

An Analysis of Targeted Single Nucleotide Polymorphisms for the Risk Prediction of Gestational Diabetes Mellitus in a Cohort of Malaysian Patients

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ABSTRACT

Recent association studies have described genetic variants among type 2 diabetes mellitus (T2DM) and their related traits. Gestational diabetes mellitus (GDM) is pathophysiologically similar to T2DM and may share genetic susceptibility. However, genetic susceptibility within GDM in our own population is still not yet explored. This study was to determine the association of GDM genetic variants in the Malaysian population. We genotyped 384 T2DM related SNPs among 174 cases of GDM and 114 controls of pregnant women using Illumina's Golden Gate genotyping assay. In this case-control study, a custom of 384-SNP plex of 236 candidate genes was designed using the Illumina's Assay Design Tool. The data analysis showed 12 SNPs had a significant association with GDM among Malaysians with p values 0.002 to 0.048 with their respective odd ratios. The SNPs rs7754840, rs10946398, rs9465871, rs7756992, rs6823091, rs7935082, rs237889, rs7903146, rs7961581 were significant under additive model while rs10811661, rs1016472, rs2270031 were associated with GDM under recessive model. Three SNPs namely rs7935082, rs1016472 and rs2270031 had reduced risk towards GDM while another nine SNPs which were rs7754840, rs10946398, rs9465871, rs7756992, rs10811661, rs6823091, rs237889, rs7903146 and rs7961581 had increased risk as much 1.75 to 2.62 times. Twelve genetic variants of T2DM were replicated in the SNP profiling among Malaysians GDM. Thus with a more significant result in a bigger sample, SNP screening is potentially a useful method in predicting the risk of gestational diabetes mellitus.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance that is first recognized during pregnancy. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests used (1). GDM is characterized with both insulin resistance and impaired insulin secretion as observed in T2DM and may share the same genetic susceptibility (2). Evidence showed that both types of DM are linked by the same risk factors such as family history of diabetes mellitus, history of abnormal glucose tolerance, excessive weight, and higher race tendency. Having similar pathophysiology between T2DM and GDM enables further exploration of genetic variants in GDM which may also serve as predictors for developing future T2DM (2).

In current clinical practice, oral glucose tolerance test (OGTT) is only offered to those who have at least one risk factor for GDM. A study performed in our centre (3) showed that the prevalence of gestational diabetes was about 24.9%. However, throughout the risk screening method, 27.7% cases of gestational diabetes mellitus had been missed. For that reason, the undiagnosed group was not treated as GDM patients until the baby was born with complications such as macrosomia. The risks for recurrence of GDM in future pregnancy include maternal weight gain and early diagnosis of GDM since 24 weeks of gestation which requires insulin treatment during pregnancy (4). Most importantly, women with history of GDM during early pregnancy have a higher risk to get T2DM compared to those diagnosed with GDM later in their pregnancy (5).

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Women with history of GDM have at least 20% risk of getting T2DM (6) after 9 years of the last affected pregnancy. In addition, babies of GDM mothers tend to be heavier and suffer from abnormal glucose tolerance since birth compared to those from non-diabetes mothers (7). Due to the exposure of maternal diabetes since in utero, the child has increased risk of obesity and getting T2DM in younger age (8). Therefore GDM does not only affect the mother but may also involve the child. So far, most of the genetic variants associated with GDM have also been implicated in determining T2DM (2). Such ability to identify pregnant women with the genetic tendency to develop T2DM in future will allow the use of targeted prevention strategies to anticipate or totally prevent complications of this condition from taking place. By recognizing the affected genes, further identification of pregnant women with higher risk will be possible and may also be used to prevent the related pregnancy problems.

Candidate gene approach studies have been carried out previously to identify susceptible genes predisposing for development of GDM. Five common polymorphisms in four genes, which previously shown to be associated with T2DM, were identified involving genes encoding potassium inwardly rectifying channel subfamily J, member 11 (*KCNJ11* E23K)(9), insulin receptor substrate 1 (*IRS1*G972R)(10), uncoupling protein 2 (*UCP2*-866GA) and calpain 10 (*CAPN10* SNP43 and SNP44)(11). An association of E23K polymorphism from *KCNJ11* that predispose to GDM was also observed in Scandinavian (12). Another study had observed significant association of *TCF7L2* variants with GDM and the polymorphisms interact with adiposity to alter insulin secretion in Mexican Americans (13). The effects of *TCF7L2* polymorphisms were also seen in Scandinavian women but other polymorphisms such as *PPARG* Pro12Ala, *PPARGC1A* Gly482Ser, *FOXO2* -512C>T, and *ADRB3* Trp64Arg were not significantly associated with GDM in their population (13).

