A peer-reviewed version of this preprint was published in PeerJ on 2 August 2019.

View the peer-reviewed version (peerj.com/articles/7429), which is the preferred citable publication unless you specifically need to cite this preprint.

Xu S, Hu A, Xie L, Liu J, Wu Q, Liu J. 2019. Age-associated changes of cytochrome P450 and related phase-2 gene/proteins in livers of rats. PeerJ 7:e7429 https://doi.org/10.7717/peerj.7429

Age-associated changes of cytochrome P450 and related phase-2 gene/proteins in livers of rats

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Cytochrome P450s (CYPs) are phase-I metabolic enzymes playing important roles in drug metabolism, dietary chemicals and endogenous molecules. Age is a key factor influencing P450s expression. Thus, age-related changes of CYP 1-4 families and bile acid homeostasis-related CYPs, the corresponding nuclear receptors and a few phase-II genes were examined. Livers from male Sprague-Dawley rats at fetus (-2 d), neonates (1, 7, and 14 d), weanling (21 d), puberty (28 and 35 d), adulthood (60 and 180 d), and aging (540 and 800 d) were collected and subjected to qPCR analysis. Liver proteins from 14, 28, 60, 180, 540 and 800 days of age were also extracted for selected protein analysis by Western-blot. In general, there were three patterns of their expression: Some of the drug-metabolizing enzymes and related nuclear receptors were low in fetal and neonatal stage, increased with liver maturation and decreased quickly at aging (AhR, Cyp1a1, Cyp2b1, Cyp2b2, Cyp3a1, Cyp3a2, Ugt1a2); the majority of P450s (Cyp1a2, Cyp2c6, Cyp2c11, Cyp2d2, Cyp2e1, CAR, PXR, FXR, Cyp7a1, Cyp7b1. Cyp8b1, Cyp27a1, Ugt1a1, Sult1a1, Sult1a2) maintained relatively high levels throughout the adulthood, and decreased at 800 days of age; and some had an early peak between 7 and 14 days (CAR, PXR, PPARα, Cyp4a1, Ugt1a2). The protein expression of CYP1A2, CYP2B1, CYP2E1, CYP3A1, CYP4A1, and CYP7A1 corresponded the trend of mRNA changes. In summary, this study characterized three expression patterns of 16 CYPs, 5 nuclear receptors, and 4 phase-II genes during development and aging in rat liver, adding to our understanding of age-related CYP expression changes and age-related disorders.

1 **Title page for PeerJ**

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11 Running Title Page

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17

18 **Abbreviations:**

19 AhR, aryl hydrocarbon receptor; BA, Bile acid; CAR, constitutive androstane receptor;

20 CYPs, cytochrome P450s; FXR, Farnesoid X receptor; NRs, nuclear receptors; PPAR,

21 peroxisome proliferator-activated receptor; PXR, pregnane X receptor (PXR); SHP,

22 small heterodimer partner; SULTs, Sulfotransferases; TCDD, tetrachlorodibenzodioxin;

23 UGTs, UDP-glucuronosyltransferases;

25 **Highlights (review only):**

26 • Livers of rats at -2, 1, 7, 14, 21, 28, 35, 60, 180, 540 and 800 days were
27 analyzed.
28 • A total of 16 cytochrome P450s, 5 nuclear receptors, 4 phase-II genes were
29 examined.
30 • Three patterns of age-associated gene expressions were characterized.
31 • Protein of CYP1A2, CYP2B1, CYP2E1, CYP3A1, CYP3A4, CYP4A1, and
32 CYP7A1 verified PCR.
33 • Age-associated P450 gene expressions in relation to diseases were discussed.

ABSTRACT

Cytochrome P450s (CYPs) are phase-I metabolic enzymes playing important roles in drug metabolism, dietary chemicals and endogenous molecules. Age is a key factor influencing P450s expression. Thus, age-related changes of CYP 1-4 families and bile acid homeostasis-related CYPs, the corresponding nuclear receptors and a few phase-II genes were examined. Livers from male Sprague-Dawley rats at fetus (-2 d), neonates (1, 7, and 14 d), weanling (21 d), puberty (28 and 35 d), adulthood (60 and 180 d), and aging (540 and 800 d) were collected and subjected to qPCR analysis. Liver proteins from 14, 28, 60, 180, 540 and 800 days of age were also extracted for selected protein analysis by Western-blot. In general, there were three patterns of their expression: Some of the drug-metabolizing enzymes and related nuclear receptors were low in fetal and neonatal stage, increased with liver maturation and decreased quickly at aging (AhR, Cyp1a1, Cyp2b1, Cyp2b2, Cyp3a1, Cyp3a2, Ugt1a2); the majority of P450s (Cyp1a2, Cyp2c6, Cyp2c11, Cyp2d2, Cyp2e1, CAR, PXR, FXR, Cyp7a1, Cyp7b1. Cyp8b1, Cyp27a1, Ugt1a1, Sult1a1, Sult1a2) maintained relatively high levels throughout the adulthood, and decreased at 800 days of age; and some had an early peak between 7 and 14 days (CAR, PXR, PPARα, Cyp4a1, Ugt1a2). The protein expression of CYP1A2, CYP2B1, CYP2E1, CYP3A1, CYP4A1, and CYP7A1 corresponded the trend of mRNA changes. In summary, this study characterized three expression patterns of 16 CYPs, 5 nuclear receptors, and 4 phase-II genes during development and aging in rat liver, adding to our understanding of age-related CYP expression changes and age-related disorders.

Keywords: Ontogeny; Aging; Cytochrome P450s; Nuclear receptors; Rat liver, mRNA/protein expression.

Introduction

Cytochrome P450s (CYPs) are phase-I metabolic enzymes playing important roles in drug metabolism, dietary chemicals, as well as endogenous molecules in the liver. CYP1, 2, 3, and 4 families are responsible for the biotransformation of most foreign substances including 70-80% of all drugs in clinical use, CYP 4 families also participate in lipid metabolism.(Cui et al. 2012; Zanger & Schwab 2013) The CYP7 families, together with CYP8 and CYP27 are important for cholesterol and bile acid metabolism and homeostasis.(Cuesta de Juan et al. 2007; Liu et al. 2014)

CYPs are regulated by many physiological, genetic, environmental, and pathological factors. For example, CYPs expression can be affected by hormones (Daskalopoulos et al. 2012), cytokines (Kot & Daujat-Chavanieu 2018), pregnancy,(He et al. 2005) sex, (Agrawal & Shapiro 2003; Das et al. 2014) and age. CYPs are subjected to age-dependent changes in cell differentiation(Czekaj et al. 2010) and epigenetic regulation,(Li et al. 2009) and age-related metabolic syndrome,(Bondarenko et al. 2016) kidney diseases,(Velenosi et al. 2012) diabetes,(Park et al. 2016) nonalcoholic steatohepatitis,(Li et al. 2017) virus hepatitis, and cirrhosis.(Kirby et al. 1996)

Age of animals greatly affects drug metabolism,(Durnas et al. 1990) alters pharmacokinetics of xenobiotics,(Matalova et al. 2016; Shi & Klotz 2011) and thus alters the sensitivity to drugs and toxicants such as acetaminophen,(Mach et al. 2014) isoniazid,(Mach et al. 2016) aflatoxin B1,(Kirby et al. 1996; Wang et al. 2018) and thioacetamide.(Kang et al. 2008) Age also influences drug-drug interactions.(Jia et al. 2014) Age-associated changes in P450 and corresponding nuclear factors are a major determinant in CYP regulation of drug metabolism, especially during development

82 (children) and in senescence (elderly).(Durnas et al. 1990; Kilanowicz et al. 2015; Shi &
83 Klotz 2011)

84 The expression and maturation of CYPs during development is a major topic of
85 research,(Kilanowicz et al. 2015) and immature rats have been proposed as a potential
86 model for xenobiotics risk evaluation for children.(McPhail et al. 2016) The ontogeny of
87 CYPs greatly affects the drug metabolism especially during the developmental
88 period,(de Zwart et al. 2008) and is the major cause of altered susceptibility to drugs
89 and toxicants in children.(Li et al. 2017; Yun et al. 2010)

90 Aging is a physiological process characterized by progressive functional decline in
91 various organs over time. Aging is an important factor leading to alterations in the
92 biotransformation, either by reduced expression or decreased function. Many
93 cytochrome P450 genes from CYP 1-3 families show decreased expression in the older
94 rats.(Yun et al. 2010) The ability of liver CYPs to metabolize xenobiotics decreases with
95 aging *in vitro* (Salmin et al. 2017) and *in vivo,*(Wauthier et al. 2007) and hepatic CYP
96 mRNA expressions are decreased with aging.(Mori et al. 2007) The ability of CYPs in
97 response to inducers such as phenobarbital is also decreased in old rats.(Agrawal &
98 Shapiro 2003) Age-associated CYP3A expression changes in the liver are more
99 remarkable as compared to that occurred in the intestine and kidney, and are tissue-
100 specific.(Warrington et al. 2004) Since P450 enzymes in humans are regulated in a
101 manner similar to that in animals.(Durnas et al. 1990; Wauthier et al. 2007) Thus, to
102 examine CYP expressions in the old laboratory animals would help evaluation of drug
103 metabolism, efficacy and toxicity in the elderly.

