycyclic aromatic hydrocarbons; anti-oxidant response element; reactive oxygen species

Introduction

The Aldo-Keto Reductases (AKRs) are a superfamily of NAD(P)H linked oxidoreductases that are generally cytosolic monomeric (37 kDa) proteins that reduce aldehydes and ketones to their corresponding primary and secondary alcohols [1-3]. They are present in prokaryotes and eukaryotes and fall into 15 families, for a complete listing the reader is referred to the AKR superfamily homepage at: www.med.upenn.edu/akr. There are thirteen human AKRs that are involved in the metabolism of sugar aldehydes, reactive lipid aldehydes, ketoprostaglandins, and ketosteroids. Xenobiotic substrates include drugs and chemical carcinogens. Since they functionalize carbonyl groups by forming alcohols for conjugation reactions, catalyzed by UDP-glucuronosyl transferases (UGTs) and sulfotransferases (SULT), AKRs can be formally be referred to as Phase I metabolizing enzymes.

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The human AKRs belong to three families AKR1, AKR6 and AKR7 (see, Table 1.) The AKR1 family contains: AKR1A1 (aldehyde reductase) [4,5]; AKR1B1 (aldose reductase); AKR1B10 (small intestine like aldose reductase) [6,7]; AKR1C1 (20 α -hydroxysteroid dehydrogenase (HSD)/dihydrodiol dehydrogenase (DD1); AKR1C2 (type 3 3 α -HSD/bile acid binding protein); AKR1C3 (type 2 3 α -HSD and type 5 17 β -HSD/prostaglandin F synthase); AKR1C4 (type 1 3 α -HSD/chordecone reductase [8,9]; and AKR1D1 (steroid 5 β -reductase) [10]. The AKR6 family contains β -subunits of the voltage-dependent shaker potassium channels (AKR6A3, AKR6A5 and AKR6A9) [11]; and the AKR7 family contains the aflatoxin aldehyde reductases (AKR7A2 and AKR7A3) [12,13]. In addition to reducing aflatoxin, AKR7A2 appears to be the major succinic semialdehyde reductase responsible for the formation of γ -hydroxybutyrate [14-15].

AKRs catalyze a ordered bi bi sequential reaction mechanism in which the binding of the obligatory cofactor NAD(P)H precedes the binding of the substrate carbonyl, reduction occurs in the central complex, followed by the release of the alcohol and NAD(P)⁺ products in that order [16,17].

In the protein-data bank (PDB) there are 84 AKR structures listed, and for several proteins there exist structures for the binary (E.NADP(H)) and ternary complexes (E.NADP(H).2nd ligand) that form in the reaction mechanism. These structures share a common protein-fold, an (α/β)₈-barrel in which eight α -helices alternate with eight β -strands, where the β -strands coalesce in the core of the structure to make up the staves of the barrel [18-20]. At the back of the structure three large disordered loops exist which undergo a conformational change upon binding cofactor and the substrate carbonyl. The cofactor binding site is highly conserved, so that the nicotinamide head group is in an *anti*-conformation with respect to the ribose sugar, and hydride transfer to the acceptor group is always 4-pro-*R*. Broad substrate specificity has evolved in these enzymes since the flexible loops can accommodate diverse structural entities. At the base of the barrel there is a highly conserved catalytic tetrad consisting of Y55, H117, D50 and K84 (numbering according to AKR1C9), where Y55 acts as the general acid/base [21,22]. The rate-determining step in the AKR reaction is enzyme dependent, it is often the rate of cofactor release, but examples exist where the chemistry step, and or the release of one or both products can contribute to rate- determination [17,23,24]. Detailed reviews of the structure-function of these enzymes have been published and this work will not be repeated here. The reader is referred to the following references [2,25,26].

There are several unique features of the human proteins. First, not all the proteins are enzymes e.g. the AKR6 family members control K⁺ ion conductance. Second, not all the enzymes are oxidoreductases, e.g. AKR1D1 is a steroid double-bond reductase, and has an altered catalytic tetrad, where the catalytic histidine is replaced by a glutamic acid. Third, not all the proteins are monomeric, e.g. the AKR6 β -subunits exist in a tetrameric arrangement at the base of the K⁺ channel; and precedence exists that the AKR7 family is also dimeric. Thus in the rat, AKR7A1 and AKR7A4 can form both homo- and hetero-dimers [27]. This article will review the function of the human enzymes, their gene regulation and the known single-nucleotide polymorphisms (SNPs).

Physiological Function of Human AKR Enzymes

Many of the human enzymes reduce common carbonyl containing substrates e.g. *p*-nitrobenzaldehyde, glyceraldehyde, 9,10-phenanthraquinone, etc., which has made the assignment of their preferred physiological substrates difficult. However, preferred roles in lipid aldehyde, steroid hormone, prostaglandin, retinoid, and chemical carcinogen metabolism are now apparent.

Detoxication of Lipid Aldehydes

Reactive oxygen species (ROS; superoxide anion, hydrogen peroxide and hydroxyl radical) produced under conditions of oxidative stress, attack polyunsaturated fatty acids (PUFA) e.g. arachidonic acid and linoleic acid, to form a series of lipid peroxides that break-down to yield reactive bifunctional electrophiles e.g. 4-hydroxy-2-nonenal (4-HNE). These electrophiles are implicated in the pathogenesis of atherosclerotic plaques [28,29] and neurodegenerative disease (Parkinson's disease and Alzheimers disease) since they can form protein cross-links [30,31]. Their bifunctionality arises since the aldehydic group can form Schiff's bases with the ϵ -amino group of lysines, and the α,β -unsaturated aldehyde can act as a Michael acceptor. 4-HNE and 4-oxo-2-nonenal can also form highly mutagenic etheno- and heptano-etheno-DNA adducts, respectively [32-34]. To limit their potential toxicity a number of enzyme systems exist to detoxify 4-HNE (e.g. ADH, ALDH, GSTs and AKRs). GST-conjugates of 4-HNE still retain the reactive aldehyde, but these conjugates are the preferred substrates for AKR1B1 [35,36]. By contrast 4-HNE can be reduced to 1,4-dihydroxy-2-nonenal (DHE) by AKR1C1-1C4 and AKR1A1, where AKR1C1 has the highest catalytic efficiency [37]. 4-HNE is usually rapidly depleted by reaction with GSH catalyzed by GSTs but under conditions in which GSH is depleted (oxidative stress), AKR1C1 may act as an effective cytosolic barrier to the genotoxic 4-HNE by catalyzing its reduction to DHE, Fig. 1. The AKR1C isoforms appear to be unique among AKRs since they detoxify the bifunctional electrophile in a single step. By reducing the aldehyde to the alcohol they remove both the aldehyde and the α,β -unsaturated carbonyl in a single reaction.

Nuclear Receptor Signaling

Human AKRs reduce a large variety of lipophilic substrates e.g. ketosteroids, ketoprostaglandins and retinals and can thereby regulate ligand access to nuclear receptors and hence affect gene transcription.

