Association between a common *KCNJ11* polymorphism (rs5219) and new-onset posttransplant diabetes in patients treated with Tacrolimus.

Beatriz Tavira[1], Eliecer Coto[1,11], Armando Torres[2,10], Carmen Díaz-Corte[3], Beatriz Díaz-Molina[4], Francisco Ortega[3,10], Manuel Arias[5,10], Juan M. Díaz[6], Rafael Selgas[7,10], Carlos López-Larrea[8,10], Marta Ruiz-Ortega[9], Alberto Ortiz,[9] Elena González[7], J.M. Campistol[10], Victoria Alvarez[1], and the Pharmacogenetics of tacrolimus REDINREN study group*.

1 Genética Molecular, Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.
2 Nefrología y Unidad de Investigación, Hospital Universitario de Canarias-Universidad de La Laguna, Tenerife. Spain.
3 Nefrología, Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.
4 Cardiología, Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.
5 Nefrología, Hospital Universitario Valdecilla, Santander, Spain.
6 Nefrología, Fundación Puigvert, Barcelona, Spain
7 Nefrología, Hospital Universitario La Paz. Madrid, Spain.
8 Inmunología, Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.
9 Cellular Biology in Renal Diseases Laboratory, Fundación Jiménez Díaz and Universidad Autónoma de Madrid, Spain.
10 Nefrología, Hospital Clinic, Barcelona, Spain.
11 Fundación Renal I. Alvarez de Toledo, Madrid, Spain

*The REDINREN pharmacogenetics of tacrolimus study group: B. Alonso, M. Díaz, A.I. Corao, E. Gomez, J.L. Rodríguez-Lambert, C. Morís, M.J. Bernardo, E.Rodrigo, I. Silva, J.M. Baltar, I. Rodríguez-Pastore, T. Ortega, M. López-Hoyos, E. Pérez-Carreño, E. Porrini, C. Jiménez , V. De la Fuente, L. Simoes dos Santos.

Correspondence:

Eliecer Coto García
Genética Molecular
Hospital Universitario Central de Asturias
33006 – Oviedo – Spain
Tel/Fax (34) 985107968
Email: eliecer.coto@sespa.princast.es

Abstract.

KCNJ11 polymorphisms have been linked to the risk of developing type 2 diabetes. Our aim was to define the contribution of *KCNJ11* to new-onset diabetes after transplantation (NODAT) among patients treated with Tacrolimus (Tac). A total of 115 NODAT and 205 non-NODAT were genotyped for rs5219 (p.E23K). AA+AG genotypes were significantly associated with NODAT-risk (p=0.004; OR=2.10). The reported effect of this *KCNJ11* polymorphism on insulin release by beta cells could explain this association.

Keywords: Postransplant diabetes; *KCNJ11*; genetic association

Introduction.

New-onset diabetes after transplantation (NODAT) is a frequent complication in patients treated with the immunosuppressive calcineurin inhibitor tacrolimus (tac; FK506) (1, 2). Patients who receive solid organs require the continuous use of this drug to avoid organ rejection and this, in addition to the increased use of organ transplantation, raised the concerns about NODAT as an important health issue. The search for biological markers to predict the risk of developing NODAT is important to define individualized therapies. Recent studies have identified single nucleotide polymorphisms (SNPs) associated with NODAT-risk among Tac-treated patients (3,4). Among candidate SNPs are those previously linked to the risk of developing type 2 diabetes (DM2) in the general population (5-9).

Recent association studies found a significant association between a *KCNJ11* (Kir6.2) SNP (rs5219; E23K) and DM2 (9,10). Moreover, activating mutations in *KCNJ11* would account for approximately 40% of permanent neonatal diabetes cases (11). *KCNJ11* encodes a component of the ATP-sensitive potassium (KATP) channel, that in pancreatic beta cells couples glucose metabolism to membrane electrical activity and insulin release (12,13). In vitro studies showed that the E23K amino acid change affected the sensitivity toward ATP and insulin release by beta cells (14).

Based on the reported association of *KCNJ11* SNPs and DM2 we hypothesized that this gene could be also implicated in the risk of developing NODAT among Tac-treated patients. To test this hypothesis, we compared the allele/genotype frequencies for several *KCNJ11* SNPs between NODAT and no-NODAT patients.

Methods.

