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Identification and validation of the microRNA response elements in the 3'-untranslated region of the UDP glucuronosyltransferase (*UGT*) *2B7* and *2B15* genes by a functional genomics approach

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Abstract

Posttranscriptional repression of UDP-glucuronosyltransferase (*UGT*) *2B7* and *2B15* expression by microRNAs (miRNAs) may be an important mechanism underlying inter-individual variability in drug glucuronidation. Furthermore, the *UGT2B15* 3'-UTR contains a common SNP (rs3100) that could influence miRNA binding. The aim of this study was to identify the complete complement of miRNAs that could regulate *UGT2B7* and *UGT2B15* expression through binding to the reference and/or variant 3'-UTRs. Luciferase reporter plasmids containing either the reference or variant 3'-UTRs were screened against a 2,048 human miRNA library to identify those miRNAs that decrease luciferase activity by at least 30% when co-transfected into HEK293 cells. Six novel miRNAs (miR-1293, miR-3664-3p, miR-4317, miR-513c-3p, miR-4483, and miR-142-3p) were identified that repressed the reference *UGT2B7* 3'-UTR, while twelve novel miRNAs (miR-770-5p, miR-103b, miR-3924, miR-376b-3p, miR-455-5p, miR-605, miR-624-3p, miR-4712-5p, miR-3675-3p, miR-6500-5p, miR-548as-3p, and miR-4292) repressed both the reference and rs3100 variant *UGT2B15* 3'-UTR. Deletion and mutagenesis studies confirmed the binding site location of each miRNA. Although the *UGT2B15* rs3100 SNP was located within the miR-376c-3p response element, there was no effect on miRNA binding. miR-142-3p, miR-3664-3p, miR-4317, miR-455-5p, miR-376c-3p, miR-770-5p, miR-3675-3p, miR-331-5p, miR-605, and miR-376b-3p transcript levels were measured by quantitative PCR and correlated with *UGT2B7* and *UGT2B15* enzyme activities in 27 human liver samples. A significant negative correlation ($R_s = -0.53$; $p = 0.005$) was demonstrated between hepatic miR-455-5p transcript levels and *UGT2B15*-mediated S-oxazepam glucuronidation activities. Thus, the *UGT2B7* and *UGT2B15* 3'-UTRs contain miRNA response elements for multiple miRNAs that may contribute to variable drug glucuronidation.

Keywords

UDP-glucuronosyltransferase; UGT2B7; UGT2B15; glucuronidation; microRNA; functional genomics

1. Introduction

The UDP-glucuronosyltransferases (UGTs) comprise a large family of conjugation enzymes capable of detoxifying a wide variety of both endogenous and exogenous substrates. The UGTs consist of 19 functional enzymes in humans that are subdivided by genetic similarity into three subfamilies, UGT1A, UGT2A, and UGT2B. The UGT2B subfamily includes 7 functional enzymes in humans (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28), located on chromosome 4q13 which are expressed as individual genes each with a unique 3'-untranslated region (3'-UTR) [1]. The UGTs are critical for the efficient elimination of the majority of the top 200 prescribed drugs in the United States, ranked second only to the cytochrome P450 enzymes [2]. UGT2B7 is the most commonly listed UGT enzyme known to contribute to the glucuronidation of the top 200 prescribed drugs [2]. Examples of clinically important drugs that are eliminated by UGT2B7-mediated glucuronidation include morphine and zidovudine (also called 3'-azido-3'-deoxythymidine or AZT) [3]. Furthermore, UGT2B7 plays a major role in the glucuronidation of endogenous compounds such as steroids and retinoids [4–6]. UGT2B15 contributes to the glucuronidation of androgenic steroids and various drugs e.g. oxazepam and lorazepam [7, 8]. Interestingly the major elimination pathway of oxazepam in humans is through stereoselective hepatic glucuronidation mediated primarily by the UGT2B15 and UGT2B7 enzymes [8]. Specifically in human liver *S*-oxazepam is mainly glucuronidated by UGT2B15 while *R*-oxazepam is glucuronidated by both UGT2B7 and UGT1A9 [8, 9]. Furthermore, the glucuronidation of this drug by human subjects is known to be polymorphic although the molecular mechanisms responsible for this variability are only partly understood [9].

We demonstrated previously that the single nucleotide polymorphism (SNP) c.735A>G (*UGT2B7*1c*; rs28365062) in the *UGT2B7* exon 2 explained only part of the relatively high interindividual variability in the clearance of a UGT2B7-specific probe substrate namely zidovudine, observed in HIV patients [10]. Furthermore, results from different groups showed that the SNPs located in the *UGT2B7* promoter and coding region cannot explain completely the observed variability in the UGT2B7 mediated glucuronidation of various drugs [11, 12]. Previous work from our lab identified SNP D85Y (253G>T, rs1902023) in exon 1 of the *UGT2B15* gene [13]. Moreover we demonstrated that the high frequency variant allele D85Y (allele frequency 47%) was a major determinant of the observed interindividual variability of oxazepam glucuronidation *in vivo* [14]. However, SNP D85Y could explain only 34% of the observed interindividual variability of hepatic oxazepam glucuronidation *in vivo* [14]. Consequently, other *trans*-acting factors could regulate UGT2B7 and UGT2B15 expression and contribute to this variability.

In recent years, microRNAs (miRNAs) have emerged as a crucial suppressive regulator of gene expression. miRNAs are a class of small non-coding RNAs that have been shown to control gene expression via imperfect base pairing to the 3' UTR of target genes [15]. Accumulating data demonstrate that miRNAs modulate the expression of drug disposition genes, including cytochrome P450 enzymes, UGTs, and transporters [16]. Recently we showed using an unbiased genome-wide screen that five novel miRNA response elements (MREs) are located in the common *UGT1A* 3'-UTR [17]. In addition two recent studies identified and validated the functional MREs of miR-376c and miR-331-5p to the 3'-UTRs of *UGT2B15* and *UGT2B17* in prostate cancer cells [18, 19]. However there is still a large gap in our knowledge regarding the role of miRNAs in the regulation of two important members of the UGT2B subfamily, namely UGT2B15 and UGT2B7. Essentially we lack knowledge of the full complement of miRNAs other than miR-376c and miR-331-5p which are involved in the direct regulation of UGT2B7 and UGT2B15. Moreover, the studies that are based on a bioinformatics scan of the target gene 3'-UTR to identify novel miRNA candidates tend to bias the results to those miRNAs that match best using available bioinformatics algorithms, which have a significant false negative discovery rate because of the imperfect binding of miRNAs to their targets. Consequently, alternate unbiased genome-wide approaches are likely needed to reveal the full complement of miRNAs regulating the UGT2B7 and UGT2B15 enzyme.

