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Research Paper
Assessing Alcohol Genes Targets in Mouse Liver

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ABSTRACT

Background: Alcohol is a risk factor for liver diseases. There is a correlation between alcohol consumption and fatty liver disease. Experiences have shown gene expression alteration in the liver following alcohol consumption. Since the molecular mechanism of connection between alcohol consumption and fatty liver disease needs a clear perspective, this study aims to explore the significant genes that are targeted by alcohol in the liver of mice.

Methods: Gene expression profiles of mice livers fed with alcohol were retrieved from the Gene Expression Omnibus (GEO) database and compared with the control samples. Data are pre-evaluated with the GEO2R program, and the significant differentially expressed genes (DEGs) are analyzed via gene expression evaluations and regulatory network assessment.

Results: Among the 25619 dysregulated genes, 78 significant DEGs were pointed out. Gene expression analysis showed that most extremely dysregulated genes are up-regulated and belong to the cytochrome *P450* genes family. Finally, cytochrome *P450* and glutathione S-transferase genes family, as well as hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 4, were introduced as the critical targeted genes.

Conclusion: In conclusion, detoxification of xenobiotics, cellular metabolism and homeostasis, the pathogenesis of some liver diseases, synthesis of several prostaglandins and steroid hormones, and inflammation fibrosis in the liver are possibly associated with alcohol consumption.

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Introduction

Alcohol is known as a risk factor for liver diseases in humans. Amount and duration of alcohol consumption are pointed out as two critical factors that impact the progression of alcoholic liver disease (ALD) [1]. Nonalcoholic fatty liver disease (NAFLD) and ALD are the leading causes of liver-related health burdens. Investigations indicate that NAFLD is possibly the key reason for mild liver disease, and ALD is the primary driver of liver-related mortality globally [2]. Alcohol-associated liver disease is highlighted as a significant risk factor for chronic liver disease. It is reported that patatin-like phospholipase domain-containing 3 (PNPLA3) and hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) are a risk factor for alcohol-associated cirrhosis in the liver of alcohol consumers. More investigation revealed that products of PNPLA3 and HSD17B13 are involved in lipid droplets in alcohol-associated cirrhosis [3, 4].

Exploring molecular mechanisms of diseases is closely tied to applied diagnostic and therapeutic methods to prevent and control diseases [5, 6]. The molecular mechanism of liver diseases has provided critical information about progression and possible correlations between liver diseases such as NAFLD, liver fibrosis, steatohepatitis, and hepatocellular carcinoma [7, 8]. Gene expression analysis is a noteworthy approach to determining the molecular mechanism of diseases [9]. Ramos-Tovar et al. reported a document about molecular mechanisms that present connections between fibrosis, inflammation, and oxidative stress in the liver [10]. Arjmand et al. published an article about the role of ALB, INS, GAPDH, CAT, IL6, and TNF as critical proteins associated with liver ischemia/reperfusion injury [11].

Evidence indicates that bioinformatics is a suitable method to explore the molecular mechanism of diseases. Wu et al. introduced *MYC*, *TGFB3*, *ADAMTS1*, *THBS1*, *RASD1*, *PCDH20*, *MAMDC4*, *CYP7A1*, *GINS2*, and *PDLIM3* as related hub genes for "NAFLD activity score" and steatosis via a bioinformatics analysis [12, 13]. Sookoian and Pirola compared nonalcoholic and alcoholic fatty liver diseases via a system biology approach. They elucidated common pathogenic mechanisms between both diseases [14]. Gene Expression Omnibus (GEO), the largest and most complete openly available resource for gene expression data, is used widely to analyze gene expression changes associated with diseases [15]. In the present study, gene expression profiles of mice liver that were treated with alcohol are extracted from the GEO database and analyzed via bioinformatics tools to find the prominent genes that are involved in liver damage following alcohol consumption.

Materials and Methods

Data collection

To find the effect of alcohol on liver function, gene expression profiles of livers of 4 male C57BL/6 mice (Leiber-Decarli ethanol diet) versus 4 controls (Lieber-DeCarli liquid control diet) were extracted from GSE40334 of GEO database [16]. The Lieber-DeCarli ethanol diet induces fatty liver in mice. The control mice were fed a Lieber-DeCarli liquid control diet (n=4). The control mice were pair-fed to the ethanol mice. After 4 weeks, a portion of the liver was used for RNA extraction.

Pre-evaluation analysis

The gene expression profiles of the alcohol group were compared with the control group via the GEO2R program to find the significant differentially expressed genes (DEGs) in response to alcohol consumption. A uniform manifold approximation and projection (UMAP) plot was applied to explore differences between the treated samples' and controls' gene expression profiles. The number of dysregulated genes and the significant DEGs were visualized via a Venn diagram.

