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Risk factors for post-transplant diabetes mellitus in renal transplant: Role of genetic variability in the CYP450-mediated arachidonic acid metabolism

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ABSTRACT

Arachidonic acid (AA) is metabolized by cytochrome P450 (CYP) enzymes to epoxyeicosatrienoic acids (EETs) and 20-hidroxyeicosatetraenoic acid (20-HETE), which play an important role both in renal transplant and diabetes mellitus (DM). We searched for associations between polymorphisms in this metabolic pathway and the risk of post-transplant diabetes mellitus (PTDM) in kidney recipients. One-hundred-sixty-four patients were genotyped for common SNPs in this route, namely *CYP2C8*3*, *CYP2C8*4*, *CYP2C9*2*, *CYP2C9*3*, *CYP2J2*7*, *CYP4A11 F434S* and *CYP4F2 V433M*. Demographic and clinical parameters were retrospectively collected at four time-points in the first year after grafting.

Thirty-four patients (20.73%) developed PTDM, which was more prevalent among older patients [OR for older age = 1.06 (1.03–1.10), $p < 0.001$] and in those with higher body mass index (BMI) [OR for higher average BMI in the first year = 1.13 (1.04–1.23); $p < 0.01$]. Creatinine clearance [OR = 0.97 (0.95–0.99); $p < 0.01$] and exposure to tacrolimus [OR = 3.25 (1.15–9.19); $p < 0.05$] were also relevant for PTDM risk. With regard to genetic variants, logistic regression analysis controlling for significant demographic and clinical variables showed that the V433M polymorphism in CYP4F2, responsible for 20-HETE synthesis, was an independent risk factor for PTDM [OR = 3.94 (1.08–14.33); $p < 0.05$].

We have shown that a genetic variant in the *CYP4F2* gene, the main gene implicated in 20-HETE synthesis, is associated with the risk for PTDM. Our findings suggest that genes in the metabolic pathways of AA may become good candidates in genetic association studies for PTDM.

1. Introduction

The cytochrome P450 (CYP) enzymes are responsible for the biotransformation of arachidonic acid into vasoactive eicosanoids. The epoxygenase branch of this pathway leads to the synthesis of epoxyeicosatrienoic acids (EETs), which have vasodilator, profibrinolytic and anti-inflammatory properties (Yang et al., 2015; Imig, 2005; Node et al., 1999). CYP2C9 and, most importantly, CYP2C8 and CYP2J2 are mostly responsible for this synthesis (Alkayed et al., 2002; Wu et al., 1997; Zeldin, 2001; Zeldin et al., 1996). In a parallel hydroxylase route, AA is also metabolized by CYP4A11 and CYP4F2 to 20-hidroxyeicosatetraenoic acid (20-HETE) (Lasker et al., 2000), which may increase blood pressure by vasoconstriction or, paradoxically, promote antihypertension by increasing sodium excretion in the kidney (Miyata and Roman, 2005; Hoopes et al., 2015). Each of these CYP genes are expressed in the kidney and present functional single nucleotide polymorphisms (SNPs) with the potential to modulate the levels of these active AA metabolites (Dai et al., 2001; King et al., 2002; Bahadur et al., 2002; Sullivan-Klose et al., 1996; Rettie et al., 1994; Stec et al., 2007; Gainer et al., 2005).

We and others have shown that these AA-derived metabolites may play a significant role in the outcome of renal transplantation (Paller and Jacob, 1994; Gervasini et al., 2013; Dolegowska et al.,

Abbreviations: 20-HETE, 20-hidroxyeicosatetraenoic acid; AA, arachidonic acid; AUC, area under the curve; CDR, concentration-to-dose ratio; CMV, cytomegalovirus; CNIs, calcineurin inhibitors; DM, diabetes mellitus; EETs, epoxyeicosatrienoic acids; HCV, hepatitis C virus; HLA, human leukocyte antigen; PTDM, post-transplant diabetes mellitus.

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2009; Gervasini et al., 2015a, 2015b; Lee et al., 2008). Moreover, an accumulating body of evidence points to these eicosanoids as compounds with an important role in both diabetes mellitus (DM) (Tessaro et al., 2015; Burgess et al., 2012a) and diabetic nephropathy (Eid et al., 2013a, 2013b). However, to our knowledge, there are no studies aimed to search for associations between genetic variability in these metabolic pathways and post-transplant diabetes mellitus (PTDM).

Being one of the most common complications in renal transplant, the occurrence of PTDM is a serious concern, as it is known to decrease graft survival and increase morbidity and mortality, mainly because of its impact on the cardiovascular system (Cosio et al., 2002). Indeed, prospective data have shown that PTDM increases the risk for cardiovascular events up to 3.3-fold compared to patients without diabetes (Hjelmesaeth et al., 2006). The determination of risk factors for this disorder is crucial for its early diagnosis and management.

In the present study we have tested the hypothesis that functional polymorphisms in the CYP-mediated AA metabolism, in combination with other demographic and clinical parameters, may affect the endogenous mechanisms involved in the development of PTDM in renal transplant recipients.