The objective of the current work was to identify the complete complement of miRNAs that could regulate UGT2B7 and UGT2B15 expression through binding to the respective 3'-UTRs. For this purpose, we employed the luciferase reporter system to perform a genome-wide screen of the *UGT2B7* and *UGT2B15* 3'-UTRs against a miRNA mimic library representing all of the available human miRNAs sequenced so far. This was followed by identification and functional validation of the miRNA response elements in the 3'-UTRs by a combination of *in silico* analysis and luciferase reporter construct assays. Furthermore we determined whether a common SNP in the *UGT2B15* 3'-UTR (rs3100) that was previously associated with *UGT2B15* mRNA allelic imbalance [14] can disrupt miRNA binding. Finally, we measured the transcript amounts of the candidate miRNAs in human liver samples to determine whether they are correlated with hepatic UGT2B7 or UGT2B15 activities.

2. Materials and methods

2.1. Chemicals and reagents.

The miRNA mimic library (miRIDIAN microRNA Mimic Library, v.19), the miRNA mimic negative controls (Negative controls 1 and 2), the short interfering RNA directed against the *UGT2B7* and *UGT2B15* 3'-UTR [positive controls: (siUGT2B7), 5' - AGA AGT CCA CTG ACA GTA T -3' - (siUGT2B15) 5' - GAC CAA ATA GGA ACA GCT CCA -3'], and the transfection reagent DharmaFECT Duo were purchased from GE Dharmacon (Lafayette, CO). The pMIR-REPORT plasmid was from Life Technologies (Grand Island, NY) and the Renilla luciferase vector pRL-CMV was purchased from Promega (Madison, WI). All synthesized DNA oligos used in this study were obtained from Integrated DNA Technologies, Inc. (Coralville, IA).

2.2. Cell lines and culture conditions.

HEK293 cells were cultured in DMEM (Life Technologies) supplemented with 10% FBS (Life Technologies). Cells were grown in a humidified chamber at 37°C and 5% CO₂.

2.3. Construction of reporter plasmids.

The pMIR-UGT2B7 and pMIR-UGT2B15 luciferase plasmids containing the entire *UGT2B7* 3'-UTR (hg38, chr4: 69,112,737–69,113,408) and *UGT2B15* 3'-UTR (hg38, chr4: 68,646,196–68,647,103; contains the rs3100 c.1761 C allele) were *de novo* synthesized by Blue Heron (Bothell, WA). For the generation of all deletion constructs and the pMIR-UGT2B15 rs3100 c.1761 T allele variant vector we used the KAPA HiFi Polymerase (Kapa Biosystems, Inc., Wilmington, MA) together with the pMIR-UGT2B15 luciferase plasmid and the respective set of primers (Table 1). All the triplicated constructs were generated using synthesized 5'-end phosphorylated oligonucleotides that were annealed with their complementary sequence (Table 1) and cloned into the pMIRREPORT vector at the *HindIII* sites directly downstream of the firefly luciferase gene. All plasmids used in this study were verified by Sanger sequencing performed at the Washington State University Genomics Core.

2.4. Luciferase reporter assays.

For all luciferase assays HEK293 cells were co-transfected with a firefly luciferase construct, the pRL-CMV Renilla luciferase control vector and the miRNA mimic or one of the mimic controls (negative or positive) as described previously [17].

2.5. Quantitative Real-time PCR (qPCR).

Total RNA was isolated from human liver samples using Trizol (Life Technologies). Mature miRNAs were isolated from individual liver samples of our human liver bank (n=27) using the mirVana™ miRNA Isolation Kit, with phenol (Life Technologies) according to the manufacturers' instructions. The source of the liver bank tissues have been published recently [20, 21]. These de-identified liver samples (Table 2) were obtained from publicly available sources, including the National Disease Research Interchange (Philadelphia, PA) and the Liver Tissue Procurement and Distribution Service (Minneapolis, MN). The use of these tissues for this study was approved by the Washington State University Institutional Review Board. mRNA and mature miRNA concentrations were determined by qPCR using reverse transcribed total RNA. For mRNA, cDNAs were first synthesized from total RNA using TaqMan Reverse Transcription Reagents (Life Technologies). qPCR was performed using TaqMan Gene Expression Master Mix and Taqman primer-probe mixes from Life Technologies, including *UGT2B15* (Hs00870076_s1) and *GAPDH* (Hs02758991_g1). Mature miRNAs were also quantified from total RNA after reverse transcription with miRNA-specific primers and MultiScribe Reverse Transcriptase (Life Technologies). qPCR was performed using TaqMan Universal Master Mix II, no UNG and Taqman miRNA assays from Life Technologies (Table 3). All assays were performed in triplicate. Gene expression was compared to an endogenous internal control (GAPDH for mRNA or the geometric mean of miR-1521 3p/miR-23b for miRNA as described previously [22]. There was not commercially available a set of primers and probes of the Applied Biosystems™ TaqMan™

microRNA assays for miR-4292 and miR-3924. Thus we used the GTEx database (<http://www.gtexportal.org/>) to determine the hepatic transcript amounts of these miRNAs. The C_t values for each miRNA were calculated this way for all of the samples in our human liver bank (n=27). Subsequently these values were normalized to the liver sample with the highest amount for each miRNA. These normalized values were then used to determine the correlation between the miRNA amounts in human liver samples and the hepatic activities of UGT2B7 and UGT2B15.

2.6. Statistical analysis.

Statistical analysis was performed with a two-tailed Student's *t* test using SigmaPlot Version 12 (Systat Software, Inc., San Jose, CA). Correlation analysis was performed by a Spearman's rank method. A *p* value of less than 0.05 was considered statistically significant.

3. Results

3.1. A genome-wide approach identifies novel miRNAs that decrease the *UGT2B7* and *UGT2B15* 3'-UTR-dependent luciferase reporter activity.

To identify candidate miRNAs involved in the regulation of UGT2B7 and UGT2B15 expression via direct binding to the respective 3'-UTRs, we used a commercially available miRNA mimic library (miRIDIAN microRNA Mimic Library, v.19) that contained all human miRNAs sequenced at that time (2,048 miRNAs as listed in miRBase version 19). We transiently co-transfected pMIR-UGT2B7 or the pMIR-UGT2B15 luciferase reporter plasmids and the pRL-CMV vector (Renilla transfection control) together with the miRNA mimics or the respective positive (siUGT2B7, siUGT2B15) and negative controls into HEK293 cells. This cell line is ideal for the heterologous expression of UGT2B7 and UGT2B15 since the mRNA transcript amounts of these enzymes in HEK293 cells are very low [23].

