Genetic polymorphisms in neuroendocrine disorder-related candidate genes associated with pre-pregnancy obesity in gestational diabetes mellitus patients by using a stratification approach

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Background: Certain candidate genes have been associated with obesity. The goal of this study is to determine the association between thirteen neuroendocrine disorder-related candidate genes and pre-pregnancy obesity among gestational diabetes mellitus (GDM) patients using the stratification approach defined the Asian and International criteria-based body mass index (BMI).

Methods: This was a post-hoc case-control exploratory sub-analysis of a cross-sectional study among GDM women to determine which candidate single nucleotide polymorphisms (SNPs) related to neuroendocrine disorders may be associated with obesity. Factors were adjusted for socio-demographic characteristics and concurrent medical problems in this particular population. Pre-pregnancy BMI and concurrent medical profiles were obtained from maternal health records. Obesity is defined as BMI of ≥27.5 kg/m^2 for Asian criteria-based BMI and >30 kg/m^2 for International criteria-based BMI. Thirteen candidate genes were genotyped using Agena® MassARRAY and examined for association with pre-pregnancy obesity using multiple logistic regression analysis. The significant difference threshold was set at P value <0.05.

Results: Three hundred and twelve GDM women were included in this study; 60.9% and 44.2% of GDM patients were obese using Asian and International criteria-based BMI, respectively. GDM patients with AA or AG genotypes in specific SNP of brain-derived neurotrophic factor (*BDNF*) (G > A in rs6265) are more likely to be obese (adjusted odd ratio =2.209, 95% CI, 1.305, 3.739, P=0.003) compared to those who carry the GG genotype in the SNP adjusted for parity, underlying with asthma, heart disease, anaemia, education background in the International criteria-based BMI stratification group. On the other hand, there were no associations between other candidate genes (*NRG1*, *FKBP5*, *RORA*, *OXTR*, *PLEKHG1*, *HTR2C*, *LHPP*, *SDK2*, *TEX51*, *EPHX2*, *NPY5R* and *ANO2*) and maternal obesity.

Conclusions: In summary, *BDNF* rs6265 is significantly associated with pre-pregnancy obesity among GDM patients. The exact role of *BDNF* adjusted for diet intake and lifestyle factors merits further investigation.

Keywords: Polymorphisms; genetic variation; obesity; brain-derived neurotrophic factor (BDNF); gestational diabetes

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Introduction

Pre-pregnancy obesity is a major burden throughout the world, especially in developing countries (1). Obesity in increasing worldwide, especially in Asia (2) and the prevalence of obesity among women is higher than in men (3). Pre-pregnancy obesity is a predictor of adverse pregnancy outcomes, with many studies reporting that pre-pregnancy obesity is associated with higher odds of having gestational diabetes mellitus (GDM) [odd ratio (OR) =3.98]; gestational hypertension disorders (OR =3.68); preeclampsia (OR =3.20), macrosomia (OR =2.17) (4-6); preterm delivery [relative risk (RR) =1.35]; and caesarean section (RR =1.66) as compared to women with normal weight (6).

Studies have reported that pre-pregnancy obesity is associated with dietary preference, sedentary lifestyle and lack of awareness in metabolic management (7,8), however the underlying mechanism for these associated factors can influence metabolism in women still remains unclear. Genetic factors are now regarded as a highly plausible explanation for explaining the association between pre-pregnancy obesity and aforementioned associated factors (9-11) as studies have shown that genetic factors had contributed e to 40% to 70% of variation in the risk of developing obesity (9-12).

Candidate gene studies are hypothesis-driven, and numerous of genes have been tested for obesity. Evidence from studies worldwide across different populations has been used to establish a human obesity gene map (13,14). Nevertheless, interest remains in the analysis of candidate genes for the reason that certain candidate genes may have overlapping functions across various traits and diseases (15). To this end, we address this issue for obesity-susceptibility by constructing a custom of single nucleotide polymorphism (SNP) array containing thirteen candidate genes that were previously tested and found to have an association with either obesity or psychiatric symptoms.

This custom SNPs provides excellent coverage of many previously tested neuroendocrine disorder-related candidate genes for obesity, including brain-derived neurotrophic factor (*BDNF*) (16,17), *FKBP5* (18), *NPY5R* (19), *EPHX2* (20) and *TPH2* (21). In contrast, genetic association studies of obesity with the following neuroendocrine disorder-related candidate genes, such as *ANO2* (22),

HTR2C (23), *LHPP* (24), *NRG1* (25), *OXTR* (26), *RORA* (27), *SDK2* (22), *TEX51* (22) and *PLEKHG1* (22) have not been evaluated. It is well known that obesity is closely related to psychiatry symptoms, since a large proportion of individuals with psychiatric symptoms such as depression or anxiety also tend to be obese (28-30); Similarly, those who are obese are at higher risk of developing depression or anxiety symptoms (28,31,32). In addition, there is increasing support for the notion that obesity is a neuroendocrine disorder in which increased leptin, insulin, glucose, triglycerides, and inflammatory cytokines lead to alterations in hypothalamic pituitary adrenal axis, serotonergic and dopaminergic system, increasing the risk of behavioural and mental health disorders (33-35). Thus, the relevance of neuroendocrine disorders-related candidate genes in predisposal for pre-pregnancy obesity is worth investigating.

The aim of the present study was to perform neuroendocrine disorder-related candidate gene analysis via mass array to evaluate the association between pre-pregnancy obesity and thirteen candidate genes adjusted for socio-demographical background, maternal and clinical profile among GDM women using a stratification approach. The association analysis between the candidate genes and pre-pregnancy obesity was as defined by Asian and International criteria-based body mass index (BMI) groups and independently analysed. We present the following article in accordance with the STREGA reporting checklist (available at http://dx.doi.org/10.21037/atm-20-1579) (36).

