Published in final edited form as:

Clin Pharmacol Ther. 2015 August ; 98(2): 205–215. doi:10.1002/cpt.145.

Age-related changes in microRNA expression and pharmacogenes in human liver

Kimberly S. Burgess, **Santosh Philips**, **Eric A. Benson**, **Zeruesenay Desta**, **Andrea Gaedigk**, **Roger Gaedigk**, **Matthew W. Segar**, **Yunlong Liu**, and **Todd C. Skaar**

Department of Pharmacology and Toxicology (K.S.B.), Department of Medicine, Division of Clinical Pharmacology (K.S.B., S.P., E.A.B., Z.D., T.C.S.), Department of Medical and Molecular Genetics (Y.L.), and Center for Computational Biology and Bioinformatics (M.W.S., Y.L.) Indiana University School of Medicine, Indianapolis, IN. Division of Clinical Pharmacology, Toxicology and Therapeutic Innovation, Children's Mercy Hospital, Kansas City, and School of Medicine, University of Missouri-Kansas City, Kansas City, MO (A.G., R.G.)

Abstract

Developmental changes in the liver can significantly impact drug disposition. Due to the emergence of microRNAs (miRNAs) as important regulators of drug disposition gene expression, we studied age-dependent changes in miRNA expression. Expression of 533 miRNAs was measured in 90 human liver tissues (fetal, pediatric (1-17 years), and adult (28-80 years); n=30 each). 114 miRNAs were upregulated and 72 were downregulated from fetal to pediatric, and 2 and 3, respectively, from pediatric to adult. Among the developmentally changing miRNAs, 99 miRNA-mRNA interactions were predicted or experimentally validated (e.g. hsamiR-125b-5p-CYP1A1; hsa-miR-34a-5p-HNF4A). In human liver samples (n=10 each), analyzed by RNA-sequencing, significant negative correlations were observed between the expression of >1000 miRNAs and mRNAs of drug disposition and regulatory genes. Our data suggest a mechanism for the marked changes in hepatic gene expression between the fetal and pediatric developmental periods, and support a role for these age-dependent miRNAs in regulating drug disposition.

Keywords

liver; fetus; pediatric; age; development; ontogeny

Introduction

Developmental changes in drug disposition create the need for age-appropriate pharmacotherapy.[1-4] Although there have been many approaches for estimating pediatric drug dosing (e.g. extrapolation from adult data), these approaches are not adequate for many

Todd C. Skaar 950 W Walnut Street R2 402 Indianapolis, IN 46202-5274 Phone: 317-274-2810 Fax: 317-274-2766 tskaar@iu.edu.

Author Contributions

K.S.B., Z.D., A.G., and T.C.S wrote the manuscript; K.S.B., S.P., Z.D., A.G., Y.L., and T.C.S. designed the research; K.S.B., S.P., and R.G. performed the research; K.S.B., E.A.B., M.W.S., and Y.L. analyzed the data.

Conflict of Interest/Disclosure

None.

children, particularly for the very young patients (infants vs children).[5] Developmental changes in drug response are difficult to predict at the individual patient level, in part due to our poor understanding of the mechanisms that regulate these developmental changes. Consequently, the high off-label use of drugs in this pediatric population is associated with an increased number of adverse events.[6] Although more clinical studies are now being conducted in pediatric populations as a result of the Pediatric Exclusivity and the Best Pharmaceuticals for Children Acts, a fundamental understanding of the ontogenesis of drug disposition genes is still needed to develop more accurate dosing guidelines for both the clinical trials and clinical care in the young.

The majority of drug disposition genes are known to change during early and late developmental stages in humans; however, little is understood about the mechanisms that control these changes. The most well characterized age-associated drug disposition genes involve expression of drug metabolizing enzymes in the liver.[7-10] These include phase I and II enzymes that are responsible for the modification and elimination of many drugs. The most notable of the phase I enzymes are the cytochrome P450s (CYPs) and many developmental changes have been observed in their expression patterns.[7-10] For example, the predominant *CYP* gene expressed in fetal liver, *CYP3A7*, peaks shortly after birth and becomes undetectable in most children and adults. In contrast, *CYP1A1*, *2C9*, *2C19*, *2D6*, and *2E1* are undetectable or expressed extremely low in the fetus, but are expressed at high levels after birth.[11-15] A well-documented example of phase II enzyme ontogeny is the delayed onset of a member of the *UGT2B* family responsible for “gray baby” syndrome in neonates as an adverse event of chloramphenicol therapy.[16] There is also data indicating that *UGT1A6* and *UGT1A9* function increases in older children and adults compared to younger children.[17] Recently, developmental patterns of drug transporter expression indicates that hepatic *MDR1* (*ABCB1*), *MRP* (*ABCC2*), *OATP1B1* (*SLCO1B1*) and *OATP1B1* (*SLCO1B3*) expression is increased in adults compared to fetal and neonatal periods.[18]

To elucidate the mechanisms underlying these developmental changes, this study focused on the developmentally-regulated expression of hepatic microRNAs (miRNAs). miRNAs are small noncoding RNAs approximately 17 to 22 nucleotides in length that bind to mRNAs and regulate translation and stability. The majority of miRNAs bind imperfect complimentary sequences in the 3’UTRs of target mRNAs.[19-21] In addition, a minority of miRNAs target other regions of mRNA such as the 5’UTR and coding regions to repress translation, and in some cases, have even increased gene expression.[22-24] The 3’UTR ‘seed sequence’ located at the 5’ end from bases 2-8 is a critical feature required for miRNA binding to its target mRNA.[25] With over 2500 mature miRNAs (miRBase Registry version 20) and a seven base pair seed sequence, miRNAs have already been shown to regulate a large number of genes, including some involved in drug metabolism and disposition.[26-30]

Hepatic miRNA expression is altered under a variety of conditions. Hepatic diseases, such as liver cancer, cirrhosis, and hepatitis C infection, are associated with altered miRNA expression[31-33] We have shown that rifampin, a known inducer of drug metabolism genes [34,35], alters miRNA expression patterns in primary human hepatocytes.[36] Altered hepatic miRNA expression patterns may contribute to rifampin-mediated drug interactions. Furthermore, in rats, aging has been associated with alterations in the hepatic miRNA

expression, and pulmonary expression of several miRNAs that are known to regulate susceptibility to carcinogens.[37,38]

In this study, we hypothesized that miRNA expression in human liver tissue changes with age and that this variable expression contribute to the developmental changes in drug metabolism and disposition genes. To test this hypothesis, we measured and analyzed miRNA and mRNA expression in human liver tissues from subjects in three different age groups (fetal, pediatric, and adult). Our results suggest that miRNAs are likely to regulate these genes either directly or indirectly through upstream regulatory genes.

Results

miRNA expression changes with developmental periods

To determine miRNA expression patterns across the fetal, pediatric, and adult developmental periods, we measured expression of 533 miRNAs in 90 liver samples using the TaqMan OpenArray platform. These are commercially available arrays that are pre-plated with 756 miRNAs that were known at the time of the array design (533 miRNAs were detected in our samples). This set of miRNAs includes those that have been shown to be important in the liver. We also measured four of these miRNAs, each in a unique set of 20 samples using single TaqMan assays to validate their expression patterns in the OpenArray platform (Supplemental Figure 1). miRNAs that did not generate threshold cycle values in at least 6 samples in a given developmental period were excluded from analysis unless that miRNA was not expressed in the group at all. Only miRNAs that were statistically significant after false discovery rate (FDR) correction are discussed.

