ENPP1/PC-1 K121Q and Other Predictors of Posttransplant Diabetes

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Abstract

Diabetes mellitus is a major cause of morbidity and mortality among transplanted patients. This study evaluated the role of the *ENPP1* K121Q polymorphism and other variables known to affect diabetes risk in 115 nondiabetic and unrelated patients who underwent kidney transplant at our institution and had consented for use of genetic material (30% whites, 48% blacks, and 22% Hispanics). Thirty-six of these patients (30%) developed posttransplant diabetes mellitus (PTDM) within 1 year of observation from transplant. Black race, *ENPP1* K121Q polymorphism, age, body mass index (BMI), and immunosuppressive medications were found to have the strongest associations with PTDM in the logistic regression and receiver operator characteristic (ROC) analysis. However, because *ENPP1* K121Q is more common in Hispanics and in blacks, who also have higher PTDM prevalence, the studied genetic polymorphism did not exert independent predictive effect, whereas ethnicity, specifically black versus non-black, was the most robust predictor of PTDM. The model with the largest ROC area under the curve (AUC) of 0.80 was comprised of black/non-black, age, BMI, and tacrolimus treatment as significant predictors. A reduced model containing only ethnicity (black/non-black) and age as predictors yielded similar results (ROC AUC 0.78). We conclude that black race and age are major and not modifiable risk factors for PTDM. The specific role of *ENPP1* K121Q on ethnic susceptibility to PTDM deserves further investigation in larger cohorts of transplanted patients.

Introduction

THE DEVELOPMENT OF DIABETES mellitus after solid-organ transplant is a common complication that has significant effects on development of cardiovascular disease, decreased transplant survival, and mortality.^{1–3} The United Renal Data System study showed that the cumulative incidence of posttransplant diabetes mellitus (PTDM) is 9.1%, 16.0%, and 24.0% at 3, 12, and 36 months posttransplant, respectively.⁴ Previous studies have identified several risk factors that increase a patient's susceptibility to developing diabetes after transplant. These include age >40 years, higher body mass index (BMI), African-American race, Hispanic ethnicity, male gender as donor, hepatitis C virus (HCV) infection, increasing human leukocyte antigen (HLA) mismatches, family history of diabetes, cadaver donor, presence of the metabolic syndrome, impaired glucose tolerance pretransplantation, and the use of corticosteroids or tacrolimus as immunosup-

pressive agents.^{5,6} More recently, specific genetic polymorphisms have also been linked to PTDM.^{7–9} Further defining and characterizing risk factors leading to PTDM should help clinicians focusing on developing strategies and treatment approaches designed for prevention of PTDM.

Previously, we have shown that among genetic factors that increase susceptibility to type 2 diabetes, the polymorphic *ENPP1* K121Q plays a significant role through increasing insulin resistance.¹⁰ Because we have also described that this genetic variant is particularly frequent in ethnic minorities, including Hispanics and blacks, also known to have increased risk for PTDM, we conducted a genetic case-control study in a cohort of patients who did not have diabetes before kidney transplant and were followed at the University of Texas Southwestern Medical Center in Dallas. Besides *ENPP1* K121Q genetic polymorphism, we also analyzed the role of previously known risk factors in predicting onset of PTDM within 1 year of follow up.

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Research Design and Methods

Subjects

Patient with kidney transplant followed at the transplant clinic at University of Texas Southwestern Medical Center at Dallas, who had volunteered to use their DNA for studies in diabetes where included in this investigation. Only patients with no diabetes at the time of transplant were selected for this study. Diabetes was identified based on the history, use of hypoglycemic agents, fasting plasma glucose (≥ 126 mg/dL), and 2 h plasma glucose (≥ 200 mg/dL) when available. The study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center at Dallas. Data were collected from chart review and laboratory results available at the time of patient clinic visit. Presence or absence of diabetes was adjudicated at 1 year from kidney transplant.

