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THREE PATTERNS OF CYTOCHROME P450 GENE EXPRESSION DURING LIVER MATURATION IN MICE

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Running Title: Developmental gene expression patterns of mouse Cyps

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Introduction: 742 words

Discussion: 1042 words

Abbreviations: Cyp, Cytochrome P450

Abstract

The neonatal period of liver development is an often overlooked phase of development. For instance, ontogeny of xenobiotic metabolizing enzymes can markedly affect biotransformation as the liver matures. To systematically examine the ontogenic gene expression patterns of cytochrome P450 genes (*Cyps*) in mice, the gene expression profiles of 19 xenobiotic metabolizing *Cyps* in *Cyp1-4* families were determined. The mRNA levels in C57BL/6 mouse livers were quantified using branched DNA technology at the following ages: gestational day 17 (2 days before birth), and postnatal days 0, 1, 3, 5, 10, 15, 20, 30, and 45. Among the 13 *Cyp* genes expressed in mouse livers, three distinct ontogenic expression patterns were identified by cluster analysis. Genes in Group 1 (*Cyp3a16* as well as *3a41b* in male) were expressed in the perinatal period, but were essentially non-detectable by 30 days of age. Genes in Group 2 (*Cyp2e1, 3a11*, and *4a10* as well as *3a41b* in female) quickly increased after birth and reached maximum expression levels by day 5. Genes in Group 3 (*Cyp1a2*, *2a4*, *2b10*, *2c29*, *2d22*, *2f2*, *3a13*, and *3a25*) were expressed at low levels until day 10-15, but markedly increased at day 20 to a high and stable level. In conclusion, the developmental expression of *Cyps* in mouse liver can be divided into three patterns, suggesting different mechanisms responsible for the expression of *Cyps* during liver maturation.

Introduction

About three-quarters of drugs primarily cleared via metabolism are biotransformed by members of the cytochrome P450 (CYP) superfamily. Humans have approximately 57 functional CYP enzymes, of which about 20 members in CYP1, 2, 3, and 4 families are likely involved in drug metabolism. It has been known since the 1950's that CYP enzymes are not mature at birth (Fouts and Adamson, 1959), however at that time it was thought that there was only one, or perhaps two CYP enzymes. Since the cloning of the *CYP* genes, ontogenic expression of some *CYP* genes in human livers has been reported (de Wildt *et al,* 1999; Koukouritaki *et al,* 2004; Blake *et al,* 2005; Leeder *et al,* 2005; Gaedigk *et al,* 2006; Hines, 2007). Based on hepatic ontogenic gene expression patterns, human drug metabolizing *CYP* genes have been categorized into three developmental patterns (Hines, 2007). Considerable differences in the ontogenic gene expression contribute to variation in drug metabolism between children and adults. These differences result in children being less responsive to drug therapy and higher risks for adverse reactions for some drugs metabolized by CYP enzymes.

Variation in *CYP* gene expression among different individuals at different ages are due not only to ontogeny, but also to genetic polymorphisms, environmental factors (e.g. diet, toxicants), and therapeutic agents (e.g. drugs). Moral, ethical, and technical limitations for studying human fetal and neonatal samples have precluded an in-depth understanding of the mechanisms controlling ontogenic expression of human *CYP* genes (Rowell and Zlotkin, 1997). Therefore, to understand the factors and mechanisms governing the developmentally programmed *CYP* gene expression patterns, it would be advantageous to have a laboratory animal model that can minimize the influences of genetics, environment, and scarcity of tissues.

Mice have been used to study ontogenic expression of *Cyp* genes (Choudhary *et al*, 2003, 2005). These studies examined the expression of 40 *Cyp* genes in embryonic developmental stages from E7- E17 and in eight adult tissues. Some xenobiotic metabolizing *Cyp* genes are not expressed in embryonic developmental stages, but expressed in adult livers. However, there is a lack of expression data for the xenobiotic metabolizing *Cyp* genes during the postnatal period (i.e. after birth but before adult).

Mouse liver originates from the gut endoderm on embryonic day 8.5 (Douarin, 1975; Houssaint, 1980). One day later, the liver assumes the morphological appearance of hepatoblasts, capable of differentiating into either hepatocytes or bile duct epithelial cells (Rogler, 1997; Shiojiri and Mizuno, 1993). Hematopoietic stem cells migrate to the fetal liver at embryonic day 10.5 (E10.5) to generate numerous hematopoietic cells (Medvinsky and Dzierzak, 1996; Mukouyama *et al*, 1998; Sanchez *et al*, 1996). Thus, from E10.5 to E15, fetal liver is predominately a hematopoietic organ. By E14.5, immature hepatoblasts begin differentiate into hepatocytes and bile-duct epithelial cells (Douarin, 1975; Houssaint, 1980). At gestation day 17, only 30% of liver is devoted to hematopoiesis (Marie and Cresteil, 1989; Sasaki and Sonoda, 2000), although hematopoietic stem cells are still observed in liver until postnatal day 5 (Apte *et al*, 2007). From the day of birth (19 days post coitus) to postnatal day 9, the liver mass increases >3 folds (Sasaki and Sonoda, 2000). Rapid cellular proliferation between postnatal day 5 and day 20 has also been reported, and by postnatal day 30, mouse livers are histologically identical to livers of mice at 3 months (Apte *et al*, 2007). However, much is not known about how and when the xenobiotic metabolizing *Cyp* genes become activated or suppressed after hematopoiesis but before adulthood. Understanding the regulation of ontogenic expression is important since

ontogeny affects both the expression and inducibility of xenobiotic metabolizing genes during postnatal liver development (Fouts and Adamson, 1959; Rich and Boobis, 1997).

Mice have over 102 CYP enzymes (Nelson *et al*, 2004), however the exact number involved in drug metabolism is not entirely clear. In the present study, 19 mouse *Cyp* genes from families *1-4* were selected based on their sequence homology to the 19 human drug metabolizing *CYP* genes (Table 1). Gene expression levels were quantified from gestational day 17 (day -2) to day 45 of ages using a branched DNA signal amplification assay. Because of lack of specific antibodies against most of the mouse Cyp proteins, gene expression at the protein level was not determined in this study. The data indicate that, in mice, there are also three developmental patterns of *Cyp* expression. Some mouse *Cyp* genes are similar, but others are not similar to their human homologous *CYP* genes.

