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ORIGINAL ARTICLE

MTNR1B gene on susceptibility to gestational diabetes mellitus: a two-stage hospital-based study in Southern China

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Abstract

Large-scale studies on genetic risk loci for melatonin receptor 1B (*MTNR1B*) gene and GDM risk have not been well generalized to the Chinese population. In this study, we performed two-stage case–control study: 1.429 pregnant women: 753 GDM/676 controls in the Southern Chinese population by genotyping 5 SNPs (rs10830963, rs1387153, rs2166706, rs1447352, and rs4753426) in *MTNR1B*. Genotypes were determined using the Sequenom MassARRAY platform and TaqMan allelic discrimination assay. Interactions between genetic variants and age/BMI as predictors of GDM risk were evaluated under the logistic regression model. In the first stage, the SNP rs10830963 was discovered to be potentially related to GDM risk (additive model: OR = 1.27, 95%CI = 1.05–1.55, $P = 0.025$), which was further confirmed in the second stage with a similar effect (additive model: OR = 1.53, 95%CI = 1.19–1.98, $P = 0.005$). In the combined stage, the G allele of rs10830963 was potentially associated with GDM risk (additive model: OR = 1.36, 95%CI = 1.17–1.59, $P < 0.001$; dominant model: OR = 1.45, 95%CI = 1.15–1.83, $P = 0.005$). The rs10830963 interacted with age and BMI to contribute to GDM risk in the combined participants. And, the similar interactive effects for the other four SNPs also exist. These findings offer the potential to improve our understanding of the etiology of GDM, and particularly of biological mechanisms.

Keywords Single nucleotide polymorphism · Gestational diabetes mellitus · Susceptibility · Melatonin receptor 1B gene

Introduction

Gestational diabetes mellitus (GDM), one of the most common complications of pregnancy, occurs to women who have no diabetes history. The prevalence of GDM is increasing globally, with about 14% of pregnant women affected by GDM (Xie et al. 2019). GDM is related to perinatal complications and presents an overwhelmingly increased risk of metabolic disease in both mothers and their children. Women with GDM are inclined to be attacked by depressive disorder, obesity, type 2 diabetes (T2D), and cardiovascular disease. Offspring also

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have a future risk of several abnormities (e.g., macrosomia) and various medical conditions such as stillbirth, fetal prematurity, obesity, and T2D across the whole life course (Zhu and Zhang 2016).

Presumable evidence suggests that pregnant women with advanced maternal age, family history of diabetes, obesity, and unhealthy lifestyle are inclined to develop GDM (Zhang et al. 2016). Based on the candidate gene strategy, previous studies have predominantly demonstrated associations of some T2D susceptibility genes with GDM risk, including transcription factor seven like two (*TCF7L2*), melatonin receptor 1B (*MTNR1B*), insulin-like growth factor two mRNA binding protein 2 (*IGF2BP2*), and CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) (Zhang et al. 2013). Of these, to date, only one Genome-Wide Association Studies (GWAS) in the Asian population has been conducted and implicated rs10830962 near *MTNR1B and* rs7754840 *in CDKAL1* as susceptibility loci of GDM (Kwak et al. 2012).

MTNR1B gene, located in chromosome region 11q14.3, encodes the G protein-coupled melatonin receptors 1*B*. As a kind of indole hormone, melatonin participates in the regulating progress of insulin secretion, glucose metabolism, and circadian rhythms. And, melatonin must be modulated to play the function of physiological regulation through the melatonin receptors (*MTNR1A*; *MTNR1B*) (Shen and Jin 2019). Genetic variants in *MTNR1B* could lead to a higher expression of *MTNR1B* in relative tissue cells, which combine with melatonin and induce the attenuation of insulin secretion (Lane et al. 2016). The activity and expression of *MTNR1B* are probably to act as markers of active glucose homeostasis with enriched production of glucose. The polymorphism in *MTNR1B* (rs1387153, rs10830963) was linked to the impaired function of β cell and involved in the increasing risk of various phenotypes of GDM (Tarnowski et al. 2017; Alharbi et al. 2019).

To the best of our knowledge, no studies have been previously conducted to explore the possibility that SNPs of *MTNR1B* predispose to GDM risk in the Southern Chinese populations. The majority of existing observational studies has inadequately adjusted for those confounding factors, including age, BMI, and parity, which has led to inconsistent results. Given the function of the *MTNR1B* gene, we wonder whether this association signal of SNPs could be found within the complex trait of GDM or not. Therefore, the current study was undertaken to explore the following hypotheses: some candidate SNPs are associated with GDM risk by means of an association study.