As shown in the Principal Components Analysis, the expression profiles for the fetal group are distinctly separated from the pediatric and adult groups (Figure 1). Hierarchical clustering, using Spearman Rank Dissimilarity, was implemented to depict the grouping of both samples and miRNAs (Figure 2) and reveal 71% dissimilarity between the fetal group and the pediatric/adult groups. Although not as dramatic, there are notable dissimilarities among the pediatric and adult groups as well. There are also clusters of miRNAs that are significantly upregulated and downregulated together among the developmental groups. We show the top 45 developmentally-regulated miRNAs between the groups; the remaining statistically significant developmentally-regulated miRNAs are shown in the supplemental materials. During the transition from the fetal to the pediatric period, 114 miRNAs were upregulated and 72 miRNAs were downregulated (Table 1 & Supplemental Tables 1 & 2). Between the pediatric and adult periods, two miRNAs were upregulated and three were downregulated (Table 2). An example of miRNA expression changes in each category of Table 1 & 2 is provided in dot plots of individual miRNA threshold cycle (C_T) values (Supplemental Figure 2). An increase in C_T values indicate a decrease in miRNA expression. miRNAs that do not generate a C_T value are assigned a value of 40 indicating that it is undetected. Supplemental Figure 2A shows an increase in hsa-let-7a-5p between the fetal and pediatric/adult group whereas Supplemental Figure 2B shows a decrease in hsa-miR-431-5p expression between these groups. Supplemental Figure 2C & 2D depicts the gradual increase and decrease in expression of hsa-miR-34a-5p and hsa-miR-18a-5p, respectively, across the developmental periods.

Inverse correlation of expression of miRNAs and ADME mRNAs

Over 14,000 human genes were analyzed using RNA-seq in 30 samples (10 per age group). Figure 3 displays the expression of 34 absorption, distribution, metabolism, elimination (ADME) and regulatory genes that are relevant to drug disposition between the populations (Figure 3 & Supplemental Table 3). Among those genes, expression of 28 mRNAs increased and expression of three decreased from fetal to pediatric, and two increased and five decreased from pediatric to adult. *CYP2D6* and *UGT2B17* gene expression levels were among those that increased from pediatric to adult while *CYP1A1, CYP1A2, CYP2C19, GSTM1*, and *SLCO1B3* expression levels decreased from pediatric and adult. *CYP3A7, GSTP1*, and *SLC15A2* expression decreased from fetal to pediatric/adult while most of the others increased. These findings are consistent with previous findings regarding the developmental expression profiles for these ADME genes.[1,2,9,18]

A linear regression model was used to determine negative correlations between the expression levels of the developmentally changing miRNAs and the ADME and regulatory mRNA expression levels. In order to eliminate bias due to the difference in the expression levels of these miRNAs and mRNAs, we considered developmental periods as a cofactor while searching for inverse correlations between the miRNA and target genes. The negative estimates shown in Table 3 indicate the changes in mRNA expression. We observed over 1000 negative correlations (data not shown), but only the top five for each mRNA are shown based on FDR. Some of these correlations may reflect direct regulation of the mRNAs by the miRNAs, but it is also conceivable that there may be indirect regulation through transcriptional regulators, such as HNF4A and PXR.[39,40]

In silico identification of miRNAs predicted to target top ADME mRNA

Predicted miRNA target analysis was performed using Ingenuity Pathway Analysis. This program identifies predicted and experimentally validated targets from multiple databases. Among the 45 developmentally-regulated miRNAs and 34 ADME and upstream regulatory genes, 28 predictions and five experimentally validated targets were revealed involving 16 genes and 21 miRNAs (Table 4). Analysis using the remaining miRNAs that were upregulated or downregulated from fetal to pediatric to adult resulted in 65 predictions and one experimentally validated target involving 46 miRNAs and 23 of the top ADME and regulatory genes (Supplemental Table 4). Eighteen of these predictions overlapped with the negatively correlated miRNA-mRNA combinations (selected correlations are shown in Table 3). The predictions with experimentally validated results (indicated in Table 4 and Supplemental Table 4) mentioned here provide confirmation that at least some ADME mRNAs are regulated by miRNAs.

Discussion

These data indicate that miRNA expression in the human liver is age-dependent which is consistent with observations of developmental changes of miRNA expression patterns in rat.[37] Specifically, miRNAs 29c-3p, 195-5p, and 497-5p were significantly upregulated and 301a-3p, 106b-5p, 185-5p, and 539-5p downregulated between the human fetal and pediatric livers which is in agreement with miRNAs that changed with age in rat liver tissue;

exceptions were miRNAs 148b-3p and let-7a-5p, which revealed opposite findings. Other miRNAs Mimura et al. found to change in aging rat liver were consistent with our data, but not significant after FDR-corrected.

We observed large variability between genes in the extent of induction or repression in mRNA expression between developmental stages, particularly between fetal and pediatric. Since drug metabolism and disposition is enzyme and transporter specific, these differences likely contribute to the differential developmental changes in drug metabolism and disposition. Furthermore, interindividual variability in the rate of change in the expression of these genes could likely also contribute to the interindividual variability in drug efficacy and toxicity. Thus, the developmental effect on drug metabolism must be determined for each specific drug. One limitation to our study is that we do not have serial samples within individuals or broad coverage of time points during fetal development, due to ethical reasons. Hence, the exact timing of some of the changes cannot be determined from our data. Also, due to limited sample materials, we were not able to measure protein expression by Western blot analysis or activity of the drug disposition genes. Since miRNAs can affect protein translation without substantial effects on mRNA levels, our miRNA-mRNA correlations may miss some gene specific effects and will need to be tested in individual experiments.

There is strong evidence that these changes in miRNA expression throughout the developmental periods are important in regulating mRNA expression levels of target genes including phase I and II enzymes, drug transporters, and regulatory genes. This regulation can be linked to the marked changes in mRNA expression levels between the fetal and pediatric/adult group as well as changes between the pediatric and adult group. Twenty-seven miRNAs, not expressed during fetal development, were expressed in the pediatric and adults samples; this can potentially be linked to highly expressed mRNA levels in the fetal liver, which are down-regulated in pediatrics and adults. Additionally, 43 miRNAs expressed during the fetal period were not expressed in pediatric and adult livers. The changes in hepatic miRNA expression from pediatric to adult periods may also contribute to changes in hepatic ADME gene expression. Several of these miRNA changes were associated with change in hepatic mRNA expression. There was substantial variability in the expression of many of the miRNAs during each of the developmental periods. This may be a result of the interindividual variability in the timing of the developmental changes. These developmental changes in miRNA expression are most likely not due to a discrete incremental change amongst individuals but more of a continual change over time; one that likely occurs in every individual, but at various rates and to variable extents. This may contribute to the variable rates of changes in hepatic drug disposition. A limitation of this study is that each of the liver samples was from a different subject, so we were not able to determine the variability in the rates of changes within individuals. Since liver biopsies within individuals would not be ethical, such a study is not feasible in human subjects. Previously, miR-34a-5p, miR-200a-3p, and miR-200b-3p in the liver were shown to increase with age in a Caucasian, mostly adult, population.[41] In our data, miR-34a-5p was upregulated 1.7- and 2.1-fold from fetal to pediatric to adult and miR-200a-3p increased 2.7-fold from fetal to pediatric (Table 2, Supplemental Table 2 and 3). However, correlations of miRNAs and mRNAs between the two cohorts varied. For example, in both studies

miR-28-3p negatively correlated with *CYP2C9* mRNA, whereas miR-148b-3p was negatively correlated with *CYP2C9* mRNA in our study (data not shown), but was described to be positively correlated with *CYP2C9* by Rieger et al.[41] This could be due to a number of reasons as liver sample demographics and ages varied between studies.