Subsequently we determined the effect of each miRNA mimic on the *UGT2B7* and *UGT2B15* 3'-UTR-dependent luciferase activities and classified them according to the degree of decrease of the relative *UGT3'*-UTR-mediated luciferase activity. We chose to study further the miRNAs that showed at least 30% decrease in the relative *UGT3'*-UTR-dependent luciferase activity in the initial library screen [19 miRNAs for UGT2B7 (Table 4 and 20 miRNAs for UGT2B15 (Table 5)]. Although miR-331-5p and miR-376c-3p caused a 38% and 54% decrease respectively in *UGT2B15* 3'-UTR-dependent luciferase activity we did not study these miRNAs further since recent studies demonstrated that they can regulate UGT2B15 expression [18, 19]. After counter-screening with the empty pMIR-REPORT vector to exclude nonselective effects, only six miRNAs for UGT2B7 (miR-1293, miR-3664-3p, miR-4317, miR-513c-3p, miR-4483, and miR-142-3p) and twelve for UGT2B15 (miR-770-5p, miR-103b, miR-3924, miR-376b-3p, miR-455-5p, miR-605, miR-624-3p, miR-47125p, miR-3675-3p, miR-6500-5p, miR-548as-3p, and miR-4292) were found to significantly reduce the luciferase activity of cells transfected with pMIR-UGT2B7 and pMIR-UGT2B15 luciferase reporter plasmids (Fig. 1A, 1B).

3.2. The *UGT2B7* and *UGT2B15* 3'-UTRs contain functional microRNA response elements (MREs) for the novel candidate miRNAs.

miRanda and RNAhybrid are well-verified online bioinformatics programs that predict the location of MREs in the 3'-UTRs of target mRNAs [24–26]. Using these programs, we identified putative MREs in the *UGT2B7* and *UGT2B15* 3'-UTRs for each of the candidate miRNAs (Fig. 2). To confirm the functionality of the candidate MREs in the 3'-UTRs we generated constructs that carried the full length *UGT2B7* and *UGT2B15* 3'-UTRs with a deletion () of the predicted MREs cloned directly downstream of the firefly luciferase gene. We also cloned each predicted MRE sequence as a triplicate in the forward or reverse (negative control) orientation in the pMIR-REPORT firefly luciferase vector. We then transiently co-transfected the full length, deletion, triplicate constructs or the empty pMIR-REPORT vector with miRNA mimics) or the miRNA mimic negative controls into HEK293 cells and quantified the luciferase activity of each construct. As shown in Figure 3 all miRNA mimics tested significantly reduced the luciferase activity of constructs carrying the full length *UGT2B7* and *UGT2B15* 3'-UTRs (left set of columns in each panel A-T). However, these reductions were abrogated when the respective MRE was deleted (Figure 3; second set of columns from left in each panel A-T). Moreover, cells transfected with the miRNA mimics and pMIR constructs carrying the MREs in the forward orientation showed a significant decrease in the luciferase activity compared with the control, while those with MREs in the reverse orientation showed no miRNA mimic effect (Figure 3; third and fourth set of columns from left in each panel A-T). As expected, none of the miRNA mimics affected luciferase activity of the empty pMIR-REPORT vector (Figure 3; right set of columns in each panel A-T).

3.3. Effect of the rs3100 single nucleotide polymorphism (SNP) in the *UGT2B15* 3'-UTR on miRNA binding.

We next sought to determine whether the common SNP rs3100 c.1761 C/T [27] present in the *UGT2B15* 3'-UTR had an effect on binding of any of the candidate *UGT2B15*-regulating miRNAs. Interestingly, *in silico* analysis using the bioinformatics program miRanda determined that the rs3100 SNP was located within the predicted miR-376c-3p MRE (Fig. 2, panels J, K). Furthermore, the variant c.1761T allele (Fig. 2, panel K) appeared to create an additional base-pairing site between the *UGT2B15* 3'-UTR and miR-376c-3p when compared with the reference c.1761C allele (Fig. 2, panel J). Consequently, lower luciferase activities would have been expected for the pMIR-*UGT2B15*-variant construct compared with the reference pMIR-*UGT2B15*. However, as shown in Figure 4 luciferase activities were reduced by all the candidate *UGT2B15*-regulating miRNAs (including miR-376c-3p) in both the pMIR-*UGT2B15* reference and variant constructs to the same extent indicating this SNP does not affect regulation by any of these miRNAs.

3.4. Expression of candidate miRNAs in human liver bank samples and correlation with hepatic *UGT2B7* and *UGT2B15* activity.

qPCR was then used to determine which of the candidate miRNAs are normally expressed in human liver. Mean ($\pm$ SD) threshold cycle (C_t) values for each miRNA were determined

using RNA extracted from 27 of our human liver bank samples (Table 2). For the UGT2B7 candidate miRNAs relatively high transcript amounts (lowest C_t values) were observed only for miR-142-3p (25.5 ± 1.1). Relatively low transcript amounts were observed for miR-3664-3p (36.1 ± 1.5) and miR-4317 (36.4 ± 1.7), while miR-513c-3p, miR-1293, and miR-4483 showed no measurable expression in any of the liver samples tested (C_t value over 40).

For the UGT2B15 candidate miRNAs relatively high transcript amounts (low C_t values) were observed for miR-455-5p (27.8 ± 0.6), miR-376c-3p (29.9 ± 1.4), miR-770-5p (29.7 ± 0.3), and miR-3675-3p (29.9 ± 0.4). Relatively low transcript amounts (high C_t values) were observed for miR-331-5p (32.3 ± 0.8), miR-605 (35.7 ± 1), and miR-376b-3p (37.6 ± 1.4). We did not measure miR-3924 and miR-4292 transcripts since publicly available RNA-seq data (<http://www.gtexportal.org/>) indicated that both these microRNAs are not expressed in human liver. Moreover recent results from a different group demonstrated that miR-4292 is not expressed in human liver [28].

Finally, we sought to determine whether expression levels of any of the candidate miRNAs were negatively associated with glucuronidation activities selective for UGT2B7 (zidovudine glucuronidation, morphine-3-glucuronidation, and morphine-6-glucuronidation) and UGT2B15 (*S*-oxazepam glucuronidation) when measured in the same human liver bank samples. These glucuronidation activities had been previously reported in [3, 13]). As shown in Table 6 although hepatic UGT2B7 activities were not negatively correlated with any of the miRNAs assayed, UGT2B15 mediated *S*-oxazepam glucuronidation activity showed a statistically significant negative correlation ($R_s = -0.53$; $p = 0.005$) with miR-455-5p levels (Table 7, Fig. 5).