Methods

Study population

We performed a post-hoc case-control analysis of a cross-sectional study among GDM women (n=312) to check for candidate SNPs that may be associated with obesity in this particular population according to the Asian and International criteria-based BMI.

The study participants were women with GDM who were enrolled for a cross-sectional study (37). All participants were native Malaysian with GDM and residents of surrounding areas. They were recruited during second or third trimester care at two tertiary hospitals in Klang Valley,

Malaysia between 1st June 2018 and 31st October 2018. The inclusion criteria were previously described in the study by Lee *et al.*, 2019 (37). In brief, the participant must be a Malaysian woman, pregnant, 18 years of age or older and with a diagnosis of GDM according to Malaysian Clinical Practice Guidelines (38,39).

Socio-demographic background and clinical characteristics

Socio-demographic backgrounds and clinical characteristics were recorded at enrollment to obtain information related to maternal profile, past-obstetrics history, concurrent medical problems, family history and psychiatric symptoms (including depression, anxiety and stress). These data were obtained from the self-administered questionnaire and medical records.

Measurement of pre-pregnancy obesity

The anthropometric data of participants were obtained from each mother's health records. Pre-pregnancy weight and height were self-reported by the pregnant mothers and recorded by a medical assistant during the first antenatal booking. Pre-pregnancy obesity is defined as women with a BMI ≥ 30 kg/m^2 before the pregnancy visit by using the international BMI classification (40). It is calculated by dividing weight at pre-pregnancy weight in kilograms (kg) by height in meters squared (m^2) (41). BMI it is used to estimate the total body fat and assesses the risk for diseases related to increased body fat. The WHO criteria for International criteria-based BMI classifies a BMI of <18.5 kg/m^2 as underweight; 18.5–24.9 kg/m^2 (as normal); 25.0–29.9 kg/m^2 (overweight); and >30 kg/m^2 as obese (42-44).

Studies have showed that Asian people may have increased health risks at a lower BMI compared to Caucasians; therefore, the Asian criteria-based BMI was modified specifically for Asian adults. Its cut-off points are lower than those defined for International criteria. For instance, WHO recommended cut-points for Asian criteria-based BMI categories as follows: <18.5 kg/m^2 (underweight); 18.5–22.9 kg/m^2 (normal); 23.0–27.4 kg/m^2 (overweight) and $\geq$27.5 (obesity) (45,46). This categorizing scheme follows National Institute for Health and Care Excellence (NICE) recommendations for Asians (47,48).

Participants

Regarding patients and controls, we analyzed the association between candidate genes and obesity using two different criteria-based BMI categories which are the Asian and International criteria based BMI categories. Participants in control group were those patients with normal weight and those overweight as defined using BMI value, while participants in the patient group were those defined as being obese. Upon completion of sample collection and analysis, data for baseline BMI and polymorphisms of candidate genes were readily available for a total of 312 participants.

Study outcomes, predictors and potential confounders

The study outcomes were association between genetic polymorphism in neuroendocrine disorder-related candidate genes and pre-pregnancy obesity. The association was presented in crude OR and adjusted OR (95% confidence interval). The predictors in this study were neuroendocrine disorder-related candidate genes. The potential confounders were socio-demographic background and clinical characteristics.

Blood sample collection, DNA extraction and Mass-array genotyping

Detailed blood sampling and DNA extraction methods have been previously described (49). In brief, 5 mL of blood samples of participants were collected by a phlebotomist and genomic DNA was isolated by using the QIAamp Blood DNA Mini Kit (QIAGEN, Hilden, Germany). The genotyping analysis for candidate genes polymorphism was conducted using the Agene® MassARRAY platform. SNP analysis performed using a Typer Analyzer.

Bias

We performed Bonferroni correction for multiple statistical significance tests to minimize bias arising from multiple testing errors.

Sample size calculation

The sample size was calculated using the following formula:

$$n = Z_{a/2}^2 p^\wedge (1 - p^\wedge)/e^2 \quad (50) \qquad [1]$$

Let $p^\wedge$ = population proportion of class of interest, here $p^\wedge$ =0.237 (16); $Z_{a/2}$ = population distribution for one sided test; and e = maximum error allow, say 0.07 (50).

$$n = Z_{a/2}^2 p^\wedge (1 - p^\wedge)/e^2 \qquad [2]$$

If $Z_{a/2}(0.95) =1.96$; $p^\wedge =0.237$ and e =0.07, then the sample size is:

$$n =(1.96)^2 (0.237) (0.763)/(0.07)^2 \qquad [3]$$

$$n =3.8416 (0.1808)/0.005 \qquad [4]$$

$$n =0.6946/0.005 \qquad [5]$$

n =139. Thus, around 139 obese GDM women to estimate p with 95% CI was needed.

Quantitative variables

Data on socio-demographic background, clinical characteristics and candidate genes are presented in term of N (%). Dependent variables were categorized into two groups: normal or overweight group and obese group. Data on age and monthly family income are presented in mean ± standard deviation.