AKR1C enzymes play pivotal roles in steroid hormone action, Table 2. They have different ratios of 3-keto-, 17-keto- and 20-keto- steroid reductase activity, and can regulate ligand access to steroid hormone receptors in target tissues in which they are differentially expressed [9]. For example, AKR1C1 is predominantly a 20 α -HSD and will reduce progesterone (a potent progestin) to 20 α -hydroxyprogesterone (a weak progestin) and will regulate ligand access to the progesterone receptor (PR) in target tissues (e.g. endometrium) [38]. AKR1C1 is also the only known NADPH-dependent 3-ketosteroid reductase that will act as a 3 β -HSD; it will convert 5 α -dihydrotestosterone (5 α -DHT) exclusively to 3 β -androstenediol, a natural pro-apoptotic ligand for ER β in target tissues (e.g. prostate) [39]. AKR1C2 is predominately a 3 α -HSD and will reduce 5 α -DHT (a potent androgen) to 3 α -androstenediol (a weak androgen) and will regulate ligand access to the androgen receptor (AR) in target tissues (e.g., prostate) [40]. This suggests that the ratio of AKR1C1 : AKR1C2 expression could determine whether proapoptotic or anti-proliferative signals are produced from 5 α -DHT. AKR1C3 will act predominately as a 17 β -HSD and will reduce Δ^4 -androstene-3,17-dione (weak androgen) to testosterone (potent androgen) and will regulate access of testosterone to the AR in target tissues, e.g. prostate [41]. AKR1C3 will also reduce estrone (weak estrogen) to 17 β -estradiol (potent estrogen) and will regulate ligand access to ER in target tissues e.g. breast and endometrium. Because AKR1C3 will also reduce progesterone to 20 α -hydroxyprogesterone its combined action in the breast would be to produce a proestrogenic state, which may be further exacerbated by its ability to make testosterone, the substrate for aromatase [9].

Orphan Nuclear Receptors

PPAR γ —AKR1C3 may also prevent ligand access to PPAR γ [42,43]. AKR1C3 is identical to prostaglandin F synthase [44-46]. The enzyme catalyzes two reactions on PGs which have important consequences for nuclear receptor signaling and pro-inflammatory and proliferative

responses [46]. With PGH₂ the product of the AKR1C3 reaction is PGF_{2α}, however, the enzyme also has substantial 11-ketoprostaglandin reductase activity and will convert PGD₂ to 9α,11β-PGF₂, Fig. 2. In the absence of AKR1C3, PGD₂ will dehydrate and isomerize to produce 15-deoxy-Δ^{12,14}-PGJ₂ (15d-PGJ₂) a potential endogenous ligand for PPARγ. PPARγ can heterodimerize with RXR which itself is ligand activated by 9-*cis*-retinoic acid [42]. The heterodimer can then activate the transcription of genes that cause terminal differentiation and/or apoptosis. Controversy exists over whether sufficient 15d-PGJ₂ is produced *in vivo* to activate PPARγ as there are large differences between the concentrations that are required to *trans*-activate PPARγ (μM) and the amount of “free” 15d-PGJ₂ that can be detected (pM to nM) [47]. These discrepancies can arise since 15d-PGJ₂ contains a highly reactive cyclopentenone moiety that is rapidly conjugated to SH groups, making it difficult to ascertain its true concentration. Recently, 15d-PGJ₂ has been shown to covalently modify PPARγ *in vitro* [48]. Another consequence of this pathway is that activation of the FP receptor leads to stimulation of MAPK, and phosphorylation and inactivation of PPARγ [49,50]. Thus, AKR1C3 may prevent PPARγ signaling by preventing the formation of its ligand 15d-PGJ₂ and by stimulating the phosphorylation of the receptor, leading to a pro-proliferative response.

RXR—AKR1B10 (small intestine like aldose reductase) is implicated in retinoic acid signaling. Retinal is the precursor of retinoic acid, which is formed by the action of ALDH, Fig. 3. However, retinals can also be reduced to retinol by AKR1B10, whereas short-chain dehydrogenases/reductases catalyze the reverse reaction and convert retinol back to retinal. Removal of retinal as retinol by AKR1B10 would prevent the formation of 9-*cis*-retinoic acid an important ligand for RXR [51]. RXR heterodimerizes with PPARγ to affect transcription through the peroxisome proliferator response element, leading to an anti-proliferative response. It is intriguing that the combined action of AKR1C3 and AKR1B10 may be able to regulate anti-proliferative signaling by individually targeting different receptors of the heterodimeric complex that forms between PPARγ and RXR. Thus, there is a need for selective inhibitors of one or both enzymes to determine whether they could be targets for new anti-proliferative agents.

CAR, PXR, FXR-Signaling—AKR1D1 (Δ⁴-3-ketosteroid 5β-reductase) is a liver specific enzyme that will convert any Δ⁴-3-ketosteroid into a 5β-dihydrosteroid, in which the A/B ring juncture in the steroid takes on a *cis*-configuration [10]. This introduces a 90° bend in the steroid structure, which is a characteristic structural feature of all bile-acids. It is likely that in addition to its role in bile-acid biosynthesis, AKR1D1 is responsible for the synthesis of ligands for the orphan receptors: PXR (pregnane X-activated receptor); CAR (the constitutive androstane receptor); and FXR (the nuclear bile-acid receptor). The 5β-pregnanes e.g. 5β-pregnane-3,20-dione are among the most potent agonists for the binding and *trans*-activation of PXR and CAR [52,53]. CAR and PXR increase the transcription of the *CYP3A4* and *CYP2B* genes where *CYP3A4* may account for the oxidative metabolism of more than 60% of clinically used drugs [54,55]. Recently, activation of FXR (nuclear bile acid receptor) by bile-acid flux has been proposed as a primary mechanism for normal liver regeneration [56]. The only known route in humans to the A/B *cis*-ring structure found in these ligands for orphan receptors is via AKR1D1.

Aryl-Hydrocarbon Receptor (AhR)—Planar polycyclic aromatic hydrocarbon *o*-quinones e.g. benzo[*a*]pyrene-7,8-dione (products of the dihydrodiol dehydrogenase activity of AKR1A1 and AKR1C1-AKR1C4) bind to the AhR with an affinity equal to that seen with benzo[*a*]pyrene, B[*a*]P. B[*a*]P-7,8-dione activation of the AhR results in increased *CYP1A1* gene expression in human hepatoma cells (HepG2) and increased *CYP1A1/1B1* gene expression in bronchoalveolar H358 cells, [57,58]. B[*a*]P-7,8-dione does not induce the *CYP1A1* gene in Hepa1c1c cells that are deficient in either AhR or ArNT. *CYP1A1* induction

was preceded by nuclear translocation of the AhR to a xenobiotic response element (XRE) as measured by electrophoretic mobility shift assays and by immunocytochemistry using a AhR antibody [57]. Collectively, these studies support the concept that AKRs generate ligands for the AhR which results in transcription of target genes.

