

Gene Expression Profile Analysis of Type 2 Diabetic Mouse Liver

Fang Zhang, Xiang Xu, Yi Zhang, Ben Zhou, Zhishui He, Qiwei Zhai*

Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Abstract

Liver plays a key role in glucose metabolism and homeostasis, and impaired hepatic glucose metabolism contributes to the development of type 2 diabetes. However, the precise gene expression profile of diabetic liver and its association with diabetes and related diseases are yet to be further elucidated. In this study, we detected the gene expression profile by high-throughput sequencing in 9-week-old normal and type 2 diabetic db/db mouse liver. Totally 12132 genes were detected, and 2627 genes were significantly changed in diabetic mouse liver. Biological process analysis showed that the upregulated genes in diabetic mouse liver were mainly enriched in metabolic processes. Surprisingly, the downregulated genes in diabetic mouse liver were mainly enriched in immune-related processes, although all the altered genes were still mainly enriched in metabolic processes. Similarly, KEGG pathway analysis showed that metabolic pathways were the major pathways altered in diabetic mouse liver, and downregulated genes were enriched in immune and cancer pathways. Analysis of the key enzyme genes in fatty acid and glucose metabolism showed that some key enzyme genes were significantly increased and none of the detected key enzyme genes were decreased. In addition, FunDo analysis showed that liver cancer and hepatitis were most likely to be associated with diabetes. Taken together, this study provides the digital gene expression profile of diabetic mouse liver, and demonstrates the main diabetes-associated hepatic biological processes, pathways, key enzyme genes in fatty acid and glucose metabolism and potential hepatic diseases.

Citation: Zhang F, Xu X, Zhang Y, Zhou B, He Z, et al. (2013) Gene Expression Profile Analysis of Type 2 Diabetic Mouse Liver. PLoS ONE 8(3): e57766. doi:10.1371/journal.pone.0057766

Editor: Ioannis P Androulakis, Rutgers University, United States of America

Received: November 8, 2012; **Accepted:** January 24, 2013; **Published:** March 1, 2013

Copyright: © 2013 Zhang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by grants from National Natural Science Foundation of China (30825009, 30970619, 31030022, 31200591 and 81021002), National Basic Research Program of China (973 Program, 2009CB918403), Program of Shanghai Subject Chief Scientist (11XD1405800), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-R-09), Postdoctor Research Program of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (2011KIP511), and SA-SIBS Scholarship Program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: qwzhai@sibs.ac.cn

Introduction

Type 2 diabetes has become epidemic in both developed countries and developing countries with a rapid change of lifestyle [1,2]. The predominant features of type 2 diabetes include increased fasting blood glucose levels, as well as disturbed peripheral glucose utilization. The prevalence of type 2 diabetes and the limitations of currently available preventative and therapeutic options highlight the need for a more complete understanding of the pathogenesis of type 2 diabetes [3].

Liver has a key role in glucose metabolism and homeostasis. Hepatic glucose metabolism mainly includes glucose transport, glycolysis, gluconeogenesis, glycogen synthesis and glycogenolysis [4], and impaired hepatic glucose metabolism will lead to the development of type 2 diabetes [4,5]. Many key enzymes involved in hepatic glucose metabolism, such as glycogen phosphorylase (GP, *Pylg*) the rate-limiting enzymes of glycogenolysis [6], fructose-1,6-bisphosphatase (FBPase, *Fbp1*) and glucose 6-phosphatase (G6pase, *G6pc*) the major control points in the pathway of gluconeogenesis [7,8], and glucokinase (GK/HK, *Gck*) the key enzyme of glycolysis [9], are the potential targets for diabetes. Meanwhile, hepatic transcriptional factors, such as Ppar α and Hnf4 α , are well-known to regulate glucose homeostasis, and involved in the development of hyperglycemia [10,11]. Impaired

hepatic fatty acid metabolism, including fatty acid oxidation, synthesis and storage, is also involved in the development of type 2 diabetes [12,13,14]. However, an overview of the hepatic glucose and fatty acid metabolism in diabetic subjects needs to be further investigated.

Microarray technology has been applied to study the genome-wide gene expression levels in type 1 diabetic tissues, such as rat muscle and mouse liver [15,16]. Microarray technology has also been used in type 2 diabetic studies to reveal the gene expression profiles in insulin-sensitive tissues from pre-diabetic and diabetic Zucker and Goto-kakizaki rats [17,18]. Similarly, mouse hepatic gene expression profiles by microarray analysis in diet-induced obesity and diabetes reveal a transcriptional adaptation to long-term high-fat diet, and provided potential molecular mechanisms underlying the development and maintenance of obesity and diabetes [19]. Microarray experiments using type 2 diabetic mouse model also suggest a role of hepatic lipogenic capacity in diabetes susceptibility [20]. Recently, high-throughput sequencing, as an attractive alternative to microarrays for transcriptome profiling, shows major advances in robustness, resolution and reproducibility based on its relatively unbiased and direct digital readout with sequencing by synthesis method [21,22,23]. In terms of both profiling coverage and quantitative accuracy of high-throughput sequencing, the application of this new technology in transcrip-

tome profiling of diabetic samples is important to further understand the underlying mechanisms.

In this study, we detected gene expression profile in normal and type 2 diabetic db/db mouse liver by high-throughput sequencing, and showed the main diabetes-associated hepatic biological processes, pathways, key enzyme genes in fatty acid and glucose metabolism and potential hepatic diseases.

Results

High-throughput Sequencing and the Top Abundance Change and Fold Change Genes in Normal and Diabetic Mouse Liver

To survey the gene expression profile in diabetic mouse liver, high-throughput sequencing was applied in this study. Finally, we obtained over 10 and 13 million reads of high quality clean tags from 9-week-old normal and type 2 diabetic db/db mice respectively (Figure 1A). In these high quality clean tags, averagely about 91.4%, 72.9% and 41.4% reads can be mapped to annotated mouse genome, genes, and unique genes respectively (Figure 1B). Totally 12132 unique genes were detected, and 10854 and 11543 unique genes were detected and quantified from normal and diabetic samples respectively, which shared 10266 genes in common (Figure 1C). Based on the high-throughput sequencing data, the top 15 abundance change genes upregulated and downregulated respectively in diabetic mouse liver were shown in Figure 1D. *Apoa1* encoding Apolipoprotein A-I, the major protein component of high density lipoprotein and playing an important role in lipid metabolism, *Mup3* (major urinary protein 3) and *Serpina3k* (serine (or cysteine) peptidase inhibitor, clade A, member 3K) were the top 3 abundance change genes downregulated in diabetic mouse liver (Figure 1D). *Sepp1* (selenoprotein P), *Thrsp* (thyroid hormone responsive SPOT14 homolog) and *Gsta3* (glutathione S-transferase, alpha 3) were the top 3 abundance change genes upregulated (Figure 1D). In addition, we showed the top 15 fold changes of genes upregulated and downregulated respectively in diabetic mouse liver in Figure 1E. Interestingly, *Cyp2b9*, *Cyp2b13*, *Cyp2b22*, *Cyp2b10*, *Cyp4a14* and *Cyp17a1* in the superfamily of Cytochrome P450, which catalyzes the oxidation of organic substances, were in the top 15 fold change genes upregulated in diabetic mouse liver (Figure 1E). *Vdac2* (voltage-dependent anion channel 2), *Hsd3b5* (hydroxy-delta-5-steroid dehydrogenase) and *Sco1a1* (solute carrier organic anion transporter family, member 1a1) were the top 3 fold changes of downregulated genes (Figure 1E).

Differentially Expressed Genes and Clustering

Among the 10854 and 11543 unique genes detected from normal and diabetic mouse liver samples, the number of differentially expressed genes were quantified and shown in Figure S1A. To avoid the possible noise signal from high-throughput sequencing, the genes with an average transcripts per million (TPM) less than 1 were excluded. The remained 8551 genes and their abundance were shown in Table S1, and were used to calculate the fold changes and false discovery rate (FDR). In this study, the absolute fold change no less than 1.5 and FDR less than 0.001 were used to define the differentially expressed genes. According to this definition, totally 2627 genes were differentially expressed between normal and diabetic mouse liver samples (Figure S1A). To gain insights into the 2627 differentially expressed genes in diabetic mouse liver, we divided them into 2 clusters based on upregulated and downregulated pattern in diabetic mouse liver (Figure S1B). Cluster A and Cluster B

included 1933 upregulated and 694 downregulated genes in diabetic mouse liver respectively.