Methods

Animals. Eight-week old C57BL/6 breeding pairs of mice were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed according to the American Animal Association Laboratory Animal Care guidelines, and were bred under standard conditions in the Lab Animal Resources Facility at the University of Kansas Medical Center. The use of these mice was approved by the Institutional Animal Care and Use Committee. Breeding pairs were set up at 4:00 pm, and separated at 8:00 am the following day. The body weights of the females were recorded each day to determine pregnancy status. Livers from male and female offspring were collected at the following ten ages: day -2 (gestational day 17), 0, 1, 3, 5, 10, 15, 20, 30, and 45 days of age (n=5), which represents periods of prenatal (day -2), neonatal (day 0 to 10), juvenile (day 15 to 30), and young adult (day 45). Due to the difficulty in distinguishing gender

from day -2 to day 5 mice, livers from male and female offspring of the same litter were pooled at each age to achieve the desired amount of tissue. From days 10 to 45, male and female liver samples were separated (5 males and 5 females per age). Livers were frozen immediately after removal in liquid nitrogen, and stored at -80°C.

Total RNA extraction. Total RNA was isolated using RNAzol B reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's protocol. RNA concentrations were quantified using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) at a wavelength of 260 nm. The integrity of total RNA was evaluated by formaldehyde-agarose gel electrophoresis, and confirmed by visualization of 18S and 28S rRNA bands.

Cyp mRNA levels quantified by the Multi-plex Suspension Array. The mouse Cyp1a1, 1a2, 1b1, 2a4, 2b10, 2c29, 2c39, 2c66, 2d22, 2e1, 2f2, 2j6, 3a11, 3a13, 3a16, 3a25, 3a41, 4a10, and 4f18 mRNA levels were quantified by the Multi-plex Suspension Array according to the manufacturer's protocol (Panomics, Fremont, CA). Briefly, individual bead-based oligonucleotide probe sets, specific for each gene examined, were developed by Panomics (panel ID: 20044). The genes and accession numbers are listed in Table 1, and also available at www.panomics.com. Three types of oligonucleotide probes were utilized: 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. On the first day, 6 μg of total RNA was loaded in each well, and incubated over-night with bead-based probe sets. On the second day, signal amplification was mediated by 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-Phycoerythrin (SAPE). The resulting fluorescence signals were analyzed using a Bio-Plex 200

System Array reader with Luminex 100 X-MAP technology, and data were acquired using Bio-Plex Data Manager Software Version 5.0 (Bio-Rad, Hercules, CA). All data were standardized to the internal control, Gapdh, and reported as mean fluorescence intensity of specific mRNA normalized to Gapdh mRNA. The cutoff value for whether or not a gene is expressed at a detectable level was conservatively set at 1% of *Gapdh* expression level. Information on method validation is available at www.panomics.com.

Statistical analysis. Statistical differences of mRNA levels between male and female mice were determined at ages after day 5 by the Student's *t*-test (JMP v. 7.0, SAS Institute, Cary, NC). Gene expression data from either male or female mice were analyzed using a two-way hierarchical clustering method (JMP v. 7.0) using Ward's minimum variance and displayed as a dendrogram.

Results

The NCBI HomoloGene Database (http://www.ncbi.nlm.nih.gov/sites/entrez/query.fcgi?db=homologene) was interrogated to find homologous genes in mice that are conserved for human *CYP* genes involved in drug metabolism. Table 1 lists the human *CYP* genes queried and their murine homologous *Cyp* genes, GeneID, mRNA accession, protein accessions, and identity of DNA and protein sequences. Homologous sequences for human *CYP2A6* and *CYP2C9* were not annotated in mice. Most human *CYP* genes have only one homologous mouse gene, but some human *CYP* genes have multiple mouse homologues. For examples, *CYP2A13* is homologous to *Cyp2a4* and *2a5*, *CYP2C8* to *Cyp2c66* and *2c65*, *CYP3A4* to *Cyp3a11*, *3a41a*, *3a41b*, and *3a44*, *CYP3A43* to *Cyp3a25* and *3a57*, while *CYP4A11* is homologous to *Cyp4a10* and *4a32*. Only one mouse

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homologue was selected for each human *CYP* gene, except two *Cyp3a*, *Cyp3a11* and *Cyp3a41b*, for *CYP3A4*, which encodes the most important CYP enzyme for xenobiotic metabolism.

Ontogenic gene expression patterns.

Cyp1 family. The mRNAs of Cyp1a1, 1a2, and 1b1 in mouse liver samples were examined but only Cyp1a2 was detected. The developmental profile for Cyp1a2 mRNA is shown in Figure 1. Cyp1a2 expression gradually increased between 1 and 20 days of ages. Between 20 and 45 days of ages, Cyp1a2 mRNA remained relatively constant.

Cyp2 family. mRNA levels were detectable in perinatal mouse livers for Cyp2a4, 2b10, 2c29, 2d22, 2e1, and 2f2, but not for 2c29, 2c66, and 2j6. As shown in Figure 2, Cyp2a4, 2c29, and 2f2 had similar expression profiles in that they were not expressed or expressed at low levels until day 10 or 15, and then dramatically increased to reach their respective plateaus. In contrast, Cyp2b10 was low at all ages, but increased steadily over the first 20-30 days of life, reaching only ~10% of Gapdh expression at days 30 and 45. Cyp2d22 displayed a similar expression profile as Cyp1a2, with expression gradually increasing from day -2 to 20 days of age, followed by a relatively constant plateau from day 20 to day 45. A distinct developmental expression was observed for Cyp2e1 in which the mRNA increased sharply from day -2 to day 0, and remained relatively constant thereafter. Sexually dimorphic expression was observed after day 30 for Cyp2a4, with higher expression in female than in male mice. Cyp2c66, and 2j6 were non-detectable in the mouse livers at all ages.

Cyp3 family. Three general profiles of ontogenic development were also observed for the Cyp3a isoforms in mouse liver (Fig. 3). Cyp3a11 increases markedly between day -2 days and day 0, then remains constant until day 15, when it increases again to another steady level. Cyp3a13 and 3a25 show a similar increase in expression shortly after birth, decrease from days 3

to 5, and then increase to steady-state levels by day 20. Cyp3a16 is expressed around birth, increases at day 0, remains relatively constant until day 20, and then decreases to a nearly undetectable level by day 45. This expression profile was also quantified by Real-Time PCR with a similar ontogenic gene expression pattern (data not shown). Cyp3a41 in male mice have a similar expression profile as Cyp3a16, in that it is expressed high before birth and increased markedly during the first three days of life. However, the decrease in Cyp3a41b in female mice was not maintained, instead it increased at 30 days of age. The gender-difference data agree with the previous funding that Cyp3a41b is a female specific enzyme in adult mouse liver (Sakuma *et al*, 2002).

