

Case Control Study

Association of gene variants with susceptibility to type 2 diabetes among Omanis

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Abstract

AIM: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omanis.

METHODS: Using case-control design, a total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped, by an allelic discrimination assay-by-design TaqMan method on fast real time polymerase chain reaction system, for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634) *CAPN10* (rs3792267) and *HHEX* (rs1111875). T2D patients were recruited from the Diabetes Clinic ($n = 243$) and inpatients ($n = 749$) at Sultan Qaboos University Hospital (SQUH), Muscat, Oman. Adult control participants ($n = 294$) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative

with diabetes mellitus. In spite of the small number of normoglycemic controls in this study, this sample was sufficient for detection of genes and loci for common alleles influencing T2D with an odds ratio of ≥ 1.3 reaching at least 80% power. Data was collected from June 2010 to February 2012.

RESULTS: Using binary logistic regression analysis, four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219, $P = 5.8 \times 10^{-6}$, OR = 1.74), *TCF7L2* (rs7903146, $P = 0.001$, OR = 1.46), *CDKAL1* (rs10946398, $P = 0.002$, OR = 1.44) and *CDKN2A/B* (rs10811661, $P = 0.020$, OR = 1.40). The fixation index analysis of these four gene variants indicated significant genetic differentiation between diabetics and controls {[*KCNJ11* (rs5219), $P < 0.001$], [*TCF7L2* (rs7903146), $P < 0.001$], [*CDKAL1* (rs10946398), $P < 0.05$], [*CDKN2A/B* (rs10811661), $P < 0.05$]}. The highest genotype variation % between diabetics and controls was found at *KCNJ11* (2.07%) and *TCF7L2* (1.62%). This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk. However, T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the Waist-to-Hip ratio ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

CONCLUSION: Results confirmed the association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661) gene variants with susceptibility to T2D among Omani Arabs.

Key words: Type 2 diabetes; Genetics; Oman; Case-control; Association; Gene; Variants

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Core tip: To investigate the association of 10 known common gene variants with susceptibility to type 2 diabetes mellitus (T2D) among Omani Arabs using case-control design. A total of 992 diabetic patients and 294 normoglycemic Omani Arabs were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Four gene variants showed significant association with T2D risk: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The highest genotype variation % between diabetics and controls was found at *KCNJ11* and *TCF7L2* gene variants.

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INTRODUCTION

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. Insulin resistance is, mostly, an early event due to environmental factors, such as obesity. Decline in β -cell function is gradual but generally a late event^[1]. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D^[2].

Candidate gene approach identified few T2D susceptibility gene variants: *Pro12Ala* (rs1801282) in the coding region of peroxisome proliferator-activated receptor γ gene and it is the more common proline allele that is associated with T2D^[3]; *E23K* (rs5219) in the coding region of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β -cells (*KCNJ11*)^[4] and a series of polymorphisms and haplotypes (UCSNP-43 or rs3792267; UCSNP-19 or rs3842570 and UCSNP-63 or rs5030952) in the coding region of the cysteine protease calpain 10 (*CAPN10*)^[5].

Genome-wide association studies (GWAS) of T2D susceptibility genes and loci and their meta-analysis identified a large number of gene variants and confirmed the previously discovered ones^[6]. The common intronic variants within the transcription factor 7-like 2 (*TCF7L2*) gene was reported as the strongest genetic risk factor for T2D^[7]. Other loci most consistently associated with T2D risk include variants within or near the solute carrier family 30/zinc transporter (*SLC30A8*), hematopoietically expressed homeobox (*HHEX*), cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*), insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*), a genomic region between cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) and fat mass and obesity associated protein (*FTO*)^[6,8].

In total, approximately, forty four common T2D susceptibility gene variants and loci have been identified to-date, but all these variants could only explain approximately 10%-15% of the heritability of T2D; which suggests that more variants remain to be discovered^[9]. Rare large-effect mutations have recently been recognized as causes of many complex diseases^[10-12].

According to the international diabetes federation, six out of the world's top ten countries with the highest prevalence (%) of T2D among adults aged 20-79 years, in 2011, are in the Middle East and North Africa region; Kuwait (21.1%), Lebanon (20.2%), Qatar (20.2%), Saudi Arabia (20.0%), Bahrain (19.9%) and

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United Arab Emirates (19.2%). Small studies were conducted among Arabs, with a limited number of participants, to investigate genetic susceptibility for T2D^[7,13-24].