4. Discussion

The most important novel findings of our study were the discovery and validation of 6 novel MREs for miR-1293, miR-3664-3p, miR-4317, miR-513c-3p, miR-4483, and miR-142-3p in the *UGT2B7* 3'-UTR, and 12 novel MREs for miR-770-5p, miR-103b, miR-3924, miR-376b-3p, miR-455-5p, miR-605, miR-624-3p, miR-4712-5p, miR-3675-3p, miR-6500-5p, miR-548as-3p, and miR-4292 in the *UGT2B15* 3'-UTR. The location of each MRE in the *UGT2B7* and *UGT2B15* 3'-UTRs are shown in Fig. 6 as well as the MREs for miR-376c and miR-331-5p, which have been previously characterized [18, 19].

We showed that half of the miRNAs we identified were expressed in human liver samples. More specifically 3 out of 6 candidate miRNAs for *UGT2B7* and 7 out of 14 candidate miRNAs for *UGT2B15* had detectable transcript amounts in human liver samples. Correlation analysis demonstrated that UGT2B7 activity did not show statistically significant correlation with any of the miRNAs while UGT2B15 activity showed a significant negative correlation with miR-455-5p hepatic levels (Fig. 5). While this suggests that miR-455-5p may contribute to inter-individual variability in hepatic *UGT2B15* activity, the number of liver samples used for this analysis was limited ($n=27$). Thus, it will be necessary to study more human liver samples to clarify the contribution of miRNAs to the variable hepatic UGT2B7 and UGT2B15 activities *in vivo*.

Previous results from our laboratory demonstrated that miR-103b and miR-376b-3p have functional MREs in the common *UGT1A* 3'-UTR [17]. In the current work we showed that miR-103b and miR-376b-3p were also among the miRNAs with functional MREs in the *UGT2B15* 3'-UTR. Thus, it is plausible that a network of miRNAs contributes to the regulation of the expression of various UGT isoforms. A genome-wide approach is required to validate this hypothesis and evaluate the role of specific miRNAs in the observed interindividual variability in the expression of UGT enzymes.

UGT2B7 activity, protein, and mRNA levels were previously demonstrated to be significantly lower in livers and kidneys obtained from diabetic patients when compared matched tissues from non-diabetic patients [29]. A meta-analysis of miRNA expression profiling studies identified miR-142-3p among the top miRNAs that were significantly and consistently upregulated in blood samples of patients with type 2 diabetes [30]. Taken together these results imply that the observed decrease of hepatic and renal UGT2B7 activity and expression in diabetic patients could be due to increased miR-142-3p expression. However, a detailed analysis of the hepatic and renal UGT2B7 activity and expression and the blood and/or tissue concentrations of miR-142-3p in a large number of samples would be required to prove the correlation between UGT2B7 altered expression and the increased levels of this miRNA in diabetes.

In recent work we used the same *in vitro* system to perform a genome-wide screen of the miRNA mimic library with the pMIR-REPORT luciferase vector carrying the reference *UGT1A* 3'-UTR or the variant 3'-UTR [17]. This way we demonstrated that SNP rs10929303 C>T located in the common *UGT1A* 3'-UTR created a novel MRE for miR-21-3p. Reporter gene assays by another group showed that the *UGT2B15* 3'-UTR rs3100 c.1761C (reference) allele resulted in lower UGT2B15-dependent luciferase reporter activity compared with the (variant) c.1761T allele in liver, breast, colon, and prostate cancer cell lines [27]. Those authors suggested that this SNP might be located within a miRNA binding site that could influence *UGT2B15* expression. Consequently we had hypothesized that the rs3100 c.1761T allele destroys the MRE of a miRNA that normally binds to the *UGT2B15* 3'-UTR rs3100 c.1761C allele. After screening for miRNAs that bind to the rs3100 c.1761C or c.1761T alleles, only a single miRNA (miR-376c-3p) was identified with a predicted MRE that included the rs3100 SNP. However, *in silico* analysis showed that the c.1761T allele (Fig. 2, panel K) enhances rather than reduces base-pairing to miR-376c-3p when compared with the c.1761C allele (Fig. 2, panel J). Despite this *in silico* prediction we were not able to experimentally demonstrate a significant difference in *UGT2B15*-dependent luciferase activity between the rs3100 c.1761C and c.1761T constructs when coexpressed with miR-376c-3p, or with any other miRNA demonstrated to bind to the *UGT2B15* 3'-UTR (Fig. 4). This lack of effect of rs3100 on miR-376c-3p binding may be because it is not located within the critical miRNA seed sequence (nucleotides 2–7). Regardless, it is still possible that rs3100 affects *UGT2B15* expression through influencing binding of another (yet to be identified) miRNA, or perhaps indirectly through another mechanism.

There are several limitations to our study. We used a 30% cutoff for luciferase reporter inhibition in the initial library screen to focus our efforts on those miRNAs most likely to influence *UGT2B7* and *UGT2B15* expression. This threshold was sensitive enough to

identify miR-376c-3p and miR-331-5p, which were the only other miRNAs with a functionally validated MRE [18, 19]. This cutoff also allowed us to minimize the significant amount of counter-screening that was needed since approximately 80% of candidate miRNAs were subsequently found to inhibit reporters lacking the UGT 3'-UTRs (i.e. false positives). However, it is possible that some of the miRNAs evaluated in our initial screen that showed less than 30% inhibition of reporter activity could also be important regulators of *UGT2B7* and *UGT2B15* expression. Furthermore, overexpression and knock-down experiments of these miRNAs in the appropriate cell line and in primary human hepatocytes are required to demonstrate their functional role in the regulation of *UGT2B7* and *UGT2B15* expression and enzyme activity.

In summary we used a genome-wide approach to identify functional MREs in the *UGT2B7* and *UGT2B15* 3'-UTRs. The genome-wide approach we employed could be further used to identify candidate miRNAs that bind directly in the 3'-UTR of other hepatic genes of pharmacological importance.

