ABCA1 C69T gene polymorphism and risk of type 2 diabetes mellitus in a Saudi population

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Type 2 diabetes mellitus (T2DM) is a disease induced by complex interactions between environmental factors and certain genetic factors. Genetic variants in the Adenosine Binding Cassette Transporter Proteins 1 (*ABCA1*) have been associated with abnormalities of serum lipid levels of high-density lipoprotein (HDL-C). Decreased serum levels of HDL-C have often been observed in T2DM cases, and this condition has been considered to be involved in the mechanism of insulin resistance (IR). Therefore, we investigated possible association between *ABCA1* C69T gene polymorphism and T2DM in a Saudi population. This study was carried out with 380 healthy control subjects and 376 T2DM patients. Genotyping of *ABCA1* C69T polymorphism was carried out by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique. We observed that the frequency of the T allele of the *ABCA1* C69T gene was significantly higher in healthy subjects compared to T2DM patients (0.28 vs 0.45; $p<0.0001$; OR (95% CI) = 0.4624 (0.3732–0.5729), and therefore the T allele may be a protective factor against T2DM in the Saudi population.

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1. Introduction

Type 2 diabetes mellitus (T2DM) has a substantial genetic component and is thought to be polygenic in nature (Jenkins *et al*. 2013). Several genes influence the underline level of glucose tolerance and thereby contribute to overall susceptibility to T2DM (Lorenzo *et al*. 2013). Genetic linkage analysis and association studies have identified several candidate genes contributing to T2DM. However, given the ethnic differences in life style, environmental factors as well as in the genetic background, it is important to examine polymorphisms related to T2DM in each ethnic group (Yamada *et al*. 2006; Wilson *et al*. 2007). ATP-binding cassette transporter 1 (*ABCA1*) has been reported to play an important role in cholesterol metabolism especially in high-density lipoprotein cholesterol (HDL-C) (Daimona *et al*. 2005; Saleheen *et al*. 2006).

Keywords. *ABCA1*; PCR-RFLP; Saudi population; type 2 diabetes mellitus (T2DM)

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Many common genetic variants in the *ABCA1* gene have been shown to be associated with decreased serum levels of HDL-C as well as a risk for coronary artery disease (CAD)/ atherosclerosis (Daimon *et al*. 2003). Decreased serum levels of HDL-C have often been observed in T2DM patients and this condition is considered to be involved in the mechanism for insulin resistance (IR) (Daimon *et al*. 2003). Therefore, *ABCA1* gene variants have been studied in association with the development of T2DM in various ethnic groups (Daimon *et al*. 2003; Balderelli *et al*. 2009). However, there are no studies about the role of *ABCA1* genetic variants in the development of T2DM in the Saudi population. Therefore, the present study was carried out with an aim to evaluate the association of *ABCA1* C69T polymorphism with lipid concentrations and T2DM in a Saudi population from Riyadh.

2. Methods

2.1 *Subjects*

A total of 376 T2DM patients (Male:Female=225:151) evaluated in the Diabetic Clinic of King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia, were included in the study. The study was approved by the ethical committee of the study hospital. As a control group, 380 healthy individuals with normal glucose levels and without any other clinical components were recruited from the same demographic area. The controls healthy were volunteers belonging to the same ethnic group. T2DM diagnosis was carried out by authorized physicians in accordance with the criteria of World Health Organization and considering fasting glucose level of 126 mg/dL or >7.0 mmol/L. Venous blood samples (5 mL each) were collected from each subject: 3 mL of serum sample was used for biochemical analysis (collected in a plain vacutainer) and 2 mL of EDTA sample was used for genotyping analysis.

T2DM patients had fasting plasma glucose 7.0 mmol/L and had developed the disease more than 5 years ago. The individuals with a history of ketoacidosis or exocrine pancreatic disease or with other metabolic disorders or with severe liver or renal dysfunction were excluded from the study. Non-diabetic individuals were defined as older than 35 years and without any known complications. The studied individuals were confirmed to be unrelated for three generations. An informed written consent was developed and sought from all the members who participated in this study.

2.2 *Clinical and biochemical measurements*

Clinical and anthropometric parameters including blood pressure, BMI (height and weight), hip and waist circumferences were measured following standard procedures. Body mass index (BMI) was calculated as weight/height (kg/m^2). Fasting blood samples were collected, and the plasma glucose, triglyceride (TG), total cholesterol (TC) and HDL-C levels were measured by an auto-analyser (Konelab, Espoo, Finland) and concentrations of LDL-C were calculated using Friedwald's formula.

2.3 *Molecular analysis*

Genomic DNA was extracted from peripheral blood leukocytes using Norgen DNA extraction kit (Norgen Biotek corp, Canada). DNA samples were stored at −80°C. Molecular analysis work was performed at the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh and Saudi Arabia.

