Tissue Distribution and Gender-Divergent Expression of 78 Cytochrome P450 mRNAs in Mice

Helen J. Renaud,* Julia Yue Cui,* Mohammed Khan,† and Curtis D. Klaassen*1

*Department of Pharmacology, Toxicology, and Therapeutics and †Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160

1To whom correspondence should be addressed at Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Room 4004 KLSIC, 3901 Rainbow Boulevard, MS-1018, Kansas City, KS 66160. Fax: (913) 588-7501. E-mail: cklaasse@kumc.edu.

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Cytochrome P450 (Cyp) enzymes from the first four families (Cyp1–4) play a major role in metabolizing xenobiotics, affecting drug pharmacokinetics and chemical-induced toxicity. Due to cloning of the mouse genome, many novel Cyp isoforms have been identified, but their tissue distribution of expression is unknown. This study compared the tissue distribution of all 78 Cyps from the Cyp1–4 families in C57BL/6 mice providing not only an indication of which tissues novel Cyps may have their greatest importance but also a cohesive comparison of the tissue distribution of all Cyp1–4 isoforms. Transcripts of the 78 Cyps were quantified by multiplex suspension arrays and quantitative real-time PCR in 14 tissues. Hierarchical clustering indicated that in male mice, 52% of the Cyp species were expressed highest in liver, 10% in kidney, 10% in duodenum/jejunum, 10% in testes, 5% in lung, and < 4% in colon, brain, heart, and stomach. Female mice had a similar pattern of Cyp messenger RNA expression; however, compared with males, females had 7% more Cyps that were liver predominant, 2% more Cyps that were stomach predominant, but 1% less Cyps that were kidney and lung predominant. Differences in gender expression were observed in 29 of the Cyps, with 24 being higher in females than males. Additionally, the data suggest a correlation between the spatial arrangement of genes within a gene cluster and their organ-predominant expression, indicating a common regulatory mechanism may be present within these clusters. In conclusion, this study provides novel data on the tissue distribution and gender-divergent expression of 78 functional mouse Cyp isoforms.

Key Words: cytochrome P450; mRNA; mice; tissue distribution.

The cytochrome P450 (Cyp) superfamily is a large and diverse group of enzymes that catalyze the metabolism of endogenous and exogenous molecules in the body. The substrates of Cyp enzymes range from lipids and steroidal hormones to xenobiotics, such as drugs and environmental chemicals. Currently, 57 human and 102 mouse putatively functional full-length Cyp genes have been identified (Nelson et al., 2004). Due to a highly conserved 26-residue region near the carboxy-terminus of all Cyp proteins across species, it is thought that all P450 genes diverged from a single ancestral gene more than 2 billion years ago. Assessment of Cyp evolution indicates an “explosion” of new Cyp genes during the past 800 million years, likely reflecting the emergence of animal-plant warfare. As animals began to ingest plant matter, plants evolved to enhance their survival by synthesizing new toxic compounds, and animals responded by developing new enzymes to process the plant-derived toxicants (Nebert et al., 1989).

The human body is constantly exposed to chemicals, such as airborne pollutants and pesticides, as well as drugs. Considering that many drugs and chemicals such as digitoxin, warfarin, ergotamine, quinine, and ellipticine are derived from plant-based compounds, it is not surprising that animals possess sufficiently diverse Cyp enzymes to handle essentially any newly synthesized drug or chemical. Therefore, Cyp enzymes play a very important role in determining the intensity and duration of therapeutic drugs. Cyp enzymes are also involved in many adverse drug-drug interactions as well as activation of xenobiotics to toxic and/or carcinogenic metabolites. Thus, Cyp enzymes are crucial in evaluating pharmacokinetics, degree of toxicity, teratogenicity, and/or carcinogenicity of a new drug or chemical. Therefore, examining the tissue-specific patterns of Cyp gene expression provides valuable information toward understanding drug and chemical kinetics, metabolism, and toxicity in the adult organism.

In humans, there is evidence that gender-divergent CYP expression plays a role in some clinically relevant adverse drug reactions (Anderson, 2005; Tran et al., 1998). For example, CYP1A2, which is involved in the oxidation of theophylline, clozapine, and caffeine, has constitutively higher activity in males than females in both Caucasian and Chinese populations (Anderson, 2005). Moreover, women suffer a higher incidence of eosinophilia after taking clozapine than men (Banov et al., 1993), which may be a result of differences in CYP1A2 activity. Thus, studying the gender-divergent expression of
CYP enzymes may help better predict gender-specific adverse drug reactions, and clinically, there is a need for this type of research.

Laboratory mice are typically used for mechanistic research and initial screening of drugs for chemical efficacy and/or toxicity; therefore, knowledge of mouse Cyp expression across organs is imperative. Additionally, due to the homology between many mouse and human Cyp genes, knowledge of mouse Cyp tissue distribution can provide insight into CYP tissue distribution in humans. Using semiquantitative PCR, previous studies measured tissue distribution of 40 Cyp genes representing each known Cyp family in male Balb/c mice (Choudhary et al., 2003). However, there is a lack of expression data for many novel xenobiotic-metabolizing Cyp isoforms as well as whether gender differences exist. Cyp enzymes involved in xenobiotic transformation primarily belong to Cyp1, Cyp2, and Cyp3 families. Substrates for Cyp enzymes from the Cyp4 family are mainly fatty acids and eicosanoids but also include some xenobiotics. Therefore, this study focused on the tissue distribution and gender differences of the 78 mouse Cyp isoforms from Cyp1, Cyp2, Cyp3, and Cyp4 families.

