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Association between rs7901695 and rs7903146 polymorphisms of the TCF7L2 gene and gestational diabetes in the population of Southern Poland

Daria Gorczyca-Siudak[1], Małgorzata Michalak-Wojnowska[2], Tomasz Gorczyca[3], Barbara Mosiewicz[1], Anna Kwaśniewska[3], Agata Filip[4], Jerzy Mosiewicz[1]

[1]*Department of Internal Medicine, Independent Public Teaching Hospital No 1, Medical University of Lublin, Poland*
[2]*Department of Cancer Genetics, Medical University of Lublin, Poland*
[3]*Department of Obstetrics and Pathology of Pregnancy, Independent Public Teaching Hospital No 1, Medical University of Lublin, Poland*
[4]*Department of Cancer Genetics with Cytogenetics Laboratory, Medical University of Lublin, Poland*

ABSTRACT

Objectives: The etiology of gestational diabetes mellitus (GDM) remains to be fully elucidated. Elevated risk for type 2 diabetes in patients with history of GDM and for GDM in women with familial history of diabetes may suggest that GDM and type 2 diabetes share a common genetic and environmental background. The *TCF7L2* (Transcription Factor 7 Like 2) gene is one of the most important genetic factors of the established correlation with type 2 diabetes, and it may also play a role in the pathophysiology of GDM.

The aim of the study was to assess the influence of two polymorphisms of the *TCF7L2* gene (rs7901695 and rs7903146), which are associated with the development of type 2 diabetes, in women with GDM.

Material and methods: The study included 50 women with glucose tolerance disorders diagnosed for the first time during the current pregnancy. Single nucleotide polymorphisms (SNPs) were genotyped using allelic discrimination. The results were confirmed using the sequencing method. Selected clinical parameters were also analyzed.

Results: No correlation between the studied polymorphisms of the *TCF7L2* gene and GDM was observed. Glycemic control with diet or diet and insulin was associated with better control of the weight gain during pregnancy.

Conclusions: No correlation between rs7903146 and rs7901695 polymorphisms of the *TCF7L2* gene and GDM was found. Glycemic control with diet or diet and insulin is associated with better control of the weight gain during pregnancy.

Key words: genetic polymorphism, gestational diabetes mellitus, *TCF7L2* gene

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INTRODUCTION

According to the Polish Gynecological Society and to the Polish Diabetes Association, gestational diabetes mellitus (GDM) is defined as glucose tolerance disorders of various intensity, diagnosed for the first time during pregnancy [1]. The incidence of GDM has been estimated at 3–5% and 5–10% in the European and Asian populations, respectively [1, 2], and a steady increase has been reported [3]. Higher incidence rates of GDM result from the changing diagnostic criteria as a universal diagnostic scheme for GDM has not been designed yet. The main differences in the diagnostic process concern varying glycemic values and different inclusion criteria.

Glucose tolerance disorders are associated with complications affecting both, the mother and the fetus [4]. GDM increases the risk of preeclampsia, gestational hypertension, infections, and surgical delivery. In the first weeks of pregnancy, hyperglycemia may be teratogenic or lead to miscarriage. In the second trimester, hyperglycemia may cause macrosomia, organomegaly, and diabetic fetopathy.

Corresponding author:
Małgorzata Michalak-Wojnowska
Department of Cancer Genetics, Medical University of Lublin
Radziwiłłowska St. 11, 20–080 Lublin, Poland
e-mail: michalak.malgorzata@gmail.com

An association between fetal complications and maternal glycemia was confirmed by the prospective randomized HAPO (Hyperglycemia and Adverse Pregnancy Outcomes) study [5].

Women with GDM present 7-fold higher risk for developing type 2 diabetes in 5–10 years after the delivery [6], while their children have higher risk of developing glucose tolerance disorders in the future [7]. GDM risk factors include: maternal age of > 35, history of the following: neonatal birth weight of > 4000 g, congenital malformations, miscarriage, hypertension, overweight or obesity, GDM in previous pregnancies, polycystic ovary syndrome, multiparity, and familial history of type 2 diabetes [1].

The exact etiology of GDM has not been fully discovered yet. Results of the studies so far have demonstrated its multifactorial pathogenesis, in which genetic predisposition and environmental factors play important roles [8]. Increased risk for type 2 diabetes in patients with history of GDM and of GDM in women with familial history of diabetes may suggest that GDM and type 2 diabetes share a common genetic and environmental background [6, 9].

