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Single Nucleotide Polymorphism for Certain Genes Involved in Gestational Diabetes with Risk Factors and Complications Positive

(Polimorfisme Nukleotida Tunggal bagi Gen Tertentu yang Terlibat dalam Faktor Risiko dan Komplikasi Positif Diabetes Gestasi)

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ABSTRACT

Gestational Diabetes Mellitus (GDM) is associated with pregnancy complications, however its mechanism has not been fully understood. The aim of this study was to investigate the single nucleotide polymorphism (SNP) for identifying candidate genes involve in risk factors and complications of GDM. A total of 174 pregnant women with GDM and 114 healthy pregnant women were genotyped with 384 SNPs from 236 genes. The SNPs identified were rs10946398 (CDKAL1) in GDM risk factors; rs328 (LPL) and rs1042778 (OXTR) in complications of caesarean section; rs5404 (SLC2A2), rs5400 (SLC2A2) and rs13306465 (IRS1) for neonatal intensive care admission. Whereby SNPs rs12255372, rs7901695 and rs7903146 from TCF7L2 gene had six times higher risk (OR, 6.40-6.53) for T2DM at postpartum. In conclusion, although the above SNPs were identified with GDM risk factors and complications among pregnant Malaysian women with GDM, a larger study is needed to ascertain this candidate genes actual association.

Keywords: Complications; gestational diabetes mellitus; single nucleotide polymorphisms; risks; Malaysia

ABSTRAK

Diabetes Melitus Gestasi (GDM) mempunyai perkaitan dengan komplikasi kehamilan, namun demikian mekanismenya masih belum dikenal pasti sepenuhnya. Kajian ini adalah untuk mengkaji polimorfisme nukleotida tunggal (SNP) bagi mengenal pasti gen yang terlibat dalam faktor risiko GDM dan komplikasinya. Sejumlah 174 wanita hamil dengan GDM and 114 wanita hamil yang sihat telah digenotip dengan 384 SNPs daripada 236 gen. SNPs yang dikenal pasti adalah rs10946398 (CDKAL1) dalam faktor risiko GDM; rs328 (LPL) dan rs1042778 (OXTR) dalam komplikasi pembedahan caesarean; rs5404 (SLC2A2), rs5400 (SLC2A2) dan rs13306465 (IRS1) untuk kemasukan wad rawatan rapi neonatal. Sementara SNPs rs12255372, rs7901695 dan rs7903146 daripada gen TCF7L2 mempunyai enam kali lebih tinggi risiko (OR, 6.40-6.53) untuk T2DM semasa postpartum. Sebagai kesimpulan, walaupun SNPs di atas telah dikenal pasti dengan faktor risiko GDM dan komplikasinya dalam kalangan wanita Malaysia yang hamil dengan Diabetes Gestasi, satu kajian yang lebih besar adalah perlu bagi memastikan penglibatan sebenar gen tersebut.

Kata kunci: Diabetes melitus gestasi; komplikasi; polimorfisme nukleotida tunggal; risiko; Malaysia

INTRODUCTION

Globally Diabetes Mellitus (DM) is currently recognized as a 'critical' disease which is also witnessed in Malaysia. Among the Malaysian adults aged 30 years and above, with an increase of 80% over 10 years, the prevalence had arose from 8.3% in 1996 to 14.9% in 2006 (Letchuman et al. 2010). The concern arises when this involves those women in the reproductive age. Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance that begins or first recognized during pregnancy. It is also known to be associated with maternal and fetal morbidities, however the comprehensive mechanisms are yet to be discovered. DM and GDM have similar pathophysiology and are proven worldwide to poses similar genetic susceptibility including among the Malaysian women (Nor Khatijah et al. 2011). They also have common predisposing risk factors to both diseases namely family history of diabetes, ethnicity preponderance and excessive body weight.

The pregnancy complications include urinary tract infections, polyhydromnios, preeclampsia, fetal malformations, caesarean sections, postpartum haemorrhage, neonatal hypoglycemia, hyperbilirubinemia and an increased rate of infant's admission to neonatal ward (Chris et al. 2003). Not only those known possible complications, it was also shown that those who are diagnosed earlier with GDM (before 20 weeks of gestation) has an increased need for insulin treatment during pregnancy and a higher risk of developing type 2 diabetes mellitus (T2DM) in six years' time (Illka et al. 2006; Jens et al. 2001). Thus, it is equally important to know who among the pregnant mothers are more prone to GDM. These factors include clinical features of obesity, personal

history of GDM, glycosuria and a strong family history of diabetes (American Diabetes Association 2002). Early and thorough education about the effects of disorder on possible pregnancy complications and fetal problems are necessary. Complications of GDM are mostly preventable, if proper glycaemic control is implemented; based on dietary control, regular home glucose monitoring and judicious use of insulin therapy.

