

The transmission of hepatitis B virus (HBV) infection from mother-to-infant (MTI) and the susceptibility of offspring to hepatitis B under intrauterine exposure to HBsAg

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Abstract. – OBJECTIVE: Hepatitis B virus (HBV) causes long-term injury to the liver in patients with chronic hepatitis B. It was reported that nearly half of this disease's cases now result from mother-to-infant (MTI) transmission. Therefore, intervention during this period of transmission of HBV could effectively prevent HBV infection in infants.

MATERIALS AND METHODS: This study employed bioinformatics methods to analyze the datasets of MTI hepatitis B transmission obtained from the Gene Expression Omnibus (GEO) database. Through this analysis, we extracted valuable information to identify genes exhibiting differential expression and uncover the associated signal pathways. Ultimately, our investigations into alterations in immune function shed light on the underlying mechanisms of MTI HBV transmission.

RESULTS: There were 593 genes that were significantly differentially expressed (512 up-regulated genes and 81 down-regulated genes) in the offspring CD8+T cells with Hepatitis B surface antigen (HBsAg) intrauterine exposure. The pathways enriched for differentially expressed genes have been revealed. Furthermore, we performed a correlation analysis between differentially expressed genes and maternal hepatitis B inheritance *via* the weighted gene co-expression network analysis (WGCNA) and eventually found a high correlation between the cyan module and the shape. Among them, there were 166 genes in the cyan module, which were mainly enriched in the phosphatidylinositol signaling system, glycerolipid metabolism, and other types of O-Glycan biosynthesis.

CONCLUSIONS: Therefore, we speculated that these signaling pathways and the genes within may be closely related to hepatitis B susceptibility and maternal hepatitis B inheritance. In this study, we showed that differentially expressed genes and signaling pathways may be valuable in preventing MTI transmission of HBV.

Key Words:

Hepatitis B, MTI transmission, Immune system, Signaling pathways.

Introduction

Among 7.2 billion people worldwide, about two billion have had previous HBV infections, and 350 million are chronically infected with it^{1,2}. About 30-50% of chronic HBV infections are transmitted from mother to child^{3,4}. The Hepatitis B vaccine (HBVAc) has effectively controlled prenatal and postpartum HBV transmission, but intrauterine HBV infection remains the leading cause of failure in neonatal immune blocking^{5,6}. With few exceptions, the majority of newborns who acquire intrauterine infection will become lifelong carriers of HBV. Eventually, approximately 20-25% of these cases will develop cirrhosis and liver cancer, which can be attributed to the persistence of chronic hepatitis B. Currently, there are still a significant number of HBV-infected females of childbearing age in China, leading to the transmission of HBV to their newborns through mother-to-infant (MTI) transmission. Among these cases, 5-40% of MTI transmissions are caused by intrauterine infection. This issue poses a social problem that not only jeopardizes the quality of the birth population but also impacts the harmony and stability of families and society in China⁷⁻⁹.

Despite the significant efficacy of active and passive immunoblockings in preventing MTI hepatitis B, the poor response to the hepatitis B vaccine in some pregnant patients remains a primary reason for the failure in completely ceasing

the newborns' MTI hepatitis B transmission¹⁰⁻¹². Besides, newborns' immune tolerance and/or other factors resulting from intrauterine HBV infection might also lead to neonatal immunization failure. Contextually, the optimal means to avoid fetal intrauterine HBV infection would be a timely and accurate rapid diagnosis. Previous evidence^{13,14} pointed out that pregnant patients' DNA level of serum HBV and their positive hepatitis B E antigen (HBeAg) are the indexes closely relevant to the HBV MTI transmission risk, and these two factors are the dominant players in the course of the intrauterine HBV transmission through chorionic capillary endothelial cells. The occurrence of MTI hepatitis B transmission happens in all newborns when their mothers are HbeAg-positive, especially when the mothers' viral loads are higher than 10^8 copies/mL. Both positive maternal HBV DNA and HbeAg are risk factors for HBV intrauterine transmission^{15,16}. It was reported that pregnant women with viral loads $>10^5$ copies/mL have a notably higher intrauterine transmission incidence than those with viral load $<10^5$ copies/mL. A debate still exists regarding the effect of delivery mode on the MTI transmission of HBV^{17,18}. The cesarean section would effectively reduce the risk of HBV intrauterine transmission^{19,20}.

