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STAT5A and STAT5B have opposite correlations with drug response gene expression

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Abstract

Introduction: STAT5A and STAT5B are important transcription factors that play a key role in regulation of several important physiological processes including proliferation, survival, mediation of responses to cytokines and in regulating gender differences in drug response genes such as the hepatic cytochrome P450s (CYPs) that are responsible for a large majority of drug metabolism reactions in the human body. STAT5A and STAT5b have a high degree of sequence homology and have been reported to have largely similar functions. Recent studies have, however, indicated that they can also often have distinct and unique roles in regulating gene expression.

Objective: In this study, we evaluated the association of STAT5A and STAT5B mRNA expression levels with those of several key hepatic cytochrome P450s (CYPs) and hepatic transcription factors (TFs) and evaluated the potential roles of STAT5A and 5b in mediating gender differences in these CYPs and TFs.

Methods: Expression profiling for major hepatic CYP isoforms and transcription factors was performed using RNA sequencing (RNA-seq) in 102 human liver samples (57 female, 45 male). Real time PCR gene expression data for selected CYPs and TFs was available on a subset of 50 human liver samples (25 female, 25 male) and was used to validate the RNA-seq findings.

Results: While STAT5A demonstrated significant negative correlation with expression levels of multiple hepatic transcription factors (including NR1I2 and HNF4A) and DMEs such as CYP3A4 and CYP2C19, STAT5B expression was observed to demonstrate positive associations with several CYPs and TFs analyzed. As STAT5A and STAT5B have been shown to be important in regulation of gender differences in CYPs, we also analyzed STAT5A and 5b associations with CYPs and TFs separately in males and females and observed gender dependent differential associations of STATs with several CYPs and TFs. Results from the real time PCR validation largely supported our RNA-seq findings.

Conclusions: Using both RNA sequencing and real time PCR, we examined the association of STAT5A and STAT5B mRNA expression with CYP and TF gene expression. While STAT5A demonstrated significant negative correlations with expression levels of multiple hepatic TFs

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(including NR1I2 and HNF4 α) and CYPs (eg. CYP3A4, CYP2C19), STAT5B expression was observed to demonstrate positive association with most of the CYPs/TFs analyzed suggesting that STAT5A and STAT5b have potentially different and distinct roles in regulating expression of hepatic drug response genes. Further studies are needed to elucidate the potential roles of STAT5A and 5b in regulation of CYPs/TFs and the potential implications of these findings.

Keywords

Cytochrome P450; mRNA expression; Transcription; Gender

1. Introduction

Hepatic drug metabolizing enzymes such as cytochromes P450s (CYPs), demonstrate substantial inter-individual variability in expression, thus impacting drug metabolism/clearance in individuals and often leading to either reduced efficacy or drug related toxicity. Moreover, several CYPs have been shown to demonstrate gender differences in their expression. Gender differences in hepatic gene expression in general and in CYPs in particular have been shown to be regulated by the differences in patterns of growth hormone secretion in males and females. These, in turn, lead to activation of transcription factors such as STAT5A and STAT5B that can activate downstream target genes and lead to gender biased expression of several hepatic CYPs.

Whereas the role of nuclear hormone receptors such as PXR and CAR and liver specific transcription factors such as HNF4 α and HNF3 β has been well established in transcriptional regulation of CYPs, the roles of transcription factors such as STAT5A and 5b and their roles in contributing to variability of cytochrome P450s are still to be explored systematically.

STAT5A and STAT5B are important transcription factors that play a key role in mediating transcriptional signals for a number of cytokine factors including growth hormone receptor. They have been shown to play a role in a number of diverse cellular processes such as proliferation, differentiation and apoptosis as part of hematopoiesis, immunoregulation, reproduction, drug response and lipid metabolism [1]. They act through the so-called JAK-STAT pathway. Upon activation by the binding of GH, JAK2 kinases recruit STAT5A or 5B hetero- or homodimers [2]. The activated STAT dimers migrate to the nucleus where they can bind to promoters of specific target genes with conserved binding sites and activate transcription [1].

STAT 5A and 5B genes (along with STAT3) are present in a cluster on chromosome 17q [2]. STAT 5a and STAT5B are located in opposite orientation around 11 kb apart. The cDNA sequences of the two genes are highly (~94%) similar and the protein domains are almost identical with minor differences [1]. Although STAT5A and B have often been reported to play redundant or overlapping roles in mediating regulatory signaling, gene knockout mouse models investigating their roles in mediating GH regulation of gender-biased hepatic gene expression have showed that STAT5A and STAT5B have certain distinct, non-overlapping functions [3]. STAT5A has been seen to involved in regulation of female-biased hepatic genes in female livers predominantly and is expressed at lower levels. STAT5B, in contrast,

has a broader regulatory influence in both male and female livers, but is primarily involved in regulation of male-biased hepatic genes in male livers [4,5].