Xenobiotic Function of Human Aldo-Keto Reductases

AKRs are implicated in the metabolism of three classes of chemical carcinogen polycyclic aromatic hydrocarbons, aflatoxin, and nicotine derived nitosamino-ketones e.g., NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone).

Polycyclic Aromatic Hydrocarbons

PAH are ubiquitous environmental pollutants that require metabolic activation to exert their carcinogenic effects. Pathways of activation are best understood with the representative compound B[a]P, Fig. 4. A major route involves monooxygenation by P4501A1/1B1 on the terminal benzo-ring to form the 7*R*,8*S*-arene oxide which is hydrolyzed by epoxide hydrolase to form (-)-7*R*,8*R*-dihydroxy-7,8-dihydro-B[a]P (B[a]P-7,8-dihydrodiol). B[a]P-7,8-dihydrodiol can then be further monooxygenated to yield (+)-*anti*-B[a]P-diol-epoxide (*anti*-B[a]PDE) which is highly mutagenic, carcinogenic and tumorigenic [59-62]. Alternatively, the dihydrodiol dehydrogenase activity of the human AKRs (AKR1A1, 1C1-1C4), can oxidize the (-)-B[a]P-7,8-dihydrodiol to yield the corresponding ketol, which rearranges to form a catechol [63-65]. The catechol is air-sensitive and will undergo a 1 electron oxidation step to yield the *o*-semiquinone anion radical, and a subsequent 1 electron oxidation step to yield the fully oxidized *o*-quinone, with the concomitant production of reactive oxygen species [66]. The *o*-quinone is a reactive Michael acceptor and will react with DNA, RNA, protein, GSH, and amino acids. It is also redox-active, and in the presence of NADPH will be reduced back to the catechol for a further round of autooxidation. This establishes a futile redox-cycle in which trace amounts of *o*-quinone can redox-cycle, deplete cellular NADPH, and amplify ROS production. A similar mechanism of metabolic activation has been proposed for the catechol estrogens [67].

The electrophilic and redox active *o*-quinones can form a spectrum of DNA-adducts and evidence exists for stable bulky adducts formed by 1,4-Michael addition (which subsequently hydrate and cyclize) [68-70], the formation of depurinating DNA-adducts [71], and the formation of 8-oxo-dGuo [72]. *In vitro* measurements of DNA-adduct formation suggest that the prominent lesions are those that result from oxidative damage of DNA [73]. Furthermore, PAH *o*-quinones were most mutagenic in an *in vitro* p53 mutagenicity assay when they were allowed to redox-cycle in the presence of NADPH and CuCl₂. In the same assay (±)-*anti*-B[a]PDE was 80 times less mutagenic than B[a]P-7,8-dione [74]. The mutations observed with PAH *o*-quinones were G to T transversions and were abolished with ROS scavengers consistent with 8-oxo-dGuo being the lesion responsible for mutation.

A consistent theme is emerging that AKR isoforms most involved in the formation of PAH *o*-quinones are important in the etiology of lung cancer. Differential display of 317/372 NSCLC patients showed that AKR1C1/AKR1C2 was over expressed by up to 50-fold in tumor tissue versus the adjacent normal tissue and that this was indicative of poor prognosis [75]. Three independent studies showed that AKR1C1 was over expressed in non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma, bronchial epithelial cells of NSCLC, and oral cancer cells by Affymetrix microarray [76-78]. Expression levels were among the highest of the 30,000 genes examined. We validated these studies in human lung adenocarcinoma (A549) cells which were shown to over express AKR1C1-AKR1C3 based on RT-PCR, Northern analysis, and functional enzyme assay. Lysates from these cells effectively converted PAH *trans*-dihydrodiols to *o*-quinones when supplemented with NADP⁺ [65]. 1-

Hydroxychavicol the active ingredient of Acrea Quid (ingested with chewing tobacco), was found to act as a monofunctional inducer (a xenobiotic that functionally induces NAD(P)H: quinone oxidoreductase without affecting the AhR [79]) in buccal cells and induce AKR1C1.

Treatment of these cells with B[a]P led to a reduction in stable (+)-*anti-trans*-B[a]PDE-N²-dGuo adducts and an increase in 8-oxo-dGuo [80]. Additionally, SNPs in *AKR1C3* and *hOGG1* (human 8-oxoguanosine glycosylase) were associated with a higher incidence of lung cancer in individuals exposed to smoky coal [81,82]. These studies suggest that the AKR pathway and resultant oxidative damage of DNA may be a component of PAH-induced lung carcinogenesis.

Nicotine Derived Nitrosaminoketones (NNK)

Nicotine in tobacco smoke gives rise to NNK which is a lung carcinogen in the A/J mouse lung model [83]. NNK is bioactivated via α -methylene hydroxylation by P450 2A6 and P450 3A4 to yield a diazo-hydroxide, Fig. 5. The diazo-hydroxide rearranges to yield a methyl carbonium ion, nitrogen and water, with the result that guanine bases are O⁶-methylated [83]. Alternatively, NNK can be bioactivated via α -methyl hydroxylation by P450 2A6 and P450 3A4 to yield a pyridyloxybutylaldehyde (PoB), which forms two stable adducts O⁶-pyridyloxybutyl-dGuo (O⁶-PoB-dGuo) and O²-pyridyloxybutyl-dThd (O²-PoB-dThd) and two depurinating adducts 7- pyridyloxybutyl-Gua and O²-pyridyloxybutyl-Cyt [84,85].

Mutation avoidance can be accomplished by detoxication of NNK, which involves its reduction to nicotine derived nitrosamino-alcohol (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; *R*- and *S*-NNAL). Although, NNAL can still undergo α -methylene and α -methyl hydroxylation, the presence of the alcohol allows NNAL to be glucuronidated and eliminated. The *S*-NNAL has a carcinogenic potency equivalent to NNK which suggests that the more relevant isomer for glucuronidation is *R*-NNAL. The reductases implicated in *S*-NNAL formation are the AKR1C1, 1C2, and 1C4 and would prevent glucuronidation by the favored route since the incorrect enantiomer is formed. Moreover, *S*-NNAL can be oxidized back to NNK. By contrast the formation of *R*-NNAL is catalyzed by 11 β -hydroxysteroid dehydrogenase (11 β -HSD type 1 or carbonyl reductase) [86,87].

Aflatoxin-dialdehyde

Aflatoxin is a potent rodent and human hepatocarcinogen which is metabolically activated by P450 3A4 to yield the aflatoxin B₁-8,9-epoxide which is an ultimate carcinogen [88]. The epoxide can be hydrated by epoxide hydrolase to form the aflatoxin-8,9-dihydrodiol which undergoes ring opening to yield the cytotoxic dialdehyde which is capable of forming protein adducts via reaction with the ϵ -amino group of lysines [89]. AKR7A family members (also referred to as aflatoxin aldehyde reductases (AFAR) reduce the dialdehyde sequentially to form the mono- and dialcohols, Fig. 6. Two enzymes can perform this reaction in humans and correspond to AKR7A2 and AKR7A3, and likely protect against aflatoxin mediated hepatotoxicity [12,13].

