original article

MTNR1B genetic polymorphisms as risk factors for gestational diabetes mellitus: a case-control study in a single tertiary care center

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BACKGROUND: Gestational diabetes mellitus (GDM) is a metabolic disease in pregnancy that causes carbohydrate intolerance and hyperglycemia. Genome-wide association studies and meta-analyses have found that the single nucleotide polymorphisms (SNPs) rs1387153 and rs10830963 of the melatonin receptor 1B (*MTNR1B*) gene are associated with GDM. No studies on the *MTNR1B* gene effect on GDM have been performed in Saudis, other Arabs, or other Middle Eastern populations.

OBJECTIVES: Investigate the association of genotype or allele frequencies of the two SNPs with GDM and with clinical parameters related to GDM.

DESIGN: Case-control study.

SETTINGS: Tertiary care center, Riyadh.

PATIENTS AND METHODS: We recruited 400 pregnant Saudi women ages 18-45 years (200 were diagnosed with GDM, and 200 were healthy controls). Biochemical assays were performed, and rs1387153 and rs10830963 polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism analysis and real-time polymerase chain reaction with TaqMan genotyping.

MAIN OUTCOME MEASURES: The association of *MTNR1B* gene (rs1387153 and rs10830963 polymorphisms) with GDM and with biochemical parameters related to GDM.

SAMPLE SIZE: 200 GDM cases and 200 non-GDM controls.

RESULTS: Differences in allele frequencies for GDM vs non-GMD were statistically significant or nearly significant for both SNPs after adjustment for age and body mass index. In a logistic regression analysis, genotype TT was positively associated with post-prandial blood glucose (P=.018), but other associations were not statistically significant.

CONCLUSION: The odds ratios for the associations between the rs1387153 and rs10830963 SNPs and GDM exceeded 1.5-fold, which is higher than typically reported for diseases with complex genetic background. These effect sizes for GDM suggest pregnancy-specific factors related to the *MTNR1B* risk genotypes.

LIMITATIONS: Only two SNPs were studied.

CONFLICT OF INTEREST: None.

Gestational diabetes mellitus (GDM) is a metabolic disorder in pregnant women, characterized by carbohydrate intolerance, which causes hyperglycemia.[1,2] GDM is first diagnosed in the second or third trimester of pregnancy, and cannot be absolutely defined as either type 1 diabetes mellitus or type 2 diabetes mellitus.[3] GDM, initially established as a clinical entity in 1964,[4] is observed in 9.2% of all pregnancies, resulting in complications such as cesarean delivery, hypoglycemia, infant macrosomia, and trauma during pregnancy and delivery.[5] GDM screening and treatment may prevent the development of adverse outcomes.[6] Risk factors for GDM include advanced maternal age, ethnicity, body mass index (BMI), family history of GDM, presence of type 2 diabetes, and a history of giving birth to macrosomic infants.[7] Also, there is strong evidence suggesting that in women who develop GDM, the disease can also progress to complications such as type 2 diabetes, and chronic cardiovascular and metabolic disease.[8] Pregnant women with a parental history of diabetes have a 2.3-fold increased risk of developing GDM, compared to those without such a history. Moreover, women who have siblings with diabetes have an 8.4-fold elevated GDM risk, compared to those without such siblings.[9] GDM and type 2 diabetes share similar pathophysiology and are associated with both insulin resistance and impaired insulin secretion. New reports indicate that GDM is a forerunner of type 2 diabetes in women predisposed to metabolic disorders in pregnancy.[10]