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Abbreviations:

UGT,	UDP-glucuronosyltransferase
3'-UTR	3'-untranslated region
SNP	single nucleotide polymorphism
miRNA	microRNA
qPCR	quantitative real-time PCR
MRE	miRNA response element

6. References

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Figure 1. miRNAs identified through a functional genomics library screen that reduce *UGT2B7* (1A) and *UGT2B15* 3'-UTR (1B) luciferase reporter (pMIR-UGT2B7 and pMIR-UGT2B15) activities by at least 30% without affecting activity of the empty vector (pMIR). Shown are relative luciferase activities (firefly/Renilla ratio normalized to the pMIR control ratio) of HEK293 cells co-transfected with the pMIR-UGT2B7/ pMIR-UGT2B15 or pMIR and with each miRNA mimic, a positive control (siUGT2B7, siUGT2B15: short interfering RNAs directed against the *UGT2B7* and *UGT2B15* 3' UTRs respectively) or the mimic negative

control. Bars represent mean $\pm$ one S.D. from three independent experiments performed in quadruplicate. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Figure 2. microRNA response elements identified in the *UGT2B7* 3'-UTR (A-F) and *UGT2B15* 3'-UTR (G-U) by *in silico analysis* for the miRNAs shown to reduce the *UGT2B7* and *UGT2B15*-dependent 3'-UTR luciferase reporter activity by at least 30% without affecting an empty luciferase reporter. Nucleotide positions in the mRNA are indicated relative to the last nucleotide of the stop codon. The binding free energy values (G°) between the miRNAs and the *UGT2B7* and *UGT2B15* 3'-UTRs are expressed in kcal/mol and were calculated using RNAhybrid.

Figure 3.

Functional validation of novel miRNA response elements (MREs; shown in Figure 2) in the *UGT2B7* and *UGT2B15* 3'-UTRs. For each miRNA evaluated luciferase reporter constructs were generated containing either the full length *UGT2B7* (panels A-E) or *UGT2B15* 3'-UTR (panels F-T), a deletion () of the predicted MRE, or the triplicated MRE in the forward or reverse (negative control) orientation. Shown are relative luciferase activities (firefly/Renilla ratio normalized to the miRNA control) of HEK293 cells co-transfected with the luciferase reporter and either the miRNA mimic or the mimic negative control. Bars

represent mean $\pm$ one S.D. from three independent experiments performed in quadruplicate.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Figure 4.
Effect of the common *UGT2B15* 3'-UTR rs3100 SNP on the binding of miRNAs to the reference 3'-UTR.

Figure 5.

Scatter plot showing a negative correlation between transcript levels of miR-455-5p measured by quantitative PCR in total RNA extracted from 27 human livers and UGT2B15-mediated *S*-oxazepam glucuronidation activities measured in microsomes prepared from the same livers. Also shown is the Spearman correlation coefficient (R_s) and associated p -value.

A **B** **Figure 6.**

Locations of known miRNA response elements (MREs) in the reference *UGT2B7* 3'-UTR (A) and *UGT2B15* 3'-UTR (B). Indicated are the positions of the novel MREs validated in this study (red triangles) and the MREs for miR-376c-3p and miR-331-5p, which had been reported previously [18, 19]. Also indicated is the position of the *UGT2B15* 3'-UTR rs3100 SNP.

Table 1.

List of primers used in this study. Underlined are shown the *HindIII* restriction sites used for cloning in the pMIR-REPORT vector. Bold are shown the respective MREs for each miRNA. All oligonucleotides used for the generation of the triplicate and deletion constructs were 5'-end phosphorylated.

Primer	Sequence (5' – 3')	Comment
MRE miR-142–3p F	GAAAATAAAAAATAAGATAAAGCCTTATGAGCTCG	Used to generate the pMIR-UGT2B7 142 construct.
MRE miR-142–3p R	GCCACAAAATATTTCTAACCATGAACCTGGG	
miR-142–3p MRE 3× F	<u>AGCTT</u> AATGAAGAAAACACTACG TATGTCAATGAAGAAAACACTACG TATGTCAATGAAGAAAACACTACG <u>A</u>	Used to generate the 142 3× F/R constructs.
miR-142–3p MRE 3× R	<u>AGCTT</u> CGTAGTGTTCCTTCATTGAC ATACGTAGTGTTCCTTCATTGACATACG TAGTGTTCCTTCATT <u>A</u>	
MRE miR-513c-3p F	TGAACTTATATTGAAATTTGTTGTTCTAATTCAC	Used to generate the pMIR-UGT2B7 513 construct.
MRE miR-513c-3p R	TCATAAGGCTTTATCTTATTTTATTTTCCG	
miR-513c-3p MRE 3× F	<u>AGCTT</u> GCTCGTATTGAAATTTGTGAGCTA GCTCGTATTGAAATTTGTGAGCTA GCTCGTATTGAAATTTGT <u>A</u>	Used to generate the 513c 3× F/R constructs.
miR-513c-3p MRE 3× R	<u>AGCTT</u> ACAAATTTCAATACGAGCTAGCTC ACAAATTTCAATACGAGCTAGCTC ACAAATTTCAATACGAGC <u>A</u>	
MRE miR-1293/4483 F	GTTTCATGGTTAGAAATATTTGTGGCAATG	Used to generate the pMIR-UGT2B7 1293/4483 construct.
MRE miR-1293/4483 R	AAATTTTTATTTGACAAAGTAAATTTTGAAAAG	
miR-1293/4483 MRE 3× F	<u>AGCTT</u> GTTTTTCAGAGATTACCACCCA TATGTCGTTTTTCAGAGATTACCACCCA TATGTCGTTTTTCAGAGATTACCACCCA <u>A</u>	Used to generate the 1293/4483 3× F/R constructs.
miR-1293/4483 MRE 3× R	<u>AGCTT</u> TGGGTGGTAAATCTCTGAAAAAC GACATATGGGTGGTAAATCTCTGAAAAAC GACATATGGGTGGTAAATCTCTGAAAAAC <u>A</u>	
MRE miR-3664–3p F	CAAAAAAAAAAAGAAAAAAAAATCTTTTCAAATTTAC	Used to generate the pMIR-UGT2B7 3664 construct.
MRE miR-3664–3p R	ATCACAATCTTCTTGCTGGAATAAAGT	
miR-3664–3p MRE 3× F	<u>AGCTT</u> GCAAGATTTCTTCTCTGAGA TATGTCGCAAGATTTCTTCTCTGAGA TATGTCGCAAGATTTCTTCTCTGAGA <u>A</u>	Used to generate the 3664 3× F/R constructs.
miR-3664–3p MRE 3× R	<u>AGCTT</u> TCTCAGGAAGAAGAAATCTTGC GACATATCTCAGGAAGAAGAAATCTTGC GACATATCTCAGGAAGAAGAAATCTTGC <u>A</u>	
MRE miR-376c-3p F	AATGATTTTAAAGCTATGAAAAATACAATGGG	Used to generate the pMIR-UGT2B15 376c construct.
MRE miR-376c-3p R	AGCTAAAGTACGTATTAAATCCCTGG	
miR-376c-3p MRE 3× F	<u>AGCTT</u> GAATTATTCTATGTCCCGGAA GAATTATTCTATGTCCCGGAA GAATTATTCTATGT <u>C</u> <u>A</u>	Used to generate the 376c 3× F/R constructs.
miR-376c-3p MRE 3× R	<u>AGCTT</u> GACATAGAATAATCTTCCGG GACATAGAATAATCTTCCGGGACATAGAAT AATTC <u>A</u>	

