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ORIGINAL ARTICLE

Apolipoprotein C3 Gene Variants and Risk of Developing Type 2 Diabetes in Saudi Subjects

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Abstract

Background: Apolipoprotein C3 (ApoC3) is a major constituent of VLDL and is a modulator of triglyceride metabolism. Recent genetic studies have implicated several ApoC3 gene polymorphisms in the development of insulin resistance and type 2 diabetes mellitus (T2DM). Considering the high prevalence of T2DM in Saudi Arabia, we sought to examine the possible association of ApoC3 gene variants with diabetes risk in Saudi population.
Methods: The 3238C > G and −482C > T polymorphisms of ApoC3 gene were studied in 268 T2DM patients and 255 healthy controls by TaqMan probe based real time polymerase chain reaction assays.
Results: Diabetic patients displayed significantly increased systolic blood pressure, fasting plasma glucose, insulin resistance, and dyslipidemia compared to control. Patients also had markedly elevated plasma VLDL levels. Genotype distribution of 3238C > G polymorphism was significantly different between patients and control. Consistently, this variant was found to be significantly associated with T2DM risk. Contrastingly, no significant relationship was found between −482C > T polymorphism and T2DM risk. Association of disease risk with 3238C > G polymorphism remained significant even after accounting for the established risk factors. Genotype-based stratification revealed a significant correlation of GG genotype of 3238C > G with elevated plasma triglycerides, insulin resistance, and VLDL, whereas the TT genotype of −482C > T correlated with elevated triglyceride and VLDL levels.
Conclusions: Thus, 3238C > G polymorphism of ApoC3 gene appears to augment the propensity to develop T2DM, while −482C > T to negatively affect lipid metabolism in Saudi subjects.

Introduction

Type 2 diabetes mellitus (T2DM) is a major public health concern with considerable morbidity and mortality. The etiology of T2DM is multifactorial involving both genetic and environmental factors. Recent studies have indicated the role of apolipoprotein C3 (ApoC3) in increasing the insulin resistance and T2DM risk. The ApoC3 is an abundant, circulating 79 amino acid glycoprotein synthesized in the liver and is a major constituent of chylomicrons and very low density lipoprotein (VLDL) and is present in smaller amounts in HDL.[1] ApoC3 is a natural lipoprotein lipase inhibitor and plays a significant role in the modulation of triglyceride rich lipoprotein metabolism and hepatic clearance of remnant particles.[2,3] Due to its inhibitory action on lipoprotein lipase, ApoC3 levels are shown to positively correlate with plasma triglyceride levels.[4–6] Consistently, mice overexpressing ApoC3 demonstrated increased plasma triglyceride levels, while ApoC3 deficient mice showed reduced triglyceride levels as compared to those in wild type mice.[7,8]

Recent studies have described an association between increased ApoC3 expression and insulin resistance as well as susceptibility to develop T2DM. The altered triglyceride metabolism due to increased ApoC3 expression was found to be the triggering event in the development of insulin resistance and T2DM. For example, in a prospective study increased serum ApoC3 levels are reported to be strong and independent predictors of T2DM in both men and women.[9] Similarly, higher ApoC3 levels are instrumental in increasing insulin resistance and T2DM risk.[10,11] Human ApoC3 overexpressing mice on a high fat diet exhibited increased hepatic triglyceride levels and insulin resistance.[12] These studies indicate that there is a strong relationship among ApoC3 over expression, altered triglyceride metabolism, and increased diabetes risk. Consistent with the modulatory effects of ApoC3 on insulin resistance and

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T2DM, several single nucleotide polymorphisms (SNPs) in ApoC3 gene are reported which have been shown to affect ApoC3 protein levels, triglyceride metabolism, insulin resistance, and diabetes risk. For instance, the two ApoC3 gene promoter polymorphisms, −455T > C and −482C > T of ApoC3 are shown to be associated with elevated ApoC3 and triglyceride levels and enhanced diabetes risk.[13] Likewise, a coding region 1100C > T polymorphism and a 3238C > G (SstI) polymorphism in 3′ UTR region of ApoC3 gene have also been described to increase ApoC3 expression and to increase insulin resistance and T2DM risk.[14–17]

