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Research Article

Association of MTNR1B Gene Polymorphisms and Expression with Gestational Diabetes Mellitus (GDM) Incidence: A Case-Control Study

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Abstract

Objective: This study aims to explore the relationship between MTNR1B gene expression and specific genetic polymorphisms, and their association with an increased incidence of Gestational Diabetes Mellitus (GDM).

Methods: A case-control study was conducted with 150 women diagnosed with GDM and 150 healthy controls. MTNR1B gene expression was quantified using quantitative PCR, and genetic polymorphisms were analyzed through SNP genotyping. Statistical evaluations were performed to determine the associations between MTNR1B variants and GDM incidence.

Results: The study found significant alterations in MTNR1B gene expression in GDM patients compared to healthy controls. Notably, the rs10830963 polymorphism in MTNR1B was significantly associated with a higher risk of GDM. Additionally, MTNR1B gene expression levels were found to correlate with the presence of this polymorphism.

Conclusions: The findings emphasize the crucial role of MTNR1B in the pathophysiology of GDM. Variations in MTNR1B and its altered expression levels could potentially serve as biomarkers for assessing GDM risk and provide insights into its molecular mechanisms.

Keywords: MTNR1B Gene, Gestational Diabetes Mellitus (GDM), Genetic Polymorphisms

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INTRODUCTION

Background

Gestational Diabetes Mellitus (GDM) represents a prevalent complication during pregnancy, manifesting as glucose intolerance that arises during gestation. Epidemiological data indicate that GDM affects approximately 7-10% of pregnant women globally, which translates into significant public health implications due to its association with adverse maternal and fetal outcomes [1]. Women with GDM are at a heightened risk of developing Type 2 Diabetes Mellitus (T2DM) later in life, while their offspring may experience various metabolic disorders, including obesity and glucose intolerance [2].

The pathophysiology of GDM involves complex interactions between genetic predisposition and environmental factors. Despite the growing body of research, the exact mechanisms through which genetic factors contribute to GDM remain only partially understood. Identifying genetic markers that contribute to the risk of GDM is crucial for improving early detection and personalized management strategies.

MTNR1B Gene

The MTNR1B gene encodes the melatonin receptor 1B, which plays a significant role in regulating glucose metabolism and insulin secretion. Melatonin, a hormone secreted by the pineal gland, is known to influence various physiological processes, including sleep-wake cycles and glucose homeostasis [3]. Variations in the MTNR1B gene have been implicated in the regulation of glucose levels and insulin sensitivity, with several studies linking MTNR1B polymorphisms to an increased risk of T2DM [4]. Recent evidence suggests that MTNR1B may also impact the development of GDM [5].

Understanding the role of MTNR1B in GDM involves exploring how genetic variations and changes in gene expression affect the receptor's function and its contribution to glucose dysregulation during pregnancy. By examining these factors, researchers aim to uncover potential biomarkers for early risk assessment and targeted intervention strategies.

Objectives

This study is designed with the following objectives:

To examine the relationship between MTNR1B gene expression levels and GDM.

To identify specific MTNR1B polymorphisms associated with an increased risk of GDM.

To explore the potential of MTNR1B as a biomarker for GDM risk assessment.

Methods

Study Design

A case-control study was conducted to investigate the association between MTNR1B gene expression, genetic polymorphisms, and GDM incidence. The study enrolled 150 pregnant women diagnosed with GDM and 150 age-matched healthy controls, attending Jan Sewa Hospital, Sriganganagar, Rajasthan, India. Ethical approval for the study was obtained from the Institutional Review Board, and informed consent was acquired from all participants to ensure adherence to ethical research practices.

The case-control design allowed for a comparison between women with GDM and those without, facilitating the identification of differences in MTNR1B gene expression and genetic variants associated with the disease. This approach also enabled the examination of potential correlations between genetic markers and GDM risk.

Sample Collection

Blood samples were collected from participants during their routine antenatal visits. The samples were processed to isolate plasma and peripheral blood mononuclear cells (PBMCs) for subsequent analyses. The use of PBMCs was crucial for accurate measurement of gene expression levels, as these cells can be readily obtained from blood and are representative of systemic changes in gene expression.

The collection protocol ensured that samples were handled and stored under optimal conditions to maintain RNA integrity and prevent degradation. Plasma samples were used for potential future analyses of biomarkers and metabolic parameters related to GDM.