2. Patients and methods

From an initial group of 175 renal transplant recipients, 164 Caucasian patients who received a single kidney at the Infanta Cristina Hospital (Badajoz, Spain) from deceased donors were included in the study. Eleven subjects were excluded because a history of pretransplant DM as revealed by clinical records. All participants gave written consent for their participation. The study was approved by the Ethics Committee of the Infanta Cristina Hospital and was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Transplant recipients were treated with anticalcineurin inhibitors (CNIs) [either cyclosporine (n = 68) or tacrolimus (n = 96)] along with mycophenolate mofetil (2 g/day) and a tapering schedule of corticoids (500 mg IV methylprednisolone at the time of surgery, 125 mg intravenously (IV) the following day and then 20 mg of oral prednisone daily, progressively tapered to 5 mg daily at 2 months after transplantation). Tacrolimus initial dose was 0.1 mg/kg each 12 h, whilst cyclosporine dosage was 4–10 mg/kg/day divided into two administrations. Further doses of CNIs were subsequently adjusted according to blood concentrations. Tacrolimus and cyclosporine blood concentrations were routinely measured using an immunoassay performed on a Cobas Mira Plus analyzer (Roche Diagnostics). Concentration to dose ratios (CDRs) (ng/ml per mg/kg/day) were calculated by dividing trough blood levels (ng/ml) by the previous daily weight-adjusted dose (mg/kg).

The occurrence of PTDM was established according to the American Diabetes Association Criteria by the retrospective revision of clinical records and was defined as two fasting plasma glucose values $\geq$ 126 mg/dL or symptoms of diabetes plus casual plasma glucose concentrations $\geq$200 mg/dL throughout the first year (Davidson and Wilkinson, 2004).

Renal function was assessed by retrieving creatinine clearance values (mL/min) at one week, one month, five months and one year after grafting. Creatinine clearance was calculated from measurement of creatinine in a 24-h urine sample.

2.1. Genotype analysis

Genomic DNA was isolated from whole blood samples with a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). Seven common, functional SNPs, namely *CYP2C8*3* (rs10509681), *CYP2C8*4* (rs1058930), *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910), *CYP2J2*7* (rs890293), *CYP4A11 F434S* (rs1126742) and *CYP4F2 V433M* (rs2108622) in the genes governing the epoxygenase and hydroxylase pathways of AA were identified by RT-PCR using commercially available probes from Life Technologies (Maryland, USA).

2.2. Statistical analyses

Fisher's exact or Pearson's X^2 test were used for the univariate analysis of the associations between categorical data. The ANOVA/t-Student's or Mann–Whitney/Kruskal–Wallis tests were used to compare mean values of quantitative variables between groups, as appropriate. Logistic binary regression was used to determine the combined influence of genetic, demographic and clinical variables on PTDM risk In order to collectively assess the risk of PTDM attributable to high exposure to either cyclosporine or tacrolimus, the first quartile was set as cut-off point to stratify into high-exposed and low-exposed patients.

The statistical power of the study was evaluated analyzing the frequency for carriers of the variant alleles with an arbitrarily established effect size set at 2.5 (type I error = 0.05). With the available study sample and the reported incidence of PTDM, the power for detecting associations with this complication ranged from 0.69 to 0.89 depending on the minor allele frequency (Quanto software v. 1.2.4, University of Southern California). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows (SPSS Inc., Chicago, Ill. USA). In all instances differences were considered to be significant when p values were lower than 0.05.

3. Results

A total of 164 renal transplant recipients (65 women) with a mean age of 44.61 ± 14.31 years at the time of transplantation were studied. Underlying diseases for renal failure were classified as follows: glomerulonephritis (37.80%), chronic interstitial nephritis (12.19%), polycystic kidney disease (10.98%) and uncertain (29.7%). Other causes accounted for 9.31% of cases. Additional clinical characteristics of the patients are shown in Table 1.

Thirty-four renal transplant recipients (20.73%) developed PTDM in the first 12 months after grafting. The incidence of this complication in our series did not significantly differ between patients on tacrolimus or cyclosporine [22.60 vs. 17.65%, respectively; odds ratio (OR) for higher risk in patients on tacrolimus: 1.39 (0.63–3.04); p = 0.441].

3.1. Genetic study

Genotypic frequencies of the seven studied polymorphisms did not show statistically significant differences from those expected by the Hardy-Weinberg principle (p > 0.05 in all cases). Minor allele frequencies ranged from 0.019 (*CYP2J2*7*) to 0.345 (*CYP4F2* V433M) (Table 2).

The risk for PTDM was assessed with a dominant model of inheritance, i.e. carriers of the variant allele vs. non-carriers, in order to make it up for the low number of subjects carrying allelic variants in homozygosity. Crude analyses shown in Table 3 did not reveal any significant association with higher risk of PTDM. Only a statistical trend was observed for *CYP2C8*3* carriers, as they accounted for 43.75% of PTDM cases but only for 28.68% of patients that did not develop this complication (p = 0.08). In the case of the polymorphisms in *CYP4A11* and *CYP4F12*, with a substantial number of mutant homozygous carriers, the recessive model was also tested but no significant results were observed either [OR values for