Metabolic Effect and Mechanism of Gestational Diabetes Mellitus on Offspring of Different Sexes

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Research

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Abstract

Introduction

Gestational diabetes mellitus (GDM) is a common pregnancy-related complication that can seriously endanger the health of the mother and child. Studies have reported that offspring have varying sensitivities to high blood sugar in utero based on their sex. However, the underlying pathogenesis of metabolic diseases is still largely unknown. Therefore, this study aims to study the metabolic influence and mechanism of gestational diabetes on male and female offspring, which is beneficial in preventing or reducing the possibility of metabolic diseases among the offspring of mothers with GDM through long-term medical monitoring.

Methods

Research samples meeting the experimental ideas were evaluated and selected from GEO database. After sample pretreatment, enrichment analysis was performed using R software to further enrich the differentially expressed genes (DEGs), and further research on the biological processes and molecular pathways related to these genes was conducted through GO analysis and KEGG analysis. Following this, a protein–protein interaction (PPI) network of the DEGs in the STRING database was constructed and then refined using Cytoscape software. The CytoHubba software was then used to screen out the top 10 hub genes. At last, Gene set enrichment analysis (GSEA) was performed using GSEA software (v. 4.0) to further understand the molecular mechanism of the disease.

Results

A total of 718 different genes were selected from GSE150621, including 454 and 264 genes with up-regulated and down-regulated expressions, which were statistically significant. Based on the data from the STRING database, the top 10 genes with the highest degree of connectivity, including OAS1, OAS2, OAS3, RSAD2, MX1, IFIT1, IFIT2, IFIT3, XAF1, and ISG15, were selected. The relative expression levels of IFIT1, OSA1, and ISG15 are relevant to the prognosis of GDM patients and the potential occurrence of some metabolic diseases in their offspring.

Conclusions

The accumulation of OAS1, IFIT1, and ISG15 genes suggests that a chronic inflammatory response is a requisite part of the GDM process. However, this is not clearly related to the metabolic mechanisms of different gender offspring of mothers with GDM; therefore, this is subject to further research.

Introduction

Gestational diabetes mellitus (GDM) refers to the varying degrees of abnormal glucose metabolism that first occur or are discovered during pregnancy ^[1]. There are quite a few causes for this disease, such as inflammation, immune-mediated disease, irritability, and metabolic abnormalities ^[4]. The incidence of

GDM varies from country to country, ranging from 1 percent to 14 percent. In particular, the prevalence of gestational diabetes among women of reproductive age in the United States is 9%, of which an estimated 2–5% are GDM. In Europe and China, the incidences of GDM are 2–6% and 1–5%, respectively [2]. Regrettably, the number continues to rise each year.

Gestational diabetes has adverse offspring outcomes in the short and long term, and the mechanism is relatively complex. From the existing research, it is clear that because a large amount of glucose is more easily transferred to the placenta, the fetus maintains the blood glucose level by producing and releasing more insulin from the pancreas. It is the development of fetal hyperinsulinemia that triggers most fetal problems, which are collectively referred to in medicine as diabetic malformations. Among them, it may be the growth-promoting activity of fetal insulin that results in disproportionate fetal growth. This gives rise to a large subcutaneous fat increase and shoulder width, such that the fetus is found to have shoulder dystocia at birth. In addition, the offspring of mothers with gestational diabetes are more likely to suffer from respiratory distress syndrome and other preterm birth problems, possibly requiring abortion.

Nevertheless, the influence of GDM on offspring does not end upon delivery. Studies have confirmed that GDM affects the long-term health of offspring in many aspects, such as glucose metabolism, body weight, blood pressure, and neurocognition. However, this effect is not uniform in all offspring of pregnant women with GDM. Lingwood Be et al. [5] have reported that, due to the metabolic differences between male and female newborns, different susceptibility to hyperglycemia in utero may also lead to long-term gender and metabolic differences. However, there is a lack of research on whether the different effects of GDM on gene expression will lead to variations in cell metabolism, and thus cause different effects, between male and female offspring. This is despite some speculation and research suggesting that mothers and children are more likely to develop certain diseases in the future, such as diabetes and obesity. However, the underlying pathogenesis of these metabolic diseases is still largely unknown, and there are no effective preventive measures as a result.

Considering the above, this study aims to present the metabolic influence and mechanism of gestational diabetes on offspring of different genders, thus preventing or reducing the possibility of metabolic diseases among the offspring of mothers with GDM through long-term medical monitoring and simultaneously minimizing the impact of potential diseases.

