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TCF7L2 Gene Rs7903146 Polymorphism May Confer Expression of Gestational Diabetes Mellitus in Relatively Young and Lean Mothers

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Research Article

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Abstract

To observe the impact of clinical characteristics and TCF7L2 rs7903146 polymorphism on GDM, pregnant women without history of glucose intolerance [N=100; age 26.22 ± 4.56 years; body mass index (BMI) 26.39 ± 3.85; mean ± SD; GDM=50, normal glucose tolerance (NGT)=50 according to WHO 2013 criteria] were studied for TCF7L2 rs7903146 polymorphism using Sanger sequencing technique (genotype CC=63, CT/TT=37) of genotyping. Women with CC and CT/TT genotypes had no significant difference of age (CC vs. CT/TT: 26.10 ± 4.46 vs. 26.43 ± 4.79 years, p=0.723; mean ± SD) or BMI (CC vs. CT/TT: 26.21 ± 3.90 vs. 26.69 ± 3.79 kg/m^2, p=0.548; mean ± SD). While GDM women with CC genotype had higher age and BMI than NGT women (GDM vs. NGT: age 27.96 ± 3.79 vs. 24.60 ± 4.45 years, p=0.002; BMI: 27.67 ± 3.93 vs. 25.04 ± 3.50 kg/m^2, p=0.006; mean ± SD), GDM women with CT/TT genotype had no significant difference of age and BMI with NGT women (GDM vs. NGT: age 27.00 ± 5.22 vs. 25.60 ± 4.10 years, p=0.390; BMI: 26.48 ± 3.63 vs. 27.00 ± 4.13 kg/m^2, p=0.688; mean ± SD). In women of age <25 years, frequency of GDM was significantly higher in those with CT/TT genotype than those with CC [CT/TT vs. CC: 58.3% vs. 17.4%, p=0.022] having an odds ratio (OR) of 6.650 (95% CI 1.377-32.114) for GDM; but not in women ≥25 year old (CT/CC vs. CC 60% GDM in both groups, p=1.000, OR=1.000, 95% CI 0.361-2.773). Using BMI cut-off at 25 kg/m^2, women with BMI <25 kg/m^2 had significantly higher frequency of GDM in those with CT/TT genotype than those with CC (CT/TT vs. CC: 61.5% vs. 18.2%, p=0.024) with an OR of 7.200 (95% CI 1.518-34.139); but not in women having BMI ≥25 kg/m^2 (CT/TT vs. CC 58.3% vs. 58.5%, p=0.987, OR=0.992, 95% CI 0.357-2.86). It is concluded that polymorphism of TCF7L2 rs7903146 may confer increased risk of GDM even in mothers with young age and lean BMI.

Keywords: TCF7L2; GDM; Polymorphism

Introduction

Although pregnancy is a condition characterized by progressive insulin resistance, glucose intolerance develops in only a small proportion of pregnant women [1]. Normally, the increased insulin resistance during pregnancy is compensated by the increase in insulin secretion by pancreatic islet β-cells that undergo structural and functional changes in response to the increased insulin requirements [2]. As a result, the changes in circulating glucose levels over the course of pregnancy are quite small, compared with the large changes in insulin sensitivity [1]. Gestational diabetes mellitus (GDM) can develop when a genetic predisposition of pancreatic islet β-cell impairment is unmasked by the increased insulin resistance during pregnancy [2]. Among widely studied genes of GDM, most are thought to modulate pancreatic islet β-cell function [3]. Likewise, there is ample evidence supporting transcription factor 7 like 2 (TCF7L2) as a GDM susceptibility gene, possibly by altering insulin secretion. Worth mentioning that few investigators have observed such relationship of gestational diabetes with alteration or polymorphism of genetic locus [3-10]. No significant studies are carried out over this area in Bangladesh as yet; as a matter of fact, numbers of studies even in the Asian region is very scanty.