DNA amplification by PCR and assay of the ENPP1 polymorphism

Genomic DNA was isolated from whole blood using commercial DNA isolation kits from QIAGEN (Chatsworth, CA). *ENPP1* K121Q was detected by PCR restriction fragment-length polymorphism analysis, as previously described.¹¹ To assure that the genotyping was of sufficient quality, we performed random duplicates in about 10% of the samples, and we carried controls from carriers and noncarriers in each genotyping assay. The assays were performed by a technician who was blind to the phenotype. No genotype errors were detected in the random duplicates.

Statistical analysis

Categorical variables were compared between subjects with and without PTDM with the Fisher exact test. Logistic regression analysis was conducted to identify predictors of PTDM using univariate and multivariable models evaluating demographic, genetic, and medication variables as predictive factors. Ethnicity was modeled as dummy variables for black, Hispanic, or non-Hispanic white status. Receiver operating characteristic (ROC) curves were generated from the logistic regression models and the area under the ROC curves (AUC) compared using a nonparametric approach.¹² Statistical analysis was performed with SAS version 9.2 (SAS Institute, Cary, NC).

Results

Table 1 summarizes the general characteristics of the study subjects based on gender, race, BMI, blood pressure, medications, and hepatitis status. Two-thirds of the participants were male. There was similar representation of blacks, Hispanics, and whites. The mean BMI was in the overweight category and the group was prehypertensive based on systolic blood pressure. Three-quarters of the subjects were taking prednisolone, while about half were either on cyclosporine, mycophenolate mofetil, or tacrolimus.

Table 2 compares the general characteristics of the group of transplanted patients who developed diabetes within 1 year of observation (PTDM) with those who did not (remained normoglycemic). PTDM developed in 30% of our subjects, which falls in the expected range based on previous

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY SUBJECTS

	115 (77/38)
Number of participants (male/female)	Mean $\pm$ SD
Age (years)	44 $\pm$ 12
BMI (kg/m ²)	29 $\pm$ 7
SBP (mmHg)	131 $\pm$ 16
DBP (mmHg)	77 $\pm$ 13
Race/ethnicity <i>n</i> (% of total study subjects)	
White	34(29%)
Hispanic	42(37%)
Black	34(39%)
History of hepatitis <i>n</i> (% of total study subjects)	
HBV	3(3.5%)
HCV	15(10%)
Medications <i>n</i> (% of total study subjects)	
Prednisolone	92(80%)
Prednisone	2(2%)
Imuran	13(11%)
Cyclosporin	47(41%)
Cellcept	74(64%)
Tacrolimus	50(43%)
Sirolimus	5(4%)

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBV, hepatitis B virus; HCV, hepatitis C virus.

studies. Blacks had the highest rate of PTDM, whereas Hispanics and whites had similar rates. The difference in the incidence of PTDM seen in blacks was statistically significant when compared to the other two groups. Age and BMI were

TABLE 2. GENERAL CHARACTERISTICS OF POSTTRANSPLANT PATIENTS GROUPS WHO DID AND DID NOT DEVELOP POSTTRANSPLANT DIABETES MELLITUS (PTDM)

	No PTDM	Yes PTDM	P value ^a
Male/Female (<i>n</i>)	53/26	24/12	<0.001
Age (years)	42 $\pm$ 12 ^b	51 $\pm$ 10 ^b	<0.001
BMI (kg/m ²)	28 $\pm$ 7	31 $\pm$ 7	0.06
SBP (mmHg)	130 $\pm$ 16	132 $\pm$ 16	0.52
DBP (mmHg)	78 $\pm$ 13	75 $\pm$ 14	0.23
Race/ethnicity (<i>n</i>)			0.008
White	26	8	
Hispanic	18	7	
Black	35	21	
History of hepatitis (<i>n</i>)			
HBV	1	2	0.21
HCV	7	8	0.07
Medications (<i>n</i>)			
Prednisolone	59	33	0.04
Prednisone	2	0	1.0
Azathioprine	9	4	1.0
Cyclosporin	34	13	0.4
Mycophenilate	48	26	0.30
Tacrolimus	31	19	0.23
Sirolimus	3	2	0.64

^aP values were determined with independent samples *t*-test for comparisons of groups means and with the Fisher exact test for comparisons of group frequencies.

^bMean $\pm$ standard deviation.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBV, hepatitis B virus; HCV, hepatitis C virus.