Previous genome wide association (GWA) studies had described reproducible gene variants associations with T2DM including *CDKALI*, *CDKN2A/B*, *SLC30A8*, *HHEX*, *TSPAN8*, *IGF2BP2* and *FTO* (14-18), however, the association of these variants with GDM was not known. A recent study in Korea, which represented an Asian population, discovered an association between the T2DM genetic variants with GDM including *CDKALI*, *CDKN2A/B*, *HHEX*, *IGF2BP2*, *SLC30A8*, and *TCF7L2* genes (19), however, this may not be the same in the Malaysian pregnant women as prevalence of GDM is highly dependent on ethnicity (1).

Differential contribution of certain genetic loci to GDM across the population is due to the differences of environmental risk profiles, body composition and genetic backgrounds (20). Among Asians, diabetes tends to develop with a lesser degree of obesity at a younger age, more diabetic complications and the death age is sooner than people in other regions (21). A discrepancy in the pathophysiology of diabetes mellitus among Asians is due

to a multiracial population and this is especially unique to individual ethnic group (22). Consequently, it is important to have one's own population data on the association of those SNPs with GDM. Thus, the aim of the study was to analyze the association of common genetic variants with GDM in pregnant women at our centre.

MATERIALS AND METHODS

SUBJECTS

This study was carried out in a tertiary hospital for 24 months since April 2007 until March 2009. Regardless of their risk factors for GDM, a universal screening for 704 primigravidae and multiparae was done between 16 and 28 weeks of gestation by using the 75 g modified glucose tolerance test (MGTT). Exclusion criteria included women with chronic disease or metabolic syndrome during pregnancy or multiple pregnancies. After an overnight fast, all subjects underwent MGTT and venous blood samples were taken at 0 hour (fasting blood glucose, FBS) and 2 hours. In this study, 2-hours post-prandial (2-HPP) blood sugar levels of ≥ 7.8 mmol/l or fasting level of > 6 mmol/l were regarded as abnormal and indicative of GDM (1). Written informed consent was obtained from the participants and the Institutional Research and Ethical Committee approved this study.

GENE AND SNP SELECTION

Two hundred thirty six candidate genes for T2DM or GDM were identified from previous association studies or recent diabetes GWA studies. Literature review was done using online journal databases through search terms as gestational, type 2 diabetes, SNP, polymorphism or association study to get the appropriate articles. The SNPs with significant association with gestational, type 2 diabetes or related traits in all population were listed as candidate SNPs in the present study. Criteria of SNP selections were based on the SNP locations, functional significance, minor allele frequency more than 10% and reproducibility of the SNPs in more than one population. The list of selected SNPs was then submitted to techsupport@illumina.com to be scored using Gene list, RS list, Sequence list or Region list. We finally selected 384 SNPs that have significant association with T2DM in other populations.

GENOTYPING

DNA was extracted from 10 ml of peripheral blood using the salt extraction method. The genomic DNA samples were genotyped for 384 T2DM related SNPs. A total of 174 subjects with GDM and 114 healthy pregnant women were included for genotyping. Genotyping of SNPs was performed using the Illumina GoldenGate assay (Illumina, San Diego, CA, USA) according to manufacturer's

genotyping protocol. Briefly, the whole genome DNA samples were amplified, fragmented and hybridized overnight onto the allele-specific oligonucleotides on the bead arrays. Non-specific fragments were then removed by washing. The remaining specifically hybridized DNA fragments were fluorescently labeled by a single base extension reaction and detected using a BeadArray scanner. Genotyping data for genotypes were then imported and analyzed using BeadStudio Genotyping Module v3.0 (Illumina, San Diego). Individual SNPs were filtered based on three criteria, including SNP call rate > 95%, minor allele frequency > 0.1 (using BeadStudio Genotyping Module v3.0) and absence of deviation from Hardy Weinberg equilibrium in either cases or controls. Finally, SNPs which qualified those criteria were used for further disease marker

association analysis while the other SNPs which did not pass the criteria were excluded.

STATISTICAL ANALYSIS

Power calculation was performed using the PS-Power and sample size calculation software (23) to calculate the sample size based on comparing two proportions. Our study provided at least 70% power to detect the effect size odds ratio (OR) ≥ 1.65 , given a minor allele frequency (MAF) $\geq 10\%$ with a Type 1 error rate of 5% (Figure 1). Agreement with Hardy-Weinberg equilibrium was tested for all SNPs in cases and controls separately using χ^2 analysis. Statistical analyses were conducted using SPSS for Windows, version 15.0 (SPSS, Chicago, IL, USA).