Primer	Sequence (5' – 3')	Comment
MRE miR-3924 F	TAGCTAACCTGCACGTTGTGCACATGTACCC	Used to generate the pMIR-UGT2B15 3924 construct.
MRE miR-3924 R	TGTTGGCGTGCTGCATCCAGTAACTCGTC	
miR-3924 MRE 3× F	<u>AGCTTTGGCACATGTATACATATGGATCCT</u> <u>TGGCACATGTATACATATGGATCCT</u> <u>TGGCACATGTATACATATGA</u>	Used to generate the 3924 3× F/R constructs.
miR-3924 MRE 3× R	<u>AGCTTCATATGTATACATGTGCCAAGGATC</u> <u>CATATGTATACATGTGCCAAGGATCCATATGTAT</u> <u>ACATGTGCCAA</u>	
MRE miR-548as-3p F	ATGGAGGATTTCCTTTTTCTGTGACAAAAC	Used to generate the pMIR-UGT2B15 548 construct.
MRE miR-548as-3p R	GAAATAAAGGAGGAGTCCCATCTTTCAGTC	
miR-548as-3p MRE 3× F	<u>AGCTTAGCATGGAGGGTTTTAAGACATA</u> <u>AGCATGGAGGGTTTTAAGACATA</u> <u>AGCATGGAGGGTTTTAA</u>	Used to generate the 548as 3× F/R constructs.
miR-548as-3p MRE 3× R	<u>AGCTTTTAAACCCCTCCATGCTTATGTC</u> <u>TTAAACCCCTCCATGCTTATGTC</u> <u>TTAAACCCCTCCATGCTA</u>	
MRE miR-455–5p F	ATACATATGTAGCTAACCTGCACGTTGTGC	Used to generate the pMIR-UGT2B15 455 construct.
MRE miR-455–5p R	AGTAACCTCGTCATTTAACATTAGGTATATCTCCAAATGC	
miR-455–5p MRE 3× F	<u>AGCTTGGATGCAGCACGCCAACATGGCACATGTGACATA</u> <u>GGATGCAGCACGCCAACATGGCACATGTGACATA</u> <u>GGATGCAGCACGCCAACATGGCACATGT</u>	Used to generate the pMIR-UGT2B15 455 construct.
miR-455–5p MRE 3× R	<u>AGCTTACATGTGCCATGTTGGCGTGCTGCATCC</u> <u>TATGTCACATGTGCCATGTTGGCGTGCTGCATCC</u> <u>TATGTCACATGTGCCATGTTGGCGTGCTGCATCCA</u>	
MRE miR-624–3p F	AAGACAAAATTTATTTTCCAGGGATTTAATACGTAC	Used to generate the pMIR-UGT2B15 624 construct.
MRE miR-624–3p R	GTGAAAAGATGTTTTGTACAGGAAAAAGGAAATCC	
miR-624–3p MRE 3× F	<u>AGCTTAACCTACCTTGTTGACAACTTACCTTGTT</u> <u>GACAACTTACCTTGTTA</u>	Used to generate the 624 3× F/R constructs.
miR-624–3p MRE 3× R	<u>AGCTTAACAAGGTAAGTTTGTCAACAAGGTAAGTTT</u> <u>GTCACAAGGTAAGTTA</u>	
MRE miR-6500–5p F	TTTGGAGATATACCTAATGTTAAATGACGAGTTACTGG	Used to generate the pMIR-UGT2B15 6500 construct.
MRE miR-6500–5p R	TTTCATAGCTTAAAAATCATTGACATAGAATAATTCAGC	
miR-6500–5p MRE 3× F	<u>AGCTTAATACAATGGGGGGAAGGATAGCAGACATA</u> <u>AATACAATGGGGGGAAGGATAGCAGACATA</u> <u>AATACAATGGGGGGAAGGATAGCA</u>	Used to generate the 6500 3× F/R constructs.
miR-6500–5p MRE 3× R	<u>AGCTTTGCTATCCTTCCCCCATTGTATTATGTC</u> <u>TGCTATCCTTCCCCCATTGTATTATGTC</u> <u>TGCTATCCTTCCCCCATTGTATTA</u>	
MRE miR-4712–5p F	GCAGCACGCCAACATGGCACATGTATAC	Used to generate the pMIR-UGT2B15 4712 construct.
MRE miR-4712–5p R	AACATTAGGTATATCTCCAAATGCTATCCTTCCC	
miR-4712–5p MRE 3× F	<u>AGCTTAAATGACGAGTTACTGGATGACATA</u> <u>AAATGACGAGTTACTGGATGACATA</u> <u>AAATGACGAGTTACTGGAT</u>	Used to generate the 4712 3× F/R constructs.