The Main Biological Processes and Pathways Altered in Diabetic Mouse Liver

To investigate the possible biologic functions of the genes affected in diabetic mouse liver, the Cluster A and B with 1933 genes upregulated and 694 genes downregulated respectively were analyzed by Gene Ontology (Figure 2A). Cluster A genes were strongly enriched in cellular ketone and organic acid metabolic process, especially in their subprocess oxoacid metabolic process and its downstream carboxylic acid metabolic process, and even more specifically in monocarboxylic acid (Figure 2B). Moreover, lipid metabolic process and its subprocess cellular lipid metabolic process, and cofactor metabolic process and its subprocess coenzyme metabolic process were enriched in Cluster A (Figure 2B). Surprisingly, Cluster B genes were mainly enriched in immune-related processes, such as adaptive immune response and lymphocyte mediated immunity, especially their downstream process B cell mediated immunity and its subprocess immunoglobulin mediated immune response (Figure 2B). These data implicate that diabetes is associated with immune response. The 2627 differentially expressed genes in diabetic mouse liver including Cluster A and Cluster B mainly enriched in metabolic processes, which is similar with Cluster A (Figure 2B). These data suggest that metabolic processes are the major processes altered in diabetic mouse liver.

Furthermore, KEGG pathway analysis was performed to further elucidate the biological functions of the gene clusters affected in diabetic mouse liver. The top 5 KEGG pathways significantly correlated with type 2 diabetes were shown in Table 1 with associated genes. These pathway analysis data further confirmed that metabolic pathways in liver are the main biological processes altered in diabetic mouse liver. Besides, the downregulated genes were enriched in complement and coagulation cascades and pathway in cancer, suggesting that diabetes is correlated with immunity and cancer.

The Upregulation of Key Enzyme Genes in Fatty Acid and Glucose Metabolism in Diabetic Mouse Liver

The differentially expressed genes were enriched in lipid metabolic process and fatty acid metabolic process (Figure 2), and it is well known that fatty acid metabolism is impaired in diabetic liver [12,13]. So we analyzed the transcriptional level of enzyme genes participated in fatty acid metabolism. Schematic of three major fatty acid metabolism subprocesses including fatty acid oxidation, fatty acid synthesis and fatty acid storage were shown in Figure 3A. Surprisingly, none of these detected enzyme genes was downregulated in diabetic mouse liver, and most of the enzyme genes in fatty acid oxidation and fatty acid storage were significantly upregulated (Figure 3A–C). For example, *Cpt1a* and *Cpt2* encoding the rate-limiting enzyme carnitine-palmitoyl transferase in fatty acid oxidation were upregulated to 1.36-fold and 1.66-fold respectively in diabetic mouse liver, and *Ehhadh* encoding the key enzyme catalyzing two steps in fatty acid oxidation was increased to 3.99-fold (Figure 3B). Moreover, the enzyme genes *Elovl6*, *Scd1*, *Gpat*, *Dgat1* and *Dgat2*, encoding fatty acid storage enzymes long-chain elongase (ELOVL6), stearoyl-CoA desaturase 1 (SCD1), mitochondrial glycerol 3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT), were upregulated to 3.01-, 1.89-, 2.37-, 6.67- and 1.48-fold respectively (Figure 3C). Whereas the enzyme genes involved in fatty acid synthesis,

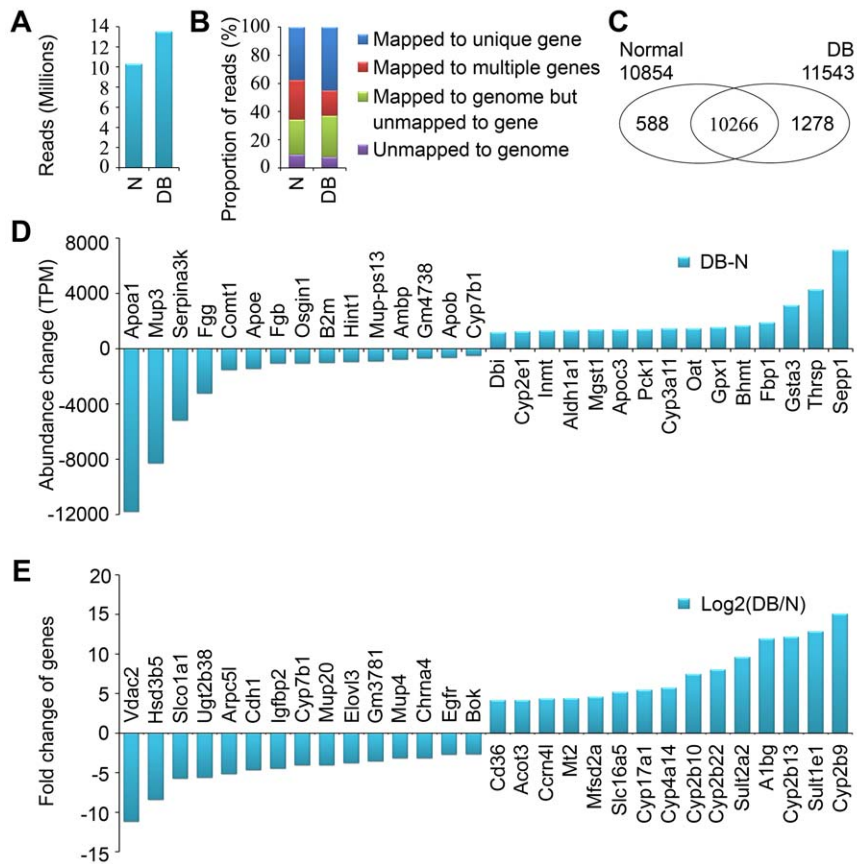


Figure 1. Sequencing and mapping messages of hepatic mRNA profiling of normal and diabetic mice. (A) The high-quality clean reads from high-throughput sequencing. Total liver RNA from 9-week-old normal (N) and diabetic (DB) db/db mice was used to prepare the high-throughput sequencing library. (B) The proportions of high-quality clean reads unmapped and/or mapped to unique genes, multiple genes and genome. (C) The number of genes detected in normal and diabetic mouse liver. (D) The top 15 abundance change genes downregulated or upregulated in diabetic mouse liver. (E) The top 15 fold change genes downregulated or upregulated in diabetic mouse liver. doi:10.1371/journal.pone.0057766.g001

including *Acy* and *Fasn* showed no significant change (Figure 3C). These results suggest that fatty acid oxidation and storage are enhanced in 9-week-old diabetic db/db mouse liver.

Diabetes is featured with impaired glucose metabolism [4,5], so we also analyzed the transcriptional level of enzyme genes participated in glucose metabolism. Schematic of four major hepatic glucose metabolism subprocesses including glycolysis, gluconeogenesis, glycogen synthesis and glycogenolysis were shown in Figure 4A and 4D. Surprisingly, none of these detected enzyme genes was downregulated in diabetic mouse liver, and numerous enzyme genes in glycolysis, gluconeogenesis and glycogenolysis were significantly upregulated (Figure 4A–E). For example, *Pfkfb3*, encoding the rate-limiting enzymes pyruvate kinase (PK) in glycolysis [24], was upregulated to 2.66-fold. Meanwhile, both *Fbp1* and *Pck1*, encoding the rate-limiting enzymes FBPase and phosphoenolpyruvate carboxykinase (PEPCK) in gluconeogenesis pathway were upregulated to 3.09- and 3.06-fold respectively. The role of glycogen metabolism is to maintain blood-glucose levels through glycogenesis and glycogenolysis [25]. We found that *Pylg*, encoding the rate-limiting enzyme glycogen phosphorylase in glycogenolysis, was significantly upregulated to 1.49-fold (Figure 4D,E). These results suggest that glycolysis, gluconeogenesis and glycogenolysis are enhanced in 9-week-old diabetic db/db mouse liver.

Taken together, these results suggest that fatty acid and glucose metabolism are markedly enhanced in 9-week-old diabetic db/db mouse liver.