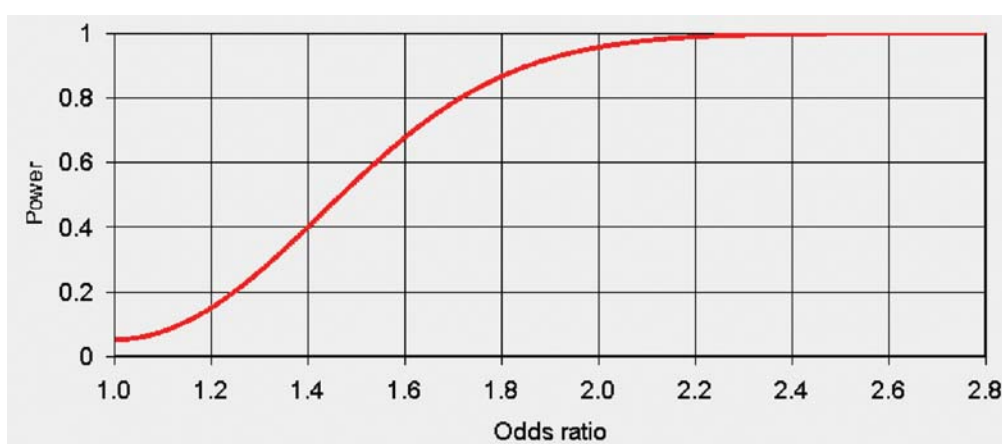


Figure 1. Power calculation

Power of the current Case-Control Study to detect associations with risk allele of varying frequencies and with a Type 1 Error rate of 5%. Graphs were plotted with the PS power and sample-size program (available at <http://www.mc.vanderbilt.edu/prevm/ps>; DuPont and Plummer 1997).

Logistic regression was used to determine the genetic effects of association between GDM and each individual SNP, measured by the ORs and its corresponding 95% confidence intervals (CI). The association analyses were performed assuming dominant, recessive and additive model for each polymorphism. Dominance was defined in terms of allele 2 (minor allele) effects; in the dominant allele 2 models, homozygous allele 1 subjects were compared with allele 2 carriers; in the recessive allele 2 models, homozygous allele 2 subjects were compared to allele 1 carriers. The genotypes were assumed as having additive effects where alleles were coded as 0 = 11, 1 = 12 and 2 = 22. The p value less than 0.05 were considered statistically significant. The associations between individual SNP and fasting blood sugar (FBS) and 2-hour post prandial (2HPP) were tested using ANOVA (SPSS v15.0).

RESULTS

A total of 384 SNPs were genotyped in 174 subjects with GDM and 114 controls with normal pregnancy. The

characteristics of the study samples were summarized in Table 1. The frequency of family history of DM, maternal weight exceeding 80 kg, age at study and birth weight was slightly higher, while gestation week at delivery in GDM group were lower than those of the control group. There was a significant difference between the two groups in terms of weight exceeding 80 kg and age at study. However, both groups of women were below 30 years old and similarly had lesser risk for GDM. Among the participants, 83.9% (146) women were treated using diet control only whereby 16.1% (28) were given insulin injection as well as dietary control to manage their diabetes.

During SNP normalization, 77 SNPs which did not pass the three criteria, were excluded from the extended analysis. Data from our study showed that 12 SNPs had significant association with GDM namely: *CDKALI* (rs7754840, rs10946398, rs9465871 and rs7756992), *CDKN2A/2B* (rs10811661), *FBXW7* (rs6823091), *MS4A7* (rs7935082), *OXTR* (rs237889), *TCF7L2* (rs7903146, rs7961581), *TRIM27* (rs1016472) and *WNT5B* (rs2270031). The best model for each SNP is shown in Table 2. Three genes, *CDKN2A/2B* (rs10811661), *TRIM27* (rs1016472) and *WNT5B* (rs2207731),

Table 1. Clinical characteristics of the study participants (n = 288)

Trait	Case (n = 174) n %	Control (n = 114) n %	P value
Anthropometric			
Race			0.627
Malay	117 (60.6)	76 (39.4)	
Chinese	42 (57.5)	31 (47.5)	
Indian	15 (68.2)	7 (31.8)	
Family history of diabetes mellitus	96 (33.3)	55 (19.1)	0.250
Maternal weight > 80 Kg	41 (14.3)	16 (5.6)	0.042
Age at study ^a	29.7 ± 4.7	28.5 ± 3.6	<0.0001
Biochemical measurements			
Fasting blood sugar (mmol/l) ^a	5.0 ± 1.2	4.4 ± 0.3	<0.0001
2 hour post prandial (mmol/l) ^a	8.7 ± 2.1	5.9 ± 0.9	<0.0001
Clinical			
Gestational week at delivery	38 weeks 3 days	38 weeks 6 days	0.252
Birth weight ^a	3.09 ± 0.5	3.04 ± 0.5	0.221
Treatment			
Diet control treatment	146 (83.9)		<0.0001
Insulin treatment	28 (16.1)		

^aData presented as mean ± standard deviation (SD).