Primer	Sequence (5' – 3')	Comment
miR-4712–5p MRE 3× R	<u>AGCTTATCCAGTAACTCGTCATTTTATGTC</u> <u>ATCCAGTAACTCGTCATTTTATGTC</u> <u>ATCCAGTAACTCGTCATTTA</u>	
MRE miR-3675–3p F	TACCTAATGTTAAATGACGAGTTACTGGAdTGC	Used to generate the pMIR-UGT2B15 3675 construct.
MRE miR-3675–3p R	TTGTATTTTTCATAGCTTAAAAATCATTGACATAGAATAATTC	
miR-3675–3p MRE 3× F	<u>AGCTTTGGGGGGAAGGATAGCATTGGAGATACATAGC</u> <u>TGGGGGGAAGGATAGCATTGGAGATACATAGC</u> <u>TGGGGGGAAGGATAGCATTGGAGATAA</u>	Used to generate the 3675 3× F/R constructs.
miR-3675–3p MRE 3× R	<u>AGCTTTATCTCCAAATGCTATCCTTCCCCCAGCTATG</u> <u>TATCTCCAAATGCTATCCTTCCCCCAGCTATG</u> <u>TATCTCCAAATGCTATCCTTCCCCCAA</u>	
MRE miR-605–5p F	TACGTACTTTAGCTGAATTATTCTATGTCAATG	Used to generate the pMIR-UGT2B15 605 construct.
MRE miR-605–5p R	AGGTAAGTTGTGAAAAGATGTTTTGTACACAGG	
miR-605–5p MRE 3× F	<u>AGCTTTGTTAAGACAAAATTTATTTTCCAGGGATTTAA</u> <u>GCTAGCTGTTAAGACAAAATTTATTTTCCAGGGATTTAA</u> <u>GCTAGCTGTTAAGACAAAATTTATTTTCCAGGGATTTAA</u>	Used to generate the 605 3× F/R constructs.
miR-605–5p MRE 3× R	<u>GCTTTTAAATCCCTGGAAAATAAATTTTGTCTTAACA</u> <u>GCTAGCTTAAATCCCTGGAAAATAAATTTTGTCTTAACA</u> <u>GCTAGCTTAAATCCCTGGAAAATAAATTTTGTCTTAACAsA</u>	
MRE miR-376b–3p F	AATACAATGGGGGGAAGGATAGCATTG	Used to generate the pMIR-UGT2B15 376b construct.
MRE miR-376b–3p R	TGACATAGAATAATTCAGCTAAAGTACG	
miR-376b–3p MRE 3× F	<u>AGCTTATGATTTTAAAGCTATGAAAGAGTGC</u> <u>ATGATTTTAAAGCTATGAAAGAGTGC</u> <u>ATGATTTTAAAGCTATGAAA</u>	Used to generate the 376b 3× F/R constructs.
miR-376b–3p MRE 3× R	<u>AGCTTTTTTCATAGCTTAAAAATCATGCACTC</u> <u>TTTCATAGCTTAAAAATCATGCACTC</u> <u>TTTCATAGCTTAAAAATCATA</u>	
MRE miR-770–5p F	TGCAGCACGCCAACATGGCACATGTATACATATG	Used to generate the pMIR-UGT2B15 770 construct.
MRE miR-770–5p R	ATTAGGTATATCTCCAATGCTATCCTTCCCC	
miR-770–5p MRE 3× F	<u>AGCTTGTTAAATGACGAGTTACTGGAGATACT</u> <u>GTTAAATGACGAGTTACTGGAGATACT</u> <u>GTTAAATGACGAGTTACTGGAA</u>	Used to generate the 770 3× F/R constructs.
miR-770–5p MRE 3× R	<u>AGCTTTCCAGTAACTCGTCATTTAACAGTATC</u> <u>TCCAGTAACTCGTCATTTAACAGTATC</u> <u>TCCAGTAACTCGTCATTTAACA</u>	
MRE miR-4292 F	TTTAATACGTACTTTAGCTGAATTATTCTATGTCAATG	Used to generate the pMIR-UGT2B15 4292 construct.
MRE miR-4292 R	AATAAATTTTGTCTTAACAAGGTAAGTTGTGAAAAG	
miR-4292 MRE 3× F	<u>AGCTTTTCCAGGGATAGCTTCCAGGGA</u> <u>TAGCTTCCAGGGATAGCA</u>	Used to generate the 4292 3× F/R constructs.
miR-4292 MRE 3× R	<u>AGCTTGCTATCCCTGGAAGC</u> <u>TATCCCTGGAAGCTATCCCTGGAAA</u>	
MRE miR-103b F	AGCATTGAGATATACCTAATGTTAAATGACG	Used to generate the pMIR-UGT2B15 103b construct.
MRE miR-103b R	AAAAATCATTGACATAGAATAATTCAGCTAAAGTACG	

Primer	Sequence (5' – 3')	Comment
miR-103b MRE 3× F	<u>AGCTTTTTTTAAGCTATGAAAAATACAATGGGGGG</u> AAGGATTGTACCTTTTTAAGCTATGAAAAATACAATGGGGGG AAGGATTGTACCTTTTTAAGCTATGAAAAATACAATGGGGGG AAGGATA <u>A</u>	Used to generate the 103b 3× F/R constructs.
miR-103b MRE 3× R	<u>AGCTTATCCTTCCCCCATGTATTTTTTC</u> ATAGCTTAAAAAGGTACAATCCTTCCCCCATGTATTTTTTC ATAGCTTAAAAAGGTACAATCCTTCCCCCATGTATTTTTTC ATAGCTTAAAA <u>A</u>	
MRE miR-331–5p F	ATGTTAAATGACGAGTTACTGGATGC	Used to generate the pMIR-UGT2B15 331 construct.
MRE miR-331–5p R	TTCCCCCATGTATTTTTTCATAGC	
miR-331–5p MRE 3× F	<u>AGCTTGGATAGCATTGGAGATATACCTAACGCCTT</u> GGATAGCATTGGAGATATACCTAACGCCTT GGATAGCATTGGAGATATACCTAA <u>A</u>	Used to generate the 331 3× F/R constructs.
miR-331–5p MRE 3× R	<u>AGCTTTTAGGTATATCTCCAAATGCTATCCAAGGCG</u> TTAGGTATATCTCCAAATGCTATCCAAGGCG TTAGGTATATCTCCAAATGCTATCCA <u>A</u>	
rs3100 F	GGGATTTAATACGTACTTTAGTTGAATTATTCTATGTCAATG	Used to generate the pMIR-UGT2B15 rs3100 construct.
rs3100 R	CATTGACATAGAATAATTCAACTAAAGTACGTATTAAATCCC	
rs7660719 F	GAACTCCACTCATAGTATGAACATTGTTTTGCAAATAC	Used to generate the pMIR-UGT2B15 rs7660719 construct.
rs7660719 R	GTATTGCAAAACAATGTTCACTATGAGTGGAGTTC	
rs13145346 F	GGTGCAGCACACTGTGTGTGAGCTGAAGCAGGGC	Used to generate the pMIR-UGT2B15 rs13145346 construct.
rs13145346 R	GCCCTGCTTCAGCTCACACACAGTGTGCTGCACC	

Table 2.

Demographics of liver donors (n=27).

