Contents available at Sciverse ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Lack of association between genetic polymorphisms within KCNQ1 locus and type 2 diabetes in Tunisian Arabs

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ARTICLE INFO

Article history:
Received 8 August 2012
Received in revised form
22 September 2012
Accepted 3 October 2012
Published on line 27 October 2012

Keywords:
Allele
Haplotype
KCNQ1
Tunisia
Type 2 diabetes

ABSTRACT

Aims: Polymorphisms of *KCNQ1* were previously associated with type 2 diabetes (T2DM) in select Caucasian and non-Caucasian populations. We investigated the association of rs231361, rs231359, rs151290, rs2237892, rs2283228, rs2237895, and rs2237896 *KCNQ1* polymorphisms with T2DM in Tunisian Arabs.

Subjects and methods: Subjects comprised 900 T2DM patients and 600 normoglycemic controls. *KCNQ1* genotyping was done by allelic discrimination (real-time PCR) and PCR-RFLP methods; the contribution of *KCNQ1* polymorphisms to T2DM were analyzed by Haploview and regression analysis.

Results: Minor allele frequency (MAF) of the 7 tested *KCNQ1* variants was comparable between T2DM cases and controls. Mild association of rs2237892 genotypes with T2DM was seen ($P = 0.014$), highlighted by the significant association of the C/T genotype with increased T2DM risk (OR, 2.11; 95%CI, 1.25–3.53), after adjusting for BMI, gender, systolic and diastolic blood pressure, and serum lipid profile. Heterogeneity in linkage disequilibrium pattern between tested *KCNQ1* variants analyzed was seen. Two-locus (rs231361 and rs231359) and 5-locus (remaining 5 SNPs) haplotype analysis did not reveal any significant association with any of the haplotypes contained in either block 1 or block 2.

Conclusion: These results indicate that there was no evidence for an association of *KCNQ1* polymorphisms with T2DM in Tunisian Arabs.

1. Introduction

Type 2 diabetes (T2DM) is a global public health problem, and is characterized by chronic hyperglycemia stemming from insulin resistance and progressive impaired pancreatic β-cell function [1]. The prevalence of T2DM has increased dramatically over the last 2 decades [2], and is predicted to increase from 8.9% in 2011 to 11.8% in 2030 in Tunisia [3]. Interaction between hereditary and lifestyle/environmental factors

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0168-8227/$ – see front matter
http://dx.doi.org/10.1016/j.diabres.2012.10.006

contribute to T2DM pathogenesis [4]. Recent genome-wide association studies (GWAS) identified several candidate genes to be implicated in T2DM pathogenesis, which included *CDKAL1*, *CDKN2A/B*, *PPARγ*, *HHEX*, *IGF2BP2*, *KCNJ11*, *SLC30A8*, *TCF7L2*, and *WFS1* [5–9]. However, the functional significance of most of these loci remains to be seen.

Three independent GWAS confirmed *KCNQ1* (potassium voltage-gated channel, KQT-like subfamily, member 1) as T2DM susceptibility candidate in East Asians [10–12]. *KCNQ1* is located on chromosome 11p15.5, with 19 exons and spanning over 400 kb [13]. *KCNQ1* encodes KvLQT1, which controls cardiac ventricular repolarization [14]. *KCNQ1* is ubiquitously expressed, especially in epithelial cells and exocrine and endocrine pancreas [15], and in insulin-secreting INS-1 cells, where inhibition of this potassium channel significantly increased insulin secretion [16]. *KCNQ1* was confirmed as T2DM susceptibility gene in two independent GWAS on Han Chinese and European populations [17,18], with the association of the *KCNQ1* variants (rs2237892, rs2237895, rs2237897, rs22832228) with T2DM being replicated in Asian [10,19–21] and European populations [10,11,22,23]. Additional *KCNQ1* variants were subsequently reported, which included rs151290 in Japanese [10] and Europeans [24], and rs231361 and rs231359 in Han Chinese [17].

The carriage of *KCNQ1* at-risk alleles was reportedly associated with impaired pancreatic β-cell function and consequently insulin secretion [10,17,24,25]. This was highlighted by the finding that *KCNQ1* rs2237892 variant was associated with increased risk of T2DM, and reduced insulin secretion and elevated fasting glucose in Japanese, Chinese and Europeans [10,24–27]. A Danish case–control demonstrated that *KCNQ1* rs2237895 was associated with reduced insulin secretion [17]. In this study, we explored the association of *KCNQ1* at-risk variants identified in GWAS (rs151290, rs231359, rs231361, rs2237892, rs2237895, rs2237896, and rs2283228) in 900 Tunisian T2DM patients and 600 normoglycemic control subjects. This is the first study to examine the association of these seven *KCNQ1* variants with T2DM among Arab population.