In this study, we evaluated the potential roles of STAT5A and STAT5B in regulating expression of several key hepatic cytochrome P450s and their transcriptional regulatory genes. Using RNA sequencing and real time PCR based gene expression profiling of mRNA expression of major hepatic CYP isoforms involved in drug metabolism and major hepatic transcription factors (and other genes implicated in CYP regulation) in human male and female human liver tissue specimens, we have shown for the first time that STAT5A and STAT5B have distinctive and potentially important roles in regulation of hepatic drug response genes as well as in contributing to gender-related differences in expression of hepatic CYPs and TFs.

2. Materials and methods

2.1. Study samples

102 de-identified normal human liver samples (57 female, 45 male) from Caucasian subjects that had been flash frozen and stored in liquid nitrogen were obtained from University of Minnesota's Biological Materials Procurement Network facility (BIONET). The study was in accordance with Helsinki declaration.

2.2. RNA isolation for mRNA expression profiling

Total RNA was isolated from 102 normal liver tissues (45 males and 57 females) using AllPrep DNA/RNA mini kit (Qiagen, Valencia, CA, USA) for mRNAs per the manufacturers' protocols. The RNA quality and yield of each total RNA sample was obtained from A260 measurements using a NanoDrop 2000 (Thermo Fischer, Waltham, MA, USA). The RNA integrity number (RIN) was tested with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

2.3. RNA sequencing analysis

Illumina HiSeq 2000 was used to carry out 50bp single end basic RNA-seq at 10× Sequencing depth and a yield of ~10–20 million reads per sample. The count level data was analyzed and corrected for batch effects. Briefly, the data was pre-processed in the following steps: (i) Converting the NGS count data to continuous data through a Bayesian hierarchical model; (ii) adjusting the batch effect using a regression approach; and (iii) transforming the data to be Gaussian using the semi-parametric normalization method.

Expression profiling data was extracted for major hepatic CYP isoforms involved in drug metabolism reactions including CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A7, CYP2B6, CYP2C18, CYP2C19 CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A4, CYP3A43, CYP3A5, CYP3A7, CYP4A22 CYP4F11, CYP4F12 CYP4F2 CYP4F22, CYP4F3 and major hepatic genes/transcription factors suggested to be involved in drug metabolism (CEBPA, CEBPB, CEBPD, CEBPE, CEBPG, FOXA1, FOXA2, FOXA3, FOXO1, HNF1A, HNF1B, HNF4A, HNF4G, NCOA1, NCOA2, NCOA3, NCOA4, NCOA5, NCOA6, NCOA7, NCOR1, NCOR2, NR0B2, NR1D1, NR1D2, NR1H2, NR1H3,

NR1H4, NR1I2, NR1I3, NR2C1, NR2C2, NR3C1 NR3C2 NR4A1 NR4A2 NR4A3 NR5A1 NR5A2 NR6A1 PPARA, PPARD, PPARG, PPARGC1A, PPARGC1B, RXRA, RXRB and RXRG).

2.4. Statistical statistical analysis

GraphPad Prism 7 for Mac OS X was used for analysis of the RNA-seq gene expression data. The data was Log10 transformed and correlations and two tailed p-values were calculated. Although Pearson's correlations are reported throughout the paper for consistency, we also calculated Nonparametric Spearman correlation values (data not shown, available upon request). Additional statistical calculations were performed by use of the statistical program R [<http://www.R-project.org>]. Gender differences in expression levels were analyzed parametrically using the *t*-test with unequal variances and non-parametrically using the Wilcoxon rank-sum test. No adjustments have been made for multiple testing.

2.5. Real-time PCR validation

Real time PCR data was obtained for STAT5A and STAT5B along with hepatic CYPs and TFs on a subset of the samples. Part of this data and the methods were reported previously [6] and details are not presented here. Briefly, for mRNA targets, first-strand cDNA preparation and real time PCR was carried out using 384 well TLDA (Taqman Low Density Array) plates. SDS 2.3 and RQ manager (Applied Biosystems) were used for data analysis and relative quantification was carried out using ddCT method.

3. Results

3.1. Data QA/QC assessment for RNA sequencing (RNA-seq) analysis

The phred quality score for base calling was observed to be quite good for the RNA sequencing analysis. For almost all the samples, more than 80% of the reads could be mapped uniquely to human genome (hg19 assembly). Although a batch effect was observed and corrected for, the genes differentially expressed between the two sequencing batches seemed not related to hepatic functions (by IPA analysis, data not shown).

