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A cluster of eight hydroxysteroid dehydrogenase genes belonging to the aldo-keto reductase supergene family on mouse chromosome 13

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Abstract A subclass of hydroxysteroid dehydrogenases (HSD) are NADP(H)-dependent oxidoreductases that belong to the aldo-keto reductase (AKR) superfamily. They are involved in prereceptor or intracrine steroid modulation, and also act as bile acid-binding proteins. The HSD family members characterized thus far in human and rat have a high degree of protein sequence similarity but exhibit distinct substrate specificity. Here we report the identification of nine murine AKR genes in a cluster on chromosome 13 by a combination of molecular cloning and in silico analysis of this region. These include four previously isolated mouse HSD genes (Akr1c18, Akr1c6, Akr1c12, Akr1c13), the more distantly related Akr1e1, and four novel HSD genes. These genes exhibit highly conserved exon/intron organization and protein sequence predictions indicate 75% amino acid similarity. The previously identified AKR protein active site residues are invariant among all nine proteins, but differences are observed in regions that have been implicated in determining substrate specificity. Differences also occur in tissue expression patterns, with expression of some genes restricted to specific tissues and others expressed at high levels in multiple tissues. Our findings dramatically expand the repertoire of AKR genes and identify unrecognized family members with potential roles in the regulation of steroid metabolism.—Vergnes, L., J. Phan, A. Stolz, and K. Reue. **A cluster of eight hydroxysteroid dehydrogenase genes belonging to the aldo-keto reductase supergene family on mouse chromosome 13. *J. Lipid Res.* 2003.** 44: **503–511.**

Supplementary key words gene identification • gene expression • alternative splicing

Aldo-keto reductases (AKRs) represent a superfamily of monomeric oxidoreductases that catalyze the NADP(H)-dependent reduction of a wide variety of substrates (1, 2). These include simple carbohydrates, steroid hormones, endogenous prostaglandins, and other aliphatic aldehydes and ketones, as well as many xenobiotic compounds. The identification of AKRs in vertebrates, plants, protozoa, fungi, eubacteria, and archaebacteria suggests that this is an ancient superfamily of enzymes (3). Currently there are more than a hundred known AKR proteins classified into 12 families (4) (www.med.upenn.edu/akr). The largest family, AKR1, can be broadly subdivided into the aldose reductase, aldehyde reductase, and hydroxysteroid dehydrogenase (HSD) subfamilies. The aldehyde reductase and aldose reductase subfamilies have been studied most extensively, with aldose reductase proteins being implicated in ocular lens development (5) and in the neurological complications of diabetes (6). Recent attention, however, has focused on the HSD subfamily members (AKR1C) because of their selective metabolism of essential steroid hormones as well as their implicated role in xenobiotic metabolism (1, 2). Notable examples include the 3α-HSD isoenzymes, 20α-HSD, and 17β-HSD type V. 3α-HSD catalyses the inactivation of androgens by converting 5α-dihydrotestosterone to 3α-androstanediol, where excess 5α-dihydrotestosterone has been implicated in prostate disease (1, 7, 8). 20α-HSD catalyses the reduction of progesterone to its inactive metabolite 20α-hydroxyprogesterone and plays an important role in the termination of pregnancy and initiation of parturition by reduction of progesterone levels in the serum and placenta (1, 9, 10). 17β-HSD type V catalyses the conversion of

Manuscript received 9 October 2002, in revised form 17 October 2002, and in re-revised form 19 November 2002.

Published, JLR Papers in Press, December 1, 2002.
DOI 10.1194/jlr.M200399-JLR200

Abbreviations: AKR, aldo-keto reductase; EST, expressed sequence tag; HSD, hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid-dehydrogenase (E.C. 1.1.1.213); 20α-HSD, 20α-hydroxysteroid dehydrogenase (E.C. 1.1.1.149); 17β-HSD type V, 17β-hydroxysteroid dehydrogenase (E.C. 1.1.1.62); RACE, rapid amplification of cDNA ends; UTR, untranslated region.

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4-androstenedione into testosterone (1, 11). Thus, HSDs may regulate intracellular levels of steroid hormones, and are potential therapeutic targets for modulating the activation of steroid-hormone receptors.

