RESEARCH

The association between expression of lncRNAs in patients with GDM

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Abstract

Objective: Gestational diabetes mellitus (GDM) is common worldwide and seriously threatens maternal and infant health. The expression of non-coding (ncRNA) is tissue-specific and highly stable in eukaryotic cells and the circulatory system, which can act as an early molecular marker of GDM.

Methods: The differential expression of lncRNA and mRNA in the peripheral blood of patients with GDM (experimental group) and healthy pregnant women (control group) was analysed via lncRNA gene chip. Employing biological function clustering and KEGG signalling pathway analysis, we selected the mRNAs and lncRNAs closely related to the insulin signalling pathway of GDM to analyse the possible regulatory mechanism in the pathogenesis of GDM. The sequencing results were further verified via quantitative PCR (Q-PCR).

Results: LncRNA microarray analysis revealed 7498 genes (3592 upregulated, 3906 downregulated) differentially expressed in the GDM group and healthy pregnant women control group, including 1098 differentially expressed lncRNAs (609 upregulated, 489 downregulated). According to the regulatory pathway of the lncRNA mRNA network, 6 lncRNAs and 4 mRNAs were found to play a significant role in insulin resistance.

Conclusions: The lncRNAs ERMP1, TSPAN32 and MRPL38 form a co-expression network with TPH1, which is mainly involved in the tryptophan metabolism pathway and in the development of GDM. Moreover, lncRNA RPL13P5 forms a co-expression network with the TSC2 gene via the PI3K-AKT and insulin signalling pathways, which are involved in the process of insulin resistance in GDM.

Introduction

The burgeoning epidemic of gestational diabetes mellitus (GDM) threatens maternal and infant health. GDM is characterized by glucose intolerance, which causes poorly controlled diabetes during pregnancy. Insulin resistance is an early determinant of declining β-cell function (1); however, the differential expression of genes underlying these phenomena is not fully understood. Differences in genetic background likely explain these differences in gene expression (2, 3). After a critical point, insufficient insulin secretion and increased insulin requirements could lead to consequent hyperglycaemia in GDM (4). Although routine examination (via fasting or postprandial blood glucose) can be fast-evolving, this is rarely effective in making an accurate and timely diagnosis. A combination of one or more molecular markers is urgently needed to monitor insulin resistance in its early stages (5).

During pregnancy, glucose is a primary source of foetal energy. As the pregnancy progresses, a foetus’ need for glucose gradually increases. Therefore, this increase in maternal glucose consumption puts the mother in a state...
of ‘accelerated hunger’. Regulatory mechanisms, such as lipodieresis and glyconeogenesis, are needed by the mother to elevate foetal blood sugar during hypoaalimentation or a reduction in blood sugar. During the first trimester of pregnancy, only slight changes in blood sugar and insulin sensitivity are observed, whereas insulin secretion is higher and anabolic processes are prioritized, allowing for the storage of more fat and energy. In late pregnancy, the i.v. glucose tolerance test shows that insulin in the first and second secretory phases increases three-fold after stimulation possibly to compensate for insulin resistance and decreased sensitivity.

In DNA-templated organic synthesis, only 2% of the genome is transcribed into proteins, whereas the remaining 98% are called ncRNA (6). Conversely, IncRNAs are a collection of long noncoding exons (>200 nt). In the past, very few tools were available for large-scale sequencing of IncRNA (7). Thus, IncRNA was once seen as irrelevant ‘transcription noise,’ but it has since been widely implicated in regulating many of the genes that are responsible for metabolic processes. It does so by adjusting related protein-coding genes through a variety of ways at different levels; IncRNA and DNA bases can be inserted within three base pairs, thus influencing the expression of target genes (8).

Information on molecules that regulate gene expression allows for gene expression ranking. These molecules are ubiquitous at the epigenetic, transcriptional and post-transcriptional processes. For example, IncRNAs take part in almost all physiological and pathophysiological processes in an organism (9, 10, 11); they exhibit a tissue-specific expression and are stable when expressed in eukaryotic cells. Thus, they can be used as an early molecular marker of GDM.