MATERIALS AND METHODS

Animals. Five male and five female C57BL/6 mice, aged 7 weeks, were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Mice were allowed to acclimate to the facility for 1 week before use and were housed according to the American Animal Association Laboratory Animal Care guidelines at the University of Kansas Medical Center. At 8 weeks of age, the following organs were collected: liver, brain, lung, heart, stomach, duodenum, jejunum, ileum, colon, kidney, testes/ovaries, and uterus. For all organs, n = 5/6 gender, except ovary, where n = 4. Eight weeks of age was selected because both male and female mice are sexually mature at this age, and it serves as a representative age for young adulthood, as we previously described (Ahouti and Klaassen, 2006, 2008; Cheng and Klaassen, 2009; Cheng et al., 2005; Cui et al., 2009). Additionally, it should be noted that each ovary sample is composed of tissue pooled from multiple mice in order to obtain sufficient RNA. The small intestine was longitudinally dissected, rinsed in saline, and divided into three equal-length sections (duodenum, jejunum, and ileum) before being frozen in liquid nitrogen. Placentas were collected from a previous study (Aleksunes et al., 2008) from pregnant mice on gestational day 17. All tissues were frozen in liquid nitrogen immediately after removal and stored at –80°C. Studies were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

RNA isolation. RNA was isolated with RNA-Bee Reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer’s protocol. RNA concentrations were determined by the 260/280 nm ratio obtained with a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA integrity was evaluated by visualization of 18S and 28S ribosomal RNA bands after formaldehyde-agarose gel electrophoresis.

CyP messenger RNA quantified by the Bio-Rad multiplex suspension array. The Bio-Rad multiplex suspension array was used to quantify the majority of the Cyp targets (Supplementary table 1), and the methods followed were according to the manufacturer’s protocol (Affymetrix/Panomics, Santa Clara, CA). This assay is based on xMAP technology (multianalyte profiling beads) that enables the detection and quantification of multiple RNA targets in a single sample. Quantification of RNA is achieved through flow cytometric analysis of fluorescently dyed microspheres (beads) that are hybridized to target RNA transcripts. Bead-based oligonucleotide probe sets specific for each Cyp examined were developed by Panomics and can be found at www.panomics.com (panel identification: 21211, 21212, 21213). Three types of oligonucleotide probes were used: capture extenders, ligation extenders, and blockers. The capture extenders discriminate the target RNA via cooperative hybridization among the different capture beads within the bead array. Into each well of a 96-well hybridization plate, 400 ng of RNA were loaded and incubated overnight with the bead-based probe sets. Signal amplification was obtained by incubation with amplification molecules that hybridize to the tail of the ligation extenders. Each amplification unit contains multiple hybridization sites for biotinylated label probes that bind streptavidin-conjugated R-phycocerythrin (Affymetrix/Panomics). Fluorescence was analyzed with a Bio-Plex 200 System Array reader with Luminex xMAP technology, and data were acquired with Bio-Plex Data Manager Software Version 5.0 (Bio-Rad, Hercules, CA). Background values were subtracted from target values and subsequently all data were standardized to the internal control, ribosomal protein L13A (Rpl13a), which encodes a ribosomal protein that is a component of the 60S subunit. Information on method validation is available at www.panomics.com.

Quantitative real-time PCR. Reverse transcription of RNA to complementary DNA (cDNA) was performed with the Applied Biosystems High Capacity Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). Briefly, equal volumes of 50 ng/μl RNA and 2× reverse transcriptase and random primers reaction mix were combined and placed in an Eppendorf Mastercycler under the following conditions: 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min. Subsequently, quantitative PCR (qPCR) was performed on the resulting cDNA. Primers for qPCR were designed with Primer-Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/tools/primer-blast), and the bit scores and e values were determined by a BLAST search (www.blast.ncbi.nlm.nih.gov/Blast.cgi) of the mouse genomic and transcript database. The criterion for primer target specificity was whether the off-target sequence matches had a bit score difference of >6 bits compared with the target sequence. In this regard, it should be noted that the primers for Cyp3a59 (bit score = 40.1; e value = 0.013) matched the off-target sequence of Cyp3a25 with a bit score = 36.1 and e value = 0.21. All primers (Supplementary table 2) were synthesized by Integrated DNA Technologies (Coralville, IA). Briefly, the PCR reaction mix contained per reaction: 12.5 μl of Applied Biosystems SYBR Green PCR Master Mix, 2.5 μl of 3 μM forward and reverse primer mix, 5 μl RNase-free H2O, and 5 μl of 2 ng/μl cDNA. Reactions were seeded in a 96-well optical reaction plate (Applied Biosystems), and fluorescence was quantified in real time with the Applied Biosystems 7300 Real-Time PCR System under the following conditions: 50°C for 2 min, 95°C for 10 min (95°C for 15 s, 60°C for 1 min) × 40 cycles. Melt curves were conducted for every reaction. Relative standard curves were generated for each target to determine the relative amount of transcript present in the sample. Relative amounts of Cyp transcript were then normalized to the housekeeping transcript Rpl13a (Supplementary figure 2).

