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

Khalid Khalaf Alharbi, Abdulrahman Mohammed Al-Sulaiman, Muath Khalid Bin Shedaid, Ali M. Al-Shangiti, Mohammed Marie, Yazeed A. Al-Sheikh, Imran Ali Khan

From the Department of Clinical Laboratory Sciences, King Saud University, Riyadh, Saudi Arabia; Department of Medical and Molecular Virology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia; Ministry of Health, Riyadh, Saudi Arabia; Saudi Society of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Correspondence: Dr. Imran Ali Khan · Department of Clinical Laboratory Sciences, King Saud University, Riyadh 11433, Saudi Arabia · T: +966114693851 · imkhan@ksu.edu.sa · ORCID: https://orcid.org/0000-0002-9746-5300

Citation: Alharbi KK, Al-Sulaiman AM, Bin Shedaid KM, Al-Shangiti AM, Marie M, Al-Sheikh YA, et al. MTNR1B genetic polymorphisms as risk factors for gestational diabetes mellitus: a case-control study in a single tertiary center. Ann Saudi Med 2019; 39(5): 309-318. DOI: 10.5144/0256-4947.2019.309

Received: May 23, 2018
Accepted: June 2, 2019
Published: October 3, 2019

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-GDM 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=0.018), 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. 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. GDM, initially established as a clinical entity in 1964, is observed in 9.2% of all pregnancies, resulting in complications such as cesarean delivery, hypoglycemia, infant macrosomia, and trauma during pregnancy and delivery. GDM screening and treatment may prevent the development of adverse outcomes. 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. 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. 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. 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.

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. 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. 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. However, Bonnefond et al. 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. The prevalence of GDM in Saudi women is 18.7%, 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%. According to a recent meta-analysis, 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 rs10830963 as 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.

**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.
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'-GGGCCTAAGAGCCTCCATT-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 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.

**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.

**Table 1.** Cut-off values for diagnosis of gestational diabetes mellitus with a 100-g oral glucose tolerance test.22

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

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

| Genotype   | GDM (n=200) | Non-GDM (n=200) | Unadjusted OR (95% CI) | P       | Adjusted OR (95% CI) | P       |
|------------|-------------|-----------------|------------------------|---------|----------------------|---------|
| rs1387153a |             |                 |                        |         |                      |         |
| 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    |

| rs10830963b |             |                 |                        |         |                      |         |
| CC         | 64 (32.0)   | 96 (48.0)       | Reference              |         |                      |         |
| CG         | 87 (43.5)   | 65 (32.5)       | 2.00 (1.29–3.15)       | .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); aHardy-Weinberg equilibrium: chi-square = 3.69; P= .06; bHardy-Weinberg equilibrium: chi-square = 18.41, P<.001; GDM: gestational diabetes mellitus. Adjusted OR is adjusted for age and body mass index.

**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 (fasting), OGTT (1, 2, and 3), TC, TG, 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 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 (OR) (length of 95% CI) | P | TT (OR) (length of 95% CI) | P | CG (OR) (length of 95% CI) | P | GG (OR) (length of 95% CI) | P |
|----------------------|-----------------------------|---|-----------------------------|---|-----------------------------|---|-----------------------------|---|
| FBG (mmol/L)         | 0.04 (0.12)                 | .716 | 0.00 (0.15)                 | .993 | 0.03 (0.12)                 | .792 | 0.12 (0.14)                 | .401 |
| PPBG (mmol/L)        | 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) | 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)          | 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); 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.29 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.26 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.52 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, all meta-analyses have indicated a positive association. So far, all genetic case-control studies have identified positive associations between MTNR1B gene polymorphisms and GDM. 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.