GDM is caused by disorders in responsiveness to insulin and its secretion [10]. Genes associated with insulin secretion, insulin resistance, glucose and lipid metabolism have been extensively studied over the past decades. However, the results of these studies were often inconclusive, incoherent, lacked statistical significance, with small sample size, and only moderate influence of the investigated genes [11].

The *TCF7L2* gene encodes a high mobility group-box (HMG box), containing a transcription factor implicated in the Wnt signaling pathway. The TCF7L2 protein has been found to be important for blood glucose homeostasis [12].

OBJECTIVES

The aim of the study was to assess the influence of two polymorphisms of the *TCF7L2* gene which play a role in the pathophysiology of type 2 diabetes, with the development of GDM.

MATERIAL AND METHODS

The study included 76 women: 26 healthy controls and 50 women with glucose tolerance disorders, diagnosed for the first time during the current pregnancy, hospitalized at the Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, from April 2013 to September 2014. Local Ethics Committee approved of the study (KE-0254/290/2012). Oral glucose tolerance test result outside the normal reference range was the inclusion criterion. The diagnosis of GDM was based on the Polish Gynecological Society criteria, which were adopted as follows: until July 2014: glycemia at 0 h ≥ 5.5 mmol/L (100 mg/dL), at 1 h ≥ 10 mmol/L (180 mg/dL), at 2 h ≥ 7.8 mmol/L (140 mg/dL), and since July 2014: at 0 h ≥ 5.1 mmol/L (92 mg/dL), glycemia at 1 h ≥ 10 mmol/L (180 mg/dL), glycemia at 2 h ≥ 8.5 mmol/L (153 mg/dL). Out of the 50 women, 40 were diagnosed with type 1 GDM, and the remaining 10 with type 2 GDM.

Table 1. Primer sequences

Primer	Sequence	Annealing temperature	Amplicon length (bp)
7903146F	TTTTAAATGGTGACAAATTC	51°C	324
7903146R	ACTATGTATTGTTGCCAGTC		
7901695F	ATTTGAGGTGTACAATTGAG	51°C	400
7901695R	TGTGCAAAATGTTCATAGTA		

The control group included 26 healthy pregnant women who were admitted to the Department for the delivery. Informed consent was obtained from all patients.

Five ml blood samples were collected from each patient and stored at –70°C until needed. Genomic DNA was isolated from blood with Qiamp DNA Blood Mini Kit (Qiagen). Purity and concentration were tested with NanoDrop 2000 UV Vis-Spectrophotometer. Single nucleotide polymorphisms were genotyped using allelic discrimination with Fast 7500 Real-Time PCR System (Life Technologies, USA). Sets of probes and primers TaqMan SNP Genotyping Assay (Life Technologies) and TaqMan Genotypic Master Mix (Life Technologies) were used in the tests. All procedures were performed in accordance with the producer's instruction. All samples were genotyped twice in the presence of controls. The results for randomly selected samples were confirmed using the sequencing method. DNA was amplified with RAPID kit (A & A Biotechnology, Poland), primer sequence and reaction conditions were designed at the Department of Cancer Genetics, Medical University of Lublin (Table 1). Both DNA strands were sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit. Capillary Electrophoresis was performed using POP7 polymer, 50 cm long capillary and ABI 3130 (Life Technologies) sequencer. The concordance was > 99%.

Statistical analysis was performed using Statistica for Windows 10.0.1011.7. The frequency of the selected genes in each group (controls and GDM) was analyzed with the Odds Ratio. Quantitative results in each group were analyzed with the Mann-Whitney U test, qualitative results were analyzed with the chi-squared test. The p-values of < 0.05 and < 0.01 were considered as statistically significant, and extremely statistically significant, respectively. The Hardy-Weinberg equilibrium (HWE) was evaluated using an on-line application of the Institute of Human Genetics (Helmholtz Center Munich, Germany) (http://ihg.gsf.de/).

RESULTS

The characteristics of the study population are presented in Table 2.

