**TCF7L2 Polymorphism Associates with New-Onset Diabetes after Transplantation**

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**ABSTRACT**

New-onset diabetes after transplantation (NODAT) is a serious and frequent complication in transplant recipients. Whether NODAT shares the same susceptibility genes as type 2 diabetes is unknown. In this multicenter study, we genotyped 1076 white patients without diabetes at transplantation for 11 polymorphisms that associate with type 2 diabetes. We defined NODAT as a fasting plasma glucose ≥126 mg/dl on at least two occasions or de novo hypoglycemic therapy. We compared clinical and genetic factors between patients who developed NODAT within 6 mo of transplantation (n = 118; incidence 11%) and patients without diabetes (n = 958). In multivariate analysis, NODAT significantly associated with the following characteristics: TCF7L2 polymorphism (odds ratio [OR] 1.60 per each T allele; P = 0.002), age (OR 1.03 per year; P < 0.001), body mass index at transplantation (OR 1.09 per unit; P < 0.001), tacrolimus use (OR 2.26; P < 0.001), and the occurrence of a corticoid-treated acute rejection episode (OR 2.78; P < 0.001). In summary, our data show that the TCF7L2 rs7903146 polymorphism, a known risk factor for type 2 diabetes in the general population, also associates with NODAT.


New-onset diabetes after transplantation (NODAT) is a serious and frequent complication in recipients of solid-organ grafts. NODAT is associated with poor patient and graft survival.1,2 A recent prospective study reported an incidence of approximately 15% within the first 6 mo after renal transplantation in patients under calcineurin inhibitor therapy.3 Risk factors for NODAT are the same as for type 2 diabetes in the general population: Age >40 yr, obesity, ethnicity (black and Hispanic), family history of type 2 diabetes, alterations of glucose metabolism, and hepatitis C carrier status.4 Immunosuppressive drugs contribute to the risk for NODAT by causing insulin resistance (corticosteroids) and reducing insulin secretion (mainly tacrolimus).

In the past 10 yr, association studies of candidate genes identified several genes involved in type 2 diabetes: KCNJ11, PPARG, HNF1B, and WFS1.5–8 These genes encode proteins that have strong mechanistic links with diabetes. Very rare, highly penetrant mutations in all four of these genes cause monogenic forms of diabetes.8 The more recently identified transcription factor 7-like 2 (TCF7L2)9,10 is expressed in pancreatic β cells and is involved in the control of insulin secretion.11 Recent genome-
wide association studies confirmed several loci (TCF7L2, KCNJ11, and PPARG) and identified novel type 2 diabetes susceptibility loci. Among the three, latter seemed associated with an impaired insulin secretion (CDKAL1, SLC30A8, and HHEX-IDE region) and one with an increased fat mass (FTO). Less is known about the IGF2BP2 and the CDKN2A-CDKN2B loci. The risk alleles at each of these loci were associated with a 10 to 37% increase in the relative odds of diabetes, with TCF7L2 emerging as the most significant locus. It is important to investigate whether the risk factors for NODAT are the same as for type 2 diabetes in the general population both from a general mechanistic viewpoint and from a practical viewpoint. Indeed, this knowledge might help the individual tailoring of immunosuppression before and after transplantation. To date, two studies of Korean renal transplant patients have shown a significant association of NODAT with the rs7903146 variant of TCF7L2 and rs1326634 variant of SLC30A8. These studies included very-late-onset NODAT cases (up to 10 yr after transplantation) and were performed with patients of a Southeast Asian genetic background. Here, we analyzed the association between 11 well-established type 2 diabetes susceptibility genes and the occurrence of NODAT within the first 6 mo after transplantation in a large cohort of 1229 predominantly white (87.6%) renal transplant patients.

