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Differences in Cytochrome p450 Enzyme Expression and Activity in Fetal and Adult Tissues

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Abstract

Introduction: Human cytochrome p450 (CYP) enzyme expression and activity is lower in the fetus as compared to the adult; however, limited quantitative data exists regarding the specific differences in magnitude or the degree of inducibility due to environmental factors.

Methods: We utilized a combination of *in silico*- and molecular-based approaches to profile and compare CYP expression/activity in human adult liver and fetal tissues. Using public datasets, we evaluated human CYP expression between: 1) placenta vs. adult livers; 2) fetal vs. adult livers; or 3) five compartments of the human placenta. We generated new experimental data, characterizing expression levels of nine CYPs in placenta/fetal liver vs. adult liver. In a subset of samples, we evaluated CYP3A4 activity. Finally, we summarized evidence of human fetal CYP expression/activity and environmental exposures during pregnancy.

Results: *In silico*, CYPs were predominately expressed at higher levels in the adult liver vs. fetal tissues, with a few noted exceptions. Sixty percent of CYP enzymes were expressed at nominal levels in the placenta. In wet-lab analyses, we observed significant CYP-specific differences in expression/activity between adult and fetal tissues; CYP2E1 and −3A4 were expressed significantly lower in fetal vs. adult livers, while CYP2J2 levels were similar.

Discussion: We provide a qualitative review of the expression of the CYP enzyme family in critical sites of xenobiotic distribution during human pregnancy and novel quantitative data regarding fetal CYP expression and activity during mid-gestation. Data outputs may be a resource for modeling predictions of chemical distribution and sensitivity.

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Keywords

Cytochrome p450; Enzyme; Expression; Fetal; Liver; Placenta; Transcriptome

Introduction

During human pregnancy, the developing fetus comes into contact with thousands of foreign compounds including environmental contaminants and pharmaceuticals [1, 2]. Human biomonitoring studies suggest that pregnant women are exposed to multiple chemicals [3–5]. Toxicokinetic/toxicodynamic (TK/TD) studies in rodents, and to a lesser degree, humans, have provided data how chemicals can distribute in the maternal and fetal tissues [6]. However, the metabolic capacity of the human fetus remains undefined due to the: 1) challenges in studying environmental chemical distribution in the human embryo/fetus; and 2) the metabolic differences between animal models and human.

The transfer of compounds from the mother to the baby depends on many variables, including: chemical properties (*e.g.,* lipophilicity, half-life), gestational age and exposure route. Most compounds involving oral exposure are initially absorbed through the intestinal tract and transported via portal-vein circulation to the liver where they undergo first-pass metabolism before entering the systemic circulation ([7]; Figure 1). Compound exposures which occur via inhalation or dermal absorbance bypass this initial step. During the earliest stages of pregnancy when the placenta is immature, compounds transfer passively from the mother to the extraembryonic layers and the embryo proper. However, as the embryo/fetus develops, it increasingly relies on the maternal blood supply and more sophisticated active transport mechanisms. Maternal and fetal blood sources do not intermix, with the placenta serving as the critical junction between the two units, where compounds must pass through several matrices to reach the fetus. At term, the volume of placental blood flow reaches ~600–700 ml/minute [8] powered by an estimated 120 maternal spiral arteries of the endometrium which deliver blood to the intervillous space of the placenta [9]. Despite these known physical barriers between the maternal and fetal compartments, numerous foreign compounds (and their metabolites) are found to cross the placenta and accumulate in the fetus.

In the maternal liver, a variety of specialized Phase I-III enzymes are responsible for compound metabolism and promotion of excretion. The majority of exogenous compounds first undergo Phase I enzymatic transformation to more polar forms by introducing or revealing functional groups (*e.g.,* -OH). In some cases, Phase I enzymes may convert substances into more toxicologically active forms. Cytochrome P450 (CYP) enzymes account for the majority of Phase I enzymes in the body (115 gene and pseudogenes [10]) and play critical roles in metabolism of xenobiotics and endogenous pathways (*e.g.,* carbohydrate, fatty acid, cholesterol, retinoic acid, and steroid metabolism/biosynthesis). CYPs are predominately expressed in the liver, but are also expressed in other organs which directly encounter foreign substances (*e.g.,* small intestine, gut, kidneys, lung, and adipose tissues).

During pregnancy, the fetus is rapidly developing and is generally immature as compared to the adult in its ability to metabolize compounds. Attempts have been made to characterize the expression of specific CYPs across development in specific organs in rodent [11], and to a lesser extent, in human [11, 12]. Recent advances in technology have enabled these assessments on global epigenetic [13], transcriptomic, proteomic, and activity levels [14, 15]. While these initial studies have been critically important in identifying relative CYP expression levels between select maternal vs. fetal tissues as well as changes across development, higher quantitative resolution is needed to predict developmental risk(s) linked to chemical exposures. Undefined variables include: 1) developmental and tissue dynamics in expression/activity in the embryo/fetus; 2) population variability; and 3) sex-dependent differences.

While compounds can be metabolized via resident CYPs, compounds may also induce the abundance of CYPs through activation of xenobiotic sensing mechanisms [16] to further promote elimination and protect the human body from potential harm. Due to their inducible nature, CYPs have been proposed as correlates of environmental exposure(s) and other stressors in relation to adverse birth outcomes. Defining the influence of environmental factors on expression/activity of CYPs in human fetal tissues is warranted to properly determine risk to xenobiotic exposures *in utero*.

Therefore, in this study, we used *in silico* and molecular-based approaches to evaluate and compare CYP expression/activity in human placenta, and fetal and adult livers. We first identified and assessed existing data transcriptomic data to compare adult and fetal liver CYP expression. We then measured expression of eight CYPs involved in xenobiotic metabolism and CYP19A1 in placenta (2nd trimester), fetal liver (2nd trimester) and adult liver using qRT-PCR. Finally, to estimate the influence of environmental factors in this context, we reviewed public research studies evaluating the relationship between chemical exposures and human fetal CYP expression/activity.

Materials and methods

***In silico* identification and analysis of transcriptomic datasets—**To evaluate global transcriptomic differences in CYP expression between adult liver and fetal liver and/or placenta, we searched two public databases, National Center for Biotechnology Information (NCBI) Gene Expression Omnibus and European Bioinformatics Institute (EBI) ArrayExpress repositories. We used the following criteria to select transcriptomic datasets for analysis: 1) human adult and fetal liver samples; or 2) adult liver and placenta samples in the same experiment and RNA-sequencing (RNA-seq) and/or Affymetrix or Illumina microarray studies with at least 3 samples per group analysis.