The prevalence of T2DM is Saudi Arabia ranks seventh in the world, with 31.6% of the population are affected. Remarkably, the incidence of diabetes has doubled over the last two decades in the country and is attributed mainly to the sedentary life style.[18,19] Therefore, it is imperative to gain a better understanding of the underlying genetic factors for T2DM risk in the Saudi population. Previously we have shown that ApoE gene polymorphisms augment the diabetes risk in the Saudi population.[20] Considering the known association of ApoC3 gene polymorphisms with insulin resistance and enhanced diabetes risk, we aimed to examine whether ApoC3 gene is a genetic risk factor for T2DM risk in Saudi population. In this study we evaluated the frequency of 3238C > G (also referred as SstI and 3u386) polymorphism of 3′UTR and −482C > T polymorphism of promoter region of ApoC3 gene in T2DM patients and healthy controls of Saudi origin.

Materials and Methods

Subjects

This study was performed with the approval from the Ethics Committee of King Khalid University Hospitals, Riyadh, Saudi Arabia. This is a cross-sectional case-control study involving 268 T2DM patients and 255 normal healthy controls. All T2DM subjects had developed the disease more than 5 years prior to their enrollment, and all had a fasting glucose level of 126 mg/dL or >7.0 mmol/L, following standards established by the World Health Organization. Individuals with a history of other metabolic disorders apart from T2DM were excluded from the study. Control subjects comprise of normal healthy individuals who volunteered for the study. Informed consents were obtained from both patients and controls. All the subjects recruited in this study were of Saudi lineage.

Anthropometric and biochemical measurements

The weight, height, waist and hip circumferences, systolic and diastolic blood pressures were measured by standard procedures.[21] Body mass index was calculated by the formula: weight (kg)/height (m^2). The fasting blood samples were collected and plasma was separated and stored at −80°C until analyzed. The plasma concentrations of fasting blood sugar, insulin, total cholesterol, triglycerides, HDL, LDL, and VLDL cholesterols were measured by automated clinical chemistry analyzer using commercially available kits following the manufacturer's instructions (KoneLab, Espoo, Finland). Insulin resistance was assessed by homeostasis model assessment-insulin resistance (HOMA-IR) method using the formula: insulin (μU/mL) × glucose (mmol/L)/22.5.

Genotyping

The genomic DNA was extracted using the commercially available kits following the manufacturer's instructions (Norgen Biotech, Canada). The genotyping for 3238C > G (rs5128) and −482 C > T (rs2854117) variants of ApoC3 gene was carried out by TaqMan assay on Real Time-PCR instrument (Applied Biosystems, Foster city, CA). Primers and probes were obtained from Applied Biosystems as Assays-by-Design™. All assay reactions were carried out in a 96 well format.

Statistical analysis

The statistical analysis was carried out using SPSS. All continuous variables were presented as mean ± standard

Table 1. Anthropometric and Biochemical Parameters in Type 2 Diabetes Mellitus Patients and Normal Controls

Parameters	*Controls* (n = *255*)	*T2DM* (n = *268*)	P-*value*
Age (years)	45.9 ± 7.8	53.6 ± 10.8	< 0.001
M/F	106/94	116/84	0.11
BMI (kg/m^2)	29.2 ± 5.6	29.8 ± 6.3	0.09
SBP (mmHg)	114.5 ± 8.3	124.4 ± 10.8	< 0.01
DBP (mmHg)	75.9 ± 6.3	77.7 ± 6.2	0.82
Glucose (mmol/L)	5.2 ± 0.6	12.9 ± 4.6	< 0.001
Insulin (μU/ml)	10.4 ± 2.5	15.68 ± 3.2	< 0.01
HOMA-IR	4.3 ± 2.8	7.21 ± 2.8	< 0.01
Triglycerides (mmol/L)	1.62 ± 0.87	2.24 ± 1.66	< 0.001
Cholesterol (mmol/L)	5.03 ± 0.96	8.63 ± 1.26	< 0.001
HDL cholesterol (mmol/L)	0.83 ± 0.31	0.65 ± 0.22	< 0.001
LDL cholesterol (mmol/L)	3.64 ± 0.22	4.78 ± 1.04	< 0.001
VLDL cholesterol (mmol/L)	0.93 ± 0.41	1.53 ± 0.52	< 0.01

Anthropometric and clinical parameters were documented by standard procedures. Biochemical parameters were estimated by automated clinical chemistry analyzer.