Gene expression analysis

The identified DEGs were grouped based on the sturgess equation to find the distribution of DEGs considering amounts of |logFC| value. The extremely dysregulated DEGs were identified and discussed based on the |logFC| value distribution.

Action map analysis

Binding and regulatory relationships, including inhibition, expression, and catalysis actions between the significant DEGs, were identified via CluePedia application of Cytoscape software, version 3.7.2.

Statistical analysis

The significant DEGs were identified based on adjusted $P<0.05$. The significant DEGs were grouped based on the Sturgess equation into 7 groups.

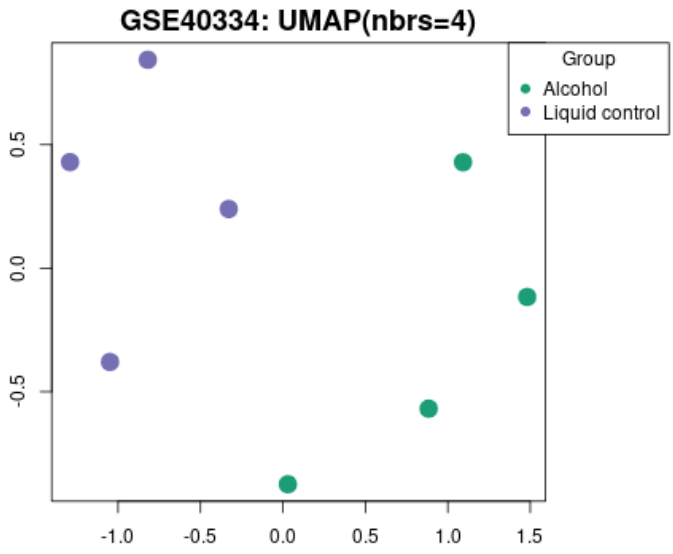

Figure 1. Uniform manifold approximation and projection presentation for analysis of gene expression comparison between treated samples with alcohol and controls

Results

The UMAP plot indicates that gene expression profiles of the livers of mice treated with alcohol are different from controls (Figure 1). The samples are separated. As shown in Figure 2, 25619 genes are dysregulated by alcohol in the livers of mice. About 78 DEGs are pointed out among the dysregulated genes. Since two DEGs were not characterized, 76 DEGs were considered for more analysis. Based on the Sturgess equation, the query DEGs were clustered in 7 groups (Figure 3). As depicted in Figure 3, amounts of |logFC| are less than 2 for 47 *DEGs*. Therefore, *Cyp2b10, Cyp2b13, Gsta1, Cyp2b9, Cyp2b23, Gstm3, Gsta2, Cyp26a1, Cdkn1a, Cyp2a5, Cbr3, Ifi27l2b, Lpin1, Ppl, Adam11, Ttc39a, Cbr3, Cyp3a11*, *Cyp2a5*, *Raet1b*, *Raet1a*, *Gstm1*, *Rab30*, *Paqr9*, *Hsd3b4*, *G0s2*, *Tsc22d1*, *Sqle*, and *Cyp7a1* were identified as the extremely dysregulated DEGs. Figure 4 shows logFC for the extremely dysregulated DEGs.

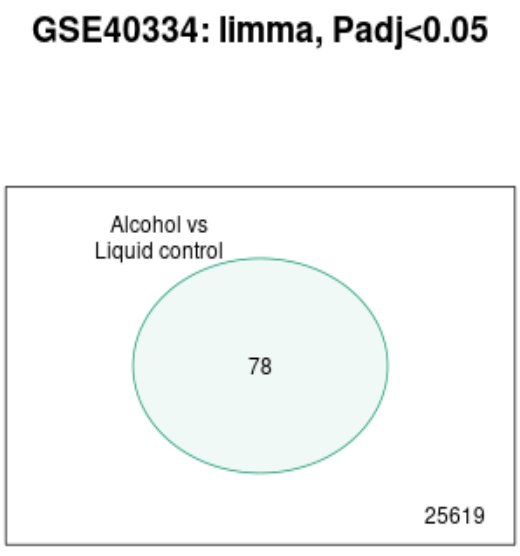

Figure 2. Venn diagram presentation for analyzing gene expression comparison between treated samples with alcohol and controls