Genetic studies such as genome-wide association studies (GWAS) have successfully identified single-nucleotide polymorphisms (SNPs) and genes representing new susceptibility loci, or disease-causing variants, for human disorders.[11] In addition to GWAS, meta-analysis, linkage analysis, and candidate case-control studies have also implicated various genes in the etiology of metabolic diseases; however, in general, few SNPs or genes have been identified by both GWAS and meta-analysis. Of those genes that are consistently identified by different genetic screening approaches, melatonin receptor 1B (*MTNR1B*) (600804 OMIM) is associated with both GDM and type 2 diabetes.[12-16] *MTNR1B* maps to chromosome 11q21-q22 comprise 2 exons and 1 intron and encodes the MT2 protein, a 362-amino acid melatonin receptor. *MTNR1B* is strongly expressed in the brain, retina, and β-cells, and MT2 regulates blood glucose homeostasis through the regulation of insulin release via the melatonin signaling pathway. The variant allele in rs10830963 will increase the islet *MTNR1B* expression through increased FOXA2-bound enhancer activity and NEUROD1 binding in islet cells.[17] However, Bonnefond et al[18] suggested that the mechanism by which the G allele in rs10830963 of the *MTNR1B* gene impairs insulin secretion is still not clear and proposed that it is a secondary effect of a central dysfunction. Abnormal *MTNR1B* variants and SNPs can contribute to the pathogenesis of GDM through this pathway. The human genome project discovered *MTNR1B* gene SNPs, including rs1387153 (chr11:92940662) and rs10830963 (chr11:92975544), that were associated with an increased risk of diabetes development.[19] The prevalence of GDM in Saudi women is 18.7%,[20] and the major factors contributing to the risk of GDM in this population are a lack of exercise and poor dietary control; the prevalence of obesity in Saudi Arabia is documented at 68%.[1] According to a recent meta-analysis,[14,15] no studies on the *MTNR1B* gene have been conducted in Saudi populations, or in other Arab/Middle Eastern populations. Therefore, our study aimed to assess the association of the *MTNR1B* polymorphisms rs1387153 and rs10830963as identified by GWAS and meta-analysis, with GDM in Saudi women, using a case-control study design.

PATIENTS AND METHODS

Study participants

In this case-control study, we recruited 400 women attending KKUH during their pregnancy: 200 had been diagnosed with GDM and 200 did not have GDM (healthy controls). All the women were selected based on their interest in the study after completing a proforma and providing a signed consent form. Inclusion criteria were Saudi nationality and the presence of a positive result on the glucose challenge test (GCT) and oral glucose tolerance tests (OGTTs). Exclusion criteria were the presence of type 1 or 2 diabetes before pregnancy, and non-Saudi nationality. The study was approved by the IRB of King Khalid University Hospitals in Riyadh, Saudi Arabia. Sample size was based on data from our previous study.[1]

Anthropometric, biochemical, and clinical data on study participants

The anthropometric measurements collected were age, gestational age, height (cm), and weight (kg). Initially, pregnant women at the 24–28th gestational week were screened with a 50-g GCT, and those with positive results subsequently underwent a 100-g OGTT. Prior to these tests, pregnant women were advised to fast overnight (for a minimum of 8 hours). If more than two abnormal values for GCT and OGTT were observed, the women were diagnosed with GDM. American Diabetes

Association criteria were used as the cutoff values for diagnosis of GDM during pregnancy (**Table 1**). Abnormal values were followed-up for GDM confirmation. More than 95% of the patients with GDM used only dietary control as treatment until delivery, and the remaining patients were treated with insulin therapy until delivery. None of the women with GDM received treatment with other medication. Peripheral blood samples (5 mL) were routinely collected from pregnant women and separated for biochemical (3 mL) and molecular (2 mL) analyses. Serum samples (3 mL) were collected for measuring fasting blood glucose (FBG), postprandial blood glucose (PPBG), and lipid profile parameters, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).[21] Biochemical serum samples were assessed with the oxidase-peroxidase method using radioimmunoassay.[22]