MTNR1B Gene Expression Analysis

Quantitative PCR (qPCR) was employed to measure MTNR1B gene expression levels. The qPCR technique allows for precise quantification of gene expression by measuring the amount of specific mRNA transcripts present in the PBMCs. The process involved several key steps:

RNA Extraction: Total RNA was extracted from PBMCs using the RNeasy Mini Kit (Qiagen). This step involved lysing the cells and isolating RNA through a series of purification steps to ensure high-quality RNA suitable for downstream analyses.

cDNA Synthesis: Complementary DNA (cDNA) was synthesized from the extracted RNA using the Superscript VILO Master Mix (Thermo Fisher Scientific). The conversion of RNA to cDNA is essential for the qPCR process, as it allows for the amplification of specific gene transcripts.

qPCR Amplification: qPCR was conducted using SYBR Green Master Mix (Applied Biosystems) with primers specific to the MTNR1B gene. The primers used for amplification were designed to target the MTNR1B gene sequence and facilitate accurate measurement of gene expression levels. The qPCR amplification plot provided insights into the relative abundance of MTNR1B mRNA in the samples.

Table 1: Primer Sequences for MTNR1B Gene Expression Analysis

Gene	Primer Forward (5' to 3')	Primer Reverse (5' to 3')
MTNR1B	CCTGCTGACATCCACAGTGG	GCTCTTGGTCTGCTTGTTCCG

Genetic Polymorphism Analysis

To investigate genetic variations, polymorphisms in the MTNR1B gene were analyzed using SNP genotyping. Single nucleotide polymorphisms (SNPs) are common genetic variations that can influence gene function and contribute to disease susceptibility. The following SNPs in MTNR1B were examined:

rs10830963
rs10830964
rs10830965

Table 2: SNPs Analyzed in MTNR1B Gene

SNP	rsID	Chromosome	Position
MTNR1B SNP1	rs10830963	11	67892255
MTNR1B SNP2	rs10830964	11	67905123
MTNR1B SNP3	rs10830965	11	67918233

SNP genotyping was performed using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific). This method allows for the accurate detection of specific SNPs by utilizing fluorescent probes that bind to the SNP variants.

Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (IBM) to evaluate the significance of differences and associations. Key statistical tests included:

Student's t-test: Used to compare MTNR1B gene expression levels between the GDM and control groups.

Logistic Regression Analysis: Employed to assess associations between MTNR1B polymorphisms and GDM risk, adjusting for potential confounders such as age, BMI, and ethnicity.

Results

Demographic Characteristics

The demographic characteristics of the study participants are summarized in the following table:

Table 3: Demographic Characteristics of Study Participants

Characteristic	GDM Group (n=150)	Control Group (n=150)	p-value
Age (years)	32.5 ± 4.7	31.8 ± 4.5	0.23
BMI (kg/m²)	29.4 ± 3.2	23.9 ± 2.8	<0.001
Parity (number of pregnancies)	2.1 ± 1.0	1.8 ± 0.9	0.12
Family History of Diabetes	90 (60%)	60 (40%)	<0.001
Pre-pregnancy Weight (kg)	70.2 ± 8.5	62.4 ± 7.9	<0.001

The demographic data reveal that the GDM group had a higher mean BMI compared to the control group, which is consistent with previous research linking obesity with an increased risk of GDM. There were no significant differences between the groups in terms of age or parity, indicating that these factors were well-matched in the study design. Also, mean pre-pregnancy weight was higher in the GDM group and this difference was statistically significant. Moreover, 60 % participants in the GDM group had family history of diabetes as compared to 40 % participants in the control group.

MTNR1B Gene Expression

The analysis of MTNR1B gene expression levels revealed a significant increase in expression among women with GDM compared to healthy controls. The p-value for this difference was less than 0.001, indicating a robust statistical significance.

Table 4: Distribution of MTNR1B Gene Expression Levels in GDM vs. Control Groups

Statistic	GDM Group	Control Group
Range	0.8 - 2.5	0.5 - 1.8
Standard Deviation	0.35	0.25
Median	1.6	1.1
Mean	1.65	1.12
Interquartile Range (IQR)	0.6	0.4

MTNR1B Polymorphisms and GDM Risk

The distribution of MTNR1B polymorphisms between the GDM and control groups was analyzed to determine their association with GDM risk.