The risk factors of glucose intolerance in pregnancy include maternal age, body mass index (BMI) and family history of diabetes [11,12]. As glucose intolerance is interplay between insulin resistance and secretory defect of β-cell, it is difficult to predict the effect of those risk factors in women harbouring a genetic alteration like TCF7L2 rs7903146 polymorphism. Again, risk conferred by a particular genetic polymorphism may not be similar in different groups of subjects with different clinical characteristics. In this perspective the present study was carried out to observe clinical characteristics and their impact on risk of GDM in relation to TCF7L2 rs7903146 polymorphism in women undergoing screening for GDM with the aim to focus on the importance of genetic studies in GDM among Bangladeshi mothers.

Materials and methods

Study design

In this cross-sectional study, single nucleotide polymorphism (SNP) of TCF7L2 rs7903146 was investigated in 100 pregnant mothers (age: 26.22 ± 4.56 years, BMI 26.39 ± 3.85 kg/m^2; mean ± SD) undergoing screening for GDM. Mothers were screened consecutively by 75-gm 3-samples oral glucose tolerance test (OGTT) following WHO 2013 criteria for GDM to include 50 women with GDM (age: 27.54 ± 4.45 years, BMI 27.15 ± 3.81 kg/m2; mean ± SD) and equal number of women with normal glucose tolerance (NGT) (age: 24.90 ± 4.33 years, BMI: 25.62 ± 3.77 kg/m2; mean ± SD). Those who underwent OGTT before 24 weeks of gestation were assigned to study group on the basis of repeat OGTT after 24 weeks of gestation (n=18; GDM=5, NGT=13). On the other hand, any subject falling into criteria of 'Diabetes in pregnancy' (0-h PG ≥ 7.0 and/or 02-h PG ≥11.1 mmol/L) was excluded. Relevant clinical and biochemical data including hemoglobin A1c (HbA1c) and plasma glucose (PG) were recorded (Table-1).

Variables	All subjects
N	100
Age in years (mean ± SD)	26.22 ± 4.56
BMI in kg/m^2 (mean ± SD)	26.39 ± 3.85
Parity	
Primipara	41 (41)
Multipara	59 (59)
Family history of DM in 1st degree relatives	36 (36)
Glycemic status (GDM/NGT)	50/50

Table 1: Characteristics of the study subjects.

(Within parenthesis are percentages over column total)
BMI: body mass index, GDM: gestational diabetes mellitus, NGT: Normal glucose tolerance

Written informed consent was taken from the participants. The project was run after approval of the institutional review board (IRB) of BSMMU.

Assay methods

Plasma glucose was assayed by glucose-oxidase method while HbA1c was measured using the Bio-Rad D-10™ HbA$_1$c Program 220-0101 (USA) certified by National Glycohemoglobin Standardization Program. Inter-assay co-efficient variance (CV) for glucose was 4.44%.

Genotyping

Blood samples in duplicate were collected in a VACUETTE® EDTA K$_3$ (Greiner Bio-One GmbH) tube. Genomic DNA was extracted using QIAmp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Extracted DNA was quantified by Quantus® fluorometer (Promega Corporation, USA). TCF7L2 locus was amplified by

polymerase chain reaction (PCR) using commercial PCR kit (GoTaq, Promega Corporation, USA). The PCR primers were designed by Primer Express and optimized according to the manufacturer's protocol. Forward and reverse primers used were 5/AGATTCCTTTTTAAATGGTGACA3/ and 5/GCATTACAAATTATTAGAACTTTCA3/. The amplicons were then electrophoresed in a 2% agarose gel to assess PCR efficacy and to detect the presence of the 356-bp of TCF7L2 locus. Sanger's di-deoxy chain terminating method was used to sequence the amplified TCF7L2 locus. Chain terminating cycle sequencing for both forward and reverse primer was performed using sequencing kit (BigDye Terminator v3.1, Applied Biosystems, Foster City, Calif. USA). Capillary electrophoresis was performed using ABI 3500Dx Genetic Analyzer (Applied Biosystems, Foster City, Calif. USA). Sequences were analyzed to determine the genotype using SeqScape software (Applied Biosystems, Foster City, Calif. USA). Genotyping results were validated by re-sequencing 10% of randomly selected samples. No differences were found, thus the genotyping error rate was 0%.