Molecular analysis

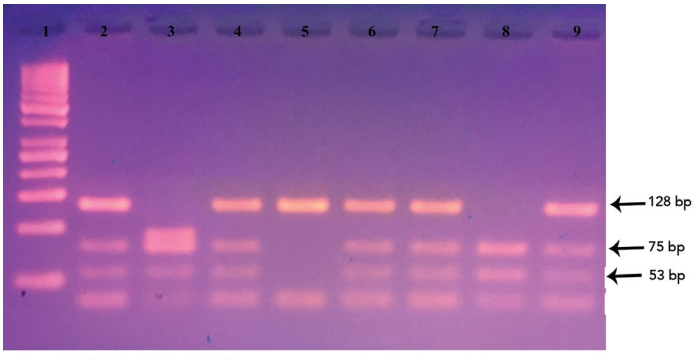
Genomic DNA was extracted from blood samples (2 mL) collected in EDTA-coated tubes using an AccuVis DNA extraction kit (AccuVis Bio, UAE) and stored at -80°C for future use. The oligonucleotide sequences for the amplification of the rs1387153 variant were 5′-ACCATTCTCAGTGGTCCTTACT-3′ and 5′-GGGCCTAAGAGCCTCCATTT-3′. The primers were synthesized by Bioserve Biotechnologies Limited (Telangana, India). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR was performed in a total volume of 25 µL, containing 5× PCR master mix, a 50 ng of genomic DNA (quantified using a NanoDrop spectrophotometer), and 100 picomoles of the reverse and forward primers. Initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 45 seconds, and then a single final extension at 72°C for 5 minutes and a 4°C hold stage. The 165-bp PCR products were digested with the BstNI (CC↓GAA) restriction enzyme (Thermo Fisher Scientific, Dubai, UAE) at 37°C for 2 hours. The digestion of products with the wild-type (CC) genotype generated fragments of 75, 53, and 37 bp, whereas the homozygous variant genotype (TT) generated fragments of 128 and 27 bp; digestion of the samples with the heterozygous (CT) genotype resulted in fragments of 128, 75, 53, and 27 bp (**Figure 1**). We ran the confirmation band of unrelated band sizes to confirm the gel run with 3% agarose (QA-Agarose TM, Cat#AGAH0500, USA) stained with ethidium bromide. Molecular weight markers and digested PCR products were separated by electrophoresis on 3% agarose gel. Sanger sequencing was performed for 10% of the samples for quality control analysis. The results of PCR-RFLP and DNA sequencing were 100% concordant (**Figure 2**). Genotyping of the rs10830963 (C_3256858_10; Catalog number 4351379, Thermofisher Scientifics, USA) SNP was performed using TaqMan allelic discrimination assay, as previously described.[16,23] The annealing temperature was 62°C, and a genotyping success rate of 96% was achieved for rs10830963; sample quality issues were determined as major reasons for the failure in achieving 100% concordance for the genotyping of this polymorphism.

Table 1. Cut-off values for diagnosis of gestational diabetes mellitus with a 100-g oral glucose tolerance test.[20]

	mmol/L	mg/dL
Fasting	5.3	95
First hour	10.0	180
Second hour	8.6	155
Third hour	7.8	140

Statistical analysis

Data were analyzed using IBM SPSS (Armonk, NY: IBM Corp) version 21. Continuous data are presented as mean, standard deviation and range. Categorical data are presented as frequencies and percentages.



Lane 1: DNA Size Ladder (50 bp)
Lane 2, 4, 6, 7, and 9: Heterozygous CT genotype (128/75/53 bp)
Lane 3: Confirmation band
Lane 5: Homozygous TT genotype (128 bp)
Lane 8: Homozygous CC genotype (75/53 bp)

Figure 1. A 3% agarose gel electrophoresis of digested PCR products of the rs1387153 polymorphism in *MTNR1B* gene.