SNPs in the 3'UTR of drug metabolism and disposition genes have been predicted to interfere with miRNA-mRNA interactions.[42,43] The cytochrome P450s analyzed in our current data set were shown to contain SNPs predicted to alter miRNA targeting in these two studies. miR-34a-5p has been previously validated to target hepatic nuclear factor 4α (*HNF4A*).[39,40] Furthermore, a SNP in the 3'UTR of *HNF4A* (rs11574744) abolished the miRNA binding sites of both miR-34a-5p and another miRNA validated as a target, miR-449a. This SNP prevented the ability of miR-34a-5p to downregulate *HNF4A* and appeared to be associated with altered *CYP2D6* activity in a cohort previously phenotyped with the *CYP2D6* probe drug, dextromethorphan.[39] This SNP is present in only African Americans, revealing the degree of complexity to miRNA regulation as we know that these SNPs occur at differing frequencies among populations. This knowledge reveals the importance in miRNA regulation of drug disposition and regulatory genes as there is a need to determine the extent of age-dependent miRNA regulation on these genes.

In this study, we have shown that miRNA expression in the human liver changes with age. These changes are most marked between fetal and pediatric ages; however, there were also notable changes between the pediatric and adult periods. Several of these miRNAs have either been validated to target ADME genes and upstream regulatory genes, as shown in Table 4, or are predicted to target these genes. These data strongly suggest that hepatic miRNA expression contributes to the clinical variability in hepatic drug disposition. The marked change in miRNA expression between the fetal and pediatric/adult groups may contribute to the high susceptibility of fetuses to adverse drug events. These developmental changes in miRNA expression may also contribute to changes in hepatic functions other than drug metabolism, such as cholesterol metabolism and xenobiotic detoxification. Collectively, this information suggests that age-dependent miRNAs significantly impact a variety of human liver functions. Additional studies are warranted to: validate the hepatic miRNA-mRNA interactions; and determine indirect effects of miRNAs on ADME genes as well as the effects of SNPs on creating or abolishing miRNA binding sites that control ADME genes.

Methods

Tissue samples and RNA preparation

Human liver tissue specimens were obtained from the NIH-supported tissue programs: the Liver Tissue Cell Distribution System (LTCDS) (n=22), the Minnesota and Pittsburg collection centers (n=35) and the Laboratory of Developmental Biology at the University of Washington (Seattle, WA; n=30). Three liver tissue samples were provided by XenoTech, LLC (Lenexa, KS). Liver tissues were stored at -80°C. This study was approved by the Indiana University Institutional Review Board.

Total RNA was extracted from 90 human liver samples (fetal, pediatric, and adult; n=30 each). Estimated fetal ages ranged from 15-24 weeks (average=18.8 weeks), pediatric ages from 1-17 years (average=8.6 years), and adult ages 28-80 years (average=54.5 years). This cohort consisted of tissue specimen from 43 male and 35 female donors (the sex of the remaining samples was unknown). Of the 90 tissue samples, 16, 10, 1 and 63 samples were from Caucasian, African American, Hispanic or donors of unknown ethnicity, respectively.

Total RNA, including small RNAs, was extracted with the Qiagen miRNAeasy Mini Kit (Valencia, CA). Briefly, approximately 30 mg frozen liver tissue was excised and homogenized for 30 sec with a PRO 200 rotary homogenizer (PRO Scientific, Oxford, CT) in QIAzol Lysis Reagent. Subsequently, chloroform was added, mixed, centrifuged, and the aqueous phase transferred to a new tube and mixed with 100% ethanol. The sample was applied onto an RNeasy Mini spin column and washed as recommended by the protocol. To avoid any DNA contamination, an on-column DNase digest was performed. After 2 additional washes of the column, RNA was eluted twice with 30 μL of RNase-free water. RNA quality (RQI; RNA quality index) was determined on an Experion StdSens RNA chip (Bio-Rad, Hercules, CA) and concentration measured with a NanoDrop 1000 instrument (Thermo Scientific, Wilmington, DE). The RQI values of the RNA preparations ranged between 7.0 and 9.9. Seventy-six RNA samples had RQI values above 8.1. RNA preparations were stored at -80°C until analysis.

miRNA expression profiling

Expression of 533 microRNAs (miRNA) was measured using TaqMan OpenArray Human miRNA Panel using the NT Cycler (Applied Biosystems, Foster City, CA). The threshold cycles were set manually based on visual inspection of real-time amplification curves of each miRNA.

Bioinformatics analysis of the miRNA expression data

The miRNA expression data was quantile normalized using the threshold cycle values of miRNA data obtained from the TaqMan OpenArray. Differential expression of the miRNAs was determined by *p* value comparisons between the 60 fetal and pediatric samples and between the 59 pediatric and adult samples (one outlying adult sample was removed from statistical analyses). They were corrected for by false discovery rate (FDR) using the Benjamini Hochberg equation.[44]The top absorption, distribution, metabolism, elimination (ADME) genes (http://www.pharmaadme.org/joomla/) that were predicted to be targeted by the differentially expressed miRNAs in this study were determined using Ingenuity Pathway Analysis. The IPA program extracts predicted and experimentally validated miRNA targets from TargetScan, miRecords, TarBase, and text mining through Ingenuity Expert Findings and Ingenuity Expert Assisted Findings.

Measurement of mRNA expression

RNA-seq was performed as previously described.[39] Standard methods were used for RNA-sequencing library construction, EXBead preparation, and Next-Generation sequencing, based on the protocol provided for the Life Technologies SOLiD4 system. Briefly, 2μg of total RNA per sample of only 30 of the 90 total samples (fetal, pediatric, and adult; n=10)

were used for library preparation. The rRNA was depleted using RiboMinus Eukaryote Kit for RNA-Seq.

Bioinformatics analysis of RNA-seq data

RNA-seq data analysis included the following steps: quality assessment, sequence alignment, and gene expression analysis.

Data Processing and Quality Assessment—We used SOLiD Instrument Control Software and SOLiD Experiment Tracking System software for the read quality recalibration. Each sequence read was scanned for low-quality regions, and if a 5-base sliding window had an average quality score less than 20, the read was truncated at that position. Any reads less than 35 bases were discarded. Our experience suggested that this strategy effectively eliminated low-quality reads while high-quality regions were retained.[45-47]

Sequence Alignment—The BFAST (http://bfast.sourceforge.net) [48] was used as the primary alignment algorithm because it has high sensitivity for aligning reads on loci containing small insertions and deletions compared to the reference genome (hg19). NGSUtils (http://ngsutils.org/) [45], a TopHat-like strategy [49], was employed to align the sequencing reads that crossed splicing junctions. After aligning the sequence reads to a filtering index including repeats, ribosomal RNAs, and other sequences that were not of interest, a sequence alignment was conducted for three levels: genome, known junctions (University of California Santa Cruz Genome Browser), and novel junctions (based on the enriched regions identified in the genomic alignment). We restricted our analysis to the uniquely aligned sequences with no more than two mismatches.

RNA-Seq Differential Expression Analysis—Differentially expressed genes were identified using edgeR [50], a Bioconductor package for differential expression analysis of digital gene expression data, based on a negative binomial distribution. To ensure the reliable gene expression measurements, genes with less than 1 read per million mappable reads were removed. A *p* value was calculated both for differential expression of the drug disposition genes between the 20 fetal and pediatric samples and between the 20 pediatric and adult samples analyzed for RNA-seq. Benjamini and Hockberg's algorithm was used to control the FDR.[44]

Inverse Correlations—A linear regression model considering developmental periods as a cofactor was used to identify inverse correlations between the expression levels of the developmentally changing miRNAs and the drug disposition mRNA expression levels. The resultant *p* values were FDR corrected using Benjamini Hockberg.[44]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This project was funded by an NIH grant (R01GM088076; TCS). The RNA-seq assays were carried out in the Center for Medical Genomics at Indiana University School of Medicine. The Liver Tissue Cell Distribution System is funded by NIH Contract #N01-DK-7-0004/HHSN267200700004C. The project entitled “Laboratory of Developmental Biology” was supported by NIH Award Number 5R24HD0008836 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development. The content does not necessarily represent the official views of the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health. We are also grateful to XenoTech, LLC for their generous gift of tissue fractions.