Statistical analysis

We used IBM SPSS Statistics version 21.0 to perform the data analysis. A chi-square goodness-of-fit test was performed to assess the agreement of the genotype distribution among candidate genes using Hardy-Weinberg equilibrium, in which if the P value for chi-square goodness-of-fit tests is significant (P<0.05), the population is not in Hardy-Weinberg equilibrium. If the genotype distribution of candidate genes does not fit Hardy-Weinberg equilibrium based on equal distribution, the expected values for genotype distribution will be adjusted according to the global population. Univariate analysis was used to analyse the association between candidate genes and obesity among the GDM mothers. Significant difference is set at a P value <0.05. In addition, we tested the candidate gene polymorphism associations with obesity and any polymorphism adjusted for socio-demographical and clinical moderator effects. Variables with a P value of less than 0.25 in univariate analysis underwent Bonferroni correction for multiple statistical significance tests. Variables with P value of less than 0.25 after a Bonferroni adjustment were entered into the multiple logistic regression analysis (51), adjusting for the fact that a rigidly set P value at <0.05 may miss many clinically important variables (52,53). A backward stepwise regression method was used (54). All analyses were made with a 95% CI, and the level of significance was set at P<0.05.

Ethical consideration

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Medical Research Ethics Committee, Ministry of Health Malaysia (No. NMRR-17-2264-37814) and informed consent was taken from all the patients.

Results

We found that 60.9% of GDM patients were obese using the Asian criteria-based BMI higher than the percentage of GDM patients with obesity (44.2%) using the International criteria-based BMI. We found a significant association only in the association between specific SNP (rs6265) of gene *BDNF* and pre-pregnancy obesity using International criteria-based BMI but not in Asian criteria-based BMI.

Analyses of the socio-demographic characteristics, past obstetric history, concurrent medical problems and family history of the 312 participants as stratified by Asian and International criteria-based BMI were performed and is shown in *Table 1*. Among the independent variables that were investigated, a significant difference was observed only in concurrent medical problems which were asthma and anaemia after a Bonferroni adjustment in the context of family-wise error for Asian criteria-based BMI categorization among GDM women (P<0.05). Asthma (P<0.05) was the only independent variable with a significant difference after a Bonferroni adjustment in the context of family-wise error for International criteria-based BMI categorization.

Analyses of the *NRG1*, *FKBP5*, *RORA*, *OXTR*, *BDNF*, *PLEKHG1* and *HTR2C* genotypes among the GDM patients with and without obesity (n=312) as stratified by Asian and International criteria-based BMI using the univariate analysis is shown in *Table 2*. Analyses of the *LHPP*, *SDK2*, *TEX51*, *EPHX2*, *NPY5R* and *ANO2* genotype among GDM women with or without obesity that were stratified by Asian and International criteria-based BMI are shown in *Table S1*, because these candidate genes have a P value >0.25 using univariate analysis.

Notably, the proportion of the AG or AA genotypes was higher than that of the GG genotype in SNP of *BDNF* (G > A in rs6265) among obese GDM women (57.7% versus 42.3%; P=0.024 after a Bonferroni adjustment) as shown in *Table 2*. On the other hand, there were no significant associations between SNPs for candidate genes (*NRG1*, *FKBP5*, *RORA*, *OXTR*, *PLEKHG1* and *HTR2C*) and pre-pregnancy obesity (P>0.05) in both stratification groups.

The associations between specific SNP's genotype of candidate genes and pre-pregnancy obesity adjusted for

Table 1 Univariate analysis on the socio-demographic background and clinical characteristics of the participants with and without obesity (n=312)

Parameters	Category	Asian criteria-based BMI			International criteria-based BMI		
		Normal and overweight (n=122)	Obese (n=190)	P value	Normal and overweight (n=174)	Obese (n=138)	P value
Socio-demographic characteristics							
Age		31.98±5.17	32.16±4.87	0.757	32.05±4.98	32.15±5.00	0.852
Ethnicity	Malay	98 (38.6)	156 (61.4)	0.694	138 (54.4)	116 (45.7)	0.284
	Non-Malay	24 (41.4)	34 (58.6)		36 (62.1)	22 (37.9)	
Religion	Muslim	101 (39.1)	157 (60.9)	0.972	142 (55.0)	116 (45.0)	0.570
	Non-Muslim	21 (38.9)	33 (61.1)		32 (59.3)	22 (40.7)	
Education	Secondary and below	61 (37.0)	104 (63.0)	0.419	83 (50.3)	82 (49.7)	0.039[a]
	Tertiary	61 (41.5)	86 (58.5)		91 (61.9)	56 (38.1)	
Employment	Unemployed	46 (37.7)	76 (62.3)	0.685	62 (50.8)	60 (49.2)	0.158[a]
	Employed	76 (40.0)	114 (60.0)		112 (58.9)	78 (41.1)	
Monthly family income, Ringgit Malaysia		3,720.89±2,263.29	3,479.65±2,338.73	0.282	3,823.04±2,204.97	3,263.49±2,404.31	0.042
Marital status	Without husband	4 (30.8)	9 (69.2)	0.529	6 (46.2)	7 (53.8)	0.476
	With husband	118 (39.5)	181 (60.5)		168 (56.2)	131 (43.8)	
Parity	Nulliparous-Primiparous	58 (34.7)	109 (65.3)	0.089[a]	85 (50.9)	82 (49.1)	0.063[a]
	Multiparous ≥2	64 (44.1)	81 (55.9)		89 (61.4)	56 (38.6)	
Smoking habit	No	121 (39.5)	185 (60.5)	0.41	173 (56.5)	133 (43.5)	0.091[b]
	Yes	1 (16.7)	5 (83.3)		1 (16.7)	5 (83.3)	
Drink alcohol	No	120 (39.3)	185 (60.7)	0.709	171 (56.1)	134 (43.9)	0.704
	Yes	2 (28.6)	5 (71.4)		3 (42.9)	4 (57.1)	
Past obstetric history							
Abortion	No	92 (40.2)	137 (59.8)	0.519	130 (56.8)	99 (43.2)	0.555
	Yes	30 (36.1)	53 (63.9)		44 (53.0)	39 (47.0)	
Macrosomia	No	119 (39.0)	186 (61.0)	1.000	171 (56.1)	134 (43.9)	0.704
	Yes	3 (42.9)	4 (57.1)		3 (42.9)	4 (57.1)	
Gestational hypertension	No	117 (39.4)	180 (60.6)	0.639	166 (55.9)	131 (44.1)	0.846
	Yes	5 (33.3)	10 (66.7)		8 (53.3)	7 (46.7)	
Stillbirth	No	117 (39.0)	183 (61.0)	1.000	166 (55.3)	134 (44.7)	0.438
	Yes	5 (41.7)	7 (58.3)		8 (66.7)	4 (33.3)	
Preterm delivery	No	115 (38.5)	184 (61.5)	0.266	166 (55.5)	133 (44.5)	0.669
	Yes	7 (53.8)	6 (46.2)		8 (61.5)	5 (38.5)	
Gestational diabetes mellitus	No	96 (40.2)	143 (59.8)	0.486	135 (56.5)	104 (43.5)	0.645
	Yes	26 (35.6)	47 (64.4)		39 (53.4)	34 (46.6)	

