Impact of the interaction between 3′-UTR SNPs and microRNA on the expression of human xenobiotic metabolism enzyme and transporter genes

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INTRODUCTION

Xenobiotic metabolizing enzymes and transporters (XMETs) are involved in biotransformation and detoxification of carcinogens, environmental toxins, and therapeutic drugs (Carlsten et al., 2008; Korkina et al., 2009). In humans, the process of biotransformation and detoxification of xenobiotics by XMETs can be divided into three phases: modification (phase I) primarily by enzymes of the cytochromes P450 superfamily; conjugation (phase II), e.g., glucuronidation by UDP-glucuronosyl transferase; and excretion (phase III) mainly by membrane transporters. XMETs are expressed in almost all tissue types, centrally and locally protecting the entire body against the damages caused by various natural and synthetic compounds. XMETs are highly expressed in digestive tract and especially in the liver, the most important organ for central metabolism (Conde-Vancells et al., 2010).

Genetic variation in the expression of human xenobiotic metabolism enzymes and transporters (XMETs) leads to inter-individual variability in metabolism of therapeutic agents as well as differed susceptibility to various diseases. Recent expression quantitative traits loci (eQTL) mapping in a few human cells/tissues have identified a number of single nucleotide polymorphisms (SNPs) significantly associated with mRNA expression of many XMET genes. These eQTLs are therefore important candidate markers for pharmacogenetic studies. However, questions remain about whether these SNPs are causative and in what mechanism these SNPs may function. Given the important role of microRNAs (miRs) in gene transcription regulation, we hypothesize that those eQTLs or their proxies in strong linkage disequilibrium (LD) altering miR targeting are likely causative SNPs affecting gene expression. The aim of this study is to identify eQTLs potentially regulating major XMETs via interference with miR targeting. To this end, we performed a genome-wide screening for eQTLs for 409 genes encoding major drug metabolism enzymes, transporters and transcription factors, in publically available eQTL datasets generated from the HapMap lymphoblastoid cell lines and human liver and brain tissue. As a result, 308 eQTLs significantly (p < 10^{-5}) associated with mRNA expression of 101 genes were identified. We further identified 7,869 SNPs in strong LD ($r^2 \geq 0.8$) with these eQTLs using the 1,000 Genome SNP data. Among these, 8,177 SNPs, 27 are located in the 3′-UTR of 14 genes. Using two algorithms predicting miR-SNP interaction, we found that almost all these SNPs (26 out of 27) were predicted to create, abolish, or change the target site for miRs in both algorithms. Many of these miRs were also expressed in the same tissue that the eQTL were identified. Our study provides a strong rationale for continued investigation for the functions of these eQTLs in pharmacogenetic settings.

Keywords: eQTL, xenobiotic metabolism enzyme and transporter, microRNA, pharmacogenetics, 3′-UTR

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cancer risk (Selinski et al., 2012). XMETs are sensitively regulated by various nuclear receptors (NRs) and transcription factors (TFs). These trans-acting regulators play a pivotal role in mediating cellular response to exposure to xenobiotics by modulating the transcription of XMETs, thus significantly contributing to the variability in the function of XMETs (Bourgine et al., 2012).

Identifying the DNA polymorphisms leading to the variations in XMET function is a major area of interest in pharmacogenetic and genomic research. To date, numerous studies focused on individual XMET genes have discovered a large number of sequence variations, many of which alter protein coding sequence and consequently affecting the activity of XMETs (Adjei et al., 2003; Hildebrandt et al., 2004; Ji et al., 2005; Moyer et al., 2007; Mrozikiewicz et al., 2011). Meanwhile, even more variants were suggested to quantitatively modulate gene transcription (Pavek and Dvorak, 2008). Recently, genome-wide mapping for gene expression quantitative trait loci (eQTLs) in a few human tissues/cells offered unprecedented opportunities to identify the most influential single nucleotide polymorphisms (SNPs) determining gene expression level of XMETs (Gamazon et al., 2010). However, unlike the variants located in the protein coding sequences for which the causality for altered enzyme activity can be more easily understood, how eQTLs affect gene transcription is largely unknown. Understanding the underlying mechanisms will lead to identification of novel causative DNA variants for XMET function as well as reliable pharmacogenetic markers.