Donor identity	Age	Gender	Ethnicity ^a	Cause of death	Smoking	Alcohol ^b	Other drug exposure ^c
LV2	8	F	A	Head trauma	No	No	
LV3	5	M	C	Meningitis	No	No	Dopamine, penicillin
LV4	24	F	C	Auto accident			Dopamine
LV5	20	M	C	Head trauma	Yes	No	Nafcillin, mannitol, furosemide, erythromycin, nystatin, neomycin
LV6	35	F	C	Head trauma	No	No	None
LV7	21	M	A	Head trauma	Yes	Yes	DDAVP
LV16	45	F	H	Stroke	No	No	Dopamine
LV17	6	M	C	Auto accident	No	No	Dopamine
LV23	74	M	C	Colon cancer			
LV25	49	F	C	Stroke	Yes	No	Diazepam, furosemide
LV30	40	F	C	Head trauma	Yes	Yes	Mannitol, furosemide, phentolamine
LV32	45	F	C	Stroke	Yes	Yes	Prednisone, synthroid, phenytoin, naproxen, diazepam
LV36	43	M	C	Stroke	No	No	Memidrolol, dexamethasone, hydrocortisol
LV39	36	M	C	Head trauma	Yes	Yes	Cefazolin, norcuronium, phytonadione, enalapril, metoclopramide
LV40	34	M	C	Gun shot wound	Yes	No	Crack cocaine, dopamine, norcuronium, fentanyl, midazolam
LV41	43	M	C	Head trauma	No	Yes	Dopamine, furosemide, neosynephrine, acetaminophen
LV42	35	M	C	Encephalopathy	No	No	Dopamine, norepinephrine, naloxone
LV43	24	M	C	Blunt trauma	No	No	
LV45	75	F	C	Stroke	No	No	Dopamine, vasopressin, furosemide, mannitol, vecuronium
LV46	49	M	C	Stroke	Yes	Yes	Occasional crack cocaine and marijuana
LV48	68	F	C	Stroke	No	No	Synthroid, celecoxib, fluoxetine, atenolol, omeprazole
LV49	21	M	C	Head trauma	Yes	No	Crack cocaine, marijuana
LV50	37	M	C	Head trauma	Yes	Yes	Marijuana
LV51	62	M	C	Stroke	No	No	Dopamine, norepinephrine, insulin, DDAVP, cefasolin
LV53	54	M	C	Brain abscess	No	No	Simvastatin, acetaminophen, alprazolam, flurazepam, venlafaxine
LV54	63	M	C	Stroke	No	Yes	Dopamine, norepinephrine, nitroprusside
LV55	46	M	C	Stroke	No	No	

^a: C, Caucasian; A, African-American; H, Hispanic.^b: History of consumption of more than 14 drinks per week.^c: DDAVP, desmopressin acetate.

Table 3.

List of Taqman miRNA assays (Life Technologies) used to measure the hepatic concentrations of miRNAs.

miRNA	Assay ID
miR-4483	461903_mat
miR-142-3p	000464
miR-4317	243568_mat
miR-3664-3p	464880_mat
miR-1293	002298
miR-513c-3p	472280_mat
miR-152-3p	000475
miR-23b	002126
miR-331-5p	002233
miR-770-5p	002002
miR-103b	121115_mat
miR-376b-3p	001102
miR-376c-3p	002122
miR-455-5p	001280
miR-605	466447_mat
miR-624-3p	002430
miR-4712-5p	463735_mat
miR-3675-3p	461795_mat
miR-6500-5p	474759_mat
miR-548as-3p	471738_mat

Table 4.

miRNAs identified to decrease the *UGT2B7*-3'-UTR-mediated luciferase reporter activity by at least 30% through library screening. Decrease in luciferase activity is represented relative to the average luciferase activities of non-transfected HEK293 cells and cells transfected with the miRNA mimic negative controls. In bold are the 6 novel candidate miRNAs identified in this study. Other listed miRNAs were not evaluated further since they showed a similar amount of inhibition of luciferase activity in a counterscreen of cells transfected with luciferase reporter lacking the *UGT1A*-3'UTR and so were considered false positives.

miRNA	Decrease in luciferase activity relative to the miRNA negative control (%)
miR-18b-3p	65.4
miR-3687	65.1
miR-34b-5p	54.6
miR-30c-2-3p	54.5
miR-30c-1-3p	50.0
miR-1293	44.2
miR-550a-3-5p	43.9
miR-3670	40.8
miR-3664-3p	40.1
miR-4317	39.5
miR-129-5p	38.4
miR-4531	36.5
miR-513c-3p	36
miR-4483	31.6
miR-1294	33.3
miR-4259	31.5
miR-28-3p	30.6
miR-4419a	30.3
miR-142-3p	30

Table 5.

miRNAs identified to decrease the *UGT2B15*-UTR-mediated luciferase reporter activity by at least 30% through library screening. Decrease in luciferase activity is represented relative to the average luciferase activities of non-transfected HEK293 cells and cells transfected with the miRNA mimic negative controls. In bold are the 12 novel candidate miRNAs identified in this study. Underlined are miR-376-3p and miR-331-5p which had been identified in a previous study by an *in silico* approach. Other listed miRNAs were not evaluated further since they showed a similar amount of inhibition of luciferase activity in a counter-screen of cells transfected with luciferase reporter lacking the *UGT1A*-3'UTR and so were considered false positives.

miRNA	Decrease in luciferase activity relative to the miRNA negative control (%)
miR-1262	62.3
<u>miR-331-5p</u>	<u>62.1</u>
miR-770-5p	54.4
miR-103b	53.9
miR-3942-5p	53.9
miR-3924	52.9
miR-30c-2-3p	50.1
miR-376b-3p	47.8
<u>miR-376c-3p</u>	<u>46.4</u>
miR-30c-1-3p	45.1
miR-455-5p	42.6
miR-605	42.6
miR-624-3p	41.6
miR-4712-5p	40.6
miR-3675-3p	40.6
miR-6500-5p	39.7
miR-548as-3p	38.7
miR-4292	37.9
miR-744-5p	35.5
miR-3678-5p	31.1

Table 6.

Correlation between UGT2B7 activities and hepatic miRNA transcript levels in samples from a human liver bank (n=27). UGT2B7 activities including zidovudine glucuronidation, morphine-3-glucuronidation, and morphine-6-glucuronidation (results previously reported in [3]). Shown are the Spearman correlation coefficients (R_s) and the associated p values.

miRNA	UGT2B7 activity						
	Zidovudine glucuronidation		Morphine-3-glucuronidation		Morphine-6-glucuronidation		
		R_s	p	R_s	p	R_s	p
miR-142-3p	-0.2	0.31	-0.02	0.92	0.04	0.84	
miR-3664-3p	0.28	0.16	0.1	0.62	0.12	0.55	
miR-4317	0.41	0.03	-0.06	0.76	-0.04	0.84	

Table 7.

Correlation between hepatic miRNA transcript levels and UGT2B15mediated S-oxazepam glucuronidation activities in samples from a human liver bank (n=27). S-oxazepam glucuronidation activities were previously reported in [13]. Shown are the Spearman correlation coefficients (R_s) and the associated p values.

	S-oxazepam glucuronidation	
	R_s	p
miR-331-5p	-0.2	0.31
miR-376c-3p	-0.05	0.8
miR-455-5p	-0.53	0.005
miR-605	0.05	0.78
miR-770-5p	-0.05	0.8
miR-3675-3p	-0.08	0.7
miR-103b	0.03	0.9