Cyp4 family. Cyp4a10 was expressed at very low levels at day -2 and increased at day 1. This surge at day 1 gradually declined throughout the next several days until day 30, when another surge in expression was noted. Although Cyp4a10 was detected in perinatal livers at high levels (Fig. 4), Cyp4f18 was very low at all ages (data not shown).

Hierarchical Clustering. The expression data from male and female genes were analyzed to determine the correlation of gene expression patterns among the *Cyps* and to estimate the age that reflects a dramatic change in *Cyp* gene expression in liver. Of the 13 *Cyp* genes found to be expressed in liver and separated by gender, two-way clustering of the gene expression patterns with respect to age revealed three distinct clades of genes, which we simply define as Groups 1, 2, and 3.

In males, Group 1 consists of *Cyp3a16* and *3a41b*; Group 2 includes *2e1, 3a11*, and *4a10*; and Group 3 has *1a2, 2f2, 2a4, 2c29, 2d22, 3a25, 2b10,* and *3a13*. (Fig. 5A). According to these data, the largest correlation distance with respect to age (as shown by the clade separation on the dendrogram scale) is observed between days 15 and 20. The mean relative expression levels for

members of these groups are plotted on the expression/time curve in Figure 5B. On average, Group 1 genes are expressed higher than Groups 2 and 3 genes before birth (day -2), and increase substantially until day 3, but then decrease and remain relatively constant until 15 days old, and then decreasing to non-detectable levels after 30 days. Group 2 genes are also expressed at very low levels on day -2, but their gene expression increases more rapidly after birth, essentially reaching maximal expression levels by day 5. Group 3 genes are expressed at low levels at day -2, remain at low levels through day 10, but dramatically increase to a plateau at day 20.

Female mice also fall into three distinct groups. Group 1 only has Cyp3a16; Group 2 includes 2e1, 3a11, 3a41b, 4a10; Group 3 contains Cyp1a2, 2a4, 2f2, 2b10, 2c29, 2d22, 3a13, and 3a25 (Fig. 6A). Similarly as with males, the largest correlation distance of the Cyp gene expression levels on the dendrogram scale with respect to age is between 15 and 20 days of age. Most of the genes in female mouse liver clustered into the same groups as males, thus, it is not surprising that their average expression profiles are also similar (Fig. 6B). Only Cyp3a41b exhibits significant gender differences, and it belongs to Group 2 in females, but Group 1 in males.

Discussion

The ontogenic expression patterns of 19 xenobiotic metabolizing *Cyp* genes in C57BL/6 mouse liver were characterized throughout postnatal liver maturation using a sensitive and quantitative technique, without potentially biasing amplification steps. Thirteen of the 19 *Cyp* genes were classified as being expressed during postnatal liver maturation. Three expression patterns were identified in both male and female mice by two-way clustering analysis (Figs. 5B

and 6B). Group 1 genes are restricted to the perinatal period, and essentially disappear in the adult. Group 2 genes are expressed at low levels before birth, but increase soon after birth, and reach their maximum expression by postnatal day 5. Group 3 genes are expressed at low levels until day 10-15, but markedly increase at day 20 to a stable high level thereafter. Only Cyp3a41b shows different mRNA expression patterns between male and female mice, and is a female-specific enzyme in adult livers.

Based on hepatic ontogenic gene expression patterns, human *CYP* genes have also been categorized into three groups (Hines, 2007), but they are not completely same as the mouse patterns reported in this study. CYP genes in the first group, such as *CYP3A7*, are expressed at high levels during prenatal and neonatal stages, and then are silenced or expressed at low levels at 1-2 years after birth (de Wildt *et al*, 1999; Lacroix *et al*, 1997; Stevens *et al*, 2003). Mouse *Cyp* genes in Group 1 identified in this study have a similar expression pattern. Although not as highly expressed as *CYP3A7* in human fetal liver, mouse *Cyp3a16* (and *Cyp3a41b* in males) appear to be best correspondence to the human *CYP3A7* profile, but on a dramatically condensed time-scale. Human *CYP* genes in Hines' second group, including *CYP3A5* and *CYP2C19*, are expressed at highly variable levels throughout development, but generally independent of age (Wrighton *et al.*, 1990; de Wildt *et al*, 1999; Stevens *et al*, 2003). No mouse *Cyp* genes in the current study were expressed similarly to human *CYP3A5* or *CYP2C19*. Human *CYP* genes in the third group, such as *CYP2C9, CYP2D6, CYP2E1, and CYP3A4*, are not expressed or are expressed at low levels in the prenatal stage, but substantially increase their gene expression within the first 1-2 years of life (de Wildt *et al*, 1999; Hines, 2007; Koukouritaki *et al*, 2004; Stevens *et al*, 2008). Mouse *Cyp* genes in Group 2, including *Cyp2e1, 3a11, and 4a10* (*3a41b* in female), are somewhat similar to the human Group 3 albeit in a dramatically condensed time-

scale. For the majority of mouse *Cyps* examined (Group 3), the data show an alternative pattern not observed in human *CYPs,* where expression is relatively low until days 10-15, but then dramatically increases to adult levels by day 20.

Perhaps the most intriguing results are the marked differences in the expression profiles of the three groups of *Cyps* between days 15 and 20. Although purely speculative, one can envision that these gene expression changes observed between 15 and 20 days are in response to dietary changes, because mice transition from milk to chow during this period. If this hypothesis holds true, then dietary factors may serve as signaling molecules to initiate a cascade whereby the liver becomes capable of responding to its environment; highlighting the importance of diet on gene expression patterning. Further studies including measuring levels of individual Cyp protein isoforms and catalytic activities will be required when such technologies become available for mice.

The current study generates complementary knowledge on gene expression profiles during liver maturation to a previous study on the expression profiling of 40 mouse *Cyp* genes in embryonic and adult tissues (Choudhary *et al*, 2003). The previous study examined the expression patterns of 40 *Cyp* genes in four embryonic developmental stages (E7, E11, E15, and E17) and 8 adult tissues (8-12 weeks). The two sets of data together provide a complete picture of gene expression patterns of 12 *Cyp* genes through embryonic development (E7 to E17 before birth) and postnatal maturation (day 0 to day 45), to adult (older than 8 weeks). The two sets of data have good concordance in the most examined *Cyp* genes with one exception. Choudary *et al*. indicated *Cyp1a1* as being expressed in adult mouse liver, whereas, this set of data indicates it is not expressed in liver of adult mice. Further interrogation of the SymAtlas Gene expression database [http://symatlas.gnf.org/SymAtlas/ (Su *et al*, 2004)], a web-application for publishing

experimental gene expression datasets, support our findings for absence of gene expression of *Cyp1a1* in adult mouse liver. Furthermore, it is well recognized that *CYP1A1* is expressed at very low levels in human livers (Westerink *et al*, 2008), but remains highly inducible (Drahushuk *et al*, 1998; Olinga *et al*, 2008; Pushparajah *et al*, 2008). Because Choudary *et al.* used pooled samples from BALB/c mice, the possibility exists that the conflicting data is the result of different mouse strains used. The current study also provides ontogeny of gene expression during liver maturation in the *Cyp2c66, 2c39, 2e1, 2j6, 3a16, 3a41b,* and *4f18* genes, which were not included in the Choudary studies.