Western blot analysis. Liver and lung tissues from three male and three female mice were homogenized in sucrose/Tris-hydrochloride buffer (10 mM tris-base, 150 mM sucrose, and protease inhibitor, pH 7.5) and centrifuged at 10,000 × g to pellet debris. Protein concentrations were determined using the bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL). For Western blot analysis, 30 μg protein from each sample was separated by SDS-polyacrylamide gel electrophoresis using a Bio-Rad Criterion 4–12% precast polyacrylamide gel. Protein bands were transferred to nitrocellulose membrane. Subsequently, membranes were immersed in a blocking solution comprised of 5% milk dissolved in 0.5% Tween-20/PBS (PBS-T), then incubated for 1 h in 1:500 primary monoclonal mouse anti-rat Cyp2b1/2b2 antibody (also detects mouse Cyp2b10; sc-53242, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in 3% milk in PBS-T. Following a 1 h incubation with 1:5000 secondary goat anti-rabbit IgG HRP-labeled antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), Western blotting was performed using the chemiluminescence detection system (ECL-western, Amersham Biosciences) and observed with ChemiDoc XRS+ (Bio-Rad, Hercules, CA).

References

Ahouti and Klaassen, 2006, 2008; Cheng and Klaassen, 2009; Cheng et al., 2005; Cui et al., 2009.

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anti-mouse (Sigma, A4416) in 3% milk in PBS-T, protein bands were visualized using enhanced chemiluminescence according to the manufacturer’s instructions (Pierce ECL Western Blotting Substrate). For detection of β-actin, blots were stripped using Western Blot Stripping Buffer (Pierce) and reprobed for β-actin using a 1:5000 dilution of rabbit polyclonal anti-human β-actin antibody (ab8227, Abcam, Cambridge, MA) in 3% milk in PBS-T, followed by 1:2000 secondary goat anti-rabbit antibody (Sigma A4914) in 3% milk in PBS-T. The intensities of the protein bands were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij), and the relative signals from each sample were determined by comparing the intensities from Cyp2b10 bands with those obtained from β-actin.

**Statistical analysis.** Statistical differences in messenger RNA (mRNA) quantity between male and female mice were determined with an unpaired two-tailed Student’s t-test (Graphpad Prism 4.0). Densitometry data from the Cyp2b10 Western blots were analyzed using a one-way ANOVA followed by Bonferroni’s multiple comparison test. For mRNA and protein data, comparisons resulting in \( p < 0.05 \) were considered statistically significant. Two-way hierarchical clustering of male or female gene expression values was conducted with JMP 8.0 software (SAS Institute, Cary, NC) using Ward’s minimal variance and displayed as a dendrogram.

**RESULTS**

The mRNA expression patterns of 78 mouse Cyp isozymes were examined by multiplex suspension array and quantitative real-time PCR (qRT-PCR) in male and female adult mouse tissues. All of the selected 78 Cyp transcripts were detected in at least one tissue.

**Cyp1 Family**

The Cyp1 family members had very different mRNA expression patterns across organs. Cyp1a1 was highest in lung and duodenum, whereas Cyp1a2 was primarily found in liver. Cyp1b1 mRNA was highest in ovary, and moderate amounts were detected in kidneys and testes (Supplementary figure 1).

**Cyp2a Subfamily**

All four isoforms of the Cyp2a family were most highly expressed in livers of female mice. Cyp2a4 mRNA was also highly expressed in kidney; however, Cyp2a5, 2a12, and 2a22 were almost exclusively found in liver. Additionally, low amounts of Cyp2a22 were detected in testes (Fig. 1).

**Cyp2b Subfamily**

The Cyp2b family members had very different mRNA organ distribution patterns (Fig. 2). Cyp2b9 and 2b13 transcripts were almost exclusively expressed in female liver (approximately 30,000% higher in female than male), whereas Cyp2b10 was highest in lung, Cyp2b19 was detected almost entirely in testes, and Cyp2b23 was ubiquitously expressed. Apart from the lung, Cyp2b10 mRNA was also highly expressed in the duodenum with decreasing amounts in the more distal parts of the small intestine (Figs. 2 and 3).

Western blot analysis of Cyp2b10 showed parallel gender- and tissue-specific protein expression patterns as Cyp2b10 mRNA (Fig. 3); specifically, protein expression of Cyp2b10 was 600 and 86% higher in lung (average of male and female) compared with male and female liver, respectively. Additionally, Cyp2b10 protein expression was 75% higher in female compared with male liver. There was no difference in protein expression in female versus male lung.

