Aldo-keto reductases (Akrs) are a conserved group of NADPH-dependent oxido-reductase enzymes. This study provides a comprehensive examination of the tissue distribution of the 16 substrate-metabolizing Akrs in mice, their expression during development, and whether they are altered by chemicals that activate distinct transcriptional factor pathways. Akrlc6, 1c14, 1c20, and 1c22 are primarily present in liver; Akr1a4, 1c18, 1c21, and 7a5 in kidney; Akrd1 in liver and kidney; Akrb7 in small intestine; Akrb3 and Akrc1 in brain; Akrb8 in testes; Akrc14 in ovaries; and Akrc12, 1c13, and 1c19 are expressed in numerous tissues. Liver expression of Akrd1 and Akrc1 is lowest during prenatal and postnatal development. However, by 20 days of age, liver Akrd1 increases 120-fold, and Akrc1 mRNAs increase as much as 5-fold (Akrc19) to 1000-fold (Akrlc6). Treatment of mice with chemical activators of transcription factors constitutive androgen receptor (CAR), pregnane X receptor (PXR), and the nuclear factor-erythroid-2 (Nrf2) transcription factor alters liver mRNAs of Akrs. Specifically, CAR activation by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) increases mRNAs of Akrlb7, Akrlc6, Akrc19, and Akrd1, whereas PXR activation by 5-pregnenolone-16α-carbonitride (PCN) increases the mRNA of Akrlb7 and suppresses mRNAs of Akrc13 and Akrc20. The Nrf2 activator 2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide (CDDO-Im) induces mRNAs of Akrlc6 and Akrc19. Moreover, Nrf2-null and Nrf2 overexpressing mice demonstrate that this induction is Nrf2-dependent.

Tissue Distribution, Ontogeny, and Chemical Induction of Aldo-Keto Reductases in Mice

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ABSTRACT

Aldo-keto reductases (Akrs) are a conserved group of NADPH-dependent oxido-reductase enzymes with (α/β)3 barrel structural similarities (Jez et al., 1997). Most Akrs are monomeric 34–37 kDa proteins; however, multimeric proteins are found in Akr2, Akr6, and Akr7 family members. Akrs have been identified in multiple species, including eubacteria, fungi, plants, and vertebrates. Akrs metabolize a wide variety of substrates, such as steroid hormones, carbohydrates, and xenobiotics (Seery et al., 1998). The substrate specificity is dependent on three flexible loops (A, B, and C) (Hoog et al., 1994).

Three Akr families have been identified. Mammalian Akrs (Table 1) are found in the Akr1, Akr6, and Akr7 families (Jin and Penning, 2007). The Akr6 family members are involved in inactivation of voltage-gated potassium channels (Xie et al., 2011). The Akr7 family members reduce a metabolite of aflatoxin (B1-dihydrodiol), a toxin produced by the fungus Aspergillus flavus, preventing the aflatoxin metabolite from forming damaging protein-adducts (Guengerich and Johnson, 1999).

Akr1 is the largest family of Akrs. The Akr1c, 1d, and 1e families are essential in the metabolism of hormones, including androgens, estrogens, and progesterone, as well as prostaglandins (Seery et al., 1998; Penning et al., 2000). Akrla4 is involved in the reduction of d-glyceraldehyde to glycerol. The Akrlb family of enzymes (Akrlb3, 1b7, and 1b8) is responsible for the reduction of toxic aldehydes generated during lipoperoxidation and steroidogenesis (Spite et al., 2007). In addition to detoxification activity, Akr1b3 and Akr1b7 also have prostaglandin F2α synthase activity (Kabututu et al., 2009).