In humans, three patterns of drug metabolizing enzymes are proposed.(Hines 2008) The first pattern (e.g.,CYP3A7) is expressed at the highest level at their highest level during the first trimester and either remains at high concentrations or decreases during gestation, but is silenced or reduced within one to two years after birth; the 2nd pattern (e.g.,SULT1A1) is expressed at relatively constant levels throughout gestation and minimal changes are observed postnatally; and the 3rd pattern (e.g.ADH1C) is not expressed or is expressed at low levels in the fetus.(Hines 2008; Hines 2013) Age-associated changes of drug metabolism in humans, especially during stages before birth and during early development (neonate/infant/child), could be studied in laboratory animals.(Hines 2013; Saghir et al. 2012)

We have collected liver samples of Sprague Dawley (SD) rats from prenatal (-2 d), neonatal (1, 7, and 14 d), weanling (21 d), puberty (28 and 35 d), adulthood (60 and 180 d), and aging (540 and 800 d), and have published several papers related to hepatic uptake Oatp transporters(Hou et al. 2014) and hepatic efflux MRP transporters.(Zhu et al. 2017) We have also used these samples to characterize the Nrf2 antioxidant pathways,(Xu et al. 2018b) glutathione S-transferases,(Xu et al. 2018a) and the antioxidant metallothionein gene expression.(Hou WY 2014) The goals of the current study were to use these samples to quantify the expression of 16 major CYP isoforms in rat livers, 5 corresponding nuclear receptors (NRs), and 4 phase-II conjugation genes. Protein expressions of selected CYPs were also performed to confirm qPCR results. Similar to three patterns of CYP expression during mouse liver development,(Hart et al. 2009) the current study identified three patterns of CYP expression in the liver of rats at 11 time points of entire life span to help our understanding the age-associated changes

127 in these important phase-I and phase-II drug metabolism genes, and age-associated

128 disorders.

129

130 **Materials and Methods**

131 **Animals.** Adult male and female SD rats (250-300 g, 10 males and 30 females)
132 were purchased from the Experimental Animal Center of Third Military Medical
133 University (Chongqing, China; Certificate No: CXK 2007–0005). Rats were kept in a
134 SPF-grade animal Facilities with controlled environment (22 ± 1 °C, 50 ± 2% humidity
135 and a 12 h: 12 h light: dark cycle) at Key Lab for Basic Pharmacology of Ministry of
136 Education. Rats had free access to purified water and standard laboratory chow
137 (Experimental Animal Center, Chongqing, China). All animal care and experimental
138 protocols were complied with the Animal Management Guidelines of China and
139 approved by the Animal Use and Care Committee of Zunyi Medical University
140 (2012−02).

141 **Sample collection.** Rats were acclimatized for one week before timely mating
142 overnight and a positive vaginal plug next morning was considered as gestation day 1.
143 Livers of offspring male rats were collected at gestation day 19 (−2 d), at birth (1 d), at
144 the neonatal stage (7 and 14 d), at weanling (21 d), at puberty (28 and 35 d), at the
145 adulthood (60 and 180 d), and at aging (540 and 800 d). Six samples per time point
146 were collected, however, n=4-5 was used to for a 96-well qPCR plate to hold all time
147 points. Rats were anesthetized by chloral hydrate (10%, 5 mL/kg, ip), followed by
148 decapitation to minimize potential pain and distress. Liver tissues were stored at −80
149 °C prior to analysis.

150 **Real-time RT-PCR analysis.** Liver total RNA was extracted by using RNAiso
151 Plus kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The quality and quantity of
152 total RNA were determined by nanodrop and the 260/280 nm ratio > 1.8. The total RNA

153 was reverse transcribed to cDNA (Applied Biosystems, Foster City, CA) and real-time
154 RT-PCR analysis (Bio-Rad Laboratories, Hercules, CA) was conducted as described
155 (Xu et al. 2018b). Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method and
156 normalized to the house-keeping gene β-actin or GAPDH (results were similar, data not
157 shown), and expressed as relative transcript levels, setting controls as 100%. The
158 primer sequences used in this study were shown in the Supplemental Table 1.

159 **Western-blot analysis.** Liver tissues (50-100 mg) were homogenized in RIPA
160 lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing 1 mM
161 phenylmethanesulfonyl fluoride (PMSF) and freshly prepared proteinase inhibitors.
162 Protein concentrations were quantified by the BCA assay (Beyotime Institute of
163 Biotechnology, Shanghai, China) and denatured (90 °C, 10 min with Nupage Loading
164 buffer). Aliquoted proteins (30 μg) were separated on NUPAGE 10% BT gels (Thermo
165 Fisher Scientific, Waltham, MA) and transferred to PVDF membranes. After blocking
166 with 5% nonfat milk at room temperature for two hours, membranes were incubated with
167 primary mouse antibody against β-actin, rabbit polyclonal antibodies against rat
168 CYP1A2 (bs-2589R), CYP2B1 (bs-14177R), CYP2E1 (bs-4562R), CYP3A1 (bs-
169 20586R), CYP4A1 (bs-5054R) and CYP7A1 (bs-21429R) (1:1000) (Biosynthesis
170 Biotechnology Co., LTD. Beijing, China) overnight at 4 °C. After washes with TBST,
171 membranes were incubated with horseradish peroxidase conjugated anti-rabbit, anti-
172 mouse IgG secondary antibodies (1:5000) for 1 h at room temperature. Protein antibody
173 complexes were visualized using an Enhanced Chemiluminescent reagent and a
174 ChemiDoc XRS system (Bio Rad Laboratories, Inc., USA). Band intensities were semi-
175 quantified by densitometry using Quantity One® software (version 4.6.2, Bio Rad

176 Laboratories, Inc., USA) (Xu et al. 2018a).

177 **Statistical analysis.** The software SPSS version 16.0 (SPSS, Inc., Chicago, IL,
178 USA) was used for statistical analysis. Data were expressed as the mean ± SEM (n= 4-
179 5 per time point). Age associated differences were analyzed by one-way analysis of
180 variance, followed by the least significant difference post hoc test, $p < 0.05$ was
181 considered to indicate a statistically significant difference from the levels of birth.

182

183 **Results**

184

185 ***Age-related expression of CYP-1 family***

186 The expression of CYP-1 family is shown in Figure 1. Aryl hydrocarbon receptor
187 (AhR) mainly mediates the expression of CYP1A1 and CYP1A2 proteins. 2,3,7,8-
188 Tetrachlorodibenzodioxin (TCDD) is a typical CYP1A1 inducer. When TCDD is
189 combined with AhR, AhR is dissociated from the complex and transferred to the nucleus.
190 It forms heteromeric dimers with AhR nuclear transport protein, and then induces the
191 expression of target genes. In this way, TCDD and other AhR activators significantly
192 induce the expression of CYP1A genes.(Aleksunes & Klaassen 2012) AhR was low in
193 fetal livers (-2d), and gradually increase after birth, and reached the highest levels at 35
194 days of age (6.7-fold of birth), and gradually declined, and at 800 days of age, it
195 remained 4.2-fold higher than at birth (Figure 1A). Cyp1a1 was low at -2 days of age
196 through 14 days of age, and begin to increase at 21 days of age, rapidly increased 48-
197 fold at 28-60 days of age, and decline rapidly after 60 days of age, and returned to 2.4-
198 fold of the birth level at 800 days of age (Figure 1B). In contrast, Cyp1a2 followed the

199 similar pattern as AhR. Cyp1a2 increased dramatically after birth, reached 250-fold at
200 weanling (21 day), and peaked on 35 days of age (1100 fold). It was gradually declined
201 afterwards. Similar to AhR, Cyp1a2 still remained at the high level at 800 days of age
202 (880 fold of birth). CYP1A2 protein expression followed the similar pattern (Figure 1C).
203

204 ***Age-related expression of CYP-2 family***

205 The expression of CYP-2 family is shown in Figure 2. Constitutive androstane
206 receptor (CAR) is a nuclear receptor of steroid hormones. It regulates the metabolizing
207 enzymes and transporters in liver and small intestine. CAR mediates endogenous
208 hormone or exogenous drug reactions, such as phenobarbital, and transcriptionally
209 regulates CYP2 expression.(Aleksunes & Klaassen 2012) CAR was low in fetal livers (-
210 2d), and gradually increase after birth, first peaked at 7 days of age, and reached the
211 highest levels at 60 days of age (6-fold of birth), and gradually declined, and at 800
212 days of age, it remained 2.3-fold higher than at birth (Figure 2A). Cyp2b2 was low in
213 fetal livers (-2 d), and begin to increase after birth, and rapidly increased at weaning (21
214 d), reaching the peak at 35 days of age (7-fold of birth) and decreased afterwards, and
215 returned to birth level after 540 days of age (Figure 2B). The expression of Cyp2b1
216 followed the similar pattern as Cyp2b2, and the expression of CYP2B1 protein followed
217 the similar pattern (Figure 2C). Cyp2c6 increased after weanling, reached the peak of
218 liver maturation (350 fold) at 60 days of age, and remained high till 540 days of age, and
219 decreased to 250-fold over birth levels at 800 days of age (Figure 2D). Cyp2c11
220 increased 800-fold at puberty (35 days of age), but dramatically increased with liver
221 maturation (60000-fold at 60 days, 98000-fold at 180 days, and 110000-fold at 540 days