In this study, we used biological information to analyze whether intrauterine HbsAg exposure affects the immune response of offspring to HBV and its related mechanisms, which is crucial to prevent MTI transmission of HBV.

Materials and Methods

Datasets

In this study, datasets related to hepatitis B in maternal and infant cohorts were screened from the GEO database (available at: www.ncbi.nlm.nih.gov/geo). The GSE162718 dataset was chosen as the analysis object. The data set was made up of two groups: liver cells from C57BL/6 mice and liver cells from C57BL/6-TG (HBV ALB-1) Bri44 transgenic mice expressing HBS.

Variation Analysis

In this study, we fetched differentially expressed genes amid the different comparison and control groups using the R software package DESeq2 (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). DESeq2, a

method based on negative binomial distribution generalized linear model, is used for differential expression screening. During this phase, we obtained the genes from the expression profile data set whose expression value was 0 and their proportion $>50\%$ were removed. The input matrix was constructed *via* the DESeq DataSet from the Matrix function, and then these data were standardized using the DESeq function. The significance of each gene was determined based on the results obtained from the difference analysis.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

We mapped genes into the background set and used the R software package Cluster Profile R (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria) for gene set enrichment analysis. An analysis of gene set functional enrichment was conducted using GO annotations in the R software package org.hs.eg.db (version 3.5.1), and genes were mapped into the background set as a reference set. Gene set enrichment analysis was conducted using the R package Cluster Profiler (version 3.5.1)^{21,22}.

WGCNA

The aim of this section was to estimate the Median Absolute Deviation (MAD) of each gene according to its expression spectrum. After the analysis, the top 50% of genes with the smallest MAD values were disregarded or excluded from further consideration. Through the Good Samples Genes method of the R software package WGCNA, outliers and samples were removed. We combined modules with a distance <0.25 ; finally, co-expression modules were obtained. In the context of the study, the Grey module refers to a group of genes that could not be clearly associated or assigned to any specific biological function or process. These genes exhibited a level of complexity or ambiguity in their roles, making it challenging to categorize them into known gene sets with defined functions^{23,24}.

Statistical Analysis

All statistical analyses were completed using SPSS version 27 (IBM Corp., Armonk, NY, USA) and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). A *p*-value lower than 0.05 was considered the threshold for statistical significance.

Results

Intrauterine Exposure of HBsAg to Regulation of Progeny Gene Expression Profile

Our study aimed to perform a bioinformatics analysis to determine the consequence of intrauterine HBsAg exposure affecting the immune response of the offspring to HBV and to reveal the underlying mechanisms, which would be meaningful for MTI HBV transmission prevention. We found that there were 593 differentially expressed genes (512 up-regulated and 81 down-regulated genes) in the group of the offspring CD8⁺T cells with HBsAg intrauterine exposure in contrast to the control (Figure 1).

Differential Genes were Analyzed by GO and KEGG

By performing GO analysis of down-regulated genes, we found that most of these genes were enriched in macromolecule localization, protein localization, nuclear part, nuclear lumen, catalytic activity, enzyme binding, and other functional pathways. The KEGG analysis revealed that the genes *MAP2K6*, *NFATC3*, *ITPR2*, *IKBKE*, *ISG15*, *TUBB2A*, *SMYD3*, *KMT5B*, and *CD28* exhibited significant enrichment. These genes were mainly associated with various pathways, including Kaposi sarcoma-associated herpesvirus infection, PD-checkpoint pathway in cancer, L1 expression and PD-1, C-type lectin receptor signaling pathway, cellular senescence, Hepatitis B, lysine degradation, Retinoic acid-inducible gene I (RIG-I)-like receptor signaling pathway, Epstein-Barr virus infection, human immunodeficiency virus 1 infection, and Gap junction signaling pathways (Figure 2).