Regulation of Human Genes

It is clear that the regulation of human AKR genes could have a profound affect on the metabolism of endogenous mediators of nuclear receptor signaling and/or detoxication of chemical carcinogens. Several major *cis*-elements affect human gene transcription; these are the osmotic response element (ORE; found in the promoter of *AKR1B1*) [90]; the phorbol ester response (AP-1) element (TRE; found in the promoters of the *AKR1B1* and *AKR1C* genes) (H.K. Lin and Penning, unpublished); and the anti-oxidant response element (ARE; found in the promoters of the *AKR1C1*-*AKR1C3* genes) [91,92]. A TESS transcription factor consensus

sequence search across the promoters of all the human AKR genes (Fig. 7.) indicated the presence of many shared elements.

Regulation by the Osmotic Response Element

AKR1B1 (aldose reductase) is induced by osmotic stress. This enzyme converts glucose to sorbitol which is hyperosmotic, thus over expression of this enzyme will lead to osmotic balance under conditions of osmotic stress. The ORE (5'-TGGAATCA-CCGC-3') or tonicity response element (TonE), found in the promoter of the *AKR1B1* gene (-1.1 kb) binds a rel-like transcription factor the TonE-binding protein (NFAT5/TonEBP) which is related to NFκB [90,93,94]. NFAT5 also regulates the expression of TNFα genes in osmotically stressed cells [95] suggesting a connection between the TonE and NFκB signal transduction pathways [95]. An ORE consensus sequence exists in the promoter region of many human AKR genes. Of these the *AKR7A3* gene appears to be a strong potential candidate for regulation by this element since its ORE's contain a similar arrangement to that seen in the *AKR1B1* gene (this study).

Regulation by the Phorbol Ester Response Element

The phorbol ester response element or AP-1 site (5'-TGACTCA-3') is located in the promoter of the *AKR1B1* gene (-1167 and -1047) and in the *AKR1C1* gene (and in addition several imperfect AP1 sites reside between -2.0 kb and the transcription start site). Activating-protein 1 (b-leucine zipper) transcription factors (c-Jun and c-fos) can be activated by both extracellular ROS via activation of a mitogen-activated protein kinase (MAPK) cascade, or they can be redox-regulated by intracellular ROS [96-99]. The similarity between the AP-1 site consensus sequence (5'-TGACTCA-3') and the "core" anti-oxidant response element (ARE) consensus sequence originally described for the *GSTA2* gene (5'-GTGAC-NNN-GC-3') [100,101], raises the prospect that the effects of ROS on gene expression may be mediated either by AP-1 or Nrf2 and small maf (see below).

Regulation by the Anti-Oxidant Response Element

AKR1C1-AKR1C3 genes have in their promoters "core" ARE's, which are transcriptionally activated by electrophilic and oxidative stress [91,92]. Whether the "core" sequence is necessary and sufficient for gene induction may depend on the promoter context. Wasserman and Fahl found that the ARE in the mouse *GSTA1* gene was considerably longer than the "core" sequence [102] while the ARE in the murine *NQO1* gene was 16 nucleotides in length [103]. These differences have led to the concept that the ARE is relatively plastic.

The ARE is *trans*-activated by Nrf2 (nuclear factor-erythroid 2 p45 related factor) which is liberated from Keap-1 [104]. Keap-1 exists as a dimer and binds Nrf2 through two separate sites in the N-terminal domain of the transcription factor; these correspond to the high affinity "ETGE" motif and the low affinity "DLG" motif [105]. This creates a "fixed-end" model to enable Keap-1 to promote the degradation of Nrf2 by acting as a substrate adaptor protein for the cullin 3-Roc1 ubiquitin E3 ligase [105,106]. This leads to the ubiquitination of Nrf2 and signals its degradation in the 26S proteasome [107]. This activity is inhibited during electrophilic or oxidative stress. This occurs through the modification of key cysteine residues on Keap-1 which may lead to a disruption of the low-affinity interaction with Keap-1 leading to the release of Nrf2 [108]. Once released Nrf2 is recruited to the ARE where it binds with its heterodimeric partner small maf to mediate gene transcription.

In HepG2 cells *AKR1C1* is induced 10-fold by a battery of ARE inducers (e.g. ethacrynic acid, t-butylhydroquinone, 2,3-dimethoxynaphthalene-1,4-dione (DMNQ), and hydrogen peroxide) at the RNA, protein and functional level [91]. The significance of DMNQ is that it is fully substituted and can only redox-cycle suggesting that intracellular ROS are the inducing species.

This induction profile is consistent with a mechanism involving an ARE. The functional ARE was located "6.3 kb upstream from the transcription start site in the *AKR1C1* gene and an identical element was found upstream in the same region (-5.5 kb) of the *AKR1C2* gene [92]. Functionality of the AKR1C2-ARE was supported by mutational analysis of the element, by transient transfection of Nrf2 to show increased transcription from reporter gene constructs containing the AKR1C2-ARE, and the failure to observe a response in Nrf2 knockout cells. A similar characterization was performed on the AKR1C1-ARE [92]. RNase protection assays also showed that the AKR1C3 gene is induced by an ARE and the consensus sequence search locates AREs at (-1.4 and -6.8 kb) [91]. Other human genes regulated by AREs include NQO1, UGTs, γ -glutamine cysteine ligase modifier (GCLM) subunit, and heme oxygenase 1

These observations raise the prospect that ROS exposure will regulate *AKR1C1-AKR1C3* genes. The outcome of this regulation could be either beneficial or harmful. On the one hand elevated expression of *AKR1C1-AKR1C3* could lead to elevated detoxication of 4-hydroxy-2-nonenal. On the other hand, AKR1C3 (prostaglandin F synthase) is uniquely positioned to reduce PGH₂ and PGD₂ to yield PGF epimers leading to the inactivation of PPAR γ upon exposure to oxidative stress. Moreover, since the PGF epimers bind to the FP receptor, this could lead to activation of NF κ B and propagation of an inflammatory response involving induction of COX-2. Similarly, AKR1C1-AKR1C3 are involved in the metabolic activation of PAH to form reactive and redox active PAH *o*-quinones, which are mutagenic. By entering into redox –cycles the formation of ROS is amplified which would lead to further expression of AKR1C isoforms that could propagate the formation of these mutagenic metabolites.

Regulation by Other Elements

Based on the role of human AKRs in steroid hormone action and nuclear receptor signaling, it is not surprising to find that many of the genes contain half-sites for steroid response elements (SRE-1). Perhaps the surprise is *AKR1D1* (steroid 5 β -reductase) which has the potential to be regulated by an Estrogen Response Element (ERE) but not by a SRE-1, suggesting that there could be sexual dimorphism in its expression. Also, based on the role of human AKRs in chemical carcinogen metabolism the prospect arises as to whether they are regulated by a XRE. Although, XREs are found in the promoters of *AKR1A1*, *AKR1B1*, *AKR7A2* and *AKR7A3*, perhaps the surprise is the absence of this element in the promoter of *AKR1C* genes, since apart from *AKR1A1* they are the isoforms most involved in the metabolic activation of PAH *trans*-dihydrodiols.

