Original Article

Importance of glucokinase -258G/A polymorphism in Asian Indians with post-transplant and type 2 diabetes mellitus

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Summary	Type 2 diabetes mellitus (T2DM) and post-transplant diabetes mellitus (PTDM) are non-synonymous forms of diabetes. Glucokinase (GCK) plays a key role in glucose metabolism. The relationship between the *GCK* promoter and specific types of diabetes, such as PTDM and T2DM, in the Asian Indian population is unknown. We examined the occurrence of a specific *GCK* promoter variant (-258G/A) in patients with T2DM and PTDM. The case-control study enrolled 640 Asian Indian subjects, including controls (*n* = 250) and T2DM (*n* = 250), PTDM (*n* = 42), and non-post-transplant diabetes mellitus (non-PTDM) (*n* = 98) patients. Purified Deoxyribonucleic acid (DNA) was genotyped with the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. The digested PCR products were analyzed on 12% polyacrylamide gels. The anthropometric, biochemical, and clinical details of each group were documented. *GCK* -258G/A alleles and genotypes were not associated with T2DM. However, among PTDM subjects, we detected a higher frequency of heterozygotes (52.4%) and a positive association with alleles/genotypes. The results suggest that the promoter region (-258G/A) of *GCK* plays an important role in PTDM in Asian Indians.

Keywords: GCK, -258G/A, T2DM, PTDM, Asian Indians

1. Introduction

Multifactorial metabolic disorders in humans include type 2 diabetes mellitus (T2DM) and post-transplant diabetes mellitus (PTDM), an irreversible form of diabetes that occurs in men and women. Impaired β-cell function and insulin resistance are key determinants of T2DM (*1*). The etiology of T2DM is thought to involve genetic and environmental factors, and the disease is characterized by decreased insulin secretion and insulin resistance (*2*). Hyperglycemia in the fasting state is one criterion that defines the disease; it can predict hard clinical endpoints in non-diabetic individuals, as well as in T2DM patients

when corrected (*3*). PTDM, also known as new-onset diabetes after transplantation (NODAT), is a frequent complication in renal transplant (RT) patients who receive immunosuppressive therapy with calcineurin inhibitors (*4*). Recipients with PTDM/NODAT and non-PTDM receive immunosuppressive drugs such as cyclosporine (CsA) and Tacrolimus (Tac) after renal transplantation and exhibit complications similar to those of T2DM patients in the general population, but with an accelerated rate of onset (*5,6*). Previous investigations have focused on candidate genes expressed in pancreatic β-cells that modulate insulin secretion and resistance and predispose individuals to PTDM and T2DM in later life. One such gene, glucokinase (*GCK*), encodes an enzyme in the glycolysis pathway that regulates glucose-stimulated insulin secretion from pancreatic β-cells and metabolizes glucose in the liver (*7*).

Hepatic GCK activity is closely and rapidly adapted by short and long term regulatory mechanisms. Peter *et al*. (*8*) group for the first time demonstrated the positive correlation between hepatic GCK expression and liver

triglyceride content in humans. A reduction in GCK levels or activity in β-cells could increase the glucose threshold for insulin secretion, leading to atypical hyperglycaemic status (*9*). The *GCK* gene is located at chromosome 7p15.3 and has 12 exons (*10*). *GCK* genetic variants contribute to the risk of T2DM in men and women of different ethnicities, but their frequencies in Asian Indians with PTDM and T2DM have not been examined. There are no studies that have been carried out with -258G/A variants in the Indian population with any of these diseases. Hence, the current study investigated whether the -258G/A variant in the promoter region of *GCK* modifies the risk of T2DM and PTDM in Asian Indians with and without a family history of T2DM.

2. Materials and Methods

2.1. *Ethical approval*

The study was approved by the ethics committee of Kamineni Hospitals, Hyderabad, India (KHL No. e373/07). The participants signed written informed consent forms before enrolment.

2.2. *Selection of subjects*

For this study, we recruited 640 Asian Indian subjects: 250 subjects with T2DM, 250 healthy controls, 42 subjects with PTDM, and 98 subjects without PTDM (non-PTDM). The selection of T2DM subjects, controls, and PTDM subjects and the inclusion and exclusion criteria for the specific groups are described in previous publications (*1,5,11*). We collected 3 mL of serum for biochemical analysis and 2 mL of anticoagulated blood for mutational analysis. The anthropometric, biochemical, and clinical measurements for T2DM, PTDM, and non-PTDM cases and control subjects are described in our previous reports (*1,5,11*).