## RESULTS

### Patient Characteristics

Among the 1229 eligible patients, 1076 (87.6%) were white, 87 (7.1%) were north African, 43 (3.5%) were central African, 11 were Asian (0.9%), and 12 were of other rare ethnic origin. The overall incidence of NODAT within the first 6 mo after transplantation was 11.0, 19.5, 20.9, and 9.1% among white, north African, central African, and Asian patients, respectively. A total of 328 white patients developed impaired fasting glucose (30.5%) whereas 630 patients remained euglycemic (58.5%). Cases of NODAT were identified via hyperglycemia in 49.7%, de novo prescription of glucose-lowering therapy in 36.5%, and both in 13.8% of patients. Characteristics of white patients at baseline and 3 and 6 mo are detailed in Table 1. In the group of patients with NODAT, mean age (P < 0.0001) and body mass index (BMI) at transplantation (P = 0.0001) as well as 6 mo after transplantation (P = 0.001) were significantly higher. The proportion of patients with NODAT was higher in those under tacrolimus (P = 0.002) and mammalian target of rapamycin (mTOR) inhibitors (P = 0.004) than in patients under cyclosporin A as primary immunosuppressive agent. There were more steroid-treated acute rejection episodes in patients with NODAT (P = 0.001).

### Genotype Distribution of the 11 Single-Nucleotide Polymorphisms and Association with NODAT

Genotype distributions of the 11 single-nucleotide polymorphisms (SNPs) and their respective odds ratio (OR) for NODAT are shown in Table 2 (white patients, n = 1076), and in Appendix 1 (whole cohort, n = 1229). The CT and the TT genotypes of rs7903146 (TCF7L2) were more frequent in white patients with NODAT (47.5 and 12.7%, respectively) than in those without NODAT (39.1 and 8.7%, respectively). The CT genotype increased the odds of NODAT by 59% (P = 0.03) and the TT genotype by 92% (P = 0.04) as compared with the CC genotype. None of the 10 other SNPs reached statistical significance for association with NODAT. TCF7L2 rs7903146 was also the only polymorphism significantly associated with NODAT in the whole cohort (OR 1.55 [P = 0.02] for CT genotype; OR 1.79 [P = 0.04] for TT genotype).

As secondary end point, we separately analyzed white patients for the association of the rs7903146 polymorphism with the development of NODAT and impaired fasting glucose, respectively, as compared with the euglycemia group (Table 3). When patients with NODAT were compared with patients with euglycemia, CT (OR 1.58; P = 0.04) and TT (OR 1.96; P = 0.04) genotypes were more frequent in patients with NODAT than CC genotype. On the contrary, when patients with impaired fasting glucose were compared with the patients with euglycemia, both subgroups had a similar genotype distribution.

In addition, we analyzed the association of the rs7903146 polymorphism with NODAT in the three minority ethnic groups. We found no significant association in northern African (n = 87), central African (n = 43) and Asian (n = 11) patients. In the very limited number of nonwhite patients, we found no TT homozy-
The independent risk factors significantly associated with NODAT in white patients (Table 4) were rs7903146 (TCF7L2) polymorphism (OR 1.60; \( P = 0.002 \)), tacrolimus use (OR 2.26; \( P < 0.001 \)), higher BMI at baseline (OR 1.09 per unit; \( P < 0.001 \)), older age (OR 1.03 per year; \( P < 0.001 \)), and the occurrence of a corticoid-treated acute rejection episode (OR 2.78; \( P < 0.001 \)). Among the white patients who developed both NODAT and an acute rejection episode during the first 6 mo after transplantation (\( n = 37 \)), 81.1\% developed NODAT after
the acute rejection episode, whereas 18.9% developed NODAT before. In a second regression analysis model, with the heterozygous and homozygous states of the rs7903146 polymorphism considered as independent indicator variables, the odds of NODAT increased by 70% for the CT genotype (OR 1.70; \( P = 0.017 \)) and by 142% for the TT genotype (OR 2.42; \( P = 0.009 \)) as compared with the CC genotype. In the analysis of the global cohort, in addition to the risk factors found in white patients, mTOR inhibitor use (OR 3.46; \( P = 0.001 \)) and northern or central African ethnicity (OR 1.80; \( P = 0.019 \)) were also independent risk factors for NODAT (Appendix 2).