Table 5: Association Between MTNR1B Polymorphisms and GDM Risk

SNP	GDM Group (%)	Control Group (%)	Odds Ratio (OR)	95% CI	p-value
rs10830963	52	30	2.3	1.5-3.5	<0.001
rs10830964	45	40	1.3	0.9-1.9	0.22
rs10830965	30	25	1.2	0.8-1.8	0.34

The logistic regression analysis showed that the rs10830963 polymorphism was significantly associated with an increased risk of GDM, with an odds ratio of 2.3 (95% CI: 1.5-3.5) and a p-value less than 0.001. This suggests that individuals carrying the rs10830963 risk allele have more than twice the likelihood of developing GDM compared to those without this variant. In contrast, the other SNPs, rs10830964 and rs10830965, did not show significant associations with GDM risk.

Correlation Between Gene Expression and Polymorphisms

The study also assessed the relationship between MTNR1B gene expression levels and the presence of specific polymorphisms. A significant positive correlation was observed between MTNR1B gene expression levels and the rs10830963 polymorphism, with a correlation coefficient (r) of 0.45 and a p-value less than 0.001. This indicates that higher MTNR1B expression is associated with the presence of the risk allele for rs10830963.

Discussion

MTNR1B and GDM Pathophysiology

The study's findings confirm that MTNR1B gene expression is significantly altered in women with GDM compared to healthy controls. The melatonin receptor 1B, encoded by the MTNR1B gene, is involved in the regulation of glucose metabolism and insulin secretion. Elevated expression of MTNR1B may disrupt normal insulin signaling and glucose homeostasis, potentially impairing insulin sensitivity or beta-cell function during pregnancy.

Melatonin, the hormone regulated by the MTNR1B receptor, plays a role in modulating circadian rhythms and glucose metabolism. Disruptions in melatonin signaling, due to altered MTNR1B expression, could contribute to insulin resistance and impaired glucose regulation. Previous research has suggested that melatonin influences pancreatic beta-cell function and insulin secretion, which may be particularly relevant in the context of pregnancy where metabolic demands are heightened [6, 7].

Impact of Polymorphisms

The rs10830963 polymorphism in the MTNR1B gene was identified as a significant risk factor for GDM in this study. This SNP has been previously associated with T2DM and glucose dysregulation [8, 9]. The risk allele of rs10830963 may affect MTNR1B receptor function or gene expression, leading to impaired glucose regulation. Given the role of MTNR1B in glucose homeostasis, genetic variations that alter its function could have significant implications for the development of GDM.

The other SNPs analyzed, rs10830964 and rs10830965, did not show significant associations with GDM risk in this study. This may be due to the specific functional impact of rs10830963 on MTNR1B receptor activity or its influence on gene expression levels, which may not be replicated by the other variants.

Implications for Screening and Prevention

The identification of MTNR1B polymorphisms, particularly rs10830963, as risk factors for GDM has important implications for screening and prevention. Genetic screening for MTNR1B variants could help identify women at higher risk for GDM early in pregnancy. This could enable the implementation of targeted preventive measures, such as lifestyle interventions and more frequent monitoring of glucose levels, to reduce the incidence of GDM and improve maternal and fetal outcomes.

Personalized management strategies based on genetic risk could enhance the effectiveness of GDM prevention and treatment, potentially leading to better health outcomes for both mothers and their children [10]. Further research is needed to validate these findings and assess the clinical utility of genetic testing for GDM risk assessment.

Limitations

Several limitations should be considered in interpreting the results of this study. The sample size, while sufficient for identifying significant associations, may limit the generalizability of the findings. A larger cohort would be beneficial for confirming the associations and examining potential interactions with other genetic and environmental factors.

The cross-sectional design of the study provides a snapshot of gene expression and polymorphism associations but does not establish causality or the temporal relationship between MTNR1B expression and GDM development. Longitudinal

studies are needed to explore how changes in MTNR1B expression and genetic variants contribute to the onset of GDM over time.

Additionally, other genetic factors and environmental influences that may affect GDM risk were not accounted for in this study. Future research should include a more comprehensive analysis of genetic, epigenetic, and environmental factors to better understand the complex interactions involved in GDM pathogenesis [11].

Conclusion

The study highlights the significant role of MTNR1B gene expression and genetic polymorphisms in the development of Gestational Diabetes Mellitus. The rs10830963 polymorphism, in particular, is associated with increased GDM risk and correlates with elevated MTNR1B gene expression levels. These findings suggest that MTNR1B and its variants could serve as potential biomarkers for early detection and risk stratification of GDM.

Future research should focus on further elucidating the molecular mechanisms by which MTNR1B influences glucose metabolism during pregnancy and evaluating the clinical utility of genetic markers in GDM management. Such efforts will contribute to improving our understanding of GDM and developing targeted strategies for its prevention and treatment.

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