By performing GO analysis of the up-regulated genes, we found that these genes were rich in system development, nervous system development, plasma membrane part, cell projection, cytoskeletal protein binding, calcium ion binding, and other functional pathways. The up-regulated genes primarily exhibited significant enrichment in several pathways and biological processes, including axon guidance, arrhythmogenic right ventricular cardiomyopathy (ARVC), glutamatergic synapse, morphine addiction, insulin secretion, renin secretion, cell adhesion molecules (CAMs), purine metabolism, ErbB signaling pathway, and hypertrophic cardiomyopathy (HCM) (Figure 3).

WGCNA Method was Used to Analyze Differentially Expressed Genes

Subsequently, we further analyzed the differentially expressed genes through WGCNA and then divided the differentially expressed genes into multiple gene modules. We analyzed the correlation between different modules and conducted a correlation analysis between modules and samples. From the Cyan module, we extracted 166 genes (Figure 4). Subsequently, we conducted an in-depth analysis of the signaling pathway involving 166 genes within this module. Our findings revealed that these genes are significantly enriched in several biological pathways, including phosphatidylinositol signaling system, glycerolipid metabolism, mismatch repair, glycerophospholipid metabolism, Notch signaling pathway, palpation, pyrimidine metabolism, breast cancer, phospholipase D signaling pathway, and other types of O-Glycan biosynthesis pathways. Signaling pathways and genes within these pathways may be related to susceptibility to hepatitis B and maternal transmission of hepatitis B (Figure 5).

Discussion

MTI transmission of HBV refers to the transmission process of HBV from infected pregnant women to their offspring by means of intrauterine infection, birth canal infection, or postpartum transmission, also known as vertical transmission^{25,26}. This HBV transmission path was first discovered in 1975, wherein there were 63 cases of HBsAg-positive infants born to 158 HBV-carrying mothers, and 51 of the 63 infants were HBsAg positive for six months²⁷. Thus, it was confirmed that HBV is transmitted from infected mothers to their babies, i.e., HBV could be transmitted through perinatal MTI transmission. In areas with a high prevalence of HBV infection, HBV is often transmitted perinatally from mother to child, which is the main mode of transmission in China. Infants infected with HBV during the perinatal period and early childhood are prone to developing chronic hepatitis B infection because of their underdeveloped immune systems. Therefore, preventing infantile HBV infection by blocking HBV transmission from mother to child will likely reduce the occurrence of chronic hepatitis B²⁸⁻³⁰.

HBVac and hepatitis B immunoglobulin (HBIG) are two ways to stop MTI transmission.

The transmission of hepatitis B virus (HBV) infection from mother-to-infant (MTI)