Acknowledgment
The authors would like to extend their sincere appreciation to the Saudi Society of Clinical Laboratory Sciences at King Saud University for its funding of this research project.
MTNR1B GENETIC POLYMORPHISMS

REFERENCES

1. Alharbi KK, Syed R, Alharbi FK, Khan IA. Association of apolipoprotein E polymorphism with impact on overweight University Pupils. Genetic testing and molecular bi- otechnology. 2017;21(5):353-6. PubMed

2. Wu L, Cui L, Tam WH, Ma RC, Wang CC. Genetic variants associated with gesta- tional diabetes mellitus: a meta-analysis and subgroup analysis. Scientific reports. 2016;6:30539.

3. Association AD. 2. Classification and diagnosis of diabetes: standards of medi- cal care in diabetes—2018. Diabetes Care. 2018;41(Supplement 1):S13-S27.

4. Olmos PR, Borzone GR. Basalβilins insu- lin therapy reduces maternal triglycerides in gestational diabetes without modifying cho- lesteryl ester transfer protein activity. Journal of Obstetrics and Gynaecology Research. 2017;43(9):1397-404.

5. Qi X, Gong B, Yu J, Shen L, Jin W, Wu Z, et al. Decreased cord blood estradiol levels in relation to mothers with gestational dia- betes. Medicine. 2018;97(45):e13347.

6. Cho HY, Jung I, Kim SJ. The association between maternal hyperglycemia and peri- natal outcomes in gestational diabetes mel- litus patients: A retrospective cohort study. Medicine. 2016;95(11):e5656.

7. Blumberg J, Ballares V, Durbin JL. Ethnic variations on gestational diabetes mellitus and evidence-based first-line interventions. The Journal of Maternal-Fetal & Neonatal Medicine. 2018.

8. Maslova E, Hansen S, Grunnet LG, Strem M, Bjerregaard AA, Hjort L, et al. Maternal protein intake in pregnancy and offspring metabolic health at age 9–16 y: results from a Danish cohort of gestational diabetes mel- litus pregnancies and controls. The American journal of clinical nutrition. 2017;106(2):623-36.

9. Siddiqui K, George TP. Resistin role in de- velopment of gestational diabetes mellitus. Biomarkers in medicine. 2017;11(7):579-86.

10. Herath H, Herath W, Wickremasinghe R. Gestational diabetes mellitus and risk of type 2 diabetes in their offspring. The impact of gestational diabetes mellitus in Sri Lankan women—A community based retrospective cohort study. PloS one. 2017;12(6):e0179647.

11. Ko JK, Noh SW, Estram L, Boffetta P, But- terworth AS, Canocch J, Dolan SM, et al. Genome-wide association studies, field syn- opses, and the development of the knowledge edge base on genetic variation and human diseases. American journal of epidemiology. 2009;170(3):269-79.

12. Groteveld FE, Wassenius NS, Rönö K, Lai- suori H, Stach-Leipinen B, Orho-Melander M, et al. Interaction between rs1030963 polymorphism in MTNR1B and lifestyle inter- vention on occurrence of gestational dia- betes. Diabetologia. 2016;59(9):1655-6.

13. Kong X, Zhang X, Xiong X, Zhang B, Hong J, Yang W. The association of type 2 diabetes loci identified in genome-wide association studies with metabolic syndrome and its compo- nents in a Chinese population with type 2 diabetes. PLoS One. 2015;10(11):e0143607.

14. Liu Q, Huang Z, Li H, Bai J, Liu X, Ye H. Relationship between melatonin receptor 1B (rs1030963) and rs1387153) with gestational diabetes mellitus: a case-control study and meta-analysis. Archives of gynecology and obstetrics. 2016;294(1):55-61.

15. Xu L, Yao X, Yu L, Xin Y, Zhao L, Wang Z, et al. Melatonin receptor 1B gene polymorphism rs10380963 and gestational diabetes mellitus among a Chinese popula- tion—a meta-analysis and subgroup association studies. Endokrynol Pol. 2017;68(5):550-60.

