Pharmacogenetic and Clinical Risk Factors for Diabetes After Transplant in a Single Pediatric Centre

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Aim:
- To study the prevalence of post kidney transplant diabetes (NODAT) in a single Asian pediatric centre.
- To identify clinical factors and single nucleotide polymorphisms (SNPs) which may predict NODAT.

Methods:
- Kidney patients from Shaw-NKF Children's Kidney Centre, National University Hospital transplanted between Feb 1989 and Oct 2017 were recruited.
- NODAT diagnosis was based on American Diabetes Association guidelines.
- Clinical parameters were collected retrospectively up to Jan 2018.
- Blood samples were collected after consent was obtained.
- DNA was extracted from leukocytes and amplified via polymerase chain reaction using the single base extension technique.
- After amplification, DNA was genotyped using fragment analysis.
- 20 SNPs from the following genes associated with type 2 diabetes mellitus were screened; HHEX rs7923837, HHEX rs5015480, TCF7L2 rs4506565, IGF2BP2 rs4402960, CDKAL1 rs1094398, ADIPOQ rs266729, ADIPOQ rs2241766, CDKAL1 rs2280789, CCL5 rs2280789, CCL5 rs2107538, CCL5 rs3817655, ABCB1 rs1045642, CYP3A4*1G rs2242480, PAI-1 rs1799889, KCNQ1 rs2237892, KCNQ1 2237895, KCNQ1 2237897, SLC30A8 rs13266634, HHEX rs1111875, CYP3A5 rs776746 .
- Parametric tests were used for univariate analysis between groups while Cox regression was used for multivariate analysis.

Results:
- 45 kidney transplant patients (14.2 ± 6.7 years at transplant; yrs) were recruited of which 9 (20%) patients had NODAT.2 patients received a second kidney transplant.
- Mean follow-up duration was 7.0 ± 7.1 years.
- NODAT was diagnosed at 2.7 ± 3.7 years post transplant.
- 1 patient had NODAT after switching maintenance cyclosporine to tacrolimus.
- On univariate analysis, NODAT patients have significantly higher body mass indices compared to non-NODAT patients (p=0.05) (Table 1). However, this significance is lost on multivariate analysis (Table 2).
- On multivariate analysis, male patients were found less likely to develop NODAT compared to females (p 0.02, 95% CI 0.0-0.6, Table 2) after adjusting for ethnicity, body mass index, CMV and dyslipidemia status.
- At 10 years post-transplant, 77.8% of patients on tacrolimus compared to 88.9% of patients on cyclosporine as initial immunosuppression were diabetes-free.
- Kaplan-Meier survival curves showed earlier onset of NODAT in patients whose initial immunosuppression was tacrolimus vs cyclosporine (Figure 1).
- IGF2BP2 rs4402960 SNP was found to be associated with NODAT. IGF2BP2 gene codes for Insulin-like growth factor 2 mRNA-binding protein 2. The T allele is reported in 38% of the local population and reported to confer risk of type 2 diabetes in East Asian, European and South Asian populations.* Frequency of rs4402960 SNPs variants in the NODAT population was significantly different from that of non-NODAT. (p=0.008, Table 3).
- IGF2BP2 rs4402960 T/T haplotype was associated with increased risk of NODAT (HR 29, CI 2.83-296.92, p=0.02).

Table 1. Baseline characteristics of NODAT and non-NODAT patients

Characteristics	NODAT (n=9)	non-NODAT (n=36)	p value
Age at Tx (years) mean ± SD	16.3 ± 3.9	13.7 ± 7.2	0.31
Male, n (%)	1 (11.1)	16 (44.4)	0.06
Ethnicity Chinese, n (%) Malay, n (%) Indian, n (%) Other, n (%)	7 (77.8) 1 (11.1) 0 (0) 1 (11.1)	24 (66.7) 9 (25.0) 3 (8.3) 0 (0)	0.14
Dial years before Tx (years) mean ± SD	3.4 ± 3.1	3.7 ± 3.3	0.82
Body mass index (kg/m^2) mean ± SD	19.4 ± 4.5	17.0 ± 2.8	0.048
Dyslipidemia, n (%)	4 (50.0)	12 (46.2)	0.58
Initial immunosuppression Tacrolimus, n (%) Cyclosporine, n (%)	8 (88.9) 1 (11.1)	27 (75.0) 9 (25.0)	0.66

Tx: transplant; Dial: dialysis.

Table 2. Multivariate analysis of pretransplant clinical risk factors for NODAT

| Pretransplant factors	Hazard ratio	p value	CI (95%)
Male	0.05	0.02	0.0-0.6
Body Mass Index	1.1	0.17	0.9-1.3
Dyslipidemia	0.5	0.41	0.1-2.7
CMV D+R-#	3.4	0.18	0.6-20.8
Chinese	0.8	0.86	0.1-8.1
Malay	0.1	0.14	0.0-2.1
Indian	0.0	0.99	0.0

CMV D+R-: cytomegalovirus donor positive serology, recipient negative serology at transplant

Table 3. Distribution of IGF2BP2 variants in the transplant population

Genetic Variants	S'pore %*	NODAT n (%)	non-NODAT n (%)	p value
rs4402960 TT	3	1 (25)	0 (0)	
rs4402960 GT	32	1 (25)	1 (3.8)	0.008
rs4402960 GG	65	2 (50)	25 (96.2)	

* Percentage variants in the Singapore population, derived from Teo YY, Sim X, Ong RTH, et al. Singapore Genome Variation Project: A Haplotype map of three South-East Asian populations. Genome Research (In press).

Figure 1.Time from transplant to diagnosis of NODAT, comparing tacrolimus and cyclosporine as initial immunosuppression. Patients on tacrolimus were diagnosed with NODAT earlier than cyclosporine.
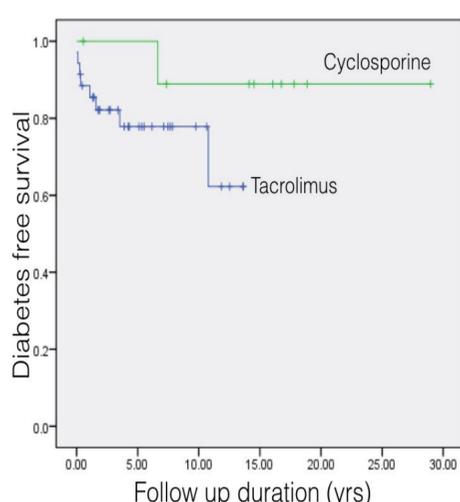
Conclusions:
- Prevalence of NODAT in our cohort was 20%.
- The female gender is a possible risk factor in NODAT.
- s4402960 SNPs in the IGF2BP2 gene were also identified as possible risk factor.
- Patients on initial immunosuppression of tacrolimus developed NODAT earlier than cyclosporine.