MicroRNAs (miRs) are single stranded, about 22-nucleotides (nt) long, evolutionarily conserved, and function as important posttranscriptional regulators of mRNA expression by binding to the 3′-UTR of target mRNAs (Ambros, 2004; Bartel, 2004). MiRs are involved in various developmental and physiological processes by negatively regulating gene expression (Zhang et al., 2007). Over 30% of all protein-coding genes were estimated to be regulated by miRs (Brennecke et al., 2003; Krek et al., 2005; Lewis et al., 2005; Lim et al., 2005). Due to the conservation of the miR target site, SNPs located in 3′-UTR sequences may abolish or create a miR target, thus significantly affecting the mRNA expression (Saunders et al., 2007). Previous studies have suggested that many XMETs are regulated by miRs (Tsuchiya et al., 2006; Takagi et al., 2010; Patron et al., 2012). Several studies also demonstrated that SNPs in XMET gene 3′-UTRs led to different levels of enzyme activity (Saunders et al., 2007; Chin et al., 2008). Hence, we hypothesized that it may be an important mechanism that common SNPs or their linkage disequilibrium (LD) proxies located in the XMET gene 3′-UTR sequences alter mRNA expression via interference with miR targeting. In order to identify these candidate SNPs that may significantly modulate XMET expression, in this study we used multiple published human eQTL datasets to perform an in silico screening for SNPs that highly correlated with mRNA level of 409 major XMET genes. The significant SNPs and/or their LD proxies located in the gene 3′-UTRs were selected to predict a potential interference with miRs. We found that 27 SNPs located in the 3′-UTR of 14 XMET genes are likely associated with gene expression via altering miR binding.

**MATERIALS AND METHODS**

**SELECTION OF eQTLs**

The general strategy for the data analysis was presented in Figure 1. We used the published eQTLs datasets generated from the HapMap lymphoblastoid cell lines (LCLs; Montgomery et al., 2010), human liver (Schadt et al., 2008), and human brain (Gibbs et al., 2010). Although additional eQTL datasets in human LCLs are also available, we chose to use the one by Montgomery et al. (2010) which utilized high-throughput sequencing for the quantification of gene expression, as this technology has been suggested to produce more accurate gene expression data. To our knowledge, all datasets were collected from tissue/cells derived from individuals of Caucasian origin. We used the online tool¹ to search statistically significant eQTLs. As our study was focused on cis-acting eQTLs, we used a cut-off of \( p = 10^{-5} \) for significance, considering the window for genomic region (500 kb) of each gene and the potential number of SNPs (1 in every 100–1,000 bp).

**SEARCH FOR SNPs IN LD WITH eQTLs**

To search SNPs in LD with significant eQTLs, we used the SNAP² program to screen the 1,000 Genome SNP data within 500 kb range of the eQTLs of interest in the CEU population with a LD level cut-off of \( R^2 = 0.8 \). Annotation for the location of eQTLs and their proxies relative to the gene structure was also collected with

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¹ http://www.ncbi.nlm.nih.gov/gtex/GTEX2/gtex.cgi
² http://www.broadinstitute.org/mpg/snap/ldsearch.php

**FIGURE 1** Schematic of the search for miRNAs and the associated SNPs from XMET genes.
In order to predict the potential SNP-miR interaction, two analyses. The PolyMiRTS program used the TargetScan; Lewis et al., 2005; Friedman et al., 2009) algorithm (Bao et al., 2007). In contrast, the MicroSNiPer program used the FASTA (Pearson and Lipman, 1988) alignment program to determine if a change in a nucleotide in 3′-UTR sequence would change the miR binding capability, based on the requirement of perfect Watson–Crick match to the seed 2–7 nt of miRs (Lewis et al., 2005). To be conservative, we used 7-mers match as the cut-off value for a positive prediction.

RESULTS

GENOME-WIDE eQTL ANALYSIS OF XMETs

Expression quantitative traits loci were screened for all 409 major XMET genes, including 144 phase I, 85 phase II and 111 phase III genes, 48 NRs, and transcription factor genes as well as another 21 genes related to drug ADME (Table A1 in Appendix). As a result, a total of 308 significant (p < 10^{-5}) eQTLs were identified from 101 XMET genes. These include nine in LCL, 83 in liver, and 221 in brain tissues. Five SNPs were found as eQTLs shared in two tissue types: rs1023252 in both LCL and brain tissues, rs11101992, rs156697, rs2071474, and rs241440 in both liver and brain tissues (Figure 2). Among the total of 308 eQTLs, 20 SNPs were found to be located in the 3′-UTR region; 3 SNPs were in the 5′-UTRs; 4 in 3′-UTR sequences, 48 in introns, and 125 in intergenic regions.