2.3. *Mutational analysis*

DNA was routinely purified at Kamineni Hospitals using a salting out procedure (*12,13*). The region 173 bp upstream of the *GCK* coding region was genotyped with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Primers were designed according to previous studies (*14*) and synthesized by BioServe Biotechnologies (Hyderabad, India). The amplified products were digested with the *Acc*I restriction enzyme (GTG↓TAC) for 2 hours, and the fragments were analyzed on 12% polyacrylamide gels stained with ethidium bromide (Figure 1).

2.4. *Statistical analysis*

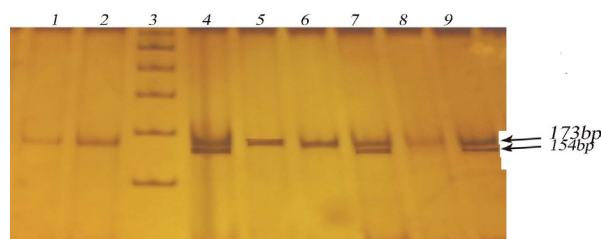Clinical data were expressed as the mean ± standard deviation (SD). Differences in genotype and allele

Figure 1: Digested products separated by 12% polyacrylamide gel electrophoresis for assessment of -258G/A GCK variants. Lane: **1**, **2**, **5**, **6**, **8** Homezygous GG 173bp; Lane: **3** Ladder (100bp); Lane: **4**, **7**, **9** Heterozygous GA 173/154bp.

frequencies were determined with the gene counting method for T2DM *vs*. controls and PTDM *vs*. non-PTDM subjects. An odds ratio (OR) with a 95% confidence interval (CI) was calculated for the -258G/A mutation. The chi-square (χ^2) test was used to compare expected and observed frequencies of categorical variables, at a significance level $p = 0.05$. The Yates correction was also used to analyze genotype frequencies. The Hardy-Weinberg equilibrium (HWE) was measured using the χ^2 test to assess goodness of fit (*15*). A value of $p < 0.05$ was considered to indicate a significant disequilibrium. Statistical calculations were performed with Openepi software.

3. Results

3.1. *Baseline characteristics of T2DM patients and controls*

The anthropometric, biochemical, and clinical characteristics of the study groups are shown in Table 1. The average age was 57.19 ± 8.22 years for T2DM patients and 53.93 ± 6.32 years for controls. The mean body mass index (BMI) was 27.5 ± 4.1 kg/m² for T2DM cases and 25.8 ± 3.9 kg/m² for controls. There were significant differences between T2DM patients and controls in the levels of fasting blood sugar, post-lunch blood sugar, triglycerides, HDL-C, and total cholesterol ($p < 0.05$). Gender, BMI, LDL-C, and family history were not significantly associated with T2DM ($p > 0.05$), although family history was closely associated with T2DM (58.4%).

3.2. *Characteristics of the PTDM and non-PTDM subjects*

The characteristics of the PTDM and non-PTDM subjects are given in Table 2. Biochemical tests were used to assess PTDM in 140 RT patients; 30% of RT recipients developed PTDM. The study included 42 patients diagnosed with PTDM and 98 patients diagnosed as non-PTDM subjects. The PTDM patient group contained 30 men and 12 women, with similar mean ages, 39.39 ± 2.12 and 40.0 1± 11.63,

Table 1. Clinical details of T2DM patients and the healthy controls

Characteristics	T2DM Cases ($n = 250$)	Healthy Controls ($n = 250$)	p value
Age (Years)	41-82 (57.19 ± 8.22)	41-60 (53.93 ± 6.32)	0.0003
Males/Females (%)	138 (55.2%)/112 (44.8%)	144 (57.6%)/106(42.4%)	0.3461
BMI (kg/m^2)	27.5 ± 4.1	25.8 ± 3.9	0.4306
T2DM Interval	13.1 ± 6.3	NA	NA
FBS (mg/dL)	143.61 ± 55.66	93.54 ± 12.13	0.0001
PPBG (mg/dL)	201.29 ± 25.25	117.29 ± 19.07	0.0001
TG (mg/dL)	156.42 ± 78.97	138.77 ± 53.69	0.0001
TC (mg/dL)	183.95 ± 51.54	175.06 ± 33.05	0.0001
HDL-C (mg/dL)	88.72 ± 23.1	82.61 ± 20.6	0.01
LDL-C (mg/dL)	38.76 ± 4.4	35.53 ± 4.1	0.2658
Family History, n (%)	146 (58.4%)	138 (55.2%)	0.3745

NA = Not Analyzed/ Not Applicable.

