Original Article

KCNJ11 and *KCNQ1* Gene Polymorphisms Are Not Associated with Post-Transplant Diabetes Mellitus in Kidney Allograft Recipients Treated with Tacrolimus

(diabetes / tacrolimus / transplantation)

E. DABROWSKA-ZAMOJCIN[1], M. TARNOWSKI[2], M. SZYDŁOWSKI[2], M. ROMANOWSKI[3], V. DZIEDZIEJKO[4], K. SAFRANOW[4], L. DOMANSKI[5], A. PAWLIK[2]

[1]Department of Pharmacology, [2]Department of Physiology, [3]Department of General and Transplantation Surgery, [4]Department of Biochemistry and Medical Chemistry, [5]Department of Nephrology and Transplantology, Pomeranian Medical University, Szczecin, Poland

Abstract. Post-transplant diabetes mellitus (PTDM) is a metabolic disorder occurring after solid organ transplantation during the therapy with calcineurin inhibitors. ATP-sensitive potassium channels KCNJ11 and KCNQ1 play an important role in the regulation of insulin secretion by β cells and development of diabetes mellitus. Numerous studies have confirmed the association between *KCNJ11* and *KCNQ1* gene polymorphisms and type 2 diabetes. The aim of this study was to examine the association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes mellitus in kidney allograft recipients treated with tacrolimus. The study included 201 patients who received kidney transplants. The patients were subdivided into two subgroups: patients with PTDM (N = 35) and patients without PTDM (N = 166). The association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes was studied in three models of univariate Cox regression analysis, i.e., additive, dominant and recessive. In these three models there were no statistically significant associations between *KCNJ11* and *KCNQ1* gene polymorphisms and PTDM. The results of this study suggest lack of association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes mellitus in kidney allograft recipients treated with tacrolimus in the Polish population.

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Corresponding author: Andrzej Pawlik, Department of Physiology, Pomeranian Medical University, Powst. Wlkp. 72, 70-111 Szczecin, Poland. E-mail: pawand@poczta.onet.pl

Abbreviations: HWE – Hardy-Weinberg equilibrium, PTDM – post-transplant diabetes mellitus.

Introduction

Post-transplant diabetes mellitus (PTDM) is a metabolic complication occurring after solid organ transplantation. This disorder is associated with the treatment with calcineurin inhibitors (Bee et al., 2011). Previous studies suggest that many of the genes associated with type 2 diabetes mellitus have also been associated with an increased risk of PTDM (McCaughan et al., 2014). The *KCNJ11* gene is a member of the potassium channel gene family encoding an inward-rectifier potassium ion channel (Kir6.2) (Gloyn et al., 2004). This channel modulates insulin production and secretion, as well as glucose metabolism, and is involved in a wide range of physiological responses (Aguilar-Bryan et al., 1998).

KCNQ1 encodes the pore-forming subunit of a voltage-gated K+ channel (KvLQT1) and is involved in triggering and maintaining glucose-stimulated insulin secretion (Jespersen et al., 2005). Previous studies suggest that genetic variants in the *KCNJ11* and *KCNQ1* genes play an important role in the predisposition to diabetes mellitus. Several studies have already indicated that there are the associations between the common variants in *KCNJ11* and *KCNQ1* genes and higher fasting plasma glucose levels, lower insulin secretion, impaired pancreatic β-cell function and development of diabetes mellitus (Liu et al., 2009; Benrahma et al., 2014). The aim of this study was to examine the association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes mellitus in kidney allograft recipients treated with tacrolimus.

Material and Methods

Patients

The study included 201 patients who received kidney transplants at the Pomeranian Medical University in

Szczecin, Poland. The patients were divided into two subgroups: patients with PTDM (N = 35) and patients without PTDM (N = 166). Patients who had a diagnosis of diabetes mellitus prior to transplantation (either as the cause of kidney disease or co-morbidity) and patients with graft failure or death within one month post-transplantation were excluded. The diagnosis of diabetes before transplantation was based on the oral glucose tolerance test. The diagnosis of diabetes was made when one of the following plasma glucose parameters was exceeded: fasting plasma glucose 5.5 mmol/l and 2 h plasma glucose 7.8 mmol/l.