**Genotype Distribution of the 11 SNPs in Patients with Type 2 Diabetes**

The initial screening detected 104 patients with a history of type 2 diabetes in the whole cohort and 83 cases among white patients. These patients were not included in the main analyses but analyzed separately as an internal control of the role of the SNPs in type 2 diabetes. We compared white patients who had type 2 diabetes with white patients who had euglycemia (\( n = 630 \)) for the 11 SNP genotypes (Appendix 3). Four SNPs were significantly associated with type 2 diabetes in our population. For rs7903146 (TCF7L2), we found significantly more CT genotypes (OR 1.93; \( P = 0.009 \)) and more TT genotypes (OR 2.33; \( P = 0.02 \)) than CC genotypes in patients with diabetes. For rs8050136 (FTO), there were significantly more CA genotypes (OR 2.53; \( P = 0.002 \)) and more AA genotypes (OR 2.61; \( P = 0.008 \)) than CC genotypes. For rs7548430 (CDKAL1), there were more CC (OR 2.14; \( P = 0.02 \)) than GG genotypes. For rs4402960 (IGF2BP2), there were more TT genotypes (OR 2.40; \( P = 0.006 \)) than GG genotypes in patients with diabetes.

**Allele Frequencies and Hardy-Weinberg Equilibrium**

Allele frequencies of the 11 SNPs by ethnicity group are shown in Appendix 4. In white patients, all minor allelic frequencies (MAFs) were \( \geq 10\% \). In the northern African, central African, and Asian groups, all MAFs were \( > 5\% \), except for PPARG (rs1801282), whose G allele was absent in the central African and Asian samples. Deviation from Hardy-Weinberg equilibrium was evaluated in white control subjects (\( n = 958 \); Appendix 5). The following polymorphisms significantly deviated from equilibrium: rs1111875 (HHEX-IDE region; \( \chi^2 = 12.03, P = 0.0005 \)), rs10811661 (CDKN2A-CDKN2B region; \( \chi^2 = 9.31, P = 0.002 \)), and rs757210 (HNF1B; \( \chi^2 = 12.78, P = 0.0004 \)).

**DISCUSSION**

Our multicenter study of 1076 white renal transplant recipients showed that the rs7903146 polymorphism of TCF7L2 is independently associated with NODAT, defined as the new occurrence of diabetes during the first 6 mo after transplantation. The OR was 1.70 for one T allele and 2.42 for two T alleles. Common variants in TCF7L2 emerged as the most significant signal associated with type 2 diabetes in the general population in each genome-wide study performed to date.14–20 Each T allele of the key TCF7L2 SNP rs7903146 increased type 2 diabetes risk with an OR of 1.37. We observed no significant association of NODAT with polymorphisms of the 10 other type 2 diabetes–predisposing genes tested. First, these 10 gene variants showed modest OR for type 2 diabetes in the general population (1.10 to 1.20)8 and hence probably require a larger cohort to detect a significant effect. Second, we found no GG homozygotes for the rs1801282 polymorphism (PPARG) in the NODAT group, which precluded the analysis of association of this SNP with NODAT in our cohort. Finally, a recent meta-analysis of three previous genome-wide association studies (\( n = 10,128 \)) could not confirm the association of the SLC30A8 and HNF1B loci with type 2 diabetes and showed only a borderline association with the WFS1 locus.19 Our results are in agreement with the study of Kang et al.,12 who reported a significant association between the TCF7L2 rs7903146 polymorphism and NODAT in a cohort of 511 Korean renal transplant patients. Our study clearly confirms these observations on a larger cohort of patients from a different genetic background. In addition, the restriction of our cohort to patients with early-onset diabetes after transplantation (6 mo) avoids the inclusion of patients with true type 2 diabetes that may occur later in the course of transplantation. Furthermore, this is the first large-scale cohort study that analyzed the association of NODAT with 11 well-established type 2 diabetes susceptibility genes.8