References

1. Hines RN. The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacology & therapeutics. 2008; 118:250–267. doi:10.1016/j.pharmthera.2008.02.005. [PubMed: 18406467]
2. Hines RN. Developmental expression of drug metabolizing enzymes: impact on disposition in neonates and young children. International journal of pharmaceutics. 2013; 452:3–7. doi:10.1016/j.ijpharm.2012.05.079. [PubMed: 22766445]
3. Kearns GL, et al. Developmental pharmacology--drug disposition, action, and therapy in infants and children. The New England journal of medicine. 2003; 349:1157–1167. doi:10.1056/NEJMra035092. [PubMed: 13679531]
4. Van Driest SL, McGregor TL. Pharmacogenetics in clinical pediatrics: challenges and strategies. Personalized medicine. 2013; 10 doi:10.2217/pme.13.70.
5. Sage DP, Kulczar C, Roth W, Liu W, Knipp GT. Persistent pharmacokinetic challenges to pediatric drug development. Frontiers in genetics. 2014; 5:281. doi:10.3389/fgene.2014.00281. [PubMed: 25221567]
6. Cuzzolin L, Atzei A, Fanos V. Off-label and unlicensed prescribing for newborns and children in different settings: a review of the literature and a consideration about drug safety. Expert opinion on drug safety. 2006; 5:703–718. doi:10.1517/14740338.5.5.703. [PubMed: 16907660]
7. Blake MJ, Castro L, Leeder JS, Kearns GL. Ontogeny of drug metabolizing enzymes in the neonate. Seminars in fetal & neonatal medicine. 2005; 10:123–138. doi:10.1016/j.siny.2004.11.001. [PubMed: 15701578]
8. Blake MJ, et al. Ontogeny of dextromethorphan O- and N-demethylation in the first year of life. Clinical pharmacology and therapeutics. 2007; 81:510–516. doi:10.1038/sj.clpt.6100101. [PubMed: 17301735]
9. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: ontogeny and drug disposition. Clinical pharmacokinetics. 1999; 37:485–505. doi:10.2165/00003088-199937060-00004. [PubMed: 10628899]
10. Hines RN. Ontogeny of human hepatic cytochromes P450. Journal of biochemical and molecular toxicology. 2007; 21:169–175. [PubMed: 17936930]
11. Lacroix D, Sonnier M, Moncion A, Cheron G, Cresteil T. Expression of CYP3A in the human liver--evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. European journal of biochemistry / FEBS. 1997; 247:625–634. [PubMed: 9266706]
12. Sonnier M, Cresteil T. Delayed ontogenesis of CYP1A2 in the human liver. European journal of biochemistry / FEBS. 1998; 251:893–898. [PubMed: 9490065]
13. Treluyer JM, Gueret G, Cheron G, Sonnier M, Cresteil T. Developmental expression of CYP2C and CYP2C-dependent activities in the human liver: in-vivo/in-vitro correlation and inducibility. Pharmacogenetics. 1997; 7:441–452. [PubMed: 9429229]
14. Treluyer JM, Jacqz-Aigrain E, Alvarez F, Cresteil T. Expression of CYP2D6 in developing human liver. European journal of biochemistry / FEBS. 1991; 202:583–588. [PubMed: 1722149]
15. Vieira I, Sonnier M, Cresteil T. Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. European journal of biochemistry / FEBS. 1996; 238:476–483. [PubMed: 8681961]

16. McCarver DG, Hines RN. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. The Journal of pharmacology and experimental therapeutics. 2002; 300:361–366. [PubMed: 11805192]
17. Miller RP, Roberts RJ, Fischer LJ. Acetaminophen elimination kinetics in neonates, children, and adults. Clinical pharmacology and therapeutics. 1976; 19:284–294. [PubMed: 1261167]
18. Mooij MG, et al. Ontogeny of Human Hepatic and Intestinal Transporter Gene Expression During Childhood: Age Matters. Drug metabolism and disposition: the biological fate of chemicals. 2014 doi:10.1124/dmd.114.056929.
19. Liu J. Control of protein synthesis and mRNA degradation by microRNAs. Current opinion in cell biology. 2008; 20:214–221. doi:10.1016/j.ceb.2008.01.006. [PubMed: 18329869]
20. Olsen PH, Ambros V. The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. Developmental biology. 1999; 216:671–680. doi:10.1006/dbio.1999.9523. [PubMed: 10642801]
21. Selbach M, et al. Widespread changes in protein synthesis induced by microRNAs. Nature. 2008; 455:58–63. doi:10.1038/nature07228. [PubMed: 18668040]
22. Duursma AM, Kedde M, Schrier M, le Sage C, Agami R. miR-148 targets human DNMT3b protein coding region. Rna. 2008; 14:872–877. doi:10.1261/rna.972008. [PubMed: 18367714]
23. Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Molecular cell. 2008; 30:460–471. doi:10.1016/j.molcel.2008.05.001. [PubMed: 18498749]
24. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:1608–1613. doi:10.1073/pnas.0707594105. [PubMed: 18227514]
25. Lambert NJ, Gu SG, Zahler AM. The conformation of microRNA seed regions in native microRNPs is prearranged for presentation to mRNA targets. Nucleic acids research. 2011; 39:4827–4835. doi:10.1093/nar/gkr077. [PubMed: 21335607]
26. Takagi S, Nakajima M, Mohri T, Yokoi T. Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. The Journal of biological chemistry. 2008; 283:9674–9680. doi:10.1074/jbc.M709382200. [PubMed: 18268015]
27. To KK, Zhan Z, Litman T, Bates SE. Regulation of ABCG2 expression at the 3' untranslated region of its mRNA through modulation of transcript stability and protein translation by a putative microRNA in the S1 colon cancer cell line. Molecular and cellular biology. 2008; 28:5147–5161. doi:10.1128/MCB.00331-08. [PubMed: 18573883]
28. Pan YZ, Morris ME, Yu AM. MicroRNA-328 negatively regulates the expression of breast cancer resistance protein (BCRP/ABCG2) in human cancer cells. Molecular pharmacology. 2009; 75:1374–1379. doi:10.1124/mol.108.054163. [PubMed: 19270061]
29. Shi XB, et al. An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:19983–19988. doi:10.1073/pnas.0706641104. [PubMed: 18056640]
30. Hong L, et al. The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. Annals of surgery. 2010; 251:1056–1063. doi:10.1097/SLA.0b013e3181dd4ea9. [PubMed: 20485139]
31. Tao ZH, et al. miR-612 suppresses the invasive-metastatic cascade in hepatocellular carcinoma. The Journal of experimental medicine. 2013; 210:789–803. doi:10.1084/jem.20120153. [PubMed: 23478189]
32. Gupta P, Cairns MJ, Saksena NK. Regulation of gene expression by microRNA in HCV infection and HCV-mediated hepatocellular carcinoma. Virology journal. 2014; 11:64. doi:10.1186/1743-422X-11-64. [PubMed: 24690114]
33. Liang Z, et al. Expression and significance of microRNA-183 in hepatocellular carcinoma. TheScientificWorldJournal. 2013; 2013:381874. doi:10.1155/2013/381874.