16. Salman M, Dasgupta S, Cholendra A, Venugopal P, Lakshimi G, Xaverio D, et al. MTNR1B gene polymorphisms and susceptibil- ity to Type 2 Diabetes: A pilot study in South Indians. Gene. 2015;566(2):189-93.

17. Gaulton KJ, Ferreira T, Lee Y, Raimond A, Maig R, Reschen ME, et al. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nature genetics. 2015;47(12):1415.

18. Bonnefond A, Froguel P. The case for too little melatonin signalling in increased diabe- tics risk. Diabetesologia. 2017;60(5):823-5.

19. Zhan Y, Li C, Gao Q, Chen J, Yu S, Liu S. Association between the rs4753426 gene polymorphism in MTNR1B with fasting plasma glu- cose level and pancreatic β-cell function in gestational diabetes mellitus. Genetics and Molecular Research. 2015;14(3):8787-85.

20. Wahabi HA, Esmaeil SA, Fayad A, Al- zeirani RA. Gestational diabetes mellitus: maternal and perinatal outcomes in King Abdulaziz University Hospital, Saudi Arabia. The Journal of the Egyptian Public Health Association. 2013;86(2):104-8. O’ Sullivan JB, Mahan MA. Criteria for the oral glucose tolerance test in pregnancy. Diabetes. 1964 Jun;13(7):288-85.

21. Al-Hakeem MM, Abotalib Z, Alharbi KK, Khan IA, Mohammed AA. Insertion and dele- tion polymorphism in the alpha-2B adre- nóceptor gene in pregnant women ripens gestational diabetes mellitus. Saudi journal of biological sciences. 2016;23(1):128-34.

22. Alharbi KK, Khan IA, Abotalib Z, Al-Ha- keem MM. Insulin receptor substrate-1 (IRS- 1) Gly927Arg: correlation with gestational diabetes mellitus in Saudi women. BioMed research international. 2014;2014.

23. Kim JY, Cheong HS, Park B-L, Baik SH, Park S, Lee SW, et al. Melatonin receptor 1B polymorphism in South Indians. Gene. 2011;14(11):666-9.

24. Chen Y-C, Sheen J-M, Tao M-M, Tain Y-L, Huang L-T. Roles of melatonin in fetal programming in compromised pregnancies. International journal of molecular sciences. 2013;14(4):3580-401.

25. Huerta-Chagoya A, Vázquez-Cárdenas P, Moreno-Macias H, Tapia-Maruni L, Rodrí- guez-Guillen R, Lopez-Vite E, et al. Genetic determinants for gestational diabetes mel- litus and related metabolic traits in Mexican women. PLoS one. 2015;10(5):126408.

26. Lee YH. Meta-analysis of genetic associa- tion studies. Annals of laboratory medicine. 2015;35(3):283-7.

27. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proença C, Sparse T, Holmikvist J, Marchand M, et al. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nature genetics. 2009;41(1):89.

28. Chambers JC, Zhang W, Zabenah D, Sehm J, Jain P, McCarthy MI, et al. Common genetic variation near melatonin receptor MTNR1B contributes to raised plasma glucose and increased risk of type-2 diabetes amongst Indian Asians and European whites. Diabetes. 2009.

29. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifson G, et al. VARIANTS IN MTNR1B INFLUENCE FASTING GLUCOSE LEVELS. Nature genetics. 2009;41(1):77.

30. Rönö T, Wen J, Yang Z, Lu B, Du Y, Groop L, et al. A common variant in MTNR1B, en- coding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glu- cose in Han Chinese individuals. Diabetology. 2009;52(5):830-3.

31. Sabatti C, Service SK, Hartikainen A-L, Poulet A, Ripatti S, Brodsky J, et al. Genome- wide association analysis of metabolic traits in a birth cohort from a founder population. Nature genetics. 2009;41(1):35.