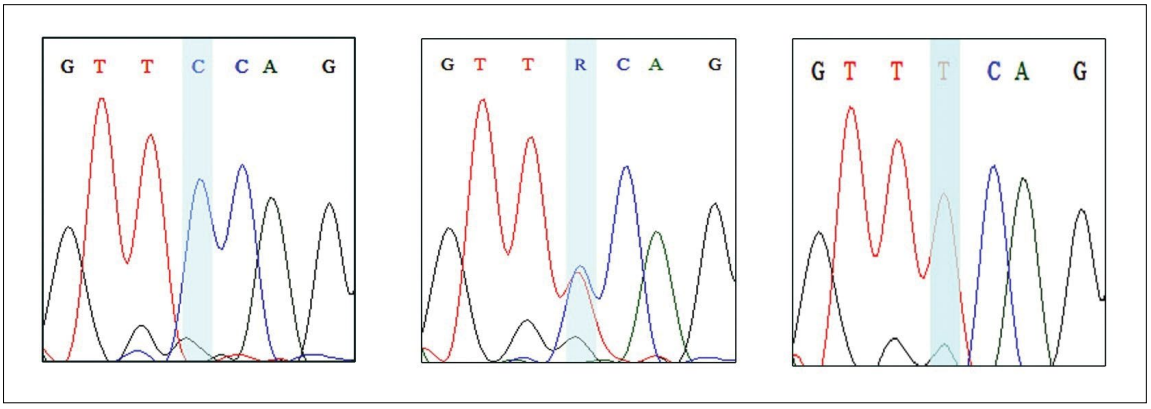


Figure 2. Chromatogram of CC, CT and TT genotypes from DNA sequencing analysis of the rs1387153 polymorphism in *MTNR1B* gene.

Table 2. Clinical characteristics of the pregnant Saudi women.

	GDM (n=200)	Non-GDM (n=200)	*P*
Age (years)	32.4 (5.8)	28.1 (6.1)	<.001
Weight (kg)	77.1 (13.3)	74.1 (12.5)	.020
Height (cm)	158.5 (6.0)	157.7 (5.2)	.15
BMI (kg/m2)	30.3 (4.7)	29.7 (4.4)	.17
FBG (mmol/L)	4.9 (0.7)	4.4 (0.5)	<.001
PPBG (mmol/L)	6.5 (5.4–7.8)	4.3 (3.9–5.0)	<.001
GCT (mmol/L)	9.5 (1.9)	6.2 (1.5)	<.001
OGTT (fasting) (mmol/L)	4.9 (4.6–5.4)	--	--
OGTT (1 h) (mmol/L)	10.8 (1.8)	7.3 (1.8)	<.001
OGTT (2 h) (mmol/L)	9.1 (1.8)	7.3 (1.8)	<.0001
OGTT (3 h) (mmol/L)	5.7 (1.7)	4.3 (1.1)	.001
TC (mmol/L)	5.8 (1.2)	5.2 (1.1)	<.001
TG (mmol/L)	1.8 (1.4–2.6)	1.5 (1.1–2.2)	<.001
HDL-C (mmol/L)	0.9 (0.4)	0.6 (0.2)	<.001
LDL-C (mmol/L)	3.7 (0.9)	3.7 (1.0)	.67

Data presented as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normal variables. FBG: fasting blood glucose; PPBG: post prandial blood glucose; GCT: glucose challenge test; OGTT: oral glucose tolerance test; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Independent sample *t* tests were performed to assess the significance between the groups for continuous variables, while chi-square tests were performed for categorical variables. Multiple logistic regression analysis was used to determine the odds of developing GDM against two SNPs and other potential risk factors. Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium were determined through a genetics package available in R software (version 3.5.2). *P* values <.05 were considered statistically significant.

RESULTS

Clinical characteristics

The mean (SD) age of the 200 GDM cases was 32.4 (5.8) years, which was significantly higher than the mean age of the 200 non-GDM participants, 28.1 (6.1) years (*P*<.001) (**Table 2**). The mean (SD) age of onset of diabetes in the GDM group was 30.2 (5.85) years. There was no significant difference in BMI between the women with and without GDM (*P*=.17). Moreover, women with GDM had significantly higher levels of FBG, PPBG, GCT, OGTT 1st hour, OGTT 2nd hour, and OGTT 3rd hour than the controls (*P*<.0001). Lipid profile investigations in all the pregnant women showed significantly higher TC, TG, and HDL-C values in women with GDM (*P*<.05); however, there was no difference between the groups in LDL-C (*P*=.67).

Genotype analyses

The genotype and allele frequencies of the *MTNR1B* gene polymorphisms in the women with GDM and healthy controls are presented in **Table 3**. The genotype frequencies of rs1387153 did not deviate from HWE with HWE (*P*=.06), while those of rs10830963 deviated from HWE (*P*<.001). The CT (46%) and TT (22%) geno-

Table 3. Genotype and allele frequencies of the *MTNR1B* gene single nucleotide polymorphisms rs1387153 and rs10830963.

