Association of the *PCK2* Gene Polymorphism With New-onset Glucose Intolerance in Japanese Kidney Transplant Recipients

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ABSTRACT

Background. New-onset diabetes mellitus after transplantation (NODAT) is a risk factor for both cardiovascular disease and poor graft survival after kidney transplantation (KTx). In this study, we identified single-nucleotide polymorphisms (SNPs) in genes involved in glucose metabolism and examined the correlation between these SNPs and glucose intolerance after KTx.

Methods. Thirty-eight patients with normal glucose tolerance before KTx were included in this study. Patients with plasma glucose levels of >140 mg/dL at 120 minutes on the 75-g oral glucose tolerance test at 1 year after KTx were classified as having new-onset impaired glucose tolerance (NIGT). We identified 8 SNPs in 7 genes that are involved in glucose metabolism among the patients included in this study, and compared the prevalence rate of NIGT among SNPs in each gene.

Results. Of the 38 patients, 11 (28.9%) were diagnosed with NIGT. For rs4982856 in the PCK2 gene, the distribution of genotypes among the total patient population was as follows: T/T, 12 (31.6%); T/C, 22 (57.9%); and C/C, 4 (10.5%). Seven of 11 patients with NIGT had the T/T genotype of rs4982856, whereas only 5 of 27 patients with normal glucose tolerance had this genotype. The T allele frequency of the rs4982856 was significantly higher in the NIGT group than in the normal group (81.8 vs 52.8%, respectively; $P = .015$).

Conclusion. Our study indicates that the T allele of the rs4982856 SNP in the PCK2 gene may be a risk factor for glucose intolerance after KTx.

DURING the last several decades, advances in immunosuppressive therapy have dramatically reduced the incidence of acute rejection episodes and improved graft survival rate after kidney transplantation (KTx) [1,2]. As a result, cardiovascular diseases (CVD) have become an emerging problem leading to graft loss, especially in kidney recipients with long-functioning grafts. New-onset diabetes mellitus after transplantation (NODAT) is one of the serious complications in patients undergoing KTx; NODAT is known to affect long-term graft function and has also been reported as a major risk factor for CVD [3]. Moreover, a recent study showed that patients diagnosed with impaired glucose tolerance (IGT), according to the definition and diagnosis of diabetes mellitus (DM) and intermediate hyperglycemia by the World Health Organization, as well as patients diagnosed with DM, are at higher risk of CVD than those with normal glucose tolerance [4,5]. Thus, identification of risk factors for IGT may be key to improving long-term patient and graft survival in KTx.

Glucocorticoids (GCs) are the primary agents used as immunosuppressive therapy for KTx. However, GCs induce insulin resistance by attenuating the ability of insulin to inhibit hepatic gluconeogenesis and as well as inhibit lipolysis in adipose tissue, leading to glucose intolerance. This insulin resistance caused by GCs is regarded as the major

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risk factor for NODAT. Therefore, the incidence of NODAT in patients who underwent KTx is reported to be as high as 46% in those treated with a standard steroid dose, but relatively low in those treated with a reduced steroid dose [6,7].

Recent studies have shown that genetic factors are involved in the onset of type 2 DM. For example, polymorphisms of genes responsible for glucose metabolism in the liver and other tissues influence the onset of type 2 DM [8–10]. However, very little is known about the influence of these genetic factors on the occurrence of NODAT or IGT after KTx.

In this study, we focused on genes encoding proteins responsible for glucose metabolism and determined their single-nucleotide polymorphisms (SNPs) in patients who underwent KTx, in order to investigate the correlation between these SNPs and abnormal glucose metabolism after KTx in the Japanese population.

MATERIALS AND METHODS

Patients

Between 2007 and 2015, 110 patients underwent KTx at Kobe University Hospital. Among them, 11 patients who were diagnosed with DM during the pretransplant period, 11 patients who were <15 years of age, and 32 patients who did not agree to participate in the study were excluded from this analysis.