Single Nucleotide Polymorphisms

A complete listing of the SNPs located in the human AKR genes can be found at the AKR web site (www.med.upenn.edu/akr) and was taken from the NCBI SNP data base (www.ncbi.nlm.nih.gov/SNP/). The nomenclature for the SNPs is based on that used for other gene superfamilies. Following the unique identifier for the gene *AKR1C1* the *1 refers to alleles which produce a protein of identical sequence to wild-type (synonymous) the “*2-n” refers to alleles which produce a protein of different sequence to wild-type and are non-synonymous. The numbering is based on order of discovery. For sub-alleles, letters are used when a different SNP produces the same protein sequence. The letters used are based on order of discovery. The number of non-synonymous SNPs in the coding regions of human AKRs so far reported are listed in parenthesis: AKR1A1 (2); AKR1B1 (8); AKR1B10 (3); AKR1C1 (3); AKR1C2 (10); AKR1C3 (9); AKR1C4 (5); AKR1D1 (although none are listed, inherited mutations are associated with bile acid deficiency [109-112]); AKR7A2 (8) and AKR7A3 (3). Since the crystal structures of many of these AKRs are available these SNPs can be mapped to these structures to predict their functional consequences.

The bulk of the SNPs do not map to the conserved cofactor-binding site, the mobile loops involved in substrate binding or the conserved catalytic tetrad. Instead, they are located either on the small loops that link α -helix to β -strand or they are on the helices or β -strands themselves. Thus, it is likely that these SNPs play a larger role in enzyme stability.

Some of the SNPs deserve a special mention. AKR1C1 differs from AKR1C2 in only seven amino acids but they have distinctive functions. The amino acids that change in going from AKR1C1 to AKR1C2 are as follows: T38V, R47H, L54V, C87S, V151M, R170H and Q172L. Several of these amino acids are highly polymorphic e.g. AKR1C1 has the following polymorphisms T38A, T38I and R47H, while AKR1C2 has the following polymorphisms, H47R, S87C, L172Q, V38I and V38A. While it is tempting to speculate that these polymorphisms may give rise to enzymes that might have intermediate AKR1C1/AKR1C2 activities, this does not appear to be the case. Only one of the seven different amino acids (L54) resides at the substrate-binding site. When the AKR1C1-L54V mutant was generated the protein now had the properties of AKR1C2 [113]. Other SNPs may also be important. The AKR1C3*2 (Q5H) has been associated with a higher incidence of lung cancer [82], and the AKR1C3*4 (E36term) will yield a truncated protein. The AKR1C4*5 (L311V) is found on the C-terminal loop and affects steroid substrate specificity and k_{cat} [114]. Although a systematic study is required to examine the effects of these allelic variants on enzyme function, this effort will likely be driven by genotyping and genome wide association studies and their correlation with a human phenotype.

Concluding Statement

Human AKRs play fundamental roles in carbonyl metabolism and utilize both natural and exogenous substrates. With the former they are poised to regulate signaling through nuclear receptors and with the later they may influence chemical carcinogenesis. More emphasis is now required on how gene regulation is achieved in a tissue specific fashion and how SNP's affect enzyme function.

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References

1. Jez JM, Flynn TG, Penning TM. *Biochemical Pharmacology* 1997;54:639–647. [PubMed: 9310340]
2. Jez JM, Bennett MJ, Schlegel BP, Lewis M, Penning TM. *The Biochemical Journal* 1997;326:625–636. [PubMed: 9307009]
3. Jez JM, Penning TM. *Chemico-Biological Interactions* 2001;130-132:499–527. [PubMed: 11306071]
4. Bohren K, Bullock B, Wermuth B, Gabbay KH. *Journal of Biological Chemistry* 1984;264:9547–9551. [PubMed: 2498333]
5. Wermuth B, Omar A, Forster A, Di Francesco C, Wolf M, Wartburg J-P, Bullock B, Gabbay KH. *Progress in Clinical Biological Research* 1987;232:297–307.
6. Cao D, Fan ST, Chung SSM. *Journal of Biological Chemistry* 1998;273:11429–11435. [PubMed: 9565553]
7. Hyndman D, Flynn TG. *Biochimica Biophysica Acta* 1998;1399:198–202.
8. Burczynski ME, Harvey RG, Penning TM. *Biochemistry* 1998;37:6781–6790. [PubMed: 9578563]
9. Penning TM, Burczynski ME, Jez JM, Hung C-F, Lin H-K, Ma H, Moore M, Palackal N, Ratnam K. *The Biochemical Journal* 2000;351:67–77. [PubMed: 10998348]
10. Kondo K, Kai M, Setoguchi Y, Eggertsen G, Sjoborn P, Setoguchi T, Okuda K, Bjorkhem I. *European Journal of Biochemistry* 1994;219:357–363. [PubMed: 7508385]