Using the above criteria, our search identified two unique transcriptomic studies: 1) an analysis of human adult (n=92) and 1st trimester fetal livers (n=14; [15]; GSE61279; Affymetrix); and 2) a comparison between human adult livers (n=4) and human term placentas (n=3; E-MTAB-1733; RNA-seq). Within these two studies, we were able to evaluate relative expression of 55 and 46 CYPs, respectively. Pseudogenes were not included in our analysis.CEL files of GSE61279 were downloaded, processed, and annotated using the Affymetrix Expression Console and Transcriptome Analysis Console (TAC) software

packages. Raw intensity values were normalized via the Robust Multi-array Average (RMA) algorithm. Pre-processed count data (Reads Per Kilobase per Million mapped reads; RPKM) of E-MTAB-1733 was acquired and values were adjusted by adding an arbitrary 1.1 units and log 2 transformed. Only CYPs with RPKM > 0 in adult liver or placenta were evaluated. One-way-ANOVA (OWA) was independently applied within each of the studies to determine differentially expressed (DE) genes between tissues. In the case of multiple probes per gene, the one with the lowest p-value, *i.e.,* most significant difference between adult vs. fetal, was used for comparison purposes. Average fold change (FC) differences in expression between adult livers and fetal tissues were defined as the difference in geometric mean of log 2 expression values. The range in CYP expression within each group was determined by calculating the 95% confidence interval range of log 2 expression. Significantly DE genes were defined as $p \leq 0.05$ and Log_2 FC ≥ 1 (absolute) as compared to the average adult liver group in each study. Hierarchical clustering of FC values was performed using average linkage and Euclidean distance (TIGR MEV [17]).

To examine mRNA expression in the compartments of the placenta, we obtained a dataset which evaluated transcript levels in amnion, basal plate, chorion, villi and isolated CTBs [18] of 2nd and term human placentas that was completed as part of the NIH Roadmap Epigenomics Mapping Consortium ([19]; GSE16368). We compared the expression of CYPs in cell/tissue samples and clustered RPKM values by the median expression level (TIGR MEV [17]). In discussing these data, a cutoff of RPKM <1.0, which represents the lower third quartile of detected transcripts (19,654 total) was selected as an arbitrary cutoff of defining genes as high versus low expression.

Targeted molecular-based analysis of CYP expression—We obtained fetal livers (n=87) and placentas (n=47) collected immediately following elective terminations (gestational week (GW) 15–22), placed in a glass conical tube on dry ice and stored at −80°C until processing. All methods were approved by the UCSF Institutional Review Board. Adult livers (n=9) were obtained courtesy of Dr. F. Peter Guengerich at Vanderbilt University. Informed consent was obtained from all donors.

RNA Isolation and cDNA Synthesis—RNA from fetal livers, adult livers, and placentas was isolated using the RNeasy Plus Mini Kit (Qiagen). Frozen fetal or adult liver (30–50mg) or placenta (200–500mg) tissue was homogenized in Buffer RLT. RNA was purified following the manufacturer's instructions. RNA amount was assessed by NanoDrop spectrometer (ThermoFisher Scientific). All samples used for this study had an absorbance (260/280nm) reading of 1.9–2.1. RNA was converted to cDNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). Relative gene expression was measured via qRT-PCR using gene-specific Taqman Gene Expression Assays (Thermo Fisher Scientific; Supplemental Table 1) with Taqman Universal Master Mix II, no UNG (Thermo Fisher Scientific). For each reaction, 10ng cDNA was used. We evaluated the relative expression of eight CYPs involved in xenobiotic metabolism: CYP1A1, −1A2, −2B6, −2C9, −2C19, −2E1, −2J2, and −3A4, and the steroid metabolizing p450 enzyme, −19A1.Relative expression was calculated using the ΔΔCT method [20]. GAPDH and ACTB expression were measured as housekeeping genes. CT values from technical replicates were averaged

and the geometric mean of housekeeping genes for each sample was subtracted to calculate the ΔCT. These values were then adjusted to the mean of the adult liver samples to determine relative expression, *i.e.,* ΔΔCT. Calculating $2^{-\Delta\Delta CT}$ yielded the relative fold change (FC) in expression. Three fetal liver samples were dropped from our analyses due to irregular housekeeping gene expression (> 2 SD ± geometric mean of gene levels). The level of detection (LOD) for qRT-PCR assays was set at 40 cycles. Each reaction was performed in triplicate, and only samples that produced CT values for at least two of the three replicates were considered to be above the LOD.

Human placenta and liver S9 extraction for CYP3A4 activity measurements— Complementary to gene expression assessments, in a subset of placenta (n=46), adult (n=6) and fetal liver (n=2) samples, we evaluated enzymatic activity of CYP3A4, a major CYP involved in the metabolism of endogenous compounds and xenobiotics; ~50% of drugs are estimated to be metabolized by −3A4 in the adult human liver [21]. In brief, pieces of frozen tissues were homogenized on ice in a 1mg:10μl diluted solution containing sucrose 0.25M; EDTA 0.1mM; Tris 3mM (pH 7.4; [22]). Supernatants were collected from samples after centrifugation at 9,000g at 4°C for 20min and stored at −80°C.CYP3A4 activity of placenta and human livers was determined using a fluorogenic-based substrate and the Vivid® Green CYP450 Screening Kit following the manufacturer's guidelines (ThermoFisher Scientific). Fluorescence was read at 485nm (excitation) and 590nm (emission) every minute over an hour duration. Values were plotted as a summation of the total fluorescence recorded over the experimental period, *i.e.,* total average rate of fluorescence units (AFU). All samples were run in parallel to eliminate technical variability and in triplicate. Significance was determined using a Student t-test and a cutoff of $p<0.05$.

Review of epidemiological evidence evaluating the influence of environmental exposures on fetal CYP expression/activity—We searched the scientific literature to identify human epidemiological studies which examined fetal CYP mRNA/protein expression, activity, and/or methylation in relation to environmental exposures in the context of disease or lack of. We searched PubMed and Google Scholar databases in July of 2018 using the following search terms:

PubMed: ((“xenobiotic” OR “xenobiotics” OR “chemical” OR “chemicals” OR “environmental chemical” OR “environmental chemicals”) AND (“metabolism” OR “expression” OR “regulation”) AND (cyp* OR P450) AND (“placenta” OR “fetal liver” OR “pregnant”)) OR ((“metabolism” OR “expression” OR “regulation”) AND (cyp* OR P450) AND (“placenta” OR “fetal liver” OR “pregnant”))

Google Scholar: ((“xenobiotic” OR “xenobiotics” OR “chemical” OR “chemicals” OR “environmental chemical” OR “environmental chemicals”) AND (“metabolism” OR “expression” OR “regulation”) AND (cyp* OR P450) AND (“placenta” OR “fetal liver” OR “pregnant”))

We conducted single-screening of title and abstracts followed by full-text review of all articles retrieved from PubMed (1,231 total) and the first 500 records (ordered by decreasing relevance) returned in Google Scholar. We included human epidemiology studies reporting

original data that examined fetal CYP mRNA/protein expression, activity, and/or methylation in relation to environmental chemical exposures. We then report and discuss study characteristics and the main findings on CYP measurements and expression and relationships to environmental exposures.

Results

In silico transcriptomic analysis of CYP expression between adult livers and fetal tissues

Based on our search and selection criteria, we identified two datasets in NCBI and EBI data repositories for analysis. Using the dataset, GSE61279, we evaluated the relative expression of 56 unique CYPs in human adult versus fetal (1st trimester) livers (Figure 2A). Forty-eight percent of CYPs (n=27, in bold) were expressed significantly higher in the mature liver as compared to the fetal liver ($p<0.05$; FC > 1, $\log_2$ scale). Specific CYPs (*i.e.,* CYP-2E1 (169 times higher in the adult vs. fetal liver, *i.e.,* 169X), −2C8 (97X), 1A2 (60X), −3A4 (60X), −2C9 (42X), −2A6 (24X), −2C18 (21X)) were over 20 times higher in the adult as compared to the fetal liver. Only three CYPs were significantly expressed higher in the fetal liver; specific steroid metabolizing enzymes (−3A7 (13X), −19A1 (7X), −51A1 (2X)). No differences in CYP expression were observed between sexes in fetal and adult livers based on the predefined significance criteria ($p>0.05$, Supplemental Table 2). CYP2E1, −1A2, −2C8, −2C9, −2A6 displayed the greatest variability across fetal liver samples ($CI_{95\%} \geq 3$ fold difference within range; Supplemental Table 3). In general, adult livers were less variable than fetal samples (80% of CYPs displayed less variability in adult vs. fetal livers), however, this could be attributed to a larger sample size (n= 92 adult vs. 14 fetal livers).