Table 2. Clinical characteristics of PTDM and non-PTDM subjects

Baseline characteristics	PTDM ($n = 42$)	non-PTDM ($n = 98$)	p value
Males / Females	0/12	75/23	0.001
Age:			
a) Males (Mean ± SD)	39.39 ± 12.12	39.55 ± 10.58	0.27
b) Females (Mean ± SD)	40.01 ± 11.63	39.26 ± 10.87	0.58
Weight:			
a) Males (Mean ± SD)	62.73 ± 15.81	66.03 ±12.73	0.08
b) Females (Mean± SD)	61.71 ± 16.93	65.49 ± 13.68	0.09
Therapy:			
a) On CsA therapy	22	58	0.01
b) On Tac therapy	20	40	0.02
Dose:			
a) CsA Dose (mg)	163.88 ± 57.4	201.29 ± 76.86	0.03
b) Tac Dose (mg)	3.15 ± 1.24	3.11 ± 1.62	0.05
Levels:			
a) C2 levels (ng/mL) CsA	750 ± 299.03	1024.8 ± 353.42	0.23
b) Trough levels (ng/mL) Tac	9.55 ± 3.38	8.0 ± 3.32	0.86
Levels:			
a) C2 levels/dose of CsA	5.24 ± 2.59	5.52 ± 1.97	0.02
b) Trough levels/dose of Tac	3.62 ± 1.96	2.98 ± 1.49	0.02

respectively. Of the 42 patients with PTDM, 22 (54%) were receiving cyclosporine A (CsA) treatment, whereas 20 (47.6%) were receiving tacrolimus (Tac). The mean age of the PTDM patients on the date of post-transplant blood sample collection was 20.4 ± 15.3 years. The t-test was performed between PTDM and non-PTDM subjects, gender, weight, subjects with cyclosporine drugs (*i.e.*, CsA, Tac) and along with the dosage were found statistically significant ($p < 0.05$).

3.3. *Hardy-Weinberg Equilibrium*

The distributions of the genotype frequencies of the -258G/A variants in case and control subjects were in accordance with the HWE (Table 3). The genotypic distribution of GCK variants and their allelic frequencies in the patients (T2DM and PTDM) and controls enrolled in this study are shown in Table 4.

3.4. *Association of the -258G/A variant with T2DM and PTDM*

The genotype and allele distributions in cases (T2DM

Table 3. Details of Hardy Weinberg equilibrium

Gene	Glucokinase
SNP ID	rs1799831
Chromosome	7p15.3
Position (Build36.3)	44,199,142
PMID	11112984
Organism	Homosapiens
Alleles 1/2	G/A
Risk allele	A
T2DM genotypes (11/12/22)	246/04/00
PTDM genotypes (11/12/22)	20/22/00
Non-PTDM genotypes (11/12/22)	92/06/00
Control Genotypes (11/12/22)	248/02/00
Risk allele frequency (T/P*)	0.8/26.2
HWE (Chi square) (T/P*)	0.02/1.73
HWE (p value) (T/P*)	0.99/0.9

(T/P*) indicates T2DM/PTDM.

and PTDM) and controls (healthy controls and non-PTDM) are shown in Table 4. The genotype frequency of GG and GA was 98.4% and 1.6% in T2DM cases and 99.2% and 0.8% in controls, respectively. In the statistical analysis, we did not find any association

Table 4. Genotype and allele distribution of -258G/A *GCK* variants in all cases (T2DM and PTDM) and controls (healthy controls and non-PTDM)

GCK	T2DM (*n* = 250)	Controls (*n* = 250)	χ^2	Odds ratio[a&b] (95% CI)	*p* value
GG	246 (98.4%)	248 (99.2%)	Reference*	-	-
GA	04 (1.6%)	02 (0.8%)	0.67	2.0 (0.36, 11.1)	0.41
AA	00 (00)	00 (00)	0.001	1.0 (0.01-51)	0.31
GA+AA	04 (1.6%)	02 (0.8%)	0.57	1.8 (0.38, 8.6)	0.70
G	496 (99.2%)	498 (99.6%)	Reference*	-	-
A	04 (0.8%)	02 (0.4%)	0.67	2.0 (0.36, 11.0)	0.41
	PTDM (*n* = 42)	Non-PTDM (*n* = 98)	χ^2	Odds ratio[a&b] (95% CI)	*p* value
GG	20 (47.6%)	92 (93.9%)	Reference*	-	-
GA	22 (52.4%)	06 (6.1%)	39.04	16.8 (6.0, 46.9)	0.0001
AA	00 (00)*	00 (00)	0.66	4.5 (0.08, 234.1)	0.63
GA+AA	22 (52.4%)	06 (6.1%)	38.35	15.6 (5.7, 42.2)	0.0001
G	62 (0.74)	190 (0.97)	Reference*	-	-
A	22 (0.26)	06 (0.03)	34.83	11.2 (4.3, 28.9)	0.0001