An overview for the Alteration of Key Genes Involved in Fatty Acid and Glucose Metabolism in Diabetic Mouse Liver

To further elucidate the change of fatty acid and glucose metabolism in diabetic mouse liver, we analyzed more related biological processes and signaling pathways. As shown in Figure 5, besides fatty acid oxidation, synthesis and storage, glycolysis, gluconeogenesis, glycogen synthesis and glycogenolysis, fatty acid and glucose transport and PPAR/RXR signaling were also analyzed and integrated into the schematic of fatty acid and glucose metabolism network in the diabetic mouse liver. Consistent with the expected enhanced fatty acid metabolism in the diabetic mouse liver, we found that the mRNA levels of fatty acid translocase CD36 (*Cd36*) and fatty acid transport protein FATP2 (*Slc27a2*) were increased to 17.7-fold and 1.45-fold respectively (Table S1), suggesting enhanced fatty acid transport in the diabetic mouse liver (Figure 5). The expected enhanced fatty acid transport is consistent with the increased expression of enzyme genes in fatty acid storage (Figure 5) [12,26]. It is reported that hepatic fatty acid stimulates PPAR/RXR signaling pathway, which can induce transcription of genes responsible for fatty acid β -oxidation

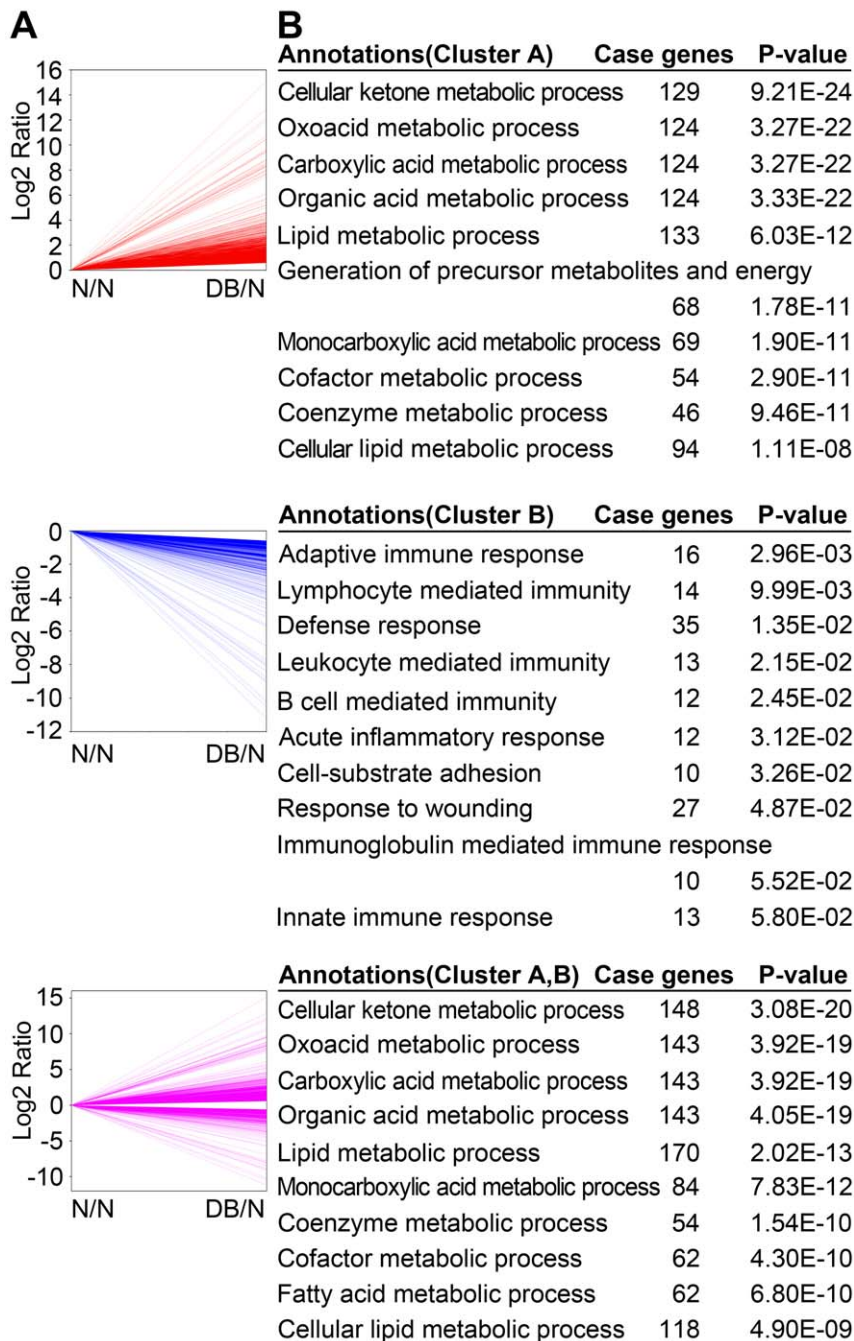


Figure 2. Genes and the related biological processes altered in diabetic mouse liver. (A) The 8551 selected genes as described in Figure S1A were separated into two distinct clusters according to the genes upregulated or downregulated in diabetic (DB) mouse liver compared with normal (N) control. Red lines indicate Cluster A including 1933 upregulated genes in diabetic mouse liver. Blue lines indicate Cluster B including 694 downregulated genes. Purple lines indicate the total 2627 altered genes, which include all genes in Cluster A and B. (B) The clustered genes were assigned to different biological processes based on Gene Ontology using the web tool DAVID. The top 10 biological functions and the case genes in each cluster ranked by *P*-value were listed (case genes ≥ 10). doi:10.1371/journal.pone.0057766.g002

[27,28]. Consistently, we showed that *PPAR α* in the diabetic mouse liver was elevated to 2.24-fold accompanied with increased expression of key enzyme genes *Cpt2* and *Ehhadh* in fatty acid β -oxidation, suggesting enhanced fatty acid oxidation in the diabetic mouse liver (Figure 5 and Table S1). Fatty acid oxidation has been reported to stimulate gluconeogenesis and suppress glycolysis [29]. Gluconeogenesis is expected to be upregulated due to the

increased expression of the two key enzyme genes *Fbp1* and *Pfk1*, however glycolysis is also expected to be increased based on the markedly increased expression of the rate-limiting enzyme gene *Pfkfb3* (Figure 5). The enhanced glycolysis might be associated with the increased glucose transport, which can be expected from the markedly increased the mRNA level of glucose transporter *Glut2* (*Slc2a2*) to 3.11-fold (Table S1). In addition, the expression

Table 1. The associated genes in the top 5 KEGG pathways significantly altered in diabetic mouse liver.

KEGG Pathway		
Cluster A Upregulated 1933 genes	Drug metabolism	P = 1.68E-13
	CYP3A25-CYP2B9-UGT2B1-GSTM5-CYP2A12-GSTM3-GSTM4-FMO1-FMO2-GSTK1-FMO3-GSTZ1-UGT2A3-GSTO1-CYP3A44-GSTA3-CYP2C55-CYP3A16-CYP3A13-LOC639023-CYP3A11-MAOB-CYP2C29-GSTT1-CYP2B13-GSTT2-CYP2B10-CYP2C50-MGST3-CYP2A22-UGT2B36-LOC100046051-AOX1-UGT2B5-CYP2A5-CYP3A59-CYP2C38-CYP2C39-MGST1	
	Valine, leucine and isoleucine degradation	P = 5.97E-11
	HSD17B10-ACADSB-EHHADH-ECHS1-ACAT2-ACAT1-ALDH3A2-AUH-MCCC2-IVD-MCEE-ACAD8-HADH-HMGCL-BCKDHA-ALDH6A1-ACAA2-ACADM-ACADS-ALDH7A1-ALDH1B1-DLD-AOX1-ABAT-HIBCH-PCCA	
	Metabolism of xenobiotics by cytochrome P450	P = 8.61E-11
CYP3A25-CYP2B9-UGT2B1-GSTM5-GSTM3-GSTM4-GSTK1-GSTZ1-GSTO1-UGT2A3-CYP3A44-GSTA3-CYP2C55-CYP3A16-CYP3A13-CYP1A1-LOC639023-CYP3A11-CYP2C29-GSTT1-EPHX1-CYP2B13-GSTT2-CYP2B10-CYP2C50-MGST3-UGT2B36-UGT2B5-CYP3A59-CYP2C38-CYP2C39-MGST1		
Cluster B Downregulated 694 genes	Retinol metabolism	P = 1.09E-09
	CYP3A25-CYP2B9-UGT2B1-ALDH1A1-CYP2A12-GM10774-ALDH1A7-UGT2A3-CYP3A44-CYP2C55-CYP3A16-CYP3A13-CYP1A1-LOC639023-CYP3A11-CYP2C29-CYP26A1-CYP2B13-CYP2B10-CYP2C50-CYP2A22-UGT2B36-DHRS4-DGAT1-UGT2B5-CYP2A5-CYP3A59-RDH16-CYP4A14-CYP2C38-CYP2C39-RETSAT	
	Oxidative phosphorylation	P = 2.53E-08
UQCRC2-ATP5D-ATP5E-NDUFB6-UQCRC1-NDUFB9-ATP6A1-ATP5B-COX7B-UQCRC5-COX5A-GM10039-UQCRCQ-ATP5G3-NDUFS7-ATP6V0E-GM4943-NDUFS8-COX6B1-ATP6V0D1-NDUFS2-ATP5K-NDUFS1-COX15-NDUFA2-NDUFB10-NDUFA9-NDUFA6-NDUFA7-COX4I1-NDUFA10-LHPP-ATP6V1D-NDUFB3-SDHA-ATP6V1C1-GM4459-NDUFV1-ATP6V1E1-ATP5A1-ATP6V0A2		
Cluster A and B 2627 genes	Complement and coagulation cascades	P = 5.50E-04
	F11-KNG2-CFB-C6-SERPING1-C1QC-PLG-C8A-C1QA-C8B-FGG-FGA-FGB-SERPINF2	
	Pathways in cancer	P = 2.13E-03
	HSP90AB1-E2F3-PPARD-CDH1-NFKB2-ITGB1-CTNNA1-ITGAV-HHIP-TPR-PIK3R1-CSF1R-EGFR-BMP4-BMP2-COL4A1-HSP90AA1-RELA-SMAD4-APPL1-STK4-FZD7-FZD6-DAPK1-VEGFB-CBLC-LAMA3-PDGFR-ATK1	
Cluster A and B 2627 genes	Drug metabolism	P = 7.44E-12
	Metabolism of xenobiotics by cytochrome P450	P = 1.46E-09
	Retinol metabolism	P = 2.75E-09
	Valine, leucine and isoleucine degradation	P = 2.23E-08
	Steroid hormone biosynthesis	P = 2.14E-06