The expression of IncRNA is stable in the blood (12), urine (13) and other body fluids and is even resistant to digestion by RNA enzymes (14). This characteristic allows it to be a non-invasive disease marker. Many IncRNAs with low expression levels are mainly located in the nucleus, and their sequence conservation has high specificity. Recent reports on IncRNAs associated with local gene regulation further support this view. In many cases, it is suggested that the regulatory locus controlling the expression of transcription or the DNA elements within the IncRNA have more activity (15). However, we found very few studies that have identified an association between insulin resistance in GDM and IncRNAs via high-throughput methods (i.e. microarray and RNA-seq).

In this study, IncRNA is monitored at the early stage of insulin resistance in GDM to explore the effects of the changes in susceptibility genes for diabetes and their expression. In our study, elevated glucose levels of oral glucose tolerance test (OGTT) were used as a diagnostic procedure for GDM. Plasma samples from women with and without GDM were collected, and a global genome microarray analysis revealed differentially expressed IncRNAs. In addition, a functional analysis of the altered molecular pathways was conducted. The potential functions of differentially expressed IncRNAs can be predicted.

Materials and methods

Study population

This research flow chart is summarized in Fig. 1. Case-control studies were conducted at the Inner Mongolia Autonomous Region People’s Hospital, Hohhot, China, from 8 October 2019 to 15 March 2020. Plasma samples were obtained from pregnant women both with and without GDM at 24–40 weeks. We measured the BMI, fasting plasma glucose (FPG) level, 1 h glucose load, 2 h glucose load, fasting insulin level, c-peptide level and glycylated haemoglobin level. In addition, a homeostatic model assessment for insulin resistance (HOMA-IR) was conducted. GDM was diagnosed according to the 2019 American Diabetes Association (ADA) criteria (16). All patients underwent an oral 75 g glucose tolerance test at 24–28 weeks with overnight fasting for 10 h. Plasma glucose test measurement was performed at 1 and 2 h after oral 75 g glucose tolerance. A GDM diagnosis was made when the plasma glucose values exceeded any of the defined thresholds (fasting: 92 mg/dL (5.1 mmol/L); 1 h: 180 mg/dL (10.0 mmol/L); and 2 h: 153 mg/dL (8.5 mmol/L)). Patients with complications of diabetes mellitus, chronic hypertension, multiple pregnancies, pre-eclampsia, obesity (BMI ≥ 30 (1)), and inflammatory diseases were excluded. This study was approved by the Institutional Ethics Committee of the Inner Mongolia Autonomous Region People’s Hospital.

Microarray data, screening and functional analysis of differentially expressed genes

The transcriptome profiles were selected from the venous plasma sample (n=6, three from GDM and three from control individuals). Venous vacuum blood collection (whole blood RNA tube, PAXgene blood collection) was performed to extract 2 mL of peripheral blood from the
patients. The total RNA from each sample was subjected to the NEB# E7335L, NEB# E6310L and NEB# E7760L Ultra Directional RNA Library Prep Kit (NEB, USA). Welch’s t-test was adopted for the analysis of microarray data and the identification of statistical specifications for significance. The differential expression of lncRNA and mRNA was screened for greater changes. A \( P \)-value of \( \leq 0.05 \) was considered statistically significant. The sequences of the clustered transcriptome assembly were compared with public databases. Gene ontology (GO) analysis was conducted for differentially expressed genes. The signalling pathways of these proteins and the functional categories of the unigenes were analysed using the KEGG database. The overall design of the four-plex experiments is illustrated in Fig. 1. Details of the procedures can be found in the supplementary materials.

