




## RESEARCH ARTICLE

# Glibenclamide, ATP and metformin increases the expression of human bile salt export pump ABCB11 [version 1; peer review: 1 approved, 2 approved with reservations]

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## Abstract

**Background:** Bile salt export pump (BSEP/ABCB11) is important in the maintenance of the enterohepatic circulation of bile acids and drugs. Drugs such as rifampicin and glibenclamide inhibit BSEP. Progressive familial intrahepatic cholestasis type-2, a lethal pediatric disease, some forms of intrahepatic cholestasis of pregnancy, and drug-induced cholestasis are associated with BSEP dysfunction.

**Methods:** We started with a bioinformatic approach to identify the relationship between ABCB11 and other proteins, microRNAs, and drugs. A microarray data set of the liver samples from ABCB11 knockout mice was analyzed using GEO2R. Differentially expressed gene pathway enrichment analysis was conducted using ClueGo. A protein-protein interaction network was constructed using STRING application in Cytoscape. Networks were analyzed using Cytoscape. CyTargetLinker was used to screen the transcription factors, microRNAs and drugs. Predicted drugs were validated on human liver cell line, HepG2. BSEP expression was quantified by real-time PCR and western blotting.


**Results:** *ABCB11* knockout in mice was associated with a predominant upregulation and downregulation of genes associated with cellular component movement and sterol metabolism, respectively. We further identified the hub genes in the network. Genes related to immune activity, cell signaling, and fatty acid metabolism were dysregulated. We further identified drugs (glibenclamide and ATP) and a total of 14 microRNAs targeting the gene. Western blot and real-time PCR analysis confirmed the upregulation of BSEP on the treatment of HepG2 cells with glibenclamide, ATP, and metformin.

**Conclusions:** The differential expression of cell signaling genes and those related to immune activity in *ABCB11* KO animals may be secondary to cell injury. We have found glibenclamide, ATP, and

## Open Peer Review

Reviewer Status   

	Invited Reviewers		
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<b>version 1</b>			
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Any reports and responses or comments on the article can be found at the end of the article.

metformin upregulates BSEP. The mechanisms involved and the clinical relevance of these findings need to be investigated.

### Keywords

BSEP/ABCB11, ABCB11-KO, Insilico, upregulation, HepG2, glibenclamide, ATP, and metformin, Nuclear Receptors

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## Introduction

The bile salt export pump (BSEP), the major bile salt transporter in the liver canalicular membrane, is coded by *ABCB11* gene, and mutations in this gene cause progressive familial intrahepatic cholestasis type- 2 (PFIC-2)<sup>1,2</sup>. Besides PFIC-2, mutations or insufficiency of BSEP is associated with a variety of diseases such as drug-induced cholestasis, pregnancy induced cholestasis, cryptogenic cholestasis, cholangiocarcinoma and hepatocellular carcinoma, which are cancers of the liver<sup>3-7</sup>. Naturally, *ABCB11* expression is induced by bile salts and is mediated by FXR- RXR heterodimer<sup>8</sup>. Here in this pilot study we explored *in silico* the interactions/networks around *ABCB11*. We wanted to identify the genes, drugs, microRNAs which might influence the expression of *ABCB11*. Drugs which could upregulate *ABCB11* expression may be useful in *ABCB11* haploinsufficiency and inhibition of the pump could result in the accumulation of toxic bile salts inside hepatocytes. Modulation of *ABCB11* expression could be clinically beneficial in a variety of medical conditions.

## Methods

### Identification of differentially expressed genes

We analyzed the microarray data set of the liver samples from *ABCB11* knockout mice (GEO accession [GSE70179](#)) using GEO2R online tool from NCBI<sup>9</sup>. All differentially expressed genes (DEGs) were filtered with two criteria:  $-1 > \log_2 FC > 1$  and adj. p-value  $< 0.05$ .

### Pathway enrichment analysis

To identify DEGs which are significant, pathway enrichment analysis was conducted using the [ClueGo](#) v2.5.5 app from Cytoscape<sup>10</sup>. [ClueGo](#) constructed and compared networks of functionally related GO terms with kappa statistics, which was adjusted at  $> 0.4$  in this study.

### Identification of hub genes and subnetwork analysis

The protein-protein interaction (PPI) networks were built by the [Search Tool for the Retrieval of Interacting Genes](#) (STRING v11.0)<sup>11</sup> and [Cytoscape](#) v3.7.1 software. The [Molecular Complex Detection](#) (MCODE v1.6), app from Cytoscape was used to screen modules of the PPI network with degree cut-off = 2, node score cut-off = 0.2, k-core = 2, and maximum depth = 100. The hub genes were identified by the [CytoHubba](#) v0.1 app. The top 10 nodes were considered as notable hub genes and displayed.