Patients with haemoglobin A1c continuously over 6.5 %, fasting blood glucose ≥ 7.0 mmol/l, or requiring treatment with oral hypoglycaemic agents or insulin for more than three months after transplantation were diagnosed as having PTDM (Davidson et al., 2003). The blood glucose levels were examined once a week. The observation period was 36 months. The mean time of follow-up was 27.2 months for the whole group and 32.4 months for the patients without PTDM. In patients with PTDM, the mean time of its diagnosis was 8.6 months since transplantation (median 1.6 months). Standard immunosuppression consisted of tacrolimus, mycophenolate mofetil and steroids. Tacrolimus was initiated at 0.1 mg/kg, with doses adjusted to maintain serum levels between 10 and 12 ng/ml in the first month after transplantation and then between 8 and 10 ng/ml. Mycophenolate mofetil was given in doses of 2 g per day, while prednisolone was given in doses of 10–20 mg per day (Romanowski et al., 2015). The study was approved by the ethics committee of the Pomeranian Medical University, Szczecin, Poland, and written informed consent was obtained from all subjects.

Methods

All samples were genotyped using allelic discrimination assays with TaqMan® probes (Applied Biosystems, Carlsbad, CA) in a 7500 Fast Real-Time PCR Detection System (Applied Biosystems). To discriminate the *KCNJ11* rs5219, *KCNQ1* rs151290 and rs2237892 alleles, TaqMan® Pre-Designed SNP Genotyping Assays were used (assay IDs: C__11654065_10; C__3075727_1; C__16171025_10), including appropriate primers and fluorescently labelled (FAM and VIC) MGB™ probes to detect the alleles. Genotypes were assigned using all of the data from the study simultaneously.

Statistical analysis

The consistency of the genotype distribution with Hardy-Weinberg equilibrium (HWE) was assessed using the exact test. Univariate and multivariate Cox proportional hazards models were used to assess the hazard of PTDM in the three years of follow-up after renal transplantation. χ^2 test or Fisher's exact test was used for the comparison of genotype and allele frequencies between the groups. $P < 0.05$ was considered statistically significant. The study sample size was sufficient to detect, with 80 % probability, the true effect size for association of the analysed alleles with the risk of PTDM measured as odds ratio (OR) equal to 0.44 or 2.09 for rs5219, 0.27 or 2.30 for rs151290, and 3.42 for rs2237892.

Results

The distributions of the studied *KCNJ11* and *KCNQ1* genotypes were in HWE ($P > 0.05$). There were no statistically significant differences in the distribution of *KCNJ11* and *KCNQ1* genotypes between patients with and without PTDM (Table 1).

The association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes was examined in three models of univariate Cox regression analysis, i.e., recessive (variant homozygotes *vs*. heterozygotes + wild-type homozygotes), dominant (variant homozygotes + heterozygotes *vs.* wild-type homozygotes) and additive (number of alleles). In these three models, there were no statistically significant associations between *KCNJ11* and *KCNQ1* gene polymorphisms and PTDM (Table 2).

We also searched for potential confounding factors associated with PTDM and found that recipients' female sex, older age, higher BMI and presence of acute rejection are significant independent predictors of PTDM (multivariate Cox model, $P < 0.01$ for BMI and $P < 0.05$ for the other variables). Therefore, we performed multivariate Cox analyses adjusted for these four parameters and found that none of the three polymorphisms showed association with PTDM after this adjustment (Table 2).

Discussion

In this study, we examined whether polymorphisms in *KCNJ11* and *KCNQ1* genes are associated with increased risk of PTDM development. Our results did not reveal any associations between *KCNJ11* and *KCNQ1* gene polymorphisms and PTDM.

Previous studies have shown that these polymorphisms may be associated with increased risk of type 2 diabetes. The minor allele frequency (MAF) of *KCNJ11* rs5219, *KCNQ1* rs151290 and *KCNQ1* rs2237892 in European population is 0.1–0.3%, 2–5% and 14–25%, respectively (Müssig et al., 2009; van Vliet-Ostaptchouk et al., 2012; Phani et al., 2014).