Materials And Methods

2.1. Materials

The original GEO dataset (GSE150621) was downloaded from the National Center for Biotechnology Information (NCBI) GEO database, which is dependable, influential, and publicly available. The dataset was compiled from a nested case-control study designed by the University of Pennsylvania. This study included a total of 14 samples quoted in platform GPL16791, which were divided into control and

infection groups. All the samples comprised amniocyte cells that were collected from expectant mothers during week 16–17 of their pregnancies and matched for offspring sex (male or female).

2.2. Microarrays and bioinformatics analysis

In the present study, the data downloaded from the GEO database was first grouped and then generally categorized as control or infected. Additionally, to explore the metabolic effects of GDM on offspring of different sexes, we grouped them as male or female. Following this, the R programming language was used to analyze the grouped sample data. Further, high-throughput sequencing codes were used for the differential expression analysis of the samples, and $P\text{-value} < 0.05$ and $|\log_2\text{FC}| > 1$ were set as the threshold. If the samples met the criteria, they were screened out as differentially expressed genes (DEGs).

2.3. GO enrichment analysis and KEGG pathway analysis

GO analysis is an effective method to annotate genes and gene products, that is, to describe genes and their products in detail. It shows the function of genes at three different levels: cellular components, molecular functions, and biological processes. This enables a preliminary understanding of the location, function, and specific biological processes of differential gene enrichment. KEGG Pathway Analysis comprises a systematic database of gene function analysis. It links differential genes with specific metabolic pathways and plays an important role in the study of disease mechanisms. The GO and KEGG analyses were mainly used for the enrichment analysis of samples during the course of the present study. $P < 0.05$ was considered a statistically significant criterion.

2.4. Mapping of the protein–protein interaction (PPI) network diagram

Exploring the functional interactions between proteins is significant for comprehending the molecular mechanisms of gestational diabetes mellitus (GDM). STRING is a fast and easy-to-use online resource (<https://string-db.org/>) that contains a large number of genes and their protein relationships. When different genes are fed into the platform, the connections between them can be instantly determined. To display the relationship between these different genes more clearly and aesthetically, the network diagram obtained via STRING was imported into Cytoscape to draw the PPI network diagram in the present study. This software allowed for changing the shape, color, line thickness, and overall frame of the protein to obtain a more closely connected and beautiful PPI network diagram. In addition, CytoHubba, a plug-in of Cytoscape, was used to select the top 10 genes as hub genes in this study.

2.5. Gene Set Enrichment Analysis (GSEA)

GO and KEGG can only locate functions, while GSEA hints whether the pathway is activated or inhibited by examining the expression level of genes in a predefined gene set in a certain state. What is more, the advantage of GSEA enrichment analysis is that it does not need a fixed threshold to filter genes. It is a method based on all genes analysis and avoids the shortcomings of traditional enrichment analysis

methods. In addition, we can understand the overall changes of pathways from GSEA analysis, and have a more comprehensive and objective understanding of the molecular mechanism of the disease.

Results

3.1. Screening of differential genes

A total of 718 different genes were chosen from GSE150621, including 454 and 264 genes with separate up-regulated and down-regulated expressions, which were statistically significant. The stated cutoff included $P\text{-value} < 0.05$ and $|\log_2FC| > 1$. All the DEGs were ranked according to the P-value, and the top 100 DEGs with $P\text{-value} < 0.05$ were selected (Figure 1). Furthermore, R language was used to create a heat map with red and blue nodes, which are associated with up or down-regulation of the above genes. Volcano plots of each group of DEGs were also generated (Figure 1).

3.2. GO enrichment analysis and KEGG pathway analysis

Within the female group, the results of the GO analysis demonstrated that the enrichment of the overlapping DEGs mainly took place in the molecular function (MF) and cell components (CC), especially in “receptor-ligand activity,” “signaling receptor ligand activity,” and “collagen-containing extracellular matrix.” As for biological process (BP), the enrichment mainly took place in “extracellular matrix organization,” “extracellular structure organization,” and “modulation of chemical synaptic transmission” (Figure 2). Within the male group, the DEGs were partly enriched in BP, such as “detoxification of copper ion,” “stress response to copper ion,” and “detoxification of inorganic compound.” Notably, there was a clear and significant down-regulation of CC, especially in “cell leading edge.” The results from the KEGG analysis of females showed that “cytokine–cytokine receptor interaction” was the most important pathway in which these genes were particularly enriched. As for males, the result showed that “sulfur metabolism” was the only pathway that was partly enriched.