34. Anderson MS, et al. Effects of Rifampin, a potent inducer of drug-metabolizing enzymes and an inhibitor of OATP1B1/3 transport, on the single dose pharmacokinetics of anacetrapib. Journal of clinical pharmacology. 2013; 53:746–752. doi:10.1002/jcph.97. [PubMed: 23670789]
35. Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. The Journal of pharmacology and experimental therapeutics. 2001; 299:849–857. [PubMed: 11714868]
36. Ramamoorthy A, et al. Regulation of microRNA expression by rifampin in human hepatocytes. Drug metabolism and disposition: the biological fate of chemicals. 2013; 41:1763–1768. doi: 10.1124/dmd.113.052886. [PubMed: 23935064]
37. Mimura S, et al. Profile of microRNAs associated with aging in rat liver. International journal of molecular medicine. 2014; 34:1065–1072. doi:10.3892/ijmm.2014.1892. [PubMed: 25118807]
38. Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S. Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J. 2009; 23:3243–3250. doi:10.1096/fj.09-135251. [PubMed: 19465468]
39. Ramamoorthy A, et al. In silico and in vitro identification of microRNAs that regulate hepatic nuclear factor 4alpha expression. Drug metabolism and disposition: the biological fate of chemicals. 2012; 40:726–733. doi:10.1124/dmd.111.040329. [PubMed: 22232426]
40. Wirsing A, Senkel S, Klein-Hitpass L, Ryffel GU. A systematic analysis of the 3′UTR of HNF4A mRNA reveals an interplay of regulatory elements including miRNA target sites. PloS one. 2011; 6:e27438. doi:10.1371/journal.pone.0027438. [PubMed: 22140441]
41. Rieger JK, Klein K, Winter S, Zanger UM. Expression variability of absorption, distribution, metabolism, excretion-related microRNAs in human liver: influence of nongenetic factors and association with gene expression. Drug metabolism and disposition: the biological fate of chemicals. 2013; 41:1752–1762. doi:10.1124/dmd.113.052126. [PubMed: 23733276]
42. Ramamoorthy A, Skaar TC. In silico identification of microRNAs predicted to regulate the drug metabolizing cytochrome P450 genes. Drug metabolism letters. 2011; 5:126–131. [PubMed: 21457141]
43. Wei R, et al. Impact of the Interaction between 3′-UTR SNPs and microRNA on the Expression of Human Xenobiotic Metabolism Enzyme and Transporter Genes. Frontiers in genetics. 2012; 3:248. doi:10.3389/fgene.2012.00248. [PubMed: 23181071]
44. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behavioural brain research. 2001; 125:279–284. [PubMed: 11682119]
45. Breese MR, Liu Y. NGSUtils: a software suite for analyzing and manipulating next-generation sequencing datasets. Bioinformatics. 2013; 29:494–496. doi:10.1093/bioinformatics/bts731. [PubMed: 23314324]
46. Juan L, et al. Potential roles of microRNAs in regulating long intergenic noncoding RNAs. BMC medical genomics. 2013; 6(Suppl 1):S7. doi:10.1186/1755-8794-6-S1-S7. [PubMed: 23369519]
47. Todd AG, Lin H, Ebert AD, Liu Y, Androphy EJ. COPI transport complexes bind to specific RNAs in neuronal cells. Human molecular genetics. 2013; 22:729–736. doi:10.1093/hmg/dds480. [PubMed: 23175440]
48. Homer N, Merriman B, Nelson SF. BFAST: an alignment tool for large scale genome resequencing. PloS one. 2009; 4:e7767. doi:10.1371/journal.pone.0007767. [PubMed: 19907642]
49. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009; 25:1105–1111. doi:10.1093/bioinformatics/btp120. [PubMed: 19289445]
50. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010; 26:139–140. doi: 10.1093/bioinformatics/btp616. [PubMed: 19910308]

Study Highlights

What is the current knowledge on the topic? The expression of hepatic drug disposition genes undergo large changes during liver development; however, the mechanisms behind these changes remain unknown. Emerging evidence implicate microRNAs in regulation of drug disposition genes.

What question did this study address? We hypothesized that developmental changes in human liver microRNAs and their variability in the rate of these changes contribute to developmental changes in drug disposition genes. We measured global miRNA and mRNA expression in fetal, pediatric, and adult human liver.

What this study adds to our knowledge? Hepatic miRNAs undergo marked age-dependent changes in expression. Significant negative correlations were observed between the developmental change in miRNA expression and the developmental change in expression of several drug disposition and regulatory genes.

How this might change clinical pharmacology and therapeutics? This study represents the first comprehensive analysis demonstrating a link between age-dependent microRNA expression in human liver and the age-dependent changes in important drug disposition genes. These findings help to further understand mechanisms of age-dependent changes in drug disposition.

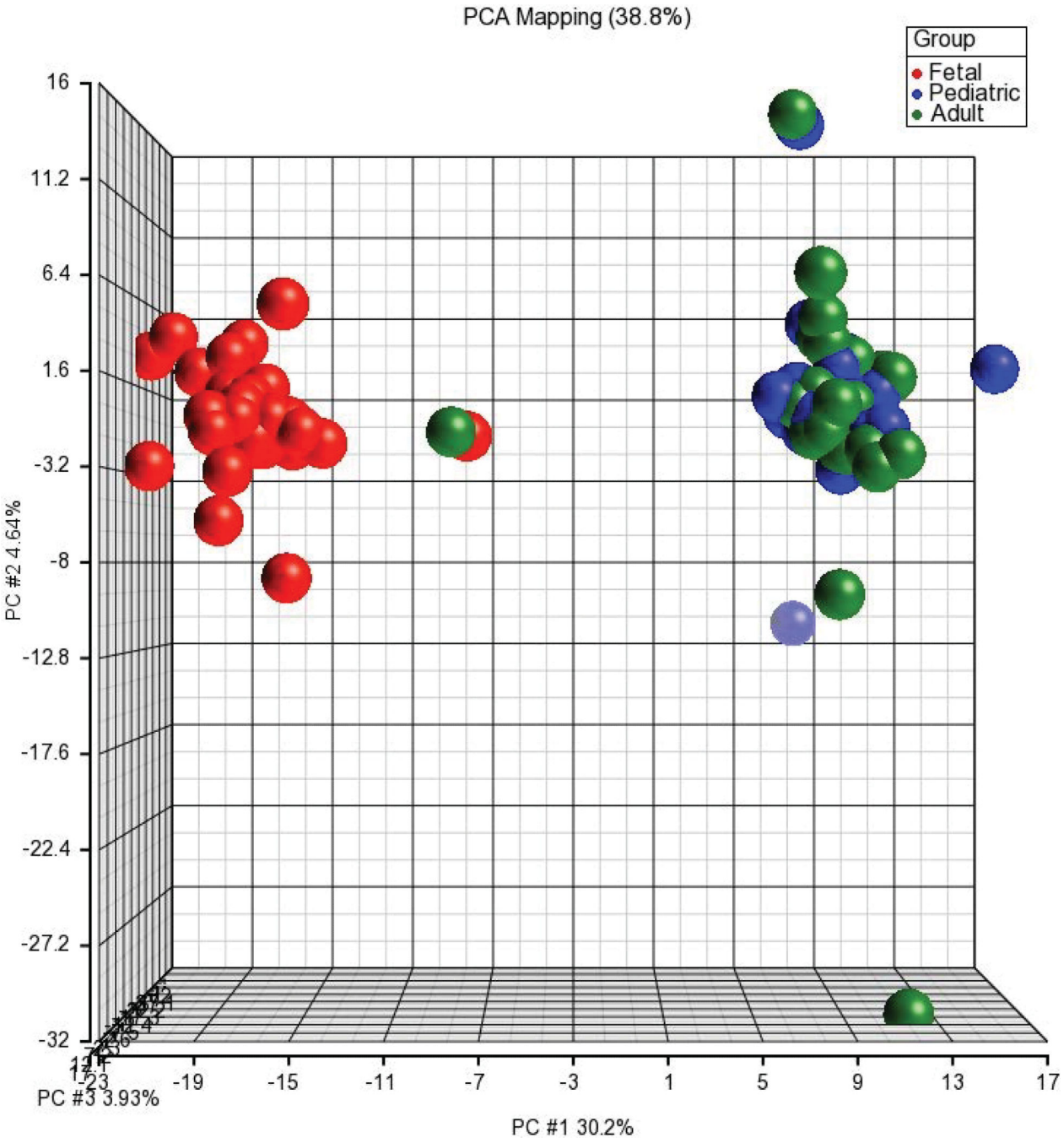

Figure 1. Principal components analysis of all miRNAs detected in human liver samples