Materials and methods

Study population and study design

In this case–control study, 1,429 pregnant women were recruited from two hospitals: the Affiliated Hospital of Nantong University (AHNU) and the Nantong Maternal and Child Health Hospital (NMCHH). Of these, 912 participants, recruited from AHNU between Jul 2017 and Jan 2019, were assigned into the first stage of the study. 517 participants, collected from NMCHH from Jun 2018 to Dec 2018, were assigned into the second stage. Women with GDM were diagnosed according to the 2010 International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria: fasting plasma glucose $\geq$ 5.1 mmol/L, and/or the 1-h plasma glucose $\geq$ 10.0 mmol/L, and/or the 2-h plasma glucose $\geq$ 8.5 mmol/L. The demographic information of all participants was collected by structured questionnaires, including age, weight, parity, family history of diabetes, etc. The obesity was categorized into three stratifications based on the Working Group on Obesity in China (WGOC) criteria recommended for the Chinese population (Zhou 2002): Underweight and Normal (BMI $<$ 24.0 kg/m^2), Overweight (24.0 kg/m^2 $\leq$ BMI $<$ 28.0 kg/m^2), Obese (BMI $\geq$ 28.0 kg/m^2).

All participants have signed written informed consent. The study protocol was reviewed and approved by the Ethics Committee of Nantong University. These processes were carried out following the approved guidelines. The women who were diagnosed with diabetes T1D and/or T2D before pregnancy, autoimmune and inflammatory diseases, neoplastic diseases, and chronic infections were excluded.

SNPs selection

We selected tag-SNPs in *MTNR1B* gene locations grounded on the Public 1000 genomes database (https://phase3browser.1000genomes.org/index.html) and NCBI database (https://www.ncbi.nlm.nih.gov/projects/SNP) with the Haploview 4.2 software. Common SNPs (Minor allele frequency, MAF $\geq$ 5% in Chinese Han population) were screened in *MTNR1B* gene regions. SNPs with low linkage disequilibrium (LD) analysis ($r^2 < 0.8$) were retained. To promote efficiency and reproducibility, we only selected the candidate SNP rs10830963 based on the Haploview software and those previously reported studies. Furthermore, several SNPs associated with GDM/plasma glucose levels in other studies were also considered and selected as candidate SNPs (rs1387153, rs2166706, rs1447352, and rs4753426) (Staiger et al. 2008; Liao et al.

2012; Liu et al. 2016). Finally, five SNPs were genotyped in this study. One SNP rs12285500 was excluded because of the call rate < 95%.

Genotyping

The genomic DNA samples were extracted from the participants' fasting peripheral venous blood samples collected during 24–28 weeks of gestation by a DNA Extraction Kit (Qiagen, Valencia, CA) and stored at − 30 ℃ for subsequent sequencing. Genotyping analysis in the first stage of this study was conducted through the iPLEX Sequenom MassARRAY platform (Sequenom, San Diego, California). A TaqMan allelic discrimination assay (Applied Biosystems, Inc.) was used for the second stage. The methods of the quality control of genotyping have been described in our previous study (Chu et al. 2016).

Statistical analysis

The χ^2 test and Student's *t*-test were used for categorical variables and continuous variables, respectively, to analyze the distribution differences of demographic characteristics, clinical variables, and genotypes between cases and controls. HWE for the distribution of each SNP was evaluated through the goodness-of-fit χ^2 test by comparing the observed genotype frequencies with the expected ones. Logistic regression (adjusted the covariates such as age, weight, parity, etc.) was performed to estimate the risk of GDM in each genotype under the additive model and dominant model and calculate the OR and their 95%CIs. Stratified analysis was conducted to further control the potential influence of age and BMI. The interaction effects between gene and age or BMI were analyzed through the dominant model and additive model. The Benjamini–Yekutieli procedure of false discovery rate (FDR) was employed to adjust the significance level of the association between SNPs and GDM risk in the additive model and dominant model (Benjamini et al. 2001). All the statistical analyses were performed with Stata 13.0 (Stata, College Station, TX).

Results

Case–control study

A flow diagram of sample selection is shown in (Fig. 1)**.** In the case–control study, a sample of 1,429 pregnancy women (912 from the first stage and 517 from the second stage) were recruited and evaluated for the genotype in *MTNR1B*. In our study, women with GDM covered elder pregnancy women than women without GDM in two stages ($P < 0.05$). There was a significant difference in BMI between cases and control groups in the first stage with *P* value 0.004, and with *P* value 0.031 in the second stage. Notably, women with GDM contain more pregnant women with a family history of diabetes compared with women without GDM ($P < 0.001$ for the first stage; $P = 0.036$ for the second stage). Also, a significant difference in weight ($P = 0.012$) and parity ($P = 0.002$) between two groups only in the first stage, while not in the second stage, and the family heredity history was similar and comparable in two stages. The basic information and stratified characteristics of the participants are separately shown in (Table 1).

