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Association of CDKAL1 gene rs7756992 A/G polymorphism with type 2 diabetes mellitus and diabetic nephropathy in the Egyptian population

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ABSTRACT

Objectives: Cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (CDKAL1) gene has been recognized as one of the type 2 diabetes mellitus (T2DM) associated genes by genome-wide association studies. Even though, the association of its rs7756992 A/G polymorphism varies in different ethnic populations. Therefore, this study was designed to evaluate the influence of single nucleotide polymorphism rs7756992 in CDKAL1 gene as a possible genetic predisposing factor for T2DM among Egyptian population. Furthermore, the association of rs7756992 variant with diabetic nephropathy as one of the most serious diabetic complications was also investigated.

Design and methods: 105 Egyptian patients with T2DM and 53 apparently healthy controls were enrolled in this study. Patients were divided into 50 T2DM patients without nephropathy and 55 T2DM patients with nephropathy. Genotyping was performed using TaqMan® allelic discrimination assay.

Results: Results of this study showed a significant association between the rs7756992 variant of CDKAL1 gene and the risk of T2DM under allelic, additive, dominant and recessive models. Moreover, this variant was found to be significantly associated with reduced risk of diabetic nephropathy under the only dominant model and one of the two additive models used.

Conclusions: Findings of this study revealed that rs7756992 variant of CDKAL1 gene is considered as a strong candidate for T2DM susceptibility whereas it is not considered as a genetic risk factor for diabetic nephropathy among Egyptian patients with T2DM.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has reached an epidemic level, with about 400 million adult patients throughout the world (Ozanne et al., 2017). In Egypt, T2DM is a fast-growing health problem that affects almost 15.6% of all adults aged 20 to 79 years, with an annual death of about 86,478 (Hegazi et al., 2015). Genetic predisposition is an important factor in the etiology of T2DM and the susceptibility to diabetic nephropathy (Fan et al., 2016; Liu et al., 2016). Accordingly, finding out new genetic risk factor contributing to T2DM in different world inhabitants would provide a better understanding of the disease, and would open the door for new treatment strategies.

Genome-wide association studies (GWAS) have identified > 60 loci encoding up to 500 different genes which increase the risk of T2DM (Rao et al., 2016). Among these genes, the cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (CDKAL1) has been identified as one of the T2DM associated genes (Saxena et al., 2007; Scott et al., 2007; Steinthorsdottir et al., 2007). Human CDKAL1 gene is one of the T2DM associated genes but this association varies in different ethnic populations (Dehwah et al., 2010). Genetic variants of CDKAL1 are associated with either defect in proinsulin conversion (Kirchhoff et al., 2008; Haupt et al., 2009; Stančáková et al., 2009) or decreased insulin response upon glucose stimulation (Saxena et al., 2007; Steinthorsdottir et al., 2007; Ruchat et al., 2009; 'tHart et al., 2010).

The CDKAL1 gene rs7756992 A/G polymorphism is associated with T2DM in many different populations (Steinthorsdottir et al., 2007; Cauchi et al., 2008; Tabara et al., 2009; Chistiakov et al., 2011; Cauchi

Abbreviations: CDKAL1, cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1; GWAS, genome-wide association studies; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; FPG, fasting plasma glucose; ACR, albumin to creatinine ratio; HbA1c, hemoglobin A1c; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence interval

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et al., 2012; Nemr et al., 2012; Chen et al., 2013; Wei et al., 2015; Kommoju et al., 2016). However, No association was observed in further populations as Austrian (Cauchi et al., 2008), Norwegian (Hertel et al., 2008) and Pima Indians(Rong et al., 2008). The impact of single nucleotide polymorphism (SNP) depends on ethnic diversity (Dehwah et al., 2010), and it would be important to perform studies for individuals with different ethnic backgrounds.

The lack of evidence for the association of CDKAL1 gene rs7756992 A/G polymorphism with either T2DM or T2DM-related complications in type 2 diabetic Egyptian patients is sufficient to warrant investigating this gene variant as a possible genetic predisposing factor in type 2 diabetic Egyptian patients.

2. Materials and methods

2.1. Subjects

This study was performed on 158 Egyptian subjects (76 males and 82 females) with mean age ± SEM (52.99 ± 0.48) ranged between (40–62) years including 105 patients with T2DM and 53 apparently healthy controls. Patients were recruited from outpatient clinics of Internal Medicine and Nephrology Department of El-Zahraa Hospital, Al-Azhar University, Cairo, Egypt, and were divided into two groups. The first group includes 50 T2DM patients without nephropathy while the second group includes 55 T2DM patients with nephropathy. All patients suffered from T2DM for > 5 years and were diagnosed according to the criteria of the American Diabetes Association; fasting plasma glucose (FPG ≥ 126 mg/dl) and hemoglobin A1c (HbA1c ≥ 6.5%) (American Diabetes Association, 2014). T2DM patients with nephropathy were diagnosed by urinary albumin to creatinine ratio (ACR > 30 mg/g) measured on at least two consecutive occasions separated by a three to six months period. On the other hand, T2DM patients with ACR < 30 mg/g were considered normal albuminuria and had no nephropathy (Yadav et al., 2008). The exclusion criteria include other types of diabetes, hepatic disease, thyroid dysfunction, advanced renal diseases other than diabetes, cancer, and smoking. Control subjects were recruited from the community with age and socioeconomic level nearly similar to patients group. They were all apparent healthy and no history for first-degree relatives with diabetes mellitus or diabetes-related symptoms (FPG < 100 mg/dl).