Table 2. Comparison of genotype frequencies between GDM cases and controls with their respective best models

Gene	SNP	Genotype	GDM n (%)	Control n (%)	Best Model	OR (95% CI)	P value ^a
<i>CDKAL1</i>	rs7754840	GG	64 (22.7)	64 (22.7)	Additive	2.19 (1.30-3.69)	0.003
		GC	81 (28.7)	37 (13.1)			
		CC	24 (8.5)	12 (14.3)			
<i>CDKAL1</i>	rs10946398	AA	67 (22.3)	64 (22.3)	Additive	2.01 (1.20-3.37)	0.008
		AC	80 (27.9)	38 (13.2)			
		CC	26 (9.1)	12 (4.2)			
<i>CDKAL1</i>	rs9465871	TT	35 (12.2)	16 (5.6)	Additive	1.75 (1.04-2.93)	0.035
		TC	85 (29.7)	43 (15.0)			
		CC	54 (18.9)	53 (18.5)			
<i>CDKAL1</i>	rs7756992	AA	54 (18.8)	52 (18.1)	Additive	2.04 (1.62-2.58)	<0.0001
		AG	87 (30.2)	48 (16.7)			
		GG	33 (11.5)	14 (4.9)			
<i>CDKN2A/2B</i>	rs10811661	TT	76 (26.4)	46 (16.0)	Recessive	2.62 (1.16-5.88)	0.02
		TC	86 (29.9)	48 (16.7)			
		CC	12 (4.2)	19 (6.6)			
<i>FBXW7</i>	rs6823091	AA	6 (5.3)	13 (7.5)	Additive	1.79 (1.08-2.97)	0.024
		AC	36 (31.6)	76 (43.7)			
		CC	72 (63.2)	85 (48.9)			
<i>MS4A7</i>	rs7935082	CC	70 (24.3)	31 (10.8)	Additive	0.51 (0.29-0.87)	0.014
		CT	71 (24.7)	62 (21.5)			
		TT	31 (10.8)	20 (6.9)			
<i>OXTR</i>	rs237889	AA	16 (14)	29 (16.7)	Additive	1.88 (1.11-3.19)	0.019
		AG	47 (41.2)	94 (54.0)			
		GG	48 (42.1)	51 (29.3)			
<i>TCF7L2</i>	rs7903146	CC	1 (0.3)	0	Additive	2.20 (1.16-4.19)	0.016
		CT	43 (15.0)	15 (5.2)			
		TT	129 (44.9)	99 (34.5)			
<i>TCF7L2</i>	rs7961581	AA	66 (57.9)	77 (44.3)	Additive	1.89 (1.15-3.10)	0.013
		AG	40 (35.1)	88 (50.6)			
		GG	8 (7.0)	8 (4.6)			
<i>TRIM27</i>	rs1016472	TT	62 (21.5)	31 (10.8)	Recessive	0.47 (0.25-0.86)	0.014
		TC	80 (27.8)	48 (16.7)			
		CC	30 (10.4)	34 (11.8)			
<i>WNT5B</i>	rs2270031	CC	101 (35.1)	59 (20.5)	Recessive	0.32 (0.12-0.91)	0.032
		CG	64 (22.2)	40 (13.9)			
		GG	8 (2.8)	14 (4.9)			

^a The p-values were not corrected for multiple testing.

showed significant association under recessive model while others showed strongest evidence of association under additive model.

Table 3 shows the association between risk allele SNPs with fasting blood sugar (FBS) and 2-hour post prandial (2HPP) glucose level. Risk allele represents the minor allele

on the marker while protective allele will be the major allele. Seven SNPs in *ALG10*, *CDKALI*, *TCEB1* and *TCF7L2* genes were associated with higher FBS while rs717120, rs3829686 and rs7961581 were associated with higher 2HPP among the GDM cases and controls.