The Akrlc subfamily has been viewed as a drug target because many Akrlc enzymes selectively metabolize steroid hormones as well as xenobiotics. In mice, nine members of the Akrlc family have been identified: Akrlc6, Akrlc12, Akrlc13, Akrlc14, Akrlc18, Akrlc19, Akrlc20, Akrlc21 and Akrlc22 (Dayashiki et al., 1995; Du et al., 2000; Matsumoto et al., 2006). Akrlc6 is required for the formation of 7 androstenedione (Rheault et al., 1999). Akrlc12 and Akrlc13 oxidize alicyclic alcohols, aliphatic alcohols, 3α-hydroxysteroids, 17β-hydroxysteroids, and 20α-hydroxysteroids (Endo et al., 2006, 2007). Akrlc18 inactivates progestosterone by reducing it to its inactive metabolite, 20α-hydroxyprogesterone (Mao et al., 1997). Akrlc20 and Akrlc21 metabolize multiple steroid hormones (Matsumoto et al., 2006; Dhagat et al., 2008).

Akrs are important for the regulation of many endogenous pathways. Akr1c19 reduces isatin in the gastrointestinal tract. Isatin is a pharmaco- logically active molecule produced by intestinal bacteria (Ishikura et al., 2005). Akr1e1 reduces anhydro-D-fructose to anhydro-D-glucitol (Ishikura et al., 2006; Dhagat et al., 2008).

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AFFILIATIONS: AFB1, aflatoxin B1; AG, 1,5-anhydro-d-glucitol; AhR, aryl hydrocarbon receptor; Akr, aldo-keto reductase; CAR, constitutive androstane receptor; CDDO-Im, 2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide; HKO, Keap1 hepatocyte knockout; HSD, hydroxysteroid dehydrogenase; KD, Keap1-knockdown; Nrf2, nuclear factor-erythroid-2; PCN, 5-pregnenolone-16α-carbonitride; PCR, polymerase chain reaction; PPARα, peroxisome proliferator-activated receptor-α; PXR, pregnane X receptor; RPL13A, ribosomal protein L13; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene.
7,8-dihydroxybenzopyrene. After several auto-oxidation steps, a 7,8-diol to form a ketol. Tautomerization of this product leads to Human Akrs 1B1 and 1B10 catalyze the oxidation of benzopyrene-carbons are byproducts of fossil fuel combustion and tobacco smoke. Polycyclic aromatic hydrocarbons (Penning et al., 1996). Polycyclic aromatic hydrocarbons (Penning et al., 1996; Mindnich et al., 2011).

Prevents AFB1-dialdehyde from forming lysine adducts (Ellis et al., 1996, 1999). AFB1-dialdehyde is cytotoxic because its ability to form protein adducts. Akr7a (aflatoxin aldehyde reductase) reduces AFB1-dialdehyde to form an alcohol, and this reduction prevents AFB1-dialdehyde from forming lysine adducts (Ellis et al., 1993).

In general, little is known about the regulation of Akrs. Therefore, in the present study, the tissue distribution of mouse Akrs was determined in adult male mice. In addition, an ontogeny study was performed to determine the pattern of Akr expression during liver and kidney development. Lastly, we sought to determine whether chemicals known to induce drug-metabolizing enzymes and transporters will also alter the mRNA expression of various Akr isoforms. The inducers include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which activates the aryl hydrocarbon receptor (AhR); 1,4-bis[2-(3,5-dichloropyridyloxy)} benzene (TCPOBOP), which activates the constitutive androstane receptor (CAR); 5-pregnenolone-16α-carbonitrile (PCN), which activates the pregnane X receptor (PXR); clofibrate, which activates the peroxisome proliferator-activated receptor (PPARα); and 2-cyano-3,12 dioxyolea-1,9 dien-28-imidazolide (CDDO-Im), which activates nuclear factor-erythroid-2 (Nrf2). Finally, those Akrs genes with expression altered by the Nrf2 agonist CDDO-Im, were further examined in genetically altered mice that have either hepatic knockout or overexpression of Nrf2.