**Construction of mRNA–lncRNA co-expression network**

The mRNA–lncRNA co-expression network was constructed based on the result of Pearson’s correlation analysis conducted on the differential expressions of lncRNAs and mRNAs. The mRNA–lncRNA pairs with a significant correlation coefficient were selected. The programme plots the fraction of the edges in a network graph with connections (edges) between all nodes in the network. The stronger the association of neighbouring genes or lncRNAs with a gene, the higher the degree and the more important the status.

A pathway interaction network named Path-Net is constructed based on interaction relationships between pathways in the KEGG database. The degree of the pathway is used as a criterion for assessing the pathway in Path-Net. 'Degree' refers to the number of relationships between a node on the network and surrounding nodes. The larger the degree, the more pathways that interact with it.

Pearson correlation analysis is carried out based on the expression differences of IncRNA and mRNA to construct an mRNA–IncRNA co-expression network. The differences in the co-expression network are used to analyse differences in expression regulatory mechanisms of these mRNA/IncRNA and identify the core positions of this mRNA/IncRNA in the co-expression network. In addition, the functions of surrounding mRNAs in the co-expression network are used to predict the function of unknown IncRNAs. mRNA–IncRNA pairs with significant correlation coefficient are selected and the expression correlation between mRNA and IncRNA is used to construct the co-expression network.

**RNA extraction and quantitative polymerase chain reaction (Q-PCR)**

Q-PCR was adopted to verify the microarray results in the GDM group (\( n = 3 \)) vs control group (\( n = 3 \)) with gene-specific primers. Total RNA was removed from the

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**Figure 1**
Research flow chart.
serum that had been stored at −80°C using Trizol. The differentially expressed lncRNAs and mRNAs were validated in an independent cohort. Q-PCR was performed using the ChamQTM SYBR qPCR Master Mix (vazyme Q311-02) and monitored using a GeneAmp PCR System 9700. The lncRNA with the best diagnostic value was selected as the biomarker.

**Statistical analysis of IncRNAs**

Data analysis was conducted using the SPSS software (version 18.0) (SPSS). All data were expressed as mean ± S.D. The expression level of the lncRNAs was calculated using the following formula: \( \Delta \Delta CT = \Delta CT \) (target gene) − \( \Delta CT \) (internal reference gene), where \( \Delta CT \) is the cycle number at which the fluorescence signal crosses the threshold, and \( \Delta \Delta CT \) is a simplified form of the relative fluorescence quantitative calculation formula that compares the difference or ratio between the different samples. \( P \)-values of \( < 0.05 \) were considered statistically significant.

**Results**

**Maternal characteristics**

A total of 44 cases were included in this study (25 patients with GDM and 19 healthy controls). The two pairs of peripheral blood samples were aged-matched. Pre-pregnancy BMI was calculated based on the self-reported weight and height before pregnancy, whereas FPG was examined on gestational week 36. No significant differences were observed between the GDM group and the control group. The sample for this data is outlined in Table 1.

**Quality of RNA data output**

The general data quality requirements for high-traffic sorting are as follows: Q30 > 85% indicates satisfactory data quality, with higher Q scores associated with a lower probability of error. The lncRNA and mRNA data of this sequencing were observed between the GDM group and the control subjects. A total of 7498 differentially expressed mRNAs (3592 upregulated and 3906 downregulated) and 1098 differentially expressed lncRNAs (609 upregulated and 489 downregulated) were found in this sequencing (Table 2). The cluster heatmap revealed the differential expression of two kinds of RNAs in the samples of the three control subjects and three patients with GDM. To specify the genes observed via differential screening, we conducted cluster analysis depending on the signal value of each gene in the sample (Fig. 2A, B, C and D).

In Fig. 2A and B, the genes identified on differential screening underwent clustering analysis according to the signal value of each gene in the samples. The absissa represents the sample names between the groups, whereas the ordinate represents the differentially expressed genes. Red indicates the high expression of differentially expressed genes, whereas green indicates the minimal expression of differentially expressed genes in the samples.