Currently, there are four recognized human HSD proteins and one known rat 3α-HSD, all of which have been classified in the AKR1C family. These five AKR1C members possess 3α-HSD activity but also exhibit distinct specificity toward other substrates (1, 12–14). Human AKR1C1 (20α-HSD) (13), AKR1C2 (3α-HSD type III, also known as human bile acid binding protein) (15, 16), AKR1C3 (17β-HSD type V, also recognized as prostaglandin D2 11-keto-reductase) (17), and AKR1C4 (3α-HSD type I, also referred to as dihydrodiol dehydrogenase 4) share more than 83% DNA sequence homology and are clustered on chromosome 10p15 (18–21). AKR1C4 may be particularly important in steroid catabolism due to its selective expression in the liver and its kinetic features (12, 13), whereas the other members are widely expressed in other tissues. AKR1C2 may have two distinct functions, depending on its site of expression. In the liver, its distinct, high affinity bile acid binding affinity suggests a role in bile acid transport along with steroid metabolism, while in other tissues it may modulate androgen activity. In rat liver, a single rat 3α-HSD (AKR1C9) is expressed, which has a role in synthesis of primary bile acids from cholesterol as well as sequestration of bile acids within the cytosol (22–25). Structural analysis of these mammalian HSDs has revealed highly conserved amino acid sequences and a three-dimensional structure consisting of an 8-chain α/β barrel (26, 27). However, significant amino acid sequence divergence does occur in the carboxyl terminus of the specific HSD proteins, which is responsible, in part, for the observed differences in substrate specificity.

In mouse, four distinct HSDs belonging to the AKR1C protein subfamily have been identified: 20α-HSD (AKR1C18) (28), 17β-HSD type V (AKR1C6) (29), AKRa (AKR1C12), and AKR1C13 (30, 31). An additional member of the AKR superfamily, AKR1E1, has also been previously identified in mouse (32). The genomic clustering of the four human HSDs suggests that these genes arose from ancient duplication events followed by divergence, and predicts that a similar genomic organization may also be present in mouse. Furthermore, the high sequence similarity among known AKR family members raises the possibility that additional, unidentified family members may also exist.

Using a combination of molecular cloning and bioinformatic analysis of available mouse genome sequence, we identified a total of nine mouse AKR gene family members in a cluster on chromosome 13. These include the four previously identified mouse HSD genes described above, four novel HSD genes, and the previously identified murine aldose reductase gene, Akr1e1. Gene structure is highly conserved among family members, and protein sequence predictions indicate ~75% amino acid similarity. Gene specific expression studies in a panel of mouse tissues revealed that some members are highly tissue specific, while others are expressed in a wide range of tissues. These results indicate that the murine HSD family has at least twice as many genes as previously thought, and the observed differences in tissue distribution and protein sequences imply that members have diverged to catalyze discrete biochemical and metabolic functions.

MATERIALS AND METHODS

Chemicals and supplies

All chemicals were of molecular biology grade or higher and were purchased from Sigma (St. Louis, MO) unless otherwise stated. Molecular biology reagents were purchased from Promega (Madison, WI), Roche Molecular Biochemicals (Indianapolis, IN), and Life Technologies (Gaithersburg, MD).

cDNA library screening

Strategene® Mouse liver cDNA ZAP library # 935302 B6CBA (C57BL/6 × CBA) constructed from a 6 to 8 weeks old female liver was screened at reduced stringency with ^{32}P random labeled partial cDNA from either rat 3α-HSD (AKR1C9) or human 20α-HSD (AKR1C1) as described, and individual clones purified to homogeneity by sequential library screening (19, 33). Each clone was sequenced in both orientations and analyzed using MacVector 6.1. For clones lacking the complete coding region, amplification of the missing 5′ region was performed by RACE and multiple clones were analyzed to verify the sequence (34).

Radiation hybrid mapping

The genes later identified as AKR1C13, AKR1C14, and AKR1C19 were mapped in a mouse-hamster radiation hybrid panel (Research Genetics, Huntsville, AL) via PCR using oligonucleotide primers derived from unique sequences within the 3′ portion of the cDNA sequence. Primers were as follows: Akr1c19-f 5′-GAGACCTGTGTCATGACTTCTAC-3′, Akr1c19-r 5′-GACTGGTGGACACAGCTCTGG-3′; Akr1c13-f 5′-TGCTGACCACCCAGAGTATCCA-3′, Akr1c13-r 5′-GTCACATCACCAGCATTATGG-3′; Akr1c14-f 5′-GATGACCATCCCAATCATCCA-3′, Akr1c14-r 5′-GGATGTGTTCAGTCACCAGT-3′. Touchdown temperature cycling was used (35), and products were resolved on 4% Metaphor agarose gels. Data were analyzed using Auto-RHMAPPER, available though the Whitehead Institute/MIT Center for Genome Research (genome.wi.mit.edu/mouse_rh/index.html) (36).

In silico identification of novel AKR genes and analysis of predicted protein sequences

AKR gene sequences were identified by using the NCBI Mouse Genome Assembly (www.ncbi.nlm.nih.gov/genome/guide/mouse) and Celera Discovery System (Celera, Rockville, MD). Specifically, we examined these mouse genome databases in the region of chromosome 13 identified by radiation hybrid mapping as harboring AKR genes. Sequences of genes predicted within a ~1 Mb region were carefully scrutinized by BLAST analysis against the cloned HSD family member sequences, and a total of nine genes with significant similarity to AKR sequences were detected. All HSD gene sequences were analyzed by BLAST against NCBI UniGene and EST databases to confirm that the novel genes correspond to expressed mRNA sequences. The gene sequences were aligned with corresponding cDNA sequences to determine exon/intron organization. Protein sequence predictions and analyses were performed with the LaserGene software suite (DNAStar, Inc., Madison, WI), including CLUSTALW for protein sequence alignments and MEGALIGN for construction of evolutionary dendrograms.