<table>
<thead>
<tr>
<th>Akr</th>
<th>Activity</th>
<th>Substrates</th>
<th>Products</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akrl4</td>
<td>Aldehyde reductase</td>
<td>β-Glucuronic acid</td>
<td>γ-Glucurono-β-lactone</td>
<td>(Takahashi et al., 2012)</td>
</tr>
<tr>
<td>Akrlb3</td>
<td>Aldose reductase</td>
<td>Prostaglandin H2</td>
<td>Prostaglandin F2α, F2β</td>
<td>(Hyndman et al., 2003)</td>
</tr>
<tr>
<td>Akrlb7</td>
<td>Aldose reductase</td>
<td>Prostaglandin H2, 3-keto bile acids</td>
<td>Prostaglandin F2α, 3β-hydroxy bile acids</td>
<td>(Hyndman et al., 2003; Schmidt et al., 2011)</td>
</tr>
<tr>
<td>Akrlb8</td>
<td>Phospholipid reductase</td>
<td>PO4/PC</td>
<td>PO4/PC</td>
<td>(Spite et al., 2007)</td>
</tr>
<tr>
<td>Akrlc6</td>
<td>HSD Testosterone synthesis</td>
<td>4-Androstenedione</td>
<td>5-Androstene-3β,17α-diol</td>
<td>(Bellemare et al., 2005)</td>
</tr>
<tr>
<td>Akrlc12</td>
<td>HSD</td>
<td>Alicyclic alcohols</td>
<td>5β-Androstane-3α,17β-diol, 4-Pregnen-17α-20α-diol-3-one, Other products</td>
<td>(Endo et al., 2006)</td>
</tr>
<tr>
<td>Akrlc13</td>
<td>Oxidation of nonsteroidal alcohols, low HSD activity</td>
<td>Alicyclic alcohols Aliphatic alcohols 3β-Hydroxysteroids 17β-Hydroxysteroids 20α-Hydroxysteroids</td>
<td>Not identified</td>
<td>(Endo et al., 2007)</td>
</tr>
<tr>
<td>Akrlc14</td>
<td>Predicted HSD</td>
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<td>Unknown</td>
<td>(Ishikura et al., 2004)</td>
</tr>
<tr>
<td>Akrlc18</td>
<td>HSD</td>
<td>Progesterone</td>
<td>20α-Hydroxyprogesterone</td>
<td>(Mao et al., 1997)</td>
</tr>
<tr>
<td>Akrlc19</td>
<td>HSD</td>
<td>Isatin</td>
<td>3-Hydroxy-5-oxoandrosterone</td>
<td>(Usami et al., 2001; Ishikura et al., 2005)</td>
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<tr>
<td>Akrlc20</td>
<td>HSD</td>
<td>α-Dicarbonyl compounds</td>
<td>Not identified</td>
<td>(Matsumoto et al., 2006)</td>
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<tr>
<td>Akrlc21</td>
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<td>5β-Androstan-17β-ol-3-one</td>
<td>Not identified</td>
<td>(Dhagat et al., 2008)</td>
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<td>Akrlc22</td>
<td>Dihydrodiol dehydrogenase</td>
<td>Not identified</td>
<td>Not identified</td>
<td>NCBI Accession# BAD02825</td>
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<td>Akrlc1</td>
<td>Anhydro-α-fructose reductase</td>
<td>Anhydro-α-fructose</td>
<td>Anhydro-α-glucitol</td>
<td>(Sakuma and Kubota, 2008)</td>
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<tr>
<td>Akrlc11</td>
<td>HSD</td>
<td>4-Cholesten-7α,ol-3-one</td>
<td>Not identified</td>
<td>(Kondo et al., 1994)</td>
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<tr>
<td>Akrlc6a</td>
<td>Shaker channel β-subunit (Kvb2)</td>
<td>1-Palmitoyl-2- arachidonoyl-3-phosphotidyl choline</td>
<td>Not identified</td>
<td>(Xie et al., 2011)</td>
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<tr>
<td>Akrlc8a</td>
<td>Shaker channel β-subunit (Kvb1)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>(Pan et al., 2008)</td>
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<tr>
<td>Akrlc14a</td>
<td>Shaker channel β-subunit (Kvb3)</td>
<td>Not identified</td>
<td>Not identified</td>
<td>NCBI Accession# NP_034729</td>
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<tr>
<td>Akrlc7a</td>
<td>Aflatoxin aldehyde reductase</td>
<td>Aflatoxin</td>
<td>Aflatoxin B1-dialdehyde</td>
<td>(Hinselwood et al., 2002)</td>
</tr>
</tbody>
</table>

HSD, hydroxysteroid dehydrogenase; NCBI, National Center for Biotechnology Information.