In Fig. 2C and D, upregulated genes with different multiples greater than 1.2 and \( P \)-values of \( \leq 0.05 \) are indicated by red dots. Those with a unique multiple less than 0.8333 and a \( P \)-value of \( \leq 0.05 \) are downregulated genes, which are indicated in green. The genes that were not significantly different are indicated by grey dots.

The differences were examined based on a \( P \)-value of \( < 0.05 \) and an absolute difference of \( +/− \) two-fold (i.e. log change > \( +/− 2.0 \)). A total of 3971 lncRNA–mRNA pairs with significant correlations in expression were screened. The functions of lncRNA were determined from the known functions of mRNA; functional enrichment analysis was conducted for significantly correlated mRNAs with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the study participants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>GDM (n = 25)</td>
</tr>
<tr>
<td>Age, years</td>
<td>31.68 ± 0.7499</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>23.79 ± 0.6881</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>5.739 ± 0.3522</td>
</tr>
<tr>
<td>1 h</td>
<td>10.82 ± 0.7044</td>
</tr>
<tr>
<td>2 h</td>
<td>9.264 ± 0.4022</td>
</tr>
<tr>
<td>INS (mU/L)</td>
<td>11.41 ± 1.129</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.62 ± 0.2714</td>
</tr>
</tbody>
</table>

| Functions of mRNA; functional enrichment analysis for significantly correlated mRNAs with differentially expressed genes. | |

To study the differences between the three control subjects and three patients with GDM, the two groups were compared, and differential gene screening was performed to methodically investigate lncRNAs in GDM. Transcriptome analysis was conducted to display the expression outlines of lncRNAs. To screen the differentially expressed genes, \( \log 2 \) fold change > 1.2 and \( P < 0.05 \) were used. The consequences of the microarray analysis established evident differences in the expression profiles of lncRNAs and mRNAs between patients with GDM and the control subjects. A total of 44 cases were included in this study (25 patients with GDM and 19 healthy controls). The two pairs of peripheral blood samples were aged-matched. Pre-pregnancy BMI was calculated based on the self-reported weight and height before pregnancy, whereas FPG was examined on gestational week 36. No significant differences were observed between the GDM group and the control group. The sample for this data is outlined in Table 1.

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differentially expressed lncRNA. Three GO terms, biological process (BP), cellular component (CC) and molecular function (MF), were utilized for the analysis using medium stringency settings. Biological process (BP) included 369 genes downregulated and 332 genes upregulated by BP, 90 downregulated and 65 upregulated by CC and 62 downregulated and 81 upregulated by MF (Fig. 3A and B). The top GO explanations were largely distributed amongst the MF, CC and BP subscriptions. Amongst the lncRNA-mRNA genes differentially sequenced, the top 10 had their expression upregulated and downregulated by BP. The functions related to selected genes are presented in Fig. 3C and D.

To create the KEGG database, Fisher’s exact test and chi-squared test were adopted to conduct pathway analysis. We analysed the significance of the pathway involved in the target genes. Pathway enrichment analyses were conducted based on the KEGG pathway analysis. Pathways with more explanations than anticipated with the differentially expressed genes (P < 0.05) are emphasized in Fig. 3E and F. Built on the KEGG analysis, 15 downregulated and 18 upregulated genes were screened.
Figure 2
Heatmap and volcano analyses of differentially expressed lncRNAs and mRNAs.

Figure 3
Microarray data, screening and functional analysis of differentially expressed genes.
The mRNA–lncRNA interaction network

The expressions of mRNA and lncRNA create differences between the lncRNA and mRNA express networks. The differences in the expressions reflect the differences in the regulatory mechanism of gene expression, pinpoint the mRNA/lncRNAs expressed in total in the core position on the network and allow the total peripheral mRNA express network to predict unknown lncRNA functions (Fig. 4). In the network, global and systematic pathway analysis can be carried out on the signal transduction relationships between significant pathways in the sample. A network map is used to show the interactions between significant pathways.