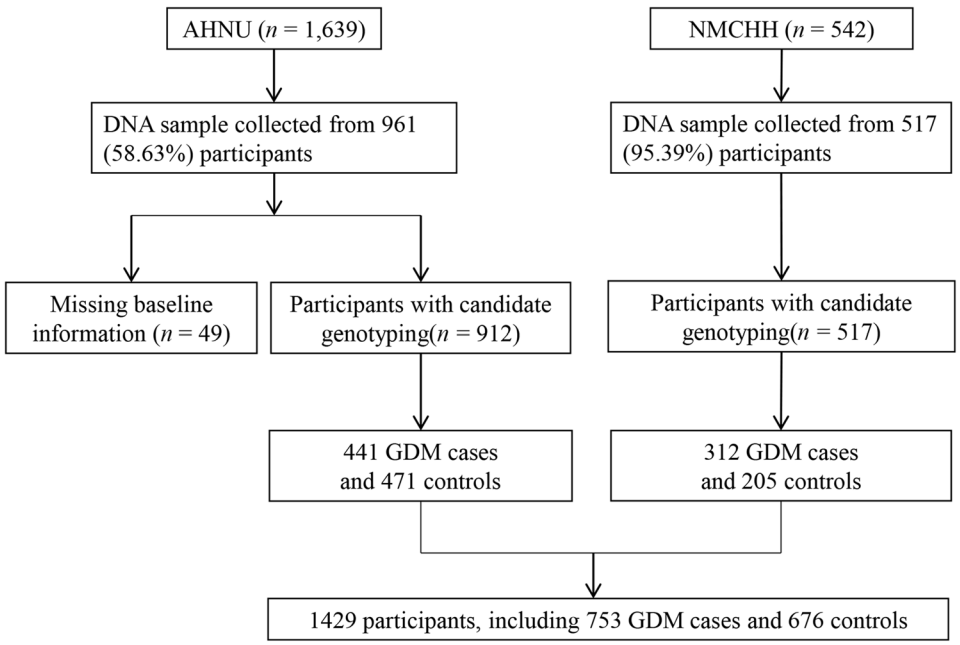

Fig. 1 The flow diagram of sample selection in the AHNU and NMCHH

Table 1 Basic characteristic of participants in the two stages of the study and stratified characteristic of participants in the two stages of the study

Variables	The first stage			The second stage		
	Cases	Controls	*P*	Cases	Controls	*P*
	(*n* = 441) *N* (%)	(*n* = 471) *N* (%)		(*n* = 312) *N* (%)	(*n* = 205) *N* (%)	
Age, year (mean ± SD)	30.15 ± 4.31	28.59 ± 3.91	**< 0.001**	29.14 ± 4.05	28.37 ± 4.30	**0.038**
Weight, kg (mean ± SD)	72.93 ± 13.44	70.85 ± 11.34	**0.012**	74.09 ± 10.60	72.15 ± 12.52	0.059
BMI, kg/m^2	27.85 ± 4.88	26.99 ± 4.15	**0.004**	28.38 ± 3.69	27.61 ± 4.34	**0.031**
Parity						
Primipara	174 (39)	235 (50)	**0.002**	136 (44)	93 (45)	0.691
Multipara	267(61)	236 (50)		176 (56)	112 (55)	
Family history of diabetes						
Yes	68 (15)	36 (7.64)	**< 0.001**	45 (14)	17 (8.29)	**0.036**
No	373 (85)	435 (92)		267 (86)	188 (92)	
Family heredity history						
Yes	82 (19)	67 (14)	0.075	51 (16)	25 (12)	0.192
No	359 (81)	404 (86)		261 (84)	180 (88)	
Variables	The first stage			The second stage		
	Cases	Controls	*P*	Cases	Controls	*P*
	(*n* = 441) *N* (%)	(*n* = 471) *N* (%)		(*n* = 312) *N* (%)	(*n* = 205) *N* (%)	
Age, year						
< 28	123 (28)	210 (45)	**< 0.001**	117 (37)	88 (43)	0.217
≥ 28	318 (72)	261(55)		195 (63)	117 (57)	
Weight, kg						
< 70	177 (40)	217 (46)	0.071	116 (37)	87 (42)	0.231
≥ 70	264 (60)	254 (54)		196 (63)	118 (58)	
BMI, kg/m^2						
< 24	80 (18)	100 (21)	**0.049**	30 (9.62)	33 (16)	**0.036**
24 ≤ BMI < 28	159 (36)	193 (41)		126 (40)	88 (43)	
≥ 28	202 (46)	178 (38)		156 (50)	84 (41)	

The genotyping success rates of all SNPs were more than 99.00%, and the genotype distribution in the control group conformed to HWE ($P > 0.05$) (See Supplementary Table 1). LD matrix of D´ values, as a common index of linkage disequilibrium, was calculated through the https://asia.ensembl.org/Homo_sapiens/Tools/LD. All 5 variants were in moderate to high LD in the study population (Supplementary Table 2) and a significant degree of LD likely does exist between the rs10830963 and all the other assessed SNPs (all $D' > 0.80$). Therefore, the rs10830963 could serve as the tag-SNP for the five candidate SNPs.