Table 2. Patient characteristics

		Study group (GDM)		Controls	p
Age (years)		30.36 (± 5.52; 20–44)		30.88 (± 5.62; 20–44)	0.5353
Weight before pregnancy [kg]		66.22 (± 11.97; 47–100)		63.42 (± 7.89; 50–81)	0.3266
Height [m²]		1.65 (± 0.05; 1.56–1.82)		1.67 (± 0.06; 1.56–1.76)	0.2698
Weight before pregnancy [kg] GDM1 vs. GDM2		63.3 (± 10.07; 47–86)	77.9 (± 12.3; 61–100)		0.0026
BMI before pregnancy [kg/m²]		24.27 (± 3.96; 16.46–33.87)		22.93 (± 3.95; 17.76 –33.28)	0.0928
BMI before pregnancy [kg/m²] GDM1 vs. GDM2		23.43 (± 3.59; 16.46–32.79)	27.65 (± 3.71; 21.61–33.87)		0.0068
Weight gain during pregnancy [kg] GDM1 vs. GDM2		11.47 (± 5.95; -3–25)		16.27 (± 4.81; 8.5–28)	0.0004
		12.87 (± 5.37; -3–25)	5.85 (4.88; 0–13)		0.0012
Week of gestation during labour		38.86 (± 1.55; 33–41)		40.15 (± 1.08; 38–41)	0.0002
Birth weight [g]		3346.53 (± 534.51; 1580–4740)		3521.92 (± 394.09; 2980–4360)	0.1204
Apgar 1st minute		9.34 (± 1.04; 6–10)		9.85 (± 0.37; 9–10)	0.0352
Way of parturition	CS	32 (64%)		13 (50%)	0.2387
	NCB	18 (36%)		13 (50%)	

BMI — body mass index; CS — caesarian section; GDM — gestation diabetes mellitus; NCB — natural child birth; p — p-value, probability value

Statistical analysis showed higher pre-pregnancy BMI in the study group as compared to controls (mean body mass 66.22 kg and BMI = 24.27 kg/m² vs. 63.42 kg and BMI = 22.93 kg/m²) but the difference was not statistically significant ($p > 0.05$; Table 2). Mean pre-pregnancy body mass of insulin-dependent patients was higher than in diet-controlled patients. Pre-pregnancy BMI was higher in patients with type 2 GDM as compared to patients with type 1 GDM (27.65 vs. 23.43 kg/m²). Weight gain during pregnancy was statistically significantly higher ($p < 0.01$) in controls as compared to the study group (16.27 kg vs. 11.47 kg). Statistically significant difference ($p < 0.01$) in weight gain was also observed in groups with type 1 and 2 GDM, weight gain was higher in diet-controlled diabetic patients as compared to insulin-controlled diabetic patients (12.87 kg vs. 5.85 kg).

Genotype frequencies of the SNPs were consistent with the Hardy-Weinberg equilibrium. Allele frequency in rs7903146 (C > T) in all of the investigated women was as follows: the frequency of alleles C and T was 67.2% (102/152) and 32.8% (50/152), respectively. In the control group, the frequency of alleles C and T was 67.3% (35/52) and 32.7% (17/52), respectively. Alleles C were found in 67% (67/100) and alleles T in 33% (33/100) of the patients with GDM. Allele distribution did not vary between the groups. Overall, 58% (29/56) of the investigated women were heterozygous, out of them 38% (18/56) were homozygous for allele C and 4% (2/56) were homozygous for allele T. Genotype distribution was identical among patients with GDM with heterozygous women constituting 58% (29/40) of the group, homozygous for allele C — 38% (19/40), and homozygous for allele T — 4% (2/40). Genotype distribution in the control group was similar (Table 3).

Allele frequency in rs7901695 (T > C) in all of the investigated women was as follows: the frequency of alleles C and T was 32.2% (49/152) and 67.8% (103/152), respectively. In the control group, the frequency of alleles C and alleles T was 34.6% (18/52) and 65.4% (34/52), respectively. Alleles C and T were found in 31% (31/100) and 69% (69/100) of the patients with GDM, respectively. Overall, 60.6% (46/76) of the women were heterozygous, out of them 36.8% (28/76) were homozygous for allele C and 2.6% (2/76) were homozygous for allele T. The results in diabetic patients and controls were similar. Among diabetic patients, heterozygous women constituted 60% (30/40) of the homozygous for allele T group — 38% (19/40) and homozygous for allele C — 2% (1/40). No correlation between rs7903146 and rs7901695 polymorphisms of the *TCF7L2* gene and the development of GDM was found.

Genotype distribution is presented in Table 3.

