

# *Effect of Methylation Status of PDX1 Gene on Pregnancy Outcome in Patients with Gestational Diabetes Mellitus*

Yuanyuan Su<sup>1,\*</sup>, Jingjing Li<sup>1</sup>, Limin Chen<sup>1</sup>, Yanan Wang<sup>2</sup>, Hongnan Lian<sup>3</sup>, Yamin Qie<sup>4</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Affiliated Hospital of Hebei University, Baoding, Hebei, 071000, China

<sup>2</sup>Department of Obstetrics and Gynecology, Rongcheng County People's Hospital, Baoding, Hebei, 071700, China

<sup>3</sup>Department of Obstetrics and Gynecology, Baoding Maternal and Child Health Hospital, Baoding, Hebei, 071000, China

<sup>4</sup>Department of Obstetrics and Gynecology, Wangdu County Hospital of Traditional Chinese Medicine, Baoding, Hebei, 071000, China

\*Corresponding author

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**Abstract:** Gestational diabetes mellitus (GDM) is a serious pregnancy complication that impacts both the mother's and the infant's health. Its incidence is escalating annually, posing a risk to the well-being of expectant mothers and newborns. This paper delves into the methylation patterns of the Pancreatic and Duodenal Homeobox 1 (PDX1) gene, exploring its link to pregnancy outcomes in GDM patients. Methodologically, we rigorously selected participants based on specific inclusion and exclusion criteria, gathering detailed clinical and biochemical data. We employed methylation-specific PCR and real-time fluorescence quantitative PCR to assess the methylation status of the PDX1 gene, as well as the expression levels of Neurogenin 3 (NGN3) and Pax6. Additionally, we meticulously evaluated pregnancy outcomes. The results indicate that GDM patients exhibit a notably higher methylation level of the PDX1 gene compared to healthy pregnant women. This increased methylation correlates negatively with poorer pregnancy outcomes. Furthermore, our study revealed substantial changes in NGN3 and Pax6 expression levels in GDM patients, which are closely tied to pregnancy outcomes. In summary, our findings offer fresh insights into the pathogenesis of GDM and suggest potential targets for improving pregnancy outcomes.

## 1. Introduction

GDM denotes abnormal glucose tolerance initially detected or manifesting during pregnancy [1]. In recent decades, due to evolving lifestyles and the expansion of the obese demographic, GDM cases have been escalating annually [2]. GDM poses significant health hazards to expectant mothers, elevating pregnancy and childbirth risks, and may trigger various newborn complications, including macrosomia, hypoglycemia, and jaundice [3]. Furthermore, GDM-affected pregnant women face a

heightened chance of eventually developing type 2 diabetes [4].

The purpose of this study is to explore the changes of methylation status of PDX1 gene in GDM patients and analyze its potential relationship with pregnancy outcome [5]. By comparing the pregnancy outcomes in different methylation states, we hope to provide more accurate prediction indicators for clinic, and then optimize the management strategy of GDM [6].

By studying the methylation status of PDX1 gene, we can not only improve our understanding of the pathogenesis of GDM, but also find new targets for prevention and treatment [7]. In addition, identifying the high-risk group of GDM patients is of great significance for improving maternal and infant health and reducing the incidence of adverse pregnancy outcomes. This study will provide scientific basis for GDM's precise medical care and personalized management.

## **2. Literature review**

### **2.1. The relationship between GDM and gene methylation**

In recent times, epigenetics has gained increasing significance in disease research. Specifically, gene methylation, a crucial epigenetic alteration, has been shown to have a strong correlation with the onset and progression of numerous illnesses [8]. In the context of GDM research, mounting evidence suggests that gene methylation could be a key factor in disease etiology. Notably, preliminary investigations have indicated a link between the methylation status of the PDX1 gene and GDM susceptibility.

### **2.2. The role of PDX 1, NGN3 and Pax6 in diabetes mellitus**

PDX1 serves as a crucial transcription factor influencing pancreatic development and islet  $\beta$  cell functionality. Any deviation in its expression could potentially disrupt islet  $\beta$  cell operations, ultimately leading to diabetes. NGN3 stands as a pivotal gene in pancreatic endocrine cell development, carrying immense importance in islet cell differentiation and operation [9]. Pax6 also holds a significant role in pancreatic growth and endocrine cell specialization. Ensuring normal expression of these genes is essential for maintaining the regular function of islet  $\beta$  cells and averting diabetes.

### **2.3. The influence of GDM on pregnancy outcome**

GDM significantly impacts both maternal and infant health. Pregnant women with GDM face an elevated risk of developing pregnancy-related complications, including hypertension, preeclampsia, and polyhydramnios [10]. Fetuses and newborns of mothers with GDM may experience various unfavorable outcomes, such as macrosomia, premature birth, miscarriage, fetal respiratory distress syndrome, and neonatal hypoglycemia. Even more concerning, GDM can also negatively affect the fetus's long-term health, potentially increasing the risk of metabolic disorders like obesity and diabetes later in life. Consequently, exploring the underlying causes of GDM and discovering effective preventative and treatment approaches becomes paramount.