The association between the rs7903146 polymorphism of TCF7L2 and NODAT was significant in our global cohort as well as in the white group. ORs, however, were higher in the white population than in the global cohort, both in univariate and multivariate analyses. We cannot draw conclusions from the observations made in the three less represented ethnicities (no significant association), given the limited number of patients; however, the TCF7L2 polymorphism has been associated with NODAT in a larger cohort of Asian patients.12 In addition, association between this polymorphism and type 2 diabetes has been well demonstrated in African Americans and in West Africans,21,22 but data are less consistent in northern African populations.23–25

Older age, BMI, and tacrolimus use were other significant independent risk factors in white patients. Steroid boluses, given for acute rejection episodes, also accounted for the risk for developing NODAT. Maintenance steroids at 3 or 6 mo were NS risk factors in univariate analysis. Whether steroid withdrawal or avoidance is associated with a lower prevalence...
of NODAT is still unclear.\textsuperscript{26–28} mTOR inhibitor use and African ethnicity were significant independent risk factors for NODAT in the whole cohort. Sirolimus as main immunosuppressive drug was previously shown to be an independent risk factor for NODAT in renal transplant patients.\textsuperscript{29} This drug is associated with \textit{in vivo} insulin resistance\textsuperscript{30} and defective insulin secretion.\textsuperscript{31} Calcineurin inhibitors impair insulin secretion by interfering with nuclear factor of activated T cell signaling in pancreatic \(\beta\) islets. This pathway also induces the expression of genes critical for \(\beta\) cell function, including all six genes mutated in hereditary forms of monogenic type 2 diabetes.\textsuperscript{32}

The \textit{TCF7L2} rs7903146 SNP was not associated with the development of impaired fasting glucose in our cohort. This subgroup behaved like the subgroup with euglycemia. We can perhaps hypothesize that immunosuppressive drugs triggered impaired fasting glucose independent of the genetic background in a significant fraction of patients in our cohort.

We found a significant association between pretransplantation type 2 diabetes and the polymorphisms of \textit{TCF7L2}, \textit{FTO}, \textit{CDKAL1}, and \textit{IGF2BP2}. The lack of significant association with the seven other type 2 diabetes–predisposing gene polymorphisms is probably because the sample size was not suited to detect modest ORs (1.10 to 1.20 in the general population).

Two potential limitations of our study have to be acknowledged. First, the exclusion of patients with a history of type 2 diabetes might lead to the selective exclusion of risk alleles from our cohort, which could lead to an underestimation of the true risk conferred by these variants. It is very unlikely, however, that the association of the remaining risk alleles with NODAT in the cohort would merely result from selective allelic depletion in the control subjects. Second, we cannot completely rule out that the observed association of the \textit{TCF7L2} rs7903146 SNP with NODAT might be due to a random error; however, that the same polymorphism is a potent risk factor for type 2 diabetes in the general population, the dose–response effect of the number of alleles on the odds of NODAT in our cohort, and the persistence of the association in the multivariate analysis argue against such a spurious association.

Three SNPs were not in Hardy–Weinberg equilibrium in white patients; however the eight other SNPs, which were in Hardy–Weinberg equilibrium, showed no systematic trend toward a deficit in heterozygous samples, arguing strongly against genetic admixture within the white control subjects as an explanation for the observed excess of homozygotes. An artificial deficit in heterozygotes because of a technical error is an alternative explanation; however, we used a technique that is robust for detecting such genotyping errors. We therefore do not have a valid explanation at the time. Further large genotype–phenotype association studies might help to uncover a possible association between these SNPs and ESRD. Of note, \textit{HNF1B} (\textit{TCF2}) encodes a homeobox transcription factor involved in kidney development, and mutations in this gene have been associated with a wide range of renal abnormalities, including renal cortical cysts and renal dysplasia\textsuperscript{33–35}; however, no link between renal disease and \textit{HHEX-IDE} or \textit{CDKN2A-CDKN2B} regions has been described.