	GDM (n=200)	Non-GDM (n=200)	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
rs1387153[a]						
CC	64 (32.0)	91 (45.5)	**Reference**			
CT	92 (46.0)	81 (40.5)	1.62 (1.04–2.50)	.032	1.54 (0.97–2.46)	.068
TT	44 (22.0)	28 (14.0)	2.23 (1.26–3.96)	.006	2.07 (1.13–3.79)	.018
CT+TT	136 (68.0)	109 (54.5)	1.77 (1.18–2.66)	.006	1.68 (1.09–2.60)	.019
C	220 (55.0)	263 (65.7)	**Reference**			
T	180 (45.0)	137 (34.3)	1.57 (1.18–2.09)	.002	1.51 (1.11–2.04)	.008
rs10830963[b]						
CC	64 (32.0)	96 (48.0)	**Reference**			
CG	87 (43.5)	65 (32.5)	2.00 (1.28–3.15)	.002	1.84 (1.14–2.98)	.012
GG	49 (24.5)	39 (19.5)	1.88 (1.11–3.19)	.018	1.74 (1.00–3.06)	.052
CG+GG	136 (68.0)	104 (52.0)	1.96 (1.31–2.94)	.001	1.81 (1.17–2.78)	.007
C	215 (53.8)	257 (64.3)	**Reference**			
G	185 (46.2)	143 (35.7)	1.55 (1.16–2.05)	.003	1.47 (1.08–1.98)	.013

Data presented as OR (95% CI); [a]Hardy-Weinberg equilibrium: chi-square = 3.69; *P*=.06; [b]Hardy-Weinberg equilibrium: chi-square = 18.41, *P*<.001; GDM: gestational diabetes mellitus. Adjusted OR is adjusted for age and body mass index.

types of the rs1387153 polymorphism were more frequently observed in the GDM cases than in the healthy controls (40.5% and 14%, respectively). The expression frequencies of the C and T alleles were 55% and 45%, respectively, in cases with GDM, compared to 65.7% and 34.3% observed in the controls. Comparisons of the minor allele frequencies and genotypes indicated that they were significantly associated with GDM using both dominant and recessive models: allele (T vs. C), P=.008; genotype, *P*=.019; for the dominant model (CT+TT vs. CC).

In patients with GDM, the frequencies of the rs10830963 CC, CG, and GG genotypes were 32%, 43.5%, and 24.5%, respectively. The G allele frequency was 46.2% (C allele, 53.8%) in cases with GDM. In healthy women, the CC, CG, and GG genotype frequencies represented as 48%, 32.5% and 19.5%, respectively, while the C and G allele frequencies were 64.3% and 35.8%, respectively. The minor allele frequencies, genotype, and dominant and recessive models were significantly different between the women with GDM and controls: allele (C vs. G), *P*=.013; *P*=.007 for the dominant model (CG+GG vs. CC)).

Correlation between glucose values, lipid profile and MTNR1B *single nucleotide polymorphisms*

One-way ANOVA was performed for the investigation of the relationship between rs1387153 and rs10830963 polymorphisms, glucose and lipid profile parameters. The genotypes and corresponding glucose values are detailed in **Table 4**. Genotype frequencies were calculated for several glucose and lipid profile parameters, including FBG, PPBG, GCT, OGTT (fasting), OGTT (1, 2, and 3), TC, TG, HDL-C and LDL-C. Apart from these, we also included age and BMI. The only association identified by one-way ANOVA was between PPBG, OGTT (fasting), and the rs1387153 SNP (*P*=.042). No associations were observed with the other variables (age, BMI, FBG, GCT, OGTT, TC, TG, HDL-C and LDL-C).

Multiple logistic regression

Adjusted logistic regression analyses were performed to assess the associations between the genotypes of both SNPs and biochemical parameters in women with GDM to estimate the contribution of genotypes to FBG, PPBG, GCT, OGTT (F, 1, 2, and 3), TG, TC, HDL-C, and LDL-C. The results are presented in **Table 5**. There was a significant interaction of the TT allele of rs1387153 with PPBG (*P*<.018).

