GENETIC VARIATIONS IN THE PANCREATIC ATP-SENTIVE POTASSIUM CHANNEL, AND SUSCEPTIBILITY TO TYPE 2 DIABETES IN TUNISIANS

A. KALLEL[1], R. DAOUED[1], Y. SEDIRI[1], B. FTOUHI[2], M. FEKI[1], H. SLIMANE[2], R. JEMAA[1], N. KAABACHI[1].
[1]- Research Laboratory LR99ES11, Biochemistry Department, La Rabta Hospital; Tunis El Manar University, Tunisia
[2]- Endocrinology Department, La Rabta Hospital, Tunis, Tunisia.

Introduction

The pancreatic beta cell adenosine triphosphate (ATP) sensitive potassium K_{ATP} channel is a key component in insulin secretion. These channels comprise two sub-units : a pore forming, K+ inward rectifer (Kir 6.2) (KCNJ11) and a sulfonylurea receptor (SUR 1) (ABCC8). Several variants genes have been associated with disorders of insulin secretion. We investigated whether common polymorphisms in the SUR1 and Kir6.2 genes are associated with incrased risk of type 2 diabetes (T2D) in a Tunisian population.

Methods

A total of 753 individuals were included in the study. A total of 391 patients with T2D were enrolled from the Department of Endocrinology, Rabta University Hospital of Tunis. The mean age of this group was 55 years (SD 8). The control group included 362 unrelated healthy volunteers. Patients and controls were homogeneous Tunisian Arab descendents who resided in Tunisia and all were from North Tunisia.
Blood samples were obtained from all subjects after an overnight fast. Plasma levels of total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) were measured by standardized enzymatic procedures on a Hitachi 912 analyser. LDL cholesterol (LDL-C) was calculated according to Friedewald's formula when the plasma TG concentrations did not exceed 4.8 mmol/L.
Genomic DNA was prepared from white blood cells by phenol extraction. The E23K (rs5219) of the KCNJ11 gene and the SUR1 gene 31 exon (rs179859) polymorphisms were performed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis (Figures 1 & 2)

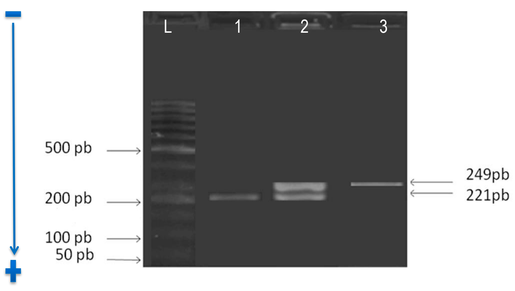

Figure 1: agarose gel electrophoresis (3%) photo for the E23K genotyping after *Eco24I* enzymatic digestion
Lane L: 50 bp DNA ladder; Lane 1 : mutant type homozygous 23K23K; Lane 2: heterozygous E23K; Lane 3 homozygous EE

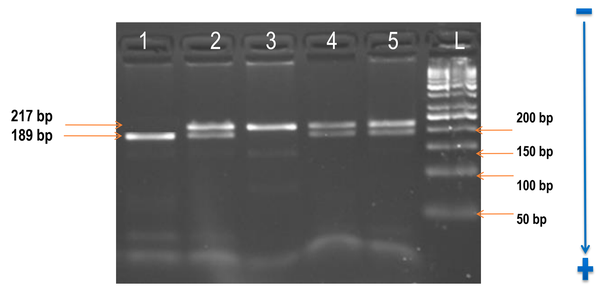

Figure 2: Gel electrophoresis of PCR amplified fragments of the 1273 G/A polymorphism after BscLI enzymatic digestion
Lane L: 50 bp DNA ladder ; Lane 3: wild type homozygous GG ; Lane 2,4,5: heterozygous G/A

Results

The clinical and biochemical characteristics of T2D patients and control subjects are shown in Table 1.

Table 1: Demographic and clinical characteristics of the study population.