222 of age), and rapidly decreased at aging of 800 days of age, but it was still 40000-fold
223 over the birth level. (Figure 2E). Cyp2d2 increased gradually after birth, reached the
224 peak (9-fold) of liver maturation at 35 -180 days of age, and decreased to 6-fold of birth
225 at 540 and 800 days of age (Figure 2F). The expression pattern of Cyp2e1 was
226 relatively stable: Cyp2e1 increased rapidly after birth, reached 30-fold of the birth levels
227 at weanling (21 days of age), and peaked on 35 days of age (35 fold). It was gradually
228 declined but remained at the high level at the age of 800 days (22 fold of the birth level).
229 CYP2E1 protein expression followed the similar pattern (Figure 2G).
230

231 ***Age-related mRNA expression of CYP-3 family***

232 The expression of CYP-3 family is shown in Figure 3. Pregnane X receptor (PXR)
233 is a highly conserved ligand-dependent transcription factor. It is mainly expressed in the
234 liver and partly expressed in the colon and small intestine. The regulation of CYP3A by
235 PXR-mediated signaling pathway is an important pathway of drug
236 metabolism.(Aleksunes & Klaassen 2012) The expression of PXR was relatively stable
237 throughout the life with approximately 2-fold variations (Figure 3A). The expression of
238 Cyp3a2 markedly increased 4 fold at 7 days of age, and rapidly increased after
239 weanling (21 days of age), reached the peak at 28 days of age (25-fold of birth) and
240 decreased gradually afterwards, and the level was still 5.4-fold of the birth level at 800
241 days of age (Figure 3B). Cyp3a1 follows similar pattern as Cyp3a2. It was markedly
242 increased after weanling, reached the peak at 28 days of age (13-fold of the birth level)
243 and decreased gradually afterwards, and the level was still 3.4-fold of the birth level at

244 800 days of age. The expression of Cyp3a1protein followed the similar patter (Figure
245 3C).
246
247 ***Age-related expression of CYP-4 family***
248 The expression of CYP-4 family is shown in Figure 4. Peroxisome proliferator-
249 activated receptors (PPARs) nuclear receptor family regulates the expression of genes
250 that control fatty acid synthesis, storage, and catabolism. PPARs mainly include PPARα,
251 PPARβ and PPARγ. The activation of PPAR can improve insulin resistance, slow down
252 atherosclerosis, and promote the metabolism of cholesterol. PPARα regulates induction
253 of CYP4A gene.(Aleksunes & Klaassen 2012) The expression of PPARα was relatively
254 stable throughout the life, except for 7 and 14 days of age (2-fold of the birth level), and
255 at 800 days of age, its levels returned to the birth level (Figure 4A). Cyp4a1 started to
256 increase after birth, with the first peak at 7 days (3.5-fold) and decreased after 14 days
257 of age, and again gradually increased after weanling and remained high throughout 540
258 days of age. At 800 days of age, it was still 1.8-fold of the birth level. The expression of
259 CYP4A1 protein followed the similar pattern (Figure 4B).
260
261 ***Age-related mRNA expression of CYPs involved in bile acids homeostasis***
262 The expression of CYPs involved in cholesterol and bile acids homeostasis is
263 shown in Figure 5. Bile acids (BAs) are the endogenous ligands of farnesoid X receptor
264 (FXR), so FXR is also called the BA receptor. Cholesterol 7α hydroxylase (Cyp7a1) is
265 important for BA synthesis. When BA overloads in the liver, toxicity to liver cells occurs,
266 including oxidative stress, inflammation, necrosis and even cirrhosis.(Cuesta de Juan et

267 al. 2007; Liu et al. 2014) The expression of FXR increased at 14 days of age (5 fold),
268 and gradually increased afterwards with age, reached peak at 180 days (13 fold), and
269 still high at 800 days (6.8 fold) (Figure 5A). Cyp7a1 started to increase at 14 days of
270 age, reached the peak at 28 days (37 fold of birth), and remained high throughout the
271 adulthood, and was still 11-fold higher than the birth level at 800 days of age. The
272 expression of CYP7A1 protein followed similar pattern (Figure 5B). The expression of
273 Cyp7b1 started to increase after birth, reached the peak at 28 days of age, and
274 decreased at 800 days of age (Figure 5C). Cyp8b1 increased at birth and decreased at
275 7 days of age. Cyp8b1 started to increase again after weanling, reached the peak at
276 adulthoods at 180 days of age, and gradually decreased thereafter. It was still higher at
277 800 days of age (Figure 5D). The expression of Cyp27a1 held a similar pattern,
278 reached the peak on 35 days of age (5.8-fold over the birth level), and remained the
279 high levels throughout 540 days of age, and decreased at 800 days of age with 3-fold
280 higher over the birth level (Figure 5E).
281
282 ***Age-related mRNA expression of UGT and SULT families***
283 The expression of UGT and SULT families is shown in Figure 6. UDP-
284 glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) are the two most
285 important phase-2 conjugation enzymes to conjugate the CYP catalyzed metabolites
286 and drugs contain functional groups such as hydroxyls and carboxylic acids.(Coughtrie
287 2015) Glucuronidation involves the reaction of uridine 5′-diphosphoglucuronic acid with
288 a number of functional groups generated from CYP metabolism and is a major
289 mechanism for the formation of water-soluble substrates for their elimination in bile or in

urine, especially for the clearance of a number of drugs in children.(Krekels et al. 2012) SULTs transfer the sulfuryl moiety from the universal donor PAPS (3'-phosphoadenosine 5'-phosphosulfate) to a wide variety of substrates with hydroxyl- or amino-groups after CYP metabolism.(Coughtrie 2016) Ugt1a1 was low in fetal livers (-2 d), and gradually increase after birth, marked increase after weanling (21 days), and reached the highest levels at 28 days of age (7.8-fold of birth), and gradually declined, and at 800 days of age, it remained 4-fold higher than the level of birth (Figure 6A). The expression of Ugt1a2 was relatively stable throughout the life, except for a small peak at day 28 and 35 days of age (2.5-fold higher than the birth level) (Figure 6B). The expression of Sult1a1 started to increase at 14 days of age, reached the peak at 180 days of age (20-fold), remained 14-fold higher than the birth levels (Figure 6C). The expression of Sult1a2 started to increase at 14 days of age, reached the first peak at weanling (15 fold), but dramatically increased after 35 days of age, reached 110-fold at 60 days of age, and 190-fold at 540 days of age. It remained 70-fold higher than the levels of birth (Figure 6D). The expression pattern of Sult1a1 was quite similar to the expression pattern of Cyp2c11.

308 **Discussion**

309 The present study characterized age-related expression of 16 CYPs, 5 NRs, and 4
310 phase-II genes in livers of rats across 11 time points from fetus (-2 d), neonates (1, 7,
311 14 and 21 d), puberty (28 and 35 d), adulthood (60 and 180 d), to aging (540 and 800 d).
312 In general, there are three patterns of their expression: (1) The expressions of AhR,
313 Cyp1a1, Cyp2b1, Cyp2b2, Cyp3a1, Cyp3a2, and Ugt1a2 were low in fetal and neonatal
314 stage, increased with liver maturation and decreased extensively after 180 days; (2) the
315 majority of CYPs and other genes maintained relatively high levels throughout the
316 adulthood and decreased at aging of 540 and/or 800 days; (3) the expression of CAR
317 PXR, PPARα, Cyp4a1, Ugt1a2 had a first peak between 7-14 days of age. The protein
318 expression of CYP1A2, CYP2B1, CYP2E1, CYP3A1, CYP4A1, and CYP7A1 followed
319 the trend of mRNA changes. Characterization of CYPs in rat entire life span provides
320 fundamental information for drug metabolism and pharmacology studies in children and
321 elderly.

322 There are three unique features of the current study: (1) Most of the studies on age-
323 related changes in hepatic CYPs are performed in mice,(Cui et al. 2012; Hart et al. 2009;
324 Li et al. 2009) this study characterized CYPs and NRs in rats, another commonly-used
325 laboratory animals; (2) Most of the age-related changes in hepatic P450 are focused on
326 the developmental stages to maturation,(Asaoka et al. 2010; Bondarenko et al. 2016;
327 Cui et al. 2012; de Zwart et al. 2008; Kilanowicz et al. 2015; Park et al. 2016) and this
328 study covered the whole life span, and (3) this study extended our efforts to
329 characterize age- and sex-related changes in hepatic drug transporters(Hou et al. 2014;
330 Zhu et al. 2017) and defense mechanisms. (Hou WY 2014; Xu et al. 2018a; Xu et al.

331 2018b)

332

333 ***P450-1 family***

334 CYP1 family is responsible for activation of toxicants and drugs. Cyp1a1 is
335 expressed very early in rodents and involved in developmental toxicity of
336 hexachloronaphthalene,(Kilanowicz et al. 2015) and immature rats has been proposed
337 as a potential model for chemical risks in children.(McPhail et al. 2016) Ontogeny of
338 hepatic CYP1A2 showed it rapidly increased after weanling, followed by significant
339 decrease during adulthood,(Elbarbry et al. 2007) However, CYP1A1/2 activity did not
340 change when fed Zuker diabetic fatty rats with high fat diet at 5 week (insulin resistant
341 stage) and 11-week (diabetic stage).(Park et al. 2016) Glycyrrhetinic acid potentiation
342 of clozapine hepatotoxicity is associated with CYP1A2 induction and suppression of
343 CYP2C11 and 2C13.(Jia et al. 2014) CYP1A1 and CYP1A2 are regulated differently,
344 CYP1A1 decreased rapidly after maturation, while CYP1A2 remained the high
345 expression levels till 104 weeks.(Yun et al. 2010) The present results agreed with the
346 literature.