DISCUSSION

The TCF7L2 protein is a transcription factor in T cells (T cell factor). The gene encoding this protein is located on chromosome 10q25, its size is 215.9 kB. It takes part in the pathogenesis of type 2 diabetes as it affects the Wnt signaling pathway, which regulates cell proliferation and differentiation [12]. Interaction between β-catenin and TCF/LEF1 transcription factors (T cell factor, lymphocyte enhancer factor-1) is the last process in this pathway. The TCF proteins bind with DNA. Mammalian cells contain four TCF proteins: TCF7 (also described as TCF1), LEF, TCF7L1-1

Table 3. The distribution of genotypes and allele frequency in the study group and the control

SNP	Genotype	Study group (GDM)		Controls		OR	95% CI	p-value	HWE p-value
		L	%	L	%				
rs7903146 *TCF7L2* C > T	C/C	19	38	10	38.462	1.02	0.38–2.70	0.9686	0.196
	C/T	29	58	15	57.692				
	T/T	2	4	1	3.846				
	%T	33		32.69					
rs7901695 *TCF7L2* T > C	T/T	19	38	9	34.615	0.86	0.32–2.32	0.7717	0.403
	T/C	30	60	16	61.538				
	C/C	1	2	1	3.846				
	%C	32		34.61					

CI — confidence interval; GDM — gestation diabetes mellitus; HWE p-value — Hardy-Weinberg equilibrium probability value of the control group; OR — odds ratio; p-value — probability value; SNP — single nucleotide polymorphism

(also described as TCF3) and TCF7L2 (also described as TCF4). Variants of the TCF7L2 gene may also affect insulin resistance by modulating glucagon-like peptide-1 secretion [13]. A study by Saxena et al. (2006), demonstrated that polymorphisms of the *TCF7L2* gene increase the risk for developing type 2 diabetes by affecting the process of insulin secretion [14]. The exact mechanism of the influence of TCF7L2 on insulin resistance remains unknown. This protein is believed to act in various signaling pathways, which is a typical feature of transcription factors.

The literature offers numerous and often conflicting reports on the relationship between the studied polymorphisms and GDM. Not only are the diagnostic criteria different, but the results of the studies concern various populations of different ethnic origins. All authors emphasize the need for further research. Our study found no correlation between the studied polymorphisms and GDM.

Rs7903146 variant of the *TCF7L2* gene is known as the polymorphism most related to GDM. A meta-analysis of 9 studies, conducted in 2013, has demonstrated that allele T in rs7903146 is related to the development of GDM [OR (95% CI) 1.44 (1.29–1.60), p < 0.001]. However, a wide variety of results of studies on this association were observed in Caucasian as compared to Asian race [15]. In 2013, the results of a long-term Finnish study on 69 polymorphisms in women with GDM were published. Rs7903146 polymorphism of the *TCF7L2* gene was, according to the authors, slightly connected with GDM [OR (95% CI) 1.30 (1.03–1.64), p = 0.028] as compared to other gene polymorphisms [16]. Table 4 shows the diagnostic criteria adopted in our study. Numerous studies conducted on the Swedish population have shown a significant correlation of particular alleles (C > T) located on rs7903146 with GDM. For instance, Papadopoulou et al. (2011), examined a representative group of 805 patients with GDM between 2000–2004. Genotypes CT and TT of rs7903146 were correlated with 1.6 times [OR (95% CI) 1.63 (1.34–1.97), p < 0.0001] and 1.9 times [OR (95% CI) 1.90 (1.37–2.64), p < 0.0001] higher risk for GDM, respectively

Table 4. Diagnostic criteria adopted in our work and aforementioned studies

Study	Criteria
Gorczyca-Siudak et al.	Until July 2014: • fasting ≥ 5.5 mmol/L (100 mg/dL) • 1st hour ≥ 10 mmol/L (180 mg/dL) • 2nd hour ≥ 7.8 mmol/L (140 mg/dL) Since July 2014: • fasting ≥ 5.1 mmol/L (92 mg/dL) • 1st hour ≥ 10 mmol/L (180 mg/dL) • 2nd hour ≥ 8.5 mmol/L (153 mg/dL)
Houpio et al. (2013)	Until September 2001: • fasting ≥ 4.6 mmol/L (82 mg/dL) • 1st hour ≥ 10 mmol/L (180 mg/dL) • 2nd hour ≥ 8.7 mmol/L (156 mg/dL) Since September 2001: • fasting ≥ 4.6 mmol/L (82 mg/dL) • 1st hour ≥ 11.2 mmol/L (200 mg/dL) • 2nd hours ≥ 9.9 mmol/L (180 mg/dL)
Papadopoulou et al. (2011)	2nd hour ≥ 9 mmol/L (162 mg/dL)
Shaat et al. (2007)	2nd hour ≥ 9 mmol/L (162 mg/dL)
Freathy et al. (2010)	• fasting > 5.8 mmol/L (105 mg/dL) • 2nd hour > 11.1 mmol/L (200 mg/dL)
Vcelak et al. (2012)	• fasting ≥ 7 mmol/L (126 mg/dL) • 2nd hour ≥ 7.8 mmol/L (140 mg/dL)
Klein et al. (2012)	• fasting ≥ 5.1 mmol/L (92 mg/dL) • 1st hour ≥ 10 mmol/L (180 mg/dL) • 2nd hour ≥ 8.5 mmol/L (153 mg/dL)
Rizk et al. (2011)	No data
Stuebe et al. (2014)	• fasting > 5,2 mmol/L (95 mg/dL) • 1st hour > 9,9 mmol/L (180 mg/dL) • 2nd hour > 8,5 mmol/L (155 mg/dL) • 3rd hour > 7,8 mmol/L (140 mg/dL)
Pagan et al. (2014)	2 of the 4 glucose values above the normal range: • fasting > 5.8 mmol/L (105 mg/dL) • 1st hour > 10,5 mmol/L (190 mg/dL) • 2nd hour > 9mmol/L (165 mg/dL) • 3rd hour > 8 mmol/L (145 mg/dL)

