P-0470 Association of single nucleotide polymorphism of KCNJ11 (E23K) gene with susceptibility to type 2 diabetes in Bangladeshi population

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Introduction:

- Type 2 diabetes mellitus (T2DM) is a multifactorial disease and its prevalence has been rapidly increasing throughout the world which is associated with several gene polymorphisms.
- KATP channels regulate insulin secretion by coupling the metabolic state of the cell to membrane potential.
- KCNJ11 gene encodes for the Kir6.2 subunit of the ATP-sensitive potassium channel in the pancreatic beta cell; thus, mutation in this gene cause impaired insulin secretion leading to T2DM.
- Elevation of blood glucose level leads to an increase in ATP/ADP ratio and a decrease in KATP channel permeability that in turn leads to membrane depolarization, activation of voltage-dependent calcium channels, Ca²⁺ influx into the cell and finally occurred insulin secretion or insulin vesicle exocytosis.

Figure 1: Etiology of KCNJ11 gene related to insulin secretion

Objective:

To investigate the association between E23K polymorphism of KCNJ11 gene and T2DM in Bangladeshi population

Materials and Method's:

Study design:

This is a prospective cross-sectional study.

Figure 2: Our Study design

Study subjects:

It was a case-control study, which was carried out with 200 unrelated subjects of 100 healthy controls and 100 diabetic patients.

A. Biochemical measurements:

- The serum fasting glucose (FSG), HbA1C, insulin (INS) and lipid profiles were estimated by GOD-PAP, HPLC, ELISA and CHOD-PAP enzymatic colorimetry methods respectively.
- HOMAB% and HOMAS% were calculated by HOMA-SIGMA Software.

B. Genomic investigations:

• DNA isolation and amplification:

DNA isolation from all patients and controls are carried out using organic method and amplified by PCR using respective primers, dNTP, and taq DNA polymerase. PCR was carried out using the following primer set:

Forward primer	5'-GAATACGTCCTGACACGCCT-3'
Reverse primer	5'-GCCAGCTGCACAGGAAGGACAT-3'

• PCR Condition for KCNJ11 (E23K)rs5219 gene

PCR Conditions for the amplification included initial step of denaturation 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 1 minute and elongation at 72°C for 1 minute and finally a step of final elongation at 72°C for 3 minutes.

Figure 3: PCR Condition for KCNJ11 (E23K) gene

• Genotyping of Subjects using RFLP:

PCR products were digested and incubated for 24 hours with Eco 241 (Ban II) at 37°C in water bath. Restriction Fragment Length Polymorphism (RFLP) digested product were directly separated by electrophoresis in 3% agarose gel. Eco 241 (Ban II) digestion produces three fragments depending on the genotype; EE: 178, 40 bp (Wild type); KK: 218 bp (Homozygous) and EK: 218, 178, 40 bp (Heterozygous).

Figure 4: RFLP detection of the E23K variant of the Kir6.2 gene.

(1) 250 bp ladder, (2) undigested sample – 218 bp, (3) wild type – 178 and 40 bp, (4) homozygous mutated – 218 bp, (5) heterozygous mutated – 218, 178 and 40 bp. The 40 bp fragments are not shown.

Statistical Analysis:

Data were analyzed using independent t-test and Chi-square or Fisher exact test, as appropriate. $p < 0.05$ was considered as significant.

Results:

- A significant differences were shown in FSG (M±SD, mmol/L, Case vs Control, 7.5±2.6 vs 5.3±0.7; $p < 0.001$) and HbA1c level (M±SD, %, Case vs Control, 7.9±1.7 vs 5.4±0.6; $p < 0.001$) between two group respectively.
- Insulin level [Med (Min- Max), μ U/mL, Case vs Control, 18.62 (3.13-44.04) vs 12.85 (1.12-26.50), $p < 0.001$] and beta cell secretory defect (HOMAB %, M±SD, Case vs Control, 128.4±79.7 vs 124.67±71.2, $p = ns$) were higher; and insulin sensitivity (HOMAS %, M±SD, Case vs control, 38.76±25.8 vs 69.9±46.5; $p = 0.002$) was significantly lower in case compared to control respectively.
- Lipid profiles were also higher in case in comparison to control but HDL level showed a significant difference between two groups (M±SD, mg/dL control 43±12 Vs case 40±8; $p < 0.001$).