Insulin resistance was assessed by homeostasis model assessment–insulin resistance (HOMA-IR) method using the formula: insulin (μU/mL) × glucose (mmol/L)/22.5.

Body mass index (BMI) was calculated by the formula: weight (kg)/height (m^2).

DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP-systolic blood pressure; T2DM, type 2 diabetes mellitus; VLDL, very low density lipoprotein.

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Table 2. Genotype Distribution of 3238C>G and −482C>T Polymorphisms of Apolipoprotein C3 Gene in T2DM Patients and Normal Controls

SNP	*Genotype*	*Control* (n=*255*)		*Patients* (n=*268*)		P-*value*
3238C>G	CC	189 (74.1%)		169 (63.1%)		
	CG	61 (23.9%)	0.14*	77(28.7%)	0.28*	0.0012†
	GG	5 (2. 0%)		22(8.2%)		
-482C>T	CC	96 (37.3%)		102 (38.06%)		
	CT	122 (47.8%)	0.51†	124 (46.27%)	0.50†	0.94
	TT	38 (14.9%)		42 (15.67%)		

The genotyping for 3238C>G (rs5128) and −482 C>T (rs2854117) variants of apolipoprotein C3 (ApoC3) gene was carried out by real time polymerase chain reaction (PCR) TaqMan assays using genomic DNA extracted from the peripheral blood. Comparison of genotypes was done by chi-squared test.

*Indicates minor allele frequency.

†*P*-values remained significant even after Bonferroni correction with new level of α set at 0.025.

SNP, single nucleotide polymorphism.

deviation. The chi-square test was used to test for the differences in frequencies between groups and genotypes. The odds ratios (95% confidence interval [CI]) were computed, testing the association of genotypes and T2DM risk, keeping wild type as the reference. Analysis of variance was done to compare the genotype-based differences in biochemical parameters. Logistic regression analysis was carried out to study the effect of potential compounders. The level of significance was given at $P<0.05$.

Results

Anthropometric data

The clinical characteristics of the T2DM patients and control subjects are shown in Table 1. Patients were significantly older and had significantly elevated systolic blood pressure, glucose, insulin, HOMA-IR, triglycerides, total LDL and VLDL cholesterols and significantly decreased HDL cholesterol compared with controls. Patients and controls were comparable for gender ratio, body mass index and diastolic blood pressure.

Genotype distribution of 3238 C>G polymorphism and diabetes risk

The distribution of genotypes and alleles of 3238C>G polymorphism in T2DM cases and controls are presented in Table 2. A significant difference ($P<0.0012$) was found in the distribution of CC, CG, and GG genotypes between cases and controls subjects. Consistent with the significant differences in genotype distributions, the odds ratio analyses revealed the GG and CG+GG genotypes to be significantly linked to diabetes risk (Table 3).

Genotype distribution of −482C>T polymorphism and diabetes risk

The distribution of genotypes and alleles of −482C>T polymorphism in T2DM cases and controls are presented in Table 2. The distribution of CC, CT, and TT genotypes in patients was found to be not significantly different ($P=0.94$) from the genotype distribution in control subjects. Corroborating with the lack of differences in the genotype distribution, the TT and CT+TT genotypes were not significantly related diabetes risk (Table 4).

Biochemical parameters according to 3238C>G and −482C>T genotypes

Since ApoC3 is a lipoprotein lipase inhibitor and its altered expression is correlated to impaired VLDL bound triglyceride metabolism and to increased insulin resistance, we aimed to assess the changes in triglyceride, HOMA-IR, and VLDL levels in accordance with the genotypes of