[17]. Shaat et al. (2007), studied the link between several polymorphisms connected with type 2 diabetes and GDM. Only the rs7903146 polymorphism turned out to be statistically significant, genotype T/T increased the risk of GDM twice [18]. Freathy et al. (2010), analyzed data from HAPO concerning the influence of rs7903146 on GDM in Caucasian women in Great Britain and Australia, versus Asian women in Thailand. In the Caucasian group, the frequency of allele T was 29.2–30.6%, and it was correlated with the risk for GDM, while in the Asian group the frequency of allele T was 4.7%, and it was not related to hyperglycemia [19].

Allele distribution in a group of 261 Czech women with GDM presented by Vcelak et al. (2012), was almost identical to our study. However, allele distribution in the control group was significantly different from our results. These authors have proven that women with allele T in rs7903146 have 1.4 times higher risk for developing GDM [%T = 33.8% vs. 26.7%, OR (95% CI) 1.41 (1.08–1.84), p = 0.0148]. Genotype distribution in women with GDM was: CC — 41.5%, CT — 49.2%, TT — 9.3% and in the control group: CC — 54.4%, CT — 39.0%, TT — 6.5%. The study revealed no increased risk of GDM in TT homozygotes as compared to healthy women [OR (95% CI) 1.33 (0.70–2.51), p = 0.476] [20].

Numerous studies have confirmed the absence of any correlation between rs7903146 and increased risk for GDM. In a study conducted in Austria on Caucasian population by Klein et al. (2012), the frequency of allele T in the group with GDM, diagnosed according to the 2007 IADPSG criteria, and the control group was similar (48.8% vs. 49.2%) [21]. Rizk et al. (2011), studied the frequency of CC, CT and TT rs7903146 genotypes of *TCF7L2* in Arab population. The study showed statistically insignificant differences between the groups with GDM and controls (39.4%, 50% and 10.6% vs. 40.6%, 43.8%, and 15.6%, respectively, p = 0.444) [22].

There are fewer studies on the rs7901695 polymorphism and their results are also incoherent. In a study conducted in Sweden by Papadopoulou et al. (2011), the TC genotype increased the risk for GDM 1.5 times [OR (95% CI) 1.56 (1.28–1.89), p < 0.0001] and CC increased the risk almost 1.9 times [OR (95% CI) 1.87 (1.36–2.57), p < 0.0001] [17]. Stuebe et al. (2014), showed that allele T in rs7901695 increases the risk for GDM among Caucasian and African-American women in the USA twice [OR (95% CI) 1.98 (1.31–2.99)] [23]. However, Pagan et al. (2014), in a study conducted on groups similar to our study (25 healthy women, 45 Caucasian women with GDM) showed no correlation between rs7901695 and the development of GDM [24].

In our study, no differences in allele distribution were observed, thus there was no correlation between this polymorphism and GDM.

CONCLUSIONS

Findings on the relationship between the investigated polymorphisms and the development of gestational diabetes mellitus continue to vary, mainly due to lack of universal diagnostic criteria, diverse populations, and different ethnic origins. In this context, various authors emphasize the need for further research. In our study, no correlation between rs7903146 (C > T) and rs7901695 (T > C) polymorphisms of the *TCF7L2* gene and gestational diabetes was observed. Additionally, glycemia normalization with diet or diet and insulin is correlated with better control over weight gain during pregnancy.

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