347

348 ***P450-2 family***

349 Hepatic CYP2C2 and CYP2C11 decreased with age, along with CYP3A enzyme
350 genes.(Mori et al. 2007) In diethyl nitrosamine-induced liver insufficiency, the altered
351 expression of cytokines might contribute to CYP2C and CYP3A isoform regulation. (Kot
352 & Daujat-Chavanieu 2018) The expression of CYP2C11 is under the regulation of
353 growth hormones,(Das et al. 2014) as well as the dopaminergic

354 receptors.(Daskalopoulos et al. 2012) The expressions of CYP2C, CYP2E1, and
355 CYP3A are influenced by metabolic syndrome.(Bondarenko et al. 2016) CYP2C11 is a
356 male-specific CYP, its expression began to increase after weanling, and at puberty
357 reached 830-fold over the birth, and further dramatically increased over 100000-fold
358 over the birth level throughout the adulthood, but rapidly decreased at aging, consistent
359 with the literature.(Agrawal & Shapiro 2003; Das et al. 2014; Yun et al. 2010)
360 Age-associated CYP2E1 expression has important implications in pharmacology
361 and toxicology. Old rats have decreased CYP2E1, altered acetaminophen
362 pharmacokinetics, resulting in less sensitive to acetaminophen toxicity.(Mach et al. 2014)
363 On the other hand, higher CYP2E1 in young rats might be a reason of increased
364 sensitivity to isoniazid toxicity.(Mach et al. 2016) The observed expression pattern for
365 CYP2B1 (marked decreases at 800 days of age) and CYP2E1 (slight decreases at 800
366 days of age) are in agreement of the literature.(Yun et al. 2010) CYP2E1 plays roles in
367 thioacetamide hepatotoxicity, as Cyp2e1-/- mice are less sensitive to liver injury.(Kang
368 et al. 2008) Age-related differences in CYP2E1 levels can have important implications
369 for the toxic and carcinogenic actions of some hydrocarbons (e.g., benzene, hexane)
370 and short-chain halocarbons, such as carbon tetrachloride.(McPhail et al. 2016)
371
372
373 ***P450-3 family***
374 CYP3 families are responsible for most drug metabolism.(Zanger & Schwab
375 2013) In rats with chronic kidney diseases, CYP3A and CYP2C mediated metabolism
376 are decreased.(Velenosi et al. 2012) Using liver microsomes (S9) from young and old

377 rats, severe metabolism impairment with aging for CYP3A and CYP2D substrates are
378 observed.(Salmin et al. 2017) CYP3A1 is higher in cells from young rats than in old
379 rats.(Czekaj et al. 2010) CYP3A in the liver are sensitive to aging with 50-70%
380 decreases, while CYP3A in the intestine is unchanged and in the kidney
381 increased.(Warrington et al. 2004) Generally speaking, hepatic CYP3A enzymes are
382 decreased with age.(Mori et al. 2007) In the present study, age-associated changes of
383 CYP3A1 and CYP3A2 followed "Pattern 1", while the expression of PXR, Cyp3a11
384 (mouse), and CYP3A4 protein followed "Pattern 2", a phenomenon in agreement of the
385 literature.(Mori et al. 2007; Yun et al. 2010)
386
387 ***3.4. P450-4 family***
388 PPARα activation induces CYP4A1, together with acyl-CoA oxidase and SREBPs,
389 that play important roles in regulating lipid metabolism, especially in rats fed with high-
390 fat-diet.(Chang et al. 2011) The herbicide propaquizafop dose-dependently activates
391 PPARα and CYP4A, leading to increased liver weight and hypertrophy as a mode of
392 action in hepato-carcinogenesis.(Strupp et al. 2018) In addition to PPARα activation, the
393 induction of CYP4A by K^+PFOS also involves CAR and PXR activation,(Elcombe et al.
394 2012) leading to hepatomegaly. In the present study, the expression of PPARα,
395 CYP4A1, CAR, and PXR followed "Pattern 3". That is the first expression peak
396 appeared at 7-14 days of age, similar to that observed in mice.(Hart et al. 2009)
397
398 ***P450-7 family and BA homeostasis***
399 Bile acid (BA) homeostasis is tightly regulated via a feedback loop operated by the

400 nuclear receptors FXR and small heterodimer partner (SHP). Loss of either FXR or SHP
401 alone, or Fxr-/-Shp-/- double knock out mice resulted in cholestasis and liver injury as
402 early as 3 weeks of age, and this dysfunction is linked to the dysregulation of bile acid
403 homeostatic key genes, particularly Cyp7a1.(Anakk et al. 2011) Hepatic CYP7A1 is a
404 rate-limiting enzyme that catabolizes cholesterol to bile acids, together with CYP8B1 as
405 the classic BA synthesis pathway, while CYP27A1 and CYP7B1 contribute to the
406 alternative pathway of BA biosynthesis.(Cuesta de Juan et al. 2007; Liu et al. 2014)
407 CYP27A1 is sensitive to inhibition by many xenobiotics.(Lam et al. 2018) In the present
408 study, the CYPs responsible for cholesterol metabolism and bile acid homeostasis
409 followed "Pattern 2", that was low at the neonatal stage, remained relative high levels
410 throughout the adulthood, and decreased at 800 days of age.
411 Taken together, CYPs are crucial enzymes in drug metabolism and
412 disposition.(Zanger & Schwab 2013) Induction or inhibition of CYPs have been
413 implicated in therapeutic efficacy and toxicity,(Jia et al. 2014; Mach et al. 2014; Mach et
414 al. 2016) and age-associated diseases.(Bondarenko et al. 2016; Velenosi et al. 2012)
415 especially in children(Kilanowicz et al. 2015; Li et al. 2017; McPhail et al. 2016) and in
416 elderly(McPhail et al. 2016; Salmin et al. 2017; Wauthier et al. 2007) Thus, a better
417 understanding of age-associated changes of CYPs is of significance for pharmacology,
418 toxicology, and therapeutics.
419
420 ***UGT and SULT***
421 Glucuronidation and sulfation are two most important phase-II reactions to conjugate
422 the CYP-catalyzed metabolites for biliary or urinary elimination. Age has significant

423 impact on hepatic activities of glucuronidation and sulfation. For example, porcine
424 hepatic glucuronidation and sulfation activities were low at birth, peaked at 5-10 weeks,
425 and then declined at 20 weeks,(Hu 2017) similar to the observations in the present
426 study.

427 In childhood and adolescence, UGT expression can be affected by hormones and is
428 a reason of individual variation to medication.(Neumann et al. 2016) Compared to adults,
429 glucuronidation is reduced in children.(Krekels et al. 2012) UGT1A1 and UGT1A6 are
430 subjected to CAR and PPARα regulation,(Osabe et al. 2008) and age-associated
431 UGT1A1 changes are paralleled with CYP3A expression alterations in rats fed high-fat
432 diet.(Kawase et al. 2015; Osabe et al. 2008)

433 Sulfation is the most highly developed pathway during fetal development where
434 glucuronidation in particular is lacking.(Coughtrie 2015) Sulfation is normally a
435 detoxification reaction to facilitate the elimination of xenobiotics, although for some
436 molecules sulfation could be bioactivation.(Coughtrie 2016) The decreased Sult1a1
437 paralleled with major CYP metabolism genes in 600-day old rats,(Mori et al. 2007)
438 similar to current observations.

439

440 ***Conclusions***

441 Overall, the present study characterized age-related changes in a total of 25 CYP
442 isoforms and relevant genes in rat livers from development to aging. In general, these
443 genes are low in neonatal stages, increase with age, but decreased in aged animals,
444 and three expression patterns are characterized. These data could help our better
445 understanding of the effects of CYPs on drug metabolism, pharmacology, and

toxicology in the context of maturation and aging.

Supplementary materials: Supplemental Table 1 of qPCR primers can be found at http://www.aspet/dmd.com

Author Contributions:

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Funding: This study is supported by the National Natural Science Foundation of China (81560592, 81560682).

Conflicts of Interest: The authors declare no conflict of interest.

461 **Figure Legends:**

462 **Figure 1. Age-related expression of CYP-1 family gene/proteins in livers of male**
463 **rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7,
464 and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and
465 at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis
466 (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-
467 blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and
468 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted
469 proteins (30 μg) were separated on NUPAGE 10% BT gels and the representative
470 western-blot was inserted into the figure (n=3). The molecular weight for CYP1A1 was
471 55 kD, and β-actin 43 kD.

472

473 **Figure 2. Age-related expression of CYP-2 family gene/proteins in livers of male**
474 **rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7,
475 and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and
476 at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis
477 (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-
478 blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and
479 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted
480 proteins (30 μg) were separated on NUPAGE 10% BT gels and the representative
481 western-blot was inserted into the figure (n=3). The molecular weight for CYP2B1 was
482 56 kD, CYP2E1 57 kD, and β-actin 43 kD.