Table 3. Associations between risk alleles and fasting blood sugar (FBS) and 2 hour post prandial

Parameter	Gene	SNP	Allele (major/minor)	Homozygous Protective allele	Heterozygous	Homozygous Risk allele	P value ^a
FBS (mmol/L)	<i>ALG10</i>	rs10466832	T/C	4.86 ± 1.1	5.25 ± 1.4		<0.0001
	<i>CDKALI</i>	rs7754840	C/G	4.82 ± 1.0	4.97 ± 1.3	4.89 ± 0.9	0.029
	<i>CDKALI</i>	rs10946398	A/C	4.89 ± 1.3	4.9 ± 1.1	4.89 ± 0.9	0.023
	<i>TCEB1</i>	rs 10504553	T/C	4.8 ± 1.1	5.07 ± 1.7	6.22 ± 2.1	0.029
	<i>TCF7L2</i>	rs7903146	C/T	4.82 ± 1.2	5.1 ± 1.2		0.001
	<i>TCF7L2</i>	rs7901695	T/C	4.84 ± 1.1	5.07 ± 1.3		<0.0001
	<i>TCF7L2</i>	rs12255372	G/T	4.83 ± 1.1	5.17 ± 1.3		<0.0001
2HPP (mmol/L)	<i>AH11</i>	rs717120	T/C	7.55 ± 2.3	7.83 ± 2.2	9.32 ± 3.5	0.046
	<i>STK11</i>	rs3829686	A/G	7.47 ± 2.2	8.4 ± 2.6	7.30 ± 1.8	0.036
	<i>TSPAN8</i>	rs7961581	T/C	7.62 ± 2.3	7.81 ± 2.4	7.52 ± 2.5	0.015

^a The p-values were not corrected for multiple testing.

DISCUSSION

The findings of this present study provided preliminary insight into the GDM genetic variants in the Malaysian population. There was an association with risk of GDM involving 12 SNPs namely, *CDKALI* (rs7754840, rs10946398, rs9465871, rs7756992), *CDKN2A/2B* (rs10811661), *FBXW7* (rs6823091), *MS4A7* (rs7935082), *OXTR* (rs237889); *TCF7L2* (rs7903146, rs7961581), *TRIM27* (rs1016472) and *WNT5B* (rs2270031) with modest effect (p value = <0.0001-0.035).

A significant p value is always used to confirm a hypothesis (24). This would enable us to choose potential markers that should be studied further in a larger sample. There is generally no accepted answer to the question of which single-SNP test to be used and we could only design optimal analysis for them. The SNP contribution to disease risk from an individual SNP is often thought to be roughly additive manner where the heterozygote risk is then intermediate between the two homozygote risks (25). Association of rs7903146 (*TCF7L2*) had been reported under three inheritance models (13). Frequencies of *TCF7L2* (rs7903146), *CDKN2A/B* (rs10811661), *CDKALI* (rs7756992), *HHEX* (rs7923837), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634) and *FTO* (rs8050136) were also compared to get the best respective model. The markers reported by earlier studies showed insignificant association under dominant model except rs7903146(20).

The subjects of the present study with genotype CC in rs717120 (*AH11*) possibly had a higher risk for GDM compared to heterozygous genotype as they had twice the effect from the risk allele. Furthermore, the mean for 2HPP

level was the highest and abnormal (> 7.8 mmol/L) for genotype rs717120 (Table 3). Therefore, it is suggested that the *AH11* gene may serve as a marker for GDM women in Malaysia although this have to be further validated in a bigger study. Meanwhile subjects with genotype AG in rs3829686 (*STK11*) and genotype TC in rs7961581 (*TSPAN8*) were predisposed to GDM due to the heterozygous effect. Table 3 explains the association of all genotypes except *TCF7L2* for a dominant model (homozygous protective allele) due to the small number of homozygous risk allele carriers. The polymorphisms within *ALG10*, *CDKALI*, *TCEB1* and *TCF7L2* gene were also found to be significant but the mean for FBS value was within the normal range. Again, this should be validated in a larger study.

The T2DM related SNPs in the *CDKALI* gene that were found consistently in recent GWA studies (16, 18, 26), were also seen with GDM in our population. In a recent study, the association was established with an increased risk of 2.04, in which the odd ratio for rs7756992 in Asians was higher than Europeans (1.26 vs 1.14) (20). The finding was similar to the Korean study (19) and the rs7756992 and rs7754840 also correlated with our study. In addition, rs7754840 and rs10946398 were also found to be associated with high fasting blood sugar level (FBS) among GDM cases (Table 3). This type 2 diabetes susceptibility allele was associated with a decreased in insulin response, decreased beta-cell glucose sensitivity (27) and was also linked to impairment of beta cell function as estimated by HOMA-beta index (28). Those effects were seen in GDM but not clearly defined in this study except the association with FBS level.

