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Type 2 diabetes mellitus susceptibility gene TCF7L2 is strongly associated with hyperglycemia in the Saudi Arabia Population of the eastern province of Saudi Arabia

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Abstract. – OBJECTIVE: We studied the association of single nucleotide polymorphisms (SNPs) rs7903146, rs12255372 and rs4506565 in type 2 diabetes mellitus (T2DM) susceptibility gene, transcription factor 7 like 2 (TCF7L2) with T2DM among the population of the Eastern Province of Saudi Arabia.

PATIENTS AND METHODS: In a case-control study, blood samples were collected from 359 T2DM patients and 351 age and sex-matched normoglycemic controls. Genotyping was done by allele specific PCR assay.

RESULTS: Our results revealed a strong association between risk T alleles in variants rs12255372 (OR: G/T=1.4233; T/T=2.0395) and rs4506565 (OR: A/T=1.6066; T/T=3.1301) and T2DM among the Saudi population of the Eastern Province of Saudi Arabia. This is the first time that this association has been identified in a Saudi population. However, a common variant, rs7903146, often found to be associated with T2DM in other populations failed to demonstrate any association to T2DM with the present population. These data further strengthens the hypothesis that Saudi populations might carry a distinct risk allele in T2DM susceptibility gene TCF7L2.

CONCLUSIONS: The present results confirm that rs12255372 and rs4506565 variants of TCF7L2 show an association, but not rs7903146, with T2DM for the Saudi population of the Eastern Province of Saudi Arabia.

Key Words:
TCF7L2, T2DM, Saudi population, Association.

Introduction

Saudi Arabia has a high incidence of diabetes mellitus with a prevalence of 24% as noted in the Diabetes Atlas, 2013 of International Diabetes Federation (IDF)[1]. The most prevalent form of this debilitating disease is Type 2 Diabetes Mellitus (T2DM), which is strongly influenced by inheritance and other factors such as age, obesity and life style. The disease is believed to be polygenic involving association with polymorphism in several genes encoding metabolic enzymes and transcription factors. However, more than any other factors, the genetic variants in the TCF7L2 (transcription factor 7 like 2) gene have been consistently associated with T2DM among several populations of various ethnicity throughout the world including France[2], Scandinavia[3], Europe[4], Japan[5], India[6], Latin America[7], West Africa[8], United States of America[9], United Kingdom[10], Lebanon[11] and Tunisia[12].

TCF7L2 encodes a transcription factor involved in Wnt/β-catenin signaling pathway that regulates cell survival, cell migration, proliferation and differentiation. The overexpression of the gene in pancreatic β cells results in impaired insulin secretion. Several of both intronic and exonic single nucleotide polymorphisms (SNPs) in TCF7L2 gene have been studied extensively with more emphasis on rs7903146 (C>T), rs12255372 (G>T) and rs4506565 (A>T). The association of the TCF7L2 genetic variant particularly rs7903146 with T2DM has consistently been replicated in various ethnic populations, but with contradicting results in case of Arab populations. Further, the data on the SNPs in TCF7L2 gene and its association with T2DM in Saudi Arabia is limited except for the report of Alsmadi et al[13] who recorded no association of rs7903146 variant with T2DM risk in the Saudi population of

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the Central Province, which is contrary to studies conducted on other populations[2-12]. Hence, with the hypothesis that the Saudi Arab population from Eastern Province with distinct ethnic origin could harbor the same SNP as a predisposing factor for development of hyperglycemia, we further investigated two more commonly encountered SNPs of TCF7L2 in T2DM, which had not been included in the previous study. Accordingly, the present study was undertaken to assess the SNPs in TCF7L2 variants rs7903146 (C>T), rs12255372 (G>T) and rs4506565 (A>T) and its association with T2DM in the population of the Eastern Province of Saudi Arabia.