**Cyp2c Subfamily**

Overall, 12 of the 15 Cyp2c isozymes were detected primarily in liver (Cyp2c29, 2c37, 2c38, 2c39, 2c40, 2c44, 2c50, 2c54, 2c67, 2c68, 2c69, and 2c70). In addition, 9 of the 12 liver-predominant Cyp2c isozymes were higher in females than males (Cyp2c37, 2c38, 2c39, 2c40, 2c44, 2c54, 2c67, 2c68, and 2c69). However, among the nine female liver-predominant isozymes, the difference between males and females varied dramatically, from 35% (Cyp2c44) to 8000% (Cyp2c69) higher in livers of females. The amount of Cyp2c44 mRNA in kidney was moderate in both sexes, however, it was 150% higher in females than males. Cyp2c55, 2c65, and 2c66 were the only three Cyp2c isoforms primarily found in tissues.

**FIG. 1.** Expression of Cyp2a subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different \(( p < 0.05 )\) from male values of that same organ. Values are expressed as means ± SEM.
Cyp2b Subfamily

Five of the eight Cyp2b isozymes were primarily found in liver (Cyp2b9, 2d10, 2d22, 2d26, and 2d40), with Cyp2d22 and 2d26 being slightly higher in females (approximately 30 and 20% higher in females than males, respectively). In contrast, Cyp2d9 was almost exclusively detected in males. Although Cyp2d26 mRNA was highest in liver, moderate amounts were detected in duodenum, jejunum, and kidney, with the latter being 100% higher in females. Cyp2d11 mRNA was high in both liver and kidney, being 115% higher in livers of females than males but 275% higher in kidneys of males than females. Cyp2d12 was highest in kidney and approximately 200% higher in males than females. Lastly, Cyp2d34 mRNA was highest in colon and low amounts were detected in ileum (Fig. 5).

Cyp2ab, e, f, and g Subfamilies

Cyp2ab1 transcript was most abundant in placenta, heart, lung, and testes. In contrast, Cyp2e1, 2f2, and 2g1 isozymes were highest in liver with Cyp2g1 being 460% higher in females. Moderate amounts of Cyp2e1 were detected in male kidney, and high levels of Cyp2f2 mRNA were detected in lung (Fig. 6).

Cyp2j Subfamily

Varying tissue distribution patterns were detected for members of the Cyp2j subfamily. One of the five members was exclusively found in male kidney (Cyp2j13), whereas Cyp2j9 was detected primarily in brain. Cyp2j5 mRNA was exclusively expressed in liver and kidney; however, Cyp2j6 was highest in the duodenum followed by stomach, jejunum, liver, and kidney. Cyp2j7 mRNA was ubiquitous, but amounts were higher in kidney, female brain, and male colon and testes (Fig. 7).

Cyp2r, s, t, u, and w Subfamilies

Cyp2r1 was primarily expressed in male liver (200% higher in male liver than in female). Cyp2s1 was highest in stomach, which is in contrast to past findings showing Cyp2s1 mRNA expression highest in lung; however, this past research did not include stomach tissue in their study (Choudhary et al., 2003). Cyp2t4...
FIG. 4. Expression of Cyp2c subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.
mRNA had a ubiquitous pattern of expression but tended to be highest in liver and testes. Cyp2u1 was primarily detected in the heart of both genders and in male liver (330% higher in male liver than in female). Cyp2w1 was highest in the distal GI tract (ileum and colon) and in testes (Fig. 8).

Cyp3a Subfamily

The Cyp3a family was detected primarily in liver and intestine. Six of the eight Cyp3a transcripts quantified were expressed highest in liver (Cyp3a11, 3a16, 3a25, 3a41a/b, 3a44, and 3a59). Cyp3a13 was one exception, with the highest amount found in

**FIG. 5.** Expression of Cyp2d subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.

**FIG. 6.** Expression of Cyp2e, f, and g subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.
the duodenum that incrementally decreased in the more distal intestinal segments. Cyp3a57 was also an exception as the highest levels of this isozyme were found in testes. Four of the seven liver-predominant isozymes were female predominant (Cyp3a16, 3a41a/b, 3a44, and 3a59), whereas the other three isozymes did not show gender divergence. Of the female liver-predominant isozymes, Cyp3a16, Cyp3a44, and 3a59 were all approximately 180% higher in females than in males, and Cyp3a41a/b was almost exclusively detected in female liver (10,000% higher in female than in male liver). Five of the seven liver-predominant Cyp3a isoforms were detected in the intestine, with decreasing amounts from proximal to distal segments (Cyp3a11, 3a13, 3a25, 3a41a/b, 3a44, and 3a59) (Fig. 9).

Cyp4a and b Subfamilies

In general, the Cyp4a family members were found primarily in liver and kidney. Cyp4a10, 4a14, 4a31, and 4a32 all had female-predominant expression in liver and kidney, although this was not statistically significant for 4a31 in kidney. It should be noted that Cyp4a14 mRNA was almost exclusively expressed in liver and kidney of females; in contrast, Cyp4a12a and 4a12b were exclusively expressed in male liver and kidney. Of the remaining Cyp4a and b family members, Cyp4a29 and Cyp4a30b mRNA were lowly and ubiquitously expressed, whereas Cyp4b1 was highest in lung and male kidney (260% higher in male than female kidney) (Fig. 10).