In general, little is known about the regulation of Akrs. Therefore, in the present study, the tissue distribution of mouse Akrs was determined in adult male mice. In addition, an ontogeny study was performed to determine the pattern of Akr expression during liver and kidney development. Lastly, we sought to determine whether chemicals known to induce drug-metabolizing enzymes and transporters will also alter the mRNA expression of various Akr isoforms. The inducers include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which activates the aryl hydrocarbon receptor (AhR); 1,4-bis[2-(3,5-dichloropyridyloxy)} benzene (TCPOBOP), which activates the constitutive androstane receptor (CAR); 5-pregnenolone-16α-carbonitrile (PCN), which activates the pregnane X receptor (PXR); clofibrate, which activates the peroxisome proliferator-activated receptor (PPARα); and 2-cyano-3,12 dioxyolea-1,9 dien-28-imidazolide (CDDO-Im), which activates nuclear factor-erythroid-2 (Nrf2). Finally, those Akrs genes with expression altered by the Nrf2 agonist CDDO-Im, were further examined in genetically altered mice that have either hepatic knockout or overexpression of Nrf2.

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Materials and Methods

Chemicals. TCPOBOP and 5-pregnenolone-16a-carbonitrile (PCN) were purchased from Sigma-Aldrich (St. Louis, MO). TCDD was a gift from Dr. Karl Rozman (University of Kansas Medical Center, Kansas City, KS). Clofibrate was purchased from Fisher Scientific (Hampton, NH). (CDDO-Im) was a gift from Reata Pharmaceuticals (Irving, TX).

Mice. Eight-week-old C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME) to determine the tissue distribution, ontogeny, and chemical induction of Akrs. Nrf2-null mice (Chan et al., 1996) were obtained from Dr. Jeffrey Chan (University of California, Irvine, CA). Keap1-knockdown (KD) mice (Okada et al., 2008) were supplied by Dr. Masayuki Yamamoto (Tohoku University, Sendai, Japan). Keap1-hepatocyte knockout (HKO) mice were engineered as described previously (Wu et al., 2011). Nrf2-null and Keap1-KD mice were backcrossed into the C57BL/6J background, and 99% congenicity was confirmed by Jackson Laboratory. The mice were housed on corncob bedding according to the Association for Assessment and Accreditation of Laboratory Animal Care guidelines. All animals were given ad libitum access to water and standard rodent chow (Harlan Teklad 8604; Harlan Teklad, Madison, WI).

For tissue distribution studies, 8-week-old male (n = 6) and female (n = 6) mice were used. Liver, kidney, lung, stomach, duodenum, jejunum, ileum, colon, brain, testes, ovaries, and heart were collected from mice. The tissues were snap-frozen in liquid nitrogen. All tissues were stored at -80°C. For the ontogeny study, liver was collected from male mice (n = 5) at postnatal days -2, 0, 1, 3, 5, 10, 20, and 45. Kidney was collected from male mice (n = 4) at postnatal days -2, 0, 10, 20, and 30. Brain was collected from male mice (n = 4) at postnatal days 0, 10, 20, and 30. For chemical induction of Akrs by Cyp inducers, the activators were administered to male mice (n = 5) for 4 days (intraperitoneally) as described previously (Cheng et al., 2005). Tissues were collected 24 hours after the final dose.

RNA Isolation, cDNA Synthesis, and Real-Time Polymerase Chain Reaction. Total RNA was isolated from 50 mg of snap-frozen liver using RNAzol B reagent purchased from Tel-Test Inc. (Friendswood, TX), and quantified using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at an UV absorbance of 260 nm. Two micrograms of total RNA was used for cDNA synthesis performed using a high-capacity cDNA synthesis kit from Applied Biosystems (Foster City, CA). Oligonucleotide primer sequences designed to be specific to 16 Akr mRNAs (detailed in Table 2). Primers were then aligned to the mouse genome to test for specificity. Real-time polymerase chain reaction (PCR) analysis was performed using a StepOnePlus instrument from Applied Biosystems. The mRNA of each gene was adjusted to the mRNA of RLP13. Relative levels of each gene were evaluated using the ΔΔCt method.