32. Takeuchi F, Katsuya T, Chakrawathy S, Yamamoto K, Fujikawa K, Szezawwa M, et al. Common variants at the GCK, GCKR, G6PC2-ABCB11 and MTNR1B loci are asso- ciated with fasting glucose in two Asian populations. Diabetesologia. 2010;53(2):299.

33. Langenberg C, Pascoe L, Mari A, Tura A, Laksso M, Frayling TM, et al. Common gen- etic variation in the melatonin receptor 1B gene (MTNR1B) is associated with decreased early-phase insulin response. Diabetesologia. 2009;52(8):1537.

34. Simas T, Bonnefond A, Anderson E, Bouatia-Naji N, Holmikvist J, Wegner L, et al. The G-allele of intronic rs10830963 in MTNR1B is associated with increased risk of impaired fasting gly- cemia and type 2 diabetes through an un- known receptor-mediated mechanism. JAMA. 2017;317(18):1829-38.
MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. Nature genetics. 2012;44(3):297.

43. Ling Y, Li X, Gu Q, Chen H, Lu D, Gao X. A common polymorphism rs3781637 in MTNR1B is associated with type 2 diabetes and lipids levels in Han Chinese individuals. Cardiovascular diabetology. 2011;10(1):27.

44. Lyssenko V, Nagorny CL, Erdos MR, Wirup N, Jonsson Å, Spiegel P, et al. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nature genetics. 2009;41(1):82.

45. Xia Q, Chen Z-X, Wang Y-C, Ma Y-S, Zhang F, Che W, et al. Association between the melatonin receptor 1B gene polymorphism on the risk of type 2 diabetes, impaired glucose regulation: a meta-analysis. PloS one. 2012;7(11):e50107.

46. Junior JPL, Frigeri HR, dos Santos-Weiss IC, de Souza EM, Rego FG, Picheth G, et al. The MTNR1B gene polymorphism rs10830963 is associated with gestational diabetes in a Brazilian population. Gene. 2015;568(1):114-5.

47. Rosta K, Al-Aissa Z, Hadarits O, Harreiter J, Nádasdi Á, Kelemen F, et al. Association study with 77 SNPs confirms the robust role for the rs10830963/G of MTNR1B variant and identifies two novel associations in gestational Diabetes Mellitus development. PloS one. 2017;12(1):e0169781.

48. Tarnowski M, Malinowski D, Safranow K, Dziedziczko V, Pavlik A. MTNR1A and MTNR1B gene polymorphisms in women with gestational diabetes. Gynecological Endocrinology. 2017;33(5):395-8.

49. Vejražková D, Lukasova P, Vankova M, Vcelak J, Bradnova O, Cirmanova V, et al. MTNR1B genetic variability is associated with gestational diabetes in Czech women. International journal of endocrinology. 2014;2014.

50. Zhang C, Bao W, Rong Y, Yang H, Bowers K, Yeung E, et al. Genetic variants and the risk of gestational diabetes mellitus: a systematic review. Human reproduction update. 2013;19(4):376-90.

51. Zhang Y, Sun C-M, Hu X-Q, Zhao Y. Relationship between melatonin receptor 1B and insulin receptor substrate 1 polymorphisms with gestational diabetes mellitus: a systematic review and meta-analysis. Scientific reports. 2014;4:6113.

52. Firneisz G, Rosta K, Al-Aissa Z, Hadarits O, Harreiter J, Nádasdi Á, et al. The MTNR1B rs10830963 Variant in Interaction with Pre-Pregnancy BMI is a Pharmacogenetic Marker for the Initiation of Antenatal Insulin Therapy in Gestational Diabetes Mellitus. International journal of molecular sciences. 2018;19(12):3734.

53. Liao S, Liu Y, Tan Y, Gan L, Mei J, Song W, et al. Association of genetic variants of melatonin receptor 1B with gestational plasma glucose level and risk of glucose intolerance in pregnant Chinese women. PloS one. 2012;7(7):e40113.