Patients. This study was approved by the ethical committee of Hospital Universitario Central Asturias (HUCA), and all the patients signed an informed consent to participate. The study included 320 Caucasian Spanish patients who received a cadaveric kidney (n=245) or heart (n=75) in the period 2006-2010 and were non diabetics at the time of

transplantation. DM2 was defined following the WHO guidelines: a fasting plasma glucose (FPG) >125 mg/dL (7.0 mmol/L) after three consecutive measurements. Oral glucose tolerance test (OGTT) was not performed to all the patients, and the definition of DM2 was thus based on the FPG values.

All the patients received a standard triple immunosuppressive therapy with Tac (Prograf TM), mycophenolate mofetil, and prednisone (15, 16) (see also the **supplementary methods**). The NODAT group consisted on 115 patients who developed DM2 in the first year post-transplant, while the non-NODAT group was composed by 205 patients who remained non-diabetics (**table 1**).

***KCNJ11* genotyping and sequencing.** DNA was obtained from blood leukocytes and SNP rs5219 was genotyped through a PCR-RFLP method (**supplementary table 1).** The *KCNJ11* coding sequence from 50 patients (25 NODAT and 25.no-NODAT) was amplified and sequenced (**supplementary table** 2). Each sequence was compared with the reference *KCNJ11* (ENSG00000187486; www.ensembl.org). All the nucleotide changes were designated according to their SNP number in the Ensembl database.

Statistical analysis. Continuous and categorical variables were compared (NODAT vs. non-NODAT) with the Student´s t and Pearson´s χ^2 tests, respectively. We compared the data at one week, six months and one year posttransplant. A univariate analysis was firstly done to determine which variables were associated with NODAT. A multivariate logistic regression was further used to define whether these variables were independent risk factors for NODAT. A $p<0.05$ was considered as statistically significant. The statistical power was calculated online (http://statpages.org/proppowr.htm).

Results.

NODAT was significantly associated with patient´s age, BMI, and the rs5219 SNP (**table 1**). Rs5219 A carriers (AA+AG genotypes) were significantly more frequent in the NODAT group ($p=0.004$; OR=2.10, 95%CI=1.27-3.48). The power of the study (at a $p=0.05$) was >85. After adjusting by age and BMI in the multivariate analysis, this

SNP remained significantly associated with NODAT (p=0.030; OR=4.12; 95%CI=1.16-13.40) (**supplementary table**).

To identify other *KCNJ11* variants in linkage disequilibrium with rs5219 that could explain this association, we sequenced the coding exons and flanking intronic nucleotides from 25 NODAT and 25 non-NODAT patients (10 AA, 5 AG, 10 GG in each group) (**supplementary figure 2**). We found a total of 6 SNPs, all reported in the SNP database (www.ensembl.org; **supplementary figure 3**).

Discussion.

We found a significant association between SNP rs5219 and NODAT. Yang et al. also found an increased frequency of the risk A allele among NODAT (n=133) compared to non-NODAT (n=170) Hispanic kidney transplant recipients (17). This polymorphism was previously linked to the risk of developing type DM2 (9, 10). *KCNJ11* encodes the kir6.2 subunit of the Potassium inwardly rectifying channel, and the activity of this K+ channel in pancreatic Beta-cells is necessary for insulin secretion (18). The isoform containing lysine 23 was linked to increased channel opening through nucleoside and decreased sensitivity towards the inhibitory effect of ATP compared to the glutamine 23 (14, 19). This suggested that E23K could predispose to DM2 by changing the channel's response to the variation of cytosolic nucleotides. In 23 K patients treated with Tac this could lead to induced insulin release and subsequent suppression of insulin transcription and release. It is however possible that this SNP was a surrogate marker for NODAT-risk, with other *KCNJ11* variants in LD with this SNP being the responsible for the observed association. To answer this question we sequenced the *KCNJ1*1 coding region from several patients. We found six previously reported variants, three of them missense amino acid changes (rs5215, rs1800467, and rs41282930). In agreement with a lack of functional effect, rs5215 (I337K) was not associated with NODAT (14, 19).

Finally, although our work supported a direct role of rs5219 on NODAT risk among Tac treated patients, we cannot exclude that LD with a variant in a nearby gene could be the responsible for the observed association (20). Our study also has two main limitations. First, it was based on a limited number of patients (although the sample size

was enough to reach a power >80). Second, OGTT was not performed to all the patients and we could thus not exclude that some non DM2 patients could be classified as diabetics based on this test.