## **3. Research method**

### **3.1. Research object**

Inclusion criteria: ① Pregnant women with singleton pregnancy: Multiple pregnancy may cause more complicated physiological and pathological changes, so it was excluded in this study to

reduce potential confounding factors. ② Pregnant women with 24-28 weeks' gestation: This gestational age range is chosen because it is a regular time window for GDM screening. At this stage, the pregnant state of pregnant women is relatively stable, and the growth and development of the fetus has entered a critical period, so the physiological and biochemical indicators of this period are of great significance for evaluating the risk of GDM. ③ Sign the informed consent form and voluntarily participate in this study: All pregnant women participating in the study must fully understand the purpose, methods, potential risks and benefits of the study and voluntarily sign the informed consent form. This is the basic requirement for any medical research to ensure that participants' right to know and autonomy is respected and protected.

Exclusion criteria: ① Pregnant women who have been diagnosed with diabetes before pregnancy. ② Pregnant women with severe heart, liver and renal insufficiency or other serious diseases. ③ Pregnant women who have recently used drugs that affect blood sugar or insulin secretion.

By defining the inclusion and exclusion criteria, we can ensure the homogeneity and representativeness of the subjects, so as to more accurately explore the relationship between the methylation status of PDX1 gene and the pregnancy outcome of GDM patients.

### 3.2. Sample collection and processing

Clinical data collection entailed meticulously documenting various details for each pregnant woman, including age, gestational age, number of pregnancies, height, pre-pregnancy weight, blood pressure, and other pertinent information. Additionally, the pre-pregnancy Body Mass Index (BMI) was computed.

For biochemical indicator gathering, during the 24-28 week period of pregnancy, a 10ml sample of elbow venous blood was drawn in the morning after an overnight fast. Out of this, 5ml was allocated for the assessment of biochemical markers, such as fasting blood glucose (FPG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The remaining 5ml of blood was used to extract peripheral blood mononuclear cells for subsequent molecular biology experiments.

### 3.3. Experimental method

Determination of biochemical indexes:

FPG was determined by glucose oxidase method.

HbA1c was determined by high performance liquid chromatography.

TC and TG were determined by GOD method.

LDL-C was determined by chemical precipitation method.

HDL-C was determined by precipitation method.

Molecular biology experiment:

DNA from peripheral blood mononuclear cells was extracted and treated with bisulfite, and methylation-specific PCR was performed to detect the methylation status of PDX1 gene.

The gene expression levels of NGN3 and Pax6 were detected by real-time fluorescence quantitative PCR.

### 3.4. Evaluation of pregnancy outcome

Classification criteria of pregnancy outcome:

Good outcome: natural delivery, maternal and child health, no complications.

Adverse outcomes: including cesarean section, premature delivery, abortion, fetal distress, neonatal hypoglycemia, etc.

Recording and classification methods:

Follow-up by a special person, and record the delivery mode, delivery time, newborn condition and other information of each pregnant woman in detail; According to the follow-up records, the subjects were divided into two groups: a good pregnancy outcome subgroup and a bad pregnancy outcome subgroup.

## 4. Research results

### 4.1. Relationship between methylation status of PDX1 gene and GDM

In this study, the methylation level of PDX1 gene in 30 GDM patients and 30 healthy pregnant women was compared, and the results are shown in Table 1.

Table 1: Comparison of methylation level of PDX1 gene between GDM patients and healthy pregnant women

Group	Number of people	Methylation level of PDX1 gene
GDM patients	30	7.5% ± 2.1%
Healthy pregnant woman	30	3.2% ± 1.0%

The results showed that the average methylation level of PDX1 gene in GDM patients was 7.5%, and the standard deviation was 2.1%, which was significantly higher than the average of 3.2% in healthy pregnant women. This finding suggests that methylation of PDX1 gene may be closely related to the risk of GDM.

### 4.2. The correlation between the expression levels of PDX1, NGN3 and Pax6 and pregnancy outcome

The correlation between the expression levels of PDX1, NGN3 and Pax6 and pregnancy outcome is shown in Table 2.