Statistics. Statistical differences between multiple groups were analyzed by a one-way analysis of variance followed by Duncan’s post hoc test. Statistical significance was considered at P < 0.05.

Results

Tissue Distribution of Mouse Akrs. The mRNAs of sixteen Akrs were quantified in 12 mouse tissues as shown in Fig. 1. Akr6 members were not quantified because they are only distantly related (15%–30% homology) to other Akrs, and they function as inactivators of voltage-gated potassium channels (McCormack and McCormack, 1994; Barski et al., 2009). Tissues from six male and six female mice were used to determine the expression of these Akr genes in liver, kidney, lung, stomach, duodenum, jejunum, ileum, colon, brain, testes, ovaries, and heart. No gender differences in expression of any Akr mRNA were detected (unpublished data). Akr1a4 mRNA was highest in kidney; however, there was also expression in liver, lungs, stomach, and small intestine. Akr1b3 mRNA was 20 times more prevalent in brain than in other tissues (Fig. 1). Akr1b7 mRNA was highest in small intestine, with similar amounts in duodenum, jejunum, and ileum (Fig. 1). There was also quantifiable Akr1b7 mRNA in kidney; however, it was only 1/20 of that in small intestine. Akr1b8 mRNA was highest in testes and second highest in stomach (Fig. 1). Akr1c6 mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1c12 and Akr1c13 were expressed in liver, stomach, duodenum, jejunum, ileum, and colon (Fig. 1). Akr1c14 was expressed at levels at least 4-fold higher in liver and ovaries than in other tissues (Fig. 1). Akr1c18 was expressed in kidneys at levels 500-fold higher than those in other tissues (Fig. 1). Akr1c19 mRNA was expressed in liver, kidney, stomach, duodenum, jejunum, ileum, and colon (Fig. 1). Akr1c20 was mainly expressed in liver, at quantities 50-fold higher than seen in other tissues (Fig. 1). Akr1c21 was expressed mainly in kidney. Akr1c22 was expressed highest in liver and stomach, with the mRNA about 20-fold higher than in other tissues (Fig. 1). Akr1d1 was expressed highest in liver and kidney, with the mRNA about 66- and 33-fold higher, respectively, than in other tissues (Fig. 1). Akr1e1 mRNA was highest in brain, with a value 100-fold higher than in other tissues (Fig. 1). Akr1f mRNA was only 1/20 of that in other tissues (Fig. 1). Akr1g mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1h mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1i mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1j mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1k mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1l mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1m mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1n mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1o mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1p mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1q mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1r mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1s mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1t mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1u mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1v mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1w mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1x mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1y mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1). Akr1z mRNA was found to be highest in liver, with levels at least 200-fold higher than those quantified in other tissues (Fig. 1).

Ontogeny of Akrs in Mouse Liver, Kidney, and Brain. A developmental study was performed to determine the pattern of Akr expression during liver, kidney, and brain development. First, the Akrs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>Akr1a</td>
<td>ATAGCCCCCTGCGCGTCTCTCTT</td>
<td>GATCTGAGCTGACATGCAAC</td>
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<tr>
<td>Akr1b</td>
<td>GGGACTTCTGCGGCCGCTGACCT</td>
<td>TGGCTGAGGCCCTGCTCTT</td>
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</table>
that were significantly expressed in livers of adult mice were examined, namely Akr1c6, Akr1c14, Akr1c19, Akr1c20, Akr1d1, Akr1e1, and Akr7a5. The mRNAs of these Akrs were quantified at postnatal days 0, 1, 3, 5, 10, 20, and 45.

Akr1c6 mRNA increased between 5 and 10 days of age and reached adult expression by 20 days of age (Fig. 2). Akr1c14 mRNA increased markedly between 10 and 20 days of age (Fig. 2). Akr1c19 and Akr1c20 mRNA also increased between 10 and 20 days of age. Akr1c19 mRNA increased 5-fold, and Akr1c20 increased more than 2-fold during this time interval (Fig. 2). Akr1e1 mRNA also increased between 10 and 20 days of age, which was about a 4-fold increase (Fig. 2). Akr1d1 mRNA was barely detectable before birth, was detectable at day 1 of age, and reached adult levels at 20 days of age (Fig. 2). The mRNA of Akr7a5 did not change markedly in livers from 2 days before birth to 45 days of age.