Despite the different degrees of LD among each SNPs, the analysis of the association between each SNPs and GDM risk was also conducted and demonstrated independently. As shown in (Table 2) significant associations were detected for the five SNPs of *MTNR1B* and GDM risk in the additive model and dominant model in the combined populations. In the first stage, logistic regression analyses revealed that the G allele of rs10830963 was potentially associated with GDM risk (additive model: OR = 1.27, 95%CI = 1.05–1.55, $P = 0.025$; dominant model: OR = 1.36, 95%CI = 1.01–1.83, $P = 0.067$). As expected, a further examination for the second stage was conducted to test the association of the SNP rs10830963 with GDM risk and the observation was consistent in additive model except for the dominant model (OR = 1.68, 95%CI = 1.14–2.47, $P = 0.045$). By exerting efforts to increase the statistic power, we combined the overall participants and found that minor allele (G) of rs10830963 was significantly associated with an increased GDM risk (additive model: OR = 1.36, 95%CI = 1.17–1.59, $P < 0.001$; dominant model: OR = 1.45, 95%CI = 1.15–1.83, $P = 0.005$). Notably, similar effects were detected for SNP rs1387153 and rs2166706 in each stage definitely. Interestingly, although no significant association was detected in the first stage, SNP rs1447352 and rs4753426 could serve as protective factors for GDM in the additive model and

Table 2 Associations between 5 SNPs in *MTNR1B* gene with GDM risk

	Genotypes	Case	Control	Crude OR (95%CI)	*P*	Adjust OR (95%CI)*	*P**	*FDR***
rs10830963								
Stage 1	CC	120	155	1		1		
	CG	228	237	1.24 (0.92–1.68)	0.156	1.27 (0.93–1.74)	0.127	
	GG	93	78	1.54 (1.05–2.26)	**0.027**	1.62 (1.09–2.41)	**0.017**	
	Dominant	321	315	1.32 (0.99–1.75)	0.058	1.36 (1.01–1.83)	**0.040**	0.067
	Additive			1.24 (1.03–1.50)	**0.025**	1.27 (1.05–1.55)	**0.015**	**0.025**
Stage 2	CC	81	78	1		1		
	CG	151	95	1.53 (1.02–2.29)	**0.039**	1.45 (0.96–2.18)	0.079	
	GG	79	31	2.45 (1.46–4.12)	**0.001**	2.39 (1.41–4.05)	**0.001**	
	Dominant	230	126	1.76 (1.20–2.57)	**0.004**	1.68 (1.14–2.47)	**0.009**	**0.045**
	Additive			1.56 (1.21–2.01)	**0.001**	1.53 (1.19–1.98)	**0.001**	**0.005**
Combined	CC	201	233	1		1		
	CG	379	332	1.32 (1.04–1.68)	**0.022**	1.31 (1.02–1.67)	**0.032**	
	GG	172	109	1.83 (1.35–2.48)	**< 0.001**	1.87 (1.37–2.56)	**< 0.001**	
	Dominant	551	441	1.45 (1.16–1.82)	**0.001**	1.45 (1.15–1.83)	**0.002**	**0.005**
	Additive			1.35 (1.16–1.57)	**< 0.001**	1.36 (1.17–1.59)	**< 0.001**	**1.6E-04**
rs1387153								
Stage 1	CC	122	163	1		1		
	CT	229	238	1.29 (0.96–1.73)	0.097	1.33 (0.98–1.81)	0.066	
	TT	89	68	1.75 (1.18–2.59)	**0.005**	1.88 (1.25–2.82)	**0.002**	
	Dominant	318	306	1.39 (1.05–1.84)	**0.023**	1.45 (1.08–1.95)	**0.012**	**0.050**
	Additive			1.32 (1.09–1.60)	**0.005**	1.36 (1.12–1.66)	**0.002**	**0.001**
Stage 2	CC	91	80	1		1		
	CT	146	94	1.37 (0.92–2.03)	0.124	1.31 (0.87–1.97)	0.190	
	TT	74	29	2.24 (1.33–3.79)	**0.003**	2.25 (1.32–3.83)	**0.003**	
	Dominant	220	123	1.57 (1.08–2.28)	**0.017**	1.54 (1.05–2.25)	**0.027**	0.059
	Additive			1.47 (1.15–1.90)	**0.003**	1.47 (1.14–1.89)	**0.003**	**0.008**
Combined	CC	213	243	1		1		
	CT	375	332	1.29 (1.02–1.63)	**0.035**	1.29 (1.02–1.66)	**0.034**	
	TT	163	97	1.92 (1.40–2.62)	**< 0.001**	2.01 (1.46–2.76)	**< 0.001**	
	Dominant	538	429	1.43 (1.14–1.79)	**0.002**	1.46 (1.16–1.84)	**0.001**	**0.005**
	Additive			1.37 (1.18–1.59)	**< 0.001**	1.40 (1.19–1.63)	**< 0.001**	**1.1E-04**
	Genotypes	Case	Control	Crude OR (95%CI)	*P*	Adjust OR (95%CI)*	*P**	*FDR***
rs1447352								
Stage 1	AA	235	219	1		1		
	AG	174	207	0.78 (0.59–1.03)	0.080	0.78 (0.59–1.04)	0.092	
	GG	32	42	0.71 (0.43–1.17)	0.175	0.75 (0.45–1.24)	0.261	
	Dominant	206	249	0.77 (0.59–1.00)	0.051	0.78 (0.59–1.02)	0.067	0.084
	Additive			0.82 (0.63–1.07)	0.145	0.83 (0.67–1.02)	0.081	0.101
Stage 2	AA	169	97	1		1		
	AG	113	85	0.76 (0.52–1.11)	0.159	0.72 (0.49–1.05)	0.091	
	GG	28	22	0.73 (0.39–1.35)	0.314	0.77 (0.42–1.44)	0.414	
	Dominant	141	107	0.76 (0.53–1.08)	0.122	0.73 (0.51–1.05)	0.086	0.086
	Additive			0.82 (0.63–1.07)	0.145	0.82 (0.63–1.08)	0.145	0.152
Combined	AA	404	316	1		1		
	AG	287	292	0.77 (0.62–0.96)	**0.019**	0.74 (0.59–0.93)	**0.010**	
	GG	60	64	0.73 (0.50–1.07)	0.111	0.77 (0.52–1.14)	0.188	
	Dominant	347	356	0.76 (0.62–0.94)	**0.011**	0.75 (0.61–0.93)	**0.008**	**0.010**
	Additive			0.82 (0.67–0.96)	**0.016**	0.82 (0.69–0.97)	**0.019**	**0.024**