3.2. Gender differences in CYP and TF expression

The RNA-seq gene expression data was analyzed for gender differences in CYP and TF expression. Significant gender differences, along with higher expression in females were observed for several CYPs (CYP2C19, CYP2E1, CYP2J2 and CYP3A4) and transcription factors (HNF1A, HNF4A, HNF4G, NCOA1, NR0B2, NR1H4) (Table 1). The most significant differences were seen for CYP2C19 (wilcox $p = 0.007986$; *t*-test $p = 0.007986$) and CYP3A4 (Wilcox $p = 0.011045$; *t*-test $p = 0.007375$), along with HNF4alpha (wilcox $p = 0.005832$; *t*-test $p = 0.006009$), HNF4gamma (wilcox $p = 0.000345$; *t*-test $p = 8.10E-05$), and NR0B2 (wilcox $p = 0.007823$; *t*-test $p = 0.003064$).

Conversely, significant gender differences along with higher expression levels in males were observed for NCOR2 (wilcox $p = 0.001689$; *t*-test $p = 0.003545$) and PPARD (wilcox $p = 0.000405$; *t*-test $p = 0.044799$). For STAT5A there was a trend towards higher expression levels in males compared to females (wilcox $p = 0.074689$; *t*-test $p = 0.022224$) while

STAT5B demonstrated non-significant but marginally higher levels in females compared to males.

3.3. Association of STAT5A with mRNA expression levels of CYPs (all subjects)

Using RNA-seq gene expression data, we observed that STAT5A had significant negative correlations with several CYPs and TFs (Table 2A and Fig. 1). Table 2A shows the correlations of STAT5A with CYPs and TFs when males and females are analyzed together, as well as when they were analyzed separately. When analyzed together, STAT5A had significant negative correlations with several CYPs (CYP2C9, CYP2E1, CYP2J2, CYP3A5) and transcription factors (AKR1D1, CEBPA, FOXA2, FOXO1, HNF1A, HNF4A, HNF4G, NR0B2, NR1H4, NR1I2, NR3C1, PPARA, PPARGC1A). Most significant negative correlations (p value < 0.0001) were observed with FOXA2, HNF4A, HNF4G and NR1I2. In contrast, STAT5A also had significant positive correlations with NCOA7 ($r = 0.264$, $p = 0.0079$) and NCOR2 ($r = 0.29$, $p = 0.0035$), two co-receptors known to play a role in regulation of drug response genes).

3.4. Association of STAT5A with mRNA expression levels of CYPs and TFs (in males and females)

Since STAT5A and STAT5B have been shown to regulate hepatic gene expression in a gender specific manner, we also analyzed the STAT5A correlations with CYPs and TFs separately in males and females (Table 2A and Fig. 1). STAT5A demonstrated significant negative correlations with CYP2C8, CYP2C9, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A43, AKR1D1, FOXA2, FOXO1, HNF4G, NR1I2 in females and with CEBPA, FOXA2, HNF1A, HNF4A, HNF4G, NR0B2, NR1H4, NR1I2, PPARA in males. Significant positive correlations were observed with NCOR2 only in females. As reported in literature STAT5A is important for female-biased hepatic gene expression in females [5], and we observed much higher number of associated genes in females than males.

3.5. Association of STAT5B with mRNA expression levels of CYPs and TFs (all subjects)

STAT5B had significant positive correlations with several CYPs and TFs (Table 2B and Fig. 1). Table 2B shows the correlations of STAT5B with CYPs and TFs when males and females are analyzed together, as well as when they were analyzed separately. When analyzed together, STAT5B had significant negative correlations with several CYPs (CYP1A1, CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP2J2, CYP3A4) as well as Transcription factors (AKR1D1, FOXO1, HNF1A, HNF4A, NR1H3, NR1H4, NR1I2, NR1I3, NR3C1, PPARA, RXRA) (See Tables 3 and 3BA).

Most significant negative correlations (p value < 0.0001) were observed with CYP2C19, NR1I3 and PPARA followed by AKR1D1 and RXRA ($p = 0.0002$). In contrast, STAT5B also had significant positive correlations with NCOA7 ($r = -0.32$, $p = 0.0012$), PPARD ($r = -0.313$, $p = 0.0015$) and POR ($r = -0.216$, $p = 0.0312$).

3.6. Association of STAT5B with mRNA expression levels of CYPs and TFs (in males and females)

STAT5B has been reported to enhance Male specific gene expression in Male mice and rats while repressing female specific expression [4]. In females, STAT5B demonstrated significant positive correlations with CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP3A4, AKR1D1, CEBPA, HNF4A, HNF4G, NCOR2, NR1H4, NR1I3, NR3C1, PPARA, RXRA. In males STAT5B was significantly correlated with CYP2C19, CYP2D6, CYP3A4, AKR1D1, FOXO1, NR1H3, NR1I2, NR1I3, PPARA, RXRA (Table 2B and Fig. 1).