3.3. PPI network analysis and screening of central genes

The Hub-protein from the PPI network was first screened. Then, using CytoHubba, a plug-in of Cytoscape, the top 10 most interconnected genes were obtained, including OAS1, OAS2, OAS3, RSAD2, MX, IFIT1, IFIT2, IFIT3, XAF1, and ISG15. The results revealed three most significantly expressed and closely linked genes: IFIT1, OAS1, and ISG15.

3.4. Gene Set Enrichment Analysis

GSEA revealed a variety of significantly enriched signals and different metabolic processes, which may help to explain the underlying molecular mechanisms of GDM.

Discussion

As one of the major obstetrical diseases, the prevalence of GDM is gradually increasing worldwide, in turn leading increased mortality and morbidity rates among mothers and their offspring. Recently, more and more reports show that the occurrence of GDM is not only due to metabolic disorders during pregnancy but also related to the chronic systemic inflammatory response mediated by proinflammatory cytokines [8]. Further and foremost, increasing research evidence shows that the immune system, through immune cells and their products such as cytokines and antibodies, exerts a vital part in modulating GDM [9–10]. Studies have ascertained that in the placental villi of GDM, the increase in IL-8 and decrease in IL-10 expression are associated with fetal loss, preterm delivery, and preeclampsia. However, no association was found between the gene expression and prevalence of gestational diabetes [11–12], and its effect on metabolism in late progeny remains unclear. Therefore, it was deemed essential to study the metabolic effects and mechanisms of gestational diabetes on offspring of different sexes.

In this study, the enrichment analysis revealed 10 differential genes related to GDM, including OAS1, OAS2, OAS3, RSAD2, MX1, IFIT1, IFIT2, IFIT3, XAF1, and ISG15. The results showed that the heterogeneity of OAS1 and IFIT1 was the most significant. Thus, based on the pathway and function enrichment analysis, the DEGs may play significant roles in receptor ligand activity, signaling receptor activator activity, and extracellular organization. Extracellular tissue mainly consists of the structure organization, matrix tissue, and structure tissue containing collagen.

The IFN system performs anti-virus, immunity regulation, anti-tumor, and cell proliferation inhibitory functions. The OASs are a family of IFN- and virus-induced proteins that consist of four genes: OAS1, OAS2, OAS3, and OASL [6]. Mainly activated by the secondary structure of double- or single-stranded RNA, OAS1 is an enzyme that plays a crucial role in innate antiviral defiance. When OAS1 is activated, it catalyzes the oligomerization of ATP into 2-5A and activates latent RNase L. In turn, it degrades viral and cellular RNA and blocks protein synthesis [7]. Further, it has been reported that extracellular OAS1 can enter cells independent of RNase L and directly inhibit viral proliferation without activating the known antiviral signaling pathways. It also has strong in vivo and in vitro antiviral activity [30]. Therefore, this study proposes that women with GDM are susceptible to viral infection during pregnancy due to low immune function, resulting in the release of OAS1 protein into the extracellular matrix to act on the corresponding receptor. This in turn inhibits the cell's RNA synthesis, leading to protein synthesis disorders. Additionally, the above is consistent the results obtained from the GO analysis in the present study, that is, DEGs mainly enriched in extracellular regions mediate receptor ligand activity as well as protein interactions.

IFIT1 is one of the upregulated genes in this study and the first identified interferon-stimulated gene (ISG). The interferon-induced protein with tetratricopeptide repeats (IFIT) family—which includes four members, IFIT1, IFIT2, IFIT3, and IFIT5—is an important component of the antiviral immune response. In fact, these genes are mostly silent in the majority of cell types but are greatly induced when the body is affected by a virus infection, an increase of interferons, and so on [13]. However, a previous study found that IFIT1 is a positive regulator of the IFNB1 gene and antiviral IFN gene program. In contrast, it negatively regulates

the expression of the pro-inflammatory cytokine TNF in genome-wide siRNA screening. Further, higher levels of TNF have been detected in the placenta of diabetic women, indicating that inflammation and hyperglycemia are responsible for the production of TNF in the full-term placenta^[17]. Therefore, according to the results of the GO and KEGG analyses in the present study, it was inferred that IFIT1 plays an important role in inhibiting the increase of inflammatory cytokines such as TNF and IFN, further reducing the continuous chronic inflammatory response in GDM. Besides, with respect to the effect of the induction of IFIT1, some reports proved that it mediates PPIs and the assembly of large protein complexes^[14]. This may also be one of the reasons why we can see the DEGs mainly enriched in the collagen-containing extracellular matrix in the GO analysis.