Linkage disequilibrium analysis

Linkage disequilibrium analysis was performed for

Table 4. Comparison of allele frequencies by clinical parameters for the rs1387153 and rs10830963 polymorphisms in the *MTNR1B* gene.

Glucose variables	rs1387153				rs10830963			
	CC (n=64)	CT (n=92)	TT (n=44)	*P*	CC (n=64)	CG (n=87)	GG (n=49)	*P*
Age (years)	29.6 (6.3)	30.5 (6.5)	31.0 (6.0)	.194	29.4 (6.1	30.8 (6.3)	30.8 (6.7)	.107
BMI (kg/m^2)	29.6 (4.5)	30.3 (4.5)	30.3 (4.7)	.289	30.1 (4.5)	29.7 (4.6)	30.5 (4.6)	.454
FBG (mmol/L)	4.8 (0.7)	4.8 (0.7)	4.8 (0.6)	.859	4.7 (0.7)	4.8 (0.7)	4.9 (0.6)	.538
PPBG (mmol/L)[a]	4.9 (4.2–6.4)	5.2 (4.2–6.7)	6.0 (4.7–7.1)	.042b	4.9 (4.2–6.5)	5.5 (4.3–7.1)	5.0 (4.4–6.5)	.294
GCT (mmol/L)	7.6 (2.4)	7.7 (2.3)	8.5 (2.4)	.505	7.2 (2.2)	8.1 (2.4)	8.4 (2.5)	.177
OGTT (fasting) (mmol/L)[a]	4.9 (4.6–5.4)	5.1 (4.7–5.5)	4.8 (4.6–5.3)	.067	5.2 (4.9–5.4)	4.8 (4.5–5.3)	4.9 (4.7–5.1)	.062
OGTT (1 h) (mmol/L)	10.1 (2.0)	10.2 (2.4)	10.2 (2.1)	.954	10.0 (2.3)	10.1 (1.9)	10.6 (2.6)	.430
OGTT (2 h) (mmol/L)	8.8 (2.1)	8.5 (2.1)	9.0 (1.9)	.404	8.5 (2.3)	8.8 (1.8)	8.9 (2.2)	.510
OGTT (3 h) (mmol/L)	5.4 (1.7)	5.6 (1.7)	5.3 (1.7)	.686	5.6 (1.8)	5.5 (1.7)	5.3 (1.7)	.650
Total cholesterol (mmol/L)	5.4 (1.2)	5.5 (1.2)	5.7 (1.2)	.272	5.4 (1.2)	5.6 (1.2)	5.5 (1.2)	.243
Triglyceride (mmol/L)[a]	1.5 (1.2–2.2)	1.7 (1.2–2.4)	1.8 (1.4–2.6)	.188	1.6 (1.2–2.2)	1.5 (1.2–2.4)	1.8 (1.2–2.6)	.521
HDL-C (mmol/L)	0.8 (0.3)	0.8 (0.4)	0.8 (0.4)	.204	0.8 (0.4)	0.8 (0.4)	0.8 (0.4)	.500
LDL-C (mmol/L)	3.7 (0.9)	3.7 (0.9)	3.7 (1.0)	.942	3.7 (0.8)	3.7 (1.0)	3.7 (1.0)	.822

Data presented as mean (standard deviation) for normally distributed variables and median (interquartile range) for non-normal variables; [a]Indicates abnormal variables; [b]Indicates significance of TT from CC; FBG: fasting blood glucose; PPBG: post-prandial blood glucose; GCT: glucose challenge test; OGTT: oral glucose tolerance test; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 5. Age and body mass index-adjusted logistic regression analysis of the association between genotypes and risk factors for gestational diabetes mellitus.