Table 1: Demographic and Clinical Data of the Study Subjects

Variables	Control Group (n=100)	Diabetic Group (n=100)	P value
Male (%)	64 (64%)	49 (49%)	-
Female (%)	36 (36%)	51 (51%)	-
BMI	25.5±3.7	24.2±3.8	0.957
HbA1c (%)	5.4±0.6	7.9±1.7	0.000
Fasting Insulin	13.7±6.6	20.4±8.8	0.050
HOMA B%	124.6±71.2	123.4±79.7	0.791
HOMA S%	69.9±46.5	38.7±25.8	0.000
Triglycerides (mg/dL)	146±74	164±88	0.177
Cholesterol (mg/dL)	180±48	156±48	0.397
HDL- Cholesterol (mg/dL)	43±12	40±8	0.000
LDL- Cholesterol (mg/dL)	108±43	84±49	0.132

FBG: Fasting blood glucose; $p < 0.01$.

A statistically significant association was found in E23K heterozygous genotype between T2DM and control [$p < 0.0001$, OR (95% CI)- 4.20 (2.27-7.80)]. However, no association was found in homozygous K23K genotype [$p = 1.000$, OR (95% CI)-1.01 (0.28-3.67)]; whereas K alleles of cases were significantly higher than control [$p < 0.05$, OR (95% CI) - 1.90 (1.40-3.20)] respectively.

Table 1: Demographic and Clinical Data of the Study Subjects

	Diabetic (n=100)	Control (n=100)	p value	OR (95% CI)
Genotype				
E23E	39 (39%)	69 (69%)		1 (Reference)
E23K	57 (57%)	24 (24%)	<0.001	4.20 (2.27-7.80)
K23K	4 (4%)	7 (7%)	1.000	1.01 (0.28-3.67)
E	96	93		1
K	61	31	0.015	1.90 (1.40-3.20)

Odds ratios (OR) and 95% confidence interval (95% CI). Results are expressed as number (percentage). $p < 0.05$ was taken as level of significance.

Table 3: Distribution of KCNJ11 (E23K) Genotypes in Diabetes Group According to Gender

	Male (n=49)	Female (n=51)	p Value	OR(95% CI)
Genotype				
E23E	20 (40.82%)	19 (37.25%)		1 (Reference)
E23K	27 (55.10%)	30 (58.82%)	0.835	1.17 (0.51-2.64)
K23K	2 (4.08%)	2 (3.92%)	1.000	1.05 (0.13-8.24)

Odds ratios (OR) and 95% confidence interval (95% CI). Results are expressed as number (percentage). $p < 0.05$ was taken as level of significance.

Discussion:

- In biochemical characteristics of T2D and control, a significant association among glucose, HbA1c and HDL level were found. KCNJ11 gene was analyzed for investigation of the association between E23K polymorphism with prevalence of T2DM.
- Li 1 found E23K contributed to T2D susceptibility. However, Wang et al. 2 did not found any relationship between the E23K and T2D. Our study showed that frequency of E23K genotype is significantly ($p < 0.001$) higher in compared with diabetic patients.
- UKPDS showed frequency of EK in T2D was a little higher than glucose tolerated people3.
- Asaf et al 4 had shown the inheritance of the K allele predisposes for T2D. Moreover, we found that carriers of K allele have more risk for T2D ($P < 0.05$) which showed the higher insulin level and lower sensitivity ($p = NS$) than E variant. This finding correlates with Chistiakov et al ($P = 0.023$) and also characterized the loci for susceptibility to T2D and altered insulin secretion5.

Conclusion:

From the obtained data, it can be concluded that KCNJ11 (E23K) gene polymorphism is associated with T2DM in Bangladesh.

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