Table 3. Type 2 Diabetes Risk for 3238C>G Polymorphism of ApoC3 Gene

	CG *OR (95% CI)*	*GG* *OR (95% CI)*	*CG+GG* *OR (95% CI)*
Unadjusted	1.41 (0.95–2.09)	4.92 (1.82–13.28)**	1.67 (1.18–2.63)*
Age	1.30 (0.83–1.82)	5.12 (2.13–13.35)*	1.60 (1.16–2.55)*
BMI	1.54 (0.87–1.90)	4.86 (1.78–12.85)*	1.64 (1.16–2.57)*
SBP	1.03 (0.84–1.72)	4.90 (1.80–13.17)*	1.69 (1.20–2.65)*
Triglycerides	1.37 (0.81–2.30)	3.46 (1.64–12.48)*	1.72 (1.23–2.68)*
Total cholesterol	1.36 (0.87–2.12)	3.57 (1.67–12.50)*	1.62 (1.16–2.57)*
HDL cholesterol	1.42 (0.86–1.90)	4.88 (1.82–12.86)*	1.63 (1.14–2.45)*
LDL cholesterol	1.37 (0.84–1.87)	3.75 (1.78–12.65)*	1.70 (1.22–2.67)*

The genotyping for 3238C>G (rs5128) variant of ApoC3 gene was carried out by real time PCR TaqMan assays using genomic DNA extracted from the peripheral blood. The CC genotype of 3238C>G was considered as reference genotype.

*$P<0.01$.

**$P=0.001$.

95% CI, 95% confidence interval; OR, odds ratio.

Table 4. Type 2 Diabetes Risk for −482C>T Polymorphism of ApoC3 Gene

Genotype	*Reference genotype*	*Odds ratio (95% CI)*	P-*value*
CT	CC	0.95 (0.65–1.39)	0.81
TT	CC	1.04 (0.62–1.75)	0.88
CT + TT	CC	0.97 (0.68–1.39)	0.89

The genotyping for −482 C>T (rs2854117) variant of ApoC3 gene was carried out by real time PCR TaqMan assays using genomic DNA extracted from the peripheral blood.

3238C>G and −482C>T polymorphisms. The data are presented in Table 5. Compared with subjects carrying CC genotype the GG genotype carriers of 3238C>G polymorphism displayed marked increase in plasma triglycerides, HOMA-IR, and VLDL levels. Interestingly, despite the lack of significant association of −482C>T polymorphism with T2DM, the TT genotype carriers had significantly increased triglyceride and VLDL levels as compared with CC genotype carriers.

Discussion

In the present study we evaluated whether the distribution of −482C>T and 3238C>G SNPs of ApoC3 gene are significantly different between control and diabetic subjects and whether these differences are informative in predicting the diabetes risk in Saudi subjects. We found a significant association of 3238C>G polymorphism with increased diabetes risk in the studied population, which indicates that ApoC3 can be a potential candidate gene in diabetes pathophysiology. Diabetic patients displayed typical clinical and biochemical indices compared with normal healthy controls. Consistent with the diabetic etiology, patients had higher systolic blood pressure, waist circumference, fasting blood glucose, HOMA-IR, triglycerides, and LDL, VLDL, and total -cholesterol compared with healthy controls. On the other hand, patients had lower HDL cholesterol than healthy controls. The significant differences in the distribution of CC, CG, and GG genotypes of 3238C>G SNP as well as the odds ratio analyses for the association of genotypes with disease risk clearly indicates the increased risk of developing T2DM in subjects carrying this polymorphism. With respect to −482C>T SNP, the frequencies of CC, CT, and TT genotypes were comparable between patient and control populations indicating the lack of −482C>T SNP association with diabetes risk in the studied population. Thus, only 3238C>G polymorphism of ApoC3 gene appears to play a significant role in the diabetes pathology and also in the prediction of disease state in this population.

There are limited population studies that have examined the association of genetic alterations in Apoc3 gene with insulin resistance and T2DM and more so with regard to −482C>T and 3238C>G polymorphisms. Our findings are consistent with several studies in which −482C>T and 3238C>G SNPs are tested for their relationship to insulin resistance and T2DM. In the case of 3238C>G polymorphism, the frequency of risk allele, G is reported to be significantly higher in T2DM patients than in nondiabetics and was found to be significantly correlated with the increased plasma lipids and lipoprotein subclasses in Indian population.[22] In another study, the risk allele, G of 3238C>G SNP, was also linked to increased insulin resistance in men after a 28 day high fat diet intake.[23] Consistently, minor allele, G is found to predispose the individuals to T2DM in Chinese population.[16] The CG and GG genotype carriers of 3238C>G SNP are also reported to exhibit impaired glucose metabolism with elevated fasting glucose levels in Hispanics of Caribbean origin.[24] Although there are limited population-based case control studies, the minor allele frequency of 0.28 of 3238C>G SNP in the studied population appears to differ from Chinese (0.301)[16] and Indian (0.34)[22] populations.