In the E-MTAB-1733 dataset, 67% of CYPs (34/51) were expressed significantly higher in the adult liver as compared to the placenta ($p<0.05$; FC >1, log 2 scale; Figure 2B). This subset included well-recognized CYPs involved in xenobiotic transformation, *e.g.,* −2E1 (1,351X), −3A4 (891X), −2C9 (588X), 2A6 (478X), 2C8 (446X); and 2B6 (256X). A smaller subset of CYPs, including,−19A1 (79X), −11A1 (20X), −24A1 (5X), −26B1 (3X), 4B1 (2X), and 2W1 (2X), were expressed higher in the placenta as compared to the adult liver.

We examined transcript abundance of 55 CYPs in amnion, basal plate, chorion, villi, and purified CTBs isolated from 2nd trimester and term placentas. In general, the majority of CYPs (~73%) were generally expressed at low levels in placental cells/tissues (median expression < 1 RPKM; Figure 3). This subset included xenobiotic transformation enzymes such as −1A1, −2E1, and −3A4. In contrast, CYPs with important roles in steroidogenesis and cholesterol metabolism, *e.g.,* −11A1, −19A1, −51A1, −26B1, −2R1, −7B1, were expressed at levels > 1 RPKM. For a subset of CYPs, expression levels were tissue specific. For example, −19A1 tended to be expressed higher in the basal plate (average = 289 RPKM) and villi (506 RPKM) as compared to the smooth chorion (9 RPKM) or amnion (3 RPKM). Specific CYPs seemed to display age-dependent differences in expression (2nd vs. Term) within specific tissue compartments (*e.g.,* −26B1, −2W1, 7B1 in the amnion), however, these patterns were difficult to gauge due to a limited sample size.

Targeted molecular analysis of CYP expression in adult liver and 2nd trimester fetal tissues

From our qRT-PCR analysis we detected CYP expression above the LOD in >98% of fetal liver samples for 6 out of the 9 CYPs analyzed (Supplemental Table 1). In total, 87% and 39% of fetal livers, respectively, expressed levels of CYP1A2 and −2B6 above the LOD. All adult liver samples displayed expression levels above the LOD for all CYPs assessed. We measured significantly lower expression for all xenobiotic metabolizing CYPs in fetal versus adult livers ($p<0.05$), with the exception of −2J2 ($p>0.05$; Figure 4). For example, −1A1, −1A2, −2B6, −3A4, and −2E1 were expressed 77X, 1,528X, 1,224X, 216X, and 12,271X lower in fetal livers versus adult, respectively. In contrast, −19A1 was expressed 119X higher in the fetal liver as compared to the adult. Within fetal samples, we did not observe sex-dependent differences in expression ($p>0.05$; Supplemental Table 2) nor significant correlation with gestational age within this narrow developmental window ($p>0.05$, not shown). In general, variability was constrained ($CI_{95\%}$< 2X) in fetal livers; with the noted exception of CYP2B6, which displayed a $CI_{95\%}$ range of 5.5 in samples above LOD and 33/84 tested samples were below LOD (Supplemental Table 3).

In a subset of placenta samples for our CYP analysis (n=4), we first identified CYPs within our panel which reached levels above our designated LOD for >50% of samples (Supplemental Table 1). In the full set of placental samples, −1A1, −2C9, −2E1, −2J2, and −19A1 mRNA expression levels were evaluated as well as −3A4 as a negative control. CYP1A2, −2B6 and −2C19 were excluded. CYP1A1 was expressed in 31% of samples, in comparison to −2E1 and −2J2, expressed in 84% and 95% of tested samples. CYP2C9 and −19A1 were expressed in all placental samples. In line with comparisons between fetal and adult livers, CYP1A1 (44X) and −2E1 (4,380X) were expressed significantly higher in the adult liver as compared to the placenta ($p<0.05$; Figure 5), whereas, −19A1 was expressed higher (703X) in the placenta as compared to adult livers ($p<0.05$). CYP2C9 levels did not differ between groups ($p>0.05$). Unlike fetal vs. adult liver comparisons, CYP2J2 was expressed ~4X lower in the placenta as compared to the adult liver. Significant differences in expression were not observed between sexes (Supplemental Table 2). Variability ($CI_{95\%}$) was observed to be highest in −1A1 and −2C9 in placenta samples ($CI_{95\%}$; Supplemental Table 3).

CYP3A4 activity in adult liver vs. fetal tissues

Utilizing a fluorescence-based substrate, we evaluated the activity of CYP3A4 in adult/fetal liver and placenta. As a proxy of relative −3A4 activity, we determined the average total AFUs for each tissue group (Figure 6). CYP3A4 activity was significantly higher in the adult liver (7,054 ± 233 AFU) as compared to the fetal liver (5,560 ± 59 AFU) or placenta (5,090 ± 91 AFU; $p<0.05$). −3A4 mRNA and activity did not correlate in adult liver samples (Pearson's r= 0.13, not shown). Of note, despite measuring activity above background controls, CYP3A4 was expressed at relatively low levels in fetal liver (as compared to adult liver), and not expressed above the LOD in any of the 2nd trimester placenta samples.

Epidemiological evidence of environmental chemical exposures and altered human fetal CYP expression/activity

We screened 1,231 search results in PubMed and 500 records in Google Scholar. We identified six relevant human studies examining the relationship between CYPs and environmental exposures in the context of disease or lack of that met our inclusion criteria (Table 1). Most CYPs were only measured in the placenta at term (n=3); others were measured in the placenta or fetal liver at term or during the second trimester (n=2).The majority of studies investigated smoking exposures (n=3). Other studies involved polybrominated diphenyl ether (PBDE) (n=1) or organochlorine compounds (n=1). Associations with CYPs were either reported as odds ratios (odds of CYP expression with increases in exposure, n=1) or as a quantitative estimates of changes in methylation, mRNA expression or activity (n=5).

Associations between specific environmental exposures during pregnancy and CYP1A1 expression were consistently reported in all studies; other CYPs (−2B6, −2C9, −2C19, −2E1, −2J2, −3A4, −3A7, −4B1, −11A1, −19A1) were reported in individual studies but were not consistent across the evidence. For instance, Zota et al. [5] identified significant associations between placenta ∑PBDEs (BDE-28, −47, −99, −100, −153) and CYP2E1 (OR=5.02, 95% CI: [1.51, 16.72]) and CYP2J2 (OR=4.15, 95% CI: [1.26, 13.64]), demonstrating 4–5X increased odds between mRNA expression of these specific p450 enzymes with PBDE chemical exposures in the human fetus. Laguex et al. [23] reported a statistically significant 5 fold increase in ethoxyresorufin-O-deethylase (EROD) activity (a proxy of CYP1A1 activity) comparing smokers to non-smokers. Huuskonen et al. [24] reported a statistically significant 140-fold increase in expression of CYP1A1. All other reported associations were not statistically significant (Table 1).