Table 1 *(continued)*

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Table 1 (continued)

Parameters	Category	Asian criteria-based BMI			International criteria-based BMI		
		Normal and overweight (n=122)	Obese (n=190)	P value	Normal and overweight (n=174)	Obese (n=138)	P value
Concurrent medical problems							
Hypertension	No	118 (39.7)	179 (60.3)	0.312	168 (56.4)	130 (43.6)	0.320
	Yes	4 (26.7)	11 (73.3)		6 (42.9)	8 (57.1)	
Allergy	No	118 (38.7)	187 (61.3)	0.438	169 (55.4)	136 (44.6)	0.470
	Yes	4 (57.1)	3 (42.9)		5 (71.4)	2 (28.6)	
Asthma	No	119 (41.8)	166 (58.2)	0.002[a]	165 (57.9)	120 (42.1)	0.014[a]
	Yes	3 (11.1)	24 (88.9)		9 (33.3)	18 (66.7)	
Heart disease	No	118 (38.6)	188 (61.4)	0.214[b]	169 (55.2)	137 (44.8)	0.233[b]
	Yes	4 (66.7)	2 (33.3)		5 (83.3)	1 (16.7)	
Anaemia	No	109 (37.3)	183 (62.7)	0.014[a]	159 (54.4)	133 (45.5)	0.070[a]
	Yes	13 (65.0)	7 (35.0)		15 (75.0)	5 (25.0)	
Thalassemia	No	121 (39.2)	188 (60.8)	1.000	172 (55.7)	137 (44.3)	1.000
	Yes	1 (33.3)	2 (66.7)		2 (66.7)	1 (33.3)	
Family history							
Diabetes mellitus	No	57 (42.5)	77 (57.5)	0.281	78 (58.2)	56 (41.8)	0.452
	Yes	65 (36.5)	113 (63.5)		96 (53.9)	82 (46.1)	
Heart disease	No	104 (39.5)	159 (60.5)	0.711	148 (56.3)	115 (43.7)	0.678
	Yes	18 (36.7)	31 (63.3)		26 (53.1)	23 (46.9)	
Hypertension	No	61 (41.2)	87 (58.8)	0.467	88 (59.5)	60 (40.5)	0.212[a]
	Yes	61 (37.2)	103 (62.8)		86 (52.4)	78 (47.6)	
Gestational diabetes mellitus	No	80 (41.0)	115 (59.0)	0.369	108 (55.4)	87 (44.6)	0.860
	Yes	42 (35.9)	75 (64.1)		66 (56.4)	51 (43.6)	
Psychiatric symptoms							
Depression symptoms	Normal	163 (38.8)	257 (61.2)	0.841	228 (54.3)	192 (45.7)	0.769
	Mild-extremely severe	24 (37.5)	40 (62.5)		36 (56.2)	28 (43.8)	
Anxiety symptoms	Normal	113 (39.1)	176 (60.9)	0.799	161 (55.7)	128 (44.3)	0.531
	Mild-extremely severe	74 (37.9)	121 (62.1)		103 (52.8)	92 (47.2)	
Stress symptoms	Normal	166 (38.5)	265 (61.5)	0.876	236 (54.8)	195 (45.2)	0.790
	Mild-extremely severe	21 (39.6)	32 (60.4)		28 (52.8)	25 (47.2)	

Data are presented as either n (%) or mean ± SD. [a], Pearson Chi-square at P<0.25 entered Bonferroni adjustment before multiple regression analysis. [b], Fisher's Exact test at P<0.25 entered Bonferroni adjustment before multiple regression analysis. After a Bonferroni adjustment in the context of family-wise error for Asian criteria-based BMI categorization among GDM women, the adjusted P value for parity was 0.152, asthma (P=0.001), heart disease (P=0.226), and anaemia (P=0.031). After a Bonferroni adjustment in the context of family-wise error for International criteria-based BMI categorization among GDM women, the adjusted P value for education was 0.075, parity (P=0.109), smoking habit (P=0.109), asthma (P=0.031), heart disease (P=0.226), anaemia (P=0.129) and family history of hypertension (P=0.276). BMI, body mass index; GDM, gestational diabetes mellitus.