2.4 *C69T polymorphism*

A three step PCR-RFLP method was followed for *ABCA1* C69T (rs1800977) genotyping (Ergen *et al*. 2012). Specific primers: forward primer: 5′-CAG CGC TTC CCG CGC GTC TTA −3′; reverse primer: 5′-CCA CTC ACT CTC GTC CGC AAT TAC −3′ were synthesized by Bioserve Biotechnology, (Hyderabad, India). DNA was denatured at 95°C for 5 min, then amplified by 35 PCR cycles (95°C for 30 s, 60°C for 30 s, 72°C for 45 s) followed by final extension step at 72°C for 5 min. The reaction volume contained 100 pmol of each primer, 6 µL of sterile water, 10 µL of 2X master mix (included $MgCl_2$, 10x Taq buffer, 10 unit of Taq DNA polymerase (Norgen Biotek corp, Canada)) and 2 µL template DNA. PCR products were digested for 18 hours with *SmaI* (Fermentas, USA) at 37°C. RFLP product were directly separated by electrophoresis in non-denaturing Polyacrylamide Gel (PAGE) and visualized after silver staining. *SmaI* digestion produces three fragments depending on the genotype; CC: 310/35 bp; TT: 345 bp and CT: 345/310/35 bp.

2.5 *Statistical analysis*

Clinical data were compared between subjects with T2DM and controls by the unpaired Student's *t*-test. Clinical characteristics of all the subjects were expressed as mean±SD. Qualitative data were compared by the chi-square test. Allele frequencies were estimated by the gene counting method, and the chi-square test was used to identify departures from Hardy–Weinberg equilibrium. The genotype distribution of C69T polymorphism was compared between T2DM patients and control subjects by the chi-square test. Statistical significance was examined by two-sided tests; statistical analyses was performed with SPSS version 19.0 software.

Table 1. Demographic characteristics of the study population

	T2DM (n=376)	Controls (n=380)	*p*
Age (Years)	50.63±10.39	46.02±7.66	<0.0001
Sex: Male/Female	225 (59.8%) /151(40.2%)	202 (53.2%) /178 (46.8%)	0.003
Body mass index (kg/m^2)	29.51±5.92	29.19±5.53	0.183
Hypertension (%)	6.5%	8.9%	0.0001
Waist (cms)	94.3±22.36	91.2±20.26	0.055
Hip (cms)	104.83±21.44	94.46±7.80	<0.0001
FBS (mmol/L)	9.89±5.22	5.25±0.60	<0.0001
Triglycerides (mmol/L)	2.23±1.65	1.61±0.86	<0.0001
Cholesterol (mmol/L)	5.63±1.26	5.05±0.97	0.0004
HDL cholesterol (mmol/L)	0.93±0.75	0.64±0.23	<0.0001
LDL cholesterol (mmol/L)	3.79±1.07	3.68±0.85	0.0008
Glucose (mmol/L)	9.40±1.5	8.69±1.82	0.0001
Insulin (μU/mL)	16.2±2.2	12.3±1.7	0.0006
Homa-IR	7.12±2.4	2.87±1.7	<0.0001
Family History	376 (100%)	200 (52.6%)	<0.0001

3. Results

3.1 *Baseline characteristics*

During the study period 376 T2DM patients and 380 healthy controls were included in the study.

All the patients and controls belonged to a Saudi population from Riyadh. The clinical charecteristics of T2DM patients and controls have been presented in the table 1. The mean age was 50.63 for T2DM patients and 40.06 years for the control group. There was a significant difference in the TC, TG, LDL-C, HDL-C and FBP between patients and controls. In the present study all the T2DM cases had a family history of T2DM: However, only 52.1% of the control group had a family history of T2DM.

The genotypic distribution of *ABCA1* C69T polymorphism and frequency of C and T alleles in patients and controls have been given in the table 2. We found a significant difference between the cases and the controls [T vs C: (χ^2=50.5 (95%CI=0.3732–0.5729) p<0.0001; TT vs CT+CC: OR=0.2339 (95%CI=0.1482–0.369) p<0.0001].

We observed that the TT genotype is significantly higher in controls compared to the patients. The frequency of the T allele was higher in patients than controls [CT+TT Vs CC: χ^2=50.13 (95%CI=0.2517–0.4605) p<0.0001]. No relationship between *ABCA1* C69T genotypes and lipid profiles was observed. In addition to these, there was no significant association between HDL-C concentration and genotypes (table 3).