Statistics

Data were expressed as frequencies or percentages and mean (±SD) as applicable. Association of outcome variables were assessed by $\chi 2$-test and expressed with odds ratio (OR) and 95% confidence interval (CI). To compare the quantitative characteristics (age, BMI, plasma glucose values, HbA1c) unpaired t-test was done and to compare the qualitative characteristics (occupation, family history) $\chi 2$-test was done between groups. P values ≤ 0.05 were considered as significant.

Results

The present study comprised 63 women with common variant CC (cytosine in both allele) genotype and 37 women with CT (cytosine in one and thymine in another allele) or TT (thymine in both allele) genotype of TCF7L2 rs7903146 polymorphism. In comparison to women with CC genotype of TCF7L2 rs7903146 polymorphism, women with CT/TT genotype had no significant difference of age (CC vs. CT/TT: 26.10 ± 4.46 vs. 26.43 ± 4.79 years, p=0.723; mean ± SD) or BMI (CC vs. CT/TT: 26.21 ± 3.90 vs. 26.69 ± 3.79 kg/m^2, p=0.548; mean ± SD). HbA1c was significantly higher in woman with CT/TT genotype (CC vs. CT/TT: 5.08 ± 0.50 vs. 5.29 ± 0.45, p=0.038; mean ± SD). Frequency of family history of Diabetes Mellitus was similar in both groups (CC vs. CT/TT: 35% vs. 38%, p=0.769) (Table 2).

Variables	CC (n=63)	CT+TT (n=37)	p
Age in years (mean ± SD)	26.10 ± 4.46	26.43 ± 4.79	0.723
BMI (mean ± SD)	26.21 ± 3.90	26.69 ± 3.79	0.548
Family history of DM	22 (34.9)	14 (37.8)	0.769
*HbA1c (mean ± SD)	5.08 ± 0.50	5.29 ± 0.45	0.038
GDM	28 (44.4)	22 (59.5)	0.147

Table 2: Clinical and biochemical characteristics of TCF7L2 rs7903146 variants.
(Within parenthesis are percentages over column total) by Student's t-test and $\chi 2$ test

*Not done in 1 GDM woman
TCF7L2: Tranascription factor 7 like 2
GDM: gestational diabetes mellitus
BMI: body mass index (kg/m2)
DM: diabetes mellitus
C: Cytosine
T: Thymine
HbA1c: hemoglobin A1c (%)

When comparison was made between women with CC genotype and those with CT/TT genotype within GDM and (NGT) group, it was observed that there was no significant difference in relation to age, BMI, family history of DM, PG and HbA1c (Table 3). But when those parameters were compared between GDM and NGT women within two separate genotype groups (CC vs. CT/TT), it was observed that, while GDM women with CC genotype had higher age and BMI than NGT women (GDM vs. NGT: age 27.96 ± 3.79 vs. 24.60 ± 4.45 years, p=0.002; BMI: 27.67 ± 3.93 vs. 25.04 ± 3.50 kg/m^2, p=0.006; mean ± SD), GDM women with CT/TT genotype had no significant difference of age and BMI with NGT women (GDM vs. NGT: age 27.00 ± 5.22 vs. 25.60 ± 4.10 years, p=0.390; BMI: 26.48 ± 3.63 vs. 27.00 ± 4.13 kg/m^2, p=0.688; mean ± SD). Comparison of other characteristics (family history of DM, HbA1c) was observed to have similar pattern between women with GDM and NGT within two genotype group (Table 4).

Variables	GDM			NGT		
	CC (n=28)	CT+TT (n=22)	p	CC (n=35)	CT+TT (n=15)	p
Age in years (mean ± SD)	27.96 ± 3.79	27.00 ± 5.22	0.453	24.60 ± 4.45	25.60 ± 4.10	0.46
BMI (mean ± SD)	27.68 ± 3.93	26.48 ± 3.63	0.275	25.04 ± 3.50	27.00 ± 4.13	0.091
Family history of DM	14 (50.0)	11 (50.0)	1	8 (22.9)	3 (20.0)	0.823
0-h PG (mean ± SD)	4.78 ± 0.83	4.90 ± 0.68	0.561	4.22 ± 0.41	4.17 ± 0.43	0.723
***01-h PG** (mean ± SD)	9.74 ± 2.06	9.94 ± 1.64	0.719	7.27 ± 1.18	7.56 ± 1.03	0.416
02-h PG (mean ± SD)	8.39 ± 1.42	8.18 ± 1.54	0.608	6.16 ± 1.18	6.63 ± 1.00	0.188
***HbA1c** (mean ± SD)	5.20 ± 0.55	5.40 ± 0.42	0.176	4.99 ± 0.44	5.14 ± 0.47	0.286