Table 2 Analyses of the *NRG1*, *FKBP5*, *RORA*, *OXTR*, *BDNF*, *PLEKHG1* and *HTR2C* genotypes among the GDM patients with and without obesity (n=312)

Candidate genes	SNP	Genotype	Asian criteria-based BMI			International criteria-based BMI		
			Normal and overweight (n=122)	Obese (n=190)	P value	Normal and overweight (n=174)	Obese (n=138)	P value
NRG1	rs2919375	TT	42 (34.4)	85 (45.0)	0.115[a]	62 (35.6)	65 (47.4)	0.108[a]
		TC	60 (49.2)	84 (44.4)		88 (50.6)	56 (40.9)	
		CC	20 (16.4)	20 (10.6)		24 (13.8)	16 (11.7)	
		TT genotype	42 (34.4)	85 (45.0)	0.065[a]	62 (35.6)	65 (47.4)	0.035[a]
		C carrier	80 (65.6)	104 (55.0)		112 (64.4)	72 (52.6)	
		T carrier	102 (83.6)	169 (89.4)	0.135[a]	150 (86.2)	121 (88.3)	0.580
		CC genotype	20 (16.4)	20 (10.6)		24 (13.8)	16 (11.7)	
FKBP5	rs3800373	TT	56 (47.9)	78 (41.1)	0.408	80 (47.3)	54 (39.1)	0.289
		TG	52 (44.4)	91 (47.9)		72 (42.6)	71 (51.4)	
		GG	9 (7.7)	21 (11.1)		17 (10.1)	13 (9.4)	
		TT genotype	56 (47.9)	78 (41.1)	0.243[a]	80 (47.3)	54 (39.1)	0.149 [a]
		G carrier	61 (52.1)	112 (58.9)		89 (52.7)	84 (60.9)	
		T carrier	108 (92.3)	169 (88.9)	0.336	152 (89.9)	125 (90.6)	0.851
		GG genotype	9 (7.7)	21 (11.1)		17 (10.1)	13 (9.4)	
RORA	rs4775340	GG	79 (64.8)	118 62.4)	0.878	112 (64.4)	85 (62.0)	0.288
		GA	38 (31.1)	64 (33.9)		53 (30.5)	49 (35.8)	
		AA	5 (4.1)	7 (3.7)		9 (5.2)	6 (2.2)	
		GG genotype	79 (64.8)	118 (62.4)	0.678	112 (64.4)	85 (62.0)	0.673
		A carrier	43 (35.2)	71 (37.6)		62 (35.6)	52 (38.0)	
		G carrier	117 (95.9)	182 (96.3)	1.000	165 (94.8)	134 (97.8)	0.175[a]
		AA genotype	5 (4.1)	7 (3.7)		9 (5.2)	3 (2.2)	
OXTR	rs53576	AA	33 (27.3)	47 (24.7)	0.286	46 (26.6)	34 (24.6)	0.536
		AG	65 (53.7)	92 (48.4)		90 (52.0)	67 (48.6)	
		GG	23 (19.0)	51 (26.8)		37 (21.4)	37 (26.8)	
		AA genotype	33 (27.3)	47 (24.7)	0.618	46 (26.6)	34 (24.6)	0.696
		G carrier	88 (72.7)	143 (75.3)		127 (73.4)	104 (75.4)	
		A carrier	98 (81.0)	139 (73.2)	0.114[a]	1136 (78.6)	101 (73.2)	0.264
		GG genotype	23 (19.0)	51 (26.8)		37 (21.4)	37 (26.8)	

Table 2 *(continued)*

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Table 2 *(continued)*

Candidate genes	SNP	Genotype	Asian criteria-based BMI			International criteria-based BMI		
			Normal and overweight (n=122)	Obese (n=190)	P value	Normal and overweight (n=174)	Obese (n=138)	P value
BDNF	rs6265	GG	36 (29.8)	71 (37.62)	0.310	49 (28.3)	58 (42.3)	0.018[a]
		GA	61 (50.4)	89 (47.1)		88 (50.9)	62 (45.3)	
		AA	24 (19.8)	29 (15.3)		36 (20.8)	17 (12.4)	
		GG genotype	36 (29.8)	71 (37.6)	0.158[a]	49 (28.3)	58 (42.3)	0.010[a]
		A carrier	85 (70.2)	118 (62.4)		124 (71.7)	79 (57.7)	
		G carrier	97 (80.2)	160 (84.7)	0.306	137 (79.2)	120 (87.6)	0.051[a]
		AA genotype	24 (19.8)	29 (15.3)		36 (20.8)	17 (12.4)	
FKBP5	rs9470080	CC	60 (49.2)	76 (40.0)	0.104[a]	81 (46.6)	55 (39.9)	0.460
		CT	53 (43.4)	87 (45.8)		75 (43.1)	65 (47.1)	
		TT	9 (7.4)	27 (14.2)		18 (10.3)	18 (13.0)	
		CC genotype	60 (49.2)	76 (40.0)	0.111[a]	81 (46.6)	55 (39.9)	0.236[a]
		T carrier	62 (50.8)	114 (60.0)		93 (53.4)	83 (60.1)	
		C carrier	113 (92.6)	163 (85.8)	0.065[a]	156 (89.7)	120 (87.0)	0.459
		TT genotype	9 (7.4)	27 (14.2)		18 (10.3)	18 (13.0)	
PLEKHG1	rs9372078	AA	44 (36.7)	77 (40.7)	0.200[a]	69 (40.1)	52 (38.0)	0.817
		AT	54 (45.0)	91 (48.1)		78 (45.3)	67 (48.9)	
		TT	22 (18.3)	21 (11.1)		25 (14.5)	18 (13.1)	
		AA genotype	44 (36.7)	77 (40.7)	0.475	69 (40.1)	52 (38.0)	0.699
		T carrier	76 (63.3)	112 (59.3)		103 (59.9)	85 (62.0)	
		A carrier	98 (81.7)	168 (88.9)	0.074[a]	147 (85.5)	119 (86.9)	0.725
		TT genotype	22 (18.3)	21 (11.1)		25 (14.5)	18 (13.1)	
HTR2C	rs6318	GG	115 (95.0)	177 (93.2)	0.638	166 (96.0)	126 (91.3)	0.176[b]
		GC	6 (5.0)	12 (6.3)		7 (4.0)	11 (8.0)	
		CC	0 (0.0)	1 (0.5)		0 (0.0)	1 (0.7)	
		GG genotype	115 (95.0)	177 (93.2)	0.499	166 (96.0)	126 (91.3)	0.089[a]
		C carrier	6 (5.0)	13 (6.8)		7 (4.0)	12 (8.7)	
		G carrier	121 (100.0)	189 (99.5)	1.000	173 (100.0)	137 (99.3)	0.444
		CC genotype	0 (0.0)	1 (0.5)		0 (0.0)	1 (0.7)	