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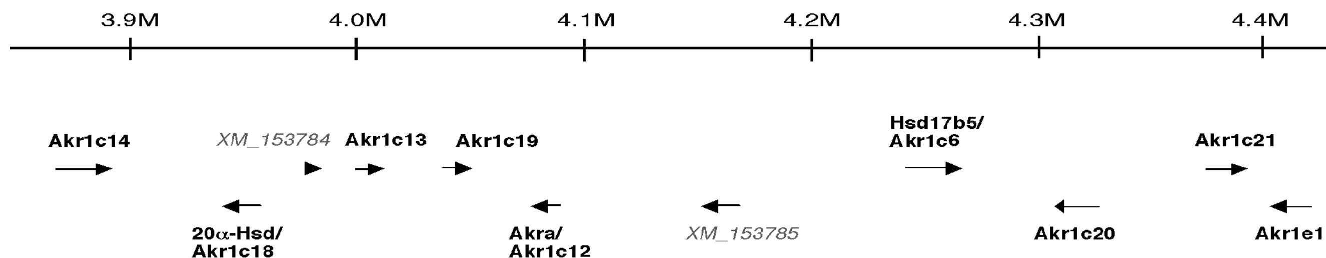

Fig. 1. Murine aldo-keto reductase (AKR) gene family members on chromosome 13. Location and orientation of nine AKR genes is shown. Gene positions are based on coordinates given in the NCBI mouse genome database. Note the presence of two nonAKR family members within this gene cluster, XM_153784 and XM_153785 (indicated by italic labels).

Northern blot and RT-PCR analysis of gene expression and gene regulation

Total RNA was extracted from C57BL/6J mouse tissues with Trizol (Invitrogen). Northern blots were prepared using 5 μg total RNA and probed with fragments of the 3′ UTR from four cloned AKR family members. These sequences had less then 60% sequence homology, allowing for selective hybridization to a single AKR family member. Probes were as follows: Akr1c6, nucleotides 876–1178 of NM_030611, Akr1c13, nucleotides 915–1184 of XM_122492, Akr1c14, nucleotides 896–1919 of XM_122485, and Akr1c19, nucleotides 203–422 of BU707256. RT-PCR analyses were performed with RNA extracted from C57BL/6J mouse tissues, as above, with the addition of mouse eye and prostate RNA purchased from Clontech. cDNA was prepared using 2 μg total RNA from each tissue (cDNA cycle kit, Invitrogen). One-twentieth of the resulting cDNA was used for PCR amplification. PCR was performed using a touchdown protocol (35) with an initial annealing temperature of 63°C falling to 53°C over 20 cycles, followed by annealing at 53°C for an additional 8–15 cycles, depending on primer set. PCR primer sequences were derived from the 3′ UTR of each gene in regions of sequence divergence to ensure amplification of the specific AKR family member. Trial amplifications were run at various cycle numbers, and conditions selected to allow detection during the exponential phase. Primer sets are listed below:

Gene Name	Forward	Reverse
Akr1c14	caatactgcgagttattttg	ggatgtgttcagtcaccagt
Akr1c18	cggtactttcctgctgatatg	agtgattggaggcggtgtgtc
Akr1c13	cagtgatgctggcaatatgacc	ttacatttatttgagatcattaat
Akr1c19	gagacctgtgtcatgacttctac	gactggtggacacagctctgg
Akr1c12	cataatgctggtgatgtgactc	tcctttttcttgaaatcatgaac
Akr1c6	gatacataagtggttctagcttta	atactcttcctatacactcttcca
Akr1c20	gatacataggtagttctatttctg	gtatcctttctatacagtcttccc
Akr1c21	gctacagagaagtggcaagtc	tatgcgatacatacctgctgc
Akr1e1	acggacctgaggctgattgtg	catggcagtagggtaggtagg
Tbp (TATA binding protein)	acccttcaccaatgactcctatg	atgatgactgcagcaaatcgc

An alternatively spliced mRNA for Akr1c12 was detected in liver cDNA prepared from C57BL/6ByJ and CAST/EiJ, and PCR amplified as above. Primers used were 5′-CTCAACAAGCCAGGACTGAAG-3′, and 5′-CATGTCCTCAGGGGACAACTG-3′. PCR products were electrophoresed in 2% metaphor agarose (FMC BioProducts, Rockland, Maine) to resolve alternative splice forms of 352 and 242 bp.