3.7. Validation of RNA-seq results

Real time PCR data for STAT5A and STAT5B along with hepatic CYPs and TFs was available on a subset of the samples and was used to validate the STAT5A and STAT5B associations observed with CYPs and TFs. As observed with RNA-sequencing data, STAT5A had significant negative correlations with several CYPs and TFs (AKR1D1, CYP2B6, CYP2C19, CYP2C9, CYP3A4, EHHADH, FOXA2, HNF4A, NR1I2, NR1I3, PPARGC1A) when males and females were analyzed together. Also, as previously seen, STAT5A had significant positive correlation with NCOR2. When males and females, were analyzed separately, STAT5A had significant negative correlations with CYP2C19, CYP3A4, MED25, NR1I2, PPARGC1A in females and CYP2C9, CYP3A4, EHHADH, FOXA2, NR1I2, NR1I3, PPARGC1A in males. It also had significant positive correlation with NCOA1 in females only.

STAT5B, as observed in the RNA-sequencing data, had overwhelmingly positive correlations with CYPs and TFs when males and females were analyzed together or separately. When males and females were analyzed together, STAT5B had significant positive correlations with AKR1D1, CEBPA, CYP2C19, CYP2C9, CYP3A4, EHHADH, HNF4A, HNF4G, NCOR2, NR1I3, NR3C1, RXRA, SLC10A1). In females alone, STAT5B was significantly associated with essentially the same genes (except CYP3A4) and in males separately STAT5B had significant positive correlations with AKR1D1, CYP2C19, CYP2C9, CYP3A4, EHHADH, HNF4A, NR1I2, NR1I3, PPARGC1A and RXRA.

4. Discussion

A number of hepatic drug response genes including cytochrome P450s demonstrate substantial inter-individual variability as well as gender-biases in their expression [7]. Among the genes suggested to be involved in contributing to gender biased CYP gene expression are transcription factors such as the Signal transducer and activators of transcription (STATs). STATs are an important family of transcription factors and are known to play an important role in several processes including metabolism of endogenous and exogenous compounds and have been shown to be involved in mediating GH regulated changes in gene expression between genders [4].

Differences in pituitary GH secretion patterns between males and females are thought to control gender biases in expression of several hepatic genes including the cytochrome P450s [8]. These effects are mediated through the JAK-STAT signaling pathway with the activated

STATs migrating to the nucleus and binding to specific target genes to regulate gene expression [9,10].

STAT5A and 5B are highly similar and encoded by genes that have ~94% sequence homology. They have been shown to be involved in carrying out similar or overlapping roles in mediating regulatory signaling. However recent studies have showed that STAT5A and STAT5B can also have distinct roles in regulating gene expression [3]. STAT5A has been seen to be involved in regulation of female-biased hepatic genes predominantly while STAT5B has been suggested to have a broader regulatory influence in both male and female livers [4,5].

However, despite their potential importance to regulating CYP expression, the roles of STAT5A and 5b in contributing to variability and gender biases in expression of human cytochrome P450s are yet to be explored systematically.

The present study explored the association of STAT5A and STAT5B mRNA levels with expression levels of common drug response genes including all the major CYPs involved in human drug metabolism as also most of the transcription factors of reported or potential importance to regulation of ADME gene expression.

We identified a number of genes that had significant gender differences in mRNA expression. While CYP2C19, CYP2E1, CYP2J2, CYP3A4, HNF1A, HNF4A, HNF4G, NR0B2 and NR1H4 were expressed at significantly higher levels in females, STAT5A, NCOA1, NCOR2 and PPARD were expressed at significantly higher levels in males (Table 1).

STAT5A expression was marginally higher in males than females (Wilcoxon p val = 0.075; t -test p val = 0.0222) and demonstrated significant negative correlations with expression of most drug response genes that it was significantly correlated with. There were more significant associations (with STAT5A) observed in females than males and the genes correlated in expression in different genders were often different, although some genes were associated in both males and females. A number of CYPs showed associations with STAT5A in females (CYP2C8, CYP2C9, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A43) while, in males, more TFs were significantly correlated with STAT5A (CEBPA, FOXA2, HNF1A, HNF4A, HNF4G, NR0B2, NR1H4 NR1I2, PPARG). Strong associations with STAT5A were seen in females in several female-biased genes (CYP3A4) while weak to no associations were observed in male-biased genes eg (PPARD, PPARG). Additionally, significant positive correlations were observed with some co-repressors such as NCOA7, Ncor2 which are known female-biased to play a role in regulating drug response genes [11].