Risk factor	CT		TT		CG		GG	
	OR (length of 95% CI)	*P*	OR (length of 95% CI)	*P*	OR (length of 95% CI)	*P*	OR (length of 95% CI)	*P*
FBG (mmol/L)	0.04 (0.12)	.716	0.00 (0.15)	.993	0.03 (0.12)	.792	.12 (0.14)	.401
PPBG (mmol/L)[a]	0.17 (0.27)	.532	0.85 (0.36)	**.018**	0.22 (0.29)	.464	-0.05 (0.32)	.878
GCT (mmol/L)	0.14 (0.59)	.818	0.84 (0.81)	.306	0.75 (0.62)	.229	1.32 (0.69)	.061
OGTT (fasting) (mmol/L)[a]	0.21 (0.12)	.090	-0.09 (0.15)	.564	-0.18 (0.13)	.149	-0.03 (0.15)	.813
OGTT (1 h) (mmol/L)	0.03 (0.38)	.947	-0.06 (0.46)	.897	-0.19 (0.38)	.613	0.34 (0.44)	.439
OGTT (2 h) (mmol/L)	-0.42 (0.35)	.238	0.08 (0.43)	.861	0.18 (0.36)	.624	0.30 (0.41)	.475
OGTT (3 h) (mmol/L)	0.15 (0.33)	.645	-0.19 (0.43)	.652	-0.20 (0.35)	.559	-0.34 (0.39)	.384
TC (mmol/L)	0.04 (0.13)	.752	0.24 (0.17)	.158	0.21 (0.14)	.123	0.09 (0.16)	.573
TG (mmol/L)[a]	0.03 (0.12)	.780	0.12 (0.15)	.426	0.11 (0.12)	.378	0.13 (0.14)	.335
HDL-C (mmol/L)	0.03 (0.04)	.517	0.07 (0.05)	.140	0.03 (0.04)	.519	0.02 (0.05)	.653
LDL-C (mmol/L)	-0.05 (0.10)	.638	-0.07 (0.13)	.597	0.04 (0.11)	.713	0.03 (0.12)	.785

Data are adjusted OR (95% CI). a indicates abnormal variables. FBG: fasting blood glucose; PPBG: post prandial blood glucose; GCT: glucose challenge test; OGTT: oral glucose tolerance test; BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

the rs1387153 and rs10830963 polymorphisms in the *MTNR1B* gene and no significant associations were identified between the haplotypes of these SNPs (*P*=.289). These data indicate that no linkage disequilibrium was significantly associated with either susceptibility to, or protection from, GDM.

DISCUSSION

In this case-control study of Saudi women with GDM and healthy controls, we investigated the associations of the *MTNR1B* polymorphisms rs1387153 and rs10830963. Consistent with the literature, this study identified a relationship of rs1387153 and rs10830963 with GDM susceptibility. Both SNPs were identified as being strongly associated with GDM in the dominant genotype (*P*=.006 and *P*=.007) and allelic (*P*=.008 and *P*=.013) models. This is the first study focusing on the *MTNR1B* gene variants in the Saudi population as well as in the Arab/Middle East population. Genetic data generated by case-control and unbiased epidemiological studies of gene polymorphisms are capable of identifying the associations between complex diseases and candidate genes. Additionally, the minor allele frequencies of common variants identified by GWAS can be used to identify the loci contributing to the susceptibility to diseases, such as GDM, in which the pathophysiological changes are similar to those observed in type 2 diabetes, characterized by peripheral insulin resistance accompanied by a defect in insulin secretion.