Table 2 (continued)

	Genotypes	Case	Control	Crude OR (95%CI)	*P*	Adjust OR (95%CI)*	*P**	*FDR***
rs2166706								
Stage 1	TT	123	162	1		1		
	TC	226	237	1.26 (0.93–1.69)	0.132	1.30 (0.96–1.76)	0.095	
	CC	91	70	1.71 (1.16–2.53)	**0.007**	1.79 (1.20–2.69)	**0.004**	
	Dominant	317	307	1.36 (1.03–1.80)	**0.033**	1.41 (1.06–1.89)	**0.020**	**0.050**
	Additive			1.30 (1.08–1.57)	**0.007**	1.34 (1.10–1.63)	**0.004**	**0.010**
Stage 2	TT	91	78	1		1		
	TC	149	95	1.34 (0.90–2.00)	0.144	1.30 (0.87–1.96)	0.205	
	CC	72	31	1.99 (1.19–3.34)	**0.009**	2.01 (1.19–3.39)	**0.009**	
	Dominant	221	126	1.50 (1.04–2.18)	**0.032**	1.48 (1.01–2.16)	**0.045**	0.059
	Additive			1.40 (1.09–1.80)	**0.009**	1.40 (1.08–1.81)	**0.010**	**0.017**
Combined	TT	214	240	1		1		
	TC	375	332	1.27 (1.00–1.60)	**0.050**	1.28 (1.00–1.63)	**0.046**	
	CC	163	101	1.81 (1.33–2.47)	**< 0.001**	1.88 (1.38–2.59)	**< 0.001**	
	Dominant	538	433	1.39 (1.11–1.74)	**0.004**	1.42 (1.13–1.79)	**0.003**	**0.005**
	Additive			1.33 (1.15–1.55)	**< 0.001**	1.36 (1.17–1.59)	**< 0.001**	**1.6E-04**
	Genotypes	Case	Control	Crude OR (95%CI)	*P*	Adjust OR (95%CI)*	*P**	*FDR***
rs4753426								
Stage 1	CC	236	227	1		1		
	CT	173	201	0.83 (0.63–1.09)	0.175	0.85 (0.64–1.13)	0.272	
	TT	31	43	0.69 (0.42–1.14)	0.148	0.73 (0.44–1.22)	0.232	
	Dominant	204	244	0.80 (0.62–1.04)	0.101	0.83 (0.64–1.09)	0.180	0.180
	Additive			0.83 (0.68–1.02)	0.076	0.86 (0.69–1.06)	0.145	0.145
Stage 2	CC	174	98	1		1		
	CT	109	87	0.71 (0.49–1.03)	0.068	1.15 (0.61–2.15)	0.671	
	TT	29	20	0.82 (0.44–1.52)	0.523	0.75 (0.39–1.43)	0.379	
	Dominant	138	107	0.73 (0.51–1.03)	0.076	0.69 (0.48–0.99)	**0.047**	0.059
	Additive			0.83 (0.63–1.08)	0.156	0.82 (0.63–1.08)	0.152	0.152
Combined	CC	410	325	1		1		
	CT	282	288	0.78 (0.62–0.97)	**0.024**	0.76 (0.61–0.95)	**0.017**	
	TT	60	63	0.76 (0.52–1.11)	0.150	0.80 (0.54–1.18)	0.262	
	Dominant	342	351	0.77 (0.63–0.95)	**0.015**	0.77 (0.62–0.95)	**0.015**	**0.015**
	Additive			0.83 (0.71–0.98)	**0.023**	0.84 (0.71–0.99)	**0.035**	**0.035**

**P* value calculated by logistic regression with adjustment for age, weight, parity and family history of diabetes

***P* value after FDR correction; $P \leq 0.05$ was considered statistically significant

dominant model in the combined population no matter whether being adjusted the confounding factors or not.