Data are presented as either n (%). [a], Pearson Chi-square at P<0.25 entered Bonferroni adjustment before multiple regression analysis. [b], Fisher's Exact test at P<0.25 entered Bonferroni adjustment before multiple regression analysis. After a Bonferroni adjustment in the context of family-wise error for Asian criteria-based BMI categorization among GDM women, the adjusted P value for NRG1 (rs2919375) was 0.129, FKBP5 (rs3800373) was 0.276, OXTR (rs53576) was 0.175, BDNF (rs6265) was 0.226, FKBP5 (rs9470080) was 0.129 and PLEKHG1(rs9372078) was 0.129. After a Bonferroni adjustment in the context of family-wise error for International criteria-based BMI categorization among GDM women, the adjusted P value for NRG1 (rs2919375) was 0.075, FKBP5 (rs3800373) was 0.226, RORA (rs4775340) was 0.226, BDNF (rs6265) was 0.024, FKBP5 (rs9470080) was 0.276, and HTR2C (rs6318) was 0.152. BMI, body mass index; SNP, single nucleotide polymorphisms; GDM, gestational diabetes mellitus.

Table 3 Multiple regression analysis between genotypes of candidate genes for obesity among the GDM patients stratified using Asian criteria-based BMI classifications adjusted for confounding factors (n=312)

Candidate genes (SNP) or factors	Genotypes	Asian criteria-based BMI	
		Crude OR (95% CI), P value	Adjusted OR (95% CI), P value
NRG1 (rs2919375)	TT	1	1
	TC/CC	1.545 (0.932, 2.560), 0.091	1.604 (0.972, 2.647), 0.065
OXTR (rs53576)	AA/AG	1.753 (0.959, 3.205), 0.068	1.785 (0.977, 3.262), 0.060
	GG	1	1
BDNF (rs6265)	GG	1	1
	AA/AG	1.259 (0.743, 2.132), 0.392	1.259 (0.743, 2.132), 0.392
FKBP5 (rs9470080)	CC/CT	2.166 (0.891, 5.263), 0.088	2.263 (0.950, 5.392), 0.065
	TT	1	1
PLEKHG1 (rs9372078)	AA/AT	1.851 (0.919, 3.726), 0.085	1.986 (0.997, 3.957), 0.051
	TT	1	1
Parity	Nulliparous-Primiparous	1.542 (0.937, 2.540), 0.089	1.598 (0.976, 2.617), 0.062
	Multiparous ≥2	1	1
Asthma	No	1	1
	Yes	6.655 (1.770, 25.020), 0.005	5.738 (1.598, 20.602), 0.007
Heart disease	No	4.105 (0.427, 39.442), 0.221	4.415 (0.460, 42.357), 0.198
	Yes	1	1
Anaemia	No	4.944 (1.685, 14.506), 0.004	5.239 (1.810, 15.171), 0.002
	Yes	1	1

Adjusted OR was determined by adjusting for socio-demographical and clinical moderators with P value <0.25 in univariate analysis. BMI, body mass index; GDM, gestational diabetes mellitus.

socio-demographic characteristics and concurrent medical problems are shown in *Table 3* for Asian criteria-based BMI classification, and *Table 4* for International criteria-based BMI classification. GDM patients with the AA or AG genotypes in specific SNP of *BDNF* (G > A in rs6265) have a 2.2 times higher odds to be obese compared to those who carry GG genotype in the SNP adjusted for parity, underlying with asthma, heart disease, anaemia, education background, smoking habit and monthly family income in the International criteria-based BMI stratification group. GDM patients with underlying asthma appeared to be significantly associated with pre-pregnancy obesity in both stratification groups, with GDM patients with underlying asthma having a 5.7 times and 2.7 times higher odds to be obese compared to those without underlying asthma in Asian and International criteria-based BMI, respectively.

We performed additional analysis to determine the association, if any between candidate gene *BDNF* (G > A in rs6265) and psychiatric symptoms (depression, anxiety and stress symptoms). The results are presented in *Table 5*. The analysis showed that there was no statistically significant association between *BDNF* (G > A in rs6265) and psychiatric symptoms among Malaysian women with GDM.