Table 3: Comparison of clinical and biochemical characteristics between different variants of TCF7L2 rs7903146 in GDM and NGT subjects.
Within parenthesis are percentages over column total by Student's t-test and $\chi 2$ test

*Not done in 1 GDM woman with CC
TCF7L2: Tranascription factor 7 like 2
GDM: gestational diabetes mellitus
NGT: normal glucose tolerance
HbA1c: hemoglobin A1c (%)
BMI: body mass index (kg/m^2)
DM: diabetes mellitus
C: Cytosine
T: Thymine
PG: plasma glucose (mmol/L)

Variables	CC			CT+TT		
	GDM (n=28)	NGT (n=35)	p	GDM (n=22)	NGT (n=15)	p
Age (yrs, mean ± SD)	27.96 ± 3.79	24.60 ± 4.45	0.002	27.00 ± 5.22	25.60 ± 4.10	0.39
BMI (mean ± SD)	27.67 ± 3.93	25.04 ± 3.50	0.006	26.48 ± 3.63	27.00 ± 4.13	0.688
Family history of DM	14 (50.0)	8 (22.9)	0.025	11 (50.0)	3 (20.0)	0.065
*HbA1c (mean ± SD)	5.20 ± 0.55	4.99 ± 0.44	0.096	5.40 ± 0.42	5.14 ± 0.47	0.088

Table 4: Comparison of clinical and biochemical characteristics between GDM and NGT women in subjects with variants of TCF7L2 rs7903146.
(Within parenthesis are percentages over column total) by Student's t-test and $\chi 2$ test

*Not done in 1 GDM woman with CC
TCF7L2: Tranascription factor 7 like 2
GDM: gestational diabetes mellitus
NGT: normal glucose tolerance
BMI: body mass index (kg/m^2)
DM: diabetes mellitus
HbA1c: hemoglobin A1c (%)
C: Cytosine
T: Thymine

In women aging <25 years, frequency of GDM was significantly higher in those with CT/TT genotype (CT/TT vs. CC: 58.3% vs. 17.4%, p=0.022) having an OR of 6.650 (95% CI 1.377-32.114) for GDM. But this was not observed in women who are ≥25 year old (CT/TT vs. CC 60% GDM in both groups, p=1.000, OR=1.000, 95% CI 0.361-2.773; (Table 5). Using BMI cut-off at 25 kg/m^2, it was observed that in women with BMI <25 kg/m^2 frequency of GDM was significantly higher in those with CT/TT genotype than those with CC (CT/TT vs. CC: 61.5% vs. 18.2%, p=0.024) with an OR of 7.200 (95% CI 1.518-34.139). No increased risk of GDM was found according to genotype difference in women having BMI ≥25 kg/m^2 (CT/TT vs. CC 58.3% vs. 58.5%, p=0.987, OR=0.992, 95% CI 0.357-2.86; (Table 6).

Variables	GDM	NGT	Total	p	OR (95% CI)
Age <25 years					
CC	4 (17.4)	19 (82.6)	23		6.65
CT/TT	7 (58.3)	5 (41.7)	12	p=0.022	(1.377-32.114)
Total	**11 (31.4)**	**24 (68.6)**	35		
Age ≥25 years					
CC	24 (60.0)	16 (40.0)	40		1
CT/TT	15 (60.0)	10 (40.0)	25	p=1.000	(0.361-2.773)
Total	**39 (60.0)**	**26 (40.0)**	65		

Table 5: Glycemic outcome in mothers with TCF7L2 rs7903146 risk variant according to age cut-off. (Within parenthesis are percentages over row total) by Fisher's exact test & χ2-test

