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Association of transcription factor 7-like 2 (*TCF7L2*) gene polymorphism with posttransplant diabetes mellitus in kidney transplant patients medicated with tacrolimus

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Abstract:
New onset posttransplant diabetes mellitus (PTDM) has a high incidence after kidney transplantation in patients medicated with tacrolimus. PTDM can adversely affect patient and graft survival. The pathophysiology of PTDM closely mimics type 2 diabetes mellitus (T2DM). One of the possible genetic factors predisposing individuals to PTDM might be a polymorphism in the transcription factor 7-like 2 gene (*TCF7L2*). This polymorphism has previously been associated with increased risk of T2DM in the general population. Therefore, the present study aimed to evaluate *TCF7L2* polymorphisms in PTDM in kidney transplant patients medicated with tacrolimus.
Non-diabetic kidney transplant patients medicated with tacrolimus (n = 234) were genotyped for the presence of *TCF7L2* gene variants (rs12255372 and rs7903146) using TaqMan probes. Of the 234 patients, 66 patients had developed PTDM and 168 had not. Frequencies of the studied single nucleotide polymorphisms (SNPs) did not differ significantly between the study groups. Moreover, haplotype analyses failed to detect any associations between *TCF7L2* haplotypes and PTDM. However, in late-onset PTDM (developed later that 2 weeks from transplantation), frequencies of the rs7903146 TT genotype and T minor allele were significantly increased compared to non-PTDM controls (17.9% *vs*. 5.9%, p = 0.017, OR: 4.13, 95% CI: 1.19–14.33 for TT genotype, 39.3% *vs*. 25.9%, p = 0.038 for T allele). If the application of *TCF7L2* rs7903146 SNPs as a marker for PTDM is confirmed by further independent studies, replacing tacrolimus with other immunosuppressants could be warranted in patients at high risk of PTDM, as diagnosed by *TCF7L2* genotyping.

Key words:
posttransplant diabetes mellitus (PTDM), *TCF7L2*, genetic polymorphism

Introduction

New onset posttransplant diabetes mellitus (PTDM) has a high incidence of onset after kidney transplantation in patients medicated with tacrolimus. PTDM can adversely affect patient and graft survival [14]. Estimates based on the use of hypoglycemic intervention have suggested that PTDM occurs in approximately 15–20% of renal transplant patients. Using American Diabetes Association (ADA) criteria, it was found that 13% of patients had PTDM by three months posttransplant and 39% had abnormal glucose metabolism. The onset of PTDM is most pronounced in the first few months posttransplant, and it continues to present at a steady rate after the first posttransplant year [16].

No clear risk factors for PTDM have been established. However, the following characteristics that appear to predispose patients to the development of PTDM have been identified: patient age of over 40 years, African American and Hispanic populations, family history, patient body weight, hepatitis C virus, and an immunosuppressive regimen with calcineurin inhibitors [2, 11].

As the pathophysiology of PTDM closely mimics type 2 diabetes mellitus (T2DM), PTDM should be considered at least as serious as onset of T2DM. Both diseases are characterized by a combination of insulin resistance and insulin hyposecretion; however, insulin hyposecretion has been a key determinant of worsening glucose tolerance following renal transplantation [3, 9].

The ability to predict a patient's risk for PTDM would be of considerable benefit in the selection of appropriate immunosuppressive regimens. Therefore, some research studies have been designed to reveal the associations of genetic factors with PTDM. Many of these studies have failed to identify such associations (including polymorphisms in *CYP3A5*, *MDR1*, *UPC2*, *PPARγ* genes), even in case of polymorphisms associated with tacrolimus dose [26]. However, Nakamura and colleagues reported that the presence of the vitamin D receptor gene *VDR TaqI t* allele might be a risk factor for PTDM. This finding suggests that genotyping diabetes-related polymorphisms could be a possible method of predicting a patient's risk for developing PTDM, and this information could be a valuable asset in the selection of appropriate immunosuppressive regimens [18]. Recently, the VNTR polymorphism in intron 4 (27-bp repeat) of the endothelial nitric oxide synthase gene (*NOS3*) has been associated with PTDM in Turkish patients [4].