FIG. 1. Percentage of study subjects with least one copy of the *ENPP1* 121Q allele is shown for the transplanted patients of this study and the general population of the Dallas Heart Study.¹³

statistically significant between groups, because those who developed PTDM were older and had a higher BMI. Both systolic and diastolic blood pressures were similar between groups.

Figure 1 shows the *ENPP1* K121Q prevalence in the study population as compared to the general population of Dallas/Fort Worth area. Gene frequency from the local population is reported based on our previous analysis of the Dallas Heart Study cohort.¹³ The prevalence was similar in blacks and Hispanics, but our study group showed an almost three-fold higher prevalence of K121Q in whites.

When comparing only the subjects with K121Q, Fig. 2A demonstrates a significant difference in those that developed PTDM and those who did not ($P=0.01$). The presence of K121Q conferred a tendency toward developing PTDM [odds ratio (OR) 2.79, 95% confidence interval (CI), 1.22–6.35]. Additionally, of the subjects who developed PTDM, a significant majority were homozygous for 121Q when compared to heterozygotes that developed PTDM and to het-

erozygotes and homozygotes that remained with normal glucose tolerance (Fig. 2B). The statistical significance of these comparisons was confounded by ethnicity. Over 90% of the black transplanted patients who had K121Q were diagnosed with PTDM, as were 43% of Hispanics, followed by only 25% of whites (data not shown).

Black race, K121Q, age, BMI, and immunosuppressive medications were found to have the strongest associations with PTDM in the logistic regression and ROC analysis. However, with the established association (confounding) between the presence of K121Q and ethnicity, K121Q did not exert an independent predictive effect, whereas ethnicity, specifically black versus non-black, was the most robust predictor in our model. The model with the largest ROC AUC of 0.80 was comprised of black/non-black, age, BMI, and tacrolimus treatment as significant predictors. Addition of *ENPP1* K121Q polymorphism to this model did not change the ROC AUC. The adjusted OR for K121Q was 1.4 (95% CI, 0.48–4.21, $P=0.52$), whereas unadjusted OR 2.8 (95% CI, 1.22–6.36, $P=0.016$). A reduced model containing only ethnicity (black/non-black) and age as predictors yielded similar results (Fig. 3).

Discussion

The *ENPP1* K121Q allele has been associated with increased risk for type 2 diabetes in several populations,¹⁴ including the Framingham cohort.¹⁵ Although induced impairment in the insulin signaling pathway seems to be the main mediator of systemic insulin resistance and susceptibility to type 2 diabetes conferred by this allele (gain of function),¹⁶ the mechanistic details whereby *ENPP1* K121Q increases diabetes risk is still under active investigation. There are no data regarding the contribution of this allele in diabetes development for special populations. Our study evaluated the role of polymorphic *ENPP1* in predicting development of diabetes in patients who undergo kidney transplant for reasons other than diabetic nephropathy. Previous studies have indicated that approximately 16% of the transplanted patients develop diabetes within 1 year.⁴ We found about 30% of the transplant patients in our cohort

FIG. 2. Percentage of study subjects with *ENPP1* X121Q polymorphism by absence or presence of posttransplant diabetes mellitus (PTDM). P values were determined with the Fisher exact test.

FIG. 3. Receiver operator characteristic (ROC) analysis. Dependent variable is posttransplantation diabetes (PTDM). (Solid line) Model 1, predictor variables: age, BMI, race (black/non-black), and tacrolimus. ROC area under the curve (AUC) = 0.80, 95% confidence interval (CI), 0.72–0.89. (Dashed line) Model 2, predictor variables: age, BMI, race (black/non-black). ROC AUC = 0.79, 95% CI, 0.69–0.88. (Dotted line) Model 3, predictor variables: age and race (black/non-black). ROC AUC = 0.78, 95% CI, 0.69–0.88.

developed diabetes. This higher frequency could be related to the high percentage of blacks participating in our study. Genetic susceptibility could interact with other known contributing variables such as age, increased BMI, and antirejection therapy to increase risk for PTDM.