RESULTS

Characterization of nine murine AKR genes on chromosome 13

To identify members of the HSD gene family in mouse, we initially employed a molecular cloning strategy of screening a mouse cDNA library with probes prepared from known AKR genes from rat (AKR1C9, corresponding to rat 3α-HSD) or human (AKR1C1, corresponding to human 20α-HSD). This resulted in the isolation of four different clones (data not shown), two of which corresponded to previously identified mouse genes (Akr1c6 and Akr1c13), and two of which were novel (Akr1c14 and Akr1c19). Akr1c13 and the two novel genes were mapped in a radiation hybrid panel (37) and found to colocalize to the proximal tip of chromosome 13 (data not shown). This region corresponds to human chromosome 10p15, which harbors the four previously identified human HSD genes, indicating that human and mouse genomes carry syntenic clusters of AKR genes.

The radiation hybrid mapping did not allow determination of the order of the mouse AKR genes on chromosome 13, indicating that these genes occur in close proximity. With the subsequent availability of mouse genome sequence, it became possible to examine the physical structure of this region. Using the BLAST tool (38), we searched chromosome 13 sequence from the NCBI mouse genome database against the four mouse HSD gene sequences. We identified genomic sequences corresponding to the four clones we had isolated, situated within 300 kb of one another on proximal chromosome 13. Unexpectedly, within this region we detected five addi-

TABLE 1. Nine mouse AKR genes analyzed in this study

Type	Name	Unigene	Accession No
Akr1c6	17β-HSD type 5	Mm.196666	NM_030611
Akr1c12	AKRa	Mm.89993	XM_127199
Akr1c13		Mm.27447	XM_122492
Akr1c14		Mm.26838	XM_122485
Akr1c18	20α-HSD	Mm.41337	XM_122486
Akr1c19		Mm.22832	XM_138403
Akr1c20		Mm.37605	BC021607
Akr1c21[a]		Mm.27085	NM_029901
Akr1e1		Mm.141365	XM_122491

[a] Provisional assignment.

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tional gene sequences having substantial sequence similarity to these HSD genes (**Fig. 1**). These genes occur in both orientations, with five genes present on the plus strand and four on the minus strand, and include the previously identified Akr1c12, Akr1c18 (20α-HSD), and Akr1e1 genes, as well as two novel HSD-related genes, provisionally named Akr1c20 and Akr1c21 (Fig. 1). Thus, within a 555 kb region, there were a total of 11 genes: nine putative HSD gene family members and two additional genes, which have no similarity to AKR family (XM_153784 and XM_153785). In addition, BLAST analysis revealed another sequence that is highly similar to HSD genes, but which appears to represent a non-expressed gene fragment, as it was not detected in EST databases and was therefore not included in further analyses.

To confirm that the novel HSD family members represent genuine expressed genes, and to determine mRNA transcript sequences for the nine AKR genes, we searched the GenBank mouse EST and Unigene databases. Unigene entries containing multiple ESTs were identified for each of the nine AKR genes, confirming that these are genuine expressed genes (**Table 1**). We determined gene structure of the nine AKR family members by alignment of the full length cDNA sequences with genomic sequences. As shown in **Fig. 2**, the nine genes have highly conserved intron/exon size and organization, although the precise size of some introns is not known due to incomplete genomic se-