The similarity in expression between mouse and human *Cyp* homologues has previously been reported for *CYP1A1, CYP1A2,* and *CYP2E1* (Choudhary *et al.*, 2005); however these comparisons were only made during fetal and adult stages, rather than determining patterns of expression occurring during the neonatal period. The ontogenic profiles of *Cyp* gene expression in this study yield insight into the functional transition period of the liver in a critical developmental stage: after hematopoiesis and before adult.

Taken together, these data indicate at least three different patterns of gene expression exist in xenobiotic metabolizing *Cyp* enzyme genes in mice. However, the mechanisms controlling the developmental patterning of *Cyp* expression has not yet been determined. It is also not clear whether human *CYP* expression is regulated through similar mechanisms as mouse *Cyps*. Nevertheless, it is interesting to ponder why some *Cyp* genes are expressed in the perinatal period (e.g. *Cyp3a16*) whereas others are not expressed until much later (e.g. *Cyp1a2, 2a4, 2f2*). No doubt, the epigenetic environment, such as DNA methylation and histone modifications, may shed clues into the observed ontogenic expression patterns. The mechanisms responsible for the

regulation of these expression patterns may be of considerable importance in understanding kinetics of xenobiotic metabolism during the neonatal period.

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Footnotes

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Figure Legends

Figure 1. Gene expression profile for *Cyp1a2*. Each bar represent the mean from n=5 animals per age. Mice aged between days -2 to 5 were not separated by gender (*see text)* and are denoted as a mixed population. Error bars indicate standard errors. Units are expressed relative to Gapdh.

Figure 2. Gene expression profiles for *Cyp2a4, 2b10, 2c29, 2d22, 2e1,* and *2f2*. Bars represent the mean from n=5 animals per age. Mice aged between days -2 to 5 were not separated by gender (*see text)* and are denoted as a mixed population. Error bars indicate standard errors. Differences ($p<0.05$) in expression levels between males and females are indicated by an asterisk.

Figure 3. Gene expression profiles for *Cyp3a11, 3a13, 3a16, 3a25,* and *3a41b*. Bars represent the mean from n=5 animals per age. Mice aged between days -2 to 5 were not separated by gender (*see text)* and are denoted as a mixed population. Error bars indicate standard errors. *$p<0.05$.

Figure 4. Gene expression profile for *Cyp4a10*. Bars represent the mean from n=5 animals per age. Mice aged between days -2 to 5 were not separated by gender (*see text)* and are denoted as a mixed population. Error bars indicate standard errors.

Figure 5. A) Hierarchical clustering of expression profiles for 13 *Cyp* genes expressed in livers of male C57/BL6 mice. The two trees describe the relationship between different gene expression profiles (left tree) and different ages (bottom tree). The dendrogram scale represents the correlation distances. Average expression from five animals per age is given by shaded

squares. Dashed lines categorize the grouping of expression patterns into either Group 1, 2, or 3. B) Ontogenic profiles of average gene expression levels among the different groups.

Figure 6. (A) Hierarchical clustering of gene expression profiles for 13 Cyp isoforms expressed in livers of female C57/BL6 mice. The two trees describe the relationship between different gene expression profiles (left tree) and different ages (bottom tree). The dendrogram scale represents the correlation distances. Average expression from five animals per age is given by shaded squares. Dashed lines categorize the grouping of expression patterns into either Group 1, 2, or 3. (B) Ontogenic profiles of the average gene expression levels among the different groups.

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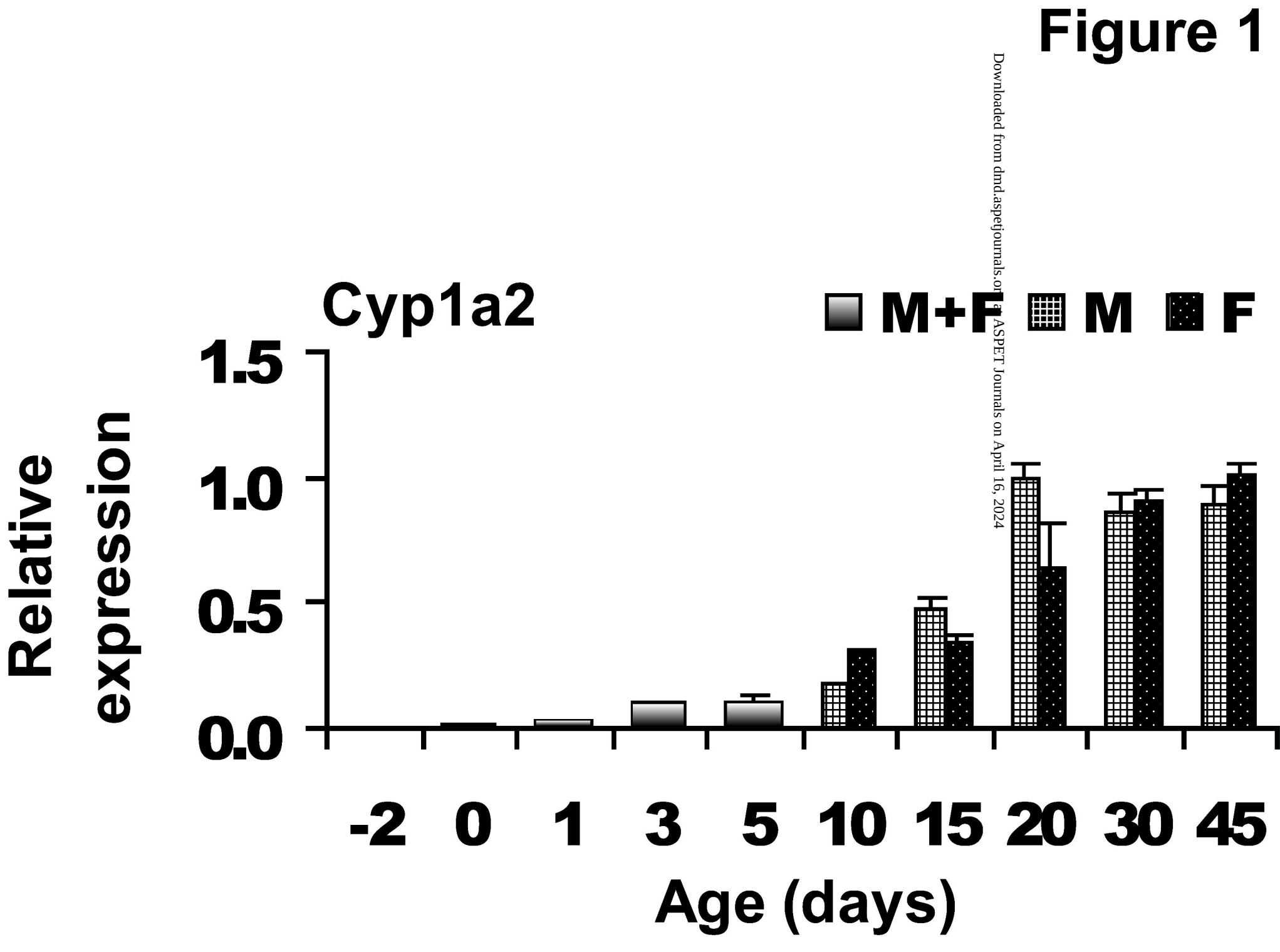
Table 1. Human *CYP* genes responsible for xenobiotic metabolism, and their homologous mouse genes.