11. McCormack K, McCormack T, Tanouye M, Rudy B, Stuhmer W. FEBS Letters 1995;370:32–36. [PubMed: 7649300]
12. Ireland LS, Harrison DJ, Neal GE, Hayes JD. The Biochemical Journal 1998;332:21–34. [PubMed: 9576847]
13. Knight LP, Primiano T, Groopman JD, Kensler TW, Sutter TR. Carcinogenesis 1999;20:1215–1223. [PubMed: 10383892]
14. Picklo MJ, Olson SJ, Hayes JD, Maresbery WR, Montine TJ. Brain Research 2001;916:229–238. [PubMed: 11597610]
15. Keenan, C.; Ghaflar, S.; Grant, AW.; Hinshelwood, A.; Li, D.; McGarvie, G.; Ellis, EM. Enzymology and Molecular Biology of Carbonyl Metabolism. Wiener; Plapp; Lindahl; Maser, editors. 12. Purdue University Press; W. Lafayette, IN: 2006. p. 388–395.
16. Askonas LJ, Ricigliano JW, Penning TM. The Biochemical Journal 1991;278:835–41. [PubMed: 1898369]
17. Grimshaw CE, Bohren KM, Lai CJ, Gabbay KH. Biochemistry 1995;34:14356–65. [PubMed: 7578039]
18. Hoog SS, Pawlowski JE, Alzari PM, Penning TM, Lewis M. Proceedings of the National Academy of Sciences USA 1994;91:2517–21.
19. Bennett MJ, Schlegel BP, Jez JM, Penning TM, Lewis M. Biochemistry 1996;35:10702–10711. [PubMed: 8718859]
20. Bennett MJ, Albert RH, Jez JM, Ma H, Penning TM, Lewis M. Structure 1997;5:799–812. [PubMed: 9261071]
21. Bohren KM, Girmshaw CE, Lai C-J, Harrison D, Ringe D, Petsko GA, Gabbay KH. Biochemistry 1994;33:2021–2032. [PubMed: 8117659]
22. Schlegel BP, Jez JM, Penning TM. Biochemistry 1998;37:3538–3548. [PubMed: 9521675]
23. Heredia VV, Penning TM. Biochemistry 2004;43:12028–12037. [PubMed: 15379543]
24. Jin Y, Penning TM. Biochemistry 2006;45:13054–63. [PubMed: 17059222]
25. Penning TM, Bennett MJ, Smith-Hoog S, Schlegel BP, Jez JM, Lewis M. Steroids 1997;62:101–111. [PubMed: 9029723]
26. Penning TM, Jin Y, Steckelbroeck S, Rizner T, Lewis M. Molecular and Cellular Endocrinology 2003;215:63–72. [PubMed: 15026176]
27. Kelly VP, Sherratt PJ, Crouch DH, Hayes JD. The Biochemical Journal 2002;366:847–61. [PubMed: 12071861]
28. Esterbauer H, Schauer RJ, Zollner H. Free Radical Biology and Medicine 1991;11:81–128. [PubMed: 1937131]
29. Esterbauer H. American Journal of Clinical Nutrition 1993;57:779S–8S. [PubMed: 8475896]
30. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. Proceedings of the National Academy of Science USA 1996;93:2696–701.
31. Zarkovic K. Molecular Aspects of Medicine 2003;24:293–303. [PubMed: 12893007]
32. Rindgen D, Nakajima M, Wehrli S, Xu K, Blair IA. Chemical Research in Toxicology 1999;12:1195–1204. [PubMed: 10604869]
33. Lee SH, Rindgen D, Bible RH Jr, Hajdu E, Blair IA. Chemical Research in Toxicology 2000;13:565–574. [PubMed: 10898588]
34. Lee SH, Oe T, Blair IA. Science 2001;292:2083–2086. [PubMed: 11408659]
35. Ramana KV, Dixit BL, Srivastava S, Balendiran GK, Srivastava SK. Biochemistry 2000;39:12172–12180. [PubMed: 11015195]
36. Srivastava S, Conklin DJ, Liu S-Q, Prakash N, Boor PJ, Srivastava S, Bhatnagar A. Atherosclerosis 2001;158:339–350.
37. Burczynski ME, Sridhar GR, Palackal NT, Penning TM. Journal of Biological Chemistry 2001;276:2890–2897. [PubMed: 11060293]
38. Rizner TL, Smuc T, Repreht R, Sinkovec J, Penning TM. Molecular and Cellular Endocrinology 2006;248:126–135. [PubMed: 16338060]

39. Steckelbroeck S, Jin Y, Gopishetty S, Oyesanmi B, Penning TM. *Journal of Biological Chemistry* 2003;279:10784–10795. [PubMed: 14672942]
40. Rizner T, Lin H-K, Peehl DM, Steckelbroeck S, Bauman DR, Penning TM. *Endocrinology* 2003;144:2922–2932. [PubMed: 12810547]
41. Penning TM, Steckelbroeck S, Bauman DR, Miller MW, Peehl DM, Fung K-M, Lin H-K. *Molecular and Cellular Endocrinology* 2006;248:182–191. [PubMed: 16417966]
42. Desmond JC, Mountford JC, Drayson MT, Walker EA, Hewison M, Ride JP, Loung QT, Hayden RE, Vanin EF, Bunce CM. *Cancer Research* 2003;63:505–512. [PubMed: 12543809]
43. Lin H-K, Jez JM, Schlegel BP, Peehl DM, Pachter JA, Penning TM. *Molecular Endocrinology* 1997;11:1971–1984. [PubMed: 9415401]
44. Matsuura K, Shirasishi H, Hara A, Sato K, Deyashiki Y, Ninomiya M, Sakai S. *Journal of Biochemistry (Tokyo)* 1998;124:940–946.
45. Suzuki-Yamamoto T, Nishizawa M, Fukui M, Okuda-Ashitaka E, Nakajima T, Ito S, Watanabe K. *FEBS Letters* 1999;462:335–340. [PubMed: 10622721]
46. Koda N, Tsutsui Y, Niwa H, Ito S, Woodward DF, Watanabe K. *Archives Biochemistry & Biophysics* 2004;424:128–136.
47. Bell-Parikh LC, Idem T, Lawson JA, McNamara P, Reilly M, FitzGerald GA. *Journal of Clinical Investigation* 2003;112:945–955. [PubMed: 12975479]
48. Soares AF, Nosjean O, Cozzzone D, D’Orazio D, Becchi M, Guichardant M, Ferry G, Boutin JA, Lagared M, Geloën A. *Biochemical Biophysics Research Communications* 2005;337:521–525.
49. Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK. *Journal of Biological Chemistry* 1997;272:5128–5132. [PubMed: 9030579]
50. Reginato MJ, Krakow SL, Bailey ST, Lazar MA. *Journal of Biological Chemistry* 1998;273:1855–1858. [PubMed: 9442016]
51. Crosas B, Hyndman D, Gallego O, Matras S, Pares X, Flynn TG. *The Biochemical Journal* 2003;373:973–979. [PubMed: 12732097]
52. Bertilsson G, Hiedrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, Berkenstam A. *Proceedings of the National Academy of Sciences USA* 1998;95:12208–12213.
53. Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Wilson TM, Collins JL, Kliewer SA. *Journal of Biological Chemistry* 2000;275:15122–15127. [PubMed: 10748001]
54. Wrighton SA, Stevens JC. *Critical Reviews in Toxicology* 1992;22:1–21. [PubMed: 1616599]
55. Shimada T, Yamazaki H, Mimura M, Wakamiya N, Ueng YF, Guengerich FP, Inui Y. *Drug Metabolism and Disposition* 1996;24:515–522. [PubMed: 8723730]
56. Hunang W, Ma K, Zhang J, Qatanami M, Cuvillier J, Liu J, Dong B, Humang X, Moore DD. *Science* 2006;312:233–236. [PubMed: 16614213]
57. Burczynski ME, Penning TM. *Cancer Research* 2000;60:908–15. [PubMed: 10706104]
58. Jiang H, Vudathala DK, Blair IA, Penning TM. *Chemical Research in Toxicology* 2006;19:68–78. [PubMed: 16411658]
59. Gelboin HV. *Physiological Reviews* 1980;60:1107–1166. [PubMed: 7001511]
60. Conney AH. *Cancer Research* 1982;42:4875–4917. [PubMed: 6814745]
61. Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, Guengerich FP. *Cancer Research* 1989;49:6304–6312. [PubMed: 2509067]
62. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. *Cancer Research* 1996;56:2979–2984. [PubMed: 8674051]
63. Penning TM, Burczynski ME, Hung C-F, McCoull KD, Palackal NT, Tsuruda LS. *Chemical Research in Toxicology* 1999;12:1–18. [PubMed: 9894013]
64. Palackal NT, Burczynski ME, Harvey RG, Penning TM. *Biochemistry* 2001;40:10901–10910. [PubMed: 11535067]
65. Palackal NT, Lee S-H, Harvey RG, Blair IA, Penning TM. *Journal of Biological Chemistry* 2002;277:24799–24808. [PubMed: 11978787]