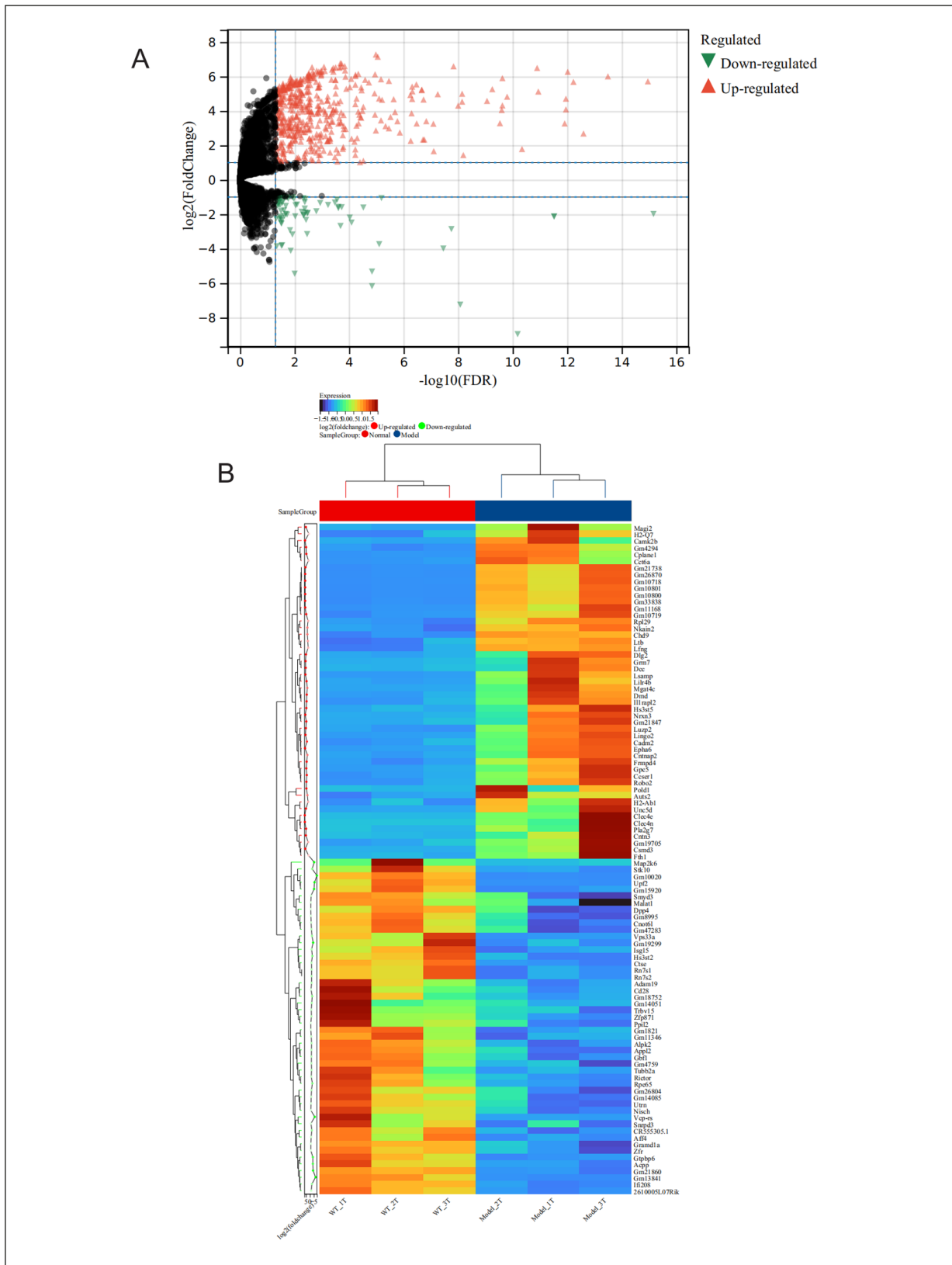


Figure 1. Analysis onto those genes of differential expression relevant to the progeny CD8⁺ T cells in MTI HBV transmission. **A**, Differentially expressed genes are plotted on a volcano plot. **B**, Differentially expressed genes heat map analysis.

