Analysis of single nucleotide polymorphisms implicate mTOR signalling in the development of new-onset diabetes after transplantation

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ABSTRACT

Introduction: Despite excellent first year outcomes in kidney transplantation, there remain significant long-term complications related to new-onset diabetes after transplantation (NODAT). The purpose of this study was to validate the findings of previous investigations of candidate gene variants in patients undergoing a protocolised, contemporary immunosuppression regimen, using detailed serial biochemical testing to identify NODAT development.
Methods: One hundred twelve live and deceased donor renal transplant recipients were prospectively followed-up for NODAT onset, biochemical testing at days 7, 90, and 365 after transplantation. Sixty-eight patients were included after exclusion for non-white ethnicity and pre-transplant diabetes. Literature review to identify candidate gene variants was undertaken as described previously.
Results: Over 25% of patients developed NODAT. In an adjusted model for age, sex, BMI, and BMI change over 12 months, five out of the studied 37 single nucleotide polymorphisms (SNPs) were significantly associated with NODAT: rs16936667:PRDM14 OR 10.57;95% CI 1.8–63.0;p = 0.01, rs1801282:PPARG OR 8.5; 95% CI 1.4–52.7; p = 0.02, rs8192678:PPARGC1A OR 0.26; 95% CI 0.08–0.91; p = 0.03, rs2144908:HNF4A OR 7.0; 95% CI 1.1–45.0;p = 0.04 and rs2340721:ATF6 OR 0.21; 95%CI 0.04–1.0; p = 0.05.
Conclusion: This study represents a replication study of candidate SNPs associated with developing NODAT and implicates mTOR as the central regulator via altered insulin sensitivity, pancreatic β cell, and mitochondrial survival and dysfunction as evidenced by the five SNPs.

General significance:

1) Highlights the importance of careful biochemical phenotyping with oral glucose tolerance tests to diagnose NODAT in reducing time to diagnosis and missed cases.
2) This alters potential genotype:phenotype association.
3) The replication study generates the hypothesis that mTOR signalling pathway may be involved in NODAT development.

1. Introduction

Despite excellent first-year outcomes in kidney transplantation, there remain significant long-term complications of premature graft loss, morbidity, and mortality related to infection and cardiovascular disease. New-onset diabetes after transplantation (NODAT) is the major form of post-transplant hyperglycaemia that is associated with such complications. Thus research has focussed on preventative measures to post-transplant hyperglycaemia development, identification of those at risk in a timely manner, and investigating molecular pathways to its development.

Investigation of single nucleotide polymorphism (SNP) variants offers such a strategy. A number of previous studies have investigated specific candidate gene variants, usually on the basis of prior evidence of association with type 2 diabetes in non-transplanted individuals. While some such SNPs have shown an association with NODAT, little attempt has been made to replicate findings in independent cohorts. More recently, the first genome-wide association study (GWAS) in the field has been reported [1]. In this study, NODAT was defined as the use of oral hypoglycaemics or insulin over a 12 year follow-up period, with the median time of NODAT onset of 100 months, and recent work highlights the growing momentum to use biochemical testing when identifying post-transplant hyperglycaemia [2,3]. It is also worth noting that 25% of patients in this GWAS study were treated with a calcineurin inhibitor-free maintenance regimen. Our group has recently shown the use of biochemical definitions of post-transplant hyperglycaemia can alter the clinical phenotype-to-genotype association to such an effect that none of the eight β 'glucotoxic' SNPs identified in the GWAS were associated with NODAT in our cohort [4].

The purpose of this study was to validate the findings of the GWAS and other candidate NODAT SNPs investigated in the Belfast study [1], but in a prospective study of patients undergoing a protocolised, contemporary immunosuppression regimen, and using detailed serial biochemical testing to identify the development of NODAT.

2. Patients and methods

From 2009 to 2012, 112 live and deceased donor renal transplant recipients were prospectively followed-up over a 12 month period in a single-centre adult tertiary centre at Queen Elizabeth Hospital Birmingham, United Kingdom. To exclude pre-existing diabetes, patients underwent glucose testing (after a minimum of 8 h fasting) immediately prior to transplantation and excluded if ≥6.1 mmol/l or HbA1c ≥6.5% (48 mmol/mol). In addition, live donor recipients underwent oral glucose tolerance testing (OGTT) with a standard 75 g glucose load within a week prior to transplantation. Following transplantation, OGTTs were then performed at 7 days, and then 3 months and 12 months in all patients except those who developed clinically manifest hyperglycaemia requiring treatment. NODAT was diagnosed if a) fasting glucose ≥7 mmol/l or 2 h OGTT was ≥11.1 mmol/l from day 7 onwards and persisted at the 3 month timepoint, b) HbA1c ≥6.5% (48 mmol/mol) from 3 months onwards, or c) requirement for institution of therapy for NODAT in which case OGTT was not undertaken (fasting clinic glucose was ≥7 mmol/l in all such patients). Exclusion criteria of pre-transplant diabetes and the requirement for exclusion of non-white patients to avoid population stratification in a genetic investigation such as this resulted in 68 patients being prospectively followed.