doi:10.1371/journal.pone.0057766.t001

of *Pyl*, encoding the rate-limiting enzyme glycogen phosphorylase in glycogenolysis was significantly upregulated, suggesting increased glycogenolysis (Figure 5). Taken together, Figure 5 provides an overview of the gene nodes involved in fatty acid and glucose metabolism and their alteration in 9-week-old diabetic db/db mouse liver.

The Potential Relationship of Diabetes with Immunity and Cancer

As shown in Figure 2, the upregulated genes were mainly enriched in metabolic processes, whereas surprisingly the down-regulated genes were mainly enriched in immune-related processes. It is well known that inflammation is one of the important factors leads to diabetes [30,31]. However, the potential relationship of other immunological processes with diabetes is still largely unknown. Here we systematically analyzed the genes in all 15 KEGG pathways in immune system. All the detected genes in the 15 KEGG immunological pathways were shown in Table S2. Notably, for the genes with an average expression level over 5 TPM in normal and diabetic mouse livers, *Asap1* (ArlGAP with SH3 domain, ankyrin repeat and PH domain1), *Cd1d1* (CD1d1 antigen), *Cd36* (CD36 antigen), *Cfl* (complement factor D), *Dntt*

(deoxynucleotidyltransferase, terminal), *H2-Q1* (histocompatibility 2, Q region locus 1) and *Zap70* (zeta-chain (TCR) associated protein kinase) in various immunological pathways were increased to over 3-fold, and *Arpc5l* (actin related protein 2/3 complex, subunit 5-like), *C1qa* (complement component 1, q subcomponent, alpha polypeptide), *C1qc* (complement component 1, q subcomponent, C chain), *C6* (complement component 6), *Cldn1* (claudin 1), *Gsn* (gelsolin), *Hspa4* (heat shock protein 4) and *Il1r1* (interleukin 1 receptor, type I) decreased to over 3-fold (Table S2). These data suggest that besides inflammatory signaling, other hepatic immune related pathways are also correlated with type 2 diabetes.

Moreover, according to KEGG analysis in Table 1, the genes downregulated in the diabetic mouse livers were also enriched in pathways in cancer, suggesting that diabetes is correlated with cancer.

To further elucidate the correlations between diabetes and hepatic diseases, we assigned the differentially expressed genes in the diabetic mouse liver to different diseases using web tool FunDO. As shown in Figure 6, 2627 differentially expressed genes were mainly related with liver cancer, hepatitis, liver disease, adenovirus infection and liver tumor. These results are in line with the correlation of diabetes with immunity and cancer [30,31,32].

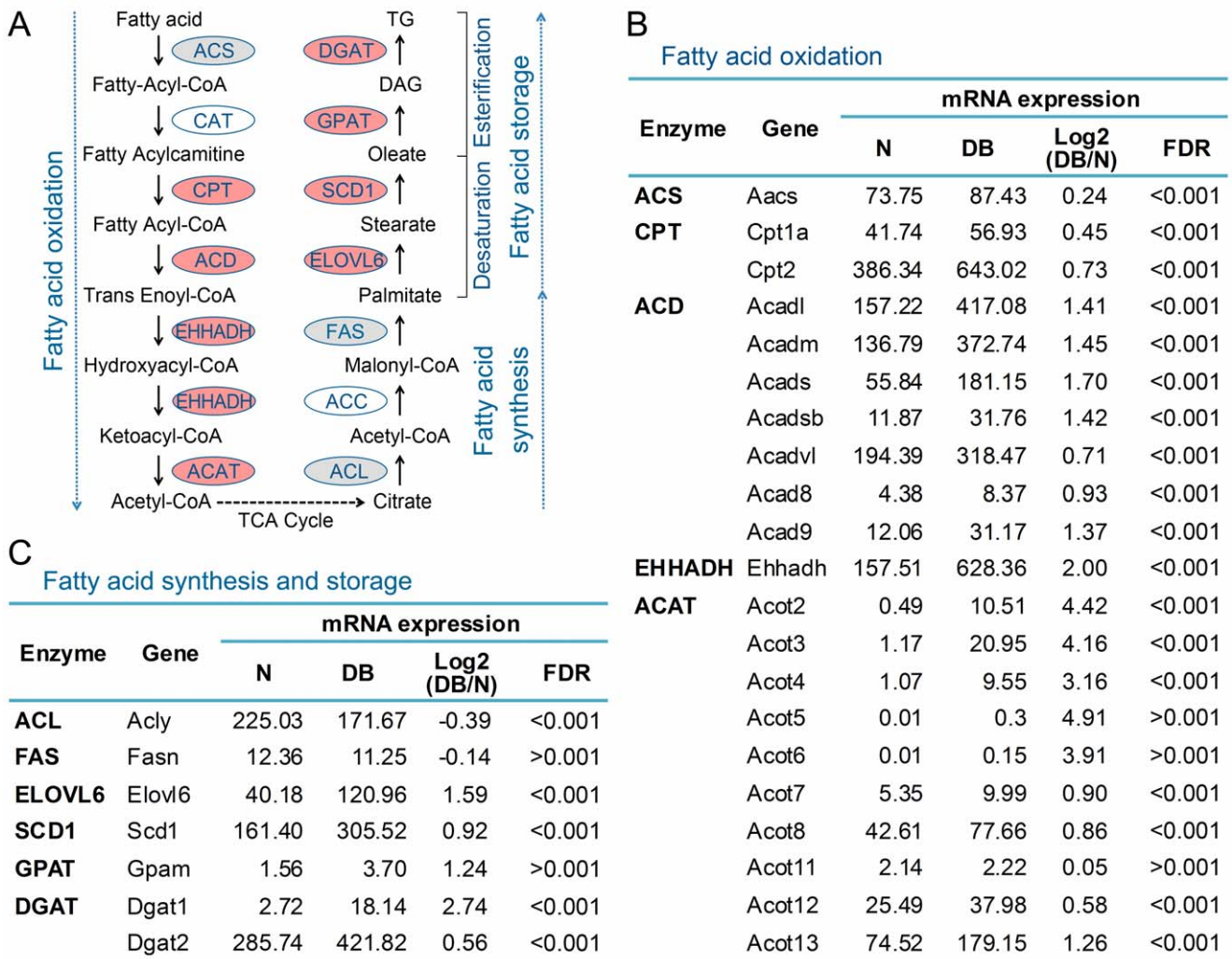


Figure 3. The expression of enzymes directly participated in fatty acid β -oxidation, synthesis and storage was increased or not significantly changed in diabetic mouse liver. (A) Schematic of fatty acid metabolism and the expression of enzymes directly participated in fatty acid oxidation, synthesis and storage in diabetic mouse liver. Red color represents the enzyme genes upregulated in diabetic mouse liver with fold change ≥ 1.5 and FDR < 0.001 , gray color indicates no significant change, and white color indicates the genes were not detected. (B–C) The mRNA levels of the enzymes directly participated in fatty acid oxidation, synthesis and storage in normal (N) and diabetic (DB) mouse liver. ACS, acetyl-CoA synthase; CAT, carnitine acyl transferase; CPT, carnitine palmitoyltransferase; ACD, acyl-CoA dehydrogenase; EHHADH, enoyl-CoA-hydratase/3-hydroxyacyl-CoA dehydrogenase; ACAT, acetyl-CoA acetyltransferase; ACL, ATP citrate lyase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; ELOVL6, long-chain elongase; SCD1, stearoyl-CoA desaturase 1; GPAT, mitochondrial glycerol 3-phosphate acyltransferase; DGAT, diacylglycerol acyltransferase. doi:10.1371/journal.pone.0057766.g003

Discussion

This study provides the gene expression profile data of type 2 diabetic mouse liver by high-throughput sequencing, and demonstrates the main biological processes, pathways, fatty acid and glucose metabolism related key enzyme genes and liver diseases correlated with type 2 diabetes. Type 2 diabetes is mainly correlated with the hepatic metabolic processes, especially fatty acid and glucose metabolism, and is also correlated cancer and immune-related liver diseases. These results should be helpful for further understanding the metabolic changes in type 2 diabetic liver and the correlation of diabetes with hepatic cancer and immunity.