Of the 112 eQTLs and proxies located in the 3′-UTR sequences, 27 SNPs were found in the 3′-UTR of 14 genes of interest. The remaining eQTLs were located in nearby genes thus were excluded from the subsequent analysis. These SNPs were all common SNPs with their minor allele frequency (MAF) ≥ 0.067. Among the 27 SNPs, 12 were found in liver, and 15 were identified in brain tissue. More detailed information for these SNPs was listed in Table A2 in Appendix.

PREDICTION of miR-SNPs INTERACTION

We focused our study on the association between miRs and these 27 SNPs in the 14 genes. After screened with the two algorithms, MicroSNiPer (Barenboim et al., 2010) and PolyMiRTS (Gong et al., 2012), all the 27 SNPs apart from rs11807 (which is not predicted to be in a target site in PolyMiRTs database) were found to potentially create, abolish, or alter the target site for miRs in both algorithms. Notably, 34 miRs were predicted by both algorithms to interact with 19 of these 27 SNPs (Table A2 in Appendix). Of these 34 overlap miRs, except for rs2480256 of CYP2E1 which is not located in the seed sequence of hsa-miR-570-3p, all the remaining SNPs were found to be located in the seed sequence of miR targets.

To further validate the interaction between miRs and SNPs, we investigated whether the identified miRs were expressed in the same tissue as the identified eQTL. We used the GEO datasets (GSE21279 and GSE26545) to screen miR expression in liver and brain tissues, respectively (Hou et al., 2011; Hu et al., 2011). Since many predicted miRs were new and not probed by the published platforms, we thus only concentrate on the list of miRs probed in the platforms. Overall, over 74% (20 out of 27) of the identified miR-SNPs were found to have at least one predicted miR co-expressed with the gene of interest in the same tissue.

We further aimed to investigate whether these 27 SNPs are more likely to be targeted by miRs especially by the co-expressed miR in liver and brain tissues, compared to random-selected 3′-UTR SNPs with similar MAF. No statistical significance were found, possibly due to the limited power caused by the small number (n = 27) of SNPs involved (data not shown).

DISCUSSION

Although a large number of DNA variants affecting the function of XMETs have been identified, and many of them have been well linked with clinical response to pharmacotherapy or disease susceptibility (Motsinger-Reif et al., 2010), genetic variations in the
activity of most XMETs remain incompletely explained. Recent studies continue to discover novel functional variants in XMET genes (Ramsey et al., 2012). Meanwhile, genome-wide association studies have found a number of XMET SNPs without previously known function significantly associated with different phenotypes in humans (Teichert et al., 2009; Estrada et al., 2012). These studies consistently suggested that additional sequence variants with fundamental role in XMET function have not been identified. Recent eQTL mapping in human tissues provided an opportunity to discover functional XMET polymorphisms at the genome-wide level. However, questions remain whether the identified eQTLs are causal for the altered gene expression and via what mechanism. Our study provides a comprehensive evaluation for this question in major human XMET genes, and generated a list of candidate SNPs that may modulate XMET genes via interference with miR targeting in multiple human tissue types.

Single nucleotide polymorphisms located in the gene 3′-UTRs could have great impact on miR targeting. It has been demonstrated that the entire 3′-UTR sequence could play important roles in miR function in addition to miR target sites (Hu and Bruno, 2011). In particular, negative selection in humans is stronger on computationally predicted conserved miR binding sites than on other conserved sequence motifs in 3′-UTRs, and polymorphisms in predicted miR binding sites are highly likely to be deleterious to the gene expression and via what mechanism. Our study provides a comprehensive evaluation for this question in major human XMET genes, and generated a list of candidate SNPs that may modulate XMET genes via interference with miR targeting in multiple human tissue types.