483

484

485 **Figure 3. Age-related expression of CYP-3 family gene/proteins in livers of male**
486 **rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7,
487 and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and
488 at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis
489 (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-
490 blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and
491 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted
492 proteins (30 μg) were separated on NUPAGE 10% BT gels and the representative
493 western-blot was inserted into the figure (n=3). The molecular weight for CYP3A1 was
494 57 kD, and β-actin 43 kD.

495

496

497 **Figure 4. Age-related expression of CYP-4 family gene/proteins in livers of male**
498 **rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7,
499 and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and
500 at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis
501 (n=4-5 for each time point). For Western-blot insert, the neonatal (14 d), at weanling
502 (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were
503 collected to extract protein. Aliquoted proteins (30 μg) were separated on NUPAGE 10%
504 BT gels and the representative western-blot was inserted into the figure (n=3). The
505 molecular weight for CYP4A1 was 59 kD, and β-actin 43 kD.

506

507

508 **Figure 5. Age-related expression of CYP-7 family gene/proteins in livers of male**
509 **rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7,
510 and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d),
511 and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis
512 (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-
513 blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and
514 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted
515 proteins (30 μg) were separated on NUPAGE 10% BT gels and the representative
516 western-blot was inserted into the figure (n=3). The molecular weight for CYP7A1 was
517 55 kD, and β-actin 43 kD.

518

519

520 **Figure 6. Age-related mRNA expression of UGT and SULT family genes in livers**
521 **of male rats.** Livers from male SD rats at the fetus (-2 d before birth), the neonatal
522 stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and
523 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by real-
524 time RT-PCR analysis (n=4-5 for each time point). *Significantly different from at birth,
525 $p < 0.05$.

526

527

528 **References**

529

530 Agrawal AK, and Shapiro BH. 2003. Constitutive and inducible hepatic cytochrome P450 isoforms in
531 senescent male and female rats and response to low-dose phenobarbital. *Drug Metab Dispos*
532 31:612-619.
533 Aleksunes LM, and Klaassen CD. 2012. Coordinated regulation of hepatic phase I and II drug-
534 metabolizing genes and transporters using AhR-, CAR-, PXR-, PPARalpha-, and Nrf2-null mice.
535 *Drug Metab Dispos* 40:1366-1379. 10.1124/dmd.112.045112
536 Anakk S, Watanabe M, Ochsner SA, McKenna NJ, Finegold MJ, and Moore DD. 2011. Combined deletion
537 of Fxr and Shp in mice induces Cyp17a1 and results in juvenile onset cholestasis. *J Clin Invest*
538 121:86-95. 10.1172/jci42846
539 Asaoka Y, Sakai H, Sasaki J, Goryo M, Yanai T, Masegi T, and Okada K. 2010. Changes in the gene
540 expression and enzyme activity of hepatic cytochrome P450 in juvenile Sprague-Dawley rats. *J*
541 *Vet Med Sci* 72:471-479.
542 Bondarenko LB, Shayakhmetova GM, Voronina AK, and Kovalenko VM. 2016. Age-dependent features of
543 CYP3A, CYP2C, and CYP2E1 functioning at metabolic syndrome. *J Basic Clin Physiol Pharmacol*
544 27:603-610. 10.1515/jbcpp-2016-0012
545 Chang CJ, Tzeng TF, Liou SS, Chang YS, and Liu IM. 2011. Kaempferol regulates the lipid-profile in high-fat
546 diet-fed rats through an increase in hepatic PPARalpha levels. *Planta Med* 77:1876-1882.
547 10.1055/s-0031-1279992
548 Coughtrie MW. 2015. Ontogeny of Human Conjugating Enzymes. *Drug Metab Lett* 9:99-108.
549 Coughtrie MWH. 2016. Function and organization of the human cytosolic sulfotransferase (SULT) family.
550 *Chem Biol Interact* 259:2-7. 10.1016/j.cbi.2016.05.005
551 Cuesta de Juan S, Monte MJ, Macias RI, Wauthier V, Calderon PB, and Marin JJ. 2007. Ontogenic
552 development-associated changes in the expression of genes involved in rat bile acid
553 homeostasis. *J Lipid Res* 48:1362-1370. 10.1194/jlr.M700034-JLR200
554 Cui JY, Renaud HJ, and Klaassen CD. 2012. Ontogeny of novel cytochrome P450 gene isoforms during
555 postnatal liver maturation in mice. *Drug Metab Dispos* 40:1226-1237. 10.1124/dmd.111.042697
556 Czekaj P, Bryzek A, Czekaj TM, Koryciak-Komarska H, Wiaderkiewicz A, Plewka D, and Sieron AL. 2010.
557 Cytochrome P450 mRNA expressions along with in vitro differentiation of hepatocyte precursor
558 cells from fetal, young and old rats. *Folia Histochem Cytobiol* 48:46-57. 10.2478/v10042-008-
559 0085-5
560 Das RK, Banerjee S, and Shapiro BH. 2014. Irreversible perinatal imprinting of adult expression of the
561 principal sex-dependent drug-metabolizing enzyme CYP2C11. *Faseb j* 28:4111-4122.
562 10.1096/fj.13-248864
563 Daskalopoulos EP, Lang MA, Marselos M, Malliou F, and Konstandi M. 2012. D(2)-dopaminergic
564 receptor-linked pathways: critical regulators of CYP3A, CYP2C, and CYP2D. *Mol Pharmacol*
565 82:668-678. 10.1124/mol.112.078709
566 de Zwart L, Scholten M, Monbaliu JG, Annaert PP, Van Houdt JM, Van den Wyngaert I, De Schaepdrijver
567 LM, Bailey GP, Coogan TP, Coussement WC, and Mannens GS. 2008. The ontogeny of drug
568 metabolizing enzymes and transporters in the rat. *Reprod Toxicol* 26:220-230.
569 10.1016/j.reprotox.2008.09.010
570 Durnas C, Loi CM, and Cusack BJ. 1990. Hepatic drug metabolism and aging. *Clin Pharmacokinet* 19:359-
571 389. 10.2165/00003088-199019050-00002
572 Elbarbry FA, McNamara PJ, and Alcorn J. 2007. Ontogeny of hepatic CYP1A2 and CYP2E1 expression in
573 rat. *J Biochem Mol Toxicol* 21:41-50. 10.1002/jbt.20156

574 Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, and Butenhoff JL. 2012. Hepatocellular
575 hypertrophy and cell proliferation in Sprague-Dawley rats from dietary exposure to potassium
576 perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors
577 PPARalpha and CAR/PXR. *Toxicology* 293:16-29. 10.1016/j.tox.2011.12.014
578 Hart SN, Cui Y, Klaassen CD, and Zhong XB. 2009. Three patterns of cytochrome P450 gene expression
579 during liver maturation in mice. *Drug Metab Dispos* 37:116-121. 10.1124/dmd.108.023812
580 He XJ, Ejiri N, Nakayama H, and Doi K. 2005. Effects of pregnancy on CYPs protein expression in rat liver.
581 *Exp Mol Pathol* 78:64-70. 10.1016/j.yexmp.2004.08.011
582 Hines RN. 2008. The ontogeny of drug metabolism enzymes and implications for adverse drug events.
583 *Pharmacol Ther* 118:250-267. 10.1016/j.pharmthera.2008.02.005
584 Hines RN. 2013. Developmental expression of drug metabolizing enzymes: impact on disposition in
585 neonates and young children. *Int J Pharm* 452:3-7. 10.1016/j.ijpharm.2012.05.079
586 Hou WY, Xu SF, Zhu QN, Lu YF, Cheng XG, and Liu J. 2014. Age- and sex-related differences of organic
587 anion-transporting polypeptide gene expression in livers of rats. *Toxicol Appl Pharmacol*
588 280:370-377. 10.1016/j.taap.2014.08.020
589 Hou WY ZD, Zhu QN, Xu SF, Wu Q, Lu YF, Liu J. 2014. Age-dependent expression of metallothionein in
590 livers of rats *Journal of Zunyi Medical College* 37:111-116.
591 Hu SX. 2017. Age-related change of hepatic uridine diphosphate glucuronosyltransferase and
592 sulfotransferase activities in male chickens and pigs. *J Vet Pharmacol Ther* 40:270-278.
593 10.1111/jvp.12355
594 Jia LL, Zhong ZY, Li F, Ling ZL, Chen Y, Zhao WM, Li Y, Jiang SW, Xu P, Yang Y, Hu MY, Liu L, and Liu XD.
595 2014. Aggravation of clozapine-induced hepatotoxicity by glycyrrhetinic acid in rats. *J Pharmacol*
596 *Sci* 124:468-479.
597 Kang JS, Wanibuchi H, Morimura K, Wongpoomchai R, Chusiri Y, Gonzalez FJ, and Fukushima S. 2008.
598 Role of CYP2E1 in thioacetamide-induced mouse hepatotoxicity. *Toxicol Appl Pharmacol*
599 228:295-300. 10.1016/j.taap.2007.11.010
600 Kawase A, Ito A, Yamada A, and Iwaki M. 2015. Age-related changes in mRNA levels of hepatic
601 transporters, cytochrome P450 and UDP-glucuronosyltransferase in female rats. *Eur J Drug*
602 *Metab Pharmacokinet* 40:239-244. 10.1007/s13318-014-0208-7
603 Kilanowicz A, Czekaj P, Sapota A, Skrzypinska-Gawrysiak M, Bruchajzer E, Darago A, Czech E, Plewka D,
604 Wiaderkiewicz A, and Sitarek K. 2015. Developmental toxicity of hexachloronaphthalene in
605 Wistar rats. A role of CYP1A1 expression. *Reprod Toxicol* 58:93-103.
606 10.1016/j.reprotox.2015.09.005
607 Kirby GM, Batist G, Alpert L, Lamoureux E, Cameron RG, and Alaoui-Jamali MA. 1996. Overexpression of
608 cytochrome P-450 isoforms involved in aflatoxin B1 bioactivation in human liver with cirrhosis
609 and hepatitis. *Toxicol Pathol* 24:458-467. 10.1177/019262339602400408
610 Kot M, and Daujat-Chavanieu M. 2018. Altered cytokine profile under control of the serotonergic system
611 determines the regulation of CYP2C11 and CYP3A isoforms. *Food Chem Toxicol* 116:369-378.
612 10.1016/j.fct.2018.04.051
613 Krekels EH, Danhof M, Tibboel D, and Knibbe CA. 2012. Ontogeny of hepatic glucuronidation; methods
614 and results. *Curr Drug Metab* 13:728-743.
615 Lam M, Mast N, and Pikuleva IA. 2018. Drugs and Scaffold That Inhibit Cytochrome P450 27A1 In Vitro
616 and In Vivo. *Mol Pharmacol* 93:101-108. 10.1124/mol.117.110742
617 Li H, Canet MJ, Clarke JD, Billheimer D, Xanthakos SA, Lavine JE, Erickson RP, and Cherrington NJ. 2017.
618 Pediatric Cytochrome P450 Activity Alterations in Nonalcoholic Steatohepatitis. *Drug Metab*
619 *Dispos* 45:1317-1325. 10.1124/dmd.117.077644