In contrast to STAT5A, STAT5B showed a trend for higher levels in females than males, although the difference was not significant. Also, STAT5B had positive correlations with expression of most drug response genes analyzed, and there were a larger number of significant associations in genes with gender-unbiased expression (in contrast to STAT5A).

More genes were correlated with STAT5B expression in males than females (some overlapped in both genders too).

Several genes with gender-unbiased expression (CYP1A1, CYP1A2, CYP2C9) showed strong association with STAT5B as also genes with gender biases in expression (eg. Female-biased genes such as CYP3A4 and male-biased genes such as NCOR2). STAT5B also demonstrated significant negative correlations with select corepressors (NCOA7) and genes (POR, PPARD).

Our findings show for the first time, the distinct and potentially opposing regulatory influences of STAT5A and 5B on cytochrome P450s and on transcription factors involved in their potential gene expression regulation. Despite the large similarities in their overall sequence and structures, there exist a number of differences in STAT5A and 5B that can potentially explain or be important for defining their distinctive roles in ADME gene expression regulation. These include structural differences in their respective C-terminal sequences and a shorter phosphotyrosyl tail segment in STAT5A [1]. The differences in C-terminus include the critical conserved tyro-sine, the phosphorylation of which is important for STAT5A and 5B activation, dimer formation and nuclear translocation [1]. Other differences include the size of first and last exons and differences in the CpG islands present in their respective promoters. Additionally, there are also two alternative two 5' exons and thus alternative promoters in STAT5B, which may be important for its tissue specific and/or differential regulation [12].

STAT5A has been reported to be expressed at much lower level in liver than STAT5B [13]. In our study too, we found STAT5A, on average to be expressed at four fold lower levels than STAT5B. Moreover STAT5A levels were higher in males than females; while STAT5B levels did not change significantly between genders, with the result that, compared to STAT5B, relatively less STAT5A was present in females than males. As STAT5A and 5B demonstrated opposite correlations with the CYPs and TFs analyzed, to explore the possibility that STAT5A and 5B ratios had any functional relevance to CYP gene expression regulation, we took the STAT5B/5A ratio and analyzed its correlations with CYP gene mRNA levels. STAT5B/5A ratios had higher correlations (more positive) with levels of most CYPs than did STAT5B alone in both males and females (data not shown).

Prior studies have shown a role for STAT5A in regulation of hepatic genes with female-biased gene expression profiles in female livers while STAT5B has been shown to play a major role in male livers [13].

We have shown for the first time that STAT5A and STAT5B have distinctive and potentially opposing roles in regulation of hepatic drug response genes as well as in contributing to gender-related differences in expression of hepatic CYPs and TFs. Future studies to elucidate the roles of STAT5A and STAT5B in regulation of drug response genes are underway in the lab.

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Abbreviations:

DME	drug metabolizing enzyme
CYP	cytochrome P450
ADME	absorption, disposition, metabolism and elimination
TF	transcription factor

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Fig. 1.

A Heat map showing correlations of STAT5A and STAT5B with CYPs and TFs in Males and Females. Positive correlations are shown in Red and negative correlations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Analyzed genes demonstrating significant gender differences in mRNA expression based on RNASeq data.

Gene	Females			Males			Gender Difference*	
	Median	Mean	SD	Median	Mean	SD	wilcox p	t-test p
STAT5A	1.68	1.867	0.36	1.922	2.088	0.60	0.074689	0.022224
CYP2C19	65.09	88.32	54.94	40.76	57.05	39.36	0.007986	0.011404
CYP2E1	3806	4528	1221.34	3081	3967	1758.52	0.007351	0.023507
CYP2J2	74.7	84.2	20.18	63.36	77.91	24.38	0.016304	0.01536
CYP3A4	1765	2281	955.37	1308	1726	714.61	0.011045	0.007375
HNF1A	5.274	6.325	1.58	4.54	5.446	1.58	0.030901	0.023665
HNF4A	43.36	49.33	10.58	36.93	41.55	11.80	0.005832	0.006009
HNF4G	2.884	3.483	0.85	2.221	2.838	0.75	0.000345	8.10E-05
NC0A1	4.202	4.607	0.66	3.942	4.37	0.62	0.086759	0.046411
NCOR2	2.336	2.586	0.54	2.709	3.002	0.66	0.001689	0.003545
NR0B2	54.46	67.7	35.18	37.17	44.94	21.39	0.007823	0.003064
NR1H4	37.17	42.75	8.56	32.72	39.52	10.81	0.039985	0.029267
PPARD	3.017	3.402	1.44	5.281	5.214	7.08	0.000405	0.044799

* Pink color indicates P value <0.05

Table 2A

Correlation of STAT5A mRNA expression to CYP and hepatic transcription factor mRNA expression as quantified using RNA sequencing (n = 100, F = 57, M = 43).