The prevalence of diabetes in Oman, in 2011, was estimated to be 10.8%; with a further 9.7% of the population at high risk of diabetes with impaired glucose tolerance, (<http://www.idf.org/diabetesatlas/content/what-is-diabetes>). Oman has a high inbred population and consanguineous marriages are about half of all marriages^[25]. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

In the present study, 10 known common gene variants, described previously, were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier GWAS studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios (ORs) among all the genes/loci discovered^[4-8,26].

MATERIALS AND METHODS

Sample size

For determining the sample size, we employed the Power Calculator for Genetic Studies developed by Skol *et al.*^[27] (2006) in their Website <http://www.sph.umich.edu/csg/abecasis/CaTS/index.html>. We used a T2D prevalence of 10% in the adult population of the Region as reported previously^[28,29]. We also anticipated disease allele frequencies of ≥ 0.25 , and assumed a multiplicative disease model^[30]. An optimum one-stage sample was deduced from the Power Calculator: 1000 cases and 1000 controls, will guarantee detection of genes and loci for common alleles influencing T2D with an OR of ≥ 1.2 reaching at least 80% power. However, we could not collect the required sample size, and only 992 cases and 294 controls were collected. This sample will guarantee detection of genes and loci for common alleles influencing T2D with an OR of ≥ 1.3 reaching at least 80% power.

Study population

A total of 992 T2D Omani Arab patients and 294 normoglycemic Omani Arab controls were included in this study. T2D patients were recruited from the Diabetes Clinic ($n = 243$) and inpatients ($n = 749$) at Sultan Qaboos University Hospital (SQUH), Muscat, Oman. A history of T2D among patients was ascertained from the diagnosis and medical history deposited in the electronic records of the hospital information system. Exclusion criteria for T2D patients included: patients diagnosed with type 1 diabetes; maturity onset diabetes of the young; positive diabetic antibodies (islet cell antibodies and glutamic acid decarboxylase antibodies) or patients diagnosed with any type of cancer. Adult

control participants ($n = 294$) were volunteers from the community and from those visiting Family Medicine Clinic at SQU, for regular medical checkup. The inclusion criteria for controls were: Omani, age ≥ 35 years, no family history of diabetes (first degree relatives) and with fasting glucose value of < 6.1 mmol/L, according to the World Health Organization 2006 criteria. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study. Almost all volunteers questioned had a relative with diabetes mellitus (DM). Data was collected from June 2010 to February 2012. Participants were informed about the project and written consents were obtained. The study was approved by the Ethics and Research Committee of the College of Medicine, Sultan Qaboos University, Muscat, Oman.

Anthropometric and biochemical parameters

T2D patients and normoglycemic control participants underwent demographic, anthropometric and biochemical investigations, summarized in Table 1. Anthropometric variables measured were: weight, height, waist and hip circumference. Obesity status was defined according to the international classification of an adult's weight (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html), [normal body mass index (BMI): 18.5-24.99 kg/m², overweight: 25.00-29.99 kg/m² and obese ≥ 30.00 kg/m²]. The biochemical investigations included: fasting glucose level and HbA_{1c}. To compare T2D patients and normoglycemic control participants' obesity status, we selected 294 T2D patients; age and sex matched with the normoglycemic control participants ($n = 294$). Waist-to-Hip ratio (WHR) was also calculated among T2D patients and control participants. Health risk based solely on the WHR, was identified according to the ranges specified at waist-to-hip ratio chart (<http://www.bmi-calculator.net/waist-to-hip-ratio-calculator/waist-to-hip-ratio-chart.php>) for males (low risk = 0.95 or below, moderate risk = 0.96-1.0, high risk= over 1.0) and females (low risk = 0.80 or below, moderate risk = 0.81-0.85, high risk = over 0.85).

Genotyping

All participants ($n = 1286$) were genotyped for the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398), *CDKN2A/B* (rs10811661), *FTO* (rs9939609 and rs8050136), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Genotyping was done by an allelic discrimination assay-by-design TaqMan method on 7500HT fast real time polymerase chain reaction system (Applied Biosystems, United States). Accuracy was achieved by duplicating approximately 10% of the samples.