High-throughput RNA sequencing approach to measure gene expression levels greatly increases our ability to quantitatively detect mRNA level in an relatively unbiased way [22]. With this

technology, we showed the top 15 abundance change genes upregulated or downregulated, and the top 15 fold change genes increased or decreased in Figure 1. To our knowledge, there is no published paper comparing the hepatic gene expression profile of wild type and db/db mice using microarray data, so we briefly compared our data with the reported microarray data from 10-week-old diabetic ob/ob mice [33]. Among the top 15 abundance change genes upregulated or downregulated, almost all these genes increased or decreased similarly, except *Pck1* encoding PEPCK, a key enzyme in gluconeogenesis. It has been reported that *Pck1* mRNA level was increased in diabetic liver [20,34], which is in line with our data. Moreover, we found that some genes such as *Comt1*, *Osgin1*, *Mup-ps13* and *Gm4738* at a relatively high abundance were quantified in this study but not found in the previous microarray data [33]. Interestingly, we also found a few largely unknown and markedly differentially expressed genes

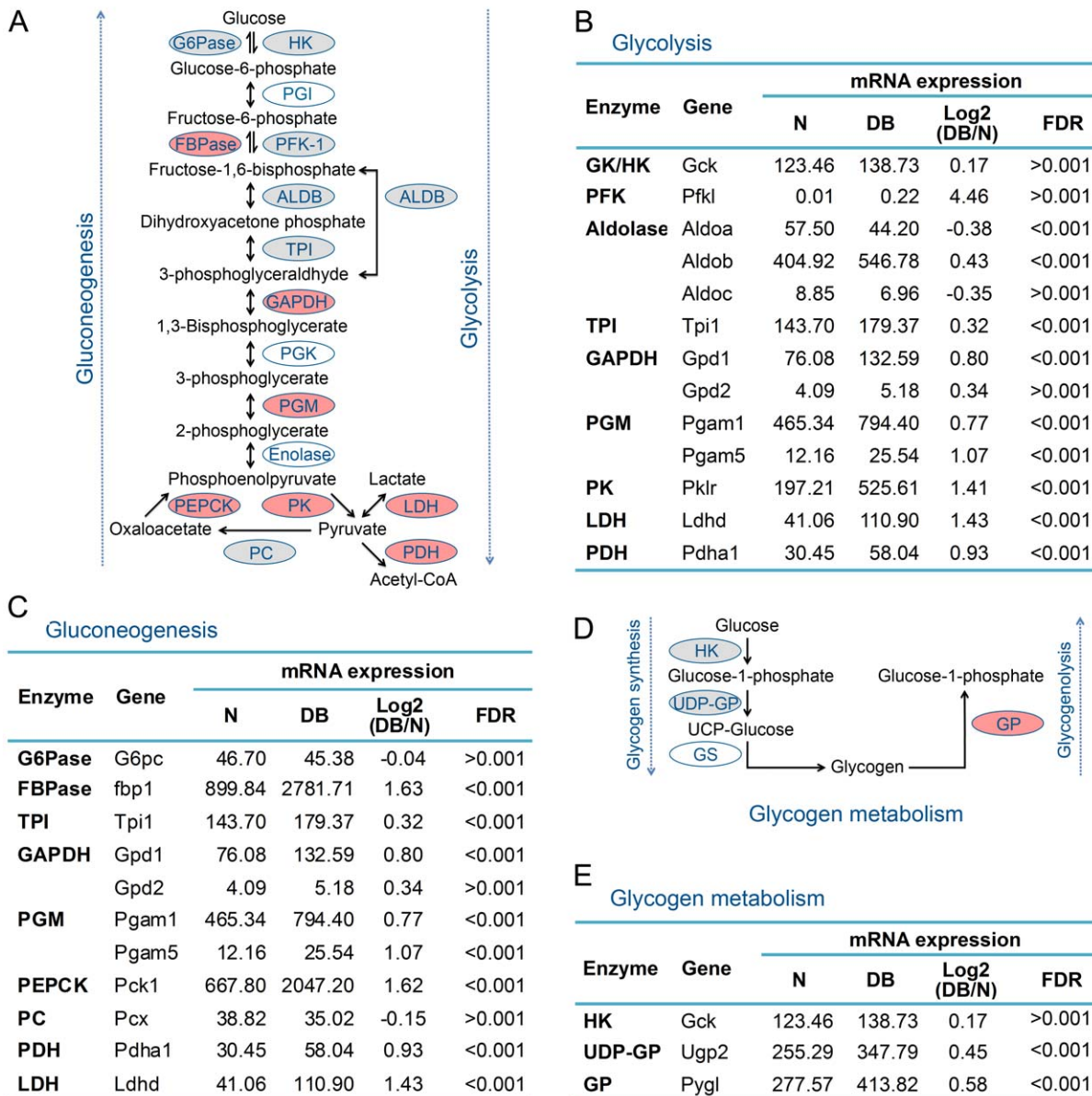


Figure 4. The expression of enzymes directly participated in gluconeogenesis, glycolysis and glycogen metabolism was increased or not significantly changed in diabetic mouse liver. (A, D) Schematic of gluconeogenesis, glycolysis and glycogen metabolism, and the expression of enzymes directly participated in these processes of diabetic mouse liver. The key enzymes include HK, PFK-1 and PK for glycolysis, G6Pase, FBPase and PEPCK for gluconeogenesis, HK, UDP-GP and GS for glycogen synthesis, and GP for glycogenolysis. Red color represents the upregulated genes in diabetic mouse liver with fold change ≥ 1.5 and FDR < 0.001 , gray color indicates no significant change, and white color indicates the genes were not detected. (B, C and E) The mRNA levels of the enzymes directly participated in glycolysis, gluconeogenesis and glycogen metabolism in normal (N) and diabetic (DB) mouse liver. HK, hexokinase; PGI, Phosphoglucoisomerase; PFK-1, phosphofruktokinase; ALDB, aldolase; TPI, triose phosphate isomerase; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerokinase; PGM, phosphoglyceromutase; PK, pyruvate kinase; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; G6Pase, Glucose-6-Phosphatase; FBPase, Fructose 1,6-bisphosphatase; PEPCK, Phosphoenolpyruvate carboxykinase; UDP-GP, Uridine diphosphoglucose pyrophosphorylase; GS, Glycogen Synthase; GP, Glycogen Phosphorylase. doi:10.1371/journal.pone.0057766.g004

including *Mup-ps13* and *Gm4738*, and the role of these genes in liver is yet to be elucidated. Similar with the top abundance change genes, the change trend of top 15 fold change genes in our observation is the same as previous microarray data [33]. Among the top 15 fold change genes upregulated or downregulated by diabetes, 15 genes were clustered in metabolic process, such as *Cyp2b9*, *Sult1e1*, *Hsd3b5* and *Cyp2b13*. Among the other 15 genes not in metabolic process, *Vdac2* has been reported to inhibit

mitochondrial apoptosis [35], and *A1bg* is specifically expressed in liver as shown in BioGPS ([http:// biogps.gnf.org](http://biogps.gnf.org)). However, the role of *Vdac2* and *A1bg* in glucose and lipid metabolism is yet to be elucidated. Future studies focused on these markedly differentially expressed hepatic genes with largely unknown effects on glucose and lipid metabolism will provide new insights into the molecular basis of type 2 diabetes.