The Akrs that were significantly expressed in kidney of adult mice were examined to determine how expression changes during development. The mRNAs were quantified at postnatal days 0, 10, 20, and 30. The mRNAs of Akr1a4 and Akr1c19 in kidneys did not change during the first month of life (Fig. 3). Akr1c18 and Akr1c21 mRNAs in kidneys of mice increased 7-fold and 200-fold, respectively, from 2 days before birth to 30 days of age (Fig. 3). Akr1d1 mRNA and Akr7a5 mRNA both increased about 3-fold from 2 days before birth to 30 days of age (Fig. 3). The mRNAs of Akr1a4 and Akr1c19 in kidneys did not change during the first month of life (Fig. 3). Akr1c18 and Akr1c21 mRNAs in kidneys of mice increased 7-fold and 200-fold, respectively, from 2 days before birth to 30 days of age (Fig. 3). Akr1d1 mRNA and Akr7a5 mRNA both increased about 3-fold from 2 days before birth to 30 days of age (Fig. 3).

The Akr that were expressed in brain of adult mice were also examined to determine whether the mRNA of the Akrs change during the first month of life. The mRNAs of Akr1b3, Akr1c18, Akr1e1, and Akr7a5 were quantified from brains of mice at postnatal days 0, 10, 20, and 30. There were no significant age-revalent changes in the mRNAs observed for any of these Akrs (Supplemental Fig. 1).

**Chemical Induction of Mouse Akrs.** Five different chemicals known to activate various transcription factors that induce cytochrome P450 enzymes as well as other drug metabolizing enzymes and transporters were used in this study (Aleksunes and Klaassen, 2012). The inducers used were: TCDD for AhR, TCPOBOP for CAR, PCN for PXR, clofibrate for PPARα, and CDDO-Im for Nrf2. Akr1a4, Akr1b3, Akr1b8, Akr1c21, Akr1e1, and Akr7a5 had low amounts of measurable mRNA in liver before and after inducer treatment (Supplemental Fig. 2). Akr1b7 mRNA was very low in liver of control mice but increased about 300-fold after TCPOBOP and 15-fold after PCN administration (Fig. 4).

Akr1c6 mRNA increased about 50% after TCPOBOP and 100% after CDDO-Im administration (Fig. 4). Akr1c12 mRNA was unresponsive to any of the activators. Akr1c13 mRNA decreased more than 50% after PCN and clofibrate treatments (Fig. 4). Akr1c14 was unresponsive to all five activators (Fig. 4). Akr1c18 mRNA increased over 400% after clofibrate administration. Akr1c19 mRNA increased more than 3-fold after TCPOBOP and more than 5-fold after CDDO-Im administration. Akr1c20 mRNA decreased 80% after TCPOBOP and 75% after treatment with PCN (Fig. 4). Akr1c22 mRNA decreased 80% after TCPOBOP and TCDD administration, and was undetectable after PCN administration. Akr1d1 mRNA more than doubled after TCPOBOP administration (Fig. 4).
Gene Dose-Response of Nrf2 on Akr1c6 and Akr1c14. Because CDDO-Im is a Nrf2 activator and increases the expression of Akr1c6 and Akr1c14, a gene dose–response model was employed to further analyze the role of Nrf2 in the regulation of these genes. Nrf2 is a short-lived protein that is readily ubiquitylated by CUL3-RBX1 and degraded by the 26S proteasome. The ubiquitylation of Nrf2 depends on Kelch-like ECH-associated protein 1 (Keap1), which functions as a substrate adaptor for CUL3-RBX1 (Kobayashi et al., 2004). If Keap1 is not present or inactivated, Nrf2 translocates into the nucleus. The four types of mice used to make the Nrf2 gene dose response were Nrf2-null, wild-type, Keap1-KD, and Keap1-HKO. In this model, Nrf2-null mice possess no Nrf2 activity, and the Keap1-HKO mice possess the highest level of Nrf2, as characterized previously (Wu et al., 2011). Mice that lack Nrf2 had a 50% decrease in Akr1c6 mRNA (Fig. 5). The Keap1-HKO mice had a 5-fold increase in Akr1c19 (Fig. 5). The transcription of a gene whose transcription was not affected by CDDO-Im treatment was also examined (not shown). The elimination of Nrf2 or Keap1 had no effect on the transcription of Akr1c14, which mirrored the results from the chemical treatment study. These results further indicate that Nrf2 is important for the regulation of Akr1c6 and Akr1c19.