	Patients N=391	Controls N= 362	P
Age (years)	55.4 ± 8.9	55.0 ± 9.2	0.553
BMI (Kg/m²)	30.9 ± 5.61	27.6 ± 5.0	< 0.001
Hypertension %	31.1	14.3	0.001
Dyslipidaemia %	39.7	18.9	< 0.001
Obesity %	48.6	32.4	< 0.001
Smokers %	21.4	16.8	<0.001
Glycemia (mom/L)	9.60 ±0.74	5.16 ±0.85	<0.001
TC (mmol/L)	4.99 ± 1.08	4.92 ± 0.98	0.322
TG (mmol/L)	1.53 ± 0.82	1.42 ± 1.07	0.113
LDL-C (mmol/L)	3.23 ± 0.82	3.03 ± 0.98	0.033
HDL-C (mmol/L)	1.11 ± 0.33	1.24 ± 0.36	< 0.001

There were significant difference for BMI (p<0.001), and the frequencies of hypertension (p=0.001), obesity (p < 0.001), smokers (p<0.001), and dyslipidemia (p<0.001) between the MI patients and control group. The baseline serum concentrations of TG, TC, and LDL-C were higher in T2D patients than controls (p<0.001). In addition,T2D patients presented lower HDL-C levels (p<0.001).

Results

The genotype distribution and allele frequencies of the E23K and 1273 G/A polymorphisms in patients and controls are shown in table 2. Genotype distribution was consistent with that predicted by Hardy-Weinberg equilibrium in patients and controls (p>0.05). For KCNJ11, carriers homozygous for 23K/23K are shown to have inceased risk for T2D (OR=1.34 [1.06-1.71], p=0.012). The type 2 diabetes patient group showed a significant higher frequency of the 23K allele compared to the controls (0.28 vs. 0.23; p= 0.012). Whereas the G/A R1273R polymorphism was not associated with higher risk of T2D (OR =1.11 [0.89-1.37], p=0.33). (Table 2).
The association of the genetic variants with T2D was also modeled by a multivariable logistic regression analysis to minimize the confounding by established risk factors, and to allow for interactions. The effect of the 23K/23K genotype dosen't remained significant also after adjustment for smoking, hypertension, dyslipidemia, and obesity.

Table 2. Genotype and allele distribution of the E23K and 1273 G/A polymorphisms in T2D patients and in controls

Genotypes	T2D patients (n = 391)	Control subjects (n = 362)	Unadjusted OR (95%CI)	P	Adjusted OR (95%CI)[a]	P
E23K (KCNJ11)						
E/E	210 (53.7%)	215 (59.4%)	1			
E/23K	140(35.8%)	129 (35.6%)	1.11 (0.81 – 1.50)	0.499		
23K/23K	41 (10.5%)	7 (5.0%)	2.33 (1.29 – 4.19)	0.005	1.18 (0.90 – 1.55)	0.211
Allele frequency (%)						
E	0.72	0.77				
23K	0.28	0.23	1.34 (1.06 – 1.71)	0.012		
1273 G/A (ABCC8)						
GG	111(28.4%)	114 (31.5%)	1			
GA	211(54.0%)	190 (52.5%)	1.14 (0.82 – 1.58)	0.430		
AA	69(17.6%)	58 (16.0%)	1.22 (0.79 – 1.89)	0.368		
Allele frequency (%)						
G	0.54	0.56				
A	0.46	0.43	1.11(0.89-1.37)	0.330		

OR = odds ratio,CI = confidence interval, SE = standard error
[a] adjusted for hypertension, dyslipidaemia and obesity

There was a significant linkage disequilibrium between the two polymorphisms in T2D patients (D' = 0.44 and p = 0.001) and in controls (D' = 0.32 and p = 0.001) .

Haplotypes H2 (E-A) and H3 (23K-G) were more commonly found in T2D patients than in controls, thus suggesting a risk effect for H2 and H3 haplotypes against T2D (Table 3).

Table 3 Comparison of the distributions of the E23K and 1273 G/A haplotypes between MI patients and controls.

Variable	Haplotypes	Controls	Patients	Unadjusted OR (95%CI)	P
H1[a]	E-G	0.421	0.335	-	-
H 2	E-A	0.355	0.377	1.33 (1.02-1.73)	0.030
H 3	23K-G	0.158	0.214	1.66 (1.18-2.34)	0.003
H 4	23K-A	0.064	0.072	1.39(0.84-2.31)	0.191

[a]Haplotype 64Val-Ins (Hap1) is chosen to be the reference haplotype. OR, odds ratio; CI confidence interval.

Conclusion

We found that the 23K allele confers inceased risk of the T2D in Tunisian population. The 23K allele carriers have been demnstrated to reduce the ATP sensitivity of the Kir 6.2/SUR1 channel complex that results in the reactivity of the KATP and subsequent supression of insulin secretion.
The functional E23K marker is a strong candidate to define T2D suceptibility.