

Association of the *PPP3CA* c.249G>A variant with clinical outcomes of tacrolimus-based therapy in kidney transplant recipients

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Background: The effects of genetic variants related to the pharmacodynamic mechanisms of immunosuppressive drugs on their therapeutic efficacy and safety have been poorly explored. This study was performed to investigate the influence of the *PPP3CA* c.249G>A variant on the clinical outcomes of kidney transplant recipients.

Patients and methods: A total of 148 Brazilian patients received tacrolimus (TAC)-based immunosuppressive therapy for 90 days post-kidney transplantation. The *PPP3CA* rs3730251 (c.249G>A) polymorphism was determined by real-time polymerase chain reaction. Single-nucleotide polymorphism (SNP) data for *CYP3A5* rs776746 (*CYP3A5**3C; g.6986A>G) were used to eliminate the confounding effects of this variant.

Results: The *PPP3CA* c.249G>A SNP did not influence early TAC exposure, renal function, or other laboratory parameters, including levels of urea, creatinine, glucose, and lipids, and blood counts. This variant also did not account for the cumulative incidence of biopsy-confirmed acute rejection or delayed graft function. Regarding adverse events, *PPP3CA* c.249A allele carriers initially had a 3.05-fold increased probability of treatment-induced blood and lymphatic system disorders compared with c.249GG genotype individuals (95% confidence interval: 1.10–8.48, $p=0.032$). However, this result was not maintained after adjusting for body weight and *CYP3A5**3C SNP status ($p=0.086$).

Conclusion: The *PPP3CA* c.249G>A variant does not influence the clinical outcomes of Brazilian patients in the early phase of TAC-based immunosuppressive regimen.

Keywords: kidney transplantation, polymorphism, *PPP3CA*, tacrolimus, adverse events, Brazilian patients

Introduction

Pharmacogenetic approaches that focus on the pharmacodynamic mechanisms of drugs have the potential to increase the therapeutic efficacy and safety.¹ For immunosuppressive drugs used in kidney transplantation, the effects of genetic variants related to these mechanisms on clinical outcomes have been poorly explored.

Tacrolimus (TAC) is an effective immunosuppressive agent. It can bind to FKBP12, and then this complex can bind to calcineurin, resulting in inhibition of the nuclear translocation of nuclear factor of activated T cells, and less activation of genes, including the one encoding interleukin-2 (IL-2).^{2,3} Consequently, both cytokine synthesis and T cell activation decrease.³

Calcineurin is a Ca²⁺/calmodulin-dependent serine/threonine phosphatase that is composed of catalytic A (CnA) and regulatory B subunits.⁴ For CnA, three isoforms encoded by *PPP3CA* (CnA α), *PPP3CB* (CnA β), and *PPP3CC* (CnA γ) have been identified.⁵ Both

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the α and β isoforms are ubiquitously expressed, whereas γ is expressed in the testis and brain tissues.^{4,6}

PPP3CA is located on human chromosome 4, and many single-nucleotide polymorphisms (SNPs) have been described at the *PPP3CA* locus.^{7,8} Several studies have explored the association of genetic variants at the *PPP3CA* locus with physiological and pathological conditions, such as athletic status, susceptibility to addiction, and cardiac hypertrophy.^{5,9,10} However, to the best of our knowledge, only two studies have evaluated the association of the *PPP3CA* SNPs with the efficacy and toxicity of calcineurin inhibitors in renal transplantation.^{11,12}

This study was designed to investigate the effect of the *PPP3CA* c.249G>A SNP on the clinical outcomes of TAC-based immunosuppression in a prospective cohort of Brazilian patients in the early phase after kidney transplantation.

Patients and methods

Patients and immunosuppressive regimens

This investigation was performed with Brazilian adult recipients ($n=148$) undergoing kidney transplantation for the first time and who had previously enrolled in a prospective core study,¹³ registered at ClinicalTrials.gov NCT01802268. The study protocol was approved by the institutional review boards of the School of Pharmaceutical Sciences, University of Sao Paulo, and the Federal University of Sao Paulo/Sao Paulo Hospital (Sao Paulo, Brazil). All subjects provided written informed consent before enrollment.

Within 48 h after the transplant surgery, all patients received TAC (0.1–0.2 mg/kg/day), enteric-coated mycophenolate sodium (MPS, initial fixed dose, 1440 mg/day), and prednisone (initial dose, 0.5 mg/kg/day; maximum, 30 mg with subsequent reduction to reach the maintenance dose of 5 mg/day by day 45). The TAC dose was adjusted to maintain a blood concentration in the range of 8–15 ng/mL in the first month and 5–15 ng/mL during the second and third months of therapy.