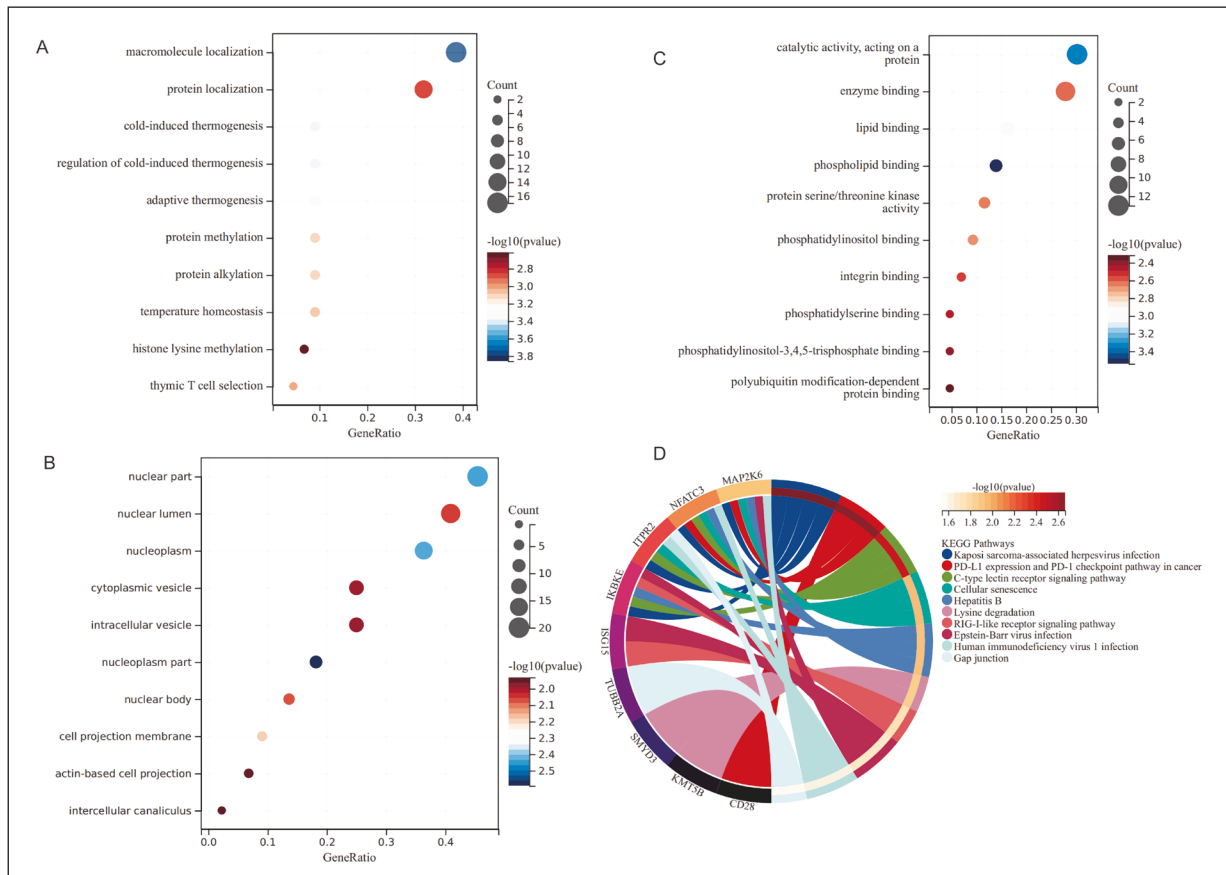


Figure 2. Go analysis onto those down-regulated genes relevant to the progeny CD8⁺ T cells in MTI HBV transmission. **A**, GO analysis, Biological Process. **B**, GO analysis, Cellular Component. **C**, GO analysis, Molecular Function. **D**, KEGG analysis results.

Blood-derived HBVac was approved for clinical application in 1981; HBVac recombinant protein was introduced in 1986 and is now used worldwide. After exposure to HBV, HBIG provides passive immunity with temporary protection (3-6 months)^{31,32}. The HBsAg immunogenic components in HBVac cannot immediately activate the immune system to produce sufficient anti-HBs, but the passive immunization with HBIG prior to this is capable of neutralizing the HBV that may come from the mother, which is an important means to block the MTI transmission. Scholars³³ have shown that the combined immunoblocking strategy of HBIG with HBVac is effective in avoiding MTI transmission after delivery. The effective rate of HBVac and HBIG combined immunoblocking is 94%, while the effective rate of HBVac or HBIG alone is 75% and 71%, respectively³³. Multiple research studies^{34,35} have demonstrated that infants born to mothers who are positive for HBsAg and HBeAg can be

effectively protected through a combination of HBIG and HBVac immunization, or through the administration of Hepatitis B vaccine alone. These studies^{34,35} have provided a scientific basis for the implementation of combined immunization strategy. However, there were still failures in immune blocking; hence, it is very important to study the effects of MTI and intrauterine transmission on the offspring's immune cells. HBsAg intrauterine exposure resulted in 593 genes with notably differential expression in the offspring CD8⁺T cells of this study. These genes may be closely related to hepatitis B susceptibility and maternal hepatitis B inheritance. Our findings enhanced our understanding towards the biological aspects of MTI HBV transmission and laid the foundation for further development of effective HBVac or prevention strategies.