Human *CYP* gene symbol	Mouse homologous gene symbol	Mouse gene ID	Mouse mRNA accession number	Mouse protein accession number	Identity (%) of DNA	Identity (%) of protein
CYP1A1	*Cyp1a1*	13076	NM 009992.3	NP 034122.1	82.7	80.6
CYP1A2	*Cyp1a2*	13077	NM 009993.3	NP 034123.1	79.9	73.0
CYP1B1	*Cyp1b1*	13078	NM 009994.1	NP 034124.1	81.8	81.0
CYP2A6	--	--	--	--		
CYP2A13	*Cyp2a4*	13086	NM 009997.2	NP 034127.2	84.3	86.6
CYP2B6	*Cyp2b10*	13088	NM 009999.3	NP 034129.1	78.1	75.8
CYP2C8	*Cyp2c66*	69888	NM 001011707.1	NP 001011707.1	78.4	72.4
CYP2C9	--	--	--	--		
CYP2C18	*Cyp2c39*	13098	NM 010003.1	NP 034133.1	79.1	74.2
CYP2C19	*Cyp2c29*	13095	NM 007815.3	NP 031841.3	79.8	74.7
CYP2D6	*Cyp2d22*	56448	NM 019823.3	NP 062797.3	79.9	75.8
CYP2E1	*Cyp2e1*	13106	NM 021282.2	NP 067257.1	79.4	78.1
CYP2F1	*Cyp2f2*	13107	NM 007817.2	NP 031843.2	82.6	81.9
CYP2J2	*Cyp2j6*	13110	NM 010008.4	NP 034138.3	78.3	76.2
CYP3A4	*Cyp3a11*	13112	NM 007818.3	NP 031844.1	78.1	72.8
CYP3A4	*Cyp3a41b*	53973	NM 017396.2	NP 059092.1	77.3	71.4
CYP3A5	*Cyp3a13*	13113	NM 007819.4	NP 031845.1	80.1	74.5
CYP3A7	*Cyp3a16*	13114	NM 007820.1	NP 031846.1	75.2	68.2
CYP3A43	*Cyp3a25*	56388	NM 019792.1	NP 062766.1	74.4	66.5
CYP4A11	*Cyp4a10*	13117	NM 010011.2	NP 034141.2	79.3	77.6
CYP4F3	*Cyp4f18*	72054	NM 024444.1	NP 077764.1	82.2	81.5