Therapy monitoring and laboratory tests

Patients were followed for 3 months posttransplantation. Clinical outcome assessments included monitoring the following variables: episodes of the biopsy-confirmed acute rejection (BCAR, according to the Banff 1997 classification¹⁴) and delayed graft function (DGF, defined as the use of dialysis within 7 days of the transplant), renal function, adverse events (AEs), and other clinical laboratory tests that were performed on blood samples that had been collected at

every visit under fasting conditions (levels of urea, creatinine, glucose, and lipids, and blood counts).

Trough TAC concentrations in blood were determined by chemiluminescent microparticle immunoassay using ARCHITECT analyzers (Abbott Diagnostics, Abbott Park, IL, USA). The TAC dose-adjusted trough concentrations in blood (C/D ratio; ng/(mL·mg)) were calculated. The estimated glomerular filtration rate (eGFR) was determined using the four-variable Modification of Diet in Renal Disease formula.¹⁵

AEs were defined as any untoward medical occurrence in a subject, with or without a causal relationship with drug treatment.^{16,17} All AEs were recorded and classified according to the System Organ Class of the Medical Dictionary for Regulatory Activities (MedDRA).¹⁸ Duplication of AEs was not considered.

Genomic DNA extraction and genotyping by real-time PCR

Genomic DNA was extracted from pretransplant whole-blood samples using the QIAcube system (Qiagen, Hilden, Germany) and quantified using a Nanodrop ND-1000 instrument (Nanodrop Technologies, Wilmington, DE, USA). DNA integrity was evaluated using 0.8% agarose gel electrophoresis.

Subjects were genotyped for the *PPP3CA* rs3730251 (c.249G>A) and *CYP3A5* rs776746 (*CYP3A5**3C; g.6986A>G) polymorphisms.

The SNP *PPP3CA* rs3730251 (c.249G>A) was selected for further investigation because it is present at the greatest global minor allele frequency among validated synonymous coding SNPs available from the Database of Single Nucleotide Polymorphisms (dbSNP) of the National Center for Biotechnology Information (NCBI).¹⁹ This variant was identified by real-time polymerase chain reaction (PCR), using a C_25618069_10 predesigned TaqMan[®] SNP genotyping assay (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification was carried out in a 7500 Fast Real-Time PCR thermocycler (Thermo Fisher Scientific) according to the manufacturer's instructions.

The SNP *CYP3A5* rs776746 (*CYP3A5**3C; g.6986A>G) was found to be the strongest predictor of TAC dosing requirements.²⁰ Genotyping results for this variant were presented in a previous study.²¹

Statistical analysis

For pharmacogenetic analyses, individuals carrying the homozygous form of the less common allele (*PPP3CA* c.249A) were grouped with heterozygous carriers.

Categorical variables were described as numbers and percentages and compared using the χ^2 or Fisher's exact test. Estimates of Hardy–Weinberg Equilibrium (HWE) were also determined using the χ^2 test.

Quantitative variables were expressed as mean \pm standard deviation. For two-group comparisons, Student's *t*-test (parametric) or Mann–Whitney test (nonparametric) was used. Stratification analyses based on *CYP3A5***3C* polymorphism were performed to eliminate the confounding effects of this variant on the results.

Logistic regression analysis was performed to assess the influence of the *PPP3CA* c.249G>A polymorphism on the risk of AEs, BCAR, and DGF. The following covariates were considered: recipient body weight, TAC concentration, MPS and prednisone doses, and *CYP3A5***3C* SNP genotype. Multicollinearity was assessed based on two closely related statistics: the tolerance level (>0.1) and variance inflation factor (<10). Goodness of fit was assessed using the Hosmer–Lemeshow test; for this test, $p > 0.05$ was regarded as satisfactory.

Statistical analyses were performed using GraphPad Prism® (GraphPad Software, La Jolla, CA, USA) and SPSS for Windows (SPSS Inc., Chicago, IL, USA). Significance level was set at $p < 0.05$.

Results

Patients and *PPP3CA* c.249G>A SNP genotype

The cohort of Brazilian kidney transplant recipients predominantly comprised young men (mean age, 45 \pm 13 years; 69.6%). The frequency of hypertension, diabetes, and dyslipidemia was 52.0%, 12.8%, and 2.7%, respectively (Table 1). The cumulative incidence rates of BCAR and DGF were 24.3% and 19.6%, respectively. After 90 days of immunosuppressive therapy, the TAC blood concentration was 5.9 \pm 2.6 ng/mL. In the same period, the MPS and prednisone doses administered were 1377.9 \pm 200.5 and 5.6 \pm 3.0 mg/day, respectively (Table 1). The donors were mostly young women (mean age, 43 \pm 10 years; 53.4%). Living donor transplants represented the major type of transplantation (58.8%; Table 1).