### Identification of transcription factor and drug target

[CyTargetLinker](#) v4.1.0 from Cytoscape was used to identify the transcription factors (TFs) and microRNAs using ENCODE and Target-scan databases, respectively. We drew *Homo sapiens* TF-target interactions linkset from database (ENCODE)<sup>12</sup> and drug-target interactions linkset from the database (DrugBank)<sup>13</sup>. The networks were visualized and analyzed using [Cytoscape](#) v3.7.1 [Cytoscape](#) app [CyTargetlinker](#) version 4.1.0[6] was used to screen the transcription factors and microRNAs

## Cell culture

HepG2 cells were grown in high-glucose DMEM (Hi-Media Lab, Mumbai, Cat. # AL111-500ML) supplemented with 10% fetal bovine serum (CellClone, Genetix Biotech Asia, New Delhi, Cat.# CCS-500-SA-U), penicillin and streptomycin (Hi-Media, Mumbai Cat. # A018-5X100ML). When cells became 80% confluent, they were individually treated with glibenclamide (500 ng/mL)<sup>14</sup>, metformin (25 mg/L)<sup>15</sup> or ATP (1 mM) for 48 h. After 48 h cells were scraped out for total protein and RNA.

## Western blot analysis

Total proteins from HepG2 cells were prepared and run on 10% SDS-PAGE and transferred to a PVDF membrane using a transfer apparatus following the standard protocols (Bio-Rad). After incubation with 5% nonfat milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Tween 20) for 1 h the membrane was washed once with TBST and incubated overnight at 4°C with rabbit antibodies against human *ABCB11* (Affinity, Catalog #DF 9278) 1: 2000 dilution; mouse anti-human  $\beta$ -actin (Santa Cruz Cat.# SC4778), dilution 1:1000. The membrane was washed three times (TBST) and incubated with a 1:5000 dilution of horseradish peroxidase-conjugated anti-rabbit (Santa Cruz Cat# SC-2004)/anti-mouse antibodies (Cat.#SC-2005) for 2 h. Blots were washed with TBST four times and developed with the ECL system (Bio-Rad, US Cat.#170-5060) according to the manufacturer's protocol. The western blot images were acquired using iBright CL1000 (Invitrogen, Thermo Fisher Scientific).

## Real-time PCR

Total RNA was isolated using NucleoZOL (Takara Cat. No. 740404.200) following manufacturer's instruction. cDNA was prepared from (deoxyribonuclease treated) total RNA using RevertAid Reverse Transcriptase (Thermo Cat. No. EP0441) following the manufacturer's instructions. Real Time PCR was done with unique oligonucleotide primers targeting *ABCB11* and *GAPDH*, Ta=60°C, in triplicates and two repeats, using GoTaq® qPCR Master Mix (Promega Cat. No. A6001) following 'manufacturer's instructions on a Veriti Thermo Cycler from Applied Biosystems Waltham, Massachusetts, USA and data was acquired using the software associated with the same machine (ViiA7 V1.2) and relative quantification was calculated using the  $2^{-\Delta\Delta C_t}$  method. Oligonucleotide primer sequences are listed in [Table 3](#).

An earlier version of this article can be found on [biorxiv.org](#) (DOI: <https://doi.org/10.1101/2020.09.01.277434>).

## Results

### Expression of 560 genes changed significantly following *ABCB11*-KO in 1.5 m mice

Gene expression profile *ABCB11* knockdown dataset GSE 70179 from GEO datasets were analysed with GEO2R tool. Genes with  $> 2$ -fold change in expression value and  $< 0.05$  adjusted p-value was filtered. Identified differentially expressed

genes (DEG) from the GSE dataset were classified in two groups - upregulated (375 genes) and downregulated (185 genes) (*Extended data*, Supp.Table-1)<sup>36</sup>. Gene ontology analysis was performed for functional analysis of DEGs by using ClueGo app from Cytoscape. PPIs of DEGs were constructed using STRING database showed an upregulation of genes related to cellular transport (pink colored nodes), and these nodes were also shared by Toll-like receptor (TLR) signalling (*Figure 1*). Downregulated genes were involved in metabolic pathways (sterol, carbohydrate, alcohol, etc.) (*Extended data*, Supp. Table-2)<sup>36</sup>. We next identified top hub genes in PPI network using CytoHubba app from Cytoscape (*Table 1*). Immunologically important genes were among the top ranked upregulated hub genes (*Figure 2a*) downregulated group majorly represents cell signaling and fatty acid metabolism (*Figure 2b*). Epidermal growth factor receptor (*EGFR*) ranked first among the genes involved in signaling pathways. Kinases play a role in the transcription, activity, or intracellular localization of ABC transporters as do protein interactions<sup>16</sup>. Proteins interacting with ABCB11 are represented in *Figure 3* which includes nuclear receptors NR1H4 and NR0B2. Most proteins were associated with bile acid metabolism and transport.

