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COMPLICATIONS
Metabolic

Pilot Study: Association of Traditional and Genetic Risk Factors and New-Onset Diabetes Mellitus Following Kidney Transplantation

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ABSTRACT

Introduction. New-onset diabetes mellitus, which occurs after kidney transplant and type 2 diabetes mellitus (T2DM), shares common risk factors and antecedents in impaired insulin secretion and action. Several genetic polymorphisms have been shown to be associated with T2DM. We hypothesized that transplant recipients who carry risk alleles for T2DM are "tipped over" to develop diabetes mellitus in the posttransplant milieu.

Methods. We investigated the association of genetic and traditional risk factors present before transplantation and the development of new-onset diabetes mellitus after kidney transplantation (NODAT). Markers in 8 known T2DM-linked genes were genotyped using either the iPLEX assay or allelic discrimination (AD)-PCR in the study cohort testing for association with NODAT. We used univariate and multivariate logistic regression models for the association of pretransplant nongenetic and genetic variables with the development of NODAT.

Results. The study cohort included 91 kidney transplant recipients with at least 1 year posttransplant follow-up, including 22 who developed NODAT. We observed that increased age, family history of T2DM, pretransplant obesity, and triglyceridemia were associated with NODAT development. In addition, we observed positive trends, although statistically not significant, for association between T2DM-associated genes and NODAT.

Conclusions. These findings demonstrated an increased NODAT risk among patient with a positive family history for T2DM, which, in conjunction with the observed positive predictive trends of known T2DM-associated genetic polymorphisms with NODAT, was suggestive of a genetic predisposition to NODAT.

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NEW-ONSET diabetes mellitus is a common complication of kidney transplantation (NODAT), with a widely dispersed reported incidence between 2% to 50%.[1] The lack of uniformity in the reported incidence is due to variations in the studied populations, varying immunosuppressive regimens, and the different definitions for diabetes ranging from the American Diabetes Association definition to diabetes being defined after institution of therapy. NODAT is associated with decreased allograft and patient survival.[2–4] Risk factors for the development of NODAT include traditional ones, such as age,[5,6] obesity,[3,7] ethnicity (African American[3,6,8] and Hispanic ethnicity,[3,9] family history of diabetes, presence of hepatitis C, and receipt of a deceased donor transplant.[6,8] Additionally, various diabetogenic immunosuppressants (corticosteroids,[10,11] calcineurin inhibitors,[3,12–14] sirolimus[15]) contribute to the development of NODAT. The diabetogenic effect of glucocorticoids is primarily caused by insulin resistance followed by enhanced gluconeogenesis in the liver and decreased glucose uptake and glycogen synthesis in skeletal muscle cells.[16,17] The pathogenesis of the diabetogenic effect of calcineurin inhibitors is attributed to both impaired insulin sensitivity and inhibition of insulin production by beta cells.[18–25]

Mechanistically, NODAT and type 2 diabetes mellitus (T2DM) have common antecedents in impaired insulin secretion and insulin action; both diseases share many of the same risk factors. It has also been shown that first-degree relatives of individuals with T2DM have up to 3.5-fold greater risk for NODAT development compared to the general population.[26] Key genes previously shown to be involved in T2DM susceptibility include transcription factor 7-like 2 (*TCF7L2*), peroxisome proliferator-activated receptor gamma (*PPARG*), potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*), solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*), cyclin-dependent kinase inhibitors *CDKN2A*/*CDKN2B,* the insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), and others.[27–35] Interestingly, it has been observed that many of the genes influence diabetes risk by affecting insulin secretion.[36–38] Moreover, recent studies have shown that the risk allele in a SNP in *SLC30A8* associated with T2DM in multiple studies was also linked with increased risk of NODAT in renal allograft patients.[39]

Type 2 diabetes mellitus is thought to be a complex polygenic disease. Because transplant-associated diabetes shares a similar clinical behavior and presentation as does T2DM, it is possible that similar nongenetic and genetic variants that increase susceptibility to T2DM may also influence NODAT development in kidney transplant recipients. We hypothesized that, in addition to risk conferred by traditional risk factors measured before transplantation, genes with known effects on T2DM susceptibility may also contribute to increased risk in the development of NODAT.

SUBJECTS AND METHODS

Study Participants

The study cohort consisted of a random sample of all patients above 18 years of age undergoing first kidney transplantations between 2003 and 2006. Patients eligible for inclusion were all those who had no diagnosis of T2DM prior to transplant (normal fasting glucose and Hb $A_{1c} < 6.0$ pretransplant and not on therapy for T2DM pretransplant) and with at least 1 year of posttransplant follow-up. Informed consent was obtained and the study was approved by our Institutional Review Board. NODAT was defined as the ongoing requirement for insulin therapy or oral diabetogenic agents beyond 1 month after transplant. We chose this time point because several factors, including the stress of surgery and introduction of immunosuppressive therapy, produce transient hyperglycemia during the first month posttransplant, which could potentially confound a NODAT diagnosis.

Single Nucleotide Polymorphism (SNP) Genotyping

Genomic DNA was extracted from saliva specimens using the Oragene kit according to the manufacturer's specifications (DNA Genotek, Ottawa, Ontario), and hydrated in TE (pH = 8.0). The DNA concentration was measured by the NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, Del), with an average yield of 13.3 μg DNA. The DNA quality was assessed using A260/A280 absorbance measurements and agarose gel electrophoresis; no visible product degradation was observed. We used this DNA to genotype variants in genes previously shown to be associated with T2DM across multiple populations including *TCF7L2, PPARG, KCNJ11, SLC30A8, CDKN2A*/*CDKN2B, IGF2BP2,* and others. All markers except *KCNJ11* rs5219 and *IGF2BP2* rs1470579 were genotyped using both the iPLEX assay in conjunction with the MassARRAY platform (Sequenom, La Jolla, Calif). In this method, primers and multiplex conditions were designed using the Assay Design v4.0 software, and DNA amplification and iPLEX primer extension were performed according to the manufacturer's protocol (Sequenom). Markers rs5219 and rs1470579 were genotyped by AD-PCR using TaqMan SNP Genotyping Assays and 7000 Sequence Detection System according to the manufacturer's protocol (Applied Biosystems). The observed genotype frequency for each SNP was assessed for deviation from that expected under Hardy-Weinberg equilibrium (HWE) using chi-square analysis, and 14 duplicate samples were used to assess data quality. Assays were considered successful and genotype data subsequently analyzed if (1) a minimum of 90% of all genotyping calls were obtained, (2) markers did not deviate significantly ($P \leq .05$) from HWE, and (3) genotyping error results were <3%.

The sample call rate per SNP ranged from 96.88% to 100%, and the rate of successful SNPs genotyped per individual ranged from 73.33% to 100%, with an average call rate of 98.89%. Of the 16 markers selected for genotyping, only rs7903146, located in the *TCF7L2* gene, failed multiple times owing to >10% missing calls. Therefore, our overall SNP success rate was 93.75%.

Data Analysis

Descriptive analyses of continuous variables were performed using Student *t* tests for continuous variables and chi-square to compare proportions. The extent to which observed genotype frequencies for each SNP deviated from that expected under the HWE was assessed; none of the markers varied significantly from the HWE. In addition, encrypted samples were used to assess data quality.

We used a logistic regression model to measure univariate and