An adjacent region near *CDKN2A/B* was associated with T2DM and cardiovascular disease (20). In comparison, the effect of genotype rs10811661 can be seen more in Asians than Europeans (1.30 vs 1.20) (20). The present study also identified an association of rs10811661 under recessive model (OR = 2.62) with GDM women in Malaysia. Meanwhile, rs7756992, rs7754840 (*CDKALI*) and rs10811661 (*CDKN2A-CDKN2B*) were associated with significant decreases in the insulin area under curve (AUC) during a 100 g OGTT performed at the time of diagnosis of GDM (19). Combination of increased maternal adiposity and insulin-desensitizing hormonal products of placenta however results in insulin resistance. During pregnancy, pancreatic β cells enhance their insulin secretion to compensate for the insulin resistance. In GDM patients however, the pancreatic β cells are either dysfunctional or the insulin supply is inadequate for the body. This is most likely where those susceptible alleles play a role in the impairment of insulin secretion as in the pathogenesis of GDM (19).

The rs7903146 polymorphism in *TCF7L2* gene has been suggested as the most powerful single genetic variant influencing T2DM risk (29) until other SNPs were recognized by GWA studies (14-18). Findings reported by a meta-analysis of 27 studies suggested a global odd ratio (OR) for *TCF7L2* variants was 1.46 [1.42–1.51] ($p = 5.4 \times 10^{-140}$) (32). The impact of *TCF7L2* was also seen in our population where rs7903146 and rs7961581 (*TCF7L2*) were significantly associated with increased risk of 2.20 and 1.89 times. These genotypes were also significant with regards to FBS in our study similar to the effect of *TCF7L2* in the elder diabetic population (32). Meanwhile other studies found the association of rs12255372 and rs7903146 with an increased level of 2-hour post prandial (2HPP) blood sugar, however no association with metabolic syndrome, or with insulin and glucose levels were noted (31, 33, 34). The meta analysis showed that T2DM was associated with the susceptible gene in *TCF7L2* at 1.42 to 1.51 times the risk of the population (32). Regardless of the sample size, the association of this important gene had consistently been identified (35-36). Even though the exact biological mechanism between *TCF7L2* and the risk of GDM is yet still unknown, this relevant finding highlighted the genetic contribution of *TCF7L2* gene in GDM.

The wnt (*WNT5B*) signaling pathway controls adipogenesis and insulin secretion (37). The gene also plays a role in the gene transcription through interaction with the *TCF7L2* nuclear factor. A study reported *WNT5B* gene to confer a significant risk of T2DM (38), while another study defined a decrease risk of T2DM with OR = 0.03135. It is probable that in combination with *TCF7L2*, the variations in *WNT5B* conferred a higher risk thus strengthening the role of wnt signaling in T2DM in dominant model (39). In current study, we identified the same interaction between these genes where there was a negative association of rs2270031 (*WNT5B*)(OR = 0.32) and positive association rs7903146 (*TCF7L2*)(OR = 2.20) with

GDM (see Table 2). With both associations in our study, the gene interaction between *WNT5B* and *TCF7L2* would also increase the risk of GDM.

In most association studies, multiple testing were carried out to reduce the false positive association that may happen due to the large genome and its massively polymorphic variants. In this study, the *P* value was given without multiple testing corrections similar to the Korean study (19) and after doing so the *P* value became non significant due to its relatively small sample size. The lack of association with other diabetic markers such as *SLC30A8*, *HHEX/IDE*, *IGF2BP2* and *FTO* with GDM in the current study is also partly due to a small sample size. With the number of cases and controls that had been used in this study, we had less than 70% power of study to detect odd ratios (OR) of 1.65. Therefore, meta-analyses or studies with larger sample sizes are vital to draw a definitive picture of such association in GDM.

Primary genome wide association study (GWAS) or microarray work should be able to reflect true reproducible associations, hence, technical validation was carried out to allow early detection of technical errors which may result in a spurious association signal (40). Technical validation refers to the reanalysis of original GWA samples using a second genotyping platform (40). Our microarray results were validated by direct sequencing using ten anonymous samples that were randomly picked. Five samples with the significant SNPs (rs7754840, rs9465871, rs7903146, rs12255372) were directly sequenced to validate their genotype. The sequence analysis confirmed the genotype as detected in the Golden Gate genotyping array (data not shown). In fact, it is suggested that the sequencing method should be made available in a bigger sample size study in future. In comparison, although microarray is timely and concise, sequencing or conventional PCR will be more cost-effective if used in a larger population.

Our current findings highlighted the important contribution of some key genetic variants to GDM in the Malaysian population. However, we consider this to be a preliminary study, with clearly a need for screening larger number of patients to help validate the results further. Locally, more information on GDM-SNP association is needed to optimize the SNP screening among pregnant women in Malaysia. The gene chip technology is now no longer a myth, and countries like China have already started the SNP screening in predicting GDM risks among pregnant women with gestational age between 38-39 weeks using this approach (41). However, it is still uncertain whether this will be cost effective for developing countries where certain ethnic groups of the population have a higher risk to GDM.