All patients received an identical immunosuppression regimen consisting of anti-CD25 monoclonal antibody induction followed by maintenance tacrolimus (target per-dose trough levels 5–8 ng/ml by liquid chromatography with tandem mass spectrometry), mycophenolate mofetil (2 g daily dose), and prednisolone (20 mg/day initially weaning to 5 mg/day by 3 months, and then continued).

Literature review to identify candidate gene variants was undertaken as described previously [1]. The eight β 'glucotoxic' SNPs that have been previously been investigated for NODAT in this cohort were excluded [4]. Genotyping were performed using Sequenom iPLEX technology.

The study received approval from the local research ethics committee (NRES West Midlands Black Country 08/H1204/103) prior to commencement and was conducted in accordance with the Declarations of Helsinki and Istanbul with patient written consent.

2.1. Statistical analysis

Data are shown as median (1st and 3rd quartiles) if not normally distributed or mean (±standard deviation) if normally distributed.

Baseline demographics were assessed using Mann–Whitney U (non-parametric data) or Student t test if normally distributed for continuous data, and Fisher exact testing for categorical data as appropriate using SPSS software, version 20 (SPSS Inc., Chicago, Illinois) for analysis. Genotype distributions were assessed for concordance with Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit test with a type 1 error rate set at 5% analysed using PLINK [5]. Genotype to phenotype associations and event analyses were conducted using logistic regression with the development of NODAT at any time during the first 12 months post-transplantation as the end measure of interest (time-to-event analysis was not undertaken due to only 2 post-transplant timepoints). Univariate genotype:phenotype relationships and then the relationship in a multivariate model fully adjusted for age, sex, baseline body mass index (BMI), and change in BMI over 12 months from transplantation (no selection process) were calculated using PLINK [5].

3. Results

Demographics of the cohort are shown in Table 1. The cohort was aged 45 years (±15), human leucocyte antigen (HLA) mismatched 2.41 (±1.43), body mass index increase of 1.0 (±2.2) with 68% undergoing live kidney transplantation. Eighteen patients (26.5%) were diagnosed with NODAT, of whom 11 patients (61.1%) were diagnosed on the basis of the result of OGTT testing alone. Patients developing NODAT were older and displayed greater changes in BMI over the first year of post-transplantation (p < 0.05 for both). There were no significant differences between patients developing and not developing NODAT for age, HLA mismatch, rejection episodes, overall steroid dose used per day, tacrolimus levels, or presence of adult polycystic kidney disease (p > 0.05 for all). No patients had a prevalent or incident hepatitis C virus infection.

Out of the remaining 42 candidate SNPs that were identified by literature review [1], 37 were successfully genotyped (rs1800961 [HNFA], rs2069763 [IL-2], rs2265917 [SHPRH], rs6903252 [intergenic], and rs7903146 [TCF7L2] were unavailable as they were not amenable to the Sequenom iPLEX genotype bundle designs). The genotype success rate for the 37 SNPs was >99%. Six SNPs (rs10117679 [GRIN3A], rs1016429 [GRIN3A], rs12255372 [TCF7L2], rs17657199 [NDST1], rs2070874 [IL-4], rs2240747 [ZNRF4]) demonstrated deviation from Hardy–Weinberg equilibrium (p < 0.05).

In the adjusted logistic regression model, five SNPs were significantly associated with NODAT: rs16936667 [PRDM14: OR 10.57; 95% CI 1.8–63.0; p = 0.01], rs1801282 [PPARG: OR 8.5; 95% CI 1.4–52.7; p = 0.02], rs8192678 [PPARGC1A: OR 0.26; 95% CI 0.08–0.91; p = 0.03], rs2144908 [HNF4A: OR 7.0; 95% CI 1.1–45.0; p = 0.04], and rs2340721 [ATF6: OR 0.21; 95% CI 0.04–1.0; p = 0.05] (Table 2).

4. Discussion

This study identifies SNP variants in common genes which are associated with the development of NODAT following kidney transplantation, thereby hypothesis generating to our understanding of mechanisms involved (discussed below) and the potential for risk-stratifying patients pre-transplantation. Some of the features of the study are relevant and worth noting in the context of its findings. Firstly, all patients underwent screening for diabetes prior to transplantation, and so the episodes of diabetes following transplantation were truly 'new onset' rather than pre-transplant diabetes which was picked up post-transplantation. Indeed, for the live donor recipients in the study, OGTTs were also undertaken to exclude pre-transplant diabetes. For the diagnosis of diabetes following transplantation, OGTTs were undertaken at serial timepoints in a carefully phenotyped prospective cohort, and so we believe that this study is particularly sensitive to the development of NODAT. Interestingly, the majority of patients were only diagnosed as a result of the OGTTs conducted specifically and additionally as part of this research study and would have been missed (or the