The risk allele frequencies in rs10830963 and rs1387153 polymorphisms vary in the global population of women with GDM and non-GDM. The Chinese population in itself presents varied risk allele frequencies in the rs10830963 polymorphism in women with GDM (G allele 45.6%, 51.7%, 45.1%, 51.2%) and those without (43.3%, 41.2%, 39.9% and 44.3%).[14,24-26] In Korea, Greece and Brazil, the risk allele frequencies vary (GDM 'G' allele-52.2%, 40.9% and 27.6%) vs non-GDM (45.3%, 27.5% & 20.2%). The rs1387153 polymorphism has been screened in a limited number of GDM studies in the world population.[14,23,27] The risk allele frequencies in the GDM (50.4%, 32.4% and 32.5%) and non-GDM (44.4%, 29.08% and 27.3%) cases were documented, and differences were observed. Previous functional studies confirmed the role of diabetogenic genes in impaired β-cell function, insulin resistance, and the abnormal utilization of glucose.[28] In pregnancy, GDM is confirmed after the detection of abnormal glucose values, as detected by GCT and OGTT tests. Up to now, only biochemical analyses have been used to identify GDM in pregnant women; no molecular tests have been documented or confirmed as disease markers in any diabetic diseases. Similar patterns have been followed for different types of diabetes, including type 1 and type 2 diabetes, and new-onset diabetes after transplantation; however, genetic polymorphisms have been identified that can explain the differences in the susceptibility to these diseases. Genetic factors with significant roles in pregnancy, such as those influencing positive pregnancy outcomes, responses to treatment, and pregnancy complications, have been identified by molecular techniques in recent studies.[29] With increased insulin resistance and beta cell dysfunction (i.e.: reduced plasticity to overcome the increased IR in 3rd trimester of pregnancy), GDM results in central physiological disturbances, with diagnoses performed in the second semester using glucose tests, such as OGTT and GCT. Finally, gaps in knowledge of and controversies surrounding GDM etiology and its diagnostic criteria remain hurdles to the disease's effective management.[30]

The plasma melatonin levels in pregnant women can be elevated; melatonin can cross the placenta and fetal blood-brain barrier, and has a major role in preventing pregnancy loss as well as in the development of fetal organs for adaption to extrauterine life.[31] GWAS are one of the most effective methods for the acceleration of the identification of type 2 diabetes risk allele variants; however, proof of the relationship between these variants and the molecular mechanisms underlying the disease is still lacking.[32] Various meta-analysis have identified the genetic risk variants for numerous diseases through the pooling of global population data. The advantage of meta-analyses is the reduction in the rates of false negative and positive results through the merging of the results of global studies on similar subjects. Finally, they provide new insights into gene-disease associations.[33] GWAS and meta-analyses have provided extensive support for genetic variants at the *MTNR1B* locus associated with fasting glucose,[34-39] insulin secretion,[40,41] and type 2 diabetes.[13,42-45] A similar genetic association was confirmed by our results for both GDM and type 2 diabetes. Previous case-control and meta-analysis studies and GWAS on global ethnically diverse populations have confirmed that the rs10830963 and rs1387153 variants in *MTNR1B* are positively associated with GDM as major risk factors.[14,15,19,23,25-28,46-51] A recent study confirmed that the G allele of rs10830963 is strongly associated with glycemic traits in GDM.[47] In addition the *MTNR1B* rs10830963 variant, in interaction with prepregnancy BMI, was reported as a predictive genetic marker for the need of antenatal insulin therapy in GDM.[52] The rs10830963 variant was associated with abnormal glucose tolerance, and the rs1387153 and

rs2166706 variants were tightly associated with glucose levels; these two variants are linked to one another. However, rs1447352 was not associated with either glucose levels or glucose tolerance;[53] all meta-analyses have indicated a positive association.[14,15,28,51] So far, all genetic case-control studies have identified positive associations between *MTNR1B* gene polymorphisms and GDM[12,14,15,24-27,46-48] The prevalence of GDM in Asian countries is now higher than that in other countries. Our study's findings are consistent with those of previous reports in concluding that both the *MTNR1B* variants tested are associated with an increase in FBG and PPBG levels.

This study has several advantages and limitations. The advantages include the participation of Saudi women, and the selection of genetic markers confirmed by meta-analysis and GWAS. The limitations of this study are the selection of only two SNPs; the relatively small sample size; and the lack of expression studies. In conclusion, we determined that the rs1387153 and rs10830963 variants are associated with GDM in Saudi women. The OR values of the associations between the *MTNR1B* rs1387153 and rs10830963 gene variants and GDM development exceeded 1.5-fold and therefore are higher than typically reported for diseases with complex genetic background. The higher genetic effect sizes for GDM development suggest pregnancy specific factors related to the *MTNR1B* risk genotypes.

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