-- indicates no data in HomoloGene

Figure 1



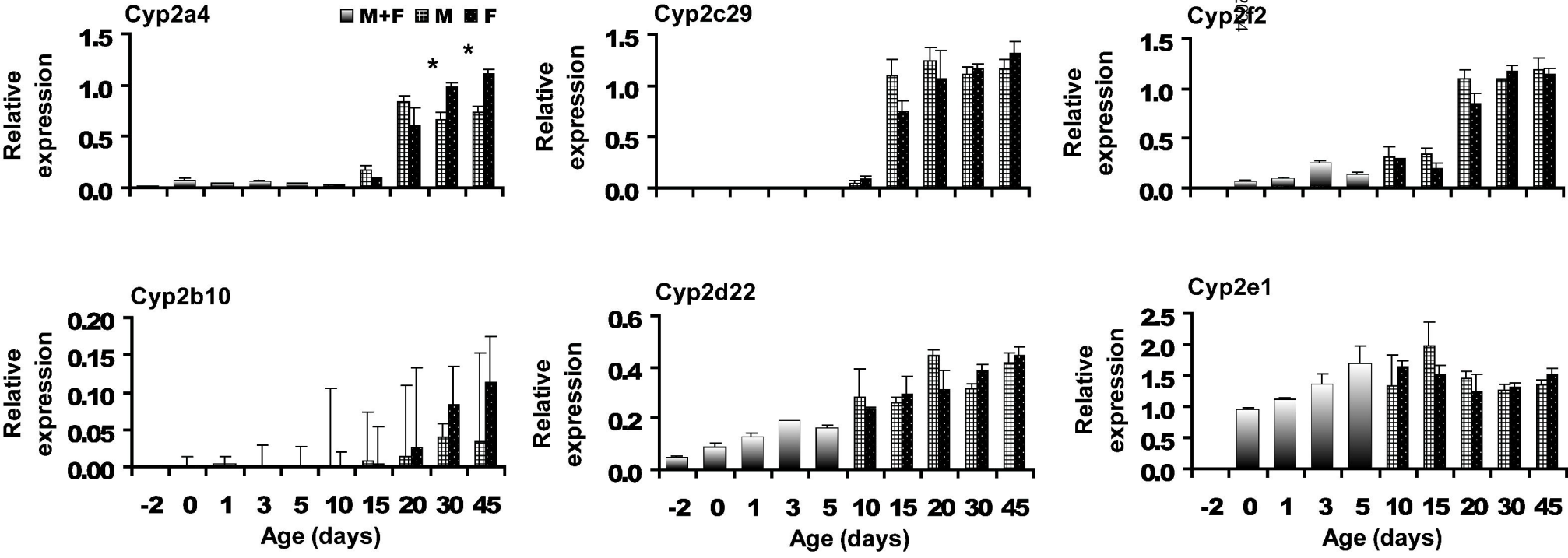


Figure 3

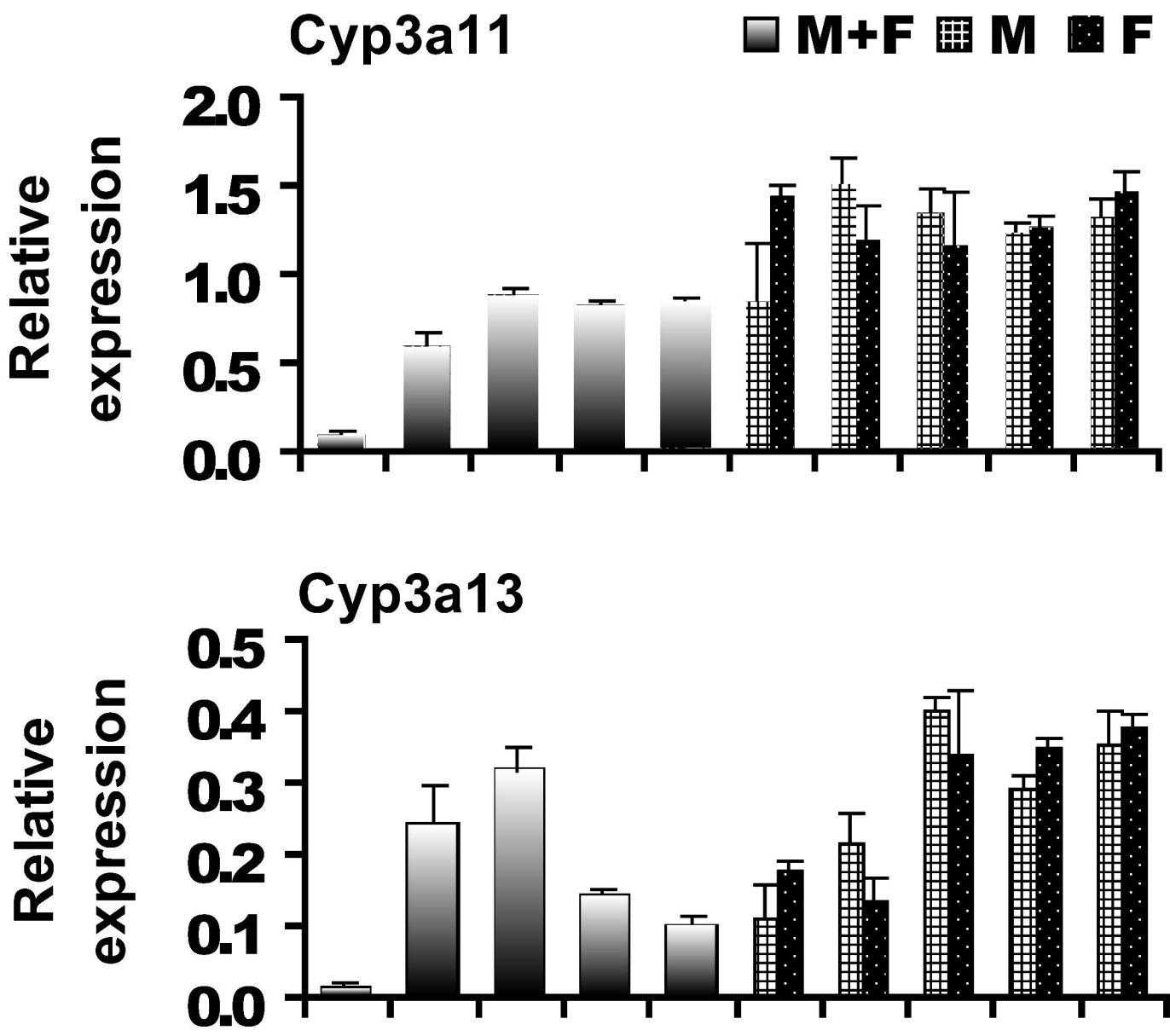


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Figure 4

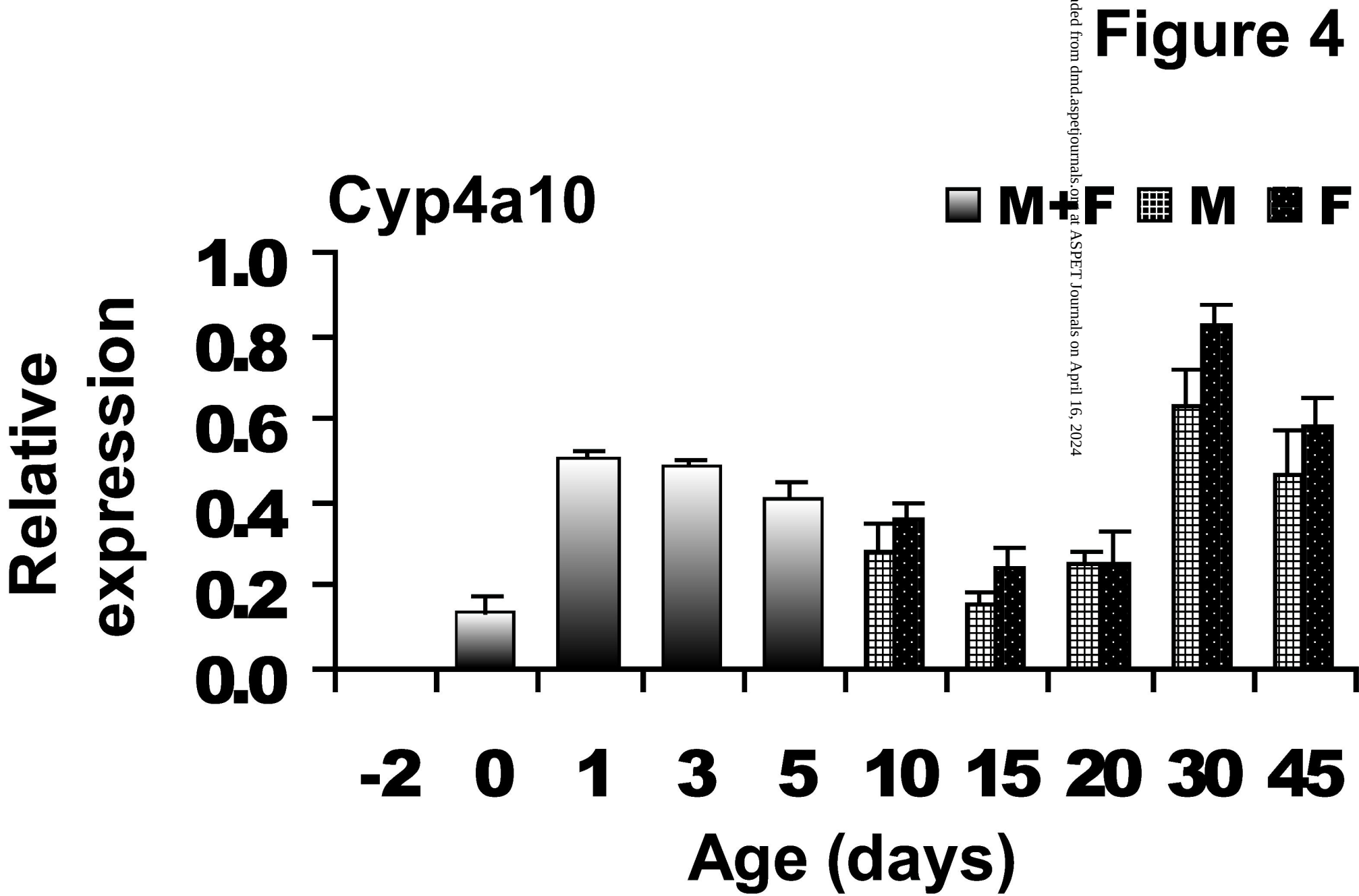