Cyp4f Subfamily

The Cyp4f family had markedly different expression patterns across the various organs. Cyp4f13 was highest in liver but relatively high amounts were observed in intestine, kidney, ovary, and uterus. Cyp4f14, 4f16, and 4f40 were all detected mainly in the small intestine. Moderate amounts of Cyp4f14 were detected in liver, which were slightly higher in females (50% higher in females than in males). Cyp4f15 was found predominantly in female liver. Cyp4f17, 4f18, and 4f37 were all detected ubiquitously across organs; however, it should be noted that Cyp4f18 was 230 and 550% higher in liver and brain of females than males, respectively. Lastly, Cyp4f39 mRNA was highest in testes and low amounts were detected in stomach and lung (Fig. 11).

Cyp4v and x Subfamilies

Expression of Cyp4v3 mRNA was highest in liver, duodenum, and jejunum and was slightly (30%) higher in livers of male than female mice. Cyp4x1 mRNA was primarily expressed in brain (Fig. 12).

Two-Way Hierarchical Clustering

In males, hierarchical clustering indicated that approximately 52% of the mRNA of mouse Cyps were highest in liver (Fig. 13). Of the remaining clusters, 10% were kidney, 10% were duodenum/jejunum, 10% were testes, 5% were lung, 4% were colon, 3% were brain, 3% were heart, and 1% were stomach predominant. In females, hierarchical clustering indicated that approximately 59% of the Cyps were highest in liver (Fig. 14). Of the remaining clusters, 10% were duodenum/jejunum, 9% were kidney, 4% were lung, 4% were colon, 3% were brain, 4% were uterus, 3% heart, 3% stomach, 1% placenta, and 1% ovary predominant.
FIG. 8. Expression of Cyp2t, u, and w subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.

FIG. 9. Expression of Cyp3a subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.
DISCUSSION

Cytochrome P450 enzymes mediate responses to environmental challenges; thus, determining P450 tissue distribution is important. Previously, the tissue-specific expression, function, and regulation of many mouse Cyp enzymes have been characterized, and the data have been compared with the human CYPs (Hrycay and Bandiera, 2009). Specifically, approximately 40 pairs of orthologous mouse-human CYP genes have been identified that encode enzymes with similar metabolic functions (Hrycay and Bandiera, 2009). The contribution of this study to the current knowledge of Cyps is that we have characterized the mRNA profiles of many more Cyp isoforms that are specialized in drug metabolism, which is by far the first systematic and the most comprehensive characterization of the drug-metabolizing Cyps across 14 critical metabolic organs. Specifically, this study determined tissue distribution and gender-divergent expression of Cyps in mice. In male and female mice, approximately 55% of Cyp isozymes were expressed highest in liver, followed by 10% in kidney, 10% in duodenum/jejunum, 5% in gonad, 5% in lung, 4% in colon, and 3% in brain. This study is the first to determine the tissue distribution patterns for 31 uncharacterized Cyps including 11 that were expressed highest in liver (Cyp2a22, 2c67, 2c68, 2c69, 2c70, 2d10, 2d40, 3a59, 2d11, 4a31, and 4a32), 5 in kidney (Cyp2d11, 4a31, 4a32, 2d12, and 2j13), 7 in intestine (Cyp2c65, 2d34, 2c66, 2w1, 3a41a/b, 3a44, and 4f40), and 2 in testes (Cyp3a57 and 4f39), with the remaining 9 being ubiquitously expressed (Cyp2ab1, 2b23, 4f17, 4f18, 4f37, 4a30b, 4a29, 2i4, and 2j7).

Liver, intestine, and kidney are the main organs involved in absorption, metabolism, and excretion of exogenous compounds; therefore, not surprisingly, the majority of Cyp enzymes are expressed most highly in these organs. What is intriguing is the enrichment of certain P450s in organs not commonly associated with xenobiotic metabolism, such as the brain. In this study, two Cyp enzymes (Cyp2j9 and 4x1) were found highest in brain.
P450s in brain are involved in essential functions such as biosynthesis and metabolism of neurosteroids, vasoactive eicosanoids, neurotransmitters (Bornheim et al., 1995; Hedlund et al., 2001; Hiroi et al., 1998), and fatty acids such as arachidonate (Rapoport et al., 2001). Cyp2j9 is highly expressed in mouse cerebellum and oxidizes arachidonic acid to an eicosanoid that possibly inhibits neurotransmitter release (Qu et al., 2001). Cyp4x1 is also highly expressed in mouse brain (Al-Anizy et al., 2006; Bylund et al., 2002), and in humans, CYP4X1 metabolizes the arachidonic acid derivative anandamide, which is an important endocannabinoid signaling molecule that mediates neurovascular function (Stark et al., 2008). Apart from their endogenous activity, the high expression of Cyp2j9 and Cyp4x1 raises questions about their role in xenobiotic metabolism in the brain.

Cyp expression in the lung is also of interest as this is the first line of defense against airborne xenobiotics. Our study corroborates that Cyp2b10 mRNA is higher in lung compared with liver (Damon et al., 1996; Honkakoski et al., 1996; Kakizaki et al., 2003), and we are the first to confirm this through protein expression. Interestingly, it is well known that Cyp2b10 is inducible by phenobarbital in liver (Audet-Walsh et al., 2009); however, Cyp2b10 is not inducible by phenobarbital in lung (Honkakoski et al., 1996; Kakizaki et al., 2003) indicating independent mechanisms of regulation in lung compared with liver. Whether Cyp2b10 has different functions in the lung versus the liver remains unknown.