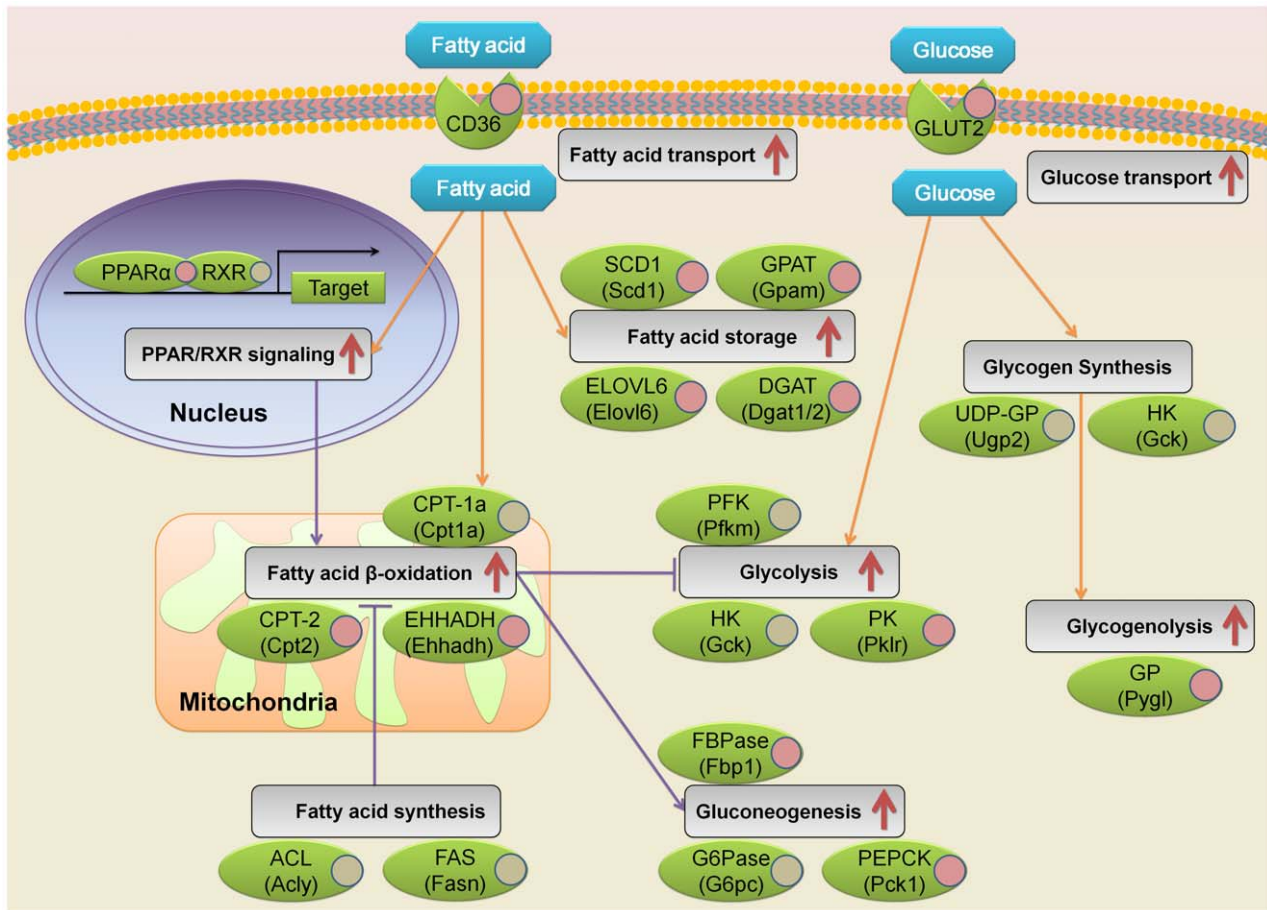


Figure 5. An overview of the key fatty acid and glucose metabolic gene nodes and their alteration in the 9-week-old diabetic db/db mouse liver. The gene nodes and their interactions were obtained from literatures, and the alteration of gene expression was from Table S1. Orange arrows indicate the metabolic flux of glucose and fatty acid. Purple arrows indicate the interaction between different metabolic processes and the indicated signaling pathway. Red arrows show that the indicated process and pathway are expected to be upregulated in diabetic mouse liver based on the detected mRNA levels. Red and gray circles labeled on each gene indicate the genes are upregulated and unaffected in diabetic mouse liver respectively. CD36, fatty acid translocase; GLUT2, glucose transporter 2.
doi:10.1371/journal.pone.0057766.g005

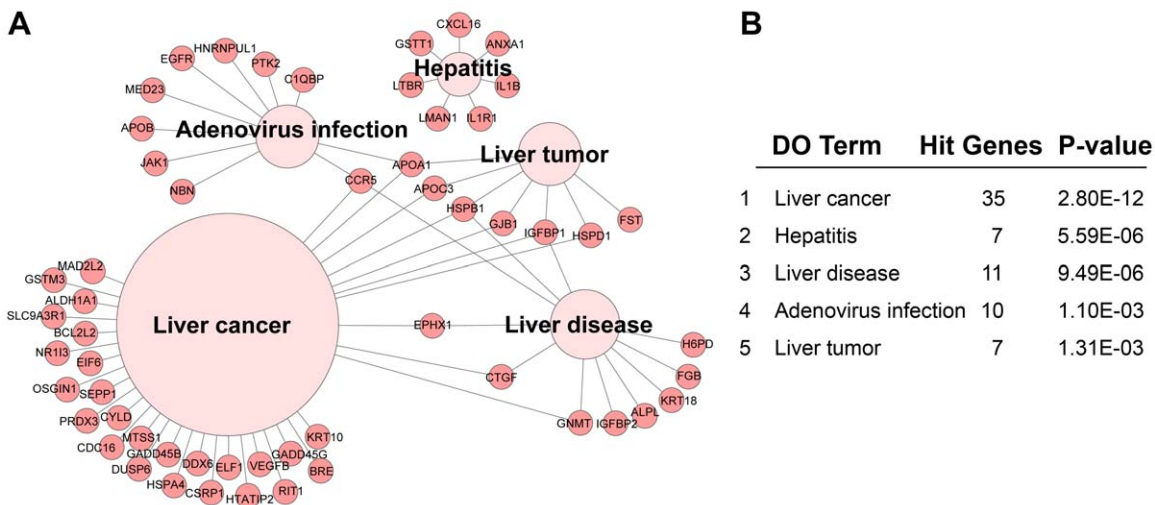


Figure 6. Diabetes is correlated with different liver diseases at a transcriptional view. (A) The map of top 5 liver diseases enriched with the genes altered in 9-week-old db/db mouse liver. 2627 altered genes were assigned to different diseases using the web tool FunDO. The sizes of the disease nodes are proportional to the number of enriched genes. (B) The number of hit genes and P-value of the top 5 enriched liver diseases in (A).
doi:10.1371/journal.pone.0057766.g006

The role of hepatic fatty acid oxidation in diabetes is complicate. Metformin, a widely used drug for type 2 diabetes, activates AMPK, induces fatty acid oxidation and inhibits lipogenesis [36]. The increased fatty acid oxidation in mice with *Aac2* deficiency prevented the onset of type 2 diabetes [37]. However, fatty acid oxidation inhibitors have been considered to treat type 2 diabetes [38]. It has been reported that increased hepatic fatty acid oxidation can enhance gluconeogenesis, and thus stimulates hepatic glucose production [38]. In this study, we found that most of the hepatic enzyme genes in fatty acid oxidation were significantly upregulated (Figure 3A,B), which is consistent with the increased expression of the gluconeogenesis enzyme genes (Figure 4A,C). The dual role of hepatic fatty acid oxidation might be based on the balance of reducing free fatty acid level and stimulating hepatic glucose production by enhancing gluconeogenesis. In addition, consistent with previous reports [39,40], the fatty acid storage enzyme genes *Elovl6*, *Scd1*, *Gpat*, *Dgat1* and *Dgat2* were markedly upregulated. Taken together, our results suggest that fatty acid metabolism is markedly enhanced in 9-week-old diabetic db/db mouse liver, implicating the diabetic mouse liver is working hard on fatty acid homeostasis and trying to solve the problem of impaired fatty acid and glucose metabolism.

It has been reported that the rate of hepatic gluconeogenesis in diabetes is elevated, [41,42,43]. Consistent with the previous reports, here we also found that the mRNA levels of rate-limiting enzymes FBPase and PEPCK in gluconeogenesis pathway were upregulated (Figure 4C). In glycolysis pathway, it is reported that the pyruvate kinase, glucokinase and phosphofructokinase (PFK) enzyme activities and the pyruvate kinase and glucokinase mRNA levels are decreased in diabetic liver [41]. Interestingly, in this study we found that the mRNA levels of glucokinase and phosphofructokinase were not significantly altered in diabetic liver, and the mRNA level of pyruvate kinase even increased (Figure 4B). The decrease of phosphofructokinase activity in diabetic rat liver is resulted from accelerated protein degradation rate while the synthetic rate remains nearly normal [44], which is consistent with no significant change of phosphofructokinase mRNA level in diabetic mouse liver in our study. The increased mRNA level of pyruvate kinase and unchanged mRNA level of glucokinase observed in this study implicate that the liver of 9-week-old db/db mice at a relative early stage of diabetes might be able to enhance glycolysis to reduce glucose level [45,46,47]. About 90% increase of hepatic glucose production in type 2 diabetic subjects is accounted by accelerated gluconeogenesis [42]. The remaining about 10% increase of hepatic glucose production in type 2 diabetic subjects is likely mainly contributed by slightly increased glycogenolysis [42], which is consistent with nearly 50% mRNA-level increase of glycogen phosphorylase (Figure 4E), the key enzyme of glycogenolysis [48]. Taken together, our data implicate that although glycolysis was enhanced to consume more glucose, gluconeogenesis and glycogenolysis were also accelerated to produce more glucose (Figure 5). Thus the 9-week-old diabetic db/db mouse liver had limited effect to improve blood glucose levels, although it showed accelerated glucose metabolism.