Transcription factor 7-like 2 (*TCF7L2*) is the gene with the largest effect on disease susceptibility discovered to date. It was identified before the genome-wide association studies (GWAS) era, and the association was rapidly replicated in GWAS across various ethnicities. [8]. TCF7L2 protein is a member of a T-cell transcription factor family that plays a critical role in the regulation of cell proliferation and differentiation through the Wnt signaling pathway. TCF7L2 is also implicated in the development and maturation of the pancreas, including the islets of Langerhans [19]. It has also been discovered that TCF7L2 exerts its influence through an impairment of insulin secretion. This impairment was reportedly due to a functional defect in the glucagon-like peptide-1 (GLP-1) signaling in β-cells and not due to defective/failing GLP-1 secretion [22]. TCF7L2 might also impact β-cell function both directly through modulating β-cell response to glucose and indirectly by modulating incretin action or secretion [17].

The variant of *TCF7L2* that has been observed to be associated with type 2 diabetes is a microsatellite marker (DG10S478) located in intron 3 [8]. Additionally, the genotypes of five single nucleotide polymorphisms (SNPs) within the same large haplotype block that correlated with DG10S478 were also associated with type 2 diabetes. The authors recommended that the two most highly correlated SNPs, rs12255372 and rs7903146, should be included in replication studies. Subsequently, these two SNPs have been associated with type 2 diabetes and impaired glucose tolerance [5, 21, 25]. The single nucleotide polymorphism rs7903146 has shown the strongest association with diabetes, and it resides in a noncoding region with no obvious mutational mechanism. It is clear, however, that the effect of the *TCF7L2* risk allele is through a defect in insulin secretion and incretin signaling [8, 22, 29]. Recent findings have documented that *TCF7L2* rs7903146 T allele carriers showed both significantly lower homeostasis model assessment for β-cell function (HOMA-B) values and higher fasting glycemia and diabetes prevalence [6]. These data indicate that SNP rs7903146:C>T might modulate the degree of insulin secretion to offset the prevailing level of insulin resistance without being a cause of insulin resistance. This effect has also been documented for other *TCF7L2* polymorphisms, such as rs12255372:G>T [12].

Therefore, the aim of the present study was to evaluate *TCF7L2* gene polymorphisms in PTDM in kidney transplant patients medicated with tacrolimus.

Materials and Methods

Patients

Non-diabetic kidney transplant patients (n = 234) were eligible for this study. Patients with diabetes mellitus prior to the transplant were excluded. All patients were Polish Caucasians. From years 2000 to 2009, subjects were recruited consecutively from patients who underwent renal transplantation in the Clinical Department of Nephrology and Dialysis of Regional Hospital, Szczecin, Poland or in the Department of Nephrology, Transplantation and Internal Medicine of Pomeranian Medical University, Szczecin, Poland. All subjects had been subsequently medicated with tacrolimus as a part of an immunosuppressive regimen. Patients who did not maintain graft function for at least one year posttransplant were excluded. For the purpose of the study, the patients were subdivided into the following two groups: patients with PTDM (n = 66), and the controls, patients without PTDM (n = 168). The characteristics of the patients are given in Table 1. Patients with hemoglobin A1c (HgA1c) levels continuously over 6.5 mg/dl, fasting plasma glucose (FPG) levels over 126 mg/dl, or who required insulin and/or oral hypoglycemic agents for more than 3 months were diagnosed as having PTDM. PTDM was diagnosed in the one-year period after transplantation, although the observation time was expanded in the case of some late-onset PTDM patients to reach 3 months from diabetes onset. PTDM onset time in the studied group varied from 1 to 50 weeks (mean ± SD: 6.0 ± 8.5 weeks). Therefore, PTDM patients were divided into the following two groups: early-onset PTDM (n = 38), for whom diabetes occurred during the first two weeks from the beginning of immunosuppressive therapy, and late-onset PDTM (n = 28), for whom diabetes was found later in the course of treatment.