With regard to −482C>T polymorphism, our finding of lack of association of this polymorphism with diabetes risk is consistent with other studies. In a multiethnic study comprising African Americans, European Americans, and Hispanics, −482C>T SNP was not a risk factor for insulin resistance.[25] The −482C>T polymorphism is also not associated with insulin resistance in obese Southern Europeans.[26] Contrastingly, several studies have found the significant relationship of −482C>T SNP with increased insulin resistance or diabetes risk. The T allele of −482C>T polymorphism is shown to increase the T2DM susceptibility in lean subjects.[13] Similarly, −482C>T polymorphism significantly correlated with insulin resistance in Asian Indians as well as in non-Asian Indians.[27] Likewise, −482C>T SNP is implicated in impaired fasting glucose levels in Caucasians, South Asians, and African Americans.[28] The risk allele T is also a significant determinant of glucose and insulin levels after oral glucose tolerance test.[14,29] Also, −482C>T SNP is a risk factor of diabetes in Turkish subjects.[30] Thus, there is considerable ambiguity in the studies relating the association of ApoC3 gene with insulin resistance and

Table 5. Distribution of Clinical Parameters According to Genotypes of 3238C>G and −482C>T Polymorphisms of ApoC3 Gene

	3238C>G				*−482C>T*			
Parameters	*CC*	*CG*	*GG*	P-*value*	*CC*	*CT*	*TT*	P-*value*
Triglycerides (nmol/L)	1.84±0.67	1.87±0.75	2.33±1.23	<0.01	1.87±.58	1.91±0.61	2.3±0.86	<0.01
HOMA-IR	3.43±2.31	3.48±2.42	4.92±2.62	<0.01	3.28±1.45	3.24±1.35	3.31±1.51	0.95
VLDL (nmol/L)	0.63±0.21	0.68±0.26	0.78±0.38	<0.01	0.71±0.38	0.73±0.41	0.97±0.62	<0.01

Biochemical parameters including triglycerides, insulin, and VLDL were estimated by automated clinical chemistry analyzer.
Insulin resistance was assessed by HOMA-IR method using the formula: insulin (μU/mL)×glucose (mmol/L)/22.5.
Analysis of variance was done to compare the genotype-based differences in biochemical parameters. The level of significance was given at $P<0.05$.

diabetes risk. The differences may presumably stem from the differences in ethnicity and environmental factors considering the multifactorial nature of diabetes pathology.[13,15,29,31] In the present study, the increased presence of triglyceride, HOMA-IR and VLDL levels in subjects carrying GG genotype of 3238C > G polymorphism is consistent with the modulating effect of ApoC3 on VLDL bound triglyceride metabolism and its effect on insulin resistance. The interesting observation is the association of TT genotype of 482C > T polymorphism with elevated triglyceride and VLDL levels. This can be explained by the possible lack of negative effects of environmental confounders, which otherwise would have rendered the subjects carrying –482C > T to develop T2DM.

The limitations of the study include the lack of a significant number of obese or overweight individuals in the studied cohort; therefore, it is unclear whether the findings of this study will also hold true if analyzed by including obese or overweight subjects. This is of importance considering that a previous study by van Hoek et al.[13] found significant association of –482C > T ApoC3 promoter polymorphism with increased T2DM risk only in lean but not in overweight subjects.

In conclusion, we found a significant association of 3238C > G polymorphism of ApoC3 gene with increased diabetes risk in Saudi subjects, underscoring the ApoC3 as potential candidate gene in diabetes etiology as well as in risk assessment. Further, our study supports the previous observations where genes linked to lipid metabolism also exert effects on glucose metabolism.

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Author Disclosure Statement

No competing financial interests exist.

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