STAT5A vs	r (AID)	P* (AID)	r (F)	P* (F)	r (M)	P* (M)
CYP1A1	-0.0942	0.3514	-0.195	0.1458	0.029	0.8534
CYP1A2	-0.153	0.1277	-0.178	0.1864	-0.09	0.566
CYP1B1	0.0934	0.3551	-0.0000202	0.9999	0.164	0.2922
CYP2A6	-0.0417	0.6803	-0.16	0.2352	0.0811	0.6052
CYP2A7	-0.183	0.0692	-0.252	0.059	-0.0715	0.6486
CYP2A13	-0.14	0.1653	-0.195	0.1468	-0.0223	0.8872
CYP2B6	-0.0545	0.5902	-0.138	0.3075	0.0253	0.8719
CYP2C8	-0.155	0.1235	-0.276	0.0376	-0.0514	0.7433
CYP2C9	-0.241	0.0157	-0.279	0.0354	-0.187	0.2303
CYP2C18	-0.0801	0.4284	0.0547	0.686	-0.134	0.3932
CYP2C19	-0.114	0.2589	-0.171	0.2024	0.0412	0.7929
CYP2D6	-0.147	0.1436	-0.136	0.3139	-0.116	0.4578
CYP2E1	-0.33	0.0008	-0.279	0.0359	-0.291	0.058
CYP2J2	-0.298	0.0026	-0.296	0.0255	-0.222	0.1522
CYP3A4	-0.143	0.1559	-0.288	0.0295	0.0503	0.7488
CYP3A5	-0.198	0.0479	-0.269	0.0432	-0.208	0.1797
CYP3A7	-0.131	0.1951	-0.213	0.1115	-0.109	0.4884
CYP3A43	-0.111	0.27	-0.35	0.0076	0.136	0.3843
CYP4F2	-0.137	0.174	-0.208	0.1207	-0.0611	0.6972
AKR1D1	-0.251	0.0117	-0.283	0.033	-0.181	0.2463
CEBPA	-0.208	0.0383	-0.0025	0.9853	-0.319	0.0372
CEBPB	-0.155	0.1226	-0.255	0.0552	-0.00956	0.9515
FOXA2	-0.405	<0.0001	-0.406	0.0017	-0.44	0.0031
FOXO1	-0.213	0.0338	-0.304	0.0215	-0.125	0.4246
HNF1A	-0.261	0.0086	-0.0368	0.7856	-0.382	0.0115

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STAT5A vs	r (AII)	P* (AII)	r (F)	P* (F)	r (M)	P* (M)
HNF1B	0.116	0.2502	0.179	0.1829	0.0472	0.7638
HNF4A	-0.403	<0.0001	-0.25	0.061	-0.446	0.0027
HNF4G	-0.468	<0.0001	-0.361	0.0058	-0.481	0.0011
MED25	0.14	0.1659	0.208	0.1206	0.0538	0.7319
NCOA1	0.0992	0.3262	0.201	0.1329	0.101	0.5188
NCOA6	0.0268	0.7912	0.00637	0.9625	-0.01	0.9491
NCOA7	0.264	0.0079	0.207	0.1232	0.289	0.0604
NCOR1	-0.0211	0.8349	-0.0749	0.5796	0.027	0.8636
NCOR2	0.29	0.0035	0.318	0.016	0.164	0.2935
NR0B2	-0.2	0.0465	0.00979	0.9424	-0.369	0.0148
NR1H3	-0.0486	0.631	0.00892	0.9475	-0.104	0.5066
NR1H4	-0.297	0.0027	-0.175	0.1939	-0.309	0.0438
NR1I2	-0.418	<0.0001	-0.317	0.0164	-0.451	0.0024
NR1I3	-0.192	0.0557	-0.195	0.1454	-0.137	0.3821
NR3C1	-0.27	0.0066	-0.222	0.0972	-0.268	0.0828
PPARA	-0.284	0.0041	-0.0988	0.4645	-0.389	0.01
PPARD	0.0423	0.6761	0.137	0.3096	-0.156	0.3174
PPARG	0.128	0.2061	0.0543	0.6881	0.142	0.3645
PPARGC1A	-0.255	0.0104	-0.248	0.0631	-0.239	0.1232
POR	-0.158	0.1168	-0.121	0.3699	-0.205	0.1878
RXRA	-0.0577	0.5685	-0.0227	0.8668	-0.0879	0.575
STAT5B	0.0301	0.7665	-0.0287	0.8321	0.138	0.3786

* p val (two tailed),

r = Pearson correlation Coefficient, orange indicates P<0.05 and r is positive; green indicates P<0.05 and r is negative

Table 2B

Correlation of STAT5B mRNA expression to CYP and hepatic transcription factor mRNA expression as quantified using RNA sequencing (n = 100, F = 57, M = 43).