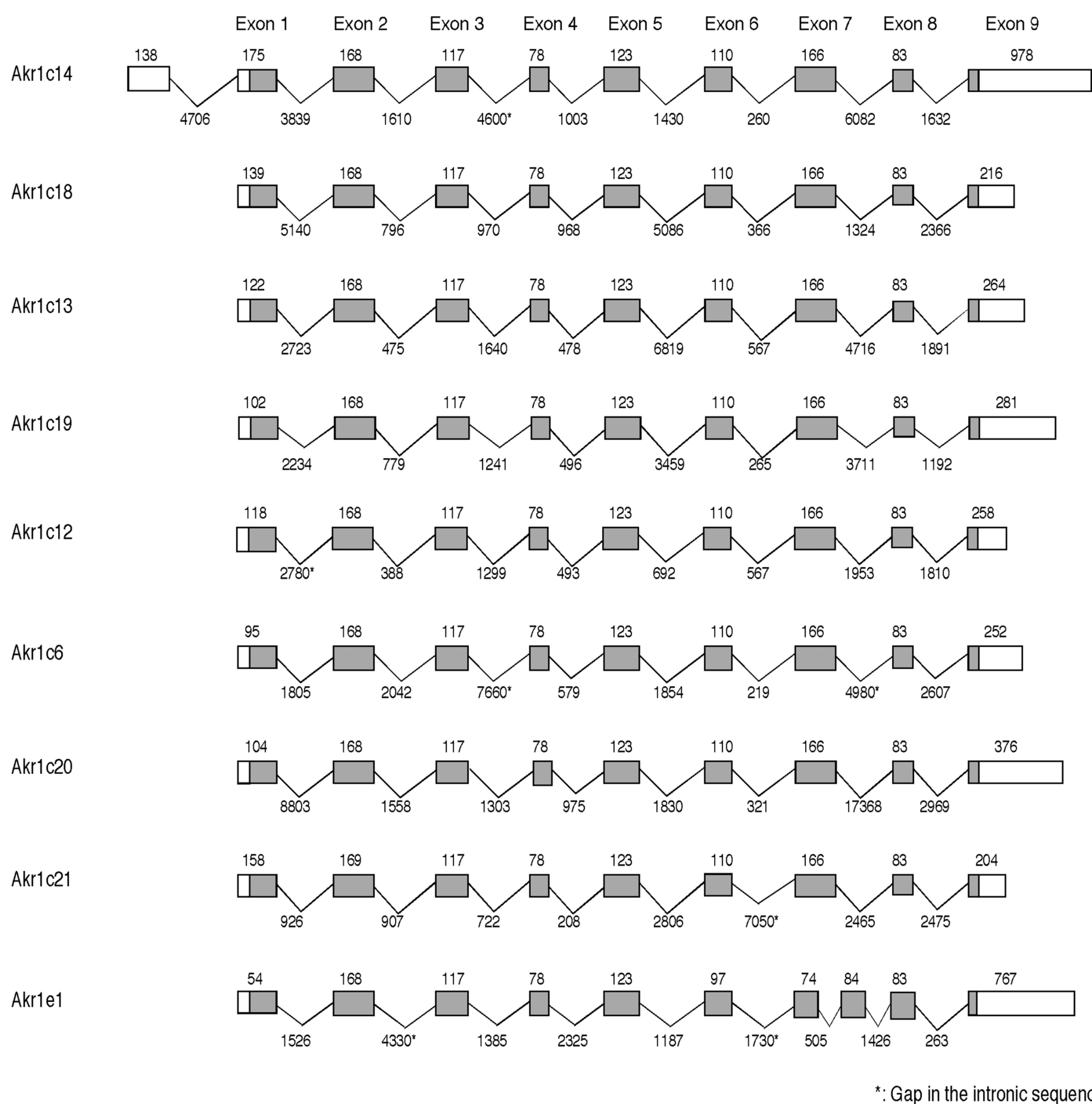

Fig. 2. Gene organization for nine mouse AKR gene family members. Exons are shown as boxes, with shaded areas representing protein coding regions and white areas representing untranslated exonic regions. Intron and exon length are shown. Note for Akr1c14, an additional, proximal exon was identified similar to that found for corresponding rat homolog, AKR1C9. Note for Akr1e1, the relatively reduced size of exon 1 and separation of exon 7 into two smaller exons, which is similar to the genomic organization of aldose reductase (AKR1B) located on murine chromosome 6.

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Fig. 3. Amino acid sequence alignment of murine hydroxysteroid dehydrogenase (HSD) family members. Predicted amino acid sequences for murine AKR family members were aligned using Megalign. Amino acids identical among all the members are highlighted in black.

quence in some regions. Seven of the genes consist of nine exons, as has been reported for previously characterized members of the HSD gene family (18, 21, 39). Two of the genes contain 10 exons; Akr1c14 contains an additional 5′ noncoding exon upstream of the position of exon 1 in the other genes, while Akr1e1 has an additional exon resulting from interruption of the prototypical exon 7 to form two exons. The additional 5′ exon and the large 3′ UTR of Akr1c14 are similar to rat 3α-HSD (AKR1C9) (40). Studies by Usui implicated alternative processing of the 5′ UTR of the gene based on detection of multiple sizes of mRNA in liver as compared with kidney or ovary (40). Lin et al. characterized the genomic organization and proximal promoter of AKR1C9 and identified two distinct initial exons similar to what we observed for the genomic organization of the Akr1c14 (41). The organization of the Akr1e1 gene is similar to the genomic organization of aldose reductase (AKR1B), a subfamily within the AKR family (42, 43).

Protein alignments were produced using the predicted amino acid sequences to identify conserved regions (**Fig. 3**). The predicted protein sequences for the nine full-length AKR genes were highly conserved and contained stretches of identical residues. Except for AKR1E1, all consist of 323 amino acids, with an overall 75% similarity and 45% identity. Based on the 3-dimensional structure of AKR members, catalytic and substrate-binding sites have been proposed (2, 27, 44). The active-site residues (Asp-50, Tyr-55 Lys-84 and His-117) are conserved in all nine murine AKRs. In contrast, residues in the A-loop (residues 120, 128, and 129), B-loop (residue 227), and C-loop (residues 306, 308, and 310), which are predicted to interact with substrates, differ among the nine AKRs suggesting different substrate specificities for these proteins (45).