The treatment protocol consisted of tacrolimus, mycophenolate mofetil and steroids. Tacrolimus was initiated at 0.1 mg/kg per day with doses adjusted to keep trough levels between 10 and 12 ng/ml in the first posttransplant month and subsequently between 8 and 10 ng/ml. An initial oral dose of 2.0 g/day of mycophenolate mofetil was given to patients once a day or in equally divided doses every 12 h. Methylprednisolone was given concomitantly. A dose of 500 mg was given on the day of surgery, tapered to 40 mg/day during the first week, 30 mg/day of prednisolone in the second week, 20 mg/day of prednisolone in the third week, 15 mg/day in the fourth week, and 10 mg/day thereafter. Total corticosteroid doses for each patient during the first year of the study were calculated, and methylprednisolone was recalculated to prednisolone using r = 1.25 cofactor.

All patients gave informed consent for this study, and the relevant ethics committee approved the study protocol.

Genotyping

Genomic DNA was extracted from 200 μl of whole blood samples using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). Pre-validated allelic discrimination TaqMan real-time PCR assays (Assay IDs: C_29347861_10, C_291484_20, Applied Biosystems, USA) were used for detection of SNPs rs7903146 and rs12255372 in the *TCF7L2* gene. Fluorescence data were captured using an ABI PRISM 7500 FAST Real-Time PCR System (Applied Biosystems, USA) after 40 cycles of PCR.

Statistical analysis

Categorical variables (allele, genotype, haplotype frequencies, and acute rejection episodes) were compared by Fisher exact tests and χ^2 tests. The EH program (Jurg Ott, Rockefeller University, New York) was used to estimate haplotype frequencies and linkage disequilibria. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using the Newcombe-Wilson method without the continuity correction. A multivariate logistic regression model was used to test independent PTDM risk factors. Variables identified as potentially significant ($p < 0.1$) were included in the multivariate analysis. A p level of less than 0.05 was considered statistically significant. The data were tested for their fit to Hardy-Weinberg equilibrium by calculating the expected frequencies of genotypes and comparing them to the observed values using χ^2 tests. All calculations were performed using Statistica 8.0 software package (Statsoft, Poland).

Tab. 1. Characteristics of patients enrolled in the study

	PTDM n = 66	No PTDM n = 168	p
Recipient age (mean ± SD) [year]	47.7 ± 10.6	43.2 ± 13.0	0.014[a]
Sex	30 (45.5%) females	78 (46.4%) females	1.000[b]
BMI (mean ± SD)	25.8 ± 4.1	24.3 ± 3.7	0.006[a]
Donor age (mean ± SD)	47.8 ± 11.8	46.0 ± 12.3	0.321[a]
Viral infections	10 (15.2%)	27 (16.1%)	1.000[b]
Acute rejection	9 (13.6%)	10 (6.0%)	0.064[b]
Steroid total dose (mean ± SD) [g]	4.20 ± 1.72	3.76 ± 2.35	0.017[c]

[a] Student *t*-test, [b] Fisher exact test, [c] Mann-Whitney U-test. Viral infections were as follows: CMV (6 *vs.* 20 in PTDM and control group, respectively, p = 0.647), HCV (2 *vs.* 4, p = 0.676), and HBV (2 *vs.* 3, p = 0.623)

Results

Mean patient age, BMI and total steroid dose were higher in PTDM group compared to non-PTDM patients (controls). No differences between groups were noted in patients' sex, donor age, viral infection frequency or acute rejection episodes (Tab. 1).

The genotype frequency distributions for both analyzed SNPs did not show significant deviations from the Hardy-Weinberg equilibrium, either in all study subjects or in the groups classified by PTDM status. Allele and genotype frequencies for SNP – rs7903146 and SNP – rs12255372 were similar in all patients (with and without PTDM) who were observed for at least one year from the transplantation (Tab. 2). The *TCF7L2* haplotype analysis revealed no association with PTDM within the studied subjects. The results of the *TCF7L2* haplotype analysis are shown in Table 3.