Understanding of specific risks as well as their complex interactions could allow identifications of risk towards GDM and its complications with the aim to further strategize GDM's management. Genetic variants in such genes are proven to increase the risk of DM in varied spectrum among various populations (Ramachandran et al. 2010; Zeggini et al. 2008), diabetic complications in adults with DM (Eero et al. 2008) and association with lower birth weight in offspring with family history of type 1 diabetes mellitus (T1DM) (Christiane et al. 2009).

We highlighted the genetic variants to GDM (Nor Khatijah et al. 2011) where twelve T2DM SNPs were replicated in Malaysian population with GDM. Eight genes with their SNPs (*CDKAL1* (rs7754840, rs10946398, rs9465871, rs7756992); *CDKN2A/2B* (rs108116610); *FBXW7* (rs6823091); *MS4A7* (rs7935082), *OXTR* (rs237889), *TCF7L2* (rs7903146, rs7961581); *TRIM27* (rs1016472); *WNT5B* (rs2270031) were involved with GDM. Thus, in this part of the study, further analysis of single nucleotide polymorphism (SNP) was performed to identify candidate genes that are involve in risk factors of GDM and its complications.

Material and Methods

This study was approved by the Institutional Research and Ethics Board. It was performed in a tertiary centre over a period of 2 years (April 2007 till March 2009). This was the second part of the study with reanalysis of SNPs for different intention, using the same study sample that was published earlier (Nor Khatijah et al. 2011). Women with chronic diseases or multiple pregnancies were excluded from the study. Written informed consent was taken from the recruited patients. A GDM universal screening of a modified glucose tolerance test (MGTT) was done between 16 and 28 weeks of gestation for all women with suitable criteria. They were fasted overnight before fasting venous blood glucose (FBS) level was taken in the morning. A 75 g glucose drink was given and 2 h post prandial (2-HPP) blood glucose was later taken. FBS values of 3.0-6.0 mmol/l and 2-HPP below 7.8 mmol/l were considered to be normal. Any women exceeded this range were diagnosed with GDM. They were managed according to the hospital protocol. After 6 weeks post partum, a repeated MGTT tests were performed among GDM women to indicate glucose intolerance level after pregnancy.

Contributing risk factors including family history of diabetes, history of abnormal or macrosomia baby and glycosuria from the antenatal history were recorded. The pregnancy outcomes include maternal complications; uterus bigger than dates, polyhydromnion, preterm delivery, pre-eclampsia, cesarean section and post-partum hemorrhagee were analysed. Fetal complications; macrosomia, anomaly, stillbirth and neonatal; hypoglycaemia, hyperbilirubinaemia, hypocalcemia, polycythaemia, birth trauma and neonatal intensive care unit (NICU) admission were documented. Demographic characteristics such as race, age, parity and maternal weight were analyzed. Controls were those women who were not diagnosed with GDM.

About 288 participants were included in the genotyping process, with 174 women with GDM and 114 healthy controls. Extractions of DNA from 10 mls of maternal peripheral blood using salt extraction method were further analyzed using Illumina GoldenGate assay (Illumina, San Diego). The SNPs genotyping was performed in batches to detect 384 SNPs that were associated with gestational diabetes mellitus and T2DM. The samples of whole genome DNA were processed to be amplified, fragmented and hybridized. The specifically hybridized DNA fragments were labeled by a flourescent dye with a single base extension reaction. Non-specific fragments were eliminated. Later, the gene chips were imaged using BeadArray scanner (Illumina). Raw genotyping data were imported using BeadStudio Genotyping Module v3.0 (Illumina, San Diego). Individual SNPs were analyzed and filtered based on SNPs call rate > 95%, minor allele frequency > 0.1 (using BeadStudio Genotyping Module v3.0) with no deviation from Hardy Weinberg equilibrium in both groups. Further disease marker association analysis was performed only on qualified SNPs as in our previous publication (Nor Khatijah et al. 2011).

Descriptive statistics of categorical variables was reported by using frequency and percentages while the mean and SD was checked for numerical variables. Logistic regression was carried out to assess the relationship between the risk factors, complications and T2DM with single nucleotide polymorphism. The crude odds ratios were obtained and the significance P-values were set at 0.05 (2-tailed). Data entry and analysis were done using SPSS for windows (version 15), produced by SPSS, Chicago, IL, USA.