To assess the relationship of these AKR members with other known family members, a phylogenetic tree was constructed of the nine mouse AKR protein sequences together with known mouse, rat, and human AKR proteins (**Fig. 4**). Aside from AKR1E1, all of the mouse proteins

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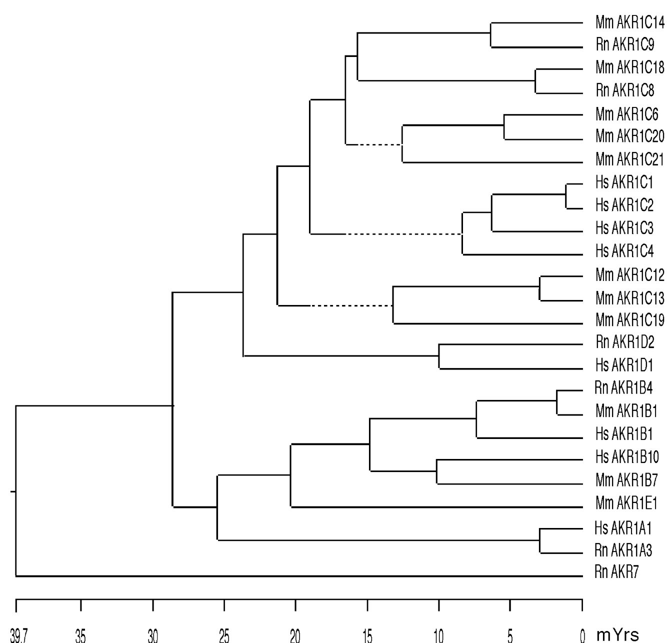

Fig. 4. Phylogenetic relationship among the human, rat and mouse AKR family members. Cluster analysis of human, rat, and mouse AKR protein sequences were performed using Megalign. Eight of the mouse genes cluster within the AKR1C1 subfamily of hydroxysteroid dehydrogenases, whereas AKR1E1 is most similar to the AKR1B1 subfamily.

are clustered with AKR1C subfamily members, indicating their similarity to the HSD subgroup. AKR1C14 and AKR1C18 (20α-HSD) were most similar to rat AKR1C9 (87% similarity) and AKR1C8 (94% similarity), respectively, and may represent the mouse homologs of these genes. The two novel proteins, AKR1C20 and AKR1C21, are closely related and are most similar to AKR1C6 (17β-HSD type V). The previously identified AKR1C12 and AKR1C13 clustered together, and are closely related to another novel protein, AKR1C19. AKR1E1 was clearly the most divergent of the nine mouse proteins, showing closest similarity to members of the AKR1B sub-family, which encodes aldose reductases.

Distinct tissue expression patterns for the nine AKR genes

The mRNA expression levels of the four clones first identified were examined by Northern blot. As illustrated in **Fig. 5A**, each clone had a distinct expression pattern. Akr1c14 mRNA was abundant in multiple tissues, while Akr1c13 and Akr1c19 expression was restricted to liver and gastrointestinal tract. Akr1c6 showed expression predominantly in liver. Due to the high nucleotide sequence similarity among the genes and concerns about possible cross-hybridization on Northern blots, we also examined tissue distribution of gene expression for the nine genes using semi-quantitative RT-PCR. PCR primer sequences were selected by comparing the nine cDNA sequences to identify sequence differences and thus ensure specificity for a single family member. PCR using primers to the ubiquitously expressed TATA-box binding protein (Tbp) was performed as a control. As shown in Fig. 5B, the re-