Finally, we used WGCNA to further analyze the association between differential genes and traits. A close relationship was observed

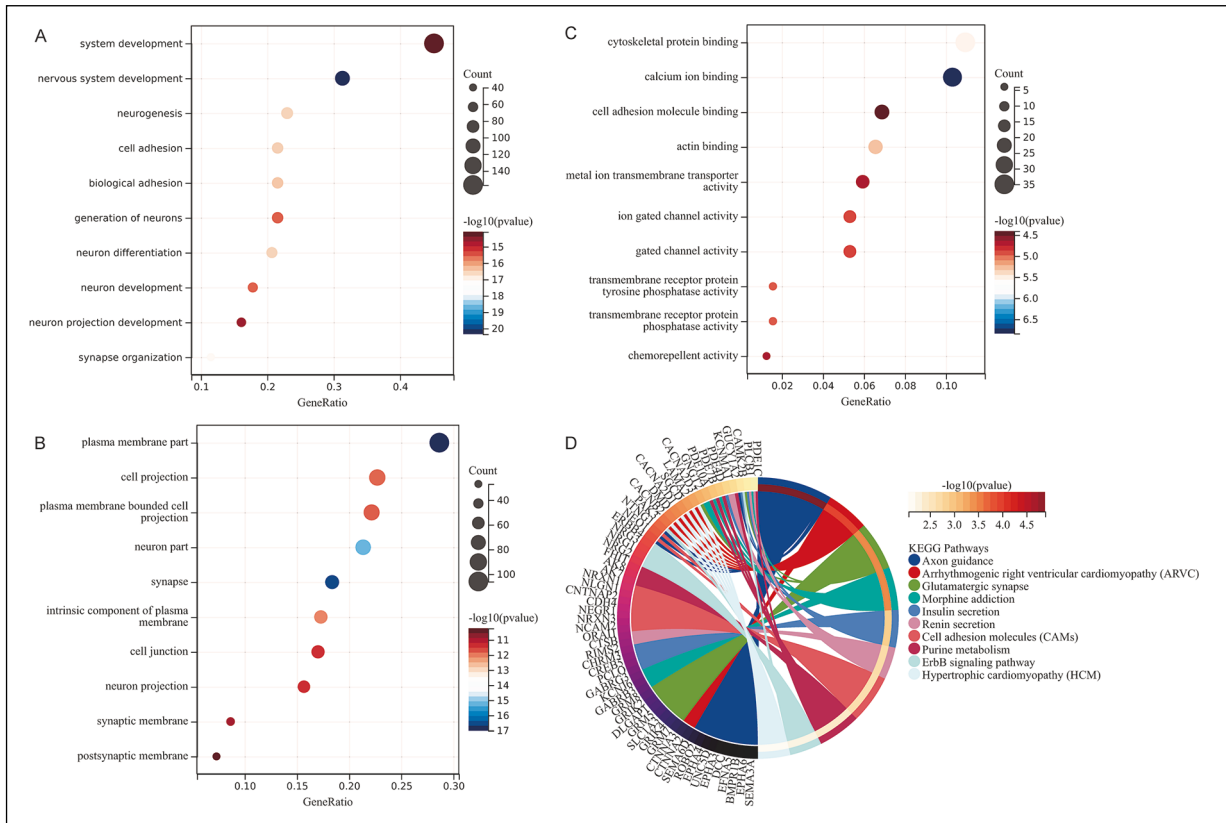


Figure 3. Go analysis onto those up-regulated genes relevant to the progeny CD8+ T cells in MTI HBV transmission. **A**, GO analysis, Biological Process. **B**, GO analysis, Cellular Component. **C**, GO analysis, Molecular Function. **D**, KEGG analysis results.

between the cyan module and these signaling pathways and the genes, indicating the role of maternal hepatitis B inheritance in hepatitis B susceptibility. Gene association patterns within different sources can be described using WGCNA, a systems biology method; in practice, this method is implemented for the identifications of highly covarying gene sets and the candidate biomarker genes or therapeutic targets in light of interconnectedness of gene sets and the relevance within gene sets and phenotypes. Unlike those research methods that only focus on gene differential expression, WGCNA is able to retrieve information from thousands or nearly 10,000 genes with the largest variation or all genes, which is an effective way of identifying the gene sets of interest and conducting significant association analysis with phenotypes. The differentially expressed genes and signaling pathways screened in this study can provide some reference for clinical diagnosis and treatment. But this is also the limitation of this study

because we have not been able to verify the expression or function of these differential genes and signaling pathways in animal models or cell models, or even clinical samples. In future research, we will focus on in-depth research in this area.