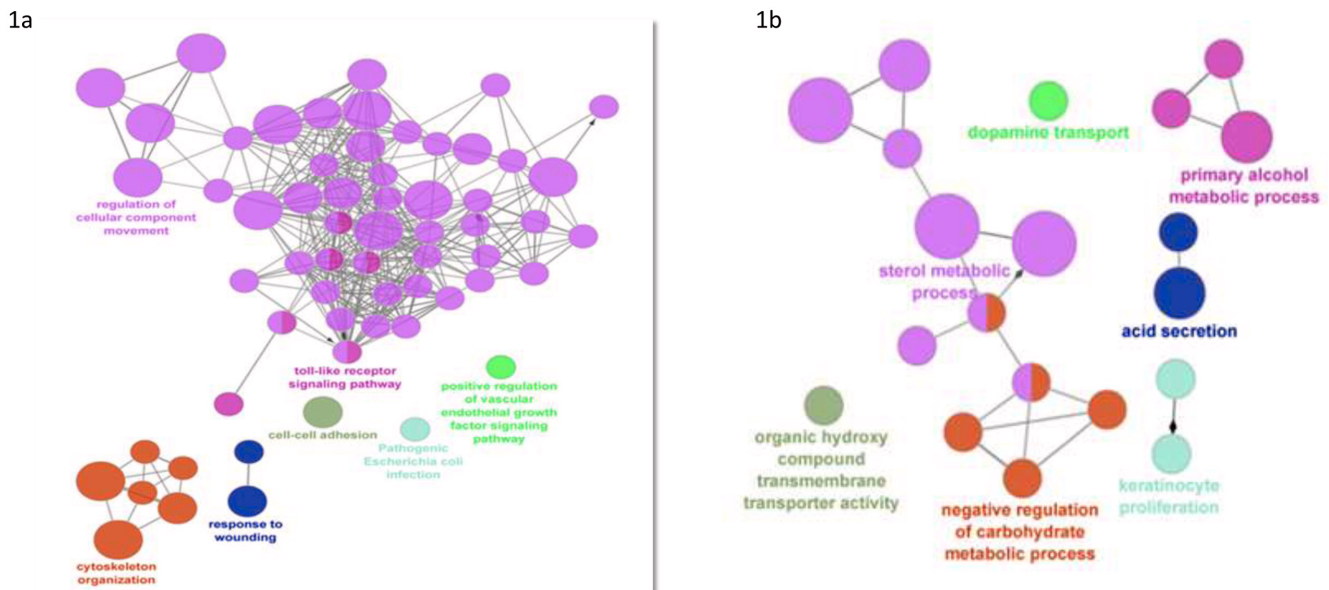
As described, sub-network analysis was performed using MCODE (*Figure 4*), and *CMPK2*, *ACTG1*, and *SSTR2* emerged

as seed nodes among upregulated genes (*Table 2*). Among downregulated gene groups, only one subnetwork was found to be significant which had three genes: *MIA3* (which codes a protein which is important in the transport of cargos that are too large to fit into COPII-coated vesicles such as collagen VII), *IGFBP4* (encoding a protein that binds to both insulin-like growth factors and modifies their functions) and *NOTUM* (encoding a carboxylesterase that acts as a key negative regulator of the Wnt signaling pathway by specifically mediating depalmitoylation of WNT proteins).

Using CyTargetLinker identified two drugs, glibenclamide, and ATP, directly targeting ABCB11. We subsequently looked for microRNAs [Target-scan database]<sup>17</sup> that were associated with ABCB11, and a total of 14 microRNAs were identified targeting the gene (*Figure 5*). Transcription factors and microRNAs targeting *ABCB11* and interacting partners are represented in *Figure 6*.

#### Glibenclamide ATP and Metformin upregulates ABCB11

We evaluated *in vitro*, the effect of three drugs, two of which were bioinformatically predicted (Glibenclamide, ATP) and one based on literature<sup>18</sup>. We found all the three compounds upregulating *ABCB11* expression based on qPCR, and this was



**Figure 1. Protein-protein interaction networks (PPIs) of DEGs were constructed using STRING database.** Gene ontology analysis was performed for functional analysis of DEGs by using ClueGo app from Cytoscape. This app allows simultaneous analysis of multiple annotation and ontology sources. Functionally grouped network is represented *Figure 1a* (upregulated genes) *1b* (downregulated genes). The node size represents enrichment significance and connections are based on kappa score (> 0.4). In upregulated gene group maximum number of nodes which are in pink color represent the cellular component movement. These nodes are shared by toll like receptor signaling pathway.

**Table 1. Genes with the greatest changes in expression.** We observed that the top ranked hub genes in PPI network which were upregulated were associated with immune activity while those downregulated are associated with cell signaling and fatty acid metabolism. *EGFR* came first in the ranking which is a critical receptor in several cell signaling pathways.

<b>(A) Upregulated genes</b>		
<b>Rank</b>	<b>Gene</b>	<b>UniportKB/Swiss-Prot Function</b>
1	<i>CXCL 10</i>	Pro-inflammatory cytokine
2	<i>IFIH1</i>	Cytoplasmic sensor of viral nucleic acids
3	<i>IFT1</i>	IFN-induced antiviral protein
4	<i>IFT2</i>	IFN-induced antiviral protein
5	<i>OASL</i>	Antiviral activity
6	<i>RSAD2</i>	Induces type 1 and type 2 interferon
7	<i>GBP2</i>	Hydrolyzes GTP TO GMP
8	<i>SAMD9L</i>	Growth factor signaling
9	<i>ISG20</i>	Interferon -induced antiviral activity
10	<i>CMPK2</i>	Participate in DUTP and dCTP synthesis in mitochondria
<b>(B) Downregulated genes</b>		
1	<i>EGFR</i>	Convert extracellular cues into appropriate cellular responses
2	<i>PPARA</i>	Ligand -activated transcription factor
3	<i>CXCL12</i>	Chemoattractant active on T-lymphocytes and monocytes
4	<i>ENPP1</i>	Nucleotide pyrophosphatase that genetrates diphosphate(ppi)
5	<i>DGAT2</i>	Triacylglycerol synthesis
6	<i>PPAP2B</i>	Lipid phosphatase activity
7	<i>LPIN1</i>	Fatty acid metabolism
8	<i>MGLL</i>	Converts mono-acylglycerides to free fatty acids and glycerol
9	<i>SYT1</i>	Calcium sensor
10	<i>MIA3</i>	Vesicle mediated transport