Subsequently, with regard to test efficiency, the associations between 5 SNPs and the susceptibility to GDM in additive model and dominant model were evaluated by stratified analysis for age and BMI. As shown in (Fig. 2), these associations for rs10830963 and rs1387153 were more evident in subjects with age ≥ 28 (for rs10830963: dominant model: OR = 1.53, 95%CI: 1.15–2.05, $P = 0.004$; additive model: OR = 1.44, 95%CI: 1.19–1.75, $P < 0.001$; for rs1387153: dominant model: OR = 1.47, 95%CI: 1.11–1.96, $P = 0.008$; additive model: OR = 1.44, 95%CI: 1.19–1.76, $P < 0.001$.). Also, this association for rs2166706 was more evident in subjects with age ≥ 28 (dominant model: OR = 1.50; 95%CI: 1.12–1.99; $P = 0.006$; additive model: OR = 1.44, 95%CI: 1.19–1.75, $P < 0.001$). Notably, the protective roles for rs1447352 were detected in subjects with age ≥ 28 (dominant model: OR = 0.67; 95%CI: 0.51–0.88; $P = 0.004$; additive model: OR = 0.77, 95%CI: 0.62–0.95, $P < 0.013$) and BMI ≥ 28 (dominant model: OR = 0.71; 95%CI: 0.51–0.98, $P = 0.038$; additive model: OR = 0.75, 95%CI: 0.58–0.97, $P < 0.031$). The protective roles for rs4753426 (OR = 0.71; 95%CI: 0.54–0.93; $P = 0.012$) could only be detected in the dominant model in subjects with age ≥ 28. No significant