Only two smoking studies investigated associations with health outcomes (birth weight)--none of the other studies investigated associations with adverse health outcomes. Neither study included quantitative estimates of the association between birth weight in the publication.

Discussion

Thousands of xenobiotics and their associated metabolites reach the embryo/fetus during pregnancy without knowing the full extent of the consequences of exposure. Determining the distribution of chemicals and associated elimination pathways during pregnancy may improve our estimation of risk. Utilizing publicly available datasets, our global profiling assessments of CYP mRNA expression in fetal and adult tissues support previous observations that, in general, CYPs, especially those involved in xenobiotic metabolism, are expressed predominately higher in the adult liver versus fetal tissues, and CYPs are consistently expressed within the extraembryonic layers of the placenta. Additionally, we report novel data supporting deficiencies in expression/activity levels of specific CYPs during human mid-gestation in fetal tissues as compared to the adult liver. In general, CYP expression was relatively consistent across fetal livers or placentas. In regards to these comparisons, we briefly discuss our findings below in the context of other CYP sub-family members.

As reviewed [25], in humans,CYP1A1 and −1A2 share a regulatory region and bidirectional promoter region, located on chromosome 15q24.1, which co-controls expression of the two enzymes. Induced by the environmentally-sensitive aryl hydrocarbon receptor (AhR) complex, CYP1A1 and −1A2 biotransform common pollutants such as polycyclic aryl hydrocarbons (PAHs), aromatic amines, and polychlorinated biphenyls into polar compounds, promoting excretion. CYP1A activity may attenuate and promote toxicity. In rodents, diminished CYP1A activity either by genetic manipulation [26] or chemical inhibitor [27] display lower sensitivity to PAH-carcinogenesis, *i.e.,* reduction in PAH-DNA adducts. The CYP1A family is also active in the endogenous metabolism of polyunsaturated fatty acids and the transformation of arachidonic acid to 19-hydroxyeicosatetraenoic acid (a signaling molecule which induces increases blood pressure, vasoconstriction and inflammatory response) and 17β-estradiol. Due to its inducibility, CYP1A1 has been investigated as a biomarker of various environmental exposures, including cigarette smoke [24, 28, 29] and polybrominated diphenyl ethers (PBDEs; [5]; Table 1), and levels of polychlorinated biphenyls (PCBs) may correlate with genetic polymorphic differences in activity [30]. CYP1A1 is known to be expressed at low levels in the fetal liver [31, 32]. Our *in silico* and qRT-PCR analyses further validate these observations. For example, 2nd trimester fetal livers displayed significantly less expression of −1A1 (77X) and −1A2 (1,528X) than adult livers; 2nd trimester placentas expressed −1A1 (44X) less than adult liver and −1A2 was detected at nominal levels (not detected; qRT-PCR; median =0.008 RPKM; *in silico*). In summary, our analyses suggest low expression of CYP1A enzymes in the fetal liver/placenta during pregnancy.

The CYP enzyme 2B6 is a major component of CYP content (1–7%) and genetic variants are suspected to account for major differences in xenobiotic clearance [33]. In particular, CYP2B6 has specific metabolizing roles in the breakdown of common flame retardants (*e.g.,* PBDEs)and interindividual differences in adult activity may account for differences in rates of hydroxylated metabolite formation [34, 35] and bioaccumulation during pregnancy [36]. Our analyses confirm previous reports indicating age-dependent differences in 2B6 expression. Adult livers expressed CYP2B61, 224 more times than the fetal liver. In post-hoc and *de novo* mRNA assessments of placenta (2nd/term), CYP2B6 was not (expressed <LOD in qRT-PCR analysis of 2nd trimester placentas) or barely detectable (median = 0.008 RPKM in term placentas).These results suggest a lower capacity of the fetus to breakdown compounds such as PBDEs, known to be metabolized by CYP2B6, possibly leading to accumulation in the placenta and fetal liver [5] and higher sensitivity.

CYP2C family members also participate in aspects of oxidative metabolism of a variety of xenobiotics, including pharmaceuticals, PAHs and organochlorines. In our combined analyses, we evaluated expression of −2C8, −2C9, −2C18, and −2C19 in fetal versus adult tissues. In comparisons between adult livers versus 1st trimester fetal liver/term placentas, all four of these isoforms were expressed at significantly higher levels in the adult liver. Limited expression of all four isoforms (median <0.2 RPKM) was observed in the placental compartments (2nd/term). In our qRT-PCR analyses, mRNA levels of −2C9 and −2C19 were detectable in 2nd trimester fetal liver but significantly lower than adult levels. In 2nd trimester placentas, −2C9 was also identified and found to be at lower levels as the adult liver, and

levels in the placenta varied ($CI_{95\%}$ = 5 fold). In general, our analyses further suggest lower expression of the 2C family members in fetal tissues vs. adult liver.

CYP2E1 has been studied extensively in the field of toxicology due to its role in breakdown of many hazardous compounds such as ethanol and benzene. The human fetus expresses −2E1 at low levels during pregnancy [37]. Our study confers with these analyses. Adult −2E1 expression was significantly higher than 1st or 2nd trimester fetal livers. Previous studies in 1st trimester tissues[38]suggest that −2E1 expression is also detectable in the placenta. Our analyses suggested −2E1 levels to be significantly lower in the placenta than adult liver and expression in 2nd trimester/term placental CTBs/tissue to be barely detected (median = 0.08 RPKM). Interestingly, based on $CI_{95\%}$, −2E1 was observed to be generally, more inherently variable than other enzymes evaluated in our analyses (Supplemental Table 3). Toxicological studies in rodent and human cells indicate the high sensitivity of −2E1 induction to environmental factors [39], implying other variables not addressed in these cumulative analyses may influence −2E1 expression in fetal tissues.

The CYP3A enzyme family consists of three isoforms in humans (−3A4, −3A5, −3A7). In the adult liver, −3A4 makes up ~40% of the CYP content and plays prominent roles in metabolism of environmental chemicals, steroids, and pharmaceuticals, *i.e.,* 50% of drugs are metabolized by −3A4 [21]. CYP3A5 and −3A7 also are involved in oxidative metabolism, however, the catalytic activity and compound-specificity differ as compared to −3A4 [40]. Our analyses expand previous reports indicating expression in placenta [41] and fetal liver [42], and significant differences in CYP3A expression/activity between adult and fetal livers [42]. In investigating public datasets, we found general differences in −3A4, −3A5 (higher in adult liver) and −3A7 (higher in fetal tissues). In comparisons between fetal liver/placenta versus adult liver, we found 3A4 to be expressed significantly higher in the adult and of general low variance in fetal tissues. Activity assessments of 3A4 in adult versus fetal tissues revealed differences, however, dramatically less in magnitude than differences in expression. While enzymatic activity may be a more appropriate indicator of functional capacity than mRNA expression; respective assays are generally less specific than mRNA analyses due to the use of non-specific substrates, other enzymes with similar activities may contribute to measured activity. Our results suggest significant differences in 3A4 expression/activity, but also, point out the complexity in determining capacity of fetal metabolism via CYP3A-mediated pathways due to the possible contribution of other enzymes.