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REFERENCES

- American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2004; 27: S5-S10.
- Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. *Genet Med* 2008; 10: 240-250.
- Shamsuddin K, Mahdy ZA, Siti Rafiaah I, Jamil MA, Rahimah MD. Risk factor screening for abnormal glucose tolerance in pregnancy. *International Journal of Gynecology & Obstetrics* 2001; 75: 27-32.
- Bottalico JN. Recurrent Gestational Diabetes: Risk Factors, Diagnosis, Management, and Implications. *Seminars in Perinatology* 2007; 31: 176-184.
- Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabetic Medicine* 2003; 21: 103-113.
- Feig DS, Zinman B, Wang X, Hux JE. Risk of development of diabetes mellitus after diagnosis of gestational diabetes. *CMAJ* 2008; 179: 229-234.
- Claesson R, Aberg A, Marsal K. Abnormal fetal growth is associated with gestational diabetes mellitus later in life: population-based register study. *Acta Obstetrica et Gynecologica* 2008; 86: 652-656.
- Westgate JA, Lindsay RS, Beattie J, Pattison NS, Gamble G, Mildenhall LFJ, Breier BH, Johnstone FD. Hyperinsulinemia in Cord Blood in Mothers With Type 2 Diabetes and Gestational Diabetes Mellitus in New Zealand. *Diabetes Care* 2006; 29: 1345-1350.
- Hansen SK, Nielsen EM, Ek J, Andersen G, Glumer C, Carstensen B, Mouritzen P, Drivsholm T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Analysis of separate and combined effects of common variation in *KCNJ11* and *PPARG* on risk of type 2 diabetes. *J Clin Endocrinol Metab* 2005; 90: 3629-3637.
- Fallucca F, Dalfra' MG, Sciuolo E, Masin M, Buongiorno AM, Napoli A, Fedele D, Lapolla A. Polymorphisms of insulin receptor substrate 1 and b3-adrenergic receptor genes in gestational diabetes and normal pregnancy. *Metabolism Clinical and Experimental* 2006; 55: 1451-1456.
- Valeriya L, Peter A, Dragi A, Marju Orho-Melander, Marketa Sjogren, Carola S, Tiinamajja T, Leif G, the Botnia Study Group. Genetic Prediction of Future Type 2 Diabetes. *PLoS Medicine* 2005; 2: 1299-1308.
- Shaat N, Ekelund M, Lernmark A, Ivarsson S, Almgren P, Berntorp K, Groop L. Association of the E23K polymorphism in the *KCNJ11* gene with gestational diabetes mellitus. *Diabetologia* 2005; 48: 2544-2551.
- Shaat N, Lernmark A, Karlsson E, Ivarsson S, Parikh H, Berntorp K, Groop L. A variant in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with an increased risk of gestational diabetes mellitus. *Diabetologia* 2007; 50: 972-979.
- Saxena R, Voight BF, Lyssenko V, Burt NP, de Bakker PIW, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D. Genome-Wide Association Analysis Identifies Loci for Type 2 Diabetes and Triglyceride Levels. *Science* 2007; 316: 1331-1336.
- Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding C, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li X, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A Genome-Wide Association Study of Type 2 Diabetes in Finns Detects Multiple Susceptibility Variants. *Science* 2007; 316: 1341-1345.
- Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nat Genet* 2007; 44: 881-885.
- Zeggini E, Scott LJ, Saxena R, Voight BF. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 2008; 40: 638-645.
- Timpson NJ, Lindgren CM, Weedon MN, Randall J, Ouwehand WH, Strachan DP, Rayner NM, Walker M, Hitman GA, Doney ASF, Palmer CNA, Morris AD, Hattersley AT, Zeggini E, Frayling TM, McCarthy MI. Adiposity-Related Heterogeneity in Patterns of Type 2 Diabetes Susceptibility Observed in Genome-Wide Association Data. *Diabetes* 2008; 58: 505-510.
- Cho YM, Kim TH, Lim S, Choi SH, Shin HD, Lee HK, Park KS, Jang HC. Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population. *Diabetologia* 2009; 52: 253-261.
- Ng MCY, Park KS, Oh B, Tam CHT, Cho YM, Shin HD, Lam VKL, Ma RCW, So WY, Cho YS, Kim H, Lee HK, Chan JCN, Cho NH. Implication of Genetic Variants near *TCF7L2*, *SLC30A8*, *HHEX*, *CDKAL1*, *CDKN2A/B*, *IGF2BP2* and *FTO* in Type 2 Diabetes and Obesity in 6719 Asians. *Diabetes* 2008.
- Yoon K, Lee J, Kim J, Cho JH, Choi Y, Ko S, Zimmet P, Son H. Epidemic obesity and type 2 diabetes in Asia. *Lancet* 2006; 368: 1681-1688.
- Ramachandran A, Ma RCW, Snehalatha C. Diabetes in Asia. *The Lancet* 2010; 375(9712): 408-418.
- DuPont WD and Plummer JWD. PS Power and sample size program available for free on the internet. *Controlled Clinical Trials* 1997; 18: 274.
- Terwilliger JD and Weiss KM. Linkage disequilibrium mapping of complex disease: fantasy or reality? *Current Opinion in Biotechnology* 1998; 9: 578-594.
- Balding DJ. A tutorial on statistical methods for population association studies. *Nature Genetics* 2006; 7: 781-791.
- Steinthorsdottir V, Thorleifsson G, Reynisdottir, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorraddottir S, Bjarnason H, Ng MCY, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So W, Ma RCY, Andersen G, Borch-Johnsen K, Jorgensen T, Vliet-Ostapchouk JVV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JCN,