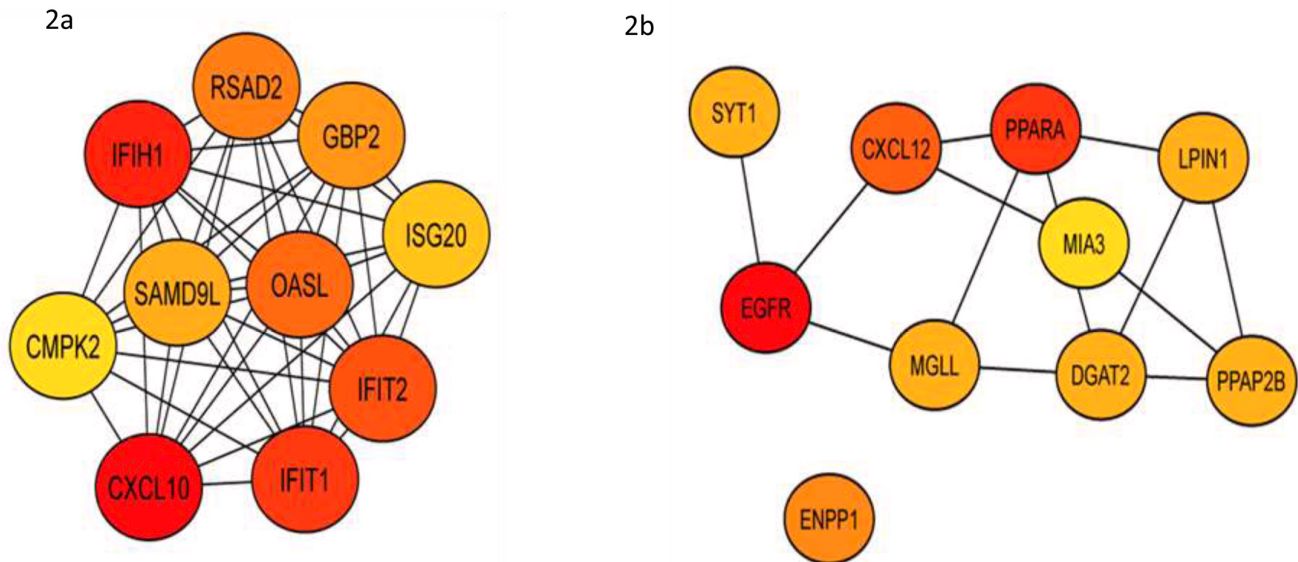
confirmed by western blot (Figure 7). Unannotated western blot images and raw qPCR Ct values are available as *Underlying data*<sup>36</sup>.

## Discussion

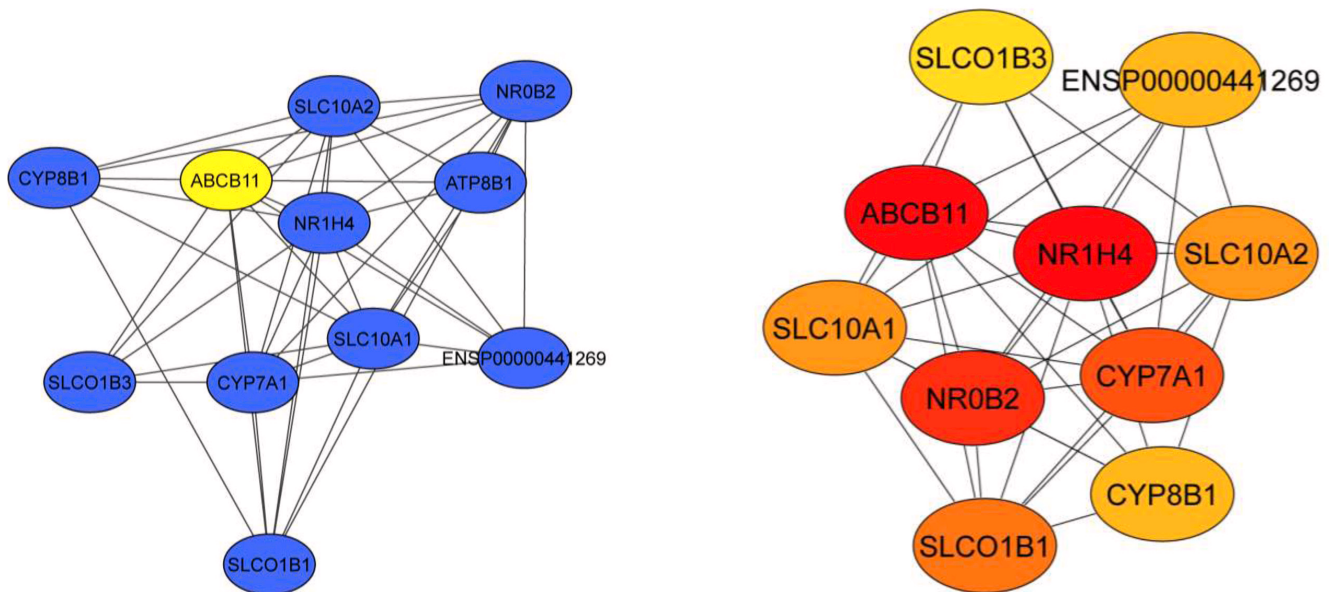
We identified several immunologically important genes being upregulated during *ABCB11* deficiency. The reason could be liver cell injury secondary to bile salt accumulation, which triggers the sterile immune response<sup>19,20</sup> and the downregulation of transport proteins and metabolically important genes could be because of decreased liver function following damage. A regenerative response follows cell injury, and a host of genes involved in regeneration are upregulated<sup>21–23</sup>; however, it appears that bile salts in the absence of BSEP hamper the regenerative response reflected by dysregulated collagen transporting protein MIA3 and NOTUM a protein involved in Wnt