The role of drug-metabolizing enzymes in ovaries and testes is another area of interest not only because these organs are not commonly associated with drug metabolism but also due to the implications of drug toxicity in the gametes. A number of P450 enzymes expressed in gonadal tissue, such as Cyp11a1 and Cyp17a1, are involved in steroidogenic hormone biosynthesis. Certain xenobiotic-metabolizing Cyp isozymes are also enriched in mouse gonads (Choudhary et al., 2003; Shimada...
et al. 2003); however, the physiological roles of these P450s in gonadal tissue are unknown (Schuppe et al., 2000). This study identified three Cyps (Cyp2b19, 3a57, and 4f39) expressed highest in testes, and one (Cyp1b1) in ovaries. The high expression of Cyp1b1 in ovary largely contributes to the development of ovarian toxicity resulting from exposure to 7,12-dimethylbenz[a]anthracene (Rajapaksa et al., 2007). Additionally, this study is the first to show the specific enrichment of Cyp3a57 and 4f39 in testis. It should be noted that past studies using semiquantitative RT-PCR showed Cyp2b19 mRNA is most abundant in mouse epidermis and was undetectable in testis (Keeney et al., 1998). This discrepancy between the current and the past studies may be explained by the increased sensitivity of qPCR compared with semiquantitative RT-PCR. However, because epidermal tissue was not evaluated in the current study, comparisons of Cyp2b19 levels between epidermis and testis cannot be made. Regardless, the present data indicate that Cyp2b19 mRNA is expressed in testes and therefore may influence certain xenobiotic-induced testicular toxicities. Cyp expressions in testes and ovaries were not compared because male and female gonads, although derived from the same embryological structure, are structurally and functionally different organs in the adult. Therefore, an apparent gender-divergent expression in the gonads may not be related to gender but actually reflects an organ-specific expression.

Cyp subfamilies have diverged somewhat differently in mice compared with humans. The mouse–human split occurred approximately 75 million years ago, allowing sufficient time for major changes in gene-cluster size and organization to accumulate (Waterston et al., 2002). A comparison of all functional homologous mouse and human Cyp1–4 genes is given in Table 1. Compared with humans, mice have undergone significant expansion of Cyp genes in seven of eight clusters (Nelson et al., 2004); the Cyp2abfgst cluster, Cyp2c cluster, Cyp2d cluster, Cyp2j cluster, Cyp3a cluster, Cyp4abx cluster, and the Cyp4f cluster

FIG. 12. Expression of Cyp4v and x subfamily isoforms in tissues of male and female C57BL/6 mice. Asterisks (*) denote female values that are statistically different ($p < 0.05$) from male values of that same organ. Values are expressed as means ± SEM.

FIG. 13. Two-way hierarchical clustering of Cyp expression in tissues of male C57BL/6 mice. Distances between isoforms reflect significance of associations. Red color represents higher and blue color represents lower expression levels, respectively.
These large Cyp gene clusters in the mouse (Fig. 15) provide a useful tool to study the spatial organization of genes in relation to gene expression patterns, giving insight into whether common spatial regulatory mechanisms exist. For example, Cyp2b10 is at the beginning of the Cyp2abfgst cluster and is highly expressed in lung; whereas the mRNA expression of downstream neighbors Cyp2b13, Cyp2b9, and Cyp2a4 are highest in liver. Genes located at the 5′ region of the Cyp2c cluster (Cyp2c55, 2c65, and 2c66) are enriched in intestine; however, the remaining downstream genes are liver specific. The Cyp2d cluster has expanded to nine genes in mice compared with one gene in humans. Considering that human CYP2D6 is noted for its exogenous drug metabolism capabilities, it is possible that the duplicated Cyp2d genes in the mouse function to metabolize foreign substrates, such as plant and dietary compounds, rather than new endogenous substrates. Data from the current study support this hypothesis, as all genes in the Cyp2d cluster were expressed in the prominent drug-metabolizing organs—liver, kidney, and intestine. The spatial organization of genes within the Cyp2d cluster may give some insight into common regulatory mechanisms as the two genes in the middle of the cluster, Cyp2d12 and Cyp2d34, are slightly segregated from the rest of the Cyp2d gene cluster and are expressed in kidney and colon, respectively. However, the remaining genes of the Cyp2d cluster, which are divided into two subclusters, are all predominantly expressed in liver and are situated more closely together compared with Cyp2d12 and Cyp2d34. The Cyp3a cluster and the Cyp4abx cluster show a similar pattern of spatial organization with respect to tissue expression. Both clusters contain an upstream gene (Cyp3a13 and Cyp4x1, respectively) that is slightly segregated from, and exhibits a different tissue distribution pattern than, the downstream liver-predominant genes. Additionally, kidney mRNA expression of genes at the terminal end of the Cyp4abx cluster gradually increase relative to the spatial arrangement on the chromosome (Cyp4a10 < Cyp4a31 = Cyp4a32 < Cyp4b1). Therefore, these data suggest a correlation exists between the spatial arrangement of genes within a cluster and their tissue-specific expression, indicating a possible common regulatory mechanism may be present within these clusters.