Statistical analysis

The SPSS statistical package software (v20.0) was

Table 1 Anthropometric and biochemical characteristics of Omani type 2 diabetes mellitus patients and controls

	T2D patients			Controls		
	Mean \pm SD or median (range) ¹			Mean \pm SD or median (range) ¹		
	Males	Females	Total	Males	Females	Total
Total number (n)	473	519	992	121	173	294
Age (yr)	56 \pm 11	56 \pm 10	56 \pm 11	41 (35-80) ¹	44 (32-79) ¹	43 (32-80) ¹
Weight (kg)	78.8 \pm 15.4 ^{NS}	75.1 \pm 17.0	76.8 \pm 16.4	76.8 \pm 14.2	70.3 \pm 13	73.7 \pm 14.7
Height (cm)	165 \pm 9 ^a	152 \pm 9	157 (106-182) ^{INS}	167 \pm 8	154 \pm 6	159 \pm 9
BMI (kg/m ²)	29.1 \pm 4.8 ^b	32.8 \pm 10.3	30 (15-58) ¹	27.6 \pm 4.3	29.5 \pm 5.2	29.3 \pm 5.8
Waist circumference (cm)	100 \pm 12	100 \pm 13	100 \pm 13	95 \pm 13	91 \pm 11	92 \pm 12
Fasting blood glucose (mmol/L)	8.7 (3-24) ¹	9.0 (3-25) ¹	8.8 (3-25) ¹	5.1 \pm 0.31	4.9 \pm 0.36	5.1 \pm 0.41
HbA _{1c} (%)	8.3 \pm 1.8	8.3 (4.9-15.5) ¹	8.2 (4.1-18.6) ¹	5.7 \pm 0.46	5.7 (4.0-7.9) ¹	5.7 \pm 0.44
Obesity status (%)						
Underweight	-	0.5	0.3	2.1	1	1.4
Normal weight	16.8	12.5	14.5	22.2	18.2	19.1
Overweight	45	30.2	36.1	43.8	34.5	38.9
Obese	38.2	56.8	49.1	29.9	45.8	39.6
Missing	-	-	-	2.1	0.5	1

¹Median (range = minimum-maximum) displayed in the table when the variable does not follow a normal distribution pattern. In all parameters, $P < 0.001$ between diabetics and controls, except: NS; ^a $P < 0.05$ between diabetics and controls; ^b $P < 0.01$ between diabetics and controls. NS: No significant difference between diabetics and controls; T2D: Type 2 diabetes mellitus; BMI: Body mass index.

used for statistical analysis of measured parameters. The measured anthropometric and biochemical parameters were tested for normal distribution using one sample Kolmogorov-Smirnov test. Independent sample *t*-test was used to test the significance of the difference in the mean values for the measured anthropometric and biochemical parameters between T2D patients and control participants with a normal distribution, while the Mann-Whitney *U* test was used for variables with skewed distribution.

The frequencies of the risk allele for each gene variant were calculated for T2D patients and normoglycemic control participants. The proportions of the genotypes of the gene variants were tested for departures from Hardy-Weinberg equilibrium (HWE) for both groups using population genetics software GenA1Ex 6.3 (Genetic analysis in Excel, version 6.3)^[31]. However, in case-control studies, HWE should be applied only to controls because a deviation from HWE in cases may indicate a genetic association^[32,33].

Genotyping data were further analyzed using GenA1Ex 6.3^[31] and Arlequin 3.1 software^[34]. For each polymorphism, GenA1Ex was used to calculate fixation index (*F*), heterozygosity (*He*) and *Fst*, which provides a measure of genetic differentiation among subpopulations (T2D patients and control populations). Arlequin was used to calculate genotype's % variation among the subpopulations and its level of significance.

Binary logistic regression analysis on the SPSS statistical package was used to test the association between each gene variant and susceptibility to T2D, adjusted for age, sex and BMI. Bonferroni correction was applied for multiple testing and adjusted *P* values were calculated to be 0.005. Beta coefficients, ORs and 95%CI were also estimated. An OR is a measure of association between an exposure and an outcome. It is measured as an exponential function of the regression

beta coefficient value ($e^{\text{beta coefficient}}$).

The association between obesity status of participants and *FTO* gene variants (rs9939609 and rs8050136) was also tested. In addition, obesity and health risk status, based on the WHR, were tested for their association with T2D risk.