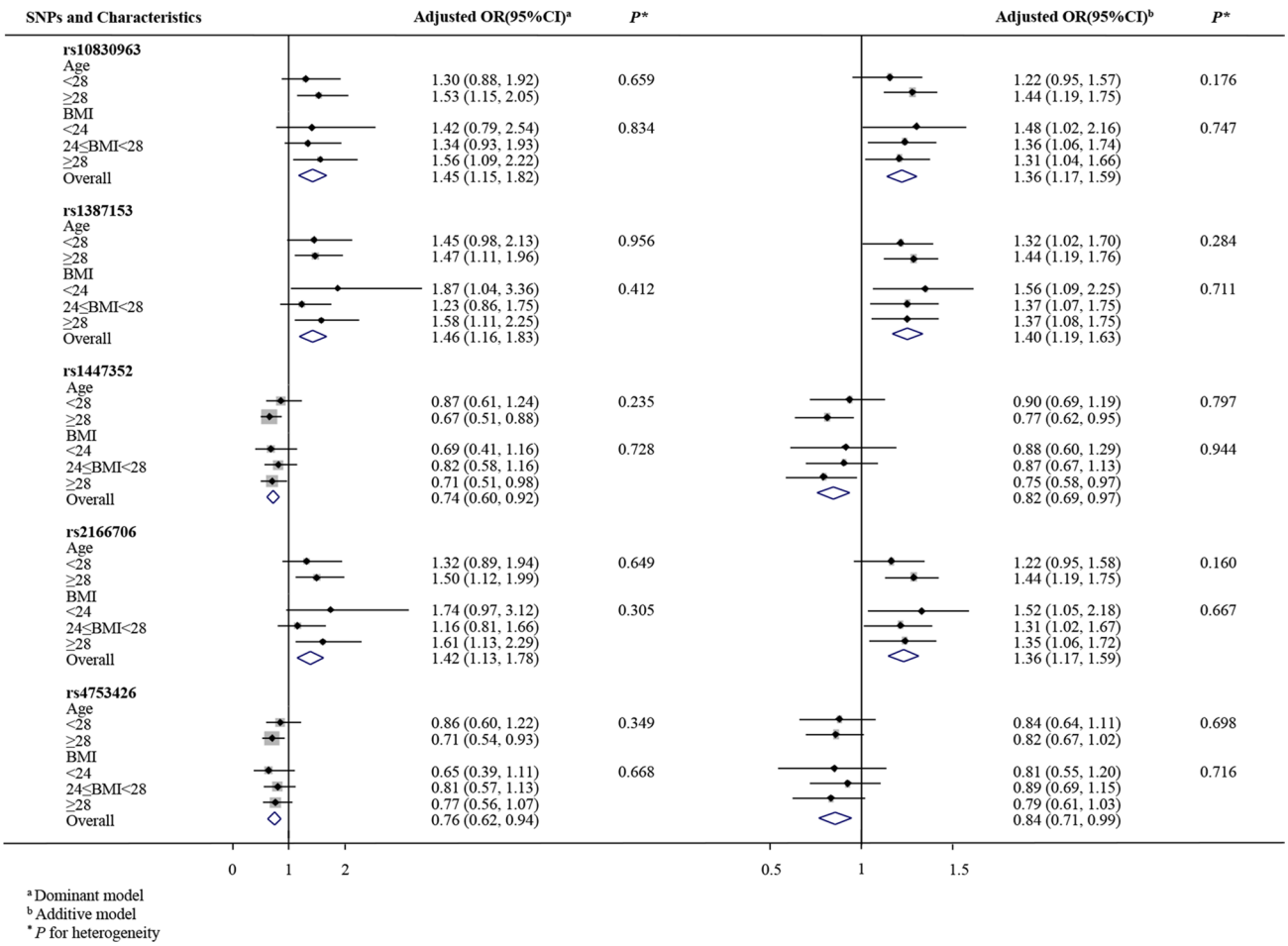

Fig. 2 Stratified analysis on the association of 5 SNPs in *MTNR1B* with GDM risk

heterogeneity was observed among the stratified subgroups. Detailed information is shown in (Fig. 2).

Considering the significant differences in the distribution of age and BMI, we further explored whether the effect of SNPs on GDM was modified by age and BMI**.** There was no multiplicative interaction between 5 SNPs and age or BMI (all $P > 0.05$). The interactive analysis showed that the rs10830963 interacted with age/BMI to contribute to GDM

Table 3 The interaction between SNP rs10830963 genotype and BMI/age on GDM risk

Factors	Genotype	Case	Control	OR (95%CI)	*P**	*P***
Age						
< 28	CC	65	100	1		0.288
< 28	CG/GG	174	197	1.34 (0.92–1.95)	0.131	
≥ 28	CC	136	133	1.50 (1.00–2.24)	0.049	
≥ 28	CG/GG	377	244	2.27 (1.58–3.25)	< 0.001	
BMI(kg/m^2)						
< 28	CC	110	142	1		0.449
< 28	CG/GG	284	271	1.34 (0.99–1.82)	0.061	
≥ 28	CC	91	91	1.26 (0.85–1.86)	0.248	
≥ 28	CG/GG	267	267	2.02 (1.46–2.78)	< 0.001	

**P* value calculated by logistic regression with adjustment for age, BMI parity and family history of diabetes

** *P* value for multiplicative interaction with adjustment for age, BMI, parity and family history of diabetes

risk (OR = 2.27, 95%CI: 1.58–3.25, $P < 0.001$; OR = 2.02; 95%CI: 1.46–2.78, $P < 0.001$) (Table 3). Similar interactive effects for the other four SNPs are shown in Supplementary table 3 and Supplementary table 4.

Discussion

Several studies have suggested that melatonin could regulate the circadian rhythm and endocrine immunity, especially insulin secretion and glucose levels (Mahanna-Gabrielli et al. 2018; Shen and Jin 2019). Variants in *MTNR1B* have been confirmed to be genetic components for GDM in multiple populations in the whole genome. The identified individual risk alleles were found to exert only moderate to small effects on the susceptibility to GDM.

In this study, without considering the LD, our results revealed that SNP rs10830963, rs1387153, and rs2166706 have significant associations with GDM risk in the Southern Chinese population. Outstandingly, SNP rs1447352, and rs4753426 could reduce the risk of GDM. This is consistent with several recent studies (Liao et al. 2012; Tarnowski et al. 2017). SNP rs10830963 and rs1387153 might reduce the function of insulin β-cells, which in turn affects the normal regulation of plasma glucose levels and leads to the occurrence of GDM (Caro-Gomez et al. 2018). And, the role of *MTNR1B* rs10830963 has been confirmed in the pathogenesis of type 2 diabetes (Gaulton et al. 2015). Furthermore, two large-scale GWAS in European and American also identified that the rs10830963 and rs1387153 pose important mechanisms of induction in the development of GDM (Rosta et al. 2017; Ding et al. 2018).

As for two SNPs, rs1447352 and rs4753426 were detected to be protective factors for GDM after adjusting potential covariates (the pooled OR = 0.82 and 0.84, respectively) in the combined stage, while not in the first stage and the second stage. Since random errors may be potential reasons for the differences in the findings among different stages. Further studies with larger sample size are warranted to test for credibility. A recent GWAS reported a significant association between rs1447352 and FPG after Bonferroni correction for multiple comparisons (Ramos et al. 2011); whereas, a contradictory conclusion was also reported (Liao et al. 2012). One study in the German population reported that rs4753426 might reduce insulin secretion and increase the level of FPG (Staiger et al. 2008). And, SNP rs4753426 was not significantly associated with GDM in white European women (Tarnowski et al. 2017). Collectively, the potential relationships between rs1447352 and rs4753426 and GDM risk have not been well documented. The possible reasons might result from differences in the genetic backgrounds of the studied population.