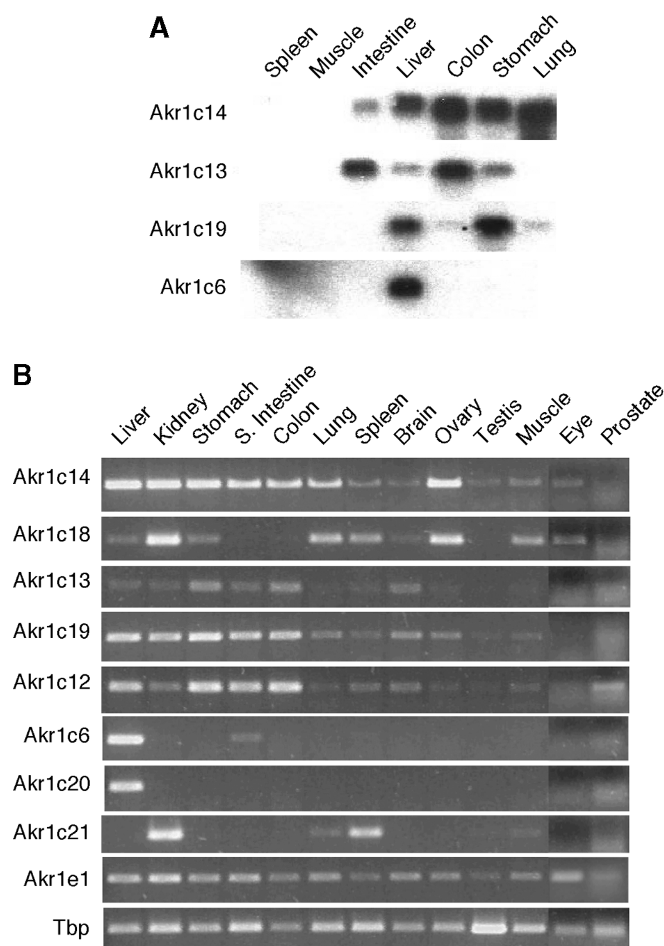

Fig. 5. Tissue expression patterns of AKR genes. RNA was prepared from C57BL/6J mouse tissues, except for eye and prostate, which were purchased from a commercial supplier. A: Northern blot analysis showing expression pattern for four AKR genes. Probes were selected in the 3′UTR region of each gene. B: RT-PCR using gene-specific PCR primers to assess expression levels in a panel of mouse tissues. Tbp (TATA binding protein) primers were used as a control showing expression in all tissues.

sults were in agreement with the northern blot studies. The nine AKR family members exhibited distinct expression patterns. Akr1e1, the only member of the aldose reductase subfamily, was expressed in all 13 tissues examined. Notably, Akr1e1 had the highest expression level in eye of any of the genes, consistent with involvement of aldose reductases in ocular lens development (5). Akr1c12, Akr1c13, and Akr1c14 were similarly expressed in multiple tissues, with highest levels in liver, kidney, and gastrointestinal tract. These results are in agreement with a previous study of Akr1c12 and Akr1c13 expression (31). Akr1c12 was unique in having relatively high levels of expression in prostate. Akr1c14 was robustly expressed in tissues of the gastrointestinal tract, but also showed prominent expression in ovary and lung. Akr1c18 was also prominent in ovary, as observed previously (28), consistent with its role in regulation of progesterone. It was also expressed at high levels in kidney, lung, and spleen. Akr1c6, Akr1c20, and Akr1c21 each exhibited a highly tis-

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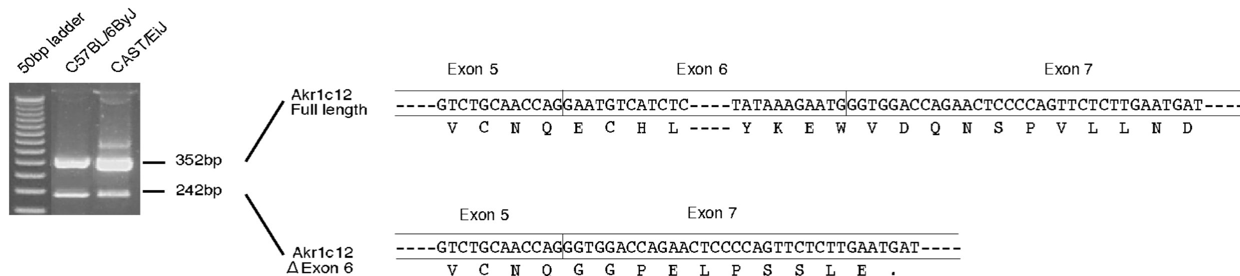

Fig. 6. Identification of an Akr1c12 alternative mRNA splice variant encoding a truncated protein. RT-PCR was performed using primers specific for sequences located in Akr1c12 exon 5 (forward primer) and exon 7 (reverse primer). PCR products of 352 bp and 242 bp were detected in liver cDNA prepared from C57BL/6ByJ and CAST/EiJ mice. Similar results were obtained using C57BL/6J cDNA samples (not shown). Sequencing of multiple PCR products revealed that the shorter form corresponds to a splice variant, which excludes exon 6 and results in a frame-shift and premature termination of protein translation.

sue-restricted expression pattern. Of the tissues analyzed here, Akr1c20 was expressed exclusively in liver, whereas Akr1c6 was expressed in liver as shown previously (11, 29), and to a lesser degree, small intestine. Akr1c21 was unique among the nine genes in showing no detectable expression in liver, but was specifically expressed in kidney and spleen, with lower levels in lung.

During the course of studies to characterize gene expression of HSD family members, we detected a novel splice variant of Akr1c12 mRNA. This is illustrated in **Fig. 6**, which shows that RT-PCR of mouse liver RNA using an internal primer set from the Akr1c12 sequence produces two distinct products. Alignment of the DNA sequences of these PCR fragments with Akr1c12 genomic sequence revealed alternative mRNA splicing, with the smaller PCR product representing a variant in which exon 6 is excluded. This exon skipping leads to the juxtaposition of exons 5 and 7 resulting in a translational frame-shift and the introduction of a premature stop codon within the exon 7 encoded sequence (Fig. 6). The alternatively spliced mRNA is thus predicted to encode a truncated AKR1C12 protein of only 200 amino acids, compared with the 323 amino acid full-length protein. This truncated protein is expected to be nonfunctional, as it lacks a third of the carboxyl terminal end, which is critical for substrate binding and protein stability. This alternatively spliced form was detected in three different mouse strains examined, two inbred *Mus musculus* strains (C57BL/6ByJ, Fig. 6 and C57BL/6J, not shown), and one *Mus castaneus* strain (CAST/EiJ, Fig. 6), indicating that this is not strain specific and occurs in multiple mouse species. The larger mRNA form containing all contiguous exons appeared to be present at higher abundance as judged by semiquantitative RT-PCR and the relative ratio of the two splice variants was not altered by dietary manipulations that included chow versus atherogenic diets, or fasted versus fed mice (data not shown).