Like IFIT1, ISG15 is also one of the ISGs that functions as an antiviral effector^[16]. ISG15 is one of nearly 20 member proteins in the ubiquitin family. The family is divided into ubiquitin and ubiquitin modifications (UBLs), and it plays an indispensable role in regulating cellular activities such as protein stabilization, cell transport, cell cycle, and immune regulation^[18].

Further, it is because ISG15 can bind to the IFA receptor firsthand even when there is no preactivated matter that it is considered an outside-in signaling molecule. Additionally, ISG15 can be secreted or released by a variety of cell types, and these secretions of ISG15 are important for activating both congenital and adaptive immune responses. This is because they can stimulate the activity of natural killer cells and T lymphocytes and promote high IFN- γ secretion (type II IFN)^[26]. Nevertheless, it is unclear how extracellular ISG15 is released, and it is not possible to detect the specific type of cell that releases it. However, some reports suggest that they may be the product of a signal accompanied by type I interferon. The above is not only consistent with our results that the overlapping DEGs principally gather in extracellular regions but also confirm the important role of the extracellular matrix in the development of GDM. Thus, it can be inferred that the secreted ISG15 act on immune cells to further enhance the innate and adaptive immune response, causing changes in the innate immune environment of GDM offspring and, thus, adverse consequences for their growth.

Therefore, the present study analyzed the metabolic influence and mechanism of gestational diabetes on offspring of different genders, which can prevent or reduce the possibility of metabolic diseases among the offspring of mothers with GDM through long-term medical monitoring. However, this study has some limitations. The sample size is too small, and the data is insufficient. Further, the data were obtained from a publicly available database, and it is unclear whether the offspring of these women with GDM have other health problems that can interfere with the outcomes.

Conclusion

In this study, the gene expression of GDM in neonates of different sexes was explored by means of a biological information analysis, and the potential of gene expression as a credible molecular biomarker for the diagnosis and prognosis of metabolic diseases in the offspring was found through enrichment analysis. IFIT1 and OAS1 were revealed to be the most important two differentially expressed genes in

this study, which can provide direction in clinical work. However, the metabolic mechanism of GDM in offspring of different sexes needs further investigation.

Declarations

Ethics approval and consent to participate

GEO belongs to public database. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open-source data, so there are no ethical issues and other conflicts of interest.

Consent for publication

All the authors have read and approved the content, and agree to submit the whole article in your journal.

Availability of data and materials

The GSE150621 dataset used during the current study is available from GEO database(<https://www.ncbi.nlm.nih.gov/gds/>).

Declaration of competing interest

All the authors declare that there are no competing interests associated with the manuscript.

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Authors' contributions

Xu-Guang Guo provided the original idea and constructed the article outline. Ke-Ying Fang used R language to complete the figures and instructed the team members to write the paper and revise the manuscript. Zi-Qi Liu used online drawing tools to complete the figures and involved in searching literature and paper writing. Qi-Lin Hu involved in [searching literature](#), paper writing and table making. Zhi-Hao Chen involved in [searching literature](#), paper writing. Yuan Cai involved in [searching literature](#) and paper writing. All the authors were responsible for data analysis and manuscript constructing. All the authors read and approve the final version of the manuscript.

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Footnotes

Not applicable.

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Identification of differentially expressed genes (DEGs) in GSE150621. (a) Volcano plot of DEGs of females. The red nodes represent up-regulated genes. The blue nodes represent down-regulated genes. $p < .01$ and $|\log(FC)| > 1$ was set as the threshold. (b) Heat map of DEGs of females. (c) Volcano plot of DEGs of males. (d) Heat map of DEGs of males.

Figure 2

GO analysis and KEGG pathway analysis of the overlapping differentially expressed genes. (a) Results of GO analysis enrichment of DEGs of females. (b) Results of KEGG pathway enrichment of females. (c) Results of GO analysis enrichment of DEGs of males. (d) Results of KEGG pathway enrichment of DEGs of males.

Figure3

Figure 3

Results of the protein–protein interaction (PPI) network analysis. (a) PPI network of overlapping differentially expressed genes in female samples. (b) PPI network of overlapping differentially expressed genes in male samples.

Figure4

Figure 4

Gene set enrichment analysis of all the genes. (a) GSEA plot of females. (b) GSEA plot of males.

Figure 5

Top 10 hub genes selected by the CytoHubba in Cytoscape based on the degree of each protein node.

Supplementary Files

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