So far, the associations between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes mellitus have not been widely investigated. Tavira et al. (2011) examined the contribution of rs5219 *KCNJ11* gene polymorphism to PTDM after transplantation among patients treated with tacrolimus. The AA and AG genotypes were significantly associated with increased risk of PTDM. In the study by Parvizi et al. (2014), the KCNJ11 KK variant was associated with an increased risk for PTDM. This study showed that polymorphisms in *KCNJ11* might predispose the patients treated with tacrolimus to development of PTDM after liver transplantation. In the study by Kurzawski et al. (2012), there was no significant association between PTDM and

Table 1. The frequency of KCNJ11 and KCNQ1 genotypes in renal allograft recipients treated with tacrolimus with and without PTDM

	No PTDM		PTDM		P value^	Compared genotypes or alleles	P value^	OR (95 % CI)
	N	%	N	%				
***KCNJ11* rs5219**								
CC	63	79.75 %	16	20.25 %	0.53	TT + CT vs CC	0.39	0.73 (0.35–1.52)
CT	74	86.05 %	12	13.95 %		TT vs CT + CC	0.72	1.18 (0.47–2.96)
TT	29	80.56 %	7	19.44 %		TT vs CC	0.92	0.95 (0.35–2.56)
						CT vs CC	0.28	0.64 (0.28–1.45)
						TT vs CT	0.45	1.49 (0.53–4.15)
Allele								
C	200	60.24 %	44	62.86 %		T vs C	0.68	0.90 (0.53–1.52)
T	132	39.76 %	26	37.14 %				
***KCNQ1* rs151290**								
CC	111	83.46 %	22	16.54 %	0.67*	AA + AC vs CC	0.65	1.19 (0.56–2.54)
AC	51	79.69 %	13	20.31 %		AA vs AC + CC	1.00*	-
AA	4	100.00 %	0	0.00 %		AA vs CC	1.00*	-
						AC vs CC	0.52	1.29 (0.60–2.75)
						AA vs AC	1.00*	-
Allele								
C	273	82.23 %	57	81.43 %		A vs C	0.87	1.06 (0.54–2.05)
A	59	17.77 %	13	18.57%				
***KCNQ1* rs2237892**								
CC	149	82.78 %	31	17.22 %	0.22*	TT + CT vs CC	0.83	1.13 (0.36–3.59)
CT	17	85.00 %	3	15.00 %		TT vs CT + CC	0.17*	-
TT	0	0.00 %	1	100.00 %		TT vs CC	0.18*	-
						CT vs CC	0.80	0.85 (0.23–3.07)
						TT vs CT	0.19*	-
Allele								
C	315	94.88 %	65	92.86 %		T vs C	0.50	1.43 (0.51–4.00)
T	17	5.12%	5	7.14 %				

^ – χ^2 test
* – Fisher's exact test

Table 2. Univariate and multivariate Cox model analysis of PTDM hazard during 3-year follow-up in relation to KCNJ11 and KCNQ1 genotypes

	Model	Univariate		Multivariate *	
		HR (95% CI)	P value	HR (95% CI)	P value
***KCNJ11* rs5219**	Additive model (number of T alleles)	0.88 (0.56–1.39)	0.59	0.78 (0.50–1.22)	0.27
	Dominant model (TT+CT vs CC)	0.73 (0.38–1.40)	0.34	0.69 (0.36–1.35)	0.28
	Recessive model (TT vs CT+CC)	1.08 (0.48–2.46)	0.85	0.72 (0.30–1.72)	0.45
***KCNQ1* rs151290**	Additive model (number of A alleles)	1.08 (0.59–1.97)	0.81	1.20 (0.64–2.25)	0.58
	Dominant model (AA+AC vs CC)	1.20 (0.62–2.34)	0.59	1.33 (0.67–2.64)	0.42
	Recessive model (AA vs AC+CC)	-	-	-	-
***KCNQ1* rs2237892**	Additive model (number of T alleles)	1.61 (0.70–3.68)	0.26	1.45 (0.64–3.29)	0.37
	Dominant model (TT+CT vs CC)	1.40 (0.55–3.60)	0.48	1.35 (0.52–3.52)	0.54
	Recessive model (TT vs CT+CC)	-	-	-	-