Gender differences in Cyp expression are of interest not only to help explain sex-dependent variation in drug therapy, but also because they provide a useful tool to investigate mechanisms of toxicity. The presence of gender differences in P450 content and activity in humans remains debatable (Gandhi et al., 2004; Smith, 1991; Tanaka, 1999; Wolbold et al., 2003); however, gender-specific expression is commonly observed in mice (Waxman and Holloway, 2009). The current study has identified 29 of the 78 mouse Cyp isoforms as having considerable gender-predominant expression, with the difference in mRNA levels between sexes being over 150% in the organ in which they were most highly expressed. Of further interest is that, of the 29 gender-predominant Cyps, 24 were highest in females (Cyp2a4, 2a5, 2a22, 2a9, 2b10, 2b13, 2c37, 2c38, 2c39, 2c40, 2c54, 2c67, 2c68, 2c69, 2g1, 3a16, 3a1a/b, 3a44, 3a59, 4a10, 4a14, 4a31, 4a32, and 4f15) and only 5 were...
predominant in males (Cyp2d9, 2j13, 2u1, 4a12a/4a12b, and 4b1). Although the sex-dependent differences in hepatic P450 content in humans is debatable, a recent meta-analysis has concluded that women have higher CYP3A activity in liver, but not intestine, compared with men (Hu and Zhao, 2010). This result correlates with our observation that female mice have higher hepatic expression of Cyp3a16, Cyp3a41a/b, 3a44, and Cyp3a59 compared with males. Overall, the large number of Cyp isoforms expressed predominantly in the female gender is interesting and yields questions regarding whether these large sex differences in mRNA expression correlate to protein expression and activity, and if so, what gender-specific functions do these enzymes have? The ability to determine protein expression of specific mouse Cyps is limited due to the high homology between Cyp enzymes, causing nonspecific antibody reactivity. Many past studies analyzing mouse Cyp mRNA, protein, and/or activity, have detected similar gender differences as the current study (Table 2). However, it should be noted that many of the studies report gender differences in protein expression and/or activity that is not specific to a single Cyp isozyme. In our study, we observed parallel gender-specific differences in Cyp2b10 protein expression patterns as

<table>
<thead>
<tr>
<th>Mouse gene name</th>
<th>Homologous human gene name</th>
<th>Percent protein</th>
<th>Percent DNA</th>
<th>Mouse gene name</th>
<th>Homologous human gene name</th>
<th>Percent protein</th>
<th>Percent DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp1a1</td>
<td>CYP1A1</td>
<td>80.6</td>
<td>82.7</td>
<td>Cyp2g1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp1a2</td>
<td>CYP1A2</td>
<td>73.0</td>
<td>79.9</td>
<td>Cyp2j5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp1b1</td>
<td>CYP1B1</td>
<td>81.0</td>
<td>81.7</td>
<td>Cyp2j6</td>
<td>CYP2J2</td>
<td>76.2</td>
<td>78.3</td>
</tr>
<tr>
<td>Cyp2a4*</td>
<td>CYP2A13</td>
<td>86.6</td>
<td>84.3</td>
<td>Cyp2j7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp2a6</td>
<td>CYP2A6</td>
<td>83.6</td>
<td>83.4</td>
<td>Cyp2j9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp2a7</td>
<td>CYP2A7</td>
<td>82.0</td>
<td>83.2</td>
<td>Cyp2j13</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp2a5*</td>
<td>CYP2A13</td>
<td>88.5</td>
<td>85.5</td>
<td>Cyp2ab1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cyp2a6</td>
<td>CYP2A6</td>
<td>85.2</td>
<td>84.6</td>
<td>Cyp2e1</td>
<td>CYP2E1</td>
<td>87.8</td>
<td>89.4</td>
</tr>
<tr>
<td>Cyp2a7</td>
<td>CYP2A7</td>
<td>83.6</td>
<td>84.3</td>
<td>Cyp2f1</td>
<td>CYP2F1</td>
<td>81.9</td>
<td>82.6</td>
</tr>
</tbody>
</table>

Note. Asterisks and superscript letters delineate mouse cytochrome P450s that are homologous to other mouse cytochrome P450s with the same demarcation.
FIG. 15. Illustration depicting the tissue predominant enrichment and chromosomal arrangement of the eight Cyp gene clusters in the mouse. Genes within a cluster that do not appear in the table were ubiquitously expressed.
compared with Cyp2b10 mRNA levels. The Cyp2b subfamily accounts for one of the prototypic examples of gender-dependent differences in hepatic P450 profiles in mice (Jarukamjorn et al., 1999, 2000, 2001; Nemoto and Sakurai, 1995). The gender-dependent expression of Cyp2b10 is thought to be regulated by both male and female sex hormones, glucocorticoids (Nemoto and Sakurai, 1995), and pituitary factors (Jarukamjorn et al., 2002). Cyp2b genes are also known to be inducible in mouse liver by phenobarbital as well as glucocorticoids through independent mechanisms (Audet-Walsh et al., 2009). From an evolutionary perspective, it is intriguing to speculate that a selective advantage was conferred by the presence of gender-dependent regulatory mechanisms and two independent induction mechanisms of mouse hepatic Cyp2b10. These elaborate mechanisms regulating Cyp2b10 expression are unlikely to be the result of chance but rather have been subject to natural selection; however, the selective pressures that led to the evolution of these regulatory mechanisms remain unknown.

