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Association between CDKAL1 Gene Polymorphism and Risk of Type 2 Diabetes in Population of Lorestan Province

Fatemeh Mohammadi Pour [1,*], Faezeh Imani Farahani [1]

[1] Personalized Medicine Research Center of AmitisGen, Tehran, Iran

** Corresponding author: Fatemeh Mohammadi Pour, Personalized Medicine Research Center of AmitisGen, Tehran, Iran. E-mail: ftm_mhp@yahoo.com*

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Abstract

Diabetes Mellitus is defined as a metabolic disorder characterized by chronic hyperglycemia. The more prevalent form, type 2 diabetes, usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. T2DM is a complex multifactorial disease in which multiple genetic variants interact with environmental factors to trigger the disease. There is sufficient evidence that T2DM has a strong genetic basis. One of the identified candidate genes associated with type 2 diabetes is CDKAL1, reduced expression of CDKAL1 would result in enhanced activity of CDK5 in β cells, which would lead to decreased insulin secretion. In this study, the association of polymorphism rs10946398 of CDKAL1 with type 2 diabetes was evaluated in 200 people by RFLP-PCR method. No significant association was found between the genotypes of polymorphism rs10946398 and type 2 diabetes in the target population. It is suggested that this polymorphism association with type 2 diabetes in larger populations evaluated.

INTRODUCTION

DM is defined as a metabolic disorder characterized by chronic hyperglycemia due to disturbances of carbohydrate, fat, and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion, insulin action, or both (1). The number of people with diabetes has increased from 180 million in 1980 to 422 million in 2014 (2). The more prevalent form, type 2 diabetes, usually begins as insulin resistance, a disorder in which the cells do not use insulin properly (3). As the need for insulin rises, the pancreas gradually loses its ability to produce it. Type 2 diabetes mellitus (T2DM) represents > 90% of the cases. T2DM is a complex multifactorial disease in which multiple genetic variants interact with environmental factors to trigger the disease. There is sufficient evidence that T2DM has a strong genetic basis (4). The concordance of T2DM in monozygotic twins is ~76%. The lifetime risk (at age 80 years) for T2DM has been calculated to be 38% if one parent had T2DM (5). If both parents are affected, the incidence of T2DM in the offspring is estimated to approach 60% by the age of 60 years (6). Advances in genotyping technology have facilitated rapid progress in large-scale genetic studies. Since 2007, genome-wide association studies (GWAS) have identified > 65 genetic variants that increase the risk of T2DM by 10–30%. Recent technological developments have allowed the successful identification of common single nucleotide polymorphisms (SNPs) contributing to diabetes susceptibility. So far, 10 SNPs have been reported in multiple studies and meta-analyses as significantly associated with increased risk of T2DM (7). One of the identified candidate genes associated with type 2 diabetes is CDKAL1. CDK5 Regulatory Subunit-Associated Protein 1-Like 1 (CDKAL1) is located on the short arm of chromosome 6 at position 22.3 (6p22.3) of the human chromosome and comprises nine exons (8). CDK5 is a small serine/threonine-protein kinase recognized as an essential molecule in the brain and has several extra-neuronal effects. CDK5 has been shown to blunt insulin secretion in response to glucose and to play a permissive role in the decrease of insulin gene expression that results from glucotoxicity, as well as in the pathophysiology of β-cell dysfunction and predisposition to type 2 diabetes (9). Thus, one can speculate that reduced expression of CDKAL1 would result in enhanced activity of CDK5 in β cells, which would lead to decreased insulin secretion (10). In agreement with this speculation, this locus was significantly associated with small decreases in insulin response to a glucose load. In this study, the association of rs10946398 polymorphism of the CDKAL1 gene with type 2 diabetes was evaluated in 120 patients with type 2 diabetes. Also, the association of this

polymorphism with BMI, weight, and age was compared, and results were compared with other studies.