signaling. It's also possible that EGFR is dysregulated via accumulating bile salts mediated by STAT3<sup>24</sup>. We have observed an upregulation of *ABCB11* in a liver cell line (HepG2) on treatment with glibenclamide, metformin, and ATP. This expression is upregulation may be a compensatory mechanism in the case of glibenclamide and metformin because these drugs are known to inhibit *ABCB11*<sup>25</sup>. Metformin is known to interfere with *ABCB11* function, mediated through AMPK-FXR crosstalk<sup>18</sup> involving metformin induced FXR phosphorylation. ATP acts through ATP receptors on hepatocytes<sup>26,27</sup>. ATP is known to cross the plasma membrane<sup>28</sup> and this can act via AMPK. However, ATP has a very short half-life<sup>29</sup>, and it may be converted to ADP, which can activate AMPK<sup>30</sup>. In a recent report, metformin was shown to suppress *ABCB11* expression, which is not in agreement with our observation, however, they performed their experiment on primary human hepatocytes,



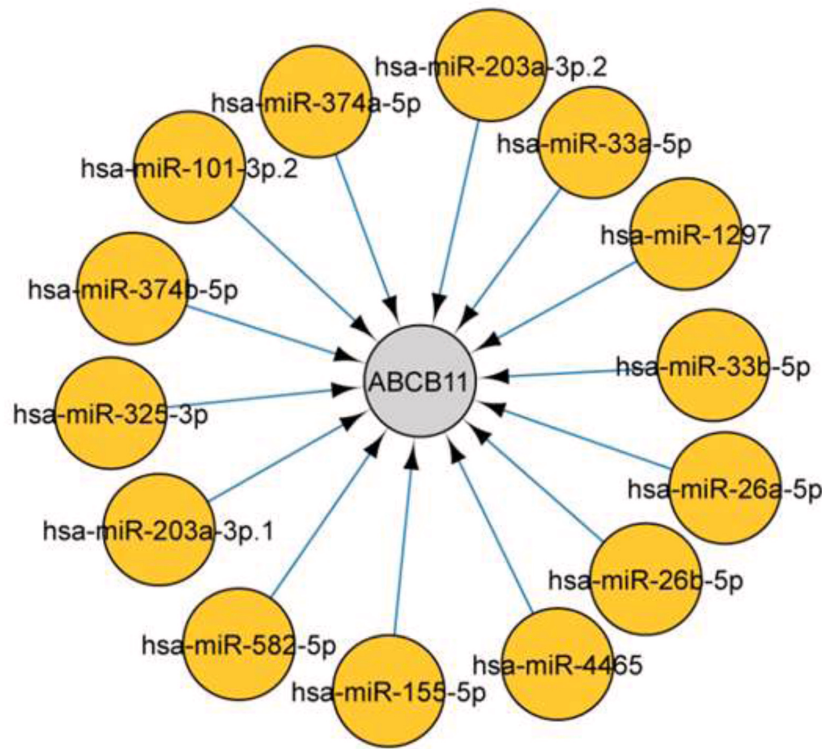


**Figure 2.** We identified top hub genes in PPI network of both upregulated and downregulated genes by using CytoHubba app from Cytoscape. We observed that the top ranked hub genes in the upregulated group were mainly related to immune activity (a). The top hub genes in downregulated group were associated with cell signaling and fatty acid metabolism. *EGFR* emerged as the top hub gene, a growth factor receptor which is crucial factor several cell signaling pathways (b).