In conclusion, we found a significant association between a common *KCNJ11* SNP and the risk of developing NODAT among heart and kidney transplanted patients treated with tacrolimus. This polymorphism could be useful to identify patients with an increased risk for NODAT.

Financial disclosure.

All the authors declare they have no conflicts of interest related with this work.

Author Contributions.

All the authors participated in the study design and contributed to the study by recruiting the patients (AT, CDC, BDM, FO, MA, JMD, RS, AO, EG, JMC), obtaining the patient´s data, or performing the laboratory work (BT, EC, VA, CLL, MRO). E.C. had full access to the data and take the responsibility for the accuracy of the data analysis.

Acknowledegements.

This work has been supported by the Instituto de Salud Carlos III-REDinREN (RETICS 0016), grant FIS-ETS-08/90008, the FEDER Funding Program from the European Union, and Sociedad Española de Cardiología (Beca Investigación Clínica).

References

1. A. Balla and M. Chobanian, New-onset diabetes after transplantation: a review of recent literature, Curr. Opin. Organ Transplant. 14 (2009) 375-379

2. E. Rodrigo, C. Piñera, M.A. de Cos, B. Sánchez, J.C. Ruiz, G. Fernández-Fresnedo, R. Palomar, J. González-Cotorruelo, C. Gómez-Alamillo, S.S. de Castro, A.L. de Francisco and M. Arias, Evolution of tacrolimus blood levels and concentration-dose ratios in patients who develop new onset diabetes mellitus after kidney transplantation, Transpl. Int. 18 (2005) 1152-1157.

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

4. L. Ghisdal, Baron C, Le Meur Y, Lionet A, Halimi JM, Rerolle JP, Glowacki F, Lebranchu Y, Drouet M, Noel C, El Housni H, Cochaux P, Wissing KM, Abramowicz D and Abramowicz M, TCF7L2 polymorphism associates with new-onset diabetes after transplantation, J. Am. Soc. Nephrol. 20 (2009) 2459-2467.

5. L.J. Scott, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS and Boehnke M, A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants, Science 316 (2007) 1341-1345

6. R. Sladek, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C and Froguel P, A genome-wide association study identifies novel risk loci for type 2 diabetes, Nature 445 (2007) 881-885

7. H. Unoki, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, Ng DP, Holmkvist J, Borch-Johnsen K, Jorgensen T, Sandbaek A, Lauritzen T, Hansen T, Nurbaya S, Tsunoda T, Kubo M, Babazono T, Hirose H, Hayashi M, Iwamoto Y,

Kashiwagi A, Kaku K, Kawamori R, Tai ES, Pedersen O, Kamatani N, Kadowaki T, Kikkawa R, Nakamura Y and Maeda S, SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations, Nat. Genet. 40 (2008) 1098-1102

8. D. Zhou, Zhang D, Liu Y, Zhao T, Chen Z, Liu Z, Yu L, Zhang Z, Xu H and He L, The E23K variation in the KCNJ11 gene is associated with type 2 diabetes in Chinese and East Asian population, J. Hum. Genet. 54 (2009) 433-435

9. L. Yang, Zhou X, Luo Y, Sun X, Tang Y, Guo W, Han X and Ji L, Association between KCNJ11 gene polymorphisms and risk of type 2 diabetes mellitus in East Asian populations: a meta-analysis in 42,573 individuals, Mol. Biol. Rep. 2011 (epub ahead of print)

10. A. Fischer, Fisher E, Mohlig M, Schulze M, Hoffmann K, Weickert MO, Schueler R, Osterhoff M, Pfeiffer AF, Boeing H and Spranger J, KCNJ11 E23K affects diabetes risk and is associated with the disposition index: results of two independent German cohorts, Diabetes Care 31 (2008) 87-89

11. E.L. Edghill, S.E. Flanagan and S. Ellard, Permanent neonatal diabetes due to activating mutations in ABCC8 and KCNJ11, Rev. Endocr. Metab. Disord. 11 (2010) 193-198

12. L.E. Fridlyand, L. Ma and L.H. Philipson, Adenine nucleotide regulation in pancreatic beta-cells: modeling of ATP/ADP-Ca2+ interactions, Am. J. Physiol. Endocrinol. Metab. 289 (2005) E839-848