Tab. 2. *TCF7L2* genotype and allele frequencies in patients with posttransplant diabetes mellitus (PTDM) compared to no-PTDM controls

	Early PTDM n = 38				Late PTDM n = 28				All PTDM n = 66				No-PTDM n = 168	
	n	(%)	OR 95% CI	p	n	(%)	OR 95% CI	p	n	(%)	OR 95% CI	p	n	(%)
rs7903146:C>T														
CC	21	(55.3)	1.05 (0.51–2.12)	0.902[a]	11	(39.3)	0.55 (0.24–1.24)	0.144[a]	32	(48.5)	0.79 (0.45–1.41)[a]	0.433[a]	91	(54.2)
CT	15	(39.5)	0.97 (0.47–2.02)	0.935	12	(42.8)	1.48 (0.62–3.56)	0.377	27	(40.9)	1.15 (0.63–2.09)	0.657	67	(39.9)
TT	2	(5.3)	0.87 (0.18–4.25)	0.860	5	(17.9)	4.14 (1.19–14.3)	0.017	7	(10.6)	1.99 (0.69–5.67)	0.191	10	(5.9)
Allele:														
C	57	(75.0)	–	-	34	(60.7)	–	-	91	(68.9)	–	–	249	(74.1)
T	19	(25.0)	–	0.872	22	(39.3)	–	0.038	41	(31.1)	–	0.259	87	(25.9)
rs12255372:G>T														
GG	21	(55.3)	0.95 (0.47–1.93)	0.885[a]	11	(39.3)	0.50 (0.22–1.12)	0.089[a]	32	(48.5)	0.72 (0.41–1.28)[a]	0.265[a]	95	(56.5)
GT	15	(39.5)	1.09 (0.52–2.28)	0.809	13	(46.4)	1.81 (0.76–4.29)	0.174	28	(42.4)	1.34 (0.73–2.44)	0.337	62	(36.9)
TT	2	(5.3)	0.82 (0.18–3.99)	0.808	4	(14.3)	3.14 (0.85–11.5)	0.073	6	(9.1)	1.62 (0.55–4.73)	0.375	11	(6.5)
Allele:														
G	57	(75.0)	–	–	35	(62.5)	–	–	92	(69.7)	–	–	252	(75.0)
T	19	(25.0)	–	1.000	21	(37.5)	–	0.050	40	(30.3)	–	0.242	84	(25.0)

Early PTDM – developed up to 14 days from transplantation, late PTDM – developed later than 14 days from transplantation; χ^2 test, calculated in relation to the "no PTDM" group, using homozygotes for a major allele or major allele count as reference; [a] homozygotes for a major allele *vs.* others

Tab. 3. *TCF7L2* haplotype and haplotype frequencies in patients with and without posttransplant diabetes mellitus (PTDM)

	Early PTDM n = 38			Late PTDM n = 28			All PTDM n = 66			No-PTDM n = 168	
	n	(%)	p	n	(%)	p	n	(%)	p	n	%
Haplotypes											
C-G	56	(73.7)	0.933	34	(60.7)	0.055	90	(68.2)	0.276[a]	246	(73.2)
C-T	1	(1.3)	0.741	0	(0.0)	0.378	1	(0.8)	0.936	3	(0.9)
T-G	0	(0.0)	0.117	2	(3.6)	0.728	2	(1.5)	0.910	6	(1.8)
T-T	19	(25.0)	0.919	20	(35.7)	0.058	39	(29.5)	0.237	81	(24.1)

Early PTDM – developed up to 14 days from transplantation, late PTDM – developed later than 14 days from transplantation; χ^2 test, calculated using major haplotype count as reference; [a] major haplotype *vs.* others; haplotypes constructed from rs7903146- rs12255372 genotyping results

Both studied *loci* (rs7903146 and rs12255372) were in strong linkage (D' = 0.948969) (calculated with 2LD program by Zhao JH). Major and minor alleles were found together in the majority of cases. They formed two common haplotypes (C-G and T-T) that stood for over 97% of all haplotypes in the studied population (calculated with EH program, Ott J).