* adjusted for recipients' age, sex, BMI and presence of acute rejection episodes
HR – hazard ratio calculated using the Cox proportional hazards model

rs5215 in the *KCNJ11* gene in kidney allograft recipients treated with tacrolimus. Tavira et al. (2012) genotyped three common *KCNQ1* SNPs in Spanish patients who received a cadaveric kidney graft and developed PTDM. In addition, the authors searched for DNA variants in the whole *KCNQ1* coding exons in these patients. SNP rs2237895 (genotype CC) was associated with an increased risk for PTDM after transplantation in the studied population, independently of other risk factors such as body mass index, recipient age, or tacrolimus dosage. Other *KCNQ1* variants were not associated with PTDM (Kang et al., 2009). Kang et al. (2009) investigated the association between the PTDM development and *KCNQ1* rs2237892 polymorphism. This polymorphism was significantly associated with PTDM in a cohort of renal allograft recipients in Korea.

ATP-sensitive potassium channels KCNJ11 and KCNQ1 play an important role in the regulation of insulin secretion by β cells and development of diabetes mellitus. Previous studies indicate that polymorphisms in *KCNJ11* and *KCNQ1* genes are associated with type 2 diabetes mellitus. Some studies suggest that these polymorphisms also are associated with PTDM. Probably, this association may depend on ethnic and genetic differences in the studied population. The results of this study suggest lack of association between *KCNJ11* and *KCNQ1* gene polymorphisms and post-transplant diabetes mellitus in kidney allograft recipients treated with tacrolimus in the Polish population. Nevertheless, this finding requires further investigations.

References

Aguilar-Bryan, L., Clement, J., Gonzalez, G., Kunjilwar, K., Babenko, A., Bryan, J. (1998) Toward understanding the assembly and structure of KATP channels. *Physiol. Rev.* **78**, 227-245.

Bee, Y. M., Tan, H. C., Tay, T. L., Kee, T. Y., Goh, S. Y., Kek, P. C. (2011) Incidence and risk factors for development of new-onset diabetes after kidney transplantation. *Ann. Acad. Med. Singap.* **40**, 160-167.

Benrahma, H., Charoute, H., Lasram, K., Boulouiz, R., Atig, R. K., Fakiri, M., Rouba, H., Abdelhak, S., Barakat, A. (2014) Association analysis of IGF2BP2, KCNJ11, and CDKAL1 polymorphisms with type 2 diabetes mellitus in a Moroccan population: a case-control study and meta-analysis. *Biochem. Genet.* **52**, 430-442.

Davidson, J., Wilkinson, A., Dantal, J., Dotta, F., Haller, H., Hernández, D., Kasiske, B. L., Kiberd. B., Krentz, A., Legendre, C., Marchetti, P., Markell, M., van der Woude, F. J., Wheeler, D. C., International Expert Panel. (2003) New-onset diabetes after transplantation: 2003 International consensus guidelines. Proceedings of an international expert panel meeting. Barcelona, Spain, 19 February 2003. *Transplantation* **75**, SS3-24.

Gloyn, A. L., Pearson, E. R., Antcliff, J. F., Proks, P., Bruining, G. J., Slingerland, A. S. (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N. Engl. J. Med.* **350**, 1838-1849.

Jespersen, T., Grunnet, M., Olesen, S. P. (2005) The KCNQ1 potassium channel: from gene to physiological function. *Physiology (Bethesda)* **20**, 408-416.

Kang, E. S., Kim, M. S., Kim, C. H., Nam, C. M., Han, S. J., Hur, K. Y., Ahn, C. W., Cha, B. S., Kim, S. I., Lee, H. C., Kim, Y. S. (2009) Association of common type 2 diabetes risk gene variants and posttransplantation diabetes mellitus in renal allograft recipients in Korea. *Transplantation* **88**, 693-698.

Kurzawski, M., Dziewanowski, K., Łapczuk, J., Wajda, A., Droździk, M. (2012) Analysis of common type 2 diabetes mellitus genetic risk factors in new-onset diabetes after transplantation in kidney transplant patients medicated with tacrolimus. *Eur. J. Clin. Pharmacol*. **68**, 1587-1594.