SNP & Variation Suite *(SVS v7.0)* was additional third party software with advance parameter testing applied to reveal SNP association mostly in genome wide association study (GWAs). Trend test was done through this software to determine association between polymorphism and disease trait in case control study under hypothesis of genetic models such as allelic model, dominant, recessive or additive. It was more conservative and did not rely on assumption of Hardy-Weinberg Equilibrium (HWE) in case and control study (Balding 2006). A trend analysis tested a linear trend of increasing effect across three groups GG, Gg and gg by coding them as 0, 1 or 2 in a logistic regression (Thakkinstian et al. 2009) and more stringent than Chi square and should be used in association testing. Further analysis was described in earlier part of this study (Nor Khatijah et al. 2011). The clinical features of risk factors and complications in GDM (n=174 women) and those

non GDM (*n*=114 women) were analysed with regards to possible SNPs and candidate genes identification.

Results

The demographic data of the participants were comparable and described in detailed during the first part of the study (Nor Khatijah et al. 2011). Among 174 women with GDM and 114 women of controls that were genotypically analysed, family history of diabetes mellitus was the highest risk predictor for GDM cases (55.2% in GDM versus 48.2% in controls) followed by glycosuria and size of uterus (Table 1). Caesarean section was higher than premature delivery (28.1% versus 17.5%, respectively), among complications that seemed likely to happen in GDM mothers. At 6 weeks post partum, 127 patients failed to carry out the MGTT and 3 were lost to follow up. For those who underwent the test, 30 patients were normal while 14 were diagnosed with T2DM after delivery. Birth weights of the babies were comparable (3.09 ± 0.5 kg in GDM, 3.04 ± 0.5 kg in normal) in both groups. However those with GDM mothers had more babies with macrosomia (6.4% in GDM versus 2.7% in controls), hyperbilirubinaemia (7.6% in GDM versus 3.6% in controls) with higher NICU admission (4.3% in GDM versus 3.6% in controls) (Table 1).

In genetic association analysis, there were SNPs discovered to be present in women with risk factors and GDM complications (Table 2). Among risk factors of GDM, *CDKAL1* gene (rs10946398) was statistically significant present in those with family history of diabetes mellitus with a protective effect (OR=0.49). In GDM complications; rs328 and rs1042778 were present among women who had caesarean sections while rs5404, rs5400 and rs13306465 were significant among cases who had their babies admitted to NICU (OR=2.25-6). The current study had also replicated the SNPs rs12255372, rs7901695 and rs7903146 (OR = 6.45, 6.4, 6.53, respectively) in *TCF7L2* gene among participants with occurrence of T2DM at six weeks postpartum. All the above SNPs are associated with T2DM and currently found to be present among those women with GDM risk factors or those with GDM complications.

Discussion

A tremendous surge of Diabetes Mellitus prevalence throughout the world including Malaysia nowadays, has also affected those in the reproductive age resulting in increment in the prevalence of Gestational Diabetes Mellitus. As GDM is recognized with its genetic potential through transgenerational effect, it is timely to address the issue of its complication and to recognize its risk factors through genetic aspect before incurring a massive public health burden.

In this current study, women with family history of DM had the highest risk factor towards GDM as seen in a previous report (Ben-Haroush et al. 2004). Genetically the presence of *CDKAL1* gene was detected here in those with family history of DM, was earlier identified as a susceptibility gene for T2DM among Europeans and Asians (Cho et al. 2009) and was also discovered in our GDM patients (Nor Khatijah et al. 2011). However, the current study showed rs10946398 in *CDKAL1* gene with a negative association (*OR* < 1) when analysed against GDM risk factor (family history of DM). With family history of DM, perhaps earlier screening and intervention to life style may contribute to explain its protective effect. SNP rs10946398 in *CDKAL1* gene was earlier reported as a CDK5 regulatory subunit-associated protein 1-like 1 (Petry 2010). No association had been reported so far on family history of DM factor with CDKAL1 gene. Whether the presence of this SNP contributes or not to the actual clinical risk factor need to be verified further in a bigger scale study.

Caesarean section was seen more than premature delivery in our GDM mothers (Table 1). However Caesarean sections could be multifactorial. As reported in earlier part of the study (Nor Khatijah et al. 2011), this smaller complication probably could be explained with a mild diabetes to start off, with fasting blood glucose < 5.3 mmol/l in our cohort of patients. Among these GDM women 83.9% were sufficed with diet modification only and the other 16.1% patients were given insulin injection in addition to the dietary control. Studies have shown that early treatment in this group of patients may result in the above (Landon et al. 2010; Nor Azlin et al. 2011). Studies (Tracy et al. 2005) have also proven that treatment reduces the rate of macrosomia, Caesarean section, fetal metabolic complications and neonatal intensive care unit days, as shown in Table 1. Among those women who had screening done at post partum, a third of them developed T2DM as previous studies had described (Ben-Haroush et al. 2004).