Data analysis of PDTM diabetes developed later than the first two weeks after transplantation revealed a significant association with the SNP – rs7903146 T allele. Both the rs7903146 T allele and the TT genotype were significantly associated with increased risk of late-onset PTDM (OR for rs7903146 TT: 4.13). That association was not observed in early-onset PTDM (OR for rs7903146 TT *vs.* CC: 0.87). For the SNP rs12255372, a similar association was observed as it reached statistical significance only for the allele analysis (Tab. 2). Similarly, nearly significant differences were observed in haplotype frequencies between late-onset PTDM and no-PTDM subjects, with 60.7% *vs.* 73.2% for the C-G haplotype and 35.7% *vs.* 24.1% for the T-T haplotype (Tab. 3).

The following variables were identified as potentially significant: patient age, BMI, acute rejection episodes, total steroid dose and rs7903146 SNP minor allele carrier status. They were included in further multivariate analyses performed in subgroups of late-onset and early-onset PTDM *vs.* no-PTDM patients. The results of the logistic regression observed that only acute rejection episodes were an independent late-onset PTDM risk factor. A trend for such an association was also observed for BMI and *TCF7L2* rs7903146 T allele carrier status (i.e., CT or TT genotype) (Tab. 4). Total steroid dose has been pointed as an independent risk factor for early-onset PTDM, but the results were not significant (p = 0.059).

Tab. 4. Multivariate logistic regression analysis of potential risk factors for early-onset and late-onset PTDM

Independent variables	Early PTDM		Late PTDM	
	OR (95% CI)	p	OR (95% CI)	p
Patient's age	1.02 (0.98–1.04)	0.305	1.03 (0.99–1.07)	0.151
BMI [kg/m^2]	1.07 (0.97–1.18)	0.174	1.10 (0.99–1.23)	0.082
Acute rejection episodes	0.68 (0.11–3.10)	0.523	4.27 (1.27–14.31)	0.019
Steroid total dose[a]	6.33 (0.93–43.1)	0.059	0.85 (0.19–3.72)	0.836
TCF7L rs7903146 T allele carrier status (CT or TT genotypes)	1.10 (0.51–2.33)	0.818	1.88 (0.80–4.24)	0.089

Early PTDM – developed up to 14 days from transplantation, late PTDM – developed later than 14 days from transplantation; OR – odds ratio; CI – confidence interval; [a] logarithmic transformation applied to fit normal distribution

Discussion

Kidney transplant recipients are particularly at risk for developing PTDM as a consequence of immunosuppression. The reported incidence rates of new-onset diabetes mellitus has been extremely variable. A recent meta-analysis reported that the incidence rate in the first year after transplantation varied from 2% to 50% [15]. This variable incidence of PTDM is likely related to differences in population demographics or differences in diagnostic criteria across the studies. In the present study, the incidence of PDTM observed among patients of Polish ethnicity fell within the range of the above cited meta-analysis.

In the current study, we observed an association between several parameters and PTDM; acute rejection episode frequency, mean patient's age, BMI, and total steroid dose were higher in a group of all PTDM patients compared to non-PTDM patients. Univariate analyses confirmed that these differences were significant. These parameters, as well as hepatitis C virus infection and a family history of diabetes, are known risk factors for PTDM in the renal transplant population [27]. We did not observe any significant differences in the frequency of viral infections between the PDTM patients and the control patients. The type of immunosuppression medication had the strongest impact on the incidence of PTDM. Both cyclosporine A and tacrolimus have been associated with increased PTDM risk. Most of the studies have reported a greater risk for tacrolimus than cyclosporin A [23, 24].