Patients and Methods

Study Patients

A case-control study was conducted on randomly selected T2DM patients (n=359) who were attending the Department of Endocrinology, King Fahd Hospital of the University, University of Dammam, Al-Khobar, Eastern Province, Kingdom of Saudi Arabia during the years 2011 and 2012. As this hospital is the referral specialist health care center in the region, the population under study represented the samples across the Eastern Province. The study group was limited to Saudi subjects, over the age of 30 years, residing in the Eastern Province. Diabetes was diagnosed as per the WHO, 2006 definitions set forth for confirming the T2DM[14]. A similar number (n=351) of age and sex matched control subjects who showed no signs of diabetes or had no family history of diabetes were also included in the study. The study was approved by the Ethical Committee of the University and written informed consent was obtained from all subjects involved.

Sample Collection and DNA Extraction

Five milliliters of peripheral blood was drawn in heparinized blood collection tubes (Vacutainer, BD Diagnostics, USA) from the study group and control group, which was immediately transported to the laboratory in an ice box. DNA was extracted from the whole blood using "*illustra blood genomicPrep mini spin kit*" (GE Healthcare, Buckinghamshire, United Kingdom) as per the manufacturer's protocol. DNA was quantified and purity was measured using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, United States of America) and stored at -80°C.

Allele Specific PCR Assay

Genotyping of the SNPs in the gene TCF7L2 was undertaken using techniques of allele specific PCR assay as described by Dutra et al[15]. The protocol is based on the Amplification of Refractory Mutation System (ARMS) method in which a set of primers has a mismatch in their 3' end. Each mismatch nucleotide is specific for one of the two variants of the polymorphic loci. A pair of wild or mutant allele specific forward primer and a common reverse primer flanking downstream of the polymorphic region were designed based on the previously established protocol[15] (Table I). A second mismatch was also main-

Table I. Primers used for genotyping of TCF7L2 SNPs of T2DM patients and control subjects

Target SNP	Primers	Sequence (5′-3′)	Reference
rs7903146 (C>T)	rs7903146-C (forward)	GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC	15
	rs7903146-T (forward)	GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT	
	rs7903146 (common reverse)	AGA TGA AAT GTA GCA GTG AAG TGC	
rs12255372 (G>T)	rs12255372 (common forward)	TTT TGT TAA TGG CTT GCA GGT	Present study
	rs12255372-G (forward)	TGC CCA GGA ATA TCC AGG CAA GAC TG	
	rs12255372-T (reverse)	GGC CTG AGT AAT TAT CAG AAT ATG ATA	
	rs12255372 (common reverse)	GCG CAT GCT AAT TTC CTG TC	
rs4506565 (A>T)	rs4506565-A (forward)	GAT ATG GCG ACC GAA GTG CTA	Present study
	rs4506565-T (forward)	GAT ATG GCG ACC GAA GTG CTT	
	rs4506565 (common reverse)	TGA GAG TGC AAC CAT CTG GA	

Mismatch bases are underlined

Table II. Description of T2DM patients and control subjects participated in the study

Parameter	T2DM Subjects (n=359)	Control Subjects (n=351)	*p* – value*
Male (%)	162 (45.12)	168 (47.86)	0.6120
Female (%)	197 (54.88)	183 (52.14)	0.6811
Mean Age (years)	50.94 ± 9.82	51.29 ± 8.03	0.9011
Age range (years)	30-83	30-86	-

*p value <0.05 was considered significant

tained at the third nucleotide from the 3' end of the forward primer, which hinders primer extension of doubly mismatched primer, thus, increasing the specificity of the allele specific PCR reaction (Table I). Standard PCR reaction mixture was formulated and amplification was performed on a MasterCycler (Eppendorf, Hamburg, Germany) with initial denaturation for 5 min at 94ºC, followed by 30 cycles of 1 min denaturation at 94ºC, 30 sec annealing at 47ºC and 1 min primer extension at 72ºC and a final extension of 5 min at 72ºC. The products were resolved on an ethidium bromide stained 1.2% agarose gel and photographed on a gel documentation system (Syngene, Cambridge, UK).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, *IBM SPSS Statistics 20.0* (IBM, New York, NY, USA). The mean age of cases and controls was compared by Students *t* test. Male and female ratio goodness of fit was analyzed by chi square (X^2) test. Genotype frequencies were tested using X^2 analysis for Hardy-Weinberg equilibrium. Subjects with CC homozygous allele were used as reference for testing Odds Ratio (OR) with 95% confidence intervals (CIs) after matching for age