Discussion

Over the years, an increasing number of polymorphisms in candidate genes related to obesity have been discovered. In this study, we performed univariate logistic regression for every candidate gene, followed by multiple logistic regressions to elucidate the association between candidate genes and pre-pregnancy obesity among GDM patients. To our knowledge, this is the first study to examine the candidate genes for pre-pregnancy obesity among

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Table 4 Multiple regression analysis between genotypes of candidate genes for obesity among the GDM patients stratified using International criteria-based BMI classifications adjusted for confounding factors (n=312)

Candidate genes (SNP) or factors	Genotypes	International criteria-based BMI	
		Crude OR (95% CI), P value	Adjusted OR (95% CI), P value
NRG1 (rs2919375)	TT	1	1
	TC/CC	1.338 (0.794, 2.253), 0.274	1.347 (0.801, 2.265), 0.262
BDNF (rs6265)	GG	1	1
	AA/AG	2.005 (1.163, 3.453), 0.012	2.209 (1.305, 3.739), 0.003
FKBP5 (rs3800373)	TT	1	1
	GG/GT	0.658 (0.388, 1.115), 0.120	0.661 (0.394, 1.109), 0.116
RORA (rs4775340)	GG/GA	3.548 (0.773, 16.277), 0.103	3.700 (0.887, 15.426), 0.073
	AA	1	1
HTR2C (rs6318)	GG	1.388 (0.454, 4.244), 0.566	1.388 (0.454, 4.244), 0.566
	CC/GC	–	–
Parity	Nulliparous-Primiparous	1.776 (1.047, 3.011), 0.033	1.672 (1.009, 2.768), 0.046
	Multiparous ≥2	1	1
Asthma	No	1	1
	Yes	3.228 (1.237, 8.420), 0.017	2.693 (1.092, 6.642), 0.031
Heart disease	No	3.850 (0.364, 40.752), 0.263	4.555 (0.434, 47.772), 0.206
	Yes	1	1
Anaemia	No	0.395 (0.120, 1.294), 0.125	0.425 (0.135, 1.336), 0.143
	Yes	1	1
Education	Secondary and below	0.776 (0.443, 1.359), 0.375	0.775 (0.443, 1.358), 0.373
	Tertiary	1	1
Smoking habit	No	1	1
	Yes	0.180 (0.018, 1.753), 0.140	0.192 (0.020, 1.835), 0.152
Monthly family income		1.000 (1.000, 1.000), 0.069	1.000 (1.000, 1.000), 0.022

Adjusted OR was determined by adjusting for socio-demographical and clinical moderators with P value <0.25 in univariate analysis. BMI, body mass index; GDM, gestational diabetes mellitus.

Table 5 Univariate analysis of the BNDF rs6265 for psychiatric symptoms among women with gestational diabetes using International criteria based BMI classifications

Psychiatric symptoms	Severity	Genotype GG	Genotype GA or AA	P value
Depressive symptoms	Normal	95 (32.1)	201 (67.9)	0.18
	Mild-extremely severe	19 (42.2)	26 (57.8)	
Anxiety symptoms	Normal	62 (31.5)	135 (68.5)	0.37
	Mild-extremely severe	52 (36.1)	92 (63.9)	
Stress symptoms	Normal	96 (31.9)	205 (68.1)	0.099
	Mild-extremely severe	18 (45.0)	22 (55.0)	

BDNF, brain-derived neurotrophic factor; BMI, body mass index.

GDM women in Malaysia. It is also the first study to use stratification approach by both Asian and International criteria-based BMI in performing the association analysis for candidate genes.

It is worth mentioning that 60.9% of GDM patients in this study were obese using the Asian-criteria-based BMI, while only around two-fifth were obese using International criteria-based BMI. Even though the percentage of obesity among GDM patient using International criteria-based BMI appeared to be lower than that when using Asian-criteria-based BMI, it is noteworthy that types of criteria-based BMI used often has an influence on the association analysis between candidate genes and obesity. For instance, we found out that there were only five candidate genes with a P value <0.25 in univariate analysis that were entered multiple regressions analysis, which included candidate genes of *NRG1*, *OXTR*, *BNDF*, *FKBP5* and *PLEKHG1* using the Asian criteria-based BMI. The five candidate genes with P value <0.25 in univariate analysis entered into the multiple regressions analysis using the International criteria-based BMI were *NRG1*, *FKBP5*, *RORA*, *BNDF* and *HTR2C*.

In this study, *BDNF* was found to have an association with pre-pregnancy obesity using the International criteria-based BMI. A possible explanation is that *BDNF* is a type of neurotrophic protein that contributes to suppressed food intake through hippocampal signalling (55,56). Polymorphism in *BNDF* gene could possibly decrease *BNDF* expression and thus assist in promoting food intake and exhibit hyperphagic behaviour which may subsequently contributes to significant weight gain (57).

The association between *BDNF* rs6265 genotypes and obesity is inconsistent among populations, as shown also in this study, where the carrier of A allele is associated with obesity in GDM patients. This finding is consistent with studies done on German (58), Belgian (16) and Estonian populations (59). However, our findings contradict the findings of studies done on American (60) and British populations (61). These studies discovered that those who carry G allele exhibited higher BMI than carriers of the A allele. These inconsistent findings may be due to differences in dietary intake and lifestyle factors, which could modify the association between genotype and obesity traits.

Study strength and limitations

This study has generated exciting findings for an association between genetic variant in SNP of *BDNF* gene and

maternal obesity, which further establishes the role of SNP of *BDNF* (rs6265) in obesity in women adjusted for socio-demographic characteristics and concurrent medical problems.

Limitations may also be present in our study. The association between candidate genes and pre-pregnancy obesity traits could be modulated by the gene-diet-lifestyle interactions; however information on diet intake, lifestyle factors and physical activity was not captured in this study. Therefore the association between candidate genes and pre-pregnancy obesity as shown in this study should be interpreted cautiously.