Current findings suggested that there was a significant correlation between respective SNPs in *LPL, OXTR, SLC2A2* and *IRS1* with caesarean sections and admittance to the NICU (Table 2). To the best of our knowledge, this has not been reported before with regards to GDM complications. The *LPL* gene which controls the lipid level in the bloodstream is reported to be involved in T2DM pathogenesis in the Korean population (Cho et al. 2009). A relationship between rs343 in *LPL* gene with T2DM-related phenotypes in total cholesterol, high density lipoprotein cholesterol (HDL) and log transformed glycosylated hemoglobin (HbA1c) was also seen in the Korean population. Susceptible polymorphism of *IRS1* gene was reported to predispose risk towards T2DM (Ekrem et al. 2006; Kalliopi et al. 2010). Furthermore, findings in French, Danish and Finnish population-based cohorts revealed the C allele of rs2943641 near *IRS1* was associated with fasting and glucose-stimulated hyperinsulinemia and also in impairment of insulin sensitivity (Ekrem et al. 2006). Taken everything together, whether these results showed the relevance of these findings where rs13306465 (*IRS1)* and rs328 *(LPL)* were significance with admission of neonates to NICU, need further verification.

The diagnosis of T2DM at six weeks post partum was detected with the presence of *TCF7L2* gene where

TABLE 1. Risk factors and complications (*n*=288)

Risk factors & complications	GDM *n* = 174 (%)	Controls *n* = 114 (%)
Family history of diabetes mellitus		
Yes	96 (55.2)	55 (48.2)
No	78 (44.8)	59 (51.8)
Uterus bigger than dates		
Yes	7 (4.0)	3 (2.7)
No	167 (96.0)	111 (97.3)
Glycosuria		
Yes	17 (9.8)	9 (7.9)
No	157 (90.2)	105 (92.1)
Maternal complications		
Premature delivery	30 (17.5)	21 (18.7)
Yes	141 (82.5)	91 (81.3)
No		
Ceaserean section		
Yes	48 (28.1)	35 (31.2)
No	123 (71.9)	77 (68.8)
Post partum diabetes (T2DM)		
Yes	14 (31.8)	
No	30 (68.2)	
Fetal complication		
Macrosomia (weight > 4kg)		
Yes	11 (6.4)	3 (2.7)
No	160 (93.6)	109 (97.3)
Neonatal complications		
Hyperbilirubinaemia		
Yes	13 (7.6)	4 (3.6)
No	158 (92.4)	108 (96.4)
NICU admission	7 (4.3)	4 (3.6)
Yes	164 (95.7)	108 (96.4)
No		

TABLE 2. Genetic relationship in risk factors and complications

Parameter	Gene	SNPs	Crude Odds ratio (95% CI)	P value*
Family history of DM	*CDKAL1*	rs10946398	0.49 (0.25,0.96)	0.006
Caesarean section	*LPL*	rs328	2.25 (1.07,4.73)	0.002
	OXTR	rs1042778	2.62 (1.28,5.36)	0.022
NICU Admission	*SLC2A2*	rs5404	6 (1.24,28.99)	0.028
	SLC2A2	rs5400	4.84 (1.01,23.10)	0.033
	IRS1	rs13306465	5.68 (1.18,27.36)	0.031
Future T2DM	*TCF7L2*	rs12255372	6.45 (2.05,20.34)	0.0004
	TCF7L2	rs7901695	6.4 (2.04,20.07)	0.0005
	TCF7L2	rs7903146	6.53 (2.04,20.83)	0.0005

*Trend test assessment

rs12255372, rs7901695 and rs7903146 were shown earlier to be associated with GDM in our pregnancy cohorts (Nor Khatijah et al. 2011). It was statistically proven that the presence of *TCF7L2* variants were able to predict the risk of gestational diabetes mellitus as much as 1.89 to 2.20 times (odd ratio) as compared to the absence of these variants (Nor Khatijah et al. 2011), which was seen again in this current study with six times higher for T2DM. Furthermore previous GWA studies had shown consistent correlation between *TCF7L2* and impairment of insulin secretion

in body (Saxena et al. 2007) and it was reproducibly associated with T2DM in various ethnic groups (Balding 2006). GDM is regarded as a 'window period', a myth prior true features of T2DM is fully developed, currently is realised to play a significant role in halting genetic propagation if discovered earlier. Inquisition of remaining gaps between GDM and its genetic link has been highlighted in various parts of the world (Petry 2010). It is timely that this is further explored in the cohort of our patients.

CONCLUSION

The genetic factor for a multifactorial disease like GDM is complex. SNPS for genes *CDKAL1, LPL, OXTR, SLC2A2, IRS1* and *TCF7L2* were discovered among pregnant Malaysian women with positive GDM risk factors and complications. Whether the presence of these SNPs in this study is just by chance or a true hallmark of a new finding warrants further scrutiny in a larger scale study.

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