66. Penning TM, Ohnishi ST, Ohnishi T, Harvey RG. *Chemical Research in Toxicology* 1996;9:84–92. [PubMed: 8924621]
67. Bolton JL, Pisha E, Zhang F, Qiu S. *Chemical Research in Toxicology* 1999;11:1113–1127. [PubMed: 9778307]
68. Shou M, Harvey RG, Penning TM. *Carcinogenesis* 1993;14:475–482. [PubMed: 8384091]
69. Balu N, Padgett WT, Lambert GR, Swank AE, Richard AM, Nesnow S. *Chemical Research in Toxicology* 2004;17:827–838. [PubMed: 15206904]
70. Balu N, Padgett WT, Nelson GB, Lambert GR, Ross JA, Nesnow S. *Analytical Biochemistry* 2006;355:213–223. [PubMed: 16797471]
71. McCoull KD, Rindgen D, Blair IA, Penning TM. *Chemical Research in Toxicology* 1999;12:237–246. [PubMed: 10077486]
72. Park J-H, Gopishetty S, Szewczuk LM, Troxel AB, Harvey RG, Penning TM. *Chemical Research in Toxicology* 2005;18:1026–1037. [PubMed: 15962938]
73. Park J-H, Troxel AB, Harvey RG, Penning TM. *Chemical Research in Toxicology* 2006;19:719–728. [PubMed: 16696575]
74. Yu D, Berlin JA, Penning TM, Field JM. *Chemical Research in Toxicology* 2002;15:832–842. [PubMed: 12067251]
75. Hsu N-Y, Ho H-C, Chow K-C, Lin T-Y, Shih C-S, Wang L-S, Tsai C-M. *Cancer Research* 2001;61:2727–2731. [PubMed: 11289154]
76. Fukumoto S-I, Yamauchi N, Moriguchi H, Hippo Y, Watanbe A, Shibahara J, Taniguichi H, Ishikawa S, Ito H, Yamamoto S, Iwanari H, Horonaka M, Ishikawa H, Niki T, Sohara Y, Kodama T, Mishimura M, Fukayama M, Doska-Akita H, Auratani H. *Clinical Cancer Research* 2005;11:1776–1785. [PubMed: 15755999]
77. Woenckhaus M, Klein-Hitpass L, Grepmeier U, Merk J, Pfeifer M, Wild PJ, Bettstetter M, Wuensch P, Blaszk H, Harrmann A, Hofstaedter F, Dietmaier W. *Journal of Pathology* 2006;210:192–204. [PubMed: 16915569]
78. Nagaraj N, Beckers S, Meansh JK, Waigel S, Vigneswaran N, Zacharias W. *Toxicology Letters* 2006;165:182–194. [PubMed: 16713138]
79. Prochaska HJ, Talalay P. *Cancer Research* 1988;48:4776–4782. [PubMed: 3409219]
80. Tang D-W, Chang K-W, Chi C-W, Liu T-Y. *Toxicology Letters* 2004;152:235–243. [PubMed: 15331132]
81. Park J, Chen L, Tockman MS, Elahi A, Lazarus P. *Pharmacogenetics* 2004;14:103–109. [PubMed: 15077011]
82. Lan Q, Mumford JL, Shen M, DeMarini M, Bonner MR, He X, Yeager M, Welch R, Channock S, Tain L, Chapman RS, Zheng T, Keohavong P, Caporaso N, Rothman N. *Carcinogenesis* 2004;25:2177–2181. [PubMed: 15284179]
83. Hecht SS. *Chemical Research in Toxicology* 1998;11:560–603.
84. Mijal R, Thomson NM, Fleischer NL, Pauly GT, Moschel RC, Kanugula S, Fang Q, Pegg AE, Peterson LA. *Chemical Research in Toxicology* 2004;17:424–434. [PubMed: 15025514]
85. Lao Y, Villata PW, Sturla SJ, Wang M, Hecht SS. *Chemical Research in Toxicology* 2006;19:674–682. [PubMed: 16696570]
86. Upadhyaya P, Kenney PMJ, Hochlter JB, Wang M, Hecht SS. *Carcinogenesis* 1999;20:1577–1582. [PubMed: 10426810]
87. Breyer-Pfaff U, Martin HJ, Ernst M, Maser E. *Drug Metabolism and Disposition* 2004;32:915–922. [PubMed: 15319331]
88. Guengerich FP, Johnson WW. *Drug Metabolism Reviews* 1999;31:141–158. [PubMed: 10065369]
89. Guengerich FP, Arneson KO, Williams KM, Deng Z, Harris TM. *Chemical Research in Toxicology* 2002;15:780–792. [PubMed: 12067245]
90. Ko B, Ruepp B, Bohren KM, Gabbay KH, Chung SS. *Journal of Biological Chemistry* 1997;272:16431–16437. [PubMed: 9195951]
91. Burczynski ME, Lin H-K, Penning TM. *Cancer Research* 1999;59:607–614. [PubMed: 9973208]
92. Lou H, Du S, Ji Q, Stolz A. *Molecular Pharmacology* 2006;69:1662–1672. [PubMed: 16478829]