Conclusions

In summary, our study found a significant association between *BNDF* rs6265 variant and pre-pregnancy obesity among GDM patients. The *BDNF* genotype appears to interact with concurrent medical problems in the Malaysian population, especially among GDM patients. The results indicate a role for *BDNF* in obesity. Larger studies considering dietary intake and lifestyle factors are required to determine whether there is a true association between *BDNF* gene and obesity.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-1579

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uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-1579). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Medical Research Ethics Committee, Ministry of Health Malaysia (No. NMRR-17-2264-37814) and informed consent was taken from all the patients.

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Supplementary

Table S1 Analyses of the *LHPP*, *SDK2*, *TEX51*, *EPHX2*, *NPY5R* and *ANO2* genotypes among the GDM patients with and without obesity (n=312)

Candidate genes	SNP	Genotype	Asian criteria-based BMI			International criteria-based BMI		
			Normal and Overweight (n=122)	Obese (n=190)	P value	Normal and Overweight (n=174)	Obese (n=138)	P value
LHPP	rs35936514	CC	57 (47.1)	95 (50.0)	0.642	85 (49.1)	67 (48.6)	0.617
		CT	50 (41.3)	79 (41.6)		69 (39.9)	60 (43.5)	
		TT	14 (11.6)	16 (8.4)		19 (11.0)	11 (8.0)	
		CC genotype	57 (47.1)	95 (50.0)	0.619	85 (49.1)	67 (48.6)	0.919
		T carrier	64 (52.9)	95 (50.0)		88 (50.9)	71 (51.4)	
		C carrier	107 (88.4)	174 (91.6)	0.359	154 (89.0)	127 (92.0)	0.371
		TT genotype	14 (11.6)	16 (8.4)		19 (11.0)	11 (8.0)	
SDK2	rs3816995	GG	74 (61.2)	113 (59.5)	0.793	100 (57.8)	87 (63.0)	0.642
		GA	41 (33.9)	64 (33.7)		62 (35.8)	43 (31.2)	
		AA	6 (5.0)	13 (6.8)		11 (6.4)	8 (5.8)	
		GG genotype	74 (61.2)	113 (59.5)	0.768	100 (57.8)	87 (63.0)	0.348
		A carrier	47 (38.8)	77 (40.5)		73 (42.2)	51 (37.0)	
		G carrier	115 (95.0)	177 (93.2)	0.499	162 (93.6)	130 (94.2)	0.837
		AA genotype	6 (5.0)	13 (6.8)		11 (6.4)	8 (5.8)	
TEX51	rs6733840	TT	76 (62.3)	118 (62.1)	0.578	108 (62.1)	86 (62.3)	0.669
		TC	42 (34.4)	61 (32.1)		56 (32.2)	47 (34.1)	
		CC	4 (3.3)	11 (5.8)		10 (5.7)	5 (3.6)	
		TT genotype	76 (62.3)	118 (62.1)	0.973	108 (62.1)	86 (62.3)	0.964
		C carrier	46 (37.7)	72 (37.9)		66 (37.9)	52 (37.7)	
		T carrier	118 (96.7)	179 (94.2)	0.312	164 (94.3)	133 (96.4)	0.384
		CC genotype	4 (3.3)	11 (5.8)		10 (5.7)	5 (3.6)	
EPHX2	rs17466684	GG	95 (77.9)	141 (74.2)	0.758	132 (75.9)	104 (75.4)	0.946
		GA	24 (19.7)	44 (23.2)		38 (21.8)	30 (21.7)	
		AA	3 (2.5)	5 (2.6)		4 (2.3)	4 (2.9)	
		GG genotype	95 (77.9)	141 (74.2)	0.463	132 (75.9)	104 (75.4)	0.919
		A carrier	27 (22.1)	49 (25.8)		42 (24.1)	34 (24.6)	
		G carrier	119 (97.5)	185 (97.4)	1.000	170 (97.7)	134 (97.1)	0.736
		AA genotype	3 (2.5)	5 (2.6)		4 (2.3)	4 (2.9)	
NPY5R	rs12501691	TT	82 (67.2)	132 (69.5)	0.849	116 (66.7)	98 (71.0)	0.640
		TA	38 (31.1)	54 (28.4)		55 (31.6)	37 (26.8)	
		AA	2 (1.6)	4 (2.1)		3 (1.7)	3 (2.2)	
		TT genotype	82 (67.2)	132 (69.5)	0.675	116 (66.7)	98 (71.0)	0.411
		A carrier	40 (32.8)	58 (30.5)		58 (33.3)	40 (29.0)	
		T carrier	120 (98.4)	186 (97.8)	1.000	171 (98.3)	135 (97.8)	1.000
		AA genotype	2 (1.6)	4 (2.1)		3 (1.7)	3 (2.2)	
ANO2	rs12579350	GG	106 (86.9)	164 (86.3)	0.975	150 (86.2)	120 (87.0)	0.677
		GA	15 (12.3)	24 (12.6)		23 (13.2)	16 (11.6)	
		AA	1 (0.8)	2 (1.1)		1 (0.6)	2 (1.4)	
		GG genotype	106 (86.9)	164 (86.3)	0.886	150 (86.2)	120 (87.0)	0.847
		A carrier	16 (13.1)	26 (13.7)		24 (13.8)	18 (13.0)	
		G carrier	121 (99.2)	188 (98.9)	1.000	173 (99.4)	136 (98.6)	0.586
		AA genotype	1 (0.8)	2 (1.1)		1 (0.6)	2 (1.4)	

Data are presented as either n (%). BMI, body mass index; SNP, single nucleotide polymorphisms; GDM, gestational diabetes mellitus.