RESULTS

Anthropometric and biochemical characteristics of all participants are summarized in Table 1. About 48% of the T2D patients and 41% of the control participants were males. The mean age of T2D patients (56 years) was higher than that of the normoglycemic control participants (45 years). T2D patients had significantly higher weight, BMI, waist circumference, fasting glucose values and HbA_{1c} % levels compared with control subjects (Table 1). Eighty five percent of the T2D Omani patients were overweight to obese in comparison to 78.5% of the control Omani participants. Half of the T2D patients and 39.6% of the control participants were obese. T2D risk was found to be significantly associated with obesity ($P = 0.002$, OR = 2.22); and with the WHR ($n = 532$, $P = 1.9 \times 10^{-7}$, OR = 2.4), [among males ($n = 234$, $P = 1.2 \times 10^{-4}$, OR = 2.0) and females ($n = 298$, $P = 0.001$, OR = 6.3)].

Among control participants, there were no significant deviation in the proportions of gene variant frequencies from HWE except in *SLC30A8* (rs13266634) ($P = 2.35 \times 10^{-4}$, $\chi^2 = 13.5$) gene variant. However, among T2D patients, there were significant deviations in: *KCNJ11* (rs5219) ($P = 4.14 \times 10^{-9}$, $\chi^2 = 34.6$), *CDKAL1* (rs10946398) ($P = 0.008$, $\chi^2 = 6.9$) and *IGF2BP2* (rs4402960) ($P = 0.038$, $\chi^2 = 4.3$) gene variants.

The risk allele frequencies of the tested variants for diabetic and control participants are summarized in Table 2. Using binary logistic regression analysis,

Table 2 Risk allele frequencies for the tested gene variants among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variant (SNPs)	Risk/non-risk allele	Risk allele frequency (f)		¹ P value	OR	95%CI for OR
			T2D patients (n = 992)	Controls (n = 294)			
<i>KCNJ11</i>	rs5219	T/C	0.320	0.222	² 5.8 × 10 ⁻⁶	1.74	1.37-2.22
<i>TCF7L2</i>	rs7903146	T/C	0.445	0.354	² 0.001	1.46	1.16-1.83
<i>CDKAL1</i>	rs10946398	C/A	0.364	0.311	² 0.002	1.44	1.15-1.80
<i>CDKN2A/B</i>	rs10811661	T/C	0.836	0.799	0.020	1.40	1.06-1.84
<i>FTO</i>	rs9939609	A/T	0.480	0.435	0.358	1.11	0.899-1.37
<i>FTO</i>	rs8050136	A/C	0.458	0.425	0.770	1.03	0.829-1.29
<i>IGF2BP2</i>	rs4402960	T/G	0.400	0.357	0.286	1.13	0.904-1.41
<i>SLC30A8</i>	rs13266634	C/T	0.857	0.855	0.329	1.16	0.859-1.57
<i>CAPN10</i>	rs3792267 (-43)	G/A	0.802	0.790	0.445	1.11	0.850-1.45
<i>HHEX</i>	rs1111875	T/C	0.301	0.280	0.636	1.06	0.839-1.33

¹P value: Level of significance; ²P value remained significant after correction for multiple testing (< 0.005). The P value, OR and 95%CI were calculated for the association between each gene variant with T2D risk. f: Frequency; T2D: Type 2 diabetes mellitus; SNPs: Single nucleotide polymorphisms.

Table 3 Fixation index, heterozygosity, Fst and % variation among Omani type 2 diabetes mellitus patients and control participants

Gene	Gene variants	T2D patients		Controls		Fst	% variation among T2D and controls	¹ P value
		F	He	F	He			
<i>KCNJ11</i>	rs5219	0.190	0.352	0.091	0.314	0.012	2.07	0.000
<i>TCF7L2</i>	rs7903146	0.017	0.486	0.000	0.476	0.009	1.62	0.000
<i>CDKAL1</i>	rs10946398	0.084	0.424	0.056	0.405	0.003	0.48	0.020
<i>CDKN2A/B</i>	rs10811661	0.000	0.275	0.003	0.320	0.002	0.37	0.042
<i>FTO</i>	rs9939609	0.000	0.502	0.000	0.497	0.002	0.31	0.050
<i>FTO</i>	rs8050136	0.006	0.493	0.000	0.503	0.001	0.12	0.147
<i>IGF2BP2</i>	rs4402960	0.066	0.448	0.000	0.476	0.002	0.23	0.089
<i>SLC30A8</i>	rs13266634	0.061	0.230	0.188	0.201	0.000	0.00	1.000
<i>CAPN10</i>	rs3792267	0.057	0.300	0.000	0.335	0.000	0.00	0.517
<i>HHEX</i>	rs1111875	0.055	0.398	0.052	0.382	0.001	0.00	0.341