Further, regarding the significant degree of LD between each SNPs and rs10830963, we found that rs10830963 could be the tag-SNP of the five candidate SNPs. And as stated, in the combined stage, the OR values of additive model and dominant model of rs10830963, rs1387153 and rs2166706 were very close; these further proved the existence of tag-SNPs. Besides, the degree of LD between rs10830963 and the other four SNPs was consistent with the discovery of Liao S and Salman M (Liao et al. 2012; Salman et al. 2015). However, the real associations between SNP rs1447352, rs4753426, and rs10830963 still need to be explored in further studies. Likewise, the interaction analysis revealed that both age and BMI interacted with rs10830963 and increased GDM risk, as the same as rs1387153 and rs2166706. In the interaction analysis, we use the BMI cut-off value of ≥ 28 kg/m^2 due to the stratified analysis. And, one study by Firneisz G et al. reported that the *MTNR1B* rs10830963 in the stratified BMI (≥ 29 kg/m^2) could predict antenatal insulin therapy initiation better (Firneisz et al. 2018), which is approximately consistent with the cut-off value in our study(≥ 28 kg/m^2). Additionally, a previous study found that *MTNR1B* rs10830963 could decrease the effect of the antenatal intervention of an individual with BMI > 30 kg/m^2 and/or prior GDM in history (Grotenfelt et al. 2016). These BMI might be vital factors that influence the incidence of GDM and the effect of early medical nutrition therapy (MNT). Most importantly, since the analysis by LD matrix of *D*' values showed that five SNPs were in moderate to high LD, the significant interaction associations and the haplotype analyses deserved to be further detected in the future study.

There are several strengths in this study. First, by exerting much effort to evaluate whether several SNPs in *MTNR1B* could be well generalized to Southern Chinese population, we systematically evaluated five SNPs in women with GDM and those without GDM, and the association for SNP rs10830963 was successfully examined in the first stage and confirmed in the second stage, and the *P* values remain significant after multiple testing in the combined stage. Second, the fact that participants were recruited from the two hospitals, representing relatively reasonable controls, might not result in potential selection bias. Third, the universal screening for GDM was conducted through OGTT in those women who enrolled in pregnancy complications screening, which avoided the relatively high misdiagnosis rate caused by the census performed only in pregnant women with potential risk factors for GDM (advanced maternal age, obesity, family history of diabetes and parity). Importantly, biological and environmental factors, such as maternal age, BMI, exercise, and diet, should be considered comprehensively to understand its biological plausibility and improve the identification of women at risk of GDM, which contribute to accurately evaluate risk stratification.

However, some limitations still need to be addressed. First, several SNPs in *MTNR1B* are located in the intron and present being silent. Considering that our study exhibiting a confirmatory manifestation in the Southern Chinese population, we selected the overlapped candidate SNP between the results of Haploview and previous studies to verify the reported association between variants of *MTNR1B* (especially the rs10830963) and GDM risk. Therefore, the other SNPs detected through the Haploview were not selected in this study. The LD matrix of D´ values indicates that all $D' \geq 0.85$ for the rs10830963, a significant degree of LD likely does exist between the rs10830963 and all the other assessed SNPs. In view of the comprehensiveness and stability of *D*' value, we most likely assessed the same single genetic effect (with five markers) and not five independent genetic effects. Therefore, *MTNR1B* rs10830963 might be the truly causal SNP that drives these associations. However, the multiple comparisons test with FDR was still employed to adjust the significance level of the association in the additive model and the dominant model. Second, discrepancy among those studies is probably attributable to ethnic diversity and differences in the genotyping methods. And, the discordant results could be attributed to different diagnostic criteria. Third, little information regarding environmental exposures and serum parameters, such as diet variables and glycated hemoglobin (HbA1c) measurement, were collected and have not been adjusted for further analysis. Nevertheless, there is sufficient assurance to believe that the findings are of considerable credibility and veracity.

In summary, our results indicated that *MTNR1B* rs10830963 is independently associated with the susceptibility to GDM and it is the tag-SNP of the other four candidate SNPs. In the meantime, the association could be altered by maternal age and BMI. Well-designed studies are warranted to seek replication and validate our findings and extend these results.

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Author contributions L.Y.J. contributed to the interpretation of results and made critical revisions. Y.L.J. drafted the protocol and wrote the final paper. X.Y.S., X.F.G., P.Z., Y.L.L, A.Y.Z., participated in the data collection. And, Y.S. reviewed the draft and made the critical revision. All authors have reviewed the final version of the manuscript and approved it for publication.

Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Compliance with ethical standards

Conflict of interest Yulong Jia declares that he has no conflict of interest. Yi Shen declares that she has no conflict of interest. Xiuying Shi declares that she has no conflict of interest. Xuefeng Gu declares that he has no conflict of interest. Peng Zhang declares that he has no conflict of interest. Yuanlin Liu declares that he has no conflict of interest. Aiyong Zhu declares that he has no conflict of interest. Liying Jiang declares that she has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Novelty statements

The rs10830963 in *MTNR1B* could modify individual susceptibility to gestational diabetes mellitus(GDM) in the study population.
The rs10830963 interacted with age and BMI to contribute to GDM risk. Similar interactive effects for the other 4 SNPs also exist.

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