DISCUSSION

Our results demonstrate that at least nine members of the AKR gene family reside in a cluster on mouse chromosome 13. These genes share similar exon/intron organization and highly conserved nucleotide sequence, suggesting that they may have arisen though gene duplication events. The predicted protein sequences for the nine AKR family members display a high degree of similarity, including conservation of the known active-site residues required for hydride transfer. However, there are also notable sequence differences among the proteins, including the identity of residues in the A-, B-, and C-loop regions, and the C terminus, all of which may influence substrate interactions. In preliminary studies, we expressed four of the mouse AKR proteins (AKR1C6, AKR1C13, AKR1C14, AKR1C19) in bacteria and examined their activity with several substrates. Initial results indicate substrate specificity differences among the four proteins, consistent with the observed amino acid differences (data not shown).

We also demonstrate that the nine AKR genes exhibit distinct expression patterns across tissues. Particularly striking are the tissue specific expression patterns detected for some genes, such as Akr1c6 and Akr1c20, which are expressed nearly exclusively in liver, and Akr1c21, which is expressed primarily in kidney and spleen. Several of the family members are abundantly expressed in tissues of the gastrointestinal tract, but even among these there are distinct differences. For example, Akr1c14 and Akr1c18 exhibit high expression levels in ovary not seen for other members, and Akr1c18 also lacks expression in small intestine and colon. The distinct tissue localization of these murine family members as compared with their presumed orthologs in other species suggest species specific function for these highly related yet distinctive genes. For example, Akr1c6 is considered to be the homolog of human AKR1C3 (17β-HSD type V), yet its lack of expression in the ovary as compared with AKR1C3 immunolocalization in humans suggests that another murine AKR may be responsible for this activity (46).

We also found evidence for alternative splicing of Akr1c12, to our knowledge a heretofore unrecognized event in the AKR family. The smaller form of Akr1c12 mRNA results from exclusion of a single exon, resulting in a translation frame-shift and early termination signal to produce a truncated protein, presumably having altered or deficient function. The alternatively spliced AKR1C12 mRNA was detected in three different mouse strains, thus

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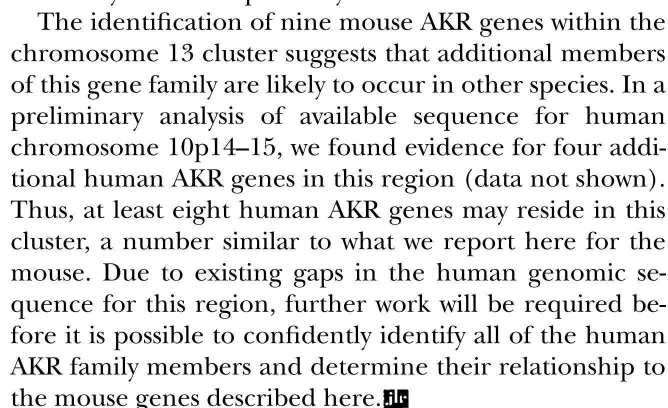

excluding the possibility that it simply represents an artifact or a strain-specific mutation. Although it is not clear at this point what the function of the truncated protein is, it is intriguing to speculate that such an alternative splice event might be a mechanism to modulate enzyme activity or modify substrate specificity.

The identification of nine mouse AKR genes within the chromosome 13 cluster suggests that additional members of this gene family are likely to occur in other species. In a preliminary analysis of available sequence for human chromosome 10p14–15, we found evidence for four additional human AKR genes in this region (data not shown). Thus, at least eight human AKR genes may reside in this cluster, a number similar to what we report here for the mouse. Due to existing gaps in the human genomic sequence for this region, further work will be required before it is possible to confidently identify all of the human AKR family members and determine their relationship to the mouse genes described here.

The authors acknowledge Maria Villalvazo and Shuo Lou for isolation of four AKR gene family members, and thank Merav Strauss for excellent technical assistance. This work was supported by National Institutes of Health Grants HL-58627 and HL-28481 (K.R.), NRSA Award GM08042 (J.P.), National Institutes of Health DK-41014, and USC Research Center for Liver Disease 5P30DK048522 (A.S.).

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