Discussion

Akrs are important enzymes for the synthesis of endogenous compounds as well as for the detoxification of xenobiotics. To better understand the role of Nrf2 in the regulation of these genes, a dose–response model was employed. Mice with different levels of Nrf2 activity were used to investigate the expression of Akr1c6 and Akr1c14. Mice lacking Nrf2 had a 50% decrease in Akr1c6 mRNA, while Keap1-HKO mice had a 5-fold increase in Akr1c19. These results suggest that Nrf2 plays a crucial role in the regulation of these genes.
understand the expression of these genes, this study first quantitatively
determined the expression of the Akrs in various tissues, although
other studies have performed limited tissue distribution studies of
a few Akrs. The present study examined more Akrs, more mice, and
contained mice of both.

The Akr1b family (Akr1b3, 1b7, and 1b8) of enzymes is re-
sponsible for the reduction of toxic aldehydes generated during lipid
peroxidation and steroidogenesis (Spite et al., 2007). Akr1b family
members (Akr1b3, Akr1b7, and Akr1b8) also possess prostaglandin
F2α synthase activity (Kabututu et al., 2009). In addition, Akr1b7 is
important for metabolizing 3-keto bile acids to 3β-hydroxy bile acids.
Toxic bile acids, such as desoxycorticosterone acetate, are converted
to less toxic 3β bile acids, such as 3βDCA, by Akr1b7 (Schmidt et al.,
2011). The present data indicates for the first time that Akr1b3 is
expressed mainly in brain. Akr1b7 was expressed mainly in intestine,
which confirms an earlier published result that was obtained using the
RNase protection assay (Lau et al., 1995). In the present study,
Akr1b8 was located mainly in testes and stomach. This finding
somewhat contradicts a previous report using qualitative reverse
transcriptase PCR of Akr1b8 in which expression was observed in
equal amounts in most tissues except brain and kidney, which
exhibited lower expression (Salabei et al., 2011). The results of the
present study may be different because the previous study did not
include stomach and was only determined after 28 cycles of PCR.

The Akr1c family members (Akr1c6, 1c12, 1c13, 1c14, 1c18, 1c19,
1c20) are viewed as drug targets because of their roles in steroid
biogenesis and xenobiotic metabolism. Previous reports have demonstrated that many of these Akrlc family members are expressed in livers of mice (Vergnes et al., 2003; Velica et al., 2009). Akrlc6 is almost exclusively expressed in liver (Fig. 1), which corresponds with previously published data (Vergnes et al., 2003; Velica et al., 2009). Akrlc12 and Akrlc13 mRNA are ubiquitously expressed. Akrlc14 expression is highest in liver and in ovaries. This finding was different from a previous report that found the highest expression of Akrlc14 in kidney, and similar expression in liver, kidney, stomach, intestine, colon, lung, and ovary (Velica et al., 2009). Akrlc18 mRNA was found mainly in the kidney and at a lower concentration in brain. This result also differed from the publication of Velica et al., which reported expression only in ovary. These differences could be the result of differences in mouse strain because Velica et al. used CD1 mice, whereas this study used C57BL6 mice. Another possible cause of the variance is that Velica et al. used pregnant and nonpregnant female mice. Pregnancy might have increased the expression of Akrlc18 mRNA.