During pregnancy, cholesterol and steroid metabolism pathways are tightly controlled by several enzymes including CYPs, sulfotransferases, hydroxysteroid dehydrogenases, and reductases [43]. Alterations in these pathways due to genetic or environmental factors can have severe consequences, including disruption of fetal brain, skeletal and reproductive development [44]. Our analyses suggest that CYPs involved in steroid/cholesterol metabolism (*e.g.,* −11A1, −19A1, −51A1) are highly expressed in fetal tissues as compared to adult liver. Furthermore, our findings confer with previous characterization studies of human placental/fetal tissues indicating the high presence of these enzymes [45], including, CYP19A1, a key enzyme involved in the rate-limiting catalyzation of the conversion of androgenic substrates to oestrogens, steroids necessary for successful fetal development and

pregnancy; CYP11A1, an enzyme involved in the early steps of steroidogenesis, specifically, the conversion of cholesterol to pregnenolone, a precursor of progesterone, a hormone critical for survival during early pregnancy; and CYP51A1 a catalyzer of removal of the C-14α methyl group from lanosterol (a precursor of cholesterol), enabling conversion to cholesterol and other steroid precursors. Our analyses further support the observation that CYPs linked with sterol metabolism are highly expressed in the fetus and provide quantitative comparisons with adult liver.

Previous analyses suggest sex-dependent differences in hepatic expression of specific CYPs in human [46–48], rodent [49, 50] and other mammalian species (reviewed, [51]). Divergent expression of CYPs are postulated to control differences in liver size between sexes. In our analyses (*in silico* and qRT-PCR), we did not observe significant differences in CYP expression between male and female liver tissues in adult and fetal samples ($p>0.05$; Supplemental Table 2). Our lack of detecting sex-dependent differences could be due to sample size, *i.e.,* a lack of power to detect, and/or our inability to address other variables suspected to influence expression such as age, ethnicity, and environmental exposures.

In our review of existing scientific literature, we identified a paucity of epidemiological studies investigating CYP expression in relation to environmental chemical exposures. While normally, CYP1A1 levels seem limited in the embryo/fetus over the course of pregnancy, initial studies suggest that 1A1 levels may serve as a sensitive biomarker for environmental compounds. CYP1A1 was consistently measured in all included studies, for a variety of different environmental chemicals ranging from cigarette smoke to industrial chemicals and pesticides, with fold induction ranging from 1–2 orders of magnitude, specific to tissue, study design and exposure. Additional CYPs were also measured, but none were measured in more than one study. Further studies are needed to provide sufficient data investigating metabolic pathways and CYP expression of a wider range of environmental chemicals, specifically in human fetal cells/tissues. This is an important existing knowledge gap currently limiting our ability to accurately assess and ensure the protection of fetal health from exposures to environmental contaminants.

In summary, using a combination of *in silico* and wet-lab approaches, we characterized from macro- and micro- viewpoints, the expression of CYP p450 metabolizing enzymes in human placenta, fetal liver, and adult liver. Inferred primarily based on RNA expression data, which ignores potential differences in protein expression and enzymatic activity, we demonstrate significant differences in metabolic competency in the CYP enzymes known for their important roles in xenobiotic metabolism. Quantitative outputs from this study may guide biomonitoring and PBPK modeling approaches aimed at understanding the distribution and disposition of environmental chemicals or pharmaceuticals during human pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Highlights

1. Characterization of global human cytochrome (CYP) p450 expression in fetal tissues.
2. New expression data of nine CYPs in 2nd trimester placenta/fetal liver.
3. Review of fetal CYPs as biomarkers in environmental pregnancy studies.
4. A resource for modeling predictions of chemical distribution and sensitivity.

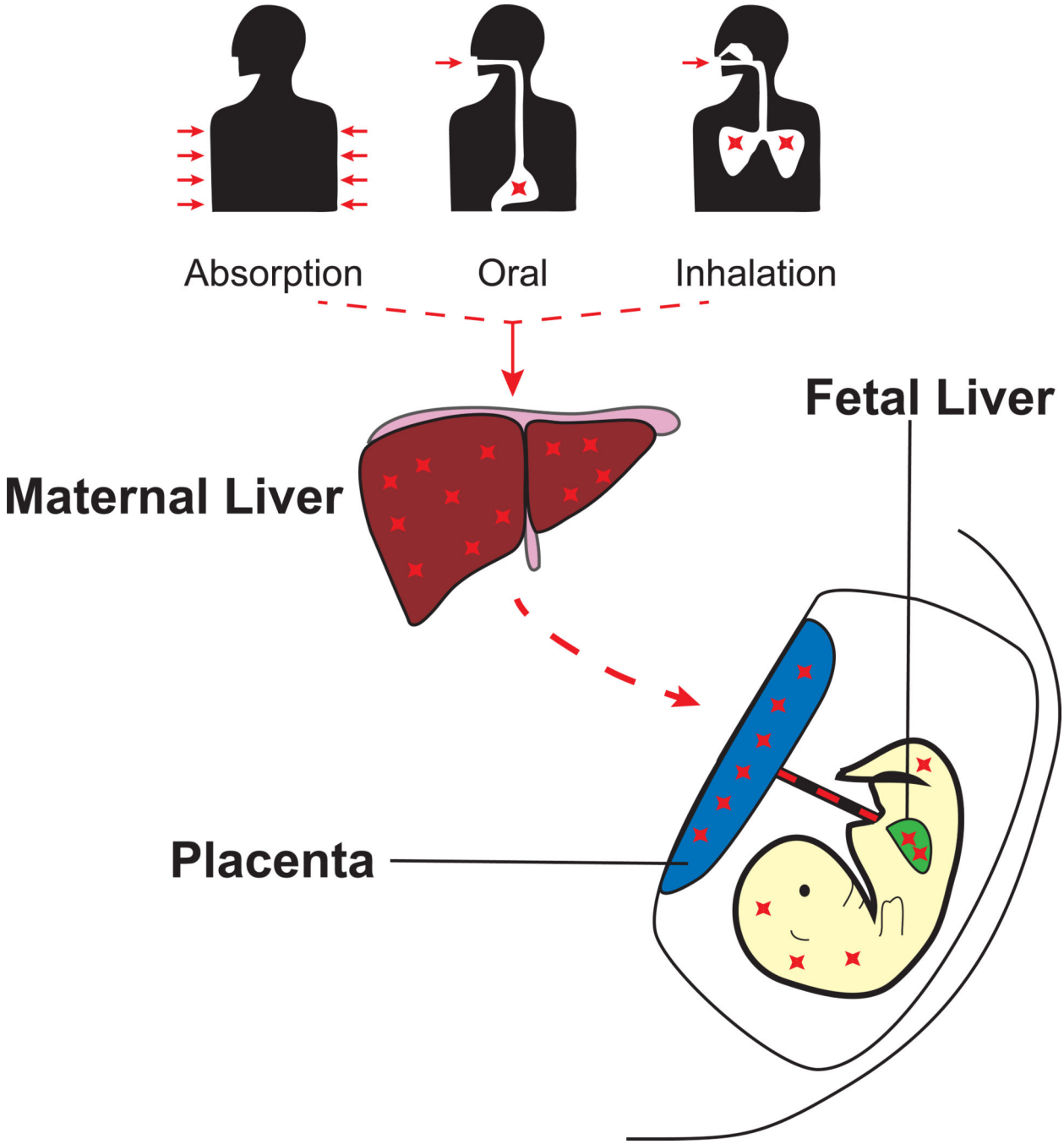

Figure 1: Routes of exposure and points of metabolism during human pregnancy.