MATERIALS AND METHODS

The target population of this study consisted of 200 men, 120 with T2D, and 80 healthy controls. All participants were between 35 and 50 years old. About 6 ml of venous blood were drawn from all study participants by venipuncture, under quality control and safety procedures. Two milliliters of the collected blood was placed into sterile ethylene diamine tetraacetic acid (EDTA) tubes for DNA extraction and consequent SNPs genotyping. Genomic DNA was isolated from blood using G-DEX™ IIb Genomic DNA Extraction Kit (iNtRON, South Korea) following the manufacturer instructions. The isolated DNA was stored at -20C° until analysis. The polymerase chain reaction was performed by specific primer: forward strand 5'-CTGCTTGCTGTTGGGGAAGA-3' and reverse strand 5'- CTCAATGCTGTTCATCAGGCAC-3', DNA genomic as PCR template and Taq DNA Polymerase Master Mix RED (Amplicon, Denmark). The PCR product was then electrophoresed by 2% agarose gel. The product is then treated with Acil restriction enzyme according to the enzyme protocol, and the product is electrophoresed on 3% agarose gel. The GG genotype has a 157 bp band, the CC genotype has 121bp and 36bp, and the three-band for CG genotype 157-121-36bp (Figure 1). The results are compared with weight, body mass index, and age. All statistical analyses were performed using SPSS v19.0 software, and data were considered significant at $P \leq 0.05$.

RESULTS

Table 1 illustrates genotypes and alleles frequencies, odds ratios, 95% confidence intervals, and P values for the CDKAL1 gene polymorphisms among T2D patients and controls. No significant association was found between the genotypes of polymorphism rs10946398 and type 2 diabetes. The results were compared with body mass index, weight, and age. There was a significant relationship between body mass index ($P = 0.021$) and gravity ($P = 0.001$), but no meaningful relationship was observed with age (Table 2).

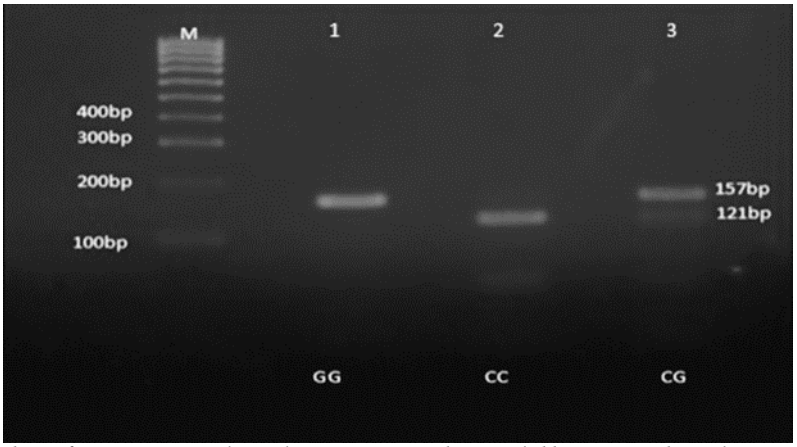

Figure 1. PCR-RFLP products of CDKAL1 gene polymorphism. Lane M: 100 bp DNA ladder. Lane 1 indicates homozygous GG (157 bp), lane 2 indicates CC genotype (121+36bp), and lane 3 indicates a heterozygous 'CG" genotype (157 +121+ 36 bp).

Table 1. The rs10946398 Genotype Frequency

Genotype	Type2 Diabetes	Control	Od ratio (85%CI)	P-value
GG	48	35	0.92	0.78
CG	50	38	0.88	0.46
CC	22	7	2.59	0.10

Table 2. The rs10946398 Genotype Frequency Relationship by Clinical Parameter

Genotype	DT2	Control	P-value
BMI			0.021*
GG	48	35	
GC	50	38	
CC	22	7	
Weight			0.001**
GG	48	35	
GC	50	38	
CC	22	7	
Age			0.812
GG	48	35	
GC	50	38	
CC	22	7	

DISCUSSION

Recent advances in human genetic research have facilitated the identification of genetic alterations conferring susceptibility to common diseases, such as T2DM, from across the entire human genome. Although the importance of CDKAL1 (rs10946398 G > C) as a susceptibility polymorphism for T2DM is well established in various populations, we could not replicate such an association in our investigated group. But we found a significant difference between case and control subjects could be established between the CDKAL1 (rs10946398 G > C) genotypes and BMI, weight. CDKAL1 (rs10946398 G > C) genotypes were significantly associated with a high risk of T2DM and β-cell function. Those associations, however, were stronger in Chinese Hans (Asians) than in individuals of European Ancestry (11, 12). Therefore, the non-significant association between CDKAL1 (rs10946398 G > C) genotypes and T2DM observed in this study could be due, among other factors, to the different genetic backgrounds of the different populations. The majority of people with T2DM are overweight or obese. Worldwide, the proportion of T2DM patients with BMI ≥ 25 kg/m2 is estimated to be 36.9 % in men and 38.0 % in women (13). It is suggested that this polymorphism association with type 2 diabetes in larger populations evaluated.

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