In the map, a circle represents a pathway, a line represents the relationship between pathways, red means the pathways where upregulated genes are located, blue means the pathways where downregulated genes are located, and yellow means a pathway containing both upregulated and downregulated genes.

We conducted an mRNA–lncRNA network analysis and created an interactive network covering the interaction between the differentially expressed lncRNAs and mRNAs. From this sub-network, six lncRNAs and four mRNAs were found to play a significant role in insulin resistance (Tables 3 and 4). The larger the degree, the more pathways that interact with it. We then designed primers to verify whether there was a differential expression (Table 5).

**Table 3** Partial results of the interaction between genes and lncRNAs.

<table>
<thead>
<tr>
<th>Gene1</th>
<th>Biotype1</th>
<th>Gene2</th>
<th>Biotype2</th>
<th>Correlation coefficient</th>
<th>Relationship</th>
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<td>TPH1</td>
<td>Coding</td>
<td>ENST00000214893.9_ERMP1</td>
<td>Noncoding</td>
<td>0.992773285</td>
<td>Positive</td>
</tr>
<tr>
<td>TPH1</td>
<td>Coding</td>
<td>ENST00000479508.5_TSPAN32</td>
<td>Noncoding</td>
<td>0.995332824</td>
<td>Positive</td>
</tr>
<tr>
<td>TPH1</td>
<td>Coding</td>
<td>ENST00000588620.5_MRPL38</td>
<td>Noncoding</td>
<td>0.996482412</td>
<td>Positive</td>
</tr>
<tr>
<td>TSC1</td>
<td>Coding</td>
<td>ENST00000632586.1_AC215522.2</td>
<td>Noncoding</td>
<td>0.998152693</td>
<td>Positive</td>
</tr>
<tr>
<td>TSC2</td>
<td>Coding</td>
<td>ENST00000412023.5_RPL13P5</td>
<td>Noncoding</td>
<td>0.989037361</td>
<td>Positive</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>Coding</td>
<td>ENST00000523340.1_SLC20A2</td>
<td>Noncoding</td>
<td>0.99553453</td>
<td>Positive</td>
</tr>
</tbody>
</table>
LncRNA expression patterns were verified via Q-PCR

After verification via Q-PCR of the GDM group (n = 3) vs control group (n = 3) with gene-specific primers (Table 5), we found that the data values (number 1) in the GDM group deviated from the other data values. This may be related to the less BMI of this person; other experimental groups were significantly small at the time of sample selection, and their insulin resistance index was also small. If we analyse this data together with the other data, the accuracy of the experimental results may be affected. Thus, we removed this outlier and conducted data analysis again.

Q-PCR was performed to compare the expression levels of lncRNAs between the GDM group (n = 2) and the control group (n = 3). As presented in Fig. 5A, Q-PCR revealed that four out of the six lncRNAs had significantly different expressions. The expressions of the lncRNAs ERMP1, TSPAN32, MRPL38 and RPL13P5 in the GDM group were significantly higher than those in the control group (P = 0.0486, 0.0096, 0.0371, 0.0075; P < 0.05). Conversely, the expressions of lncRNA AC215522.2 and SLC20A2 were higher in the GDM group than in the control group (P = 0.0840, 0.0538). There was no significant difference. Figure 5B presents the expression levels of the four mRNAs. The expression of mRNAs TSC1 and TSC2 in the GDM group was significantly greater than that in the control group (P = 0.0014, 0.0086; P < 0.05).

Discussion

Human placental lactogen (hPL) plays a role in promoting luteinizing hormone and glycogen synthesis. Prolactin (PRL) signalling has been involved in the regulation of glucose homeostatic adaptations to pregnancy (17). It has numerous biological functions, including lactation regulation, morphogenesis, reproduction, metabolism and adaptations to physiological stressors. PRL signalling regulates glucose metabolism through insulin signalling-related pathways.

hPL can stimulate insulin-like growth factor (IGF), insulin, adrenocortical hormone and pulmonary surfactant (PS) and enhance the acquisition of glucose and amino acid in the foetus to facilitate its growth and development. When glucose supply is insufficient, hPL stimulates fat decomposition, free fatty acid increase and gluconeogenesis, as well as inhibits the effect of insulin on peripheral tissues to increase blood glucose through
the reduction of its utilization, so as to ensure sufficient energy supply to the foetus. In patients with GDM, insulin resistance leads to dysglycaemia and decreased insulin sensitivity.