Compounds are transferred from the mother to the embryo/fetus dependent on several factors such as the inherent properties of the compound, gestational age, and exposure route. Compound exposures occurring via oral exposure are absorbed through the intestinal tract and transported via portal-vein circulation to the liver, undergo first-pass metabolism, and then, enter the systemic circulation. Inhalation or dermal exposures bypass this initial step. In the earliest periods of pregnancy, compounds passively transfer from the mother to the placenta and the embryo proper. Later on in pregnancy, the embryo/fetus relies on the

maternal blood supply and active transport. In humans, maternal and fetal blood do not coalesce. The placenta serves as the junction between the two units and compounds must pass through several barriers to reach the embryo/fetus.

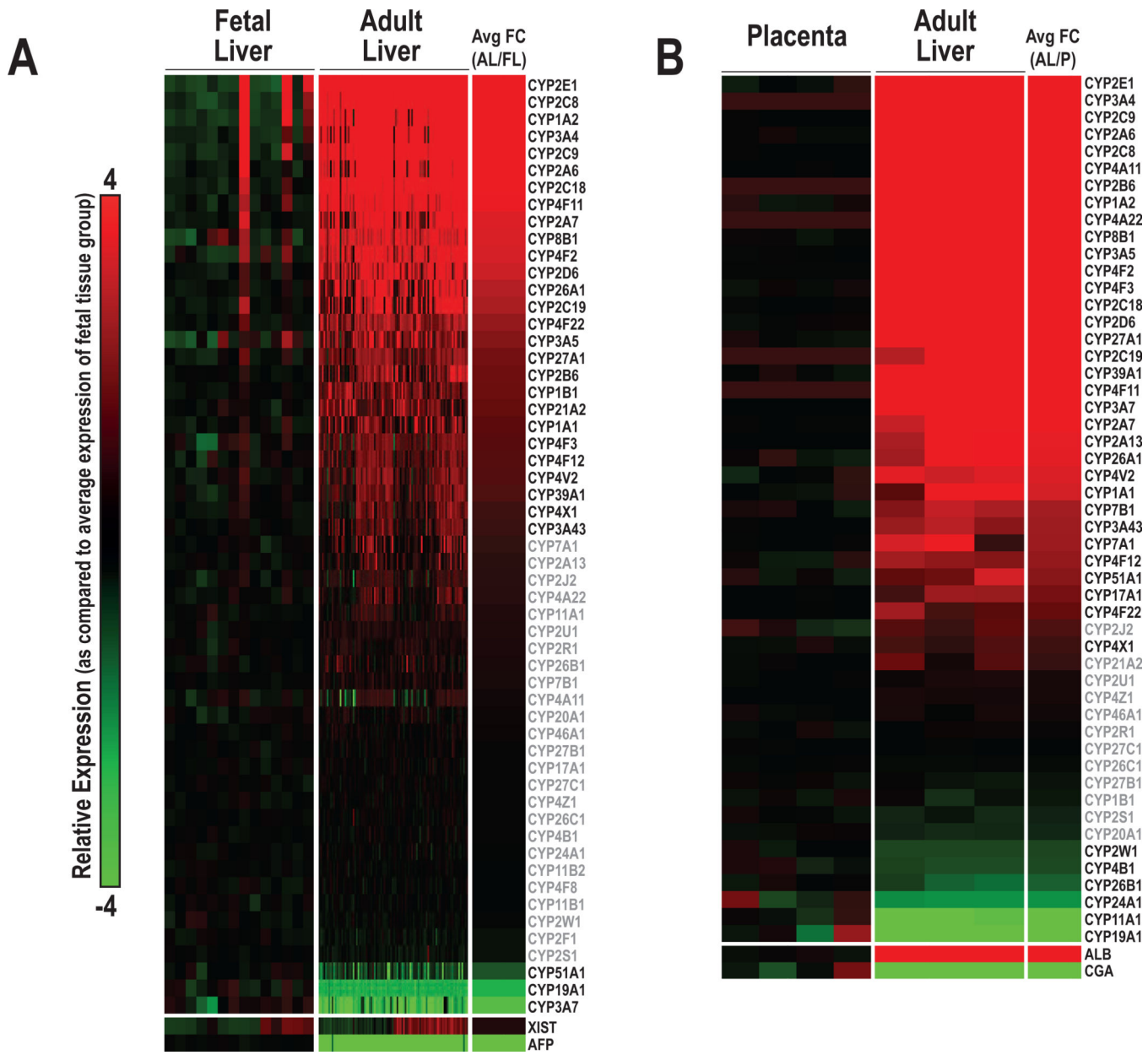

Figure 2: Global CYP expression profiles in human adult and fetal tissues.

Relative CYP expression (log 2 scale) in human 1st trimester fetal and adult livers (**A**; GSE61279;[15]); or human term placenta and adult livers (E-MTAB-1733; **B**). CYPs in bold were identified to be differentially expressed between the two compartments ($p<0.05$; absolute FC > 1, log2). The average fold change (FC) difference between adult and fetal compartments is displayed to the right of each clustering heatmap. Expression levels of additional genes, include: X Inactive Specific Transcript (XIST), a transcript expressed higher in males versus females; Alpha-fetoprotein (AFP),a marker of liver immaturity; albumin (ALB), a marker of liver maturity; and Glycoprotein Hormones, Alpha Polypeptide (CGA), a placental hormone.