We performed the 75-g oral glucose tolerance test (OGTT) before KTx and at 12 months after KTx. Eighteen patients diagnosed with either IGT or DM before KTx were excluded. Finally, 38 patients with normal glucose tolerance before KTx were eligible for the study. We divided these 38 patients into 2 groups: patients with plasma glucose level >140 mg/dL at 120 minutes in the 75-g OGTT at 1 year after KTx (new-onset impaired glucose tolerance [NIGT] group), and those with plasma glucose level <139 mg/dL (normal glucose tolerance [NOR] group). For all 38 patients, we retrospectively collected recipient and donor age at KTx, gender, body mass index (BMI), duration of dialysis, number of acute rejection episodes, family history of diabetes, type of calcineurin inhibitors, and total amount of methylprednisolone (mPSL) administered until 1 year after KTx.

Immunosuppressive therapy consisted of intravenous administration of either tacrolimus (Tac) or cyclosporine (CsA) for 3 or 4 days, followed by oral administration of either agent targeting an adequate trough concentration. The dose of Tac was determined as the mean dose achieving the targeted trough level of 8 to 10 ng/mL in the first month after KTx, 5–8 ng/mL in the second month, and 5 ng/mL in the third month or later. The dose of CsA was determined as the mean dose achieving the targeted trough level of 200 to 250 ng/mL in the first month after KTx, 150–200 ng/mL in the second month, and 100 to 150 ng/mL in the third month or later. Along with calcineurin inhibitors, mycophenolate mofetil (20 to 30 mg/kg) and mPSL (500 mg bolus on day 0, gradually decreasing to 4 mg/body at 4 weeks after transplantation), and basiliximab (20 mg/kg on days 0 and 4) were used. The maintenance dose of mPSL ranged from 2.0 to 4.0 mg/d, based on the immunosuppressive state of each patient. Acute rejection was treated with intravenous bolus of 500 mg/d mPSL for 3 days.

This study was approved by the research ethics committee of Kobe University Hospital, and written informed consent was obtained from each patient for the use of blood samples for the purpose of research.

SNP Analyses

The genomic DNA of all patients was extracted from peripheral blood mononuclear cells using the NucleoSpin Blood kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), in accordance with the manufacturer's protocol.

We identified 8 SNPs in 7 genes. rs1499821 and rs5398 in *SLC2A2* and rs4982856 in *PCK2* are the SNPs of genes encoding proteins involved in glucose metabolism in the liver, and rs4402960, rs10811661, rs1111875, rs13266634, and rs7756992 in *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8*, and *CDKAL1*, respectively, are the SNPs of genes associated with glucose metabolism in other tissues. We collected blood samples from 38 patients included in this study for routine clinical laboratory testing and detected each SNP in the samples using the TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA). Reactions were performed using an ABI PRISM 7900HT Sequence Detection System (Thermo Fisher) with the following protocol: 1 cycle at 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. After polymerase chain reaction, genotypes were determined using the 7900HT Sequence Detection System.

Statistical Analysis

Chi-square tests were used to test categorical data, which are presented as 95% confidence intervals (CIs) and odds ratios (ORs), and Mann-Whitney *U* tests were used to compare continuous values between groups. The analysis was performed using StatView version 5.0 software (Abacus Concepts, Inc, Berkeley, CA). $P < .05$ was considered statistically significant.

Table 1. Patients' Characteristics

		NIGT	NOR	*P*
No. patients	38	11	27	
Recipient age at KTx (years)	42.5 ± 13.9	37.3 ± 9.0	44.6 ± 15.0	.14
Donor age at KTx (years)	52.0 ± 8.6	50.2 ± 8.7	56.6 ± 6.7	.10
Gender (male/female)	23/15	8/3	15/12	.32
BMI (kg/m^2)	21.3 ± 3.3	22.7 ± 3.0	20.7 ± 3.3	.08
Duration of Dialysis (years)	4.2 ± 4.9	2.7 ± 5.0	4.7 ± 4.8	.26
Family history of Diabetes (+/−)	6/32	2/9	4/23	.79
Episode of acute rejection (+/−)	8/30	3/8	5/22	.54
CNI (Tac/CyA)	31/7	9/2	22/5	.98
Total amount of mPSL (g)	2.75 ± 0.69	2.66 ± 0.66	2.96 ± 0.75	.44

Abbreviations: NIGT, new-onset impaired glucose tolerance group; NOR, normal glucose tolerance group; KTx, kidney transplantation; BMI, body mass index; CNI, calcineurin inhibitor; Tac, tacrolimus; CyA, cyclosporine; mPSL, methylprednisolone.