13. D.K. Fuhrer, M. Kobayashi and H. Jiang, Insulin release and suppression by tacrolimus, rapamycin and cyclosporin A are through regulation of the ATP-sensitive potassium channel, Diabetes Obes. Metab. 3 (2001) 393-402

14. C. Schwanstecher, Meyer U and Schwanstecher M, K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channels, Diabetes 51 (2002) 875-879

15. B. Tavira, Coto E, Diaz-Corte C, Ortega F, Arias M, Torres A, Diaz JM, Selgas R, Lopez-Larrea C, Campistol JM and Alvarez V, Pharmacogenetics of tacrolimus after renal transplantation: analysis of polymorphisms in genes encoding 16 drug metabolizing enzymes, Clin. Chem. Lab. Med. 49 (2011) 825-833

16. B. Tavira, Coto E, Diaz-Corte C, Ortega F, Arias M, Torres A, Diaz JM, Selgas R, Lopez-Larrea C, Campistol JM, Ruiz-Ortega M and Alvarez V, KCNQ1 gene variants

and risk of new-onset diabetes in tacrolimus-treated renal-transplanted patients, Clin. Transplant. 25 (2011) E284-291

17. J. Yang, I.I.Hutchinson, T. Shah and D.I. Min, Genetic and clinical risk factors of new-onset diabetes after transplantation in Hispanic kidney transplant recipients, Transplantation 91 (2011) 1114-1119

18. F.M. Ashcroft, ATP-sensitive potassium channelopathies: focus on insulin secretion, J. Clin. Invest. 115 (2005) 2047-2058

19. C. Schwanstecher, Neugebauer B, Schulz M and Schwanstecher M, The common single nucleotide polymorphism E23K in K(IR)6.2 sensitizes pancreatic beta-cell ATP-sensitive potassium channels toward activation through nucleoside diphosphates, Diabetes 51 (2002) S363-367

20. J.C. Florez, Jablonski KA, Kahn SE, Franks PW, Dabelea D, Hamman RF, Knowler WC, Nathan DM and Altshuler D, Type 2 diabetes-associated missense polymorphisms KCNJ11 E23K and ABCC8 A1369S influence progression to diabetes and response to interventions in the Diabetes Prevention Program, Diabetes 56 (2007) 531-536

Table 1. Differences between the NODAT vs. Non-NODAT groups. All the continuous variables are expressed as Mean (±SD) except the Tac values (median and range).

		NO-NODAT (n=205) (mean± S.D)	NODAT (n=115) (mean± S.D)	P values
Age (years)		45±13/18-70	53±13/24-75	0.001
Gender (% male)		64%	63%	0.57
BMI (kg/m^2) at transplant		24.50±4.32 / 18-38	25.30±4.33 / 18-37	0.003
Δ BMI (0-6 months)		0.83±0.37	0.89±0.12	0.06
Δ BMI (0-12 months)		1.84±0.32	1.79±0.25	0.07
		NO-NODAT (n=205) (median/range)	NODAT (n=115) (median/range)	
One week	Tacrolimus dose (mg/day)	8 / 1-30	10 / 2-25	0.11
	Tacrolimus dose (mg/kg/day)	0.12 / 0.01-0.40	0.14 / 0.03-0.34	0.26
	Tacrolimus blood levels (ng/ml)	11 / 1.61-38.12	11.21 / 3.41-27.10	0.73
6 months	Tacrolimus dose (mg/day)	4 / 0.5-16	5 / 1.5-14	0.18
	Tacrolimus dose (mg/kg/day)	0.06 /0.01-0.29	0.07 / 0.02-0.19	0.38
	Tacrolimus blood levels (ng/ml)	8.21 / 1.50-23.60	8.17 / 3.90-18.90	0.55
12 months	Tacrolimus dose (mg/day)	5 / 0.5-15	5 / 1.52-13.20	0.16
	Tacrolimus dose (mg/kg/day)	0.07 /0.02-0.33	0.08 / 0.03-0.29	0.41
	Tacrolimus blood levels (ng/ml)	8.18 / 1.51-19.87	8.45 / 2.60-17.90	0.45
KCNJ11 rs5219 K/K (AA genotype)		25/205=12%	19/115=17%	**0.004 ***
E/K (AG genotype)		95/205=46%	67/115=58%	
E/E (GG genotype)		85/205=42%	29/115=25%	
K (A allele)		0.35	0.46	**0.017**

* AA+AG vs. GG ; p=0.004; OR=2.10, 95%CI=1.27-3.48