STAT5B vs	r (All)	P* (All)	r (F)	P* (F)	r (M)	P* (M)
CYP1A1	0.273	0.006	0.386	0.003	0.168	0.2819
CYP1A2	0.313	0.0015	0.445	0.0005	0.225	0.1469
CYP1B1	-0.0109	0.9141	-0.288	0.0296	0.183	0.2401
CYP2A6	0.255	0.0105	0.234	0.0801	0.249	0.1072
CYP2A7	0.0932	0.3564	0.0459	0.7346	0.0979	0.5322
CYP2A13	0.104	0.301	0.0542	0.689	0.105	0.5038
CYP2B6	0.106	0.2931	0.0339	0.8023	0.122	0.4354
CYP2C8	0.261	0.0087	0.219	0.1011	0.257	0.096
CYP2C9	0.303	0.0022	0.271	0.0413	0.299	0.0513
CYP2C18	0.179	0.075	0.13	0.3356	0.203	0.1925
CYP2C19	0.403	<0.0001	0.409	0.0016	0.359	0.0181
CYP2D6	0.126	0.2128	-0.128	0.3432	0.306	0.0462
CYP2E1	0.22	0.0275	0.153	0.2546	0.203	0.1919
CYP2J2	0.226	0.0239	0.23	0.0855	0.17	0.2762
CYP3A4	0.318	0.0012	0.27	0.0424	0.315	0.0396
CYP3A5	0.157	0.1186	0.133	0.3222	0.211	0.1751
CYP3A7	-0.132	0.1914	0.238	0.075	-0.385	0.0107
CYP3A43	0.148	0.1418	0.215	0.1083	0.0625	0.6905
CYP4F2	0.181	0.0716	0.0922	0.4952	0.191	0.221
AKR1D1	0.367	0.0002	0.413	0.0014	0.313	0.0407
CEBPA	0.161	0.1105	0.382	0.0034	-0.0569	0.7171
CEBPB	-0.0769	0.4471	-0.323	0.0142	0.0851	0.5874
FOXA2	-0.0169	0.8675	-0.0684	0.6133	0.0361	0.8184
FOXO1	0.248	0.0128	0.0225	0.8678	0.423	0.0047
HNF1A	0.245	0.014	0.238	0.0748	0.205	0.1883

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STAT5B vs	r (All)	P* (All)	r (F)	P* (F)	r (M)	P* (M)
HNFI1B	-0.0159	0.8753	0.0357	0.7921	-0.0553	0.7245
HNFI4A	0.312	0.0016	0.338	0.0101	0.243	0.116
HNFI4G	0.134	0.1848	0.355	0.0068	-0.133	0.3941
MED25	0.024	0.8123	0.0183	0.8926	0.0518	0.7416
NCOA1	-0.00168	0.9868	-0.0149	0.9127	-0.05	0.7504
NCOA6	-0.137	0.1756	-0.0807	0.5509	-0.147	0.3457
NCOA7	-0.32	0.0012	-0.451	0.0004	-0.202	0.1947
NCOR1	0.159	0.1148	0.0891	0.51	0.219	0.1589
NCOR2	0.0802	0.4275	0.292	0.0273	-0.00481	0.9756
NR0B2	0.115	0.2545	-0.0048	0.9717	0.186	0.2323
NR1H3	0.281	0.0046	0.185	0.1682	0.36	0.0177
NR1H4	0.208	0.0381	0.269	0.0434	0.126	0.421
NR1I2	0.296	0.0028	0.19	0.1578	0.334	0.0288
NR1I3	0.443	<0.0001	0.448	0.0005	0.424	0.0046
NR3C1	0.225	0.0245	0.399	0.0021	0.0853	0.5865
PPARA	0.515	<0.0001	0.667	<0.0001	0.365	0.0161
PPARD	-0.313	0.0015	-0.377	0.0038	-0.22	0.1569
PPAR6	-0.043	0.6713	-0.00419	0.9753	-0.0368	0.8149
PPARGC1A	0.152	0.1321	-0.0258	0.8488	0.236	0.1274
POR	-0.216	0.0312	-0.377	0.0039	-0.0809	0.606
RXRA	0.359	0.0002	0.39	0.0027	0.34	0.0255
STAT5A	0.0301	0.7665	-0.0287	0.8321	0.138	0.3786

* p val (two tailed);
r = Pearson correlation Coefficient; orange indicates P<0.05 and r is positive; green indicates P<0.05 and r is negative

Table 3A

Correlation of STAT5A mRNA expression to CYP and hepatic transcription factor mRNA expression as quantified using Taqman real time PCR (n = 64, F = 34, M = 30).