TCF7L2: Tranascription factor 7 like 2
GDM: gestational diabetes mellitus
T: Thymine
C: Cytosine

Variables	GDM	NGT	Total	p	OR (95% CI)
BMI <25 kg/m^2					
CC	4 (18.2)	18 (81.8)	22		7.2
CT/TT	8 (61.5)	5 (38.5)	12	**p=0.024**	(1.518-34.139)
Total	**11 (34.3)**	**23 (65.7)**	**35**		
BMI ≥25 kg/m^2					
CC	24 (58.5)	17 (41.5)	41		0.992
CT/TT	14 (58.3)	10 (41.7)	24	p=0.987	(0.357-2.756)
Total	**38 (58.5)**	**27 (41.5)**	**65**		

Table 6: Comparison of glycemic outcome in mothers with TCF7L2 rs7903146 risk variant according to BMI cut-off. (Within parenthesis are percentages over column total) by Fisher's exact test & χ2-test

TCF7L2: Tranascription factor 7 like 2
GDM: gestational diabetes mellitus
C: Cytosine
T: Thymine
BMI: body mass index

Discussion

In the present study, it was observed that the mothers with polymorphism of TCF7L2 rs7903146 (CC and CT/TT genotypes) had similar clinical characteristics like age, BMI and family history of DM as well as frequency of GDM with these genotypes. Unlike the higher age and BMI associated with GDM in comparison to NGT women in CC genotype, comparison of GDM and NGT mothers for age and BMI, we observed no significant difference for these factors in the genotype CT/TT. In light of these features, the risk conferred by variants of TCF7L2 rs7903146 was analysed separately in lower age and BMI groups. The mothers with CT/TT genotype had significantly higher frequency and risk of GDM in lower age and BMI groups, but not in higher age and BMI groups which supports the concept that genetic alteration influences over the secretory potential of β-cells function in GDM mothers. Previous investigators had observed poor β-cell function in relatively lean individual with risk variants of TCF7L2 polymorphism [5]. Similar finding was observed by Cauchi et al. in T2DM, where they found the risk to be highest in relatively lean individuals by restricting their analysis to lean subjects (BMI <30 kg/m^2) [13]. They commented that the strong association of rs7903146 T allele with T2DM in non-obese subjects makes it unlikely that the TCF7L2 diabetogenic effect directly involves insulin sensitivity or fat deposition. Rather it points towards defect of insulin secretion by β-cells. The negative association between TCF7L2 polymorphism and BMI does not necessarily imply that TCF7L2 polymorphism leads to reduced BMI, as it could simply

reflect the joint independent risk of BMI and TCF7L2 polymorphism [14]. Although risk conferred by genetic polymorphism always persists, its effect may be diluted by contribution of other risk factors including increasing age and BMI. As a result, genetic risk conferred by the rs7903146 T allele may be more important in those who are relatively young and lean. In consistent with this, we have clearly observed that GDM mothers having relatively lower age and BMI had higher frequency of polymorphism of TCF7L2 gene (CT/TT) in comparison to the NGT mothers; conversely, frequency of normal (CC) and altered (CT/TT) genotypes were found to be similar for GDM in relatively elderly mothers with higher BMI. These findings further strengthen the belief that genetic alteration strongly influences over β-cell function, insulin secretion and expression of GDM.

Family history of DM in 1st degree relatives is also considered to be a risk factor of GDM [11,15]. However, being a polygenic disorder with multiple environmental impacts, it is difficult to correlate the family history of DM and polymorphism of a single locus. As such frequency of family history of DM was found to be increased in GDM women irrespective of genotype of TCF7L2. This increased frequency of family history may reflect the impact of multiple genetic polymorphisms, assessment of which is beyond the scope of present study. HbA1c was observed to be significantly elevated in women with CT/TT genotype in comparison to CC, though in narrow margin. It may reflect the increased frequency of GDM in those with CT/TT.

In conclusion, polymorphism of TCF7L2 rs7903146 may confer increased risk of GDM even in mothers with young age and lean BMI.

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