Genotype frequencies for the *PPP3CA* rs3730251 (c.249G>A) variant were as follows: GG: 87.8%, GA: 10.8%, and AA: 1.4%. This genotype distribution was in HWE. The frequency of the *PPP3CA* c.249A allele (6.8%, data not shown) was in accordance to that described by the 1000 Genomes Project (for all individuals).²²

To obtain a more detailed analysis, anthropometric and clinical characteristics were evaluated in subgroups of the

Table 1 Biodemographical and clinical characteristics of Brazilian kidney transplant recipients and donors

Variables	Patients, n=148
Recipients	
Age (years)	45.4 \pm 12.9
Body weight (kg)	66.3 \pm 12.6
Sex, male	103 (69.6)
Ethnicity	
White	69 (46.6)
Mixed	53 (35.8)
African	18 (12.2)
Others	8 (5.4)
Comorbidities	
Hypertension	77 (52.0)
Diabetes	19 (12.8)
Dyslipidemia	4 (2.7)
Biopsy-confirmed acute rejection ^a	36 (24.3)
Delayed graft function ^b	29 (19.6)
Donors	
Age (years)	43.4 \pm 10.3
Sex, male	68 (45.9)
Missing data	1 (0.7)
Living donor	87 (58.8)
Ethnicity	
White	82 (55.4)
Mixed	46 (31.1)
African	14 (9.5)
Others	4 (2.7)
Missing data	2 (1.3)
Immunosuppression at 90 days	
Tacrolimus concentration (ng/mL)	5.9 \pm 2.6
Mycophenolate sodium dose (mg/day)	1377.9 \pm 200.5
Prednisone dose (mg/day)	5.6 \pm 3.0

Notes: Values are represented as n (%). Age, body weight, and concentration and doses of the immunosuppressive drugs are presented as mean \pm standard deviation. ^aCumulative incidence. ^bDefined as the use of dialysis within 7 days of the transplant.

patients based on the presence of the *PPP3CA* c.249G>A SNP. Age, type of transplantation, and frequencies of sex, ethnicity, comorbidities, BCAR, and DGF were similar between *PPP3CA* c.249A allele carriers (c.249GA+AA genotypes) and c.249GG genotype patients ($p > 0.05$, data not shown). However, *PPP3CA* c.249GG patients had higher mean body weight (67.1 \pm 12.5 kg) than c.249A allele carriers (59.7 \pm 11.4 kg, $p = 0.028$).

Associations among *PPP3CA* c.249G>A SNP, drug exposure, and treatment outcomes

As expected, the *PPP3CA* c.249G>A SNP did not influence either TAC exposure (dose, concentration, or C/D) or doses of MPS and prednisone administered during the study period (data not shown). Blood concentrations of TAC are strongly influenced by the *CYP3A5***3C* SNP;²⁰ therefore, these data were also analyzed and compared after stratification based on this variant. No significant association was identified.

Similarly, the *PPP3CA* c.249G>A polymorphism was not associated with differences in eGFR or other laboratory parameters, including levels of urea, creatinine, glucose, and lipids, and blood counts (data not shown).

Logistic regression analyses were performed to determine whether the *PPP3CA* c.249G>A SNP could account for the cumulative incidence of BCAR, DGF, or AEs in this study (Table 2).

With regard to AEs, the *PPP3CA* c.249A allele carriers initially had a 3.05-fold greater probability of treatment-induced blood and lymphatic system disorders compared with c.249GG genotype patients (95% confidence interval: 1.10–8.48, $p=0.032$; Table 2). However, this association was not

Table 2 Logistic regression analysis of association of *PPP3CA* c.249G>A polymorphism with clinical outcomes up to 90 days after kidney transplantation