Principal components analysis of all 533 miRNAs measured using TaqMan OpenArray. Axes are principal components 1, 2, and 3. Red: Fetal, Blue: Pediatric, Green: Adult

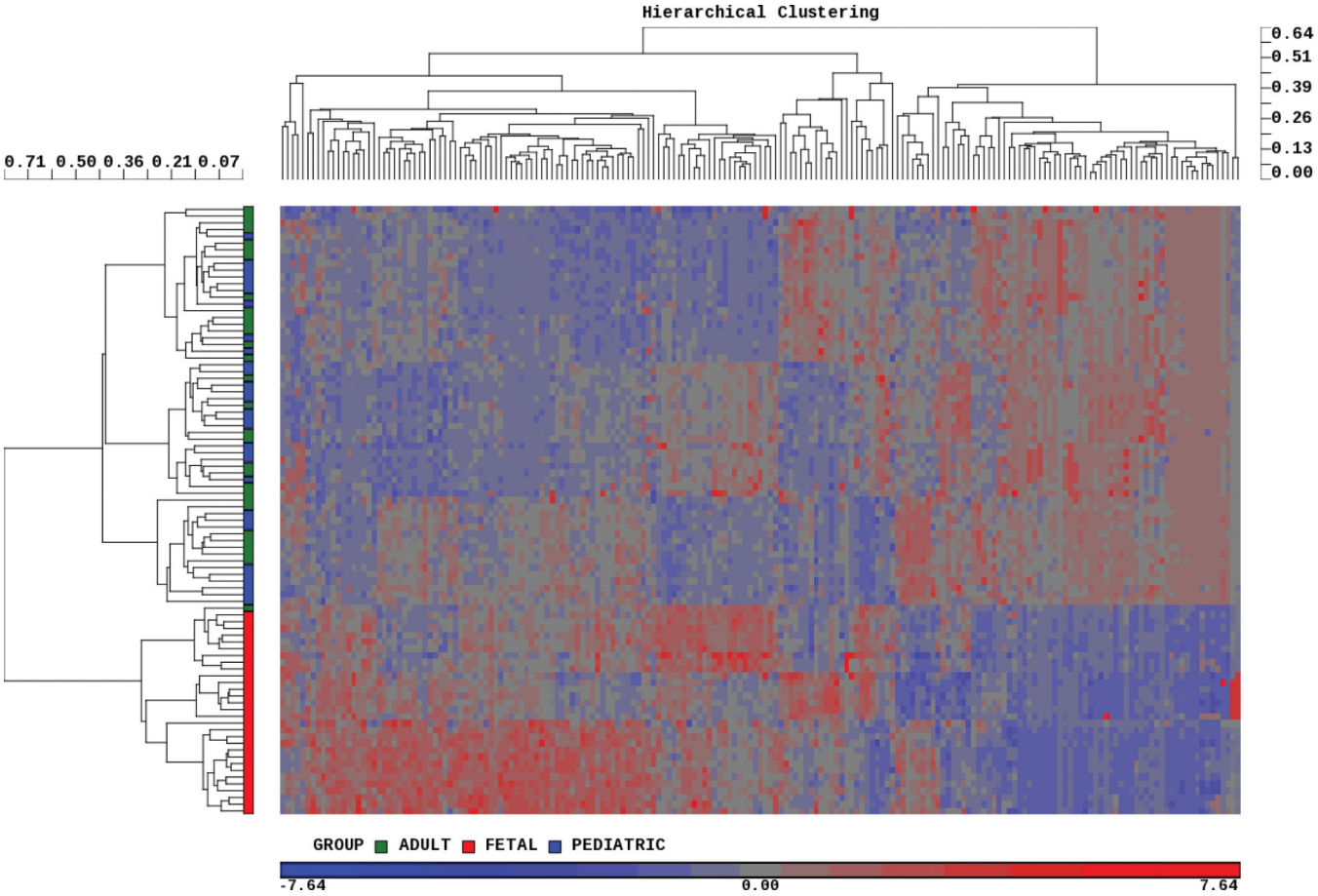

Figure 2. Heatmap of all developmentally-regulated miRNAs in human liver samples

Hierarchical clustering of miRNA expression levels using Spearman Rank Dissimilarity. X-axis: miRNAs. Y-axis: 90 samples across the 3 developmental periods (Red: Fetal, Blue: Pediatric, Green: Adult). Color scale below the figure shows increasing expression from blue to gray to red.

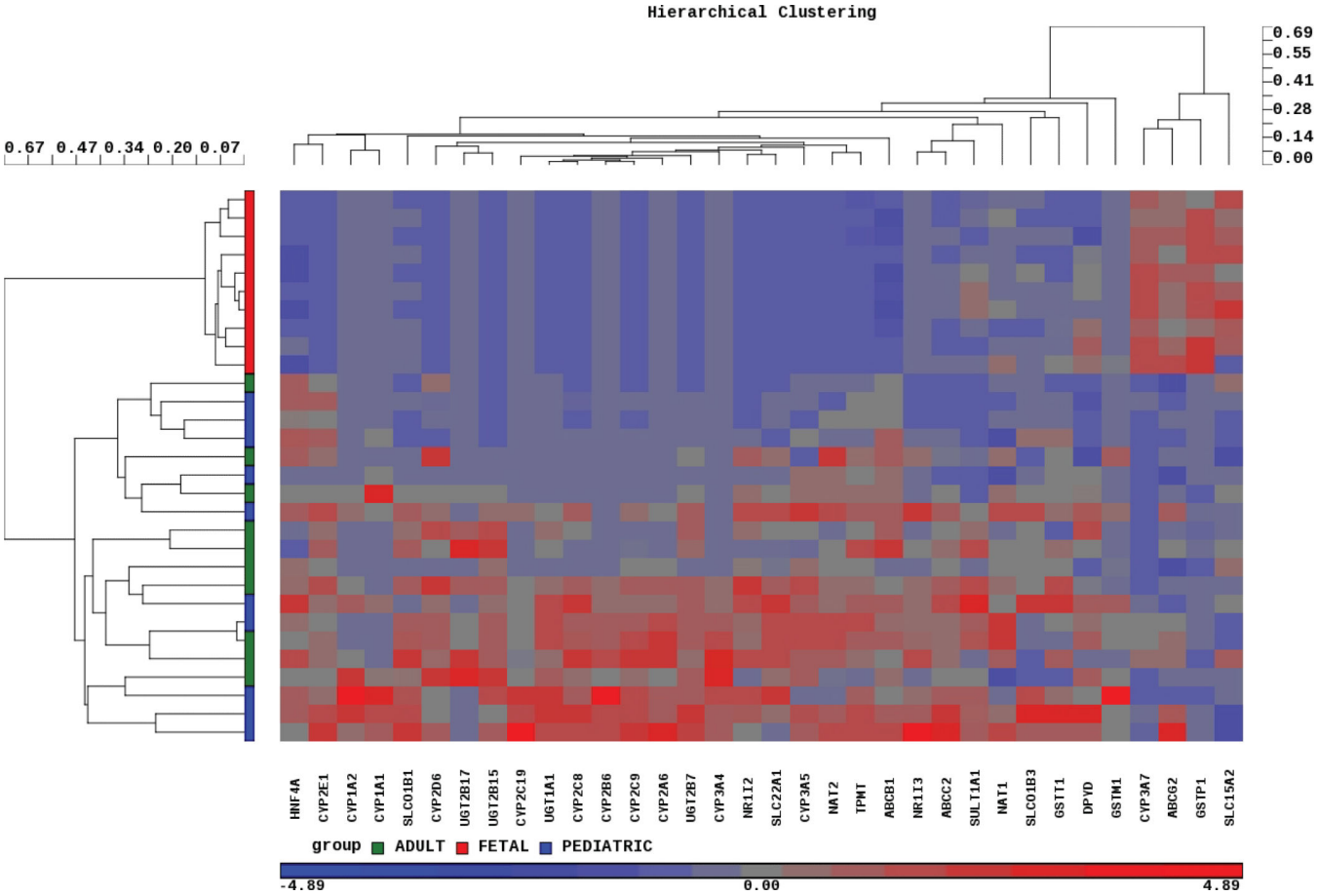

Figure 3. Heatmap of the top ADME mRNA expression across the developmental periods

Hierarchical clustering of the top ADME and regulatory gene mRNA using Spearman Rank Dissimilarity. X-axis: mRNAs. Y-axis: 90 samples across the 3 developmental periods (Red: Fetal, Blue: Pediatric, Green: Adult). Color scale below the figure shows increasing expression from blue to gray to red. **SLC22A2* and *SLC22A6* are excluded due to insufficient reads in the majority of samples.