2. Subjects and methods

2.1. Subjects

Basic clinical characteristics of the study subjects are shown in Table 1. Patients included 900 consecutive unrelated T2DM patients, who were evaluated at outpatient diabetes clinics at Farhat Hached Hospital (Sousse) and Fattouma Bourguiba Hospital (Monastir). T2DM was diagnosed based on the 1999 WHO criteria (fasting plasma glucose $\geq$ 7.0 mmol/l and/or 2-h plasma glucose $\geq$ 11.1 mmol/l). Patients with other forms of diabetes (including maturity onset diabetes of the young [MODY]), or diagnosed with T2DM before 30 years of age, were excluded. Normoglycemic control subjects (n = 600) were included if they reported no personal or family history of diabetes, and had either normal glucose tolerance (fasting plasma glucose $<$ 6.1 mmol/l and 2-h plasma glucose $<$ 7.8 mmol/l), or HbA1c levels $<$ 5.6% with fasting plasma glucose $\leq$ 6.1 mmol/l. All case and control subjects were Tunisian Arabs; non-Arab subjects (Berbers and other minorities) were excluded. Informed consent was obtained from every participant, and the study protocol was approved by local ethics committees, and was in accordance with the Declaration of Helsinki II guidelines.

Table 1 – Clinical characteristic of patients and controls.

Characteristic	Patients (900)	Controls (600)	P value[a]
Male gender[b]	334 (37.8)	399 (45.5)	0.001
Age at study (years)	61.2 ± 9.7	52.0 ± 11.9	<0.001
Mean BMI (kg/m^2)	28.4 ± 5.2	24.8 ± 3.1	<0.001
Age of onset (years)	48.4 ± 10.6	N/A[c]	N/A
Diabetes duration (years)	12.68 ± 8.1	N/A	N/A
SBP (mmHg)	142.0 ± 21.6	121.2 ± 15.9	<0.001
DBP (mmHg)	81.3 ± 12.3	75.6 ± 10.5	0.003
Glucose (mmol/L)	12.4 ± 5.5	5.0 ± 0.8	<0.001
HbA1c (%)	9.2 ± 6.3	5.3 ± 1.1	<0.001
Urea (mmol/L)	9.7 ± 8.7	5.3 ± 1.2	<0.001
HDL (mmol/L)	1.1 ± 0.5	1.4 ± 0.4	<0.001
LDL (mmol/L)	2.7 ± 1.4	3.9 ± 1.4	<0.001
Total cholesterol (mmol/L)	4.6 ± 1.3	5.0 ± 1.0	<0.001
Triglycerides (mmol/L)	1.8 ± 1.4	1.5 ± 2.9	0.002

[a] Pearson's χ^2 test (*categorical variables*), Student's *t*-test (*continuous variables*).
[b] Number (percent).
[c] Not applicable.

2.2. SNP genotyping

Total genomic DNA was isolated from peripheral blood lymphocytes by the salting out method. We selected rs231361, rs231359, rs151290, rs2237892, rs2283228, rs2237895 and rs2237896 *KCNQ1* SNPS in view of their frequency in Caucasians, and reported association with T2DM. One SNP rs2237892 was genotyped by the allelic discrimination method on StepOne real-time PCR system (Applied Biosystems, Foster City, CA), using commercially available primers obtained from the assay-on-demand system, with well-defined genotype clusters. The other six SNPs were genotyped by restriction fragment length polymorphism (PCR-RFLP) analysis, using the indicated restriction endonucleases (Table 2). Genotype frequencies of the seven SNPs were consistent with Hardy–Weinberg equilibrium (Table 3), and the minor allele frequencies (MAF) obtained were comparable to those in the HapMap CEU sample.

2.3. Statistical analyses

Data were expressed as mean ± SD (continuous variables) or as percent of total (categorical variables), and intergroup significance was assessed by Student's *t*-test (continuous variables), and χ^2 test (categorical variables). Allele frequencies were calculated by gene-counting method; each SNP was tested for Hardy–Weinberg equilibrium using SNPStats (http://bioinfo.iconcologia.net/snpstats/start.htm). After the power was computed for each SNP (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html), the overall power was calculated as the average power over the SNPs genotyped (Table 2). At α = 0.05, this sample size provided 97.2% power in detecting