STAT5A vs	r (AID)	P* (AID)	r (F)	P* (F)	r (M)	P* (M)
AKR1D1	-0.29	0.0201	-0.197	0.263	-0.352	0.0566
CEBPA	-0.0258	0.8395	0.239	0.173	-0.211	0.2634
CYP2B6	-0.255	0.0419	-0.291	0.0953	-0.242	0.1977
CYP2C19	-0.276	0.0273	-0.374	0.0293	-0.133	0.4824
CYP2C9	-0.365	0.003	-0.315	0.0692	-0.466	0.0094
CYP3A4	-0.459	0.0001	-0.512	0.002	-0.384	0.0363
EHHADH	-0.348	0.0048	-0.178	0.314	-0.446	0.0136
FOXA2	-0.347	0.005	-0.27	0.123	-0.435	0.0164
HNF4A	-0.273	0.0289	-0.201	0.2545	-0.309	0.0963
HNF4G	-0.234	0.0629	-0.153	0.3866	-0.261	0.1637
MED25	-0.194	0.1239	-0.375	0.0287	-0.0558	0.7695
NC0A1	0.194	0.124	0.375	0.0287	0.0557	0.77
NC0A6	0.216	0.0864	0.0867	0.6258	0.255	0.1743
NCOR2	0.288	0.0211	0.212	0.2284	0.31	0.0957
NR1I2	-0.531	<0.0001	-0.511	0.002	-0.529	0.0026
NR1I3	-0.359	0.0036	-0.228	0.1944	-0.449	0.0128
NR3C1	0.0186	0.8842	0.0898	0.6136	-0.0559	0.7692
PPARGC1A	-0.431	0.0004	-0.452	0.0074	-0.441	0.0147
PPIA	0.0611	0.6317	0.0971	0.5849	-0.016	0.9332
RXRA	-0.179	0.1581	-0.0497	0.7803	-0.308	0.0975
SLC10A1	-0.207	0.1007	-0.117	0.5085	-0.213	0.2588
STAT5B	0.0962	0.4495	0.257	0.1419	0.00284	0.9881

* p val (two tailed),
r = Pearson correlation Coefficient, orange indicates P<0.05 and r is positive; green indicates P<0.05 and r is negative

Table 3B

Correlation of STAT5B mRNA expression to CYP and hepatic transcription factor mRNA expression as quantified using Taqman real time PCR (n = 64, F = 34, M = 30).

STAT5B vs	r (All)	P* (All)	r (F)	P* (F)	r (M)	P* (M)
AKR1D1	0.486	<0.0001	0.509	0.0021	0.452	0.012
CEBPA	0.255	0.0419	0.486	0.0036	0.162	0.3913
CYP2B6	0.132	0.2969	0.258	0.1408	-0.0581	0.7605
CYP2C19	0.358	0.0036	0.437	0.0097	0.362	0.0492
CYP2C9	0.454	0.0002	0.388	0.0232	0.495	0.0054
CYP3A4	0.433	0.0004	0.265	0.1293	0.586	0.0007
EHHADH	0.417	0.0006	0.368	0.0321	0.387	0.0348
FOXA2	0.168	0.1854	0.173	0.3282	0.126	0.5054
HNF4A	0.404	0.0009	0.42	0.0134	0.385	0.0354
HNF4G	0.345	0.0053	0.431	0.0108	0.231	0.2195
MED25	-0.0335	0.7928	0.161	0.3621	-0.199	0.291
NCOA1	0.00108	0.9932	-0.161	0.362	0.199	0.2907
NCOA6	-0.111	0.3837	0.151	0.3944	-0.258	0.1688
NCOR2	0.29	0.0201	0.503	0.0025	0.231	0.2192
NR1I2	0.222	0.0774	0.111	0.5304	0.43	0.0178
NR1I3	0.411	0.0007	0.449	0.0077	0.441	0.0146
NR3C1	0.36	0.0034	0.572	0.0004	0.255	0.1744
PPARGC1A	0.193	0.1272	-0.136	0.4435	0.418	0.0214
PPIA	0.114	0.3691	0.448	0.0079	-0.0876	0.6453
RXRA	0.513	<0.0001	0.623	<0.0001	0.437	0.0158
SLC10A1	0.567	<0.0001	0.543	0.0009	0.356	0.0534
STAT5A	0.068	0.5932	0.257	0.1419	0.00284	0.9881

p val (two tailed), # r = Pearson correlation Coefficient, orange indicates P<0.05 and r is positive; green indicates P* <0.05 and r is negative