620 Li Y, Cui Y, Hart SN, Klaassen CD, and Zhong XB. 2009. Dynamic patterns of histone methylation are
621 associated with ontogenic expression of the Cyp3a genes during mouse liver maturation. *Mol*
622 *Pharmacol* 75:1171-1179. 10.1124/mol.108.052993
623 Liu J, Lu H, Lu YF, Lei X, Cui JY, Ellis E, Strom SC, and Klaassen CD. 2014. Potency of individual bile acids to
624 regulate bile acid synthesis and transport genes in primary human hepatocyte cultures. *Toxicol*
625 *Sci* 141:538-546. 10.1093/toxsci/kfu151
626 Mach J, Huizer-Pajkos A, Cogger VC, McKenzie C, Le Couteur DG, Jones BE, de Cabo R, and Hilmer SN.
627 2014. The effect of aging on acetaminophen pharmacokinetics, toxicity and Nrf2 in Fischer 344
628 rats. *J Gerontol A Biol Sci Med Sci* 69:387-397. 10.1093/gerona/glt095
629 Mach J, Huizer-Pajkos A, Mitchell SJ, McKenzie C, Phillips L, Kane A, Jones B, de Cabo R, Cogger V, Le
630 Couteur DG, and Hilmer SN. 2016. The effect of ageing on isoniazid pharmacokinetics and
631 hepatotoxicity in Fischer 344 rats. *Fundam Clin Pharmacol* 30:23-34. 10.1111/fcp.12157
632 Matalova P, Urbanek K, and Anzenbacher P. 2016. Specific features of pharmacokinetics in children.
633 *Drug Metab Rev* 48:70-79. 10.3109/03602532.2015.1135941
634 McPhail BT, White CA, Cummings BS, Muralidhara S, Wilson JT, and Bruckner JV. 2016. The immature rat
635 as a potential model for chemical risks to children: Ontogeny of selected hepatic P450s. *Chem*
636 *Biol Interact* 256:167-177. 10.1016/j.cbi.2016.07.005
637 Mori K, Blackshear PE, Lobenhofer EK, Parker JS, Orzech DP, Roycroft JH, Walker KL, Johnson KA, Marsh
638 TA, Irwin RD, and Boorman GA. 2007. Hepatic transcript levels for genes coding for enzymes
639 associated with xenobiotic metabolism are altered with age. *Toxicol Pathol* 35:242-251.
640 10.1080/01926230601156286
641 Neumann E, Mehboob H, Ramirez J, Mirkov S, Zhang M, and Liu W. 2016. Age-Dependent Hepatic UDP-
642 Glucuronosyltransferase Gene Expression and Activity in Children. *Front Pharmacol* 7:437.
643 10.3389/fphar.2016.00437
644 Osabe M, Sugatani J, Fukuyama T, Ikushiro S, Ikari A, and Miwa M. 2008. Expression of hepatic UDP-
645 glucuronosyltransferase 1A1 and 1A6 correlated with increased expression of the nuclear
646 constitutive androstane receptor and peroxisome proliferator-activated receptor alpha in male
647 rats fed a high-fat and high-sucrose diet. *Drug Metab Dispos* 36:294-302.
648 10.1124/dmd.107.017731
649 Park SY, Kim CH, Lee JY, Jeon JS, Kim MJ, Chae SH, Kim HC, Oh SJ, and Kim SK. 2016. Hepatic expression
650 of cytochrome P450 in Zucker diabetic fatty rats. *Food Chem Toxicol* 96:244-253.
651 10.1016/j.fct.2016.08.010
652 Saghir SA, Khan SA, and McCoy AT. 2012. Ontogeny of mammalian metabolizing enzymes in humans and
653 animals used in toxicological studies. *Crit Rev Toxicol* 42:323-357.
654 10.3109/10408444.2012.674100
655 Salmin SF, Giroux MC, Vachon P, and Beaudry F. 2017. In vitro metabolism of specific CYP2D and CYP3A
656 opioid substrates using rat liver S9 fractions and mass spectrometry reveal a severe metabolic
657 impairment with increasing age. *Biomed Chromatogr* 31. 10.1002/bmc.3786
658 Shi S, and Klotz U. 2011. Age-related changes in pharmacokinetics. *Curr Drug Metab* 12:601-610.
659 Strupp C, Bomann WH, Spezia F, Gervais F, Forster R, Richert L, and Singh P. 2018. A human relevance
660 investigation of PPARalpha-mediated key events in the hepatocarcinogenic mode of action of
661 propaquizafop in rats. *Regul Toxicol Pharmacol* 95:348-361. 10.1016/j.yrtph.2018.04.005
662 Velenosi TJ, Fu AY, Luo S, Wang H, and Urquhart BL. 2012. Down-regulation of hepatic CYP3A and CYP2C
663 mediated metabolism in rats with moderate chronic kidney disease. *Drug Metab Dispos*
664 40:1508-1514. 10.1124/dmd.112.045245
665 Wang H, Li W, Muhammad I, Sun X, Cui X, Cheng P, Qayum A, and Zhang X. 2018. Biochemical basis for
666 the age-related sensitivity of broilers to aflatoxin B1. *Toxicol Mech Methods* 28:361-368.
667 10.1080/15376516.2018.1428258

668 Warrington JS, Greenblatt DJ, and von Moltke LL. 2004. Age-related differences in CYP3A expression and
669 activity in the rat liver, intestine, and kidney. *J Pharmacol Exp Ther* 309:720-729.
670 10.1124/jpet.103.061077
671 Wauthier V, Verbeeck RK, and Calderon PB. 2007. The effect of ageing on cytochrome p450 enzymes:
672 consequences for drug biotransformation in the elderly. *Curr Med Chem* 14:745-757.
673 Xu S, Hou D, Liu J, and Ji L. 2018a. Age-associated changes in GSH S-transferase gene/proteins in livers of
674 rats. *Redox Rep* 23:213-218. 10.1080/13510002.2018.1546985
675 Xu SF, Ji LL, Wu Q, Li J, and Liu J. 2018b. Ontogeny and aging of Nrf2 pathway genes in livers of rats. *Life*
676 *Sci* 203:99-104. 10.1016/j.lfs.2018.04.018
677 Yun KU, Oh SJ, Oh JM, Kang KW, Myung CS, Song GY, Kim BH, and Kim SK. 2010. Age-related changes in
678 hepatic expression and activity of cytochrome P450 in male rats. *Arch Toxicol* 84:939-946.
679 10.1007/s00204-010-0520-1
680 Zanger UM, and Schwab M. 2013. Cytochrome P450 enzymes in drug metabolism: regulation of gene
681 expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 138:103-141.
682 10.1016/j.pharmthera.2012.12.007
683 Zhu QN, Hou WY, Xu SF, Lu YF, and Liu J. 2017. Ontogeny, aging, and gender-related changes in hepatic
684 multidrug resistant protein genes in rats. *Life Sci* 170:108-114. 10.1016/j.lfs.2016.11.022

685

Figure 1

Figure 1. Age-related expression of CYP-1 family gene/proteins in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted proteins (30 µg) were separated on NUPAGE 10% BT gels and the representative western-blot was inserted into the figure (n=3). The molecular weight for CYP1A1 was 55 kD, and β-actin 43 kD.