[a-] Crude odds ratio (95 % CI); [b-] odds ratio (95 % CI) Adjusted for Yates correction. *Reference indicated the normal allele/genotypes *i.e.* GG is normal/homozygous genotype and G allele is wild allele.

between alleles and genotypes (G *vs*. A: OR = 2.0 [95% CI: 0.36-11.0], *p* = 0.41; GA *vs*. GG: OR = 2.0 [95% CI: 0.36-11.1], *p* = 0.41). Statistically significant differences in allele and genotype frequencies were observed when PTDM and non-PTDM subjects were compared (G *vs*. A: OR = 11.2 [95% CI: 4.3-28.9], *p* < 0.0001; GA *vs*. GG: OR = 16.8 [95% CI: 6.0-46.9], *p* < 0.0001). The dominant model also showed a significant association (GA+AA *vs*. AA: OR = 15.6 [95% CI: 5.7-42.2], *p* < 0.0001).

4. Discussion

Gene identification is important for understanding the pathophysiology of disease and for improving diagnosis, prevention, and treatment (*16*). We performed a case-control study to evaluate the association between -258G/A *GCK* variants and PTDM and T2DM in the Asian Indian population. To our knowledge, this study is the first to find that alleles and genotypes are associated with an increased risk of PTDM (*p* < 0.0001). T2DM and PTDM are chronic disorders characterized by hyperglycemia, insulin resistance, and impaired insulin secretion due to pancreatic β-cell defects; the diseases have a strong genetic component (*7*). In India, 65.1 million adults have been diagnosed with T2DM (*17*). PTDM has become a serious health complication worldwide. Insulin is used to monitor glucose values for more than 6 months after transplantation, in PTDM subjects and lifelong without transplantation. In those with T2DM, the disease develops in the later thirties and early forties and progresses. Those with PTDM develop diabetes 3 months after RT and may later develop T2DM.

One of our goals was to investigate whether -258 G/A variants in the promoter region of *GCK* are associated with T2DM in the Asian Indian population. We compared T2DM cases with control subjects and found no significant differences in allele or genotype

frequencies. This was the first study carried out in this population. The discrepancy between the results of the present study (T2DM *vs*. controls) and those of Chinese studies can be attributed to the *GCK* gene in the Chinese population (*18*). Our study is consistent with three previous studies of T2DM in different races and ethnicities, which found that *GCK* was not associated with T2DM (*19-21*). Our results also showed that the -258G/A GCK variant had no significant effect on abnormal glucose levels in T2DM patients receiving noninsulin treatment. The extent to which the *GCK* promoter variants affect these traits might vary, possibly explaining the contrasting results. Mutations in *GCK* can alter the reaction. Some rare exonic mutations have been investigated previously, and the role of these mutations in the pathogenesis of reduced fetal growth and in adult insulin resistance has been established. *GCK* is associated with reduced β-cell function, impaired glucose tolerance, and T2DM (*22*). Yang *et al*. (*23*) performed a meta-analysis of the -30G>A *GCK* polymorphism in gestational diabetes mellitus (GDM) and T2DM and concluded that the A allele was a risk factor for GDM and T2DM among whites. Fu *et al*. (*7*) also performed meta-analysis studies of T2DM with 298,468 subjects, and the results agree with those of Yang *et al*. (*23*). Our study is not in agreement with these studies. The small sample size could be one reason.

In the comparison of PTDM *vs*. non-PTDM patients, promoter region variants were significantly associated with PTDM (*p* < 0.0001). Our results in PTDM subjects are in accordance with those from a study of a Korean population (*24*). In our study population, PTDM and GDM (unpublished data) were strongly associated with *GCK*. Given these results, we cannot conclude that mutations in *GCK* have no role in the Asian Indian population. The lack of any significant association between the variants and disease might be due to several

factors, including ethnicity, low sample size, and low statistical power. Of 640 subjects, none had the AA genotype, suggesting that the AA genotype is associated with disease development.

The current study has several limitations. Firstly, we enrolled a small number of PTDM (n = 42), and non-PTDM (n = 98) subjects. Secondly, only promoter region variants were selected. Thirdly, GDM data was excluded to strengthen the study. In addition, insulin levels were not measured. Finally, biochemical data for PTDM and non-PTDM subjects were not obtained. Our study suggests that large, unbiased epidemiological studies of genetic predisposition can provide insight into the *in vivo* relationship between candidate genes and diseases. The reproduction of genetic association studies can be problematic because of insufficient power, multiple hypothesis testing, population stratification, source of controls, publication bias, and phenotypic heterogeneity (*25*).

5. Discussion

The findings of this study indicate that PTDM has a strong genetic association with *GCK*. Our findings also indicate that the -258G/A variant in the promoter region of *GCK* is not an independent risk factor for the development of T2DM in Asian Indian subjects.

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