Table 1

Changes in miRNA expression from fetal to pediatric

Upregulated miRNAs*	p-value	FDR**	Fold Change
hsa-miR-139-5p	6.2E-26	2.0E-24	3.7
hsa-let-7a-5p	4.7E-16	5.5E-15	2.1
hsa-miR-10a-5p	4.9E-14	4.0E-13	3.4
hsa-miR-132-3p	2.7E-13	2.0E-12	3.7
hsa-let-7b-5p	5.0E-12	3.2E-11	> 500
hsa-miR-125b-5p	6.8E-11	3.8E-10	2.2
hsa-let-7g-5p	3.1E-08	1.3E-07	1.7
hsa-miR-1275	3.3E-08	1.3E-07	3.0
hsa-miR-122-3p	3.0E-07	1.1E-06	9.2
hsa-miR-1271-5p	2.3E-05	6.4E-05	4.2
hsa-miR-139-3p	2.4E-05	6.8E-05	19.5
hsa-miR-128a-3p	3.1E-05	8.7E-05	1.6
hsa-miR-10b-5p	5.6E-04	1.3E-03	9.1
hsa-miR-106a-5p	7.5E-04	1.8E-03	151.9
hsa-miR-126-5p	1.3E-03	3.0E-03	1.5
hsa-miR-10b-3p	2.8E-03	6.1E-03	17.3
hsa-let-7f-5p	3.5E-03	7.6E-03	1.5
hsa-miR-140-3p	4.5E-03	9.5E-03	2.2
hsa-let-7e-5p	8.8E-03	1.8E-02	1.3
hsa-miR-1244	1.8E-02	3.4E-02	1.9

Downregulated miRNAs	p-value	FDR**	Fold Change
hsa-miR-431-5p	1.0E-58	5.5E-56	< −500
hsa-miR-668-3p	5.0E-42	1.3E-39	< −500
hsa-miR-539-5p	3.3E-36	4.3E-34	−101.6
hsa-miR-411-5p	8.3E-36	8.9E-34	−18.2
hsa-miR-409-3p	2.1E-34	1.6E-32	−30.8
hsa-miR-889-3p	4.7E-32	3.1E-30	−39.0
hsa-miR-433-3p	6.0E-32	3.6E-30	< −500
hsa-miR-483-3p	2.0E-30	1.1E-28	−52.6
hsa-miR-136-3p	3.2E-30	1.5E-28	−26.1
hsa-miR-512-3p	5.5E-30	2.5E-28	< −500
hsa-miR-432-5p	5.9E-28	2.2E-26	< −500
hsa-miR-485-3p	5.6E-28	2.2E-26	< −500
hsa-miR-382-5p	8.7E-28	3.1E-26	< −500
hsa-miR-487a-3p	3.8E-25	1.1E-23	< −500
hsa-miR-519e-5p	1.2E-24	3.4E-23	< −500
hsa-miR-130a-3p	3.1E-24	8.3E-23	−3.7
hsa-miR-515-3p	7.3E-23	1.8E-21	< −500

Downregulated miRNAs	p-value	FDR**	Fold Change
hsa-miR-154-3p	1.0E-22	2.3E-21	< −500
hsa-miR-144-5p	2.6E-21	5.8E-20	< −500
hsa-miR-519a-3p	2.2E-20	4.4E-19	< −500

* The alternative nomenclature for miRNAs and TaqMan assay IDs are provided in Supplemental Table 1, to allow relation of our data to that utilized in other reports using the # designation over the −3p or −5p designations.

** False discovery rate comparing fetal vs. pediatric

Table 2

Changes in miRNA expression from pediatric to adult

Downregulated miRNA	p-value	FDR*	Fold Change
hsa-miR-18a-5p	1.4E-05	3.7E-03	−2.0
hsa-miR-20a-3p	7.8E-05	1.4E-02	−2.0
hsa-miR-219a-5p	1.7E-04	2.3E-02	−12.0

Upregulated miRNA	p-value	FDR*	Fold Change
hsa-miR-34a-5p	1.6E-06	8.3E-04	2.1
hsa-miR-30a-3p	3.2E-04	3.4E-02	1.7

*False discovery rate comparing pediatric vs. adult

Table 3

Negative correlations between developmental miRNA and top ADME and regulatory mRNA

	Gene	miRNA	p-value	FDR	Estimate*
Phase I	*CYP2A6*	hsa-miR-10a-5p	1.1E-02	4.3E-02	−44.2
	CYP2C8	hsa-miR-132-3p	1.3E-02	4.9E-02	−113.9
	CYP2D6	hsa-miR-132-3p	1.3E-03	1.0E-02	−13.3
	CYP2D6	hsa-miR-10b-3p	1.7E-03	1.2E-02	−2.3
	CYP2D6	hsa-miR-34a-5p	2.3E-03	1.5E-02	−13.3
	CYP2D6	hsa-miR-30a-3p	1.2E-02	4.7E-02	−8.5
	CYP2D6	hsa-miR-125b-5p	1.3E-02	4.8E-02	−13.0
	CYP2E1	hsa-miR-125b-5p	7.9E-05	2.0E-03	−795.4
	CYP2E1	hsa-miR-219a-5p	1.0E-04	2.3E-03	−131.8
	CYP2E1	hsa-miR-132-3p	4.6E-04	5.6E-03	−597.3
	CYP2E1	hsa-miR-34a-5p	7.5E-04	7.3E-03	−604.7
	CYP2E1	hsa-miR-30a-3p	1.0E-02	4.1E-02	−365.1
	CYP3A5	hsa-miR-132-3p	3.7E-03	2.1E-02	−30.5
	CYP3A5	hsa-miR-34a-5p	6.9E-03	3.1E-02	−30.0
	CYP3A7	hsa-miR-431-5p	3.0E-11	6.9E-08	−34.4
	CYP3A7	hsa-miR-668-3p	1.2E-10	2.2E-07	−42.0
	CYP3A7	hsa-miR-889-3p	8.1E-08	3.3E-05	−94.7
	CYP3A7	hsa-miR-539-5p	1.4E-07	4.7E-05	−93.8
	CYP3A7	hsa-miR-485-3p	1.6E-07	4.8E-05	−24.3
Phase II	*GSTP1*	hsa-miR-431-5p	3.6E-13	2.5E-09	−4.7
	GSTP1	hsa-miR-668-3p	2.6E-11	6.9E-08	−5.7
	GSTP1	hsa-miR-483-3p	5.2E-10	6.0E-07	−10.9
	GSTP1	hsa-miR-382-5p	5.1E-10	6.0E-07	−4.0
	GSTP1	hsa-miR-889-3p	5.0E-09	4.3E-06	−13.2
	NAT2	hsa-miR-132-3p	1.2E-03	9.7E-03	−4.0
	NAT2	hsa-miR-34a-5p	4.8E-03	2.5E-02	−3.7
	TPMT	hsa-miR-34a-5p	2.9E-04	4.2E-03	−2.5
	TPMT	hsa-miR-219a-5p	3.2E-04	4.5E-03	−0.5
	TPMT	hsa-miR-125b-5p	3.7E-03	2.0E-02	−2.4
	TPMT	hsa-miR-30a-3p	4.3E-03	2.3E-02	−1.6
	TPMT	hsa-miR-10a-5p	9.0E-03	3.8E-02	−1.4
	UGT1A1	hsa-miR-10a-5p	3.9E-03	2.1E-02	−42.0
	UGT1A1	hsa-miR-30a-3p	8.7E-03	3.7E-02	−39.3
	UGT1A1	hsa-miR-219a-5p	1.2E-02	4.7E-02	−9.7
	UGT2B15	hsa-miR-30a-3p	1.4E-03	1.1E-02	−10.6
	UGT2B15	hsa-miR-219a-5p	1.9E-03	1.3E-02	−2.7
	UGT2B15	hsa-miR-34a-5p	6.2E-03	2.9E-02	−12.3
	UGT2B15	hsa-miR-125b-5p	8.8E-03	3.7E-02	−2.9
	UGT2B15	hsa-miR-30a-3p	9.1E-03	3.8E-02	−1.9