This provides a measure of genetic differentiation among population; ¹P value: Level of significance; F: Fixation index; He: Heterozygosity; Fst: The inbreeding coefficient within subpopulation, relative to total; T2D: Type 2 diabetes mellitus.

four gene variants out of 10 showed statistically significant association ($P < 0.05$) with susceptibility to T2D: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661), Table 2. After correction for multiple testing, *KCNJ11*, *TCF7L2* and *CDKAL1* gene variants still showed a significant association ($P < 0.005$) with T2D.

Fst values, showed statistically significant genetic differentiation between T2D patients and controls (Table 3), in the following gene variants: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). These findings confirmed the results obtained using binary logistic regression analysis.

This study was not able to detect an association of T2D risk with gene variants of *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *CAPN10* (rs3792267) and *HHEX* (rs1111875). Moreover, no association was found between *FTO* gene variants (rs9939609 and rs8050136) and T2D risk.

DISCUSSION

In this study, four gene variants showed significant association with T2D risk using binary logistic regression analysis after adjustment for confounding factors

of age, sex and BMI: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). The association of *KCNJ11* (rs5219), *TCF7L2* (rs7903146) and *CDKAL1* (rs10946398) gene variants with T2D risk remained significant after correction for multiple testing. Fst, a measure of genetic differentiation among subpopulations (diabetics and controls), confirmed the significant risk difference between diabetics and controls at the four gene loci [*KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661)]. The highest genetic variation between diabetics and controls was found in *KCNJ11* and *TCF7L2* gene variants (Table 3). However, none of the other gene variants previously reported in GWAS were found to be associated with risk to T2D in Omanis.

KCNJ11 (rs5219) gene variant was found to be associated with T2D risk among Omani Arabs with an OR of 1.74, which is higher than that reported in previous European studies. Our findings were consistent with what was reported among Saudi Arabs (OR = 1.7), although the risk allele frequency was found to be lower among Saudis compared to Omani Arabs^[16]. However, in both Arab studies the association may be overestimated due to the small number of participants included or due to a high-inbred population.

In contrast, no association of this gene variant with susceptibility to T2D was found among Tunisian Arabs^[14]. Large scale studies and meta-analysis of the *KCNJ11* gene variants have shown that the lysine variant of the rs5219 gene loci resulting in a 1.15 times higher risk of developing T2D^[4,35] and GWAS studies confirmed this association^[26,36,37]. The rs5219 variant is located within the N-terminal of the subunit kir6.2 of the ATP-sensitive potassium channel gene of β -cells and can cause spontaneous hyperactivity of pancreatic beta-cells and reduced sensitivity of KATP channels to ATP, resulting in impaired insulin secretion^[38].

Although this study is relatively small, the association of *TCF7L2* (rs7903146) gene variant with T2D risk among Omani Arabs is consistent with previous large GWAS studies^[14,15,22,36,37,39-44]. The association of *TCF7L2* gene variants with susceptibility to T2D was marginal between the rs12255372 gene variant and T2D risk among Emirati Arabs; but not in Saudi Arabs^[13,17]. In contrast, subsequent studies among North African Arabs (Tunisians and Moroccans), Palestinians and Iranians, confirmed the association of *TCF7L2* (rs7903146) gene variant with susceptibility to T2D^[7,14,15,22]. Comprehensive genotyping studies across the *TCF7L2* gene showed that the rs7903146 variant to be consistently associated with T2D among European with an OR of 1.37 (1.28 to 1.47)^[45,46]. Meta-analysis of 27 different studies found a global OR of 1.46 (95%CI: 1.42-1.51)^[7]. The common variants in the *TCF7L2* gene predispose to T2D by reducing beta-cell function and insulin secretion. *TCF7L2* mRNA levels in human pancreatic islets increase with the number of risk alleles and are fivefold higher in T2D patients than in controls pancreatic islets, and over-expression of *TCF7L2* leads to reduced glucose stimulated insulin secretion^[47].