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### APPENDIX

**Table A1 | Major XMETs and related genes investigated in this study.**

| Phase I \( (n = 144) \) | Phase II \( (n = 85) \) | Phase III \( (n = 111) \) | Nuclear receptors and transcription factors \( (n = 48) \) | Miscellaneous genes \( (n = 21) \) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| AADAC | AANAT | ABC1 | AHR | CRABP1 |
| ABP1 | ACSL1 | ABCA1 | AHRR | CRABP2 |
| ADH1A | ACSL3 | ABCA2 | AIP | CYB5A |
| ADH1B | ACSL4 | ABCA3 | ARNT | GZMA |
| ADH1C | ACRM1 | ABCA7 | ARNT2 | GZMB |
| ADH4 | ACRM28 | ABCA8 | CREBBP | MT1A |
| ADH5 | ACSM3 | ABCB1 | EP300 | MT1B |
| ADH6 | AGXT | ABCB10 | ESR1 | MT1F |
| ADH7 | ASMT | ABCB11 | ESR2 | MT1H |
| ADHE1 | ASMT | ABCB4 | FOXA2 | MT1M |
| AKR1A1 | BAAT | ABCB5 | FOXO1 | MT1X |
| AKR1B1 | CCBL1 | ABCB6 | HIF1A | MT2A |
| AKR1B10 | CESSA | ABCB7 | HIF3A | MT3 |
| AKR1C1 | COMT | ABCB8 | HNF4A | MT4 |
| AKR1C2 | DOST | ABCB9 | HSF90AA1 | MTHFR |
| AKR1C3 | GALT | ABCC1 | KEAP1 | POR |
| AKR1C4 | GGT1 | ABC10 | NCOA1 | RBP1 |
| AKR1C1L1 | GLYAT | ABC11 | NCOA2 | RBP2 |
| AKR1D1 | GNMT | ABC12 | NCOA3 | TP53 |
| AKR1E2 | GSTA1 | ABC12 | NCO1 | TXN |
| AKR1E2 | GSTA1 | ABC2 | NCO2 | TXN2 |
| AKR1F3 | GSTA1 | ABC3 | NFE2L2 | |
| AKR1G4 | GSTA1 | ABC4 | NRG2B | |
| ABDH16A1 | GSTA5 | ABC5 | NR1H2 | |
| ABDH16B1 | GST1 | ABC6 | NR1H3 | |
| ABDH1A1 | GSTM1 | ABC8 | NR1H4 | |
| ABDH1A2 | GSTM2 | ABC9 | NR1I2 | |
| ABDH1A3 | GSTM3 | ABCD4 | NR1I3 | |
| ABDH1B1 | GSTM4 | ABCG2 | NR3C1 | |
| ABDH1L1 | GSTM5 | ABCG8 | NR3C2 | |
| ABDH2 | GSTO1 | ALD | NR5A2 | |
| ABDH3A1 | GSTO2 | AQP1 | PPARA | |
| ABDH3A2 | GSTP1 | AQP7 | PPARD | |
| ABDH3B1 | GSTT1 | AQP9 | PPAR | |
| ABDH3B2 | GSTT2 | ATP6V0C | PPARC1A | |
| ABDH4A1 | GSTT2B | ATP7A | PPARC1B | |
| ABDH5A1 | GSTZ1 | ATP7B | PPRC1 | |
| ABDH6A1 | HNMT | KCNK9 | PTGES3 | |
| ABDH7A1 | INMT | MARCKSL1 | RARA | |
| ABDH8A1 | MGST1 | MDR/TAP | RARB | |
| ABDH9A1 | MGST2 | MRP | RARG | |
| AOC2 | MGST3 | MVP | RXRA | |
| AOC3 | MPST | OABP | RXRB | |
| AOX1 | NAA20 | OATP2 | RXRG | |
| BHE | NAT1 | SLC10A1 | THRA | |
| CBR1 | NAT2 | SLC10A2 | THR8 | |
| CBR3 | NNMT | SLC15A1 | TRIP11 | |
| CBR4 | PNMT | SLC15A2 | VDR | |
| CEL | PTGES | SLC16A1 | | |