Dependent variable	Unadjusted logistic regression		Adjusted logistic regression ^a	
	OR (95% CI)	p	OR (95% CI)	p
Adverse events				
Gastrointestinal disorders	0.86 (0.30–2.45)	0.773	0.75 (0.24–2.40)	0.630
Constipation	0.54 (0.15–1.99)	0.357	0.34 (0.07–1.61)	0.172
Diarrhea	0.90 (0.30–2.69)	0.847	1.08 (0.34–3.38)	0.899
Dyspeptic syndrome	0.93 (0.25–3.48)	0.915	0.80 (0.20–3.18)	0.754
Nausea and/or vomiting	0.89 (0.19–4.24)	0.884	0.42 (0.05–3.54)	0.428
Infections and infestations	1.29 (0.46–3.66)	0.631	1.45 (0.47–4.52)	0.518
Metabolism and nutrition disorders	1.00 (0.33–3.02)	0.994	1.31 (0.41–4.17)	0.645
Nervous system disorders	0.93 (0.31–2.79)	0.899	0.96 (0.31–3.02)	0.945
Headache	1.42 (0.37–5.47)	0.606	1.25 (0.31–5.10)	0.756
Renal and urinary disorders	1.60 (0.58–4.44)	0.367	1.85 (0.63–5.45)	0.267
Blood and lymphatic system disorders	3.05 (1.10–8.48)	0.032	2.66 (0.87–8.13)	0.086
Anemia	2.92 (0.98–8.72)	0.055	2.42 (0.70–8.29)	0.161
Musculoskeletal and connective tissue disorders	1.33 (0.40–4.41)	0.642	1.27 (0.36–4.48)	0.709
Skin and subcutaneous tissue disorders	0.32 (0.04–2.57)	0.286	0.40 (0.05–3.25)	0.389
Biopsy-confirmed acute rejection	1.67 (0.58–4.82)	0.346	2.27 (0.73–6.99)	0.154
Delayed graft function	1.70 (0.55–5.22)	0.355	1.27 (0.37–4.40)	0.706

Notes: Reference: *PPP3CA* c.249GG. ^aAdjusted for body weight and *CYP3A5*3C* (rs776746) SNP status. Bold indicates significance.

Abbreviations: OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism.

maintained after adjusting for body weight and *CYP3A5*3C* (rs776746) SNP status (Table 2). Adjustments for patient body weight were necessary because previous analyses had indicated that *PPP3CA* c.249GG patients were heavier than c.249A allele carriers. Moreover, an adjustment for TAC concentrations and doses of MPS and prednisone was performed. No significant result was detected (data not shown).

Discussion

Therapeutic drug monitoring (TDM) of TAC plays an important role in patient management. The goal of TDM is to use drug concentrations to manage the medication regimen of a patient and optimize clinical outcomes.²³ However, the amount of drug measured in the blood does not accurately reflect its pharmacological effects.²⁴

Biomarkers may be useful as they can quantitatively reflect the effects of a given drug on its target and also predict the clinical response to a drug.²⁵ As the quantity of genetic data grows, genotyping will increasingly yield these useful biomarkers.²⁶

This exploratory study was the first to investigate the effects of the *PPP3CA* c.249G>A SNP on the efficacy and safety of a TAC-based regimen in Brazilian kidney transplant recipients who were followed for the first 3 months after transplantation. Moreover, it was the first investigation to evaluate the relationship of *PPP3CA* polymorphisms with different classes of AEs. In this study, the *PPP3CA* c.249G>A SNP was not associated with risk of DGF, BCAR, or AEs. Moreover, this polymorphism was not associated with eGFR, biochemical blood tests, or early drug exposure in our patient cohort.

In accord with these findings, five other SNPs (rs13146281, rs7665292, rs2201677, rs10031159, and rs13117493) and one short tandem repetition (rs45441997) in *PPP3CA* were also not associated with risk of acute or subclinical rejection, or with serious infections in renal transplant recipients treated with calcineurin inhibitors.^{11,12} Similarly, variants in other genes that encode components of the calcineurin pathway, such as *FKBP1A* (FKBP12), *PPP3CB* and *PPP3R1* (calcineurin B, α subunit), *CALMI*, 2, and 3 (calmodulin 1–3 isoforms), showed no association with the outcomes of treatment in renal patients.^{11,12}

We must note that these studies are in the preliminary stages; therefore, further investigations involving *PPP3CA* polymorphisms and responses to therapy in kidney transplantation should be conducted. Indeed, until now, all of the studies had inherent limitations, including the sample size and number of genetic variants evaluated.

Noceti et al²⁵ suggested a strong association between TAC pharmacodynamic parameters and polymorphisms in

PPP3CA. The authors reported that, in ex vivo TAC-treated peripheral blood mononuclear cells from 35 volunteers, the *PPP3CA* rs45441997 variant was associated with variability in intracellular IL-2 expression in CD4⁺ T cells.²⁵ IL-2, discovered as a T cell growth factor, is a cytokine produced after antigen activation that plays pivotal roles in the immune response.²⁷ Its transcription can be inhibited by TAC. Studies involving *IL2* polymorphisms have yielded encouraging results. The *IL2* rs2069763 and rs2069762 variants, for example, have been associated with acute rejection in Turkish and Brazilian kidney transplant recipients.^{28,29}

Conclusion

The results reported here indicate that the *PPP3CA* c.249G>A variant does not influence clinical outcomes in Brazilian patients who are in the early phase of TAC-based immunosuppressive regimen.

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Disclosure

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