Inflammation is considered as one of the important factors causing the development of type 2 diabetes [49,50,51]. Similarly, we also found many immune-related genes were markedly changed in type 2 diabetic mouse liver (Table S2). To our surprise, genes downregulated in diabetic mouse liver were mainly enriched in immune-related processes, such as adaptive immune response and lymphocyte mediated immunity (Figure 2B). These findings suggest that type 2 diabetes is associated with altered hepatic immune response. Consistently, it is reported that the frequency of hepatitis B and C virus infection is increased in type 2

diabetic patients [52,53,54], which is also in line with the association of hepatitis with type 2 diabetes (Figure 6). These findings suggest that the alteration of immune-related genes observed in this study may facilitate the infection of hepatitis B and C virus. Moreover, it has been reported that the liver has immune-related functions, such as removal of pathogens and antigens, which requires a local immune response [55,56]. Sinusoidal endothelial cells and Kupffer cells in liver can directly interact with passenger leukocytes, and should be involved in the local immune response. Selective disruption of hepatic resident macrophages, Kupffer cells, has been shown to be sufficient to improve hepatic insulin sensitivity in high-fat diet model [57,58]. These previous findings suggest that the downregulated immune-related genes observed in our study might play important roles in Kupffer cells or other hepatic immune response cells in response to the development of diabetes. Future studies focused on these downregulated immune-related genes such as in Kupffer cells might provide new insights for the development of hepatic insulin resistance and type 2 diabetes.

Epidemiological studies clearly indicate that the risk of several types of cancer including liver cancer, is increased in diabetic patients [59,60,61]. The possible biologic links between diabetes and cancer risk include hyperinsulinemia, hyperglycemia or chronic inflammation, but the underlying molecular mechanisms are still largely unknown [59]. In this study, analysis of the hepatic gene expression pattern by Fundo also suggests that type 2 diabetes is highly associated with liver cancer (Figure 6). Moreover, our study provides the genes affected by type 2 diabetes and associated with liver cancer (Figure 6). Future studies focused on these candidate genes might shed new light on the molecular mechanisms for the high risk of liver cancer in diabetic patients.

Taken together, this study shows the quantitative gene expression profile and the alteration of biological processes, pathways, and key enzyme genes in fatty acid and glucose metabolism in type 2 diabetic mouse liver. Moreover, we found that hepatic local immune response and liver cancer are associated with type 2 diabetes. These findings provide new insights for the alteration of hepatic fatty acid and glucose metabolism and involvement of hepatic immunity in the development of type 2 diabetes.

Materials and Methods

Animal Experiments

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences (Protocol number 2007-AN-9). C57BL/6 male mice and diabetic db/db male mice at the age of 7 weeks obtained from SLAC (Shanghai, China) and Model Animal Research Center of Nanjing University (Nanjing, China) were divided into two groups for 12 mice per group, and were allowed to have access to water and chow diets *ad libitum*. About 3 h after the beginning of light cycle, 9-week-old mice were sacrificed, and the livers were immediately collected and snap-frozen in liquid nitrogen.

Sample Preparation and Solexa Library Construction

Liver samples were ground in liquid nitrogen, and total RNA was extracted with Trizol reagent (Invitrogen). The integrity of total RNA was evaluated using an Agilent Bioanalyzer 2100. Solexa libraries were constructed following the manufacturer's standard according to the schematic as described previously [62].

Solexa Sequencing and Data Analysis

The image files obtained from Illumina 1G sequencer were processed to produce sequence data. Then the high-quality reads were screened from the original raw data, and the adaptors were removed from each sequence. Subsequently, high quality clean tags were compared with RefSeq database and the expression level of each gene was normalized to transcripts per million (TPM). The raw data and the processed gene expression data were deposited in GEO with a series number GSE43314, including GSM1060446 and GSM1060447 for normal and diabetic mouse liver respectively. The significance of digital gene expression profiles was analyzed as described previously [63]. The differentially expressed genes were classified into upregulated and downregulated groups as Cluster A and B respectively, and then the expression pattern of these genes was visualized using the heat-map function in the R base package [64].

Biological Process and Pathway Analysis

The clustered genes were assigned to biological processes based on Gene Ontology using the web tool DAVID (<http://david.abcc.ncifcrf.gov/home.jsp>) [65,66]. Hypergeometric test was used to select the enriched biological process in Gene Ontology for each cluster. The pathways associated with these gene clusters were analyzed by KEGG (www.genome.jp/kegg/pathway.html) [67].

FunDo Analysis

To study the correlation of disease and gene expression change, the differentially expressed genes in db/db mouse liver were assigned to different diseases based on Disease Ontology and peer-reviewed evidence from GeneRIF using the web tool FunDO (<http://django.nubic.northwestern.edu/fundo/>) [68]. Then the gene-disease interaction networks were visualized by Cytoscape v2.6.2 [69].

References

1. Yach D, Stuckler D, Brownell KD (2006) Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med* 12: 62–66.
2. Yang G, Pan C, Lu J (2010) Prevalence of erectile dysfunction among Chinese men with type 2 diabetes mellitus. *Int J Impot Res* 22: 310–317.
3. Voight BF, Scott LJ, Steinthorsdotir V, Morris AP, Dina C, et al. (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 42: 579–589.
4. DeFronzo RA (2004) Pathogenesis of type 2 diabetes mellitus. *Med Clin North Am* 88: 787–835, ix.
5. Bouche C, Serdy S, Kahn CR, Goldfine AB (2004) The cellular fate of glucose and its relevance in type 2 diabetes. *Endocr Rev* 25: 807–830.
6. Agius L (2010) Physiological control of liver glycogen metabolism: lessons from novel glycogen phosphorylase inhibitors. *Mini Rev Med Chem* 10: 1175–1187.
7. van Poelje PD, Dang Q, Erion MD (2007) Discovery of fructose-1,6-bisphosphatase inhibitors for the treatment of type 2 diabetes. *Curr Opin Drug Discov Devel* 10: 430–437.
8. McCormack JG, Westergaard N, Kristiansen M, Brand CL, Lau J (2001) Pharmacological approaches to inhibit endogenous glucose production as a means of anti-diabetic therapy. *Curr Pharm Des* 7: 1451–1474.
9. Matschinsky FM, Magnuson MA, Zelent D, Jetton TL, Doliba N, et al. (2006) The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy. *Diabetes* 55: 1–12.
10. Lefebvre P, Chinetti G, Fruchart JC, Staels B (2006) Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J Clin Invest* 116: 571–580.
11. Stoffel M, Duncan SA (1997) The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci U S A* 94: 13209–13214.
12. Lewis GF, Carpentier A, Adeli K, Giacca A (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201–229.
13. McGarry JD (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51: 7–18.

Supporting Information

Figure S1 Genes differentially expressed in normal and diabetic mouse liver. (A) 8551 genes with average TPM no less than 1 in normal and diabetic mouse liver were selected to analyze the gene expression profile. Number of genes differentially expressed in diabetic mouse liver compared with normal control according to the indicated fold change and FDR value was listed. 2627 genes with absolute fold change ≥ 1.5 and FDR < 0.001 were considered as differentially expressed genes in this study. (B) Heat-map images for the 2627 differentially expressed genes. The selected genes were classified into Cluster A or B, based on the genes upregulated or downregulated in diabetic mouse liver. Red and blue indicate genes with high and low abundance respectively. (TIF)

Table S1 Gene expression data of normal and diabetic mouse liver. The 8551 genes with average TPM no less than 1 in normal and diabetic mouse liver were listed. N_TPM, the TPM of the indicated genes in normal mouse liver; DB_TPM, the TPM of the indicated genes in diabetic mouse liver. (XLS)

Table S2 Gene expression profile of immune-related 15 pathways in KEGG database in diabetic (DB) mouse liver compared with normal (N) control. (XLS)

Author Contributions

Conceived and designed the experiments: FZ QZ. Performed the experiments: FZ YZ BZ ZH. Analyzed the data: FZ XX QZ. Wrote the paper: FZ QZ.