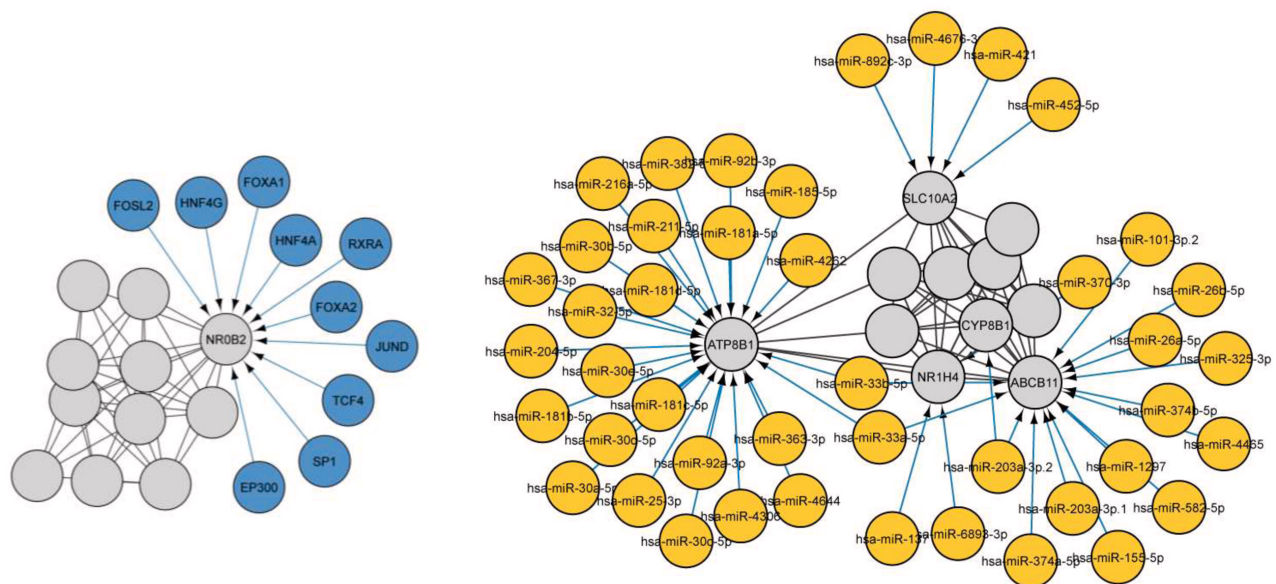


**Figure 3.** A protein-protein interaction network of the *ABCB11* gene was constructed using the STRING app in Cytoscape with confidence score > 0.4 for *Homo sapiens*. This network was constructed to analyze the relationship between *ABCB11* and other proteins. Cytohubba app was used to calculate centrality of each node by MCC method. Node colour (red to yellow) represents the significance of the centrality in the group. In this analysis, we counted 11 nodes and 42 edges. These proteins majorly involved in bile acid metabolism and transport. Most of these genes are participant of more than one pathway which was expected because these pathways intersect and coregulated. We also mapped the *NR0B2* protein, which is participate in sterol metabolism.