PRL acts on target cells by activating prolactin receptors (PRLRs). In mice, pancreatic PRLR signalling was shown to be required for pregnancy-associated changes in maternal \( \beta \) cell mass and function. PRLR gene disruption in the pancreas resulted in fewer insulin-producing cells, which were thus unable to expand appropriately during pregnancy, resulting in reduced blood insulin levels and maternal glucose intolerance (18). The function of \( \beta \) cell mass is resolved by placental lactogen (PL), lactogenic hormone and prolactin (19), which they bind to. Aside from this pathway, PRLRs can transduce their signal via the phosphatidylinositol 3-kinase (PI3K) pathways and RAS–RAF–mitogen-activated protein kinase (MAPK) (20).

A current study has demonstrated that the expressions of a large number of genes changed during pregnancy. The most significant genes induced during pregnancy are Tph1 and Tph2 (21), which encode two isomers of tryptophan hydroxylase, the rate-limiting agent for the synthesis of serotonin (5-hydroxytryptamine (5-HT)) (22). Hydroxylation of tryptophan to 5-hydroxytryptamine is catalysed by TPH. The study has demonstrated that \( \beta \) cells and serotonergic neurons shared a common gene expression programme as well as the ability to synthesize, store and secrete serotonin (22). By analysing the IncRNA–mRNA network, IncRNAs ERMP1, TSPAN32 and MRPL38 related to TPH were selected for further detailed study. In the current study, the expression profiles of IncRNAs ERMP1, TSPAN32 and MRPL38 exhibited significant differences between the GDM and control groups. The results revealed that IncRNAs ERMP1, TSPAN32 and MRPL38 all had pre-diagnostic values for GDM.

Pregnancy is accompanied by physiological changes in maternal plasma proteins. In normal pregnancy, the characterizing maternal plasma proteome is important to understand the changes in calculating pregnancy outcome. Pregnancy-associated plasma protein-A (PAPP-A) is a proteolytic enzyme that was first discovered as a placental protein of primates. It was found to increase the availability of activated IGF receptors by lysing lecithin. The insulin-like growth factor-binding proteins (IGFBPs) compete with the insulin-like growth factor receptor (IGFR) and are bound to IGFs (23), such as IGFBP4, which play a significant role in metabolism and ultimately regulate

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**Table 5** Primers for the analysis of mRNA and IncRNA by quantitative RT PCR.

