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

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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 $\chi^2$ 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 KCNJ11 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 \textit{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.

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References


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

<table>
<thead>
<tr>
<th></th>
<th>NO-NODAT (n=205)</th>
<th>NODAT (n=115)</th>
<th>P values</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(mean± S.D)</td>
<td>(mean± S.D)</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>45±13/18-70</td>
<td>53±13/24-75</td>
<td>0.001</td>
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<tr>
<td>Gender (% male)</td>
<td>64%</td>
<td>63%</td>
<td>0.57</td>
</tr>
<tr>
<td>BMI (kg/m$^2$) at transplant</td>
<td>24.50±4.32 / 18-38</td>
<td>25.30±4.33 / 18-37</td>
<td>0.003</td>
</tr>
<tr>
<td>Δ BMI (0-6 months)</td>
<td>0.83±0.37</td>
<td>0.89±0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Δ BMI (0-12 months)</td>
<td>1.84±0.32</td>
<td>1.79±0.25</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>NO-NODAT (n=205)</th>
<th>NODAT (n=115)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(median/range)</td>
<td>(median/range)</td>
<td></td>
</tr>
<tr>
<td>One week</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Tacrolimus dose (mg/day)</td>
<td>8 / 1-30</td>
<td>10 / 2-25</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus dose (mg/kg/day)</td>
<td>0.12 / 0.01-0.40</td>
<td>0.14 / 0.03-0.34</td>
<td>0.26</td>
</tr>
<tr>
<td>Tacrolimus blood levels (ng/ml)</td>
<td>11 / 1.61-38.12</td>
<td>11.21 / 3.41-27.10</td>
<td>0.73</td>
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<tr>
<td>6 months</td>
<td></td>
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<tr>
<td>Tacrolimus dose (mg/day)</td>
<td>4 / 0.5-16</td>
<td>5 / 1.5-14</td>
<td>0.18</td>
</tr>
<tr>
<td>Tacrolimus dose (mg/kg/day)</td>
<td>0.06 /0.01-0.29</td>
<td>0.07 / 0.02-0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>Tacrolimus blood levels (ng/ml)</td>
<td>8.21 / 1.50-23.60</td>
<td>8.17 / 3.90-18.90</td>
<td>0.55</td>
</tr>
<tr>
<td>12 months</td>
<td></td>
<td></td>
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<tr>
<td>Tacrolimus dose (mg/day)</td>
<td>5 / 0.5-15</td>
<td>5 / 1.52-13.20</td>
<td>0.16</td>
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<tr>
<td>Tacrolimus dose (mg/kg/day)</td>
<td>0.07 /0.02-0.33</td>
<td>0.08 / 0.03-0.29</td>
<td>0.41</td>
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<tr>
<td>Tacrolimus blood levels (ng/ml)</td>
<td>8.18 / 1.51-19.87</td>
<td>8.45 / 2.60-17.90</td>
<td>0.45</td>
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</tbody>
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|                      |                   |                |          |
| KCNJ11 rs5219        |                   |                | 0.004 *  |
| K/K (AA genotype)    | 25/205=12%        | 19/115=17%     |          |
| 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