Conclusions

Our study revealed that there were 512 and 81 genes, notably up-regulated and down-regulated, respectively, in the CD8+T cells from the offspring mice that underwent HBsAg intrauterine-exposure treatment. Out of the 166 differentially expressed genes, a significant correlation was observed between these genes and the shape-related characteristics under investigation. These genes showed prominent enrichment in various biological pathways, including the phosphatidylinositol signaling system, glycerolipid metabolism, mismatch repair, phospholip-

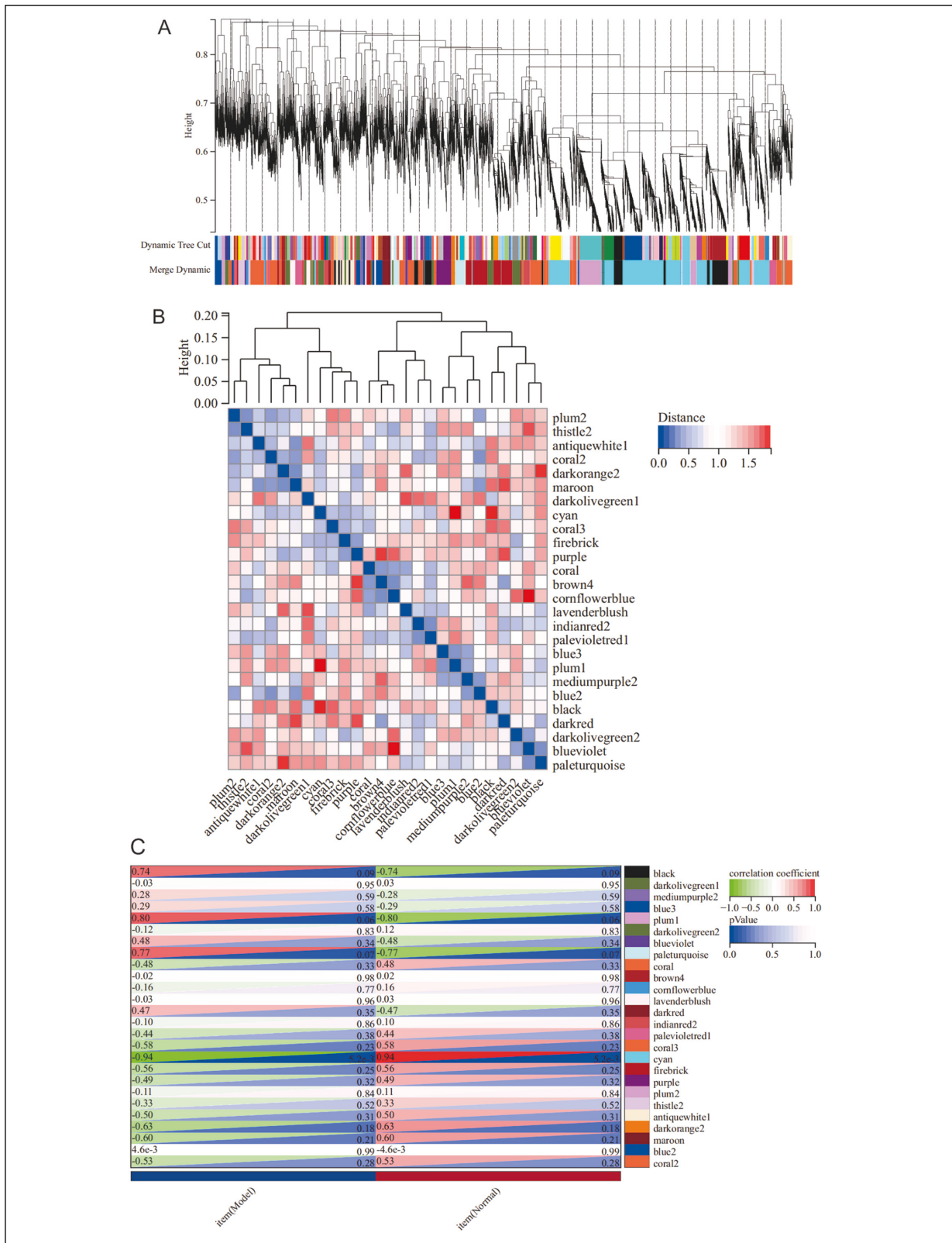


Figure 4. Associations between gene co-expression networks and traits of samples analyzed by WGCNA. **A**, On the basis of weighted correlations, hierarchical clustering was performed; and several gene modules were derived from the clustering results based on the set criteria. **B**, Co-expression module analysis. **C**, module and sample trait association analysis.