93. Lopez-Rodriguez C, Aramburu J, Rakeman AS, Rao A. Proceedings of the National Academy of Sciences U S A 1999;96:7214–7219.
94. Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM. Proceedings of the National Academy of Sciences U S A 1999;96:2538–2542.
95. Lopez-Rodriguez C, Aramburu J, Jin L, Rakeman AS, Michino M, Rao A. Immunity 2001;15:47–58. [PubMed: 11485737]
96. Abate C, Patel L, Ruascher FJ III, J F, Curran T. Science 1990;249:1157–1161. [PubMed: 2118682]
97. Abate C, Luk D, Gagne E, Roeder RG, Curran T. Molecular and Cell Biology 1990;10:5532–5535.
98. Xanthoudakis S, Miao G, Wang F, Pan Y-CE, Curran T. EMBO Journal 1992;11:3323–3335. [PubMed: 1380454]
99. Xanthoudakis S, Miao GG, Curran T. Proceedings of the National Academy of Sciences USA 1994;91:23–27.
100. Rushmore TH, Morton MR, Pickett CB. The Journal of Biological Chemistry 1991;266:11632–11639. [PubMed: 1646813]
101. Nguyen T, Rushmore TH, Pickett CB. The Journal of Biological Chemistry 1994;269:13656–13662. [PubMed: 8175801]
102. Wasserman W, Fahl WE. Proceedings of the National Academy of Sciences 1997;94:5361–5366.
103. Nioi P, McMahon M, Itoh K, Yamamoto M, Hayes JD. The Biochemical Journal 2003;374:337–348. [PubMed: 12816537]
104. Dinkova-Kostova AT, Holtzclaw WD, Kensler TW. Chemical Research in Toxicology 2005;18:1779–1791. [PubMed: 16359168]
105. McMahon M, Thomas N, Yamamoto M, Hayes JD. The Journal of Biological Chemistry 2006;281:24756–24768. [PubMed: 16790436]
106. Kobayashi AKM, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, Yamamoto M. Molecular Cell Biology 2004;24:7130–7139.
107. Furukawa M, Xiong Y. Molecular and Cellular Biology 2005;25:162–171. [PubMed: 15601839]
108. Dinkova-Kostova AT, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. Proceedings of the National Academy of Sciences U S A 2002;99:11908–11913.
109. Sumazaki R, Nakamura N, Shoda J, Kurosawa T, Tohma M. The Lancet 1997;349:329.
110. Kimura A, Kondo K-H, Okuda KI, Higashi S, Suzuki M, Kurosawa T, Tohma M, Inoue T, Nishiyori A, Yoshino M, Kato K, Setoguchi T. European Journal of Pediatrics 1998;157:386–390. [PubMed: 9625335]
111. Gonzales E, Crestel D, Buassan C, Dabadie A, Gerhardt M-F, Jacquemin E. Journal of Hepatology 2004;40:715–719. [PubMed: 15030993]
112. Lemonde HA, Custring EJ, Bouquet J, Duran M, Overmars H, Scrambler PJ, Clayton PT. Gut 2003;52:1494–1499. [PubMed: 12970144]
113. Matsuura K, Deayashiki Y, Sato K, Ishida N, Miwa G, Hara A. The Biochemical Journal 1997;323:61–64. [PubMed: 9173902]
114. Kume T, Iwasa H, Shirasahi H, Yokoi T, Nagashima K, Otsuka M, Terada T, Takagi T, Hara A, Kamataki T. Pharmacogenetics 1999;9:763–771. [PubMed: 10634139]

Fig 1.
AKR Isoforms and the Metabolism of Lipid Aldehydes

Fig 2.
AKR1C3 as Prostaglandin F Synthase

Fig 3.
AKR1B10 as a Retinal Reductase

Fig 4.
Role of Human AKRs in PAH-Activation

Fig 5.
Role of Human AKRs in NNK Metabolism

Fig 6.
Role of Human AKRs in Aflatoxin Metabolism

Fig 7.

TESS transcription factor consensus sequence search through the promoter regions of human AKR genes. Promoters (-9000 to -1.0 kb; ATG +1) of the human genes indicated were searched for matches to the following consensus sequences: AP-1 (blue; TGACTCA); ARE (red; (G) TGA(G/C)NNNGC); XRE (green; TNGCGTG); Steroid Response Element -1 (0.5 site; yellow GGTACA); ERE (gray; GGTgANNNTGACC, cGTCANNNTGACC, GGTcANNNTGACC) and ORE (black; consensus *TGGAAAATAT*, *TGGAAAATTT*, *TGGAAAATCA* (consensus sequences with a change of only one nucleotide at positions in italics are noted on the figure)). ARE = antioxidant response element, XRE = xenobiotic response element, ERE = estrogen response element, and ORE = osmotic response element.

Table 1**Human AKR members identified by HUGO**

Gene	Protein	Chromosomal Localization	Putative natural substrates and physiological role
<i>AKR1A1</i>	Aldehyde reductase L-hexonate dehydrogenase	1p33-p32	Reduction of aldehyde to alcohol (e.g. glyceraldehyde to glycerol)
<i>AKR1B1</i>	Aldose reductase	7q35	Reduction of aldehyde to alcohol (e.g. glucose to sorbitol in the polyol pathway)
<i>AKR1B10</i>	(Small intestine) Aldose reductase	7q33	Reduction of aldehyde to alcohol (e.g. retinal to retinol)
<i>AKR1C1</i>	20 α -HSD, DD1	10p15-10p14	Reduction of 20-ketosteroid to 20 α -hydroxysteroid (e.g. elimination of progesterone)
<i>AKR1C2</i>	Type 3 3 α -HSD, DD2, Bile acid binding protein	10p15-10p14	Reduction of 3-ketosteroid to 3 α -hydroxysteroid (e.g. elimination of 5 α -dihydrotestosterone)
<i>AKR1C3</i>	Type 2 3 α -HSD, Type 5 17 β -HSD, Prostaglandin F synthase, DDx	10p15-10p14	Reduction of 17-ketosteroid to 17 β -hydroxysteroid (e.g. formation of testosterone and 17 β -estradiol) Prostaglandin F synthesis (e.g. reduction of PGH ₂ to PGF _{2α})
<i>AKR1C4</i>	Type 1 3 α -HSD, DD4, Chlordecone reductase	10p15-10p14	Reduction of 3-ketosteroid to 3 α -hydroxysteroid, hepatic clearance of steroids, bile acid synthesis
<i>AKR1D1</i>	5 β -reductase	7q32-q33	Reduction of Δ^4 -3-ketosteroids to 5 β -dihydrosteroids, bile acid synthesis
<i>AKR6A3</i>	Potassium voltage gated channel, β -subunit-1	3q26.1	-
<i>AKR6A5</i>	Potassium voltage gated channel, β -subunit-1	1p36.3	-
<i>AKR6A9</i>	Potassium voltage gated channel, β -subunit-1	17p13.1	-
<i>AKR7A2</i>	Aflatoxin aldehyde reductase	1p35.1-p36.23	Reduction of aldehyde to alcohol (e.g. succinic semialdehyde)
<i>AKR7A3</i>	Aflatoxin aldehyde reductase	1p35.1-p36.23	Reduction of aldehyde to alcohol

Table 2

Regulation of Nuclear Receptors (NR) by Human AKRs

Aldo-Keto Reductase	Reduction Reaction	Nuclear Receptor
Steroid Receptors		
AKR1C1	Progesterone → 20 α -OH-Progesterone	PR [*]
AKR1C1	5 α -DHT → 3 β -androstanediol	ER β
AKR1C2	5 α -DHT → 3 α -androstanediol	AR [*]
AKR1C3	Adione → Testosterone	AR
AKR1C3	Estrone → 17 β -Estradiol	ER α
AKR1C3	Progesterone → 20 α -OH-Progesterone	PR
AKR1C3	DOC → 20 α -DOC	MR [*]
Orphan Receptors		
AKR1A1	BP-7,8-diol → BP-7,8-dione	AhR [*]
AKR1C1-4	BP-7,8-diol → BP-7,8-dione	AhR [*]
AKR1B10	Retinal → Retinol	RXR, RAR
AKR1C3	PGD ₂ → 9 α ,11 β -PGF ₂	PPAR γ
AKR1D1	Progesterone → 5 β -pregnane-3,20-dione	hPXR, hCAR

* Positive in trans-activation assays