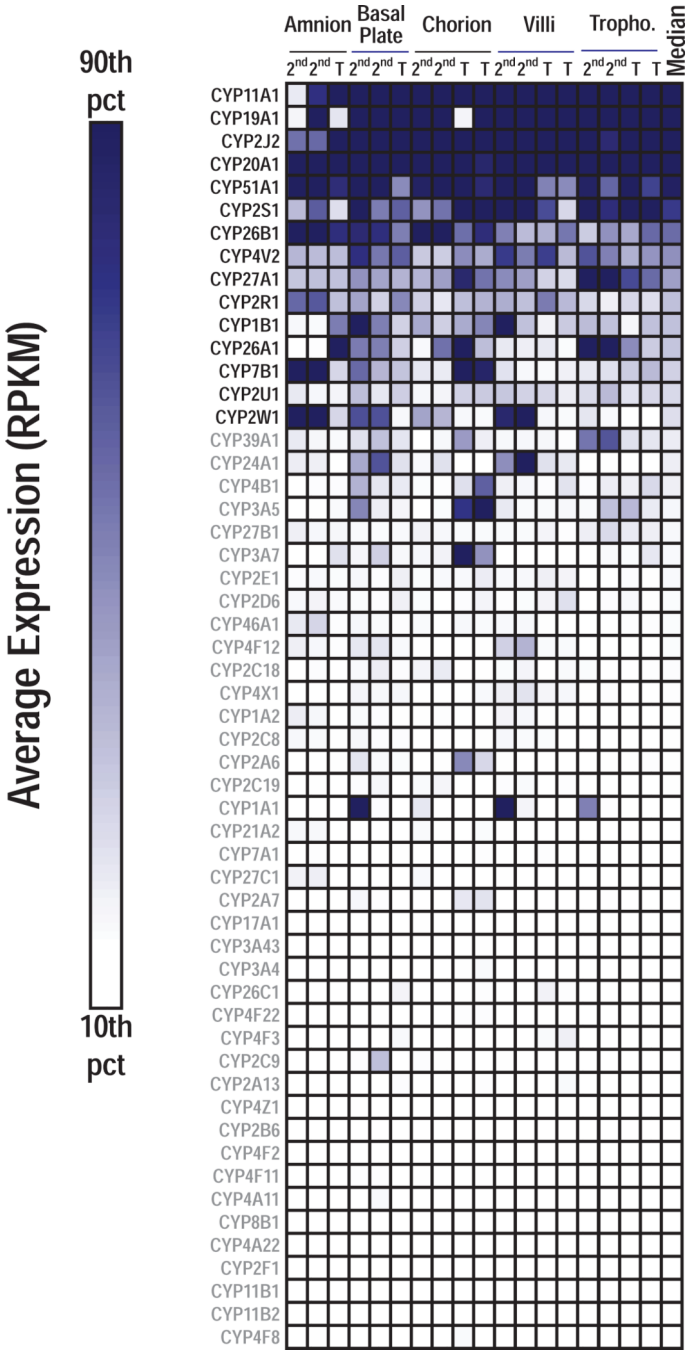

Figure 3: Global CYP enzyme expression profiles of human placental regions.

Compartment-specific expression (RPKM) of CYPs in placental compartments (amnion, basal plate, chorion, villi, cytotrophoblasts) of 2nd trimester and term human placentas (GSE16368; [19]; **A**]). Median RPKM expression across all samples is displayed (right of heatmap). CYPs expressed at median levels above 1 RPKM are in bold.

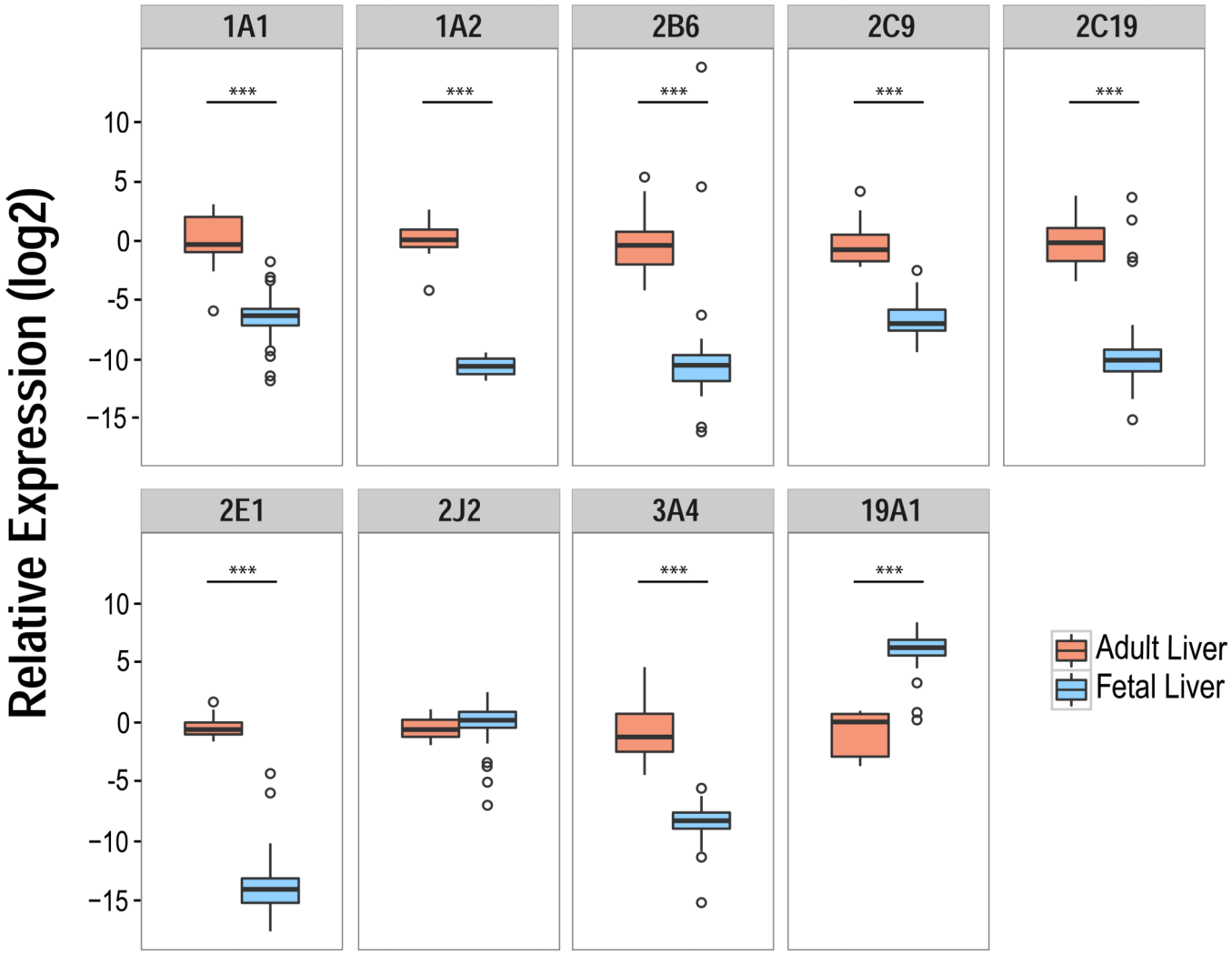

Figure 4: CYP expression in human adult livers and 2nd trimester fetal livers.

Relative CYP RNA expression (log2) in human adult (n=9) and fetal livers (n=84). Values represent the adjusted log2 average and standard error (SE) to the mean. Asterisks (*) denote significance (t-test, $p<0.05$).

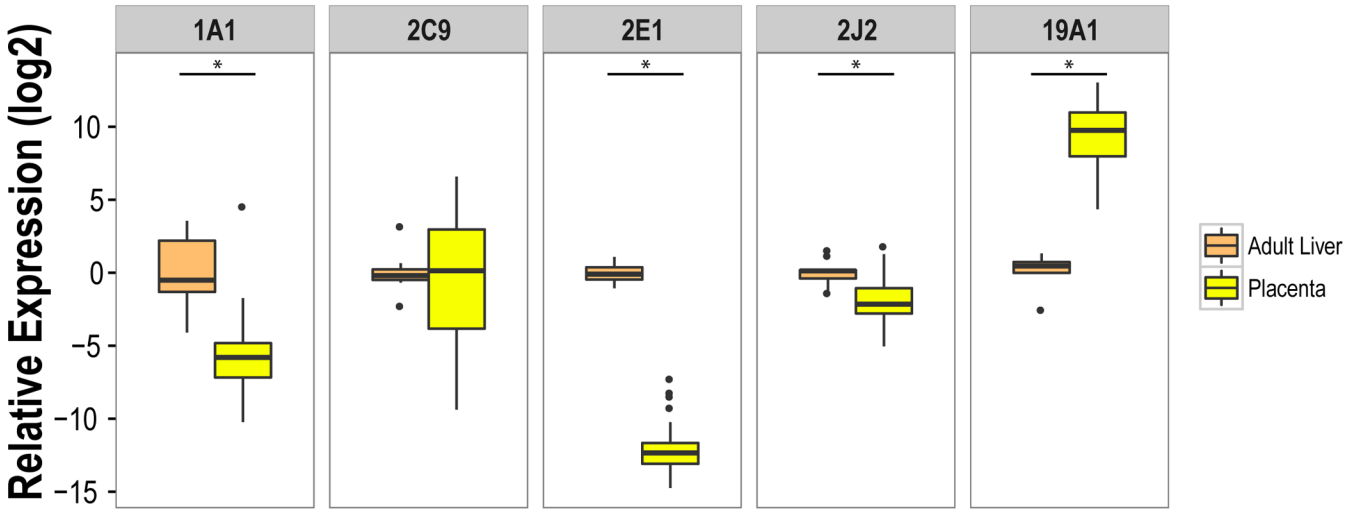

Figure 5: CYP expression in human adult livers and 2nd trimester placentas.