(Continued)
Table A1 | Continued

| Phase I  | Phase II  | Phase III | Nuclear receptors and transcription factors ($n = 48$) | Miscellaneous genes ($n = 21$) |
|----------|-----------|-----------|------------------------------------------------------|--------------------------------|
| CES1     | SAT1      | SLC18A2   |                                                      |                                |
| CES2     | SULT1A1   | SLC19A1   |                                                      |                                |
| CES3     | SULT1A2   | SLC19A2   |                                                      |                                |
| CES4     | SULT1A3   | SLC19A3   |                                                      |                                |
| CES7     | SULT1A4   | SLC1A1    |                                                      |                                |
| CYP11A1  | SULT1B1   | SLC1A2    |                                                      |                                |
| CYP11B1  | SULT1C2   | SLC1A3    |                                                      |                                |
| CYP11B2  | SULT1C3   | SLC1A6    |                                                      |                                |
| CYP17A1  | SULT1C4   | SLC1A7    |                                                      |                                |
| CYP19A1  | SULT1E1   | SLC21A5   |                                                      |                                |
| CYP1A1   | SULT2A1   | SLC22A1   |                                                      |                                |
| CYP1A2   | SULT2B1   | SLC22A11  |                                                      |                                |
| CYP1B1   | SULT4A1   | SLC22A12  |                                                      |                                |
| CYP20A1  | SULT6B1   | SLC22A16  |                                                      |                                |
| CYP21A2  | TPMT      | SLC22A2   |                                                      |                                |
| CYP24A1  | TST       | SLC22A3   |                                                      |                                |
| CYP26A1  | UGT1A1    | SLC22A4   |                                                      |                                |
| CYP26B1  | UGT1A10   | SLC22A5   |                                                      |                                |
| CYP26C1  | UGT1A3    | SLC22A6   |                                                      |                                |
| CYP27A1  | UGT1A4    | SLC22A7   |                                                      |                                |
| CYP27B1  | UGT1A5    | SLC22A8   |                                                      |                                |
| CYP27C1  | UGT1A6    | SLC22A9   |                                                      |                                |
| CYP2A13  | UGT1A7    | SLC25A13  |                                                      |                                |
| CYP2A6   | UGT1A8    | SLC28A1   |                                                      |                                |
| CYP2A7   | UGT1A9    | SLC28A2   |                                                      |                                |
| CYP2B6   | UGT2A1    | SLC28A3   |                                                      |                                |
| CYP2C18  | UGT2A3    | SLC29A1   |                                                      |                                |
| CYP2C19  | UGT2B10   | SLC29A2   |                                                      |                                |
| CYP2C8   | UGT2B11   | SLC29A3   |                                                      |                                |
| CYP2C9   | UGT2B15   | SLC29A4   |                                                      |                                |
| CYP2D6   | UGT2B17   | SLC2A1    |                                                      |                                |
| CYP2E1   | UGT2B28   | SLC31A1   |                                                      |                                |
| CYP2F1   | UGT2B4    | SLC38A1   |                                                      |                                |
| CYP2J2   | UGT2B7    | SLC38A2   |                                                      |                                |
| CYP2R1   | UGT3A1    | SLC38A5   |                                                      |                                |
| CYP2S1   | UGT3A2    | SLC3A1    |                                                      |                                |
| CYP2U1   |           | SLC3A2    |                                                      |                                |
| CYP2W1   |           | SLC47A1   |                                                      |                                |
| CYP3A1   |           | SLC47A2   |                                                      |                                |
| CYP3A4   |           | SLC5A4    |                                                      |                                |
| CYP3A43  |           | SLC6A3    |                                                      |                                |
| CYP3A5   |           | SLC6A4    |                                                      |                                |
| CYP3A7   |           | SLC7A11   |                                                      |                                |
| CYP4A1   |           | SLC7A5    |                                                      |                                |
| CYP4A11  |           | SLC7A6    |                                                      |                                |
| CYP4A22  |           | SLC7A7    |                                                      |                                |
| CYP4B1   |           | SLC7A8    |                                                      |                                |
| CYP4F11  |           | SLCO1A2   |                                                      |                                |
| CYP4F12  |           | SLCO1B1   |                                                      |                                |
| CYP4F2   |           | SLCO1B3   |                                                      |                                |
| CYP4F22  |           | SLCO1C1   |                                                      |                                |