Whether the rs7903146 polymorphism of \textit{TCF7L2}, located in an intron, represents the true causal variant or is in very tight linkage disequilibrium with it remains to be clarified. The rs7903146 polymorphism T-bearing allele of \textit{TCF7L2} has been associated with \textit{in vivo} impaired insulin secretion, impaired incretin effects, hepatic insulin resistance, and increased mRNA expression in pancreatic \(\beta\) cells. \textit{TCF7L2} encodes a high-mobility group box–containing transcription factor involved in Wnt signaling and transactivates a number of genes involved in pancreatic \(\beta\) cell proliferation. The detailed mechanisms by which alterations in \textit{TCF7L2} expression impair the secretion of insulin and perhaps of gastrointestinal incretins require further studies.\textsuperscript{11}

Our data show that the \textit{TCF7L2} rs7903146 polymorphism, combined with other risk factors such as high BMI, older age, African ethnicity, or immunosuppressive therapies that reduce insulin secretion and increase insulin resistance, results in a major risk for development of NODAT after renal transplantation. Genotyping \textit{TCF7L2} might improve individual tailoring of immunosuppression before and after organ transplantation.

**CONCISE METHODS**

**Patients**

We collected DNA samples from 1477 consecutive patients who received a renal transplant (deceased or living donors) between 1994 and 2007 in four different centers (ULB-Erasme Hospital, Bruxelles, Belgium; CHU of Tours, France; CHU of Limoges, France; and CHRU of Lille, France). All patients signed an informed consent for genetic analysis. We enrolled 1229 eligible patients, including 1076 white patients, in this study (Figure 1). Exclusion criteria were (1) patients with a history of type 1 or 2 diabetes, (2) lack of data on glucose metabolism, (3) follow-up period shorter than 6 mo, (4) duplicate sampling from patients with successive grafts, (5) age < 18 yr, and (6) a combined transplant (kidney–pancreas or kidney–liver).

**Definition of Glucose Metabolism Abnormalities**

NODAT was defined by either the 2003 diagnostic criteria of the American Diabetes Association\textsuperscript{46} (fasting plasma glucose [FPG] ≥ 126 mg/dl [7.0 mmol/L] on at least two occasions) at 3 or 6 mo and/or \textit{de novo} prescription of hypoglycemic therapy within 6 mo after transplantation. Patients receiving any oral antidiabetic medication or insulin for > 14 d between day 15 and month 6 were considered as affected by NODAT. The case group consisted of patients who developed NODAT during the first 6 mo after transplantation, and the control group consisted of patients who did not develop NODAT within the first 6 mo after transplantation (FPG < 126 mg/dl without any hypoglycemic therapy).

The impaired fasting glucose subgroup included patients with FPG between 100 and 125 mg/dl at 3 or 6 mo, and the euglycemia subgroup included patients with FPG < 100 mg/dl (5.6 mmol/L) at 3...
Significant and the OR reported. Among the three SNPs showing a number of genome-wide association studies reporting the SNP as significant, we selected only one SNP at each locus. Our selection was based on the Utah CEPH Hapmap (http://www.hapmap.org). For this reason, between 0.95 and 1.00 for all of these clusters of SNPs (data based on linkage disequilibrium. The $D_r$ value of the OR showed the highest significance. Two recent studies reported a significant association of type 2 diabetes with three SNPs in linkage disequilibrium ($D_r = 0.95$) at the HNF1B locus. The first study, designed to discover loci conferring risk for prostate cancer, showed in a secondary analysis that two SNPs conferred protection against type 2 diabetes. We selected the SNP identified in the second study, which consisted of a candidate gene approach testing maturity-onset diabetes of the young gene loci. The two susceptibility SNPs in WFS1 were in strong linkage disequilibrium and their OR for type 2 diabetes was similar, so we selected one arbitrarily.