	Gene	miRNA	p-value	FDR	Estimate*
	UGT2B17	hsa-miR-125b-5p	8.2E-03	3.5E-02	−2.6
	UGT2B17	hsa-miR-30a-3p	9.0E-03	3.8E-02	−1.7
	UGT2B17	hsa-miR-219a-5p	1.3E-02	4.9E-02	−0.4
	UGT2B7	hsa-miR-219a-5p	5.2E-03	2.6E-02	−3.3
	UGT2B7	hsa-miR-125b-5p	8.0E-03	3.5E-02	−18.7
	UGT2B7	hsa-miR-30a-3p	9.3E-03	3.8E-02	−12.0
	UGT2B7	hsa-miR-132-3p	1.2E-02	4.6E-02	−14.6
Transporters	*ABCB1*	hsa-miR-219a-5p	2.1E-06	1.9E-04	−0.9
	ABCB1	hsa-miR-34a-5p	9.2E-05	2.1E-03	−3.9
	ABCB1	hsa-miR-125b-5p	5.1E-04	5.9E-03	−4.1
	ABCB1	hsa-miR-30a-3p	1.3E-03	1.0E-02	−2.5
	ABCB1	hsa-miR-132-3p	1.5E-03	1.1E-02	−3.2
	ABCC2	hsa-miR-10a-5p	8.8E-03	3.7E-02	−13.0
	ABCG2	hsa-miR-668-3p	4.1E-03	2.2E-02	−0.2
	ABCG2	hsa-miR-431-5p	9.0E-03	3.8E-02	−0.2
	ABCG2	hsa-miR-512-3p	1.1E-02	4.3E-02	−0.1
	SLC15A2	hsa-miR-889-3p	1.2E-04	2.4E-03	−0.1
	SLC22A1	hsa-miR-30a-3p	1.7E-03	1.2E-02	−25.5
Regulatory	*HNF4A*	hsa-miR-132-3p	1.2E-04	2.4E-03	−4.3
	HNF4A	hsa-miR-30a-3p	3.8E-04	5.0E-03	−3.2
	HNF4A	hsa-miR-125b-5p	1.2E-03	9.9E-03	−4.5
	HNF4A	hsa-miR-219a-5p	3.6E-03	2.0E-02	−0.7
	HNF4A	hsa-miR-34a-5p	1.0E-02	4.0E-02	−3.2
	NR1I2	hsa-miR-30a-3p	6.0E-03	2.8E-02	−2.0
	NR1I2	hsa-miR-10b-3p	7.3E-03	3.3E-02	−0.4
	NR1I2	hsa-miR-125b-5p	1.0E-02	4.1E-02	−3.0

* Estimates indicate the changes in mRNA expressed as RPKM (reads per kilobases per million) per one threshold cycle decrease in miRNA expression. Note: Decrease in threshold cycle is an increase in miRNA expression.

Table 4

List of miRNA-mRNA predicted targets

miRNA	Seed Sequence	mRNA	Source	Confidence
hsa-let-7a-5p	GAGGUAG	*ABCC2*	TargetScan Human	Moderate (predicted)
hsa-miR-154-3p	AUCAUAC	*ABCG2*	TargetScan Human	Moderate (predicted)
hsa-miR-106a-5p	AAAGUGC	*ABCG2*	TargetScan Human	Moderate (predicted)
hsa-miR-512-3p	AGUGCUG	*ABCG2*	TargetScan Human	Moderate (predicted)
hsa-miR-519a-3p	AAGUGCA	*ABCG2*	miRecords	Experimentally Observed
hsa-miR-125b-5p	CCCUGAG	*CYP1A1*	miRecords	Experimentally Observed
hsa-miR-431-5p	GUCUUGC	*CYP1A2*	TargetScan Human	Moderate (predicted)
hsa-miR-668-3p	GUCACUC	*CYP1A2*	TargetScan Human	Moderate (predicted)
hsa-miR-1275	UGGGGGA	*CYP2B6*	TargetScan Human	Moderate (predicted)
hsa-miR-483-3p	CACUCCU	*CYP2B6*	TargetScan Human	Moderate (predicted)
hsa-miR-539-5p	GAGAAAU	*CYP3A7*	TargetScan Human	Moderate (predicted)
hsa-miR-106a-5p	AAAGUGC	*DPYD*	TargetScan Human	Moderate (predicted)
hsa-miR-34a-5p	GGCAGUG	*DPYD*	TargetScan Human	High (predicted)
hsa-miR-519e-5p	UCUCCAA	*DPYD*	TargetScan Human	Moderate (predicted)
hsa-miR-519a-3p	AAGUGCA	*DPYD*	TargetScan Human	Moderate (predicted)
hsa-miR-1271-5p	UUGGCAC	*DPYD*	TargetScan Human	Moderate (predicted)
hsa-miR-34a-5p	GGCAGUG	*HNF4A*	TargetScan Human	Experimentally Observed
hsa-miR-668-3p	GUCACUC	*HNF4A*	TargetScan Human	Moderate (predicted)
hsa-miR-18a-5p	AAGGUGC	*NR1I2*	TargetScan Human	High (predicted)
hsa-miR-219a-5p	GAUUGUC	*NR1I2*	TargetScan Human	Moderate (predicted)
hsa-miR-34a-5p	GGCAGUG	*NR1I2*	TargetScan Human	Moderate (predicted)
hsa-miR-132-3p	AACAGUC	*SLC15A2*	TargetScan Human	Moderate (predicted)
hsa-miR-519a-3p	AAGUGCA	*SLC15A2*	TargetScan Human	Moderate (predicted)
hsa-miR-1275	UGGGGGA	*SLC22A1*	TargetScan Human	Moderate (predicted)
hsa-miR-382-5p	AAGUUGU	*SLC22A2*	TargetScan Human	Moderate (predicted)
hsa-miR-431-5p	GUCUUGC	*SLC22A2*	TargetScan Human	Moderate (predicted)
hsa-miR-139-5p	CUACAGU	*SLCO1B1*	TargetScan Human	Moderate (predicted)
hsa-miR-10a-5p	ACCCUGU	*TPMT*	TargetScan Human	Moderate (predicted)
hsa-miR-125b-5p	CCCUGAG	*UGT2B15*	miRecords	Experimentally Observed
hsa-miR-382-5p	AAGUUGU	*UGT2B15*	TargetScan Human	Moderate (predicted)
hsa-miR-125b-5p	CCCUGAG	*UGT2B17*	miRecords	Experimentally Observed
hsa-miR-382-5p	AAGUUGU	*UGT2B17*	TargetScan Human	Moderate (predicted)
hsa-miR-409-3p	AAUGUUG	*UGT2B17*	TargetScan Human	Moderate (predicted)