14. Postic C, Girard J (2008) Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 118: 829–838.
15. van Lunteren E, Moyer M (2009) Gene expression profiling in the type 1 diabetes rat diaphragm. *PLoS One* 4: e7832.
16. Dhahbi JM, Mote PL, Cao SX, Spindler SR (2003) Hepatic gene expression profiling of streptozotocin-induced diabetes. *Diabetes Technol Ther* 5: 411–420.
17. Suh YH, Kim Y, Bang JH, Choi KS, Lee JW, et al. (2005) Analysis of gene expression profiles in insulin-sensitive tissues from pre-diabetic and diabetic Zucker diabetic fatty rats. *J Mol Endocrinol* 34: 299–315.
18. Zhou H, Saito S, Piao G, Liu ZP, Wang J, et al. (2011) Network screening of Goto-Kakizaki rat liver microarray data during diabetic progression. *BMC Syst Biol* 5 Suppl 1: S16.
19. Kim S, Sohn I, Ahn JI, Lee KH, Lee YS (2004) Hepatic gene expression profiles in a long-term high-fat diet-induced obesity mouse model. *Gene* 340: 99–109.
20. Lan H, Rabaglia ME, Stoehr JP, Nadler ST, Schueler KL, et al. (2003) Gene expression profiles of nondiabetic and diabetic obese mice suggest a role of hepatic lipogenic capacity in diabetes susceptibility. *Diabetes* 52: 688–700.
21. t Hoen PA, Ariyurek Y, Thygesen HH, Vreugdenhil E, Vossen RH, et al. (2008) Deep sequencing-based expression analysis shows major advances in robustness, resolution and inter-lab portability over five microarray platforms. *Nucleic Acids Res* 36: e141.
22. Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res* 18: 1509–1517.
23. Blencowe BJ, Ahmad S, Lee IJ (2009) Current-generation high-throughput sequencing: deepening insights into mammalian transcriptomes. *Genes Dev* 23: 1379–1386.
24. Hess B, Boiteux A (1971) Oscillatory phenomena in biochemistry. *Annu Rev Biochem* 40: 237–258.
25. Nordlie RC, Foster JD, Lange AJ (1999) Regulation of glucose production by the liver. *Annu Rev Nutr* 19: 379–406.
26. Bradbury MW (2006) Lipid metabolism and liver inflammation. I. Hepatic fatty acid uptake: possible role in steatosis. *Am J Physiol Gastrointest Liver Physiol* 290: G194–198.
27. Kersten S, Desvergne B, Wahli W (2000) Roles of PPARs in health and disease. *Nature* 405: 421–424.

28. Kovacs TR, Li P, An J, Akimoto T, Slentz D, et al. (2005) Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J Biol Chem* 280: 33588–33598.
29. Randle PJ (1998) Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 14: 263–283.
30. Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. *J Clin Invest* 115: 1111–1119.
31. Dandona P, Aljada A, Bandyopadhyay A (2004) Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 25: 4–7.
32. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, et al. (2010) Diabetes and cancer: a consensus report. *Diabetes Care* 33: 1674–1685.
33. Keller MP, Choi Y, Wang P, Davis DB, Rabaglia ME, et al. (2008) A gene expression network model of type 2 diabetes links cell cycle regulation in islets with diabetes susceptibility. *Genome Res* 18: 706–716.
34. Marzban L, Rahimian R, Brownsey RW, McNeill JH (2002) Mechanisms by which bis(maltolato)oxovanadium(IV) normalizes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase expression in streptozotocin-diabetic rats in vivo. *Endocrinology* 143: 4636–4645.
35. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ (2003) VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science* 301: 513–517.
36. Zhou G, Myers R, Li Y, Chen Y, Shen X, et al. (2001) Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108: 1167–1174.
37. Abu-Elheiga L, Oh W, Kordari P, Wakil SJ (2003) Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc Natl Acad Sci U S A* 100: 10207–10212.
38. Foley JE (1992) Rationale and application of fatty acid oxidation inhibitors in treatment of diabetes mellitus. *Diabetes Care* 15: 773–784.
39. Matsuzaka T, Shimano H, Yahagi N, Kato T, Atsumi A, et al. (2007) Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. *Nat Med* 13: 1193–1202.
40. Postic C, Girard J (2008) The role of the lipogenic pathway in the development of hepatic steatosis. *Diabetes Metab* 34: 643–648.
41. Pilkis SJ, Granner DK (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54: 885–909.
42. DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 15: 318–368.
43. Samuel VT, Shulman GI (2012) Mechanisms for insulin resistance: common threads and missing links. *Cell* 148: 852–871.
44. Dunaway GA, Leung GL, Thrasher JR, Cooper MD (1978) Turnover of hepatic phosphofructokinase in normal and diabetic rats. Role of insulin and peptide stabilizing factor. *J Biol Chem* 253: 7460–7463.
45. Wu C, Kang JE, Peng IJ, Li H, Khan SA, et al. (2005) Enhancing hepatic glycolysis reduces obesity: differential effects on lipogenesis depend on site of glycolytic modulation. *Cell Metab* 2: 131–140.
46. Wu C, Okar DA, Newgard CB, Lange AJ (2001) Overexpression of 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase in mouse liver lowers blood glucose by suppressing hepatic glucose production. *J Clin Invest* 107: 91–98.
47. O'Doherty RM, Lehman DL, Telemaque-Potts S, Newgard CB (1999) Metabolic impact of glucokinase overexpression in liver: lowering of blood glucose in fed rats is accompanied by hyperlipidemia. *Diabetes* 48: 2022–2027.
48. Rabitzsch G, Mair J, Lechleitner P, Noll F, Hofmann U, et al. (1995) Immunoenzymometric assay of human glycogen phosphorylase isoenzyme BB in diagnosis of ischemic myocardial injury. *Clin Chem* 41: 966–978.
49. Olefsky JM, Glass CK (2010) Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 72: 219–246.
50. Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. *J Clin Invest* 116: 1793–1801.
51. Fernandez-Real JM, Pickup JC (2008) Innate immunity, insulin resistance and type 2 diabetes. *Trends Endocrinol Metab* 19: 10–16.
52. Demir M, Serin E, Gokturk S, Ozturk NA, Kulaksizoglu S, et al. (2008) The prevalence of occult hepatitis B virus infection in type 2 diabetes mellitus patients. *Eur J Gastroenterol Hepatol* 20: 668–673.
53. Sangiorgio L, Attardo T, Gangemi R, Rubino C, Barone M, et al. (2000) Increased frequency of HCV and HBV infection in type 2 diabetic patients. *Diabetes Res Clin Pract* 48: 147–151.
54. Soverini V, Persico M, Bugianesi E, Forlani G, Salamone F, et al. (2011) HBV and HCV infection in type 2 diabetes mellitus: a survey in three diabetes units in different Italian areas. *Acta Diabetol* 48: 337–343.
55. Knolle PA, Gerken G (2000) Local control of the immune response in the liver. *Immunol Rev* 174: 21–34.
56. Liaskou E, Wilson DV, Oo YH (2012) Innate immune cells in liver inflammation. *Mediators Inflamm* 2012: 949157.
57. Lanthier N, Molendi-Coste O, Horsmans Y, van Rooijen N, Cani PD, et al. (2010) Kupffer cell activation is a causal factor for hepatic insulin resistance. *Am J Physiol Gastrointest Liver Physiol* 298: G107–116.
58. Neyrinck AM, Cani PD, Dewulf EM, De Backer F, Bindels LB, et al. (2009) Critical role of Kupffer cells in the management of diet-induced diabetes and obesity. *Biochem Biophys Res Commun* 385: 351–356.
59. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, et al. (2010) Diabetes and cancer: a consensus report. *CA Cancer J Clin* 60: 207–221.
60. Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ (2004) Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* 159: 1160–1167.
61. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R (2009) Diabetes and cancer. *Endocr Relat Cancer* 16: 1103–1123.
62. Zhang F, Xu X, Zhou B, He Z, Zhai Q (2011) Gene expression profile change and associated physiological and pathological effects in mouse liver induced by fasting and refeeding. *PLoS One* 6: e27553.
63. Audic S, Claverie JM (1997) The significance of digital gene expression profiles. *Genome Res* 7: 986–995.
64. RDC T (2010) R: A Language and Environment for Statistical Computing. Vienna Austria R Foundation for Statistical Computing.
65. Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4: 44–57.
66. Dennis G, Jr., Sherman BT, Hosack DA, Yang J, Gao W, et al. (2003) DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4: P3.
67. Kanehisa M, Goto S, Hattori M, Aoki-Kinoshita KF, Itoh M, et al. (2006) From genomics to chemical genomics: new developments in KEGG. *Nucleic Acids Res* 34: D354–357.
68. Osborne JD, Flatow J, Holko M, Lin SM, Kibbe WA, et al. (2009) Annotating the human genome with Disease Ontology. *BMC Genomics* 10 Suppl 1: S6.
69. Shannin P, Markiel A, Ozier O, Baliga NS, Wang JT, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13: 2498–2504.