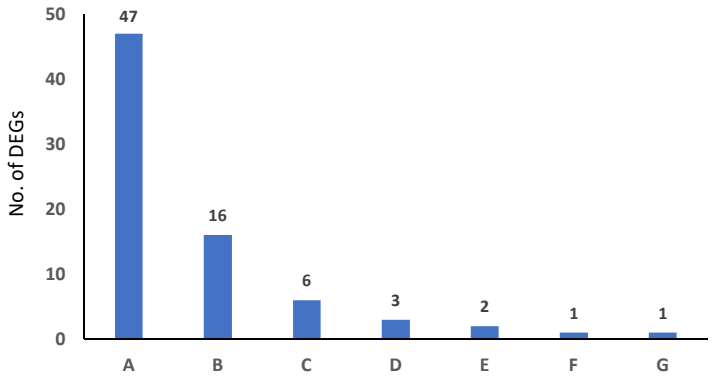

Figure 3. Grouping of significant DEGs based on amounts of |logFC|

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Notes: Amounts of |logFC| for A-G (x_i) are as follows: $1 \leq x_i < 2$, $2 \leq x_i < 3$, $3 \leq x_i < 4$, $4 \leq x_i < 5$, $5 \leq x_i < 6$, $6 \leq x_i < 7$, and $7 \leq x_i < 8$, respectively.

Figure 5 displays binding, activation, inhibition, expression, and catalysis relationships between significant DEGs. Among 76 significant DEGs, including 44 isolated individuals and 23 nodes included in 3 sub-networks, were recognized by the CluePedia application of Cytoscape software. The constructed sub-networks are presented in Figure 5. As it is depicted in Figures 4 and 5, *Cyp2b10*, *Cyp2b13*, *Gsta1*, *Cyp2b9*, *Cyp2b23*, *Gstm3*, *Gsta2*, *Cyp26a1*, *Cyp2a5*, *Cyp3a11*, *Gstm1*, *Hsd3b4*, and *Cyp7a1* are common DEGs between the main connected component of action map and the extremely dysregulated genes.

Discussion

Alcohol induces fatty liver disease and gene expression change in the mouse liver [17]. As shown in Figure 1, gene expression profiles of mouse liver in the presence of alcohol are different from controls. Seventy-eight significant DEGs were pointed out as the targeted genes by alcohol in the liver of mice (Figure 2). Up-regulation of cyclooxygenase-2 in the liver of rats in response to consumption of alcohol and induced up-regulation of hepatic diacylglycerol acyltransferase 2 following chronic alcohol feeding are reported by researchers [18, 19].

As shown in Figure 3, gene expression analysis revealed that 29 DEGs were expressed extremely. *Cyp2b10* and *Cyp7a1* are the most up- and down-regulated genes, respectively (Figure 4). Action map analysis introduced

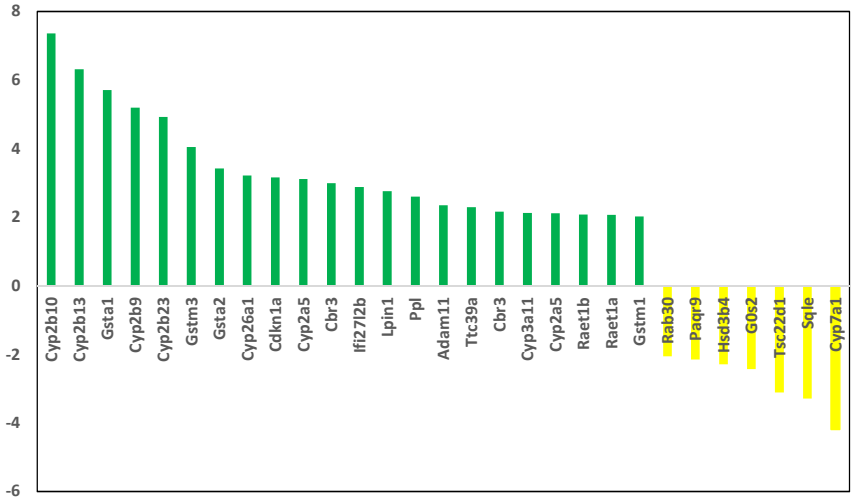

Figure 4. Amounts of logFC for members of B-G groups of DEGs

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Notes: Green and yellow colors refer to up- and down-regulation.

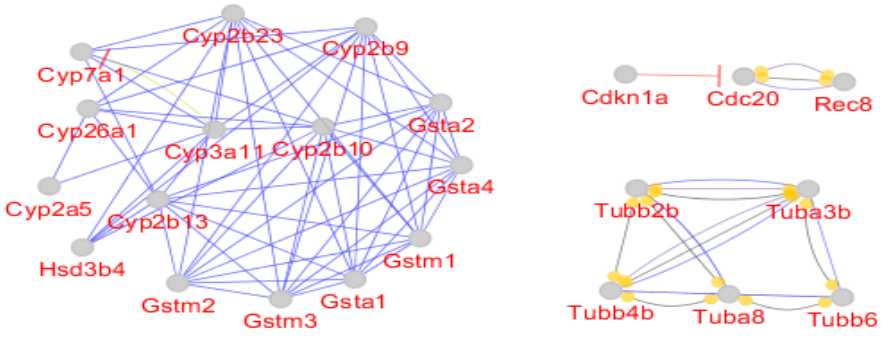

Figure 5. Action map for the significant DEGss

Notes: Forty-four isolated genes are not shown. Blue, red, yellow, and purple refer to binding, inhibition, expression, and catalysis actions.