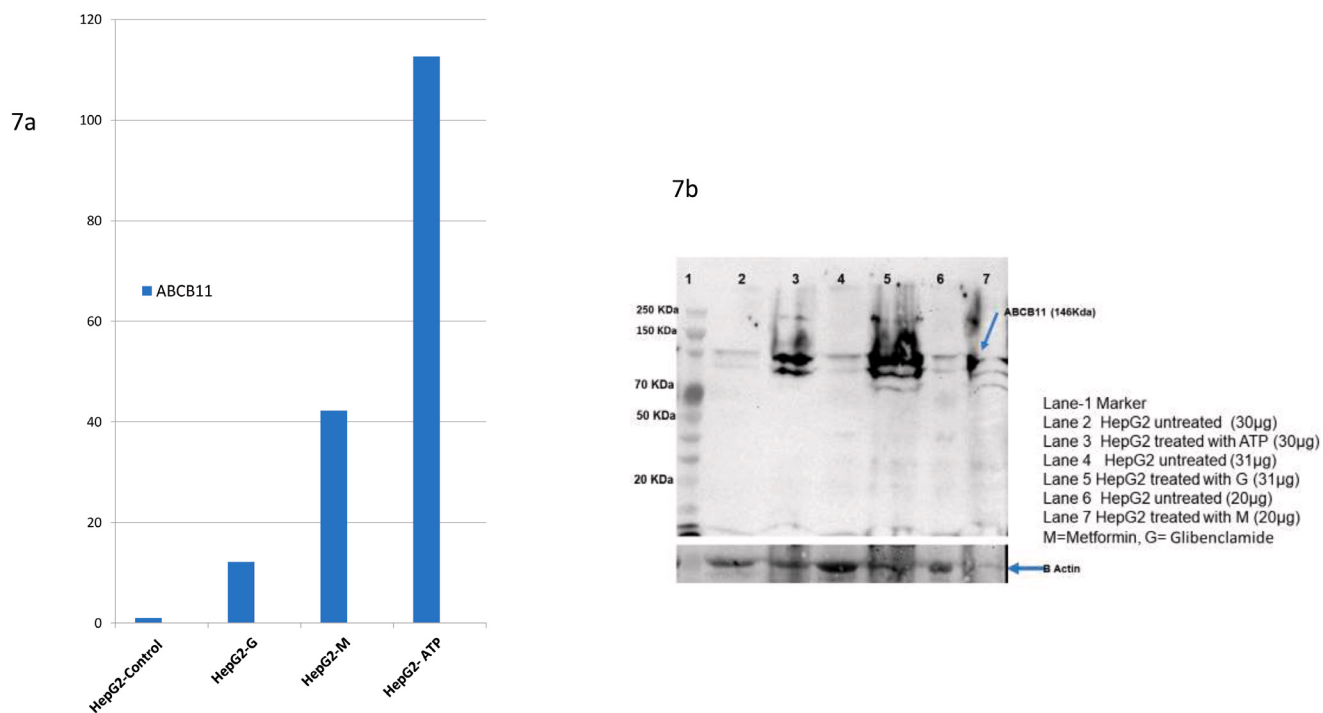


**Figure 5.** CyTargetlinker was used to screen microRNAs using the Target-scan database. We identified microRNAs that were associated with *ABCB11*. In total 14 microRNA identified targeting the gene.



**Figure 6.** Cytoscape app CyTargetlinker version 4.1.0 was used to screen the transcription factors and microRNAs using ENCODE and Target-scan databases, respectively. In the screen of transcription factors of *ABCB11* interaction network we observed 21 nodes and 52 edges. Among these transcription factors, FOXA have been suggested an important factor in bile duct development and lipid accumulation. HNF4A in the regulation dyslipidaemia and terminal liver failure and JUND in fibrosis development. Others can be investigated in future studies. We counted 55 nodes and 89 edges in the search of microRNA targeting the *ABCB11* network. Four genes (*ABCB11*, *ATP8B1*, *SLC10A2* and *NR1H4*) targeted by multiple microRNAs also some microRNAs such as has-miR-203a-3p.2 and has-miR-203a-3p.2 target more than one gene. By nature, a microRNA can regulate several pathways therefore it would be interesting to study in future the dysregulation of these microRNAs and interaction with Identified transcription factors.





**Figure 7. Three drugs, two of which were bioinformatically predicted (Glibenclamide, ATP) and one (Metformin) based on evidence from literature.** All the three compounds upregulated ABCB11 expression based on Real Time PCR data. This was further confirmed by Western Blot against anti-human ABCB11 antibody as the primary antibody. The upper image in 7b shows the PVDF membrane probed with antibody against ABCB11 and the lower image shows the same membrane stripped and probed with antibody against beta-actin (loading control).

and they have also treated their cells with dimethylsulfoxide (DMSO)<sup>31</sup>.

There are many reports stating the influence of DMSO on human gene expression. For example, Verheijen *et al.* “exposed 3D cardiac and hepatic microtissues to medium with or without 0.1% DMSO and analyzed the transcriptome, proteome and DNA methylation profiles”. They found that “in both tissue types, transcriptome analysis detected >2000 differentially expressed genes affecting similar biological processes, thereby indicating consistent cross-organ actions of DMSO”. In both tissue types, the transcriptome analysis detected over 2000 differentially expressed genes affecting similar biological processes<sup>32</sup>. Moskot *et al.* reported alterations of lysosomal ultrastructure upon DMSO treatment<sup>33</sup>. Alizadeh *et al.* reported that DMSO catalyzes hepatic differentiation of adipose tissue-derived mesenchymal stem cells<sup>34</sup>. It has been observed that “culturing pluripotent stem cells in DMSO activates the retinoblastoma protein, increases the proportion of cells in the early G1 phase of the cell cycle, and subsequently improves their competency for directed differentiation into multiple lineages in more than 25 stem cell lines”<sup>35</sup>. However, we are not sure whether the observed difference is attributed to DMSO.

In conclusion, we need more experiments to determine the mechanisms of action of these drugs on the upregulation of *ABCB11*. Many changes in gene expression following *ABCB11*

knockout could be secondary to stress, immune and regenerative responses following hepatocyte injury in mice liver.

## Data availability

### Underlying data

Harvard Dataverse: Real Time PCR for ABCB11 and few NRs. <https://doi.org/10.7910/DVN/AOYKY736>.

This project contains the following underlying data:

- 2020-09-12 092712-ViiA7-export.xls. (qPCR data following addition of ATP, metformin or glibenclamide.)
- ABCB11 WESTERN BLOT DRUG.tif. (Unannotated western blot image for ABCB11.)
- ABCB11\_WB\_Repeat\_Drugs.tif. (Unannotated repeat western blot image for ABCB11.)
- actin drug.tif. (Unannotated western blot image for β-actin.)
- Actin\_2020\_07\_11\_182456.jpg. (Unannotated western blot image, including β-actin loading control.)
- Actin\_2020\_07\_11\_182456.tif. (As above, but in tif format.)
- nisha\_qPCR\_DATA\_7142020.xls. (qPCR data for *ABCB11* and other indicated genes.)

- realtime and western blott (1).pptx. (Western blot and qPCR data pooled into a single file.)
- Repeat\_Actin\_drug\_WB.tif. (Unannotated repeat western blot image for  $\beta$ -actin.)

### Extended data

Harvard Dataverse: Real Time PCR for ABCB11 and few NRs. <https://doi.org/10.7910/DVN/AOYKY736>.