The expression of Akr genes was examined at different stages of liver, kidney, and brain development. The liver undergoes marked developmental changes after birth. Liver changes from a hematopoietic organ before birth to an organ necessary for xenobiotic metabolism after birth. The expression of the P450 enzymes and transporters in liver changes over the course of development (Hart et al., 2009; Cui et al., 2012a,b; Lu et al., 2012). An increase in the mRNA of several Akrl genes in liver was observed after birth (Fig. 2). Akrl1 mRNA increased over 100-fold between birth and day 45. Akrl20 mRNA increased over 50-fold, Akrl6 increased over 10-fold, and Akrl4 and Akrl19 increased in liver to a lesser extent between birth and day 45 (Fig. 2).

The expression of several Akr genes in kidney was also quantified in kidney at multiple ages. Similar to some of the Akr genes described previously in liver, two Akr mRNAs increase during development in kidney. Akrlc18 mRNA increased over 7-fold, and Akrlc21 mRNA increased in kidney about 200-fold from birth to 30 days of age (Fig. 3).

Various transcription pathways are known to increase various drug-metabolizing enzymes in liver, therefore we sought to determine whether these pathways also alter the expression of Akrs. AhR activation led to the increase of mRNA of Akr1b7. CAR activation led to increased mRNA of Akr1b7, which had been described previously (Liu et al., 2009), and Akrlc6. PXR activation caused an increase of Akrlb7 and a decrease in the mRNAs of Akrlc13, Akrlc20, and Akrlc22 (Fig. 4). PXR activation has been reported previously to cause a repression of Sult1e1 by decreasing the HNF4α interaction with the Sult1e1 promoter (Kodama et al., 2011). This might also be the mechanism by which PXR causes a decrease in Akrlc18, 1c20, and 1c22. Activation of PPARα led to an increase of the mRNAs of Akrlc17 and Akrlc18. PPARα activation also led to a decrease in mRNA of Akrlc13. Activation of Nrf2 resulted in the upregulation of Akrlc6 and Akrlc19 (Fig. 4). These results were further confirmed by knockdown of the Nrf2 and Keap1 genes (Fig. 5).

Akrl1d is expressed mainly in liver and kidney (Fig. 1). This discovery that Akrl1d is found in liver is not surprising because Akrl1d was previously described as serving a role in bile acid biogenesis (Gonzales et al., 2004). Akrl1d mRNA, similar to many drug-metabolizing genes, increases in liver from birth to adulthood (Fig. 2). Akrl1d mRNA was increased 3-fold by CAR activation (Fig. 4). The change in expression of Akrl1d may contribute to changes in bile acid profile in the developing organism.

Akrl1e is responsible for the reduction of 1,5-anhydro-β-D-fructose (AF), which is produced from glucose, to 1,5-anhydro-β-D-glucoitol (AG). The physiologic role that AG plays in glucose metabolism is not well understood, but patients with diabetes mellitus have elevated levels of AG in blood and urine (Sakuma and Kubota, 2008). Akrl1e was found mainly in brain (3-fold higher than other tissues) in the present study (Fig. 1), which is different from Vergnes et al. who reported expression in every tissue. They performed reverse transcriptase PCR, using between 28 and 35 cycles and only presenting the gel. The signal from some of these tissues might be out of the logarithmic range. The present study used a fluorescent system and quantified the signals at 21 cycles, which was within the logarithmic range for all of the tissues. Akrl1e mRNA increased in liver from birth to adulthood (Fig. 2).

In conclusion, the present study examined the regulation of the mRNAs of Akrs in tissues, the ontology of Akr mRNAs in mouse liver, and whether activators alter their expression. This study demonstrated that mouse Akrs mRNAs have developmental patterns similar to those of multiple drug-metabolizing enzymes; that is, the mRNAs of Akrs 1c6, 1c14, 1c19, 1c20, 1d1, and 1e1 increased, starting at birth and continuing to increase until adulthood. This study also demonstrates that AhR, CAR, PXR, PPARα, and Nrf2 transcriptional pathways influence the transcription of some Akr genes.

**Authorship Contributions**

**Participated in research design:** Pratt-Hyatt and Klaassen

**Conducted experiments:** Pratt-Hyatt and Lickteig

**Contributed new reagents:** Lickteig

**Performed data analysis:** Pratt-Hyatt and Klaassen

**Wrote or contributed to the writing of the manuscript:** Pratt-Hyatt and Klaassen

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