Relative CYP expression (log2) in human adult livers (n=9) and second trimester placentas (n=47). Asterisks (*) denote significance (t-test, $p<0.05$). Box plots represent the adjusted log2 average and standard error (SE) to the mean. Asterisks (*) denote significance (t-test, $p<0.05$).

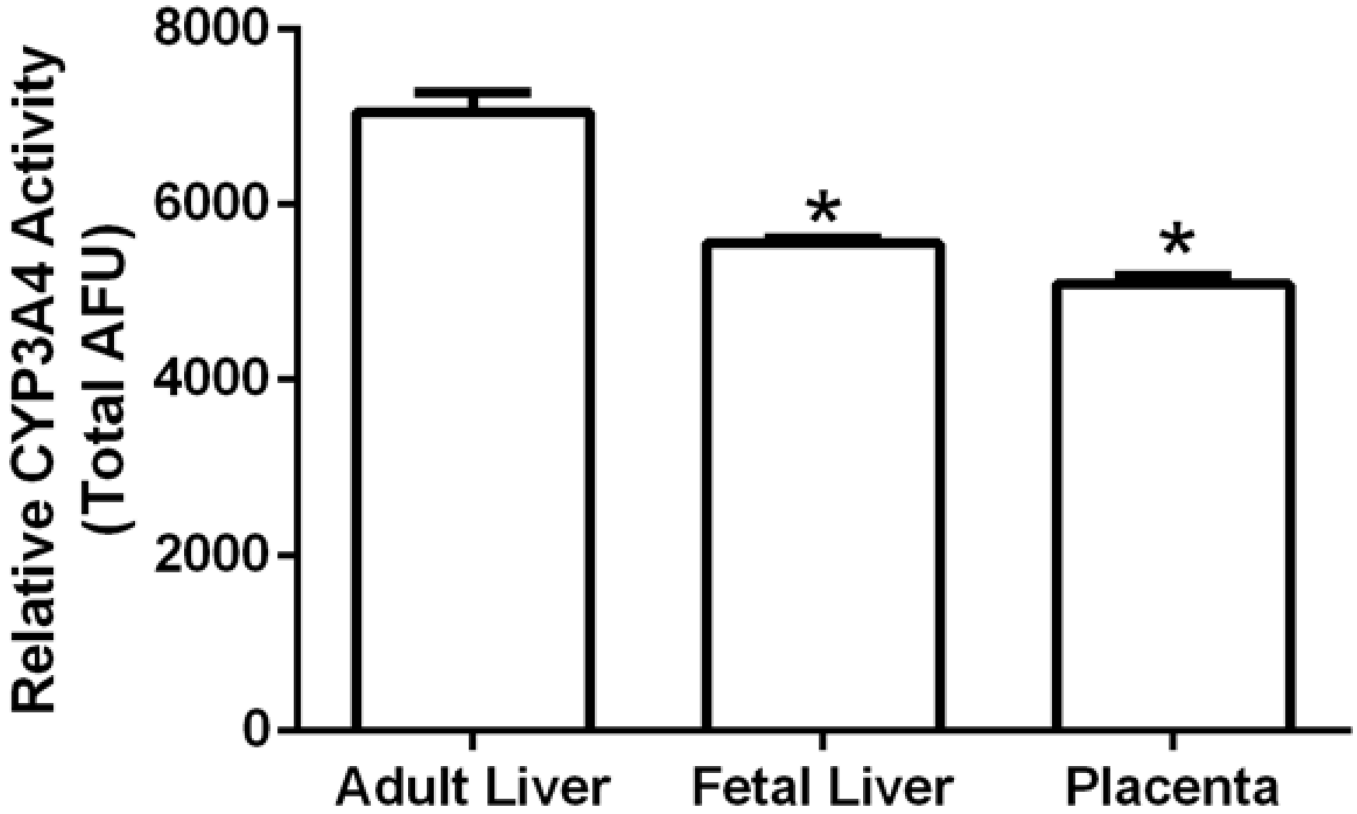

Figure 6: Activity of CYP3A4 in human placenta and adult/fetal livers.

Relative CYP3A4 activity (expressed as the total average rate of fluorescence units (AFU)).Asterisks (*) denote significance (t-test, $p<0.05$).

Table 1:

CYPs as Biomarkers of Human Environmental Chemical Exposures in Epidemiological Studies.

Reported statistically significant results are displayed in bold. Assessments of CYPs were conducted on the mRNA (R) or Protein (P), Activity (A), or Methylation (M) level as denoted. Additional abbreviations include: not determined (ND); Birth Weight (BW); Polybrominated Diphenyl Ethers (PBDEs); and Organochlorines (OCs).

Exposures	Biological Matrices	Associations with CYPs	Disease	Ref
PBDEs	Placenta (2nd trimester)	1A1 (R), OR: 1.26 95% CI: [0.44, 3.55] **2E1 (R), OR: 5.02 95% CI: [1.51, 16.72]** 2C9 (R), OR: 1.60 95% CI: [0.59, 4.32] **2J2 (R), OR: 4.15 95% CI: [1.26, 13.64]**	ND	[5]
	Fetal Liver (2nd trimester)	1A1 (R), OR: 2.36 95% CI: [0.98, 5.68] 2E1 (R), OR: 1.71 95% CI: [0.80, 3.67] 2C9 (R), OR: 0.70 95% CI: [0.34, 1.44] 2J2 (R), OR: 0.69 95% CI: [0.33, 1.45] 2C19 (R), OR: 0.91 95% CI: [0.45, 1.83] 3A4 (R), OR: 1.36 95% CI: [0.67, 2.78] 2B6 (R), OR: 1.23 95% CI: [0.59, 2.58]		
Smoking	Fetal Liver (2nd trimester)	**1A1 (A), increase in males** **3A7 (A), increase in females** No quantitative estimates available.	ND	[29]
Smoking	Placenta (term)	1A1 (M, $CpG_{1)}$, ΔM=1.75%; 95% CI: [−3.09, 6.60] 1A1 (M, $CpG_{2)}$, ΔM=0.43%; 95% CI: [−3.63, 4.49] **1A1 (M, $CpG_{3)}$, ΔM=−4.57%; 95% CI: [−7.15, −1.98]** 1A1 (M, CpG_{4}), ΔM=0.98%; 95% CI: [−2.22, 4.19]	BW	[28]
Smoking	Placenta (term)	11A1 (A) 0.49 fold decrease (p=0.702) 19A1 (A) 0.66 fold decrease (p=0.259) **1A1 (R) 142.86 fold increase (p=0.001)** 2B6 (R) 0.26 fold decrease (p=0.898) **4B1 (R) 6.88 fold increase (p=0.026)**	BW	[24]
OCs	Placenta (term)	**1A1 (A) 4.6 fold increase (p<0.05)**	ND	[23]