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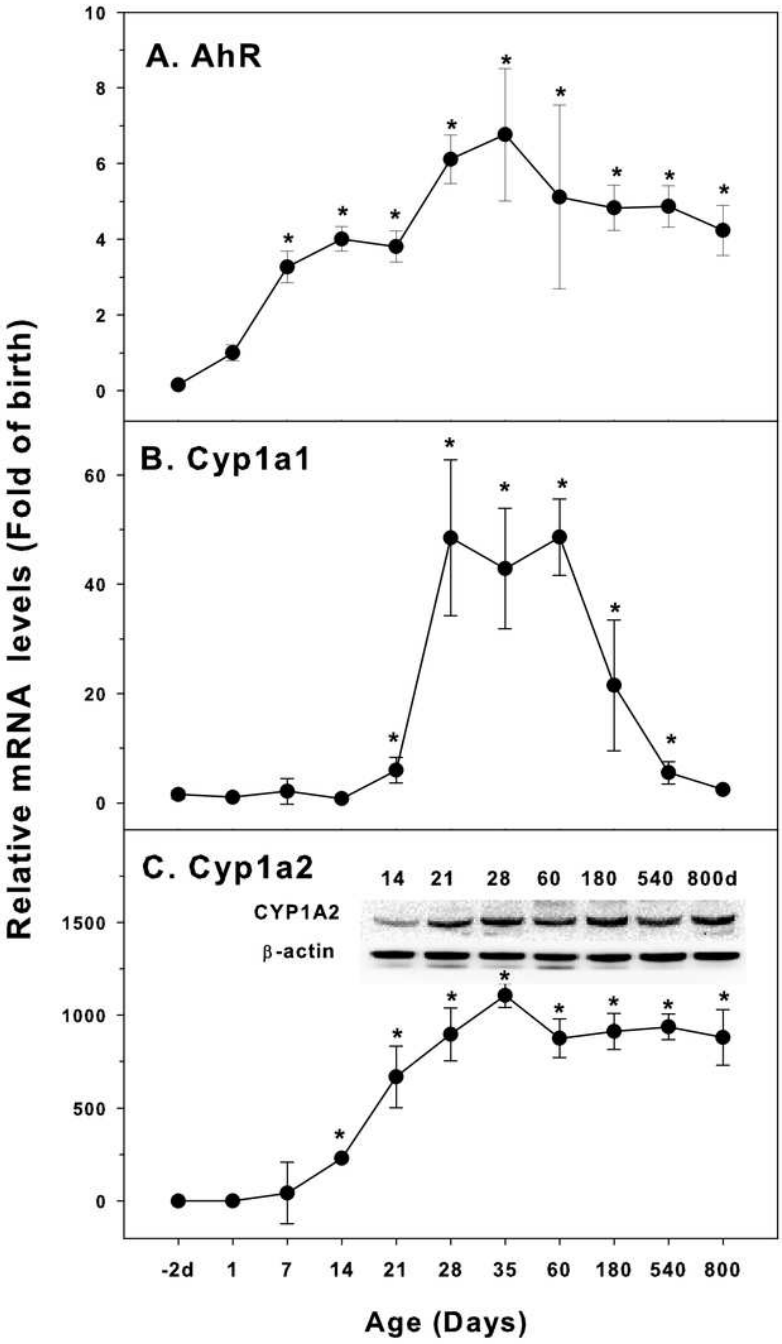
A. AhR
B. Cyp1a1
C. Cyp1a2
14 21 28 60 180 540 800d
CYP1A2
β-actin
Relative mRNA levels (Fold of birth)
Age (Days)
-2d 1 7 14 21 28 35 60 180 540 800

Figure 2

Figure 2. Age-related expression of CYP-2 family gene/proteins in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted proteins (30 µg) were separated on NUPAGE 10% BT gels and the representative western-blot was inserted into the figure (n=3). The molecular weight for CYP2B1 was 56 kD, CYP2E1 57 kD, and β-actin 43 kD.

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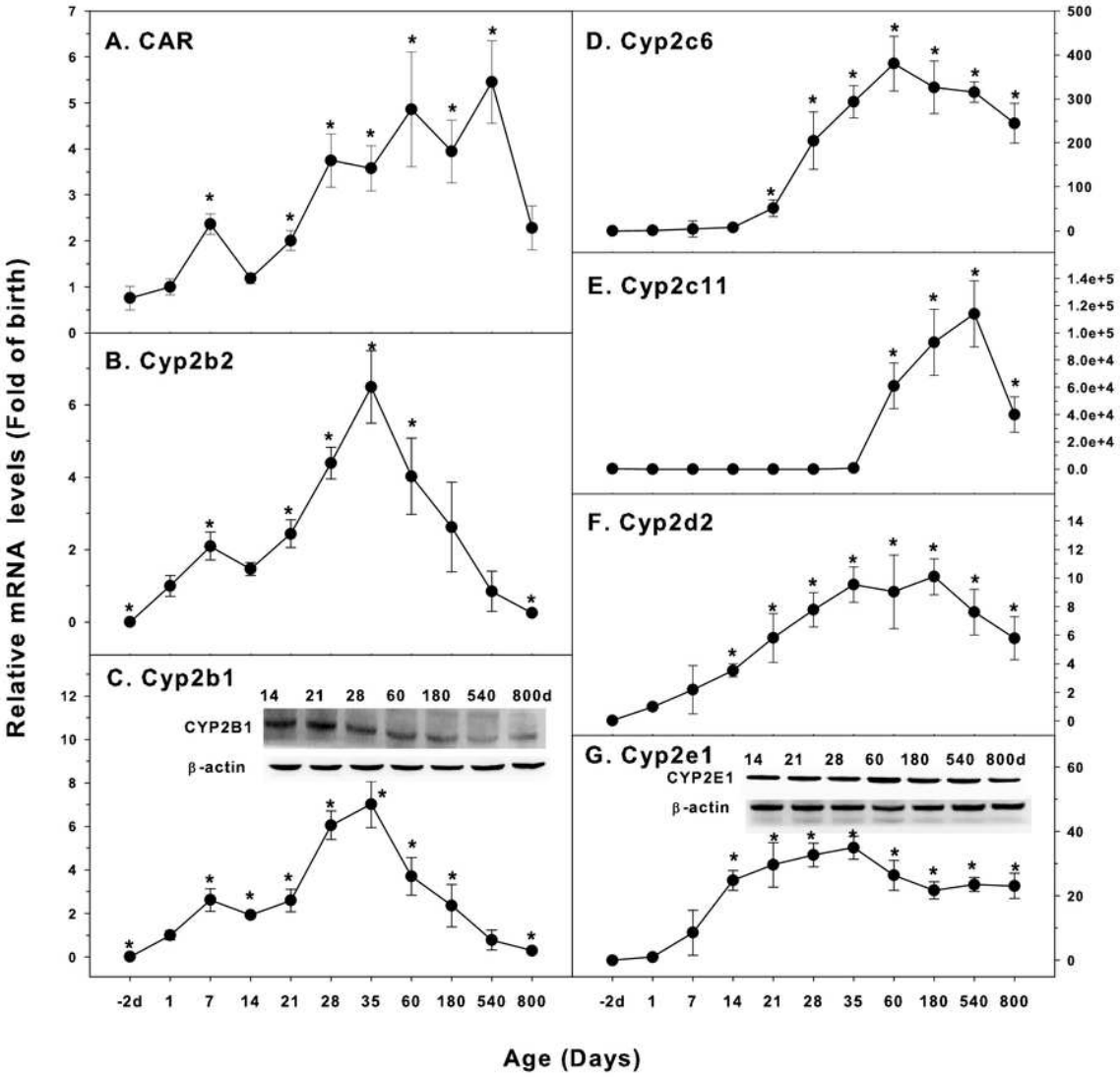
A. CAR
B. Cyp2b2
C. Cyp2b1
14 21 28 60 180 540 800d
CYP2B1
β-actin
D. Cyp2c6
E. Cyp2c11
F. Cyp2d2
G. Cyp2e1
14 21 28 60 180 540 800d
CYP2E1
β-actin
Relative mRNA levels (Fold of birth)
-2d 1 7 14 21 28 35 60 180 540 800
Age (Days)

Figure 3

Figure 3. Age-related expression of CYP-3 family gene/proteins in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted proteins (30 µg) were separated on NUPAGE 10% BT gels and the representative western-blot was inserted into the figure (n=3). The molecular weight for CYP3A1 was 57 kD, and β-actin 43 kD.

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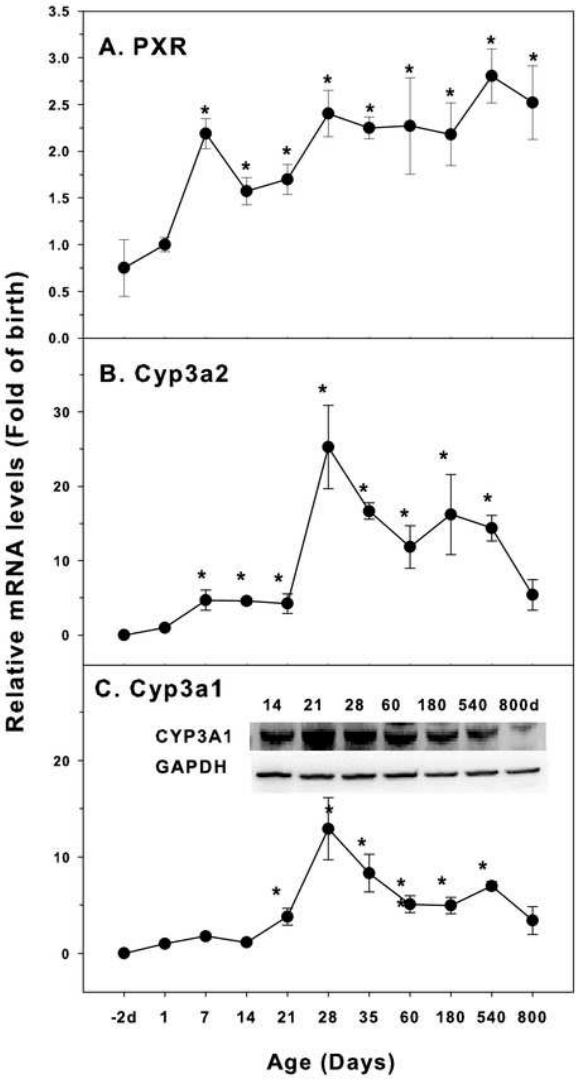
A. PXR
B. Cyp3a2
C. Cyp3a1
14 21 28 60 180 540 800d
CYP3A1
GAPDH
Relative mRNA levels (Fold of birth)
-2d 1 7 14 21 28 35 60 180 540 800
Age (Days)

Figure 4

Figure 4. Age-related expression of CYP-4 family gene/proteins in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis (n=4-5 for each time point). For Western-blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted proteins (30 µg) were separated on NUPAGE 10% BT gels and the representative western-blot was inserted into the figure (n=3). The molecular weight for CYP4A1 was 59 kD, and β-actin 43 kD.