(Continued)
Table A1 | Continued

| Phase I  
(n = 144) | Phase II  
(n = 85) | Phase III  
(n = 111) | Nuclear receptors and transcription factors (n = 48) | Miscellaneous genes (n = 21) |
|----------|----------|----------|---------------------------------|---------------------------|
| CYP4F3   | SLC02A1  |          |                                 |                           |
| CYP4F8   | SLC02B1  |          |                                 |                           |
| CYP4V2   | SLC03A1  |          |                                 |                           |
| CYP4X1   | SLC04A1  |          |                                 |                           |
| CYP4Z1   | SLC04C1  |          |                                 |                           |
| CYP51A1  | SLC05A1  |          |                                 |                           |
| CYP7A1   | SLC06A1  |          |                                 |                           |
| CYP7B1   | TAP1     |          |                                 |                           |
| CYP8B1   | TAP2     |          |                                 |                           |
| Dhrs2    | Vdac2    |          |                                 |                           |
| Dhrs4    | Vdac3    |          |                                 |                           |
| Dhrs9    |          |          |                                 |                           |
| Dpyd     |          |          |                                 |                           |
| Ephpx1   |          |          |                                 |                           |
| Ephpx2   |          |          |                                 |                           |
| Esd      |          |          |                                 |                           |
| Fmo1     |          |          |                                 |                           |
| Fmo2     |          |          |                                 |                           |
| Fmo3     |          |          |                                 |                           |
| Fmo4     |          |          |                                 |                           |
| Fmo5     |          |          |                                 |                           |
| Hsd17b10 |          |          |                                 |                           |
| Kcnab1   |          |          |                                 |                           |
| Kcnab2   |          |          |                                 |                           |
| Kcnab3   |          |          |                                 |                           |
| Kdm1a    |          |          |                                 |                           |
| Kdm1b    |          |          |                                 |                           |
| Maoa     |          |          |                                 |                           |
| Maob     |          |          |                                 |                           |
| Nqo1     |          |          |                                 |                           |
| Nqo2     |          |          |                                 |                           |
| Paox     |          |          |                                 |                           |
| Pon1     |          |          |                                 |                           |
| Pon2     |          |          |                                 |                           |
| Pon3     |          |          |                                 |                           |
| Ptgs1    |          |          |                                 |                           |
| Ptgs2    |          |          |                                 |                           |
| Spr      |          |          |                                 |                           |
| Suox     |          |          |                                 |                           |
| Tbxas1   |          |          |                                 |                           |
| Uch1     |          |          |                                 |                           |
| Uch3     |          |          |                                 |                           |
| Xdh      |          |          |                                 |                           |
Table A2 | Putative miRNAs associated with SNPs in the 3′-UTR region.

| Gene         | Classification | SNP          | Tissue | Putative miRNAs                                                                 |
|--------------|----------------|--------------|--------|-------------------------------------------------------------------------------|
|              | microSNiPer    | PolymiRTs    | Overlap|
| ALDH16A1     | Phase I        | rs1055637    | Liver  | hsa-miR-4265, hsa-miR-1231, hsa-miR-3120-5p, hsa-miR-4322, hsa-miR-4669, hsa-miR-4726-3p, hsa-miR-3151, hsa-miR-4472, hsa-miR-491-5p, hsa-miR-132-5p, hsa-miR-4669 |
|              |                |              |        |                                                                                             |
| CYP2E1       | Phase I        | rs2480256    | Liver  | hsa-miR-570, hsa-miR-4762-5p, hsa-miR-613-3p, hsa-miR-570-3p, hsa-miR-500a-5p, hsa-miR-500b, hsa-miR-500a, hsa-miR-500b, hsa-miR-500a |
|              |                |              |        |                                                                                             |
| CYP2U1       | Phase I        | rs8727       | Liver  | hsa-miR-549, hsa-miR-125b-2*, hsa-miR-549, hsa-miR-549, hsa-miR-549, hsa-miR-549 |
|              |                |              |        |                                                                                             |
| CYP3A5       | Phase I        | rs15524      | Liver  | hsa-miR-501-5p, hsa-miR-500b, hsa-miR-500a, hsa-miR-500a, hsa-miR-500a, hsa-miR-500a |
|              |                |              |        |                                                                                             |
| CYP3A7       | Phase I        | rs10211      | Liver  | N/A, hsa-miR-125a-5p, hsa-miR-125b-5p, hsa-miR-125b-5p, hsa-miR-125b-5p, hsa-miR-125b-5p |
|              |                |              |        |                                                                                             |
| EPHX2        | Phase I        | rs1042032    | Brain  | hsa-miR-4476, hsa-miR-4533, hsa-miR-432*, hsa-miR-761, hsa-miR-183, hsa-miR-3665, hsa-miR-32390 |
|              |                |              |        |                                                                                             |
| EPHX2        | Phase I        | rs1042064    | Brain  | hsa-miR-576-3p, hsa-miR-22, hsa-miR-4696, hsa-miR-4696, hsa-miR-4696, hsa-miR-4696 |
|              |                |              |        |                                                                                             |
| GSTM3        | Phase II       | rs1109138    | Brain  | hsa-miR-4766-3p, hsa-miR-2964a-3p, hsa-let-7i*, hsa-let-7i*, hsa-let-7i*, hsa-let-7i* |
|              |                |              |        |                                                                                             |
| GSTM3        | Phase II       | rs1537236    | Brain  | hsa-miR-4762-5p, hsa-miR-4470, hsa-miR-4790-3p, hsa-miR-4421, hsa-miR-3182, hsa-miR-1237, hsa-miR-486-5p, hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p |
|              |                |              |        |                                                                                             |
| GSTM3        | Phase II       | rs1537235    | Brain  | hsa-miR-4793-3p, hsa-miR-3120-5p, hsa-miR-4527, hsa-miR-29b, hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p |
|              |                |              |        |                                                                                             |
| GSTM3        | Phase II       | rs3814309    | Brain  | hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p, hsa-miR-4793-3p |
| (Continued)  |                |              |        |                                                                                             |
Table A2 | Continued