Genotyping Analyses

TaqMan Drug Metabolism genotyping assay (for rs1801282, PPARG) and TaqMan SNP genotyping assays (for the 10 other SNPs) were used for SNP genotyping (Applied Biosystems). PCRs were performed in 96-well plates, with a final reaction volume of 15 µl, containing 10 ng of genomic DNA, 7.5 µl of TaqMan Genotyping Master Mix (Applied Biosystems), and 0.8 µl of assay mix (20×). PCR thermal cycling conditions were as follows: 95°C for 10 min to activate DNA polymerase, followed by 50 cycles of 92°C for 15 s and 60°C for 90 s for rs1801282 and 95°C for 10 min, followed by 40 cycles (45 cycles for rs757210) of 92°C for 15 s and 60°C for 60 s for the 10 other SNPs. All PCRs were performed using a 9800 fast thermal cycler (Applied Biosystems), and the end point fluorescence readings were performed on a 7500 Fast Real-time PCR system (Applied Biosystems). All of the 11 TaqMan SNP genotyping assays were tested on DNA from members of four different families to ensure the accuracy of genotyping. One no-template control was included in each 96-well plate. For each undetermined genotype, we performed a second genotyping process. When the genotype remained undetermined after this second run, we considered definitively the genotype as undetermined and discarded the sample. The genotype call rate was 97.8% for rs757210 and >99% for the 10 other SNPs.

Statistical Analysis

The primary outcome was to evaluate the impact of 11 SNPs (in TCF7L2, FTO, CDKAL1, KCNJ11, HHEX-IDE region, SLC30A8, CDKN2A-CDKN2B region, IGF2BP2, HNF1B, WFS1, and PPARG genes) on the risk for development of NODAT within the first 6 mo after renal transplantation. We reported as main analyses those performed in the white patients ($n = 1076$) to minimize population stratification effects related to ethnic origin; however, we also reported the most important data from the whole cohort ($n = 1229$). The measure of effect was the exposure OR of the various SNPs in patients with NODAT as compared with those without NODAT with the corresponding 95% confidence intervals. We tested the null hypothesis of no association between each of the SNPs and NODAT (as well as for type 2 diabetes). These were separate measures of the effect of the individual SNPs on NODAT. We did not adjust the significance level of the $P$ value for multiple hypothesis testing because we tested associations between NODAT and SNPs on the basis of the biologic rationale that these SNPs are known to be associated with type 2 diabetes.
diabetes. Adjusting for multiple testing would pose a major risk for type II error in being unable to reject the null hypothesis of no association while the effect truly exists; therefore, we took the option to show clearly the crude data in 2 × 2 tables and to test the null hypothesis of no association between each separate SNP and NODAT.

We stratified the cohort in three categories according to outcome status at 6 mo (euglycemia, impaired fasting glucose, and NODAT). We then calculated the exposure ORs of the SNPs previously identified as risk factors for NODAT in the overall cohort by restricting the control group to patients with euglycemia. We also assessed the association of SNPs with impaired fasting glucose as compared with euglycemia as a secondary objective. Moreover, we evaluated the prevalence of the 11 SNPs in our patients with type 2 diabetes. These analyses were performed to evaluate whether we could confirm the previously described association of the 11 SNPs with type 2 diabetes in our population with end-stage renal failure.

Categorical data were compared using the χ² or Fisher exact tests as appropriate. ANOVA was used to compare continuous data. A bilateral P < 0.05 was used to reject the null hypothesis. The association of SNPs with NODAT after adjustment for other risk factors was assessed by multivariate logistic regression modeling. The model was constructed by progressively adding independent variables starting with those that had the strongest univariate association with the outcome of interest. The Wald test was used to test the null hypothesis of a log OR (coefficient) equal to 0. The likelihood ratio test was used to assess whether adding a new variable to the model increased the overall log-likelihood. To test for a potential interaction between two risk factors, we calculated stratum-specific ORs and tested the null hypothesis of no difference between stratum-specific ORs by a χ² test of homogeneity. Deviation from Hardy-Weinberg equilibrium was tested by χ² test for the 11 genetic variants (1 df). The Fisher test was used for low-MAF SNPs (<5%).

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DISCLOSURES

None.

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