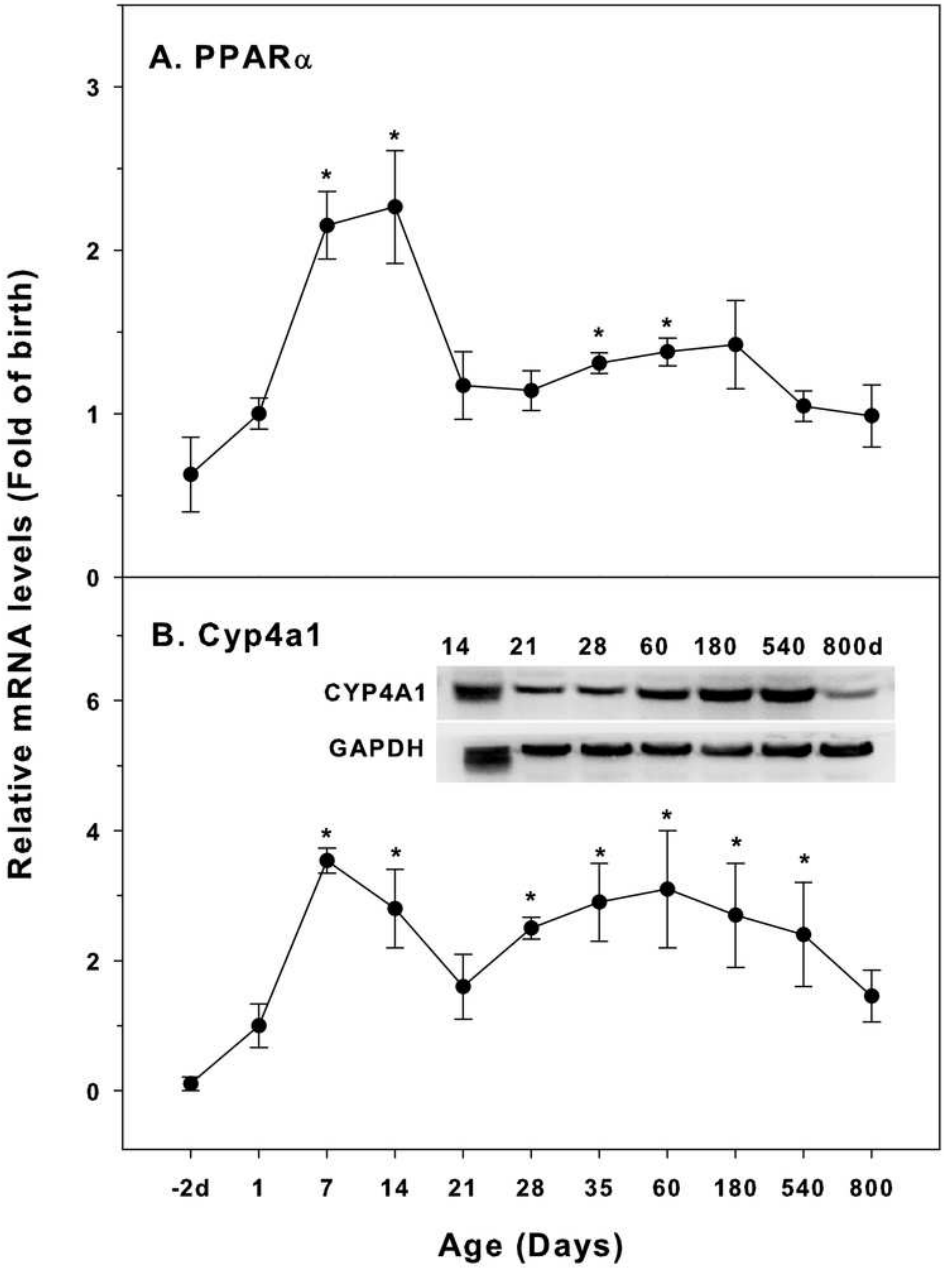
A. PPARα
B. Cyp4a1
14 21 28 60 180 540 800d
CYP4A1
GAPDH
Relative mRNA levels (Fold of birth)
Age (Days)
-2d 1 7 14 21 28 35 60 180 540 800

Figure 5

Figure 5. Age-related expression of CYP-7 family gene/proteins in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by qPCR analysis (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$. For Western-blot insert, the neonatal (14 d), at weanling (21 d), at puberty (28 d), at adult (60 and 180 d), and at aging (540 and 800 d) were collected to extract protein. Aliquoted proteins (30 µg) were separated on NUPAGE 10% BT gels and the representative western-blot was inserted into the figure (n=3). The molecular weight for CYP7A1 was 55 kD, and β-actin 43 kD.

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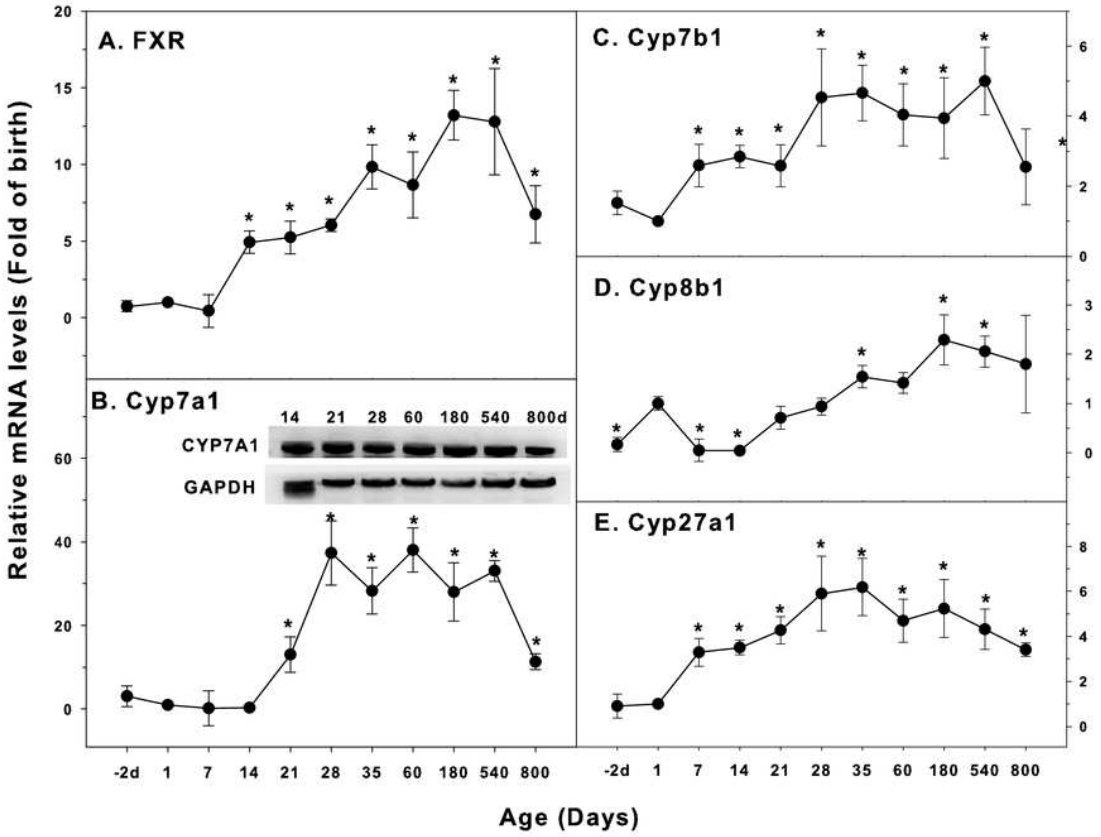

Figure 6

Figure 6. Age-related mRNA expression of UGT and SULT family genes in livers of male rats.

Livers from male SD rats at the fetus (-2 d before birth), the neonatal stage (1, 7, and 14 d), and at weanling (21 d), at puberty (28 and 35 d), at adult (60 and 180 d), and at aging (540 and 800 d), were collected to extract RNA, followed by real-time RT-PCR analysis (n=4-5 for each time point). *Significantly different from at birth, $p < 0.05$.

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