Table 2. Distribution of ABCA1 C69T genotypes and alleles of this study

Genotype and allele frequencies	Cases (n=376)	Controls (n=380)
CC	200 (53.2%)	106 (27.9%)
CT	144 (38.3%)	204 (53.7%)
TT	32 (8.5%)	70 (18.4%)
C	544 (0.72)	416 (0.55)
T	208 (0.28)	344 (0.45)

4. Discussion

ABCA1 has an important role in carrying cholesterol from peripheral tissues to liver (Ergen *et al*. 2008). Mutations in the *ABCA1* gene have been reported to induce several transport defects of HDL-C (Oram 2000). Epidemiological studies suggest that the risk of developing coronary heart disease for patient with T2DM is 2–4 times higher than their counterparts without diabetes (Mooradian 2009). Some experimental studies suggested that accumulation of cholesterol in islets reduces glucose-stimulated insulin secretion (GSIS) and impairs glucose tolerance in mice. Therefore, elevated cholesterol levels may be a risk factor for glucose intolerance and diabetes (Brunham *et al*. 2007). Previous studies have reported *ABCA1* C69T polymorphism to be associated with increased incidence of diabetes, and have suggested that it might have an important role in maintaining glucose-mediated insulin secretion. Salinas *et al*. (2007) reported the

ABCA1 modulated cholesterol accumulation within the β-cell plasma membrane, suggesting that *ABCA1* is a determinant of HDL-C and a link between diabetes, metabolic syndrome and atherosclerosis (Salinas *et al*. 2007). The authors have also demonstrated that high glucose levels can suppress the expression of *ABCA1*gene in mouse primary peritoneal macrophages (Gao *et al*. 2010).

Earlier studies have investigated the effect of *ABCA1* polymorphism on lipid-related diseases such as CAD and T2DM (Sheidina *et al*. 2004; Stefkova *et al*. 2004). Vergeer *et al*. (2010) showed that heterozygous carriers of *ABCA1* mutations displayed mild hyperglycaemia compared to noncarriers of similar age, sex and BMI (Vergeer *et al*. 2010). Patel *et al*. (2011) indicated that *ABCA1* expression and protein concentrations in leukocytes, as well as function in cultured skin fibroblasts are reduced in T2DM patients (Vergeer *et al*. 2010). Brunham *et al*. (2007) reported a new role for *ABCA1* in mediating cholesterol homeostasis and insulin secretion in pancreatic β-cells. *ABCA1* gene polymorphism study was carried out in a French cohort [n=5040] (Porchay *et al*. 2006) as a part of Insulin Resistance Syndrome study. The T allele of the C69T single nucleotide polymorphism (SNP) was associated with higher HDL-C levels in normal-weight men (BMI <25 kg/m2) (Porchay *et al*. 2006).

In the present study we investigated the association of *ABCA1* C69T polymorphism with T2DM in a Saudi population. To the best of our knowledge this is the first study investigating the association of this polymorphism with T2DM in this ethnic group. We found a significantly higher frequency of the T allele and TT genotype in the control group in comparison with the patients. Our results are in accordance with a previous study by Ergen *et al*. (2012), who also found a higher frequency of the TT genotype and T allele in the controls in comparison with T2DM (Ergen *et al*. 2012). As suggested by the authors we also contemplate that the T allele might have a protective effect against T2DM in the Saudi population from Riyadh.

We also evaluated the relationship between lipid levels and *ABCA1* C69T polymorphism in the subjects. However, we could not find a significant association between the two. Our results are similar to the Finnish study and Turkish study, where no association between this polymorphism and HDL-C concentration was reported.

In conclusion, our study shows a protective effect of the T allele in T2DM patients. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

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Table 3. Circulation of lipid profile conferring to the ABCA1 C69T genotypes in study groups

ABCA1 Genotypes	T2DM Cases (n=376)					Healthy controls (n=380)				
	CC (n=200)	CT (n=144)	TT (n=32)	Min-Max	*p*	CC (n=106)	CT (n=204)	TT (n=70)	Min-Max	*p*
TG	2.25±1.66	2.23±1.82	2.29±1.08	2.56-3.61	*p*=0.98	1.58±0.88	1.65±0.86	1.56±0.86	0.46-4.85	*p*=0.69
TC	5.63±1.26	5.64±1.21	5.53±1.57	2.81-10.38	*p*=0.91	4.97±0.92	5.08±0.99	5.10±0.98	2.43-7.49	*p*=0.62
HDL-C	0.83±0.38	0.78±0.38	0.98±0.36	0.56-2.17	*p*=0.23	0.66±0.23	0.64±0.23	0.61±0.23	0.08-1.35	*p*=0.48
LDL-C	3.78±1.05	3.90±0.99	3.50±1.39	1.08-6.57	*p*=0.15	3.59±0.80	3.69±0.88	3.78±0.86	1.36-6.35	*p*=0.35

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