| Gene    | Classification | SNP       | Tissue | Putative miRNAs                  |
|---------|----------------|-----------|--------|----------------------------------|
|         |                |           |        | microSNiPer                      |
| GSTM5   | Phase II       | rs11807   | Liver  | hsa-miR-1202                     |
|         |                |           |        | hsa-miR-1227                     |
|         |                |           |        | hsa-miR-1973                     |
| MGST3   | Phase II       | rs8133    | Liver  | hsa-miR-875-3p                   |
|         |                |           |        | hsa-miR-582-3p                   |
|         |                |           |        | hsa-miR-4698                     |
|         |                |           |        | hsa-miR-4694-3p                  |
|         |                |           |        | hsa-miR-4495                     |
|         |                |           |        | hsa-miR-411 *                    |
|         |                |           |        | hsa-miR-4694-3p                  |
|         |                |           |        | hsa-miR-411 *                    |
|         |                |           |        | hsa-miR-522-3p                   |
| ATP7B   | Phase II       | rs928169  | Liver  | hsa-miR-4734                     |
|         |                |           |        | hsa-miR-4430                     |
|         |                |           |        | hsa-miR-4430                     |
|         |                |           |        | hsa-miR-4481                     |
|         |                |           |        | hsa-miR-4472                     |
|         |                |           |        | hsa-miR-4472                     |
|         |                |           |        | hsa-miR-4430                     |
|         |                |           |        | hsa-miR-4498                     |
|         |                |           |        | hsa-miR-194 *                    |
|         |                |           |        | hsa-miR-4694-3p                  |
|         |                |           |        | hsa-miR-4734                     |
|         |                |           |        | hsa-miR-4430                     |
|         |                |           |        | hsa-miR-4481                     |
|         |                |           |        | hsa-miR-4472                     |
|         |                |           |        | hsa-miR-4472                     |
|         |                |           |        | hsa-miR-4430                     |
|         |                |           |        | hsa-miR-4498                     |
|         |                |           |        | hsa-miR-194 *                    |
| SLC31A1 | Phase III      | rs10759637| Liver  | hsa-miR-4448                     |
|         |                |           |        | hsa-miR-4448                     |
|         |                |           |        | hsa-miR-4448                     |
|         |                |           |        | hsa-miR-4461                     |
| TAP2    | Phase III      | rs13501   | Brain  | hsa-miR-3198                     |
|         |                |           |        | hsa-miR-1289                     |
|         |                |           |        | hsa-miR-1289                     |
|         |                |           |        | hsa-miR-3198                     |
|         |                |           |        | hsa-miR-4309                     |
|         |                |           |        | hsa-miR-4309                     |
|         |                |           |        | hsa-miR-317-5p                   |
| TAP2    | Phase III      | rs17034   | Brain  | hsa-miR-4772-3p                  |
|         |                |           |        | hsa-miR-4772-3p                  |
|         |                |           |        | hsa-miR-317-5p                   |
| TAP2    | Phase III      | rs241451  | Brain  | hsa-miR-1206                     |
|         |                |           |        | hsa-miR-1206                     |
|         |                |           |        | hsa-miR-1206                     |
|         |                |           |        | hsa-miR-1206                     |