We found that the frequency of *ENPP1* 121Q allele is higher in the PTDM group than in the group of transplant patients who did not develop diabetes within 1 year. We also found that the *ENPP1* K121Q variant has a strong association with PTDM in the logistic regression and ROC analysis (Fig. 3). However, because our population included three major different ethnicity/race groups, and because the risk for diabetes is related to ethnicity/race, the overall results do not allow a firm conclusion on the allele effects on PTDM risk. With the limitations derived by the relatively small number of study subjects, our study confirms several previous observations reported in regard to predictors of PTDM risk. We confirmed that age, ethnicity, BMI, and therapy with tacrolimus are significant predictors.

Larger studies on the effects of genetics in predicting onset of PTDM have been performed in a Korean population and suggested that genetic variants, such as *SLC30A8*, *TCF7L2*, *HHEX*, *CDKLA1*, *CDKN2A/B*, and *KCNQ1* are associated with increased risk for PTDM.^{7–9} The *ENPP1* K121Q-adjusted OR in our population was 1.4 for the risk of developing diabetes after transplant, which is consistent an expected clinical effect size. The width of the 95% CI from 0.5 to 4.2 (adjusted for race, age, BMI, and tacrolimus) indicates that our study was not powered to detect this size of OR. The analysis is further complicated by the much higher prevalence of the polymorphism in blacks, a well-represented

population among the transplant patients in the United States. Hence, the lack of an independent genetic effect on the risk for development of diabetes in our transplant population could be due to a type 2 statistical error. On the other hand, because the *ENPP1* 121Q allele is thought to increase diabetes risk through worsening insulin resistance, the apparent lack of a significant independent effect of this allele in our study could be related to a predominantly β -cell-dependent mechanism of hyperglycemia on PTDM.

Future studies in much larger sample size will allow discriminating the role of genetics of insulin resistance versus β -cell dysfunction and race/ethnicity in PTDM risk. Of interest in our study, the gene allele frequency within Hispanics and blacks was similar in the study subjects as compared to the general population (Fig. 1). However, the frequency of *ENPP1* K121Q was significantly higher in the white study group as compared to the Dallas Heart Study whites (Fig. 1). We do not have an explanation for this finding. However, two previous studies performed in both type 1¹⁷ and type 2 diabetes patients¹⁸ have suggested a role of the polymorphic *ENPP1* K121Q in early development of nephropathy. Eller et al.¹⁹ have also reported that in patients with end-stage renal disease receiving dialysis, *ENPP1* K121Q variant associates with higher vascular calcifications. The mechanisms involved in these findings are unclear, but they clearly suggest accelerated progression of renal disease in the presence of other initiating etiologic factors (diabetes or end-stage renal disease from other causes) in carriers of K121Q allele and could explain the increased frequency of *ENPP1* K121Q in the white group of our study cohort. Clearly, the possibility of an effect of *ENPP1* 121Q allele on kidney dysfunction leading to transplant in nondiabetic patients deserves further investigation in a much larger cohort.

A previous analysis of U.S. Renal Data System in Medicare beneficiaries who had received kidney transplants identified a PTDM relative risk increase of 68% for African Americans and 35% increase for Hispanics, when compared to the white patients.⁴ That study also identified age, BMI, HCV, and use of tacrolimus as predictors of PTDM. In our study, black race was the strongest predictor of PTDM risk. Over 90% of blacks developed PTDM within 1 year of observation. The ROC analysis depicted in Fig. 3 shows that adding the information of age to the black race provided the most robust information (ROC-AUC 0.78). Adding BMI and tacrolimus treatment did not improve prediction of risk.

In conclusion, this study conducted in a series of consecutive, unrelated patients who underwent kidney transplantation revealed black race and age to be the major determinants of risk for development of diabetes mellitus within 1 year from transplant. BMI and use of tacrolimus also contributed to increasing relative risk but the incremental effect was small. The role of *ENPP1* K121Q genetic variant remains uncertain and is not independently associated with PTDM in this study. Larger studies are needed to assess the independent contribution of genetics to development of PTDM, a major cause of morbidity, transplant failure, and overall mortality for the transplanted patient population.

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