<table>
<thead>
<tr>
<th>Amplification primer name</th>
<th>Amplification primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERMP1_F</td>
<td>GCACTGACACACTGGAATCAT</td>
</tr>
<tr>
<td>ERMP1_R</td>
<td>ACCCTGAAACCTTAGTGC</td>
</tr>
<tr>
<td>TSPAN32_F</td>
<td>ATGCTGGTCACTGCTTTT</td>
</tr>
<tr>
<td>TSPAN32_R</td>
<td>CTGGTACGGTCTGCTTCA</td>
</tr>
<tr>
<td>MRPL38_F</td>
<td>GCCGATGACCTGCTTCA</td>
</tr>
<tr>
<td>MRPL38_R</td>
<td>GCCGATGACCTGCTTCA</td>
</tr>
<tr>
<td>AC215522.2_F</td>
<td>AGCCGACACTTCCCCCTTT</td>
</tr>
<tr>
<td>AC215522.2_R</td>
<td>CAATTCAGGCTGCTGAGAGA</td>
</tr>
<tr>
<td>RPL13P5_F</td>
<td>CATGATCCTGAAAGCCACT</td>
</tr>
<tr>
<td>RPL13P5_R</td>
<td>GCCAAACTTCCAGACTGA</td>
</tr>
<tr>
<td>SLC20A2_F</td>
<td>CTCGAGGAACTTTAGGTTGAAGGTTG</td>
</tr>
<tr>
<td>SLC20A2_R</td>
<td>CCCACGAGGACCTACATC</td>
</tr>
<tr>
<td>IGFBP4_F</td>
<td>GGTCCACACACACGACTT</td>
</tr>
<tr>
<td>IGFBP4_R</td>
<td>CTCACAGCCTCATCAGACA</td>
</tr>
<tr>
<td>TSC1_F</td>
<td>ACCCTTGGTGGCTGCTTCA</td>
</tr>
<tr>
<td>TSC1_R</td>
<td>AGTGGCTACTCCGAGCTCT</td>
</tr>
<tr>
<td>TSC2_F</td>
<td>GCCATACCTTGTCTCGATGAT</td>
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<tr>
<td>TSC2_R</td>
<td>GTGTCCTGTTGGCTGTTACTT</td>
</tr>
<tr>
<td>TPH1_F</td>
<td>ATGGCAGGTATCTGCTCTG</td>
</tr>
<tr>
<td>TPH1_R</td>
<td>AGCCAGGACACTTACCCTAC</td>
</tr>
</tbody>
</table>

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**Figure 5** Q-PCR results showing relative IncRNA and mRNA expressions.
Being a stable and detectable RNA, ncRNA can be used to treat diseases by supplementing the expression of downregulated ncRNA and inhibiting the overexpression of ncRNA.

However, it should be noted that we are not sure if insulin resistance in all subjects is due to pregnancy and not obesity; more samples are needed to support this conclusion. Current research on ncRNA is not comprehensive, and its mechanism is not yet fully understood. Most of the studies lack a large sample size. Therefore, the clinical use of ncRNA for the prediction, diagnosis and prognosis of disease still needs further study and testing. Finally, the prognostic implications of our findings were not assessed in our study, as that needs long-term follow-up. We provide a new perspective to elucidate the underlying mechanism of insulin resistance in patients with GDM and, therefore, this point needs investigation in future studies.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent statement
Consent was obtained from each patient after a full explanation of the purpose and nature of all procedures used.

Author contribution statement
Li Yuanyuan, first author, contributions to this article: direct participation, including preparation and design of experiments, carrying out research, collecting data, analysing/interpreting data, statistical analysis, article writing, including drafting articles. Cheng Xingbo, corresponding author, contribution to this article: critically reviewing the intellectual content of the article, work support, technical support, guidance, supportive contribution. Li Dongmei, second author, contributions to this article: critically reviewing the intellectual content of the article, work support, technical support, guidance, supportive contribution.

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Authors contribution statement
Li Yuanyuan, first author, contributions to this article: direct participation, including preparation and design of experiments, carrying out research, collecting data, analysing/interpreting data, statistical analysis, article writing, including drafting articles. Cheng Xingbo, corresponding author, contribution to this article: critically reviewing the intellectual content of the article, work support, technical support, guidance, supportive contribution. Li Dongmei, second author, contributions to this article: critically reviewing the intellectual content of the article, work support: obtaining research funds, administrative support, guidance, supportive contribution.

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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent statement
Consent was obtained from each patient after a full explanation of the purpose and nature of all procedures used.

Author contribution statement
Li Yuanyuan, first author, contributions to this article: direct participation, including preparation and design of experiments, carrying out research, collecting data, analysing/interpreting data, statistical analysis, article writing, including drafting articles. Cheng Xingbo, corresponding author, contribution to this article: critically reviewing the intellectual content of the article, work support, technical support, guidance, supportive contribution. Li Dongmei, second author, contributions to this article: critically reviewing the intellectual content of the article, work support: obtaining research funds, administrative support, guidance, supportive contribution.

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