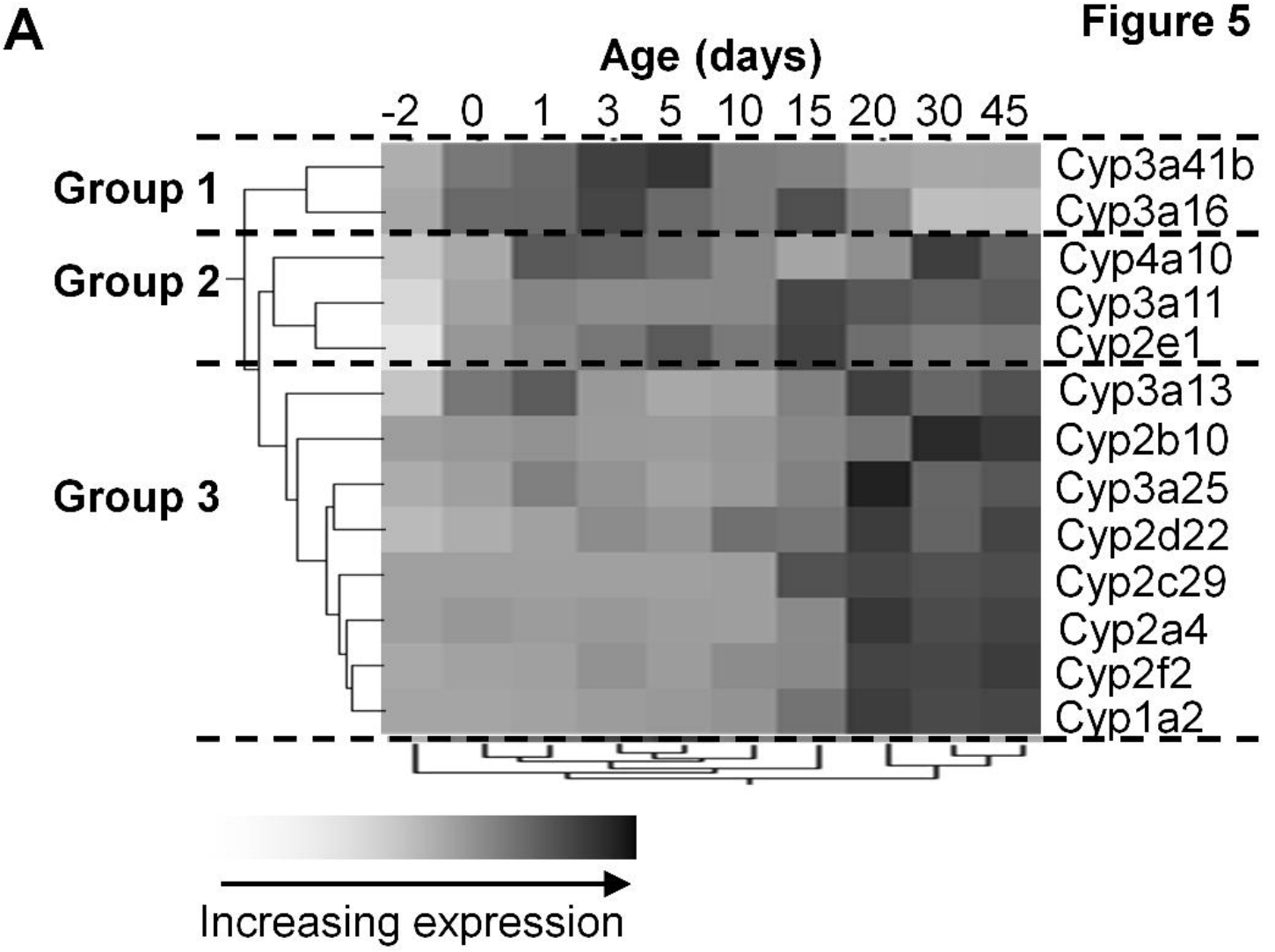


Figure 5


A
Age (days)
-2 0 1 3 5 10 15 20 30 45
Group 1
Group 2
Group 3
Cyp3a41b
Cyp3a16
Cyp4a10
Cyp3a11
Cyp2e1
Cyp3a13
Cyp2b10
Cyp3a25
Cyp2d22
Cyp2c29
Cyp2a4
Cyp2f2
Cyp1a2
Increasing expression