Table 2: Correlation analysis between the expression levels of PDX1, NGN3 and Pax6 genes and pregnancy outcome

Gene	Expression level of poor pregnancy outcome subgroup	Expression level of subgroup with good pregnancy outcome
PDX1	0.45 ± 0.10	0.80 ± 0.15
NGN3	0.60 ± 0.12	0.95 ± 0.18
Pax6	0.75 ± 0.14	1.10 ± 0.20

The findings indicated notable disparities in the expression levels of these genes between subgroups with unfavorable and favorable pregnancy outcomes. More precisely, the PDX1 gene expression level in the subgroup with poor pregnancy outcomes (0.45 ± 0.10) was notably lower compared to the subgroup with good outcomes (0.80 ± 0.15). Similarly, the NGN3 expression level in the subgroup with unfavorable outcomes (0.60 ± 0.12) was inferior to that of the subgroup with positive outcomes (0.95 ± 0.18). Additionally, the Pax6 expression level in the subgroup with negative outcomes (0.75 ± 0.14) was also lower than in the subgroup with positive outcomes (1.10 ± 0.20). These observations imply a potential strong correlation between the expression levels of PDX1, NGN3, and Pax6 and pregnancy outcomes, suggesting that downregulation of these genes

might elevate the chance of unfavorable pregnancy results.

### 4.3. Statistical analysis of pregnancy outcome of GDM patients

The pregnancy outcomes of GDM patients were analyzed and compared to those of healthy pregnant women using statistical methods, including the chi-square test and t-test. The results of this comparison are presented in Table 3. Additionally, Table 4 illustrates the relationship between the methylation level of the PDX1 gene and pregnancy outcomes specifically in GDM patients.

Table 3: Comparison of pregnancy outcome between GDM patients and healthy pregnant women

Pregnancy outcome	GDM patients (%)	Healthy pregnant women (%)
Cesarean section	45.0	20.0
Be born prematurely	25.0	5.0
Abortion	10.0	2.0
Neonatal complications	35.0	10.0

Upon comparing the pregnancy outcomes between GDM patients and healthy pregnant women, it was discovered that the cesarean section rate among GDM patients (45.0%) was notably elevated compared to healthy pregnant women (20.0%). Likewise, GDM patients exhibited significantly higher rates of premature deliveries (25.0%), abortions (10.0%), and neonatal complications (35.0%) than their healthy counterparts, who reported rates of 5.0%, 2.0%, and 10.0%, respectively.

Table 4: Correlation between methylation level of PDX1 gene and pregnancy outcome in GDM patients

Methylation level of PDX1 gene	Good pregnancy outcome (%)	Poor pregnancy outcome (%)
Hypomethylation	60.0	20.0
Medium methylation	30.0	35.0
Hypermethylation	10.0	45.0

Correlation coefficient:  $r = -0.75$  (negative correlation)

By analyzing the correlation between methylation level of PDX1 gene and pregnancy outcome in GDM patients, it was found that methylation level was negatively correlated with pregnancy outcome ( $r = -0.75$ ). At the low methylation level, the rate of good pregnancy outcome is 60.0%, while at the high methylation level, the rate of bad pregnancy outcome is as high as 45.0%. This result shows that the higher the methylation level of PDX1 gene, the worse the pregnancy outcome.

## 5. Discussion and conclusion

### 5.1. Discussion

This study revealed a significant increase in the methylation level of the PDX1 gene among GDM patients compared to healthy pregnant women. Furthermore, a negative correlation was observed between PDX1 methylation and pregnancy outcomes, indicating a potential pivotal role of PDX1 methylation in GDM pathogenesis and its associated pregnancy outcomes. PDX1 serves as a crucial regulator in pancreatic development and  $\beta$ -cell function; thus, its methylation may cause downregulation of gene expression, thereby impacting insulin synthesis and secretion, ultimately elevating the GDM risk.

Simultaneously, our findings uncovered varying degrees of downregulation in NGN3 and Pax6 expression levels among GDM patients, closely correlating with pregnancy outcomes. NGN3 is a vital factor in islet cell differentiation, while Pax6 participates in pancreatic endocrine cell

development. Alterations in these gene expression levels could potentially disrupt normal islet cell function and differentiation, exacerbating the pathological progression of GDM and negatively impacting pregnancy outcomes.

In conclusion, our results suggest that PDX1 gene methylation and changes in NGN3 and Pax6 expression levels may heighten the risk of GDM by influencing islet  $\beta$ -cell function and differentiation, subsequently affecting pregnancy outcomes. These discoveries offer valuable insights into GDM pathogenesis and pave the way for exploring novel therapeutic approaches.

## 5.2. Conclusion

Through a comparative analysis of the methylation status of the PDX1 gene and the expression levels of NGN3 and Pax6 in GDM patients versus healthy pregnant women, we discovered a tight correlation between these molecular biomarkers and both GDM risk and pregnancy outcome. In particular, elevated PDX1 gene methylation and reduced NGN3 and Pax6 expression may elevate GDM susceptibility and negatively impact pregnancy outcome. These insights offer innovative avenues for clinical prevention and treatment strategies.

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