The present study revealed a significant association between late-onset PTDM and the *TCF7L2* rs7903146 gene variant. Carriers of the *TCF7L2* rs7903146 TT genotype were significantly predisposed to late-onset PTDM. The rs7903146 T allele was significantly associated with late-onset PTDM. For the *TCF7L2* rs12255372 polymorphism, a strong, although not statistically significant, trend to the development of PDTM within the late posttransplant days was noted. In the analysis of the whole PTDM group (not subdivided into early- and late-onset PTDM) observed for one year, haplotype analyses failed to reveal any significant associations between PTDM and *TCF7L2* rs7903146 and rs12255372 genotypes and haplotypes. Our results were in keeping with other observations pointing out an association between *TCF7L2* polymorphism and PTDM. To date, one study of Korean renal transplant patients has shown a significant association of PTDM with the *TCF7L2* rs7903146 variant [10]. This study included very-late-onset PTDM cases (up to 10 years of observation after transplantation) and was performed with patients of a Southeast Asian genetic background. Chakkera and colleagues analyzed a genetic polymorphism previously associated with T2DM in a cohort of 91 kidney transplant patients. They found no association between *TCF7L2* variants and PTDM, but their sample size was too small to detect any minor effects [1]. A study by Ghisdal and colleagues [7] involved patients of predominantly Caucasian origin who developed PTDM within 6 months of transplantation [10]. The results of the study demonstrated that PTDM was associated with the *TCF7L2* rs7903146 T allele, which was an independent risk factor in the multivariate analysis (along with age, BMI, tacrolimus use and occurrence of a corticoid-treated acute rejection episode). These findings, along with the results of the present study, have indicated that the *TCF7L2* rs7903146 polymorphism is a potential marker of PTDM. It should be stated that studies by Kang and colleagues [10] and by Ghisdal and colleagues [7] involved patients who had been treated with cyclosporin, tacrolimus and sirolimus. Patients medicated with tacrolimus were not analyzed separately. However, in the study by Ghisdal and colleagues [7], tacrolimus was pointed out as an independent risk factor for PTDM.

Both the frequency of acute rejection episodes and the total steroid dose were increased in PTDM patients, compared with no-PTDM control patients. These two factors have been known to be associated with PTDM and have usually been linked because high methylprednisolone doses (pulse therapy) are injected in cases of graft rejection. In our study, the mean methylprednisolone dose received in case of acute rejection episodes was 1.9 g, which was approximately half of the mean total steroid dose received during first 12 months of therapy. In the current study, the multivariate analysis demonstrated that acute rejection episodes were an independent late-onset PTDM risk factor and that steroid dose was the only risk factor of early-onset PTDM identified with $p < 0.1$. The 28.2% of all study participants developed PTDM (16.2% – early-onset PTDM and 12% – late-onset PTDM), whereas from patients who experienced an acute rejection episode PTDM was observed in 47.5% (15.8% – early-onset PTDM and 31.6% – late-onset PTDM). However, some patients received

monoclonal antibodies instead of steroids in case of acute rejection episode, and this alternate medication could partly explain the observed differences.

On the other hand, the role of steroid withdrawal or avoidance in PTDM has not been well defined [13, 28]. The restriction of the study to patients with diabetes developed up to 1 year from transplantation avoided the inclusion of patients with true diabetes type 2. Development of true diabetes type 2 could occur later in the course of transplantation, as might have been the case in the Korean study [10]. Some studies have also postulated that higher levels of tacrolimus were associated with an increased risk of PTDM [20]. However, in the present study, tacrolimus blood concentrations were similar in both evaluated groups due to therapeutic monitoring of blood drug levels. The drug regimens administered in PTDM and no-PTDM subjects were different regarding steroid dose. As the steroid doses in therapeutic regimens applied in Poland are at highest level in early posttransplant periods (mean daily dose in studied patients in first week was 36.8 mg, second week was 22.5 mg, and after 12 months was 8.4 mg), these doses could rather predispose patients to develop early PTDM. Our study confirmed that total steroid dose was significantly associated with PDTM. However, steroid dose was not an independent factor of late-onset PDTM. With late-onset PTDM, genetic factors, including the studied *TCF7L2* polymorphism, may play more important role and could slightly increase PTDM risk.

Finally, one of the limitations of our study was its sample size, which allowed the detection of differences in genotype frequencies between PTDM and no-PTDM groups, with > 80% power, only if they exceed 21% and differences in allele frequencies over 13%. Any minor effects could have been missed. In particular, the results of analyses performed in the groups of early and late PTDM should be treated as preliminary because the number of subjects was even lower in the separate groups.

If our observations, along with findings from Asian and other European populations, are confirmed, replacing tacrolimus with other immunosuppressants could be warranted in patients at high risk of PTDM. Risk could be diagnosed by *TCF7L2* rs7903146 genotyping. Finally, the application of *TCF7L2* rs7903146 SNPs as a marker of PTDM should be verified by further independent studies.

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