- Pedersen, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Gene* 2007; 39: 770-775.
27. Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M. Common variants of the novel type 2 diabetes genes, CDKAL1 and HHEX/IDE, are associated with decreased pancreatic β -cell function. *Diabetes* 2007; 56: 3101-3104.
 28. Horikoshi M, Hara K, Ito C, Shojima N, Nagai R, Ueki K, Froguel P, Kadowaki T. Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. *Diabetologia* 2007; 50: 2461-2466.
 29. Weedon MN, McCarthy MI, Hitman G, Walker M, Groves CJ, Zeggini E, Rayner NW, Shields B, Owen KR, Hattersley AT, Frayling TM. Combining Information from Common Type 2 Diabetes Risk Polymorphisms Improves Disease Prediction. *Plos Medicine* 2006; 3: 1877-1882.
 30. Melzer D, Murray A, Hurst AJ, Weedon MN, Bandinelli S, Corsi AM, Ferrucci L, Paolisso G, Guralnik JM, Frayling TM. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Medicine* 2006; 4: 34.
 31. Saadi H, Nagelkerke N, Carruthers SG, Benedict S, Abdulkhalek S, Reed R, Lukic M, Nicholls MG. Association of TCF7L2 polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects. *Diabetes Res Clin Pract* 2008; 80: 392-398.
 32. Cauchi S, Achhab YE, Choquet H, Dina C, Krempler F, Weitgasser R, Nejjari C, Patsch W, Chikri M, Meyre D, Frogue P. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med* 2007; 85(7): 777-782.
 33. Bodhini D, Radha V, Dhar M, Narayani M, Mohan V. The rs12255372(G/T) and rs7903146(C/T) polymorphisms of the TCF7L2 gene are associated with type 2 diabetes mellitus in Asian Indians. *Metabolism Clinical and Experimental* 2007; 56: 1174-1178.
 34. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, Frayling TM, Yajnik CS. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. *Diabetologia* 2007; 50: 63-67.
 35. Humphries SE, Gable D, Cooper JA. Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. *J Mol Med* 2006; 84: 1-10.
 36. Saxena R, Gianniny L, Burt NP. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 2006; 55: 2890-2895.
 37. Jin T. The WNT signalling pathway and diabetes mellitus. *Diabetologia* 2008. 51: 1771-1780.
 38. Kanazawa A, Tsukada S, Sekine A, Tsunoda T, Takahashi A, Kashiwagi A, Tanaka Y, Babazono T, Matsuda M, Kaku K, Iwamoto Y, Kawamori R, Kikkawa R, Nakamura Y, Maeda S. Association of the Gene Encoding Wingless-Type Mammary Tumor Virus Integration-Site Family Member 5B (WNT5B) with Type 2 Diabetes. *Am J Hum Genet* 2004; 75: 832-843.
 39. Salpea KD, Gable DR, Cooper JA, Stephens JW, Hurel SH, Ireland HA, Feher MD, Godsland IF, Humphries SE. The effect of WNT5B IVS3C>G on the susceptibility to type 2 diabetes in UK Caucasian subjects. *Nutrition, Metabolism & Cardiovascular Diseases* 2008; 19: 140-145.
 40. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JPA, Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Reviews Genetics* 2008; 9: 356-369.
 41. Zhaoxia L, Minyue D, Qi C, Danqing C. Gestational diabetes mellitus screening based on the gene chip technique. *Diabetes Res Clin Pract* 2010; 89: 167-173.