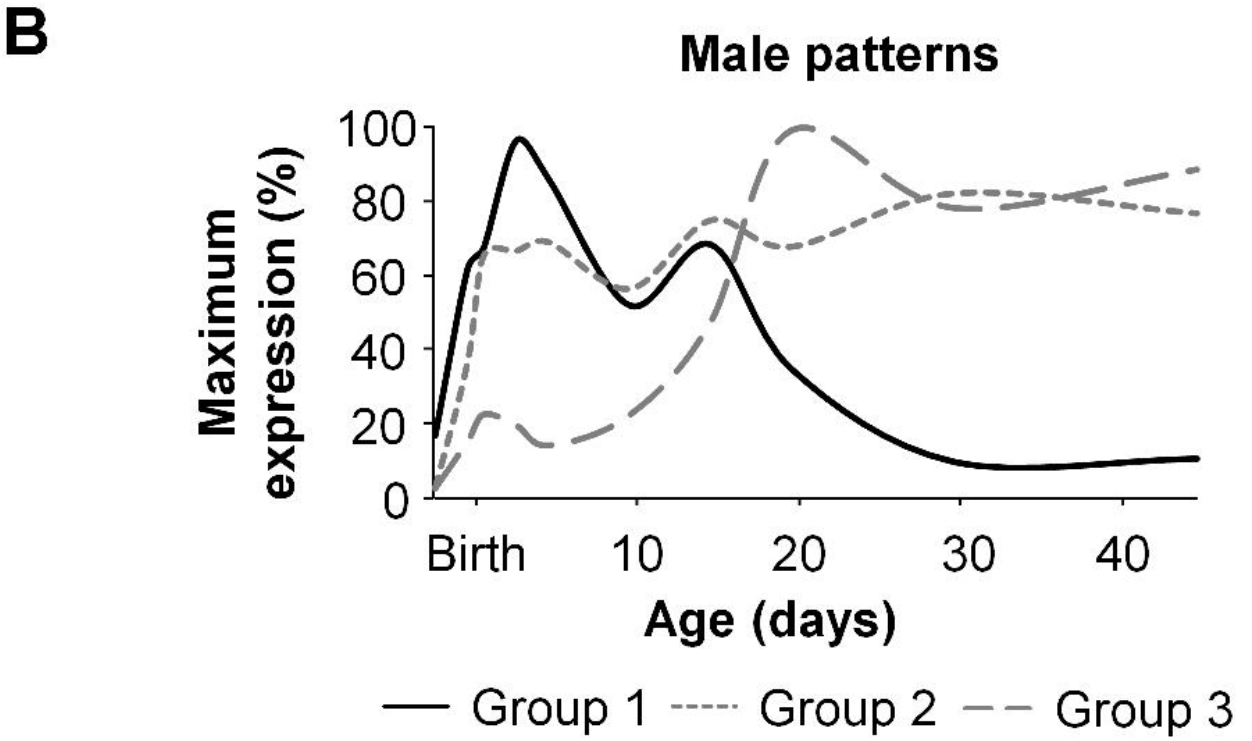
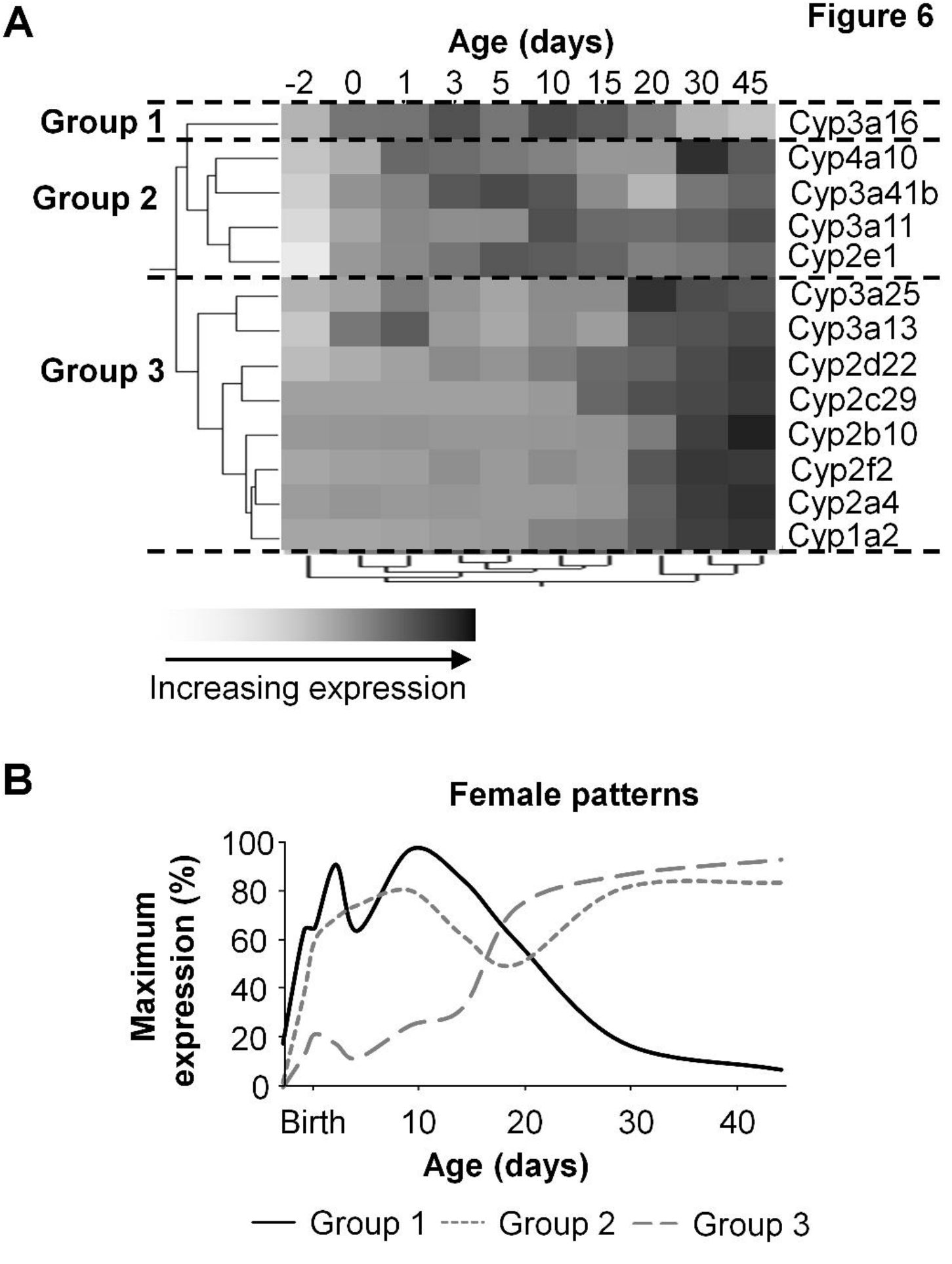

B
Male patterns
Maximum expression (%)
100
80
60
40
20
0
Birth
10
20
30
40
Age (days)
Group 1
Group 2
Group 3

Figure 6


A
Age (days)
-2 0 1 3 5 10 15 20 30 45
Group 1
Group 2
Group 3
Cyp3a16
Cyp4a10
Cyp3a41b
Cyp3a11
Cyp2e1
Cyp3a25
Cyp3a13
Cyp2d22
Cyp2c29
Cyp2b10
Cyp2f2
Cyp2a4
Cyp1a2
Increasing expression
B
Female patterns
Maximum expression (%)
100
80
60
40
20
0
Birth
10
20
30
40
Age (days)
Group 1
Group 2
Group 3