This project contains the following extended data:

- Supp-Table-1-Dysregulated genes GSE70179, GEO2R, NCBI. (Differentially expressed genes)
- Supp-Table-2-Gene ontology analysis, DAVID. (Gene ontology analysis of

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CCO 1.0 Public domain dedication).

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# Open Peer Review

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## Version 1

Reviewer Report 09 March 2021

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 **Amit Dash** 

Baylor College of Medicine, Houston, TX, USA

In the article by Vats et al., the authors used the preexisting microarray data set of the liver samples from ABCB11 knockout mice and used different bioinformatics methods to draw a relationship between ABCB11 and other proteins, microRNAs, and drugs. They validated the predicted drugs by real-time PCR and Western blot in HepG2 cells. The authors did an outstanding job in this pilot study which may be helpful for future researchers. Though their approach and methodologies are right but for better reproducibility and understanding, authors need to explain the following points

Major comments:

1. Why authors choose HepG2 cells and not other hepatoma cell lines? They need to describe it in the discussion.
2. Authors need to give justification about their chosen drug concentration. Is it the optimum concentration?
3. For more reproducibility of the data, it will be nice if authors mention the detailed protein extraction method and whether they used whole-cell extract or any fractions like nuclear and cytoplasmic extract.
4. In figure 7a, the authors need to mention what is on the Y-axis of the graph and need to put error bars and statistical significance. The method of statistical analysis needs to discuss in materials and methods. Also, it will be nice if authors make a graph for the western blot taking the averages and errors of three independent experiments and normalized with actin.
5. From their raw data, it is surprising that the endogenous control (GAPDH or 18S) is highly variable between controls and treatments. Did the authors start with an equal amount of RNA? How much RNA they used for cDNA. Also, it is confusing out of 18S and GAPDH, which they used for endogenous control. If they used 18S, they need to mention the primer



sequences.

6. Authors need to recheck the GAPDH primers as the melting point is more than one.

Minor comments:

1. Under introduction, on line "we explored *in silico* **the** interactions/networks around," "**the**" can be omitted.
2. Fig1 resolution needs to be increased.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Cancer biology, transcription, nuclear receptor, cell biology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 03 February 2021

<https://doi.org/10.5256/f1000research.29399.r76680>

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**Jayanta Roy-Chowdhury**

Department of Medicine, Montefiore Medical Center, Bronx, NY, USA

In this manuscript the authors have used the data on hepatic gene expression submitted to NCBI by Zhang Y, Neale G and Schuetz to determine the effect on the haploinsufficiency of *ABCB11* (BSEP) in mouse liver. The 375 upregulated genes included those involved in cellular transport and innate immunity (e.g. IFN signaling), whereas the 185 downregulated genes included signal transduction proteins, e.g. epidermal growth factor receptor and those involved with metabolic pathways. In addition, they have examined the effect of Glibenclamide, metformin and ATP on the expression of human *ABCB11* in the human cell line HepG2. All three chemicals were found to increase *ABCB11* expression.

Comments:

The information gene pathway analysis provides useful information as it has revealed "nodes" and "hubs" that mediate the up regulation and down regulation of the genes the expression of which is dysregulated in deficiency of *ABCB11* function. However, as the original data set had been derived from whole mouse liver, it comprises gene expression by hepatocytes as well as non-parenchymal cells. It is known that bile acid accumulation can affect gene expression in various cell types in addition to hepatocytes (such as lung cells). Perhaps, the authors can mention this in the Discussion section.

A second concern is that HepG2 cells are not the most appropriate cell line for modeling the induction of *ABCB11* in human hepatocytes, because, unlike some other human hepatoma cell lines, these cells lack the most abundant hepatocyte microRNA, miR-122. miR-122 is known to target various genes, thereby enhancing IFN signaling. On the other hand IFN downregulates the expression of miR-122. Thus, transcriptional induction of *ABCB11* in HepG2 cell may not parallel that in primary human hepatocytes. Therefore, the drug induction study should be validated in a different human hepatoma cell line, or at least, the authors should discuss this complexity in interpreting the results.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Hepatology; Genetic disorders; Cell transplantation; Gene therapy

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 25 January 2021

<https://doi.org/10.5256/f1000research.29399.r76410>

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### **Premkumar Kumpati**

Department of Biomedical Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

The authors have provided an interesting manuscript that described the role of Bile salt export pump (BSEP/ABCB1) and its relationship with other proteins, microRNAs and Drugs. Various bioinformatics tools were used to explore and carried out experiments to validate and correlate their findings.

Overall, I believe that this manuscript is well written and will provide some useful information for the scientific community. Having said that there are some concerns I have that the authors should address before it is ready/accept for indexing.

#### **Minor Comments to the Author**

1. The author can provide more information on biological mechanism of ABCB11.
2. What is the rationale for selecting concentration of drugs used glibenclamide (500 ng/mL), metformin (25 mg/L) or ATP (1 mM).
3. What is the basis for selecting Glibenclamide & ATP out of the total hits identified using bioinformatic analysis.
4. Providing Uniport accession number of each gene in table 1 could be more informative, authors may consider doing it.
5. Image quality for Fig.1 & 4 may be increased.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Genomics, Genetics, Nanotherapeutics, Cancer biology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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