CDKAL1 (rs10946398) gene variant's OR was found to be 1.44 among Omani Arabs, which may be overestimated due to the small number of participants. Variants at *CDKAL1* gene loci showed an association with T2D risk among Caucasians^[26,36,37], Asians^[48,49], African Americans^[50] and Arabs^[22,24]. A recent meta-analysis of *CDKAL1* (rs10946398) gene variant showed a significant association of this variant with susceptibility to T2D risk (OR = 1.12)^[51]. Another study among Russian population showed a significant association of the C allele with higher risk of T2D^[52]. The *CDKAL1* gene encodes cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1. The CDK5 is a serine/threonine enzyme that inhibits both Ca^{2+} efflux into the beta-cell and insulin secretion, while inhibition of this enzyme results in enhanced insulin secretion^[53].

The association of *CDKN2A/B* (rs10811661) gene variant with susceptibility to T2D risk among Omanis was also confirmed in this study (OR = 1.40), in agreement with a recent large study among North African Arabs^[22]. In contrast, this gene variant was not found to be associated with T2D risk in other Arab studies^[24,54]. Previous studies among European populations have

shown an association with an OR of 1.20^[26,36,37]. A recent meta-analysis concluded that the T allele of rs10811661 is a risk factor of T2D both in Asians and Europeans^[55]. *CDKN2A* and *CDKN2B* genes encode p16^{INK4a} and p15^{INK4b}, which inhibit CDK4 and CDK5, respectively. CDK4 and CDK5 play an important role in β cell function and regeneration^[55].

FTO gene variants (rs9939609 and rs8050136) have been shown to associate with BMI and obesity^[8,56] and GWAS studies of T2D have also suggested the involvement of *FTO* gene in T2D pathogenesis through obesity^[8,26]. It is surprising, therefore, that we could not detect an association of T2D risk with *FTO* gene variants (rs9939609 and rs8050136). This could be attributed to the fact that both diabetics and controls have similar distributions of body weight. The impact of the *FTO* gene variants on T2D risk through obesity, seen in other populations, was not observed here. Hennig *et al*^[57] tested the effect of *FTO* variants on measures of BMI in a population of lean Gambians (Africans) and also found no association. However, this study showed a significant association between obesity and T2D risk; and between health risk, based on the WHR, and T2D risk among males and females.

In spite of the small number of participants examined in this case-control study, we were able to confirm the effect of four common gene variants on T2D risk among Omani Arabs. Oman has a homogeneous population due to a high level of inbreeding and the tradition of consanguineous marriages. The difficulty in recruiting Omani participants with no family history of diabetes was the main reason behind the small number of control participants in this study, where, almost everybody has a relative with DM. This might have raised risk allele frequencies of *T2D* gene variants and made it easier to detect.

All previous GWAS identified common gene variants, which could only explain 10%-15% of the heritability of T2D. Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Limitation of this study was the lack of oral glucose tolerance test, where we could not run the test among the control group. However, the strength of this study was that the control participants were with no family history of diabetes.

This study confirmed the effect of four common gene variants on T2D risk among Omani Arabs: *KCNJ11* (rs5219), *TCF7L2* (rs7903146), *CDKAL1* (rs10946398) and *CDKN2A/B* (rs10811661). However, we could not detect the association of other known common gene variants with susceptibility to T2D.

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COMMENTS

Background

Type 2 diabetes mellitus (T2D) is one of the most common non-communicable diseases globally. Insufficient compensatory insulin secretion due to insulin resistance causes T2D. In addition to the environmental factors, there is strong evidence that genetic factors play an important role in the pathogenesis of T2D.

Research frontiers

Oman has a high inbred population and consanguineous marriages are about half of all marriages. Therefore, genetic factors might play an important role in the pathogenesis of T2D among Omanis.

Innovations and breakthroughs

In the present study, 10 known common gene variants were examined for their association with susceptibility to T2D among Omani Arabs using case-control study design. Selection of variants was predominantly based on earlier Genome-wide association studies, which extensively investigated T2D and showed a significant association of those variants with the highest odds ratios among all the genes/loci discovered.

Applications

Large studies with new strategies, other than the classic case-control study design, are required to find the hidden heritability due to rare variants behind developing T2D among Omani and other Arab populations.

Terminology

T2D is one of the most common non-communicable diseases globally, and it is a result of insufficient compensatory insulin secretion due to insulin resistance. Candidate gene approach focuses on associations between genetic variation within pre-specified genes of interest and phenotypes or disease states. Genome-wide association studies scan the entire genome for common genetic variation.

Peer-review

This is a well-written and interesting paper evaluating the association between a variety of gene polymorphisms and the risk for type 2 diabetes mellitus.

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