15 significant DEGs as the critical elements of the action map main component (the largest sub-network in Figure 5). Merging results of gene expression analysis and action map evaluation indicate that *Cyp2b10*, *Cyp2b13*, *Gsta1, Cyp2b9, Cyp2b23, Gstm3, Gsta2, Cyp26a1, Cyp2a5*, *Cyp3a11*, *Gstm1*, *Hsd3b4*, and *Cyp7a1* are the critical targeted genes by alcohol in the liver of mouse.

Cytochrome *P450* genes *(CYP)* is a superfamily of genes known from the Eukarya, Archaea, and Bacteria [20]. *Cyp2b10*, *Cyp2b13*, *Cyp2b9*, *Cyp2b23*, *Cyp26a1*, *Cyp2a5*, *Cyp3a11*, and *Cyp7a1* (highlighted as members of the final results of analyses) belong to the *CYP* gene family. Like the cytochrome *P450* genes family, glutathione S-transferase (GST) is a large supergene family of enzymes involved in the detoxication of electrophiles via glutathione conjugation [21]. As discussed, *Gsta1*, *Gstm3*, *Gsta2*, and *Gstm1* are introduced as members of final critical genes that fit *GST* family genes. Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 4 (Hsd3b4) are the last essential DEG targeted by alcohol in the liver of mice.

Based on the literature, CYP enzymes are membrane-bound hemoproteins. These enzymes' pivotal role in detoxifying xenobiotics, cellular metabolism, and homeostasis is investigated and confirmed. Several documents have discussed the involvement of cytochrome P-450 proteins in the metabolism of drugs, chemicals, and endogenous substrates. As reported, the hepatic CYPs contribute to the pathogenesis of some liver diseases [22, 23]. Fisher et al. showed significant alterations in hepatic P450 activity through progressive steps of NAFLD [24]. There is an interesting correlation between gene expression analysis and the results of action map evaluation. Based on gene expression analysis, all critical members of the *CYP* family except *Cyp7a1*, which is down-regulated highly, have been up-regulated in response to alcohol consumption. *Cyp7a1* is down-regulated by *Cyp3a11* in action map evaluation. Based on published documents about the mechanism of alcohol-induced liver injury, many hepatic toxic effects of ethanol consumption are associated with alcohol metabolism in the liver. They are correlated with cytochrome P450 2E1 level [25].

Gsta1, *Gstm3*, *Gsta2*, and *Gstm1* are the members of the glutathione S-transferase family that appear as critical genes in response to the presence of alcohol in the liver of mice. There is evidence that GSTs play a role in the catalysis of glutathione-dependent isomerization reactions. These reactions are vital for the synthesis of several prostaglandins and steroid hormones. The involvement of GSTs in response to drugs and environmental stressors has been investigated and confirmed [26, 27]. Investigations indicate a close correlation between glutathione content and the pathogenesis of liver diseases. Based on published documents on ALD, NAFLD, chronic cholestatic injury, hepatocellular carcinoma, ischemia/reperfusion damage, hepatitis B virus, and hepatitis C virus are liver diseases that are associated with liver glutathione content [28].

Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 4 (Hsd3b4), as a critical enzyme in steroid hormone metabolism, is the third critical gene that is down-regulated in mouse liver in response to consumption of alcohol. Finally, alcohol affects mainly superfamily genes such as *CYP* and *GST* to induce alcoholic liver damage.

Conclusion

In conclusion, alcohol targets several members of the cytochrome *P450* genes family, glutathione S-transferase family, and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 4 in the liver of mice. Detoxification of xenobiotics, cellular metabolism and homeostasis, metabolism of drugs, chemicals and endogenous substrates, the pathogenesis of some liver diseases, synthesis of several prostaglandins and steroid hormones, the pathogenesis of liver diseases, ALD, NAFLD, chronic cholestatic injury, hepatocellular carcinoma, ischemia/reperfusion damage, hepatitis B virus, and hepatitis C virus are liver diseases, and inflammation fibrosis in the liver are possibly associated function with alcohol consumption.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.RETECH.REC.1402.868).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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