Liu, Y., Zhou, D. Z., Zhang, D., Chen, Z., Zhao, T., Zhang, Z., Ning, M., Hu, X., Yang, Y. F., Zhang, Z. F., Yu, L., He, L., Xu, H. (2009) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes in the population of mainland China. *Diabetologia* **52**, 1315-1321.

McCaughan, J. A., McKnight, A. J., Maxwell, A. P. (2014) Genetics of new-onset diabetes after transplantation. *J. Am. Soc. Nephrol.* **25**, 1037-1049.

Müssig, K., Staiger, H., Machicao, F., Kirchhoff, K., Guthoff, M., Schäfer, S. A., Kantartzis, K., Silbernagel, G., Stefan, N., Holst, J. J., Gallwitz, B., Häring, H. U., Fritsche, A. (2009) Association of type 2 diabetes candidate polymorphisms in KCNQ1 with incretin and insulin secretion. *Diabetes* **58**, 1715-1720.

Parvizi, Z., Azarpira, N., Kohan, L., Darai, M., Kazemi, K., Parvizi, M. M. (2014) Association between E23K variant in KCNJ11 gene and new-onset diabetes after liver transplantation. *Mol. Biol. Rep.* **41**, 6063-6069.

Phani, N. M., Guddattu, V., Bellampalli, R., Seenappa, V., Adhikari, P., Nagri, S. K., D'Souza, S. C., Mundyat, G. P., Satyamoorthy, K., Rai, P. S. (2014) Population specific impact of genetic variants in KCNJ11 gene to type 2 diabetes: a case-control and meta-analysis study. *PLoS One* **9**, e107021.

Romanowski, M., Dziedziejko, V., Maciejewska-Karlowska, A., Sawczuk, M., Safranow, K., Domanski, L., Pawlik, A. (2015) Adiponectin and leptin gene polymorphisms in patients with post-transplant diabetes mellitus. *Pharmacogenomics* **16**, 1243-1251.

Tavira, B., Coto, E., Díaz-Corte, C., Ortega, F., Arias, M., Torres, A., Díaz, J. M., Selgas, R., López-Larrea, C., Campistol, J. M., Ruiz-Ortega, M., Alvarez, V., Pharmacogenetics of Tacrolimus REDINREN Study Group. (2011) KCNQ1 gene variants and risk of new-onset diabetes in tacrolimus-treated renal-transplanted patients. *Clin. Transplant*. **25**, E284-291.

Tavira, B., Coto, E., Torres, A., Díaz-Corte, C., Díaz-Molina, B., Ortega, F., Arias, M., Díaz, J. M., Selgas, R., López-Larrea, C., Ruiz-Ortega, M., Ortiz, A., González, E., Campistol, J. M., Alvarez, V., Pharmacogenetics of tacrolimus REDINREN Study group. (2012) Association between a common KCNJ11 polymorphism (rs5219) and new-onset posttransplant diabetes in patients treated with Tacrolimus. *Mol. Genet. Metab*. **105**, 525-527.

van Vliet-Ostaptchouk, J. V., van Haeften, T. W., Landman, G. W., Reiling, E., Kleefstra, N., Bilo, H. J., Klungel, O. H.,

de Boer, A., van Diemen, C. C., Wijmenga, C., Boezen, H. M., Dekker, J. M., van 't Riet, E., Nijpels, G., Welschen, L. M., Zavrelova, H., Bruin, E. J., Elbers, C. C., Bauer, F., Onland-Moret, N. C., van der Schouw, Y. T., Grobbee, D. E., Spijkerman, A. M., van der A, D. L., Simonis-Bik, A. M., Eekhoff, E. M., Diamant, M., Kramer, M. H., Boomsma, D. I., de Geus, E. J., Willemsen, G., Slagboom, P. E., Hofker, M. H., 't Hart, L. M. (2012) Common variants in the type 2 diabetes KCNQ1 gene are associated with impairments in insulin secretion during hyperglycaemic glucose clamp. *PLoS One* **7**, e32148.