All the subjects were given both oral and written information about the experimental procedures and informed consent of all the subjects was taken. This study was approved by ethics committees of all participating facilities. All patients included in the study were subjected to clinical investigations and full medical history was taken. Laboratory investigations were performed for all patients and controls.

2.2. Sampling

Fasting blood samples of total 6 ml were withdrawn from all subjects involved in this study. 2 ml were collected in vacutainer tube containing EDTA and then divided into 2 aliquots. The first aliquot was used for HbA1c estimation while the second aliquot was stored at − 80 °C until the time of DNA extraction. 1 ml was collected in vacutainer tube containing fluoride and used for FPG determination. 3 ml were collected in serum vacutainer tube and used for determination of serum creatinine, urea and C-peptide. Morning urine samples were collected and used for determination of urinary albumin and creatinine.

2.3. Laboratory investigations

Fasting plasma glucose (Trinder, 1969), serum and urinary creatinine (Bowers, 1980), and serum urea (Weatherburn, 1967) were measured using commercially available kits. Urinary microalbumin (Cambiaso et al., 1988) was measured by turbidimetric immunoassay technique using BioSystems (Spain) kit. ACR was calculated according to Khawali et al. (2002). The HbA1c (Abraham et al., 1978) was measured in whole blood colorimetrically using Stanbio Glycohemoglobin (USA) kit. Serum C-peptide (Ashby and Frier, 1981) was determined by enzyme-linked immunosorbent assay (ELISA) using DRG C-peptide enzyme immunoassay (GmbH, Germany) kit.

2.4. DNA extraction and genotyping

Genomic DNA was extracted and purified from the EDTA whole blood of participants with GeneJET™ Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA) according to the manufacturer's instructions and stored at − 80 °C in aliquots until required. The concentration of DNA was estimated by its optical density at wavelength 260 nm using NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). DNA purity was examined at a 260/280 nm and 260/230 nm absorbance ratios.

Genotyping of rs7756992 SNP of CDKAL1 was carried out using a TaqMan® allelic discrimination assay by design supplied from (Applied Biosystems, Foster City, CA, USA). This assay was carried out using Applied Biosystems StepOne™ real-time PCR system (Applied Biosystems, Foster City, CA, USA) in a reaction volume of 20 µl, according to the manufacturer's protocol and using commercially available primers and probes.

2.5. Statistical analysis

D'Agostino and Pearson omnibus normality test was used. Quantitative variables were expressed as mean ± standard error of mean (mean ± SEM). On the other hand, qualitative variables were expressed in numbers (n) and percentage (%). Analysis of the difference among various studied groups for quantitative variables was evaluated either by one way analysis of variance (ANOVA) test for parametric variables or by Kruskal-Wallis test for non-parametric variables. Additionally, the difference between two groups was evaluated by Mann-Whitney *U* test for non-parametric variables. The Chi-square test (x^2) was used to compare between qualitative variables and to test deviation or accordance with the Hardy-Weinberg equilibrium (HWE), and also used to determine the genetic association of the rs7756992 SNP with risk to T2DM and diabetic nephropathy. Odds ratios (ORs) with 95% confidence interval (95% CI) were assessed for the risk allele. Genotypes were tested for various genetic models including additive, dominant and recessive. All statistical analyses were performed using Graph pad prism (windows version 6; Graph pad software, Inc., USA). A 0.05 level of probability was used as the criterion for significance ($p < 0.05$).

3. Results

Clinical characteristics of controls and patients are summarized in Table 1. All the studied groups were matched for age and sex. Also, no significant difference between the patient groups regarding the duration of T2DM.

Genotype distribution of the CDKAL1 rs7756992 A/G SNP was in agreement with Hardy–Weinberg equilibrium ($p > 0.05$). Analysis of genotype distribution in all studied subjects showed that recessive homozygous genotype (GG) was 12.03%, heterozygous genotype (AG) was 48.10%, and dominant homozygous genotype (AA) was 39.87%. In addition, 64% had the A allele frequency, while 36% had the G allele frequency, and thus minor allele frequency (MAF) was 0.36.

The statistical data showed a significant association between the rs7756992 variant of CDKAL1 gene and the risk of T2DM (OR = 3.48, 95% CI = 2–6.09, $p < 0.0001$). Moreover, significant association with the risk of T2DM was observed under the different genetic models used in this study which include two additive models (AG versus AA, OR = 4.69, 95% CI = 2.23–9.85, $p < 0.0001$ and GG versus AA,