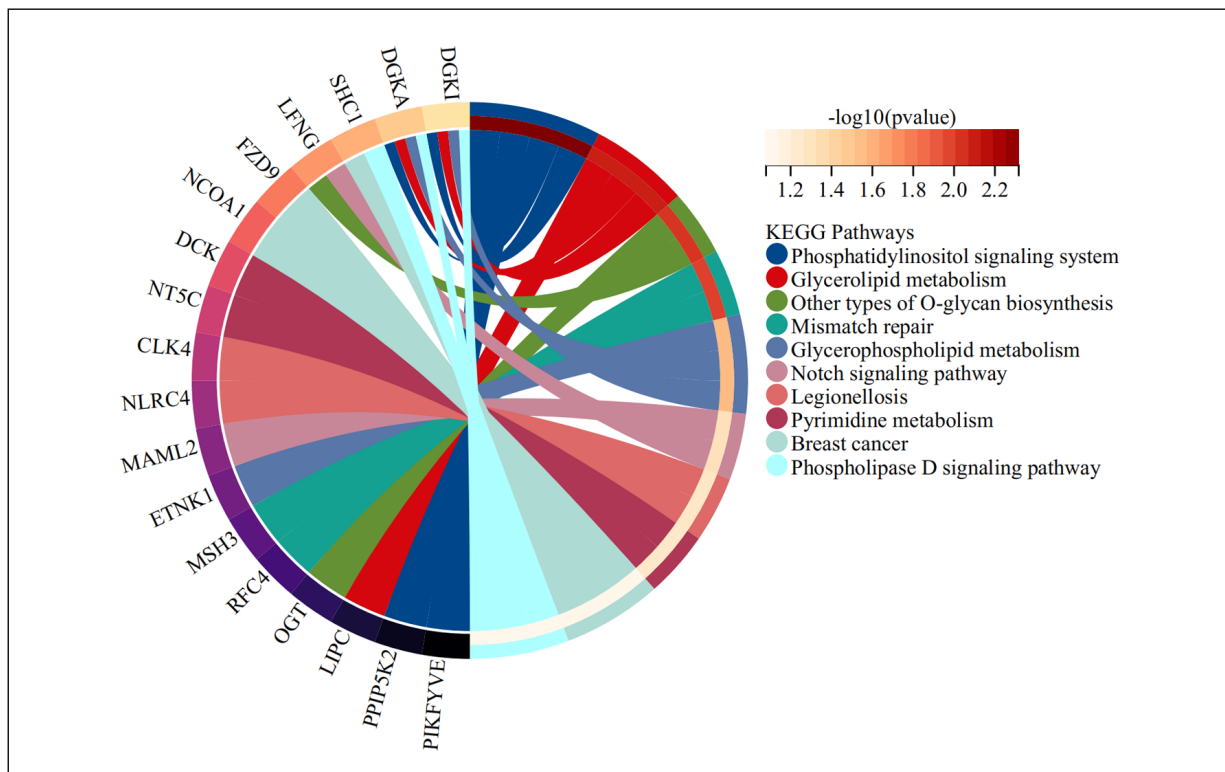


Figure 5. KEGG analysis of hub genes. The KEGG pathway enrichment circle diagram shows the hub genes and their signaling pathways.

id metabolism, Notch signaling pathway, legionellosis, pyrimidine metabolism, breast cancer, phospholipase D signaling pathway, and other types of O-Glycan biosynthesis. Those signaling pathways and the genes were considered relevant and pinpointed a correlation between hepatitis B susceptibility and infection of an infant with HBV from its mother. And we also believed that our findings would be a valuable clue for MTI HBV transmission prevention.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

MZ and HW contribute equally to this study. ML designed the experiments. MZ and HW performed data analysis. MZ and HW wrote the manuscript.

Ethics Approval and Informed Consent

Not applicable.

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