It should be noted that previous studies report that Cyp2c29 mRNA displays a female-predominant pattern in mouse livers (Lofgren et al., 2009). However, in that study, 11-week-old C57/6OlaHsd mice were used, in contrast to 8-week-old C57BL/6 mice in the present study. Therefore, the discrepancy of the results is likely due to age difference and/or mouse strain. In

<table>
<thead>
<tr>
<th>Cyp</th>
<th>Gender</th>
<th>Tissue</th>
<th>Reference</th>
<th>Example from literature of Cyp gender differences in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp2a4</td>
<td>Female</td>
<td>Liver</td>
<td>Burkhart et al. (1985)</td>
<td>mRNA, protein, and activity</td>
</tr>
<tr>
<td>Cyp2a5</td>
<td>Female</td>
<td>Liver</td>
<td>van Iersel et al. (1994)</td>
<td>Activity</td>
</tr>
<tr>
<td>Cyp2a22</td>
<td>Female</td>
<td>Liver</td>
<td>Jarukamjorn et al. (2001)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp2b9</td>
<td>Female</td>
<td>Liver</td>
<td>Wiwi et al. (2004)</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>Cyp2b10</td>
<td>Female</td>
<td>Liver</td>
<td>Hernandez et al. (2006)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp2b13</td>
<td>Female</td>
<td>Liver</td>
<td>Wiwi et al. (2004)</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>Cyp2c37</td>
<td>Female</td>
<td>Liver</td>
<td>Hernandez et al. (2009)</td>
<td>mRNA showed no gender difference</td>
</tr>
<tr>
<td>Cyp2c38</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2c39</td>
<td>Female</td>
<td>Liver</td>
<td>Lofgren et al. (2009)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp2c40</td>
<td>Female</td>
<td>Liver</td>
<td>Hernandez et al. (2009)</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>Cyp2c54</td>
<td>Female</td>
<td>Liver</td>
<td>Wang et al. (2004)</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>Cyp2c67</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2c68</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2c69</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2d9</td>
<td>Male</td>
<td>Liver and kidney</td>
<td>Wiwi et al. (2004)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp2g1</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2j13</td>
<td>Male</td>
<td>Kidney</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp2u1</td>
<td>Male</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp3a16</td>
<td>Female</td>
<td>Liver</td>
<td>Pass et al. (2002)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp3a41a/b</td>
<td>Female</td>
<td>Liver</td>
<td>Holloway et al. (2007)</td>
<td>mRNA</td>
</tr>
<tr>
<td>Cyp3a44</td>
<td>Female</td>
<td>Liver</td>
<td>Hernandez et al. (2009)</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>Cyp3a59</td>
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<td>Liver</td>
<td>N/A</td>
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<tr>
<td>Cyp4a10</td>
<td>Female</td>
<td>Liver</td>
<td>Heng et al. (1997)</td>
<td>mRNA showed no gender difference</td>
</tr>
<tr>
<td>Cyp4a12a/4a12b</td>
<td>Male</td>
<td>Liver and kidney</td>
<td>Muller et al. (2007)</td>
<td>Kidney—mRNA, protein, and activity</td>
</tr>
<tr>
<td>Cyp4a14</td>
<td>Female</td>
<td>Liver and kidney</td>
<td>Heng et al. (1997)</td>
<td>Liver—protein</td>
</tr>
<tr>
<td>Cyp4a31</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp4a32</td>
<td>Female</td>
<td>Liver and kidney</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cyp4b1</td>
<td>Male</td>
<td>Kidney</td>
<td>Imaoka et al. (1995)</td>
<td>mRNA and activity</td>
</tr>
<tr>
<td>Cyp4f15</td>
<td>Female</td>
<td>Liver</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
another study, Cyp2j5 mRNA and protein, detected by northern blot analysis and RNA PCR as well as immunoblotting, were observed to be abundant in the kidney, but they were found in the liver at relatively lower levels (Ma et al., 1999). In our study, we showed that Cyp2j5 mRNA is high in both liver and kidney at comparable levels. The discrepancy might be due to the age (16 weeks of age in the previous report vs. 8 weeks of age in the present study). Although our study provides a large data set of Cyp mRNA tissue distribution in mice, it is important to mention that mRNA data does not always predict protein expression and activity and should therefore be interpreted with caution until confirmed by protein expression or activity. However, this extensive analysis of Cyp mRNA levels provides information on the tissue and/or gender in which specific Cyps may be involved in xenobiotic metabolism and toxicity.

In conclusion, by examining the tissue- and gender-dependent patterns of Cyp mRNA expression, our study provides valuable information about the tissues in which these Cyps may have their greatest importance. In addition, these data may also prove helpful in predicting the pharmacokinetics of administered drugs.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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