(Continued)
| Gene       | Classification | SNP          | Tissue   | Putative miRNAs                                                                 |
|------------|----------------|--------------|----------|-------------------------------------------------------------------------------|
|            |                |              |          | microSNiPer                      | PolymiRTs                  | Overlap                      |
| TAP2       | Phase III      | rs241453     | Brain    | hsa-miR-4298                     | hsa-miR-1302               | hsa-miR-1302                 |
|            |                |              |          | hsa-miR-4298                     | hsa-miR-1302               | hsa-miR-4298                 |
| TAP2       | Phase III      | rs241454     | Brain    | hsa-miR-4476                     | hsa-miR-1302               | hsa-miR-1302                 |
|            |                |              |          | hsa-miR-4476                     | hsa-miR-4298               | hsa-miR-4476                 |
| TAP2       | Phase III      | rs241455     | Brain    | hsa-miR-130a*                    | hsa-miR-130a-5p            | hsa-miR-130a-5p              |
|            |                |              |          | hsa-miR-323-3p                   | hsa-miR-23a-3p             | hsa-miR-23a-3p               |
|            |                |              |          | hsa-miR-4779                     | hsa-miR-23b-3p             | hsa-miR-23b-3p               |
| TAP2       | Phase III      | rs241456     | Brain    | hsa-miR-3940-5p                  | hsa-miR-2110               | hsa-miR-4450                 |
|            |                |              |          | hsa-miR-4507                     | hsa-miR-4450               | hsa-miR-4450                 |
|            |                |              |          | hsa-miR-92a-1*                   | hsa-miR-450a-3p            | hsa-miR-450a-3p              |
|            |                |              |          | hsa-miR-92a-1*                   | hsa-miR-1270               | hsa-miR-1270                 |
|            |                |              |          | hsa-miR-23a-3p                   | hsa-miR-3676-6p            | hsa-miR-3676-6p              |
|            |                |              |          | hsa-miR-3609                     | hsa-miR-4531               | hsa-miR-4531                 |
|            |                |              |          | hsa-miR-4798-3p                  | hsa-miR-4683               | hsa-miR-4683                 |
|            |                |              |          | hsa-miR-620                      | hsa-miR-530                | hsa-miR-530                  |
| TAP2       | Phase III      | rs2857101    | Brain    | hsa-miR-944                      | hsa-miR-126-5p             | hsa-miR-944                  |
|            |                |              |          | hsa-miR-4795-3p                  | hsa-miR-4795-3p            | hsa-miR-4795-3p              |
|            |                |              |          | hsa-miR-183*                     | hsa-miR-944               | hsa-miR-944                  |
| UGT2A1     | Phase II       | rs4148312    | Liver    | hsa-miR-548t                     | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-548ah                    | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-3662                     | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-3662                     | hsa-miR-3609               | hsa-miR-3609                 |
|            |                |              |          | hsa-miR-548c-3p                  | hsa-miR-3609               | hsa-miR-3609                 |
|            |                |              |          | hsa-miR-3646                     | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-3646                     | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-3609                     | hsa-miR-548c-3p            | hsa-miR-548c-3p              |
|            |                |              |          | hsa-miR-3609                     | hsa-miR-548 ah             | hsa-miR-548 ah              |
|            |                |              |          | hsa-miR-3609                     | hsa-miR-548 ah             | hsa-miR-548 ah              |
|            |                |              |          | hsa-miR-340                      | hsa-miR-548n               | hsa-miR-548n                 |
|            |                |              |          | hsa-miR-1245                     | hsa-miR-548t-6p            | hsa-miR-548t-6p              |
|            |                |              |          | hsa-miR-106a                     | hsa-miR-4716-5p            | hsa-miR-4716-5p              |
| ARNT       | Nuclear receptors | rs11552229 | Liver    | hsa-miR-4716-5p                  | hsa-miR-4716-5p            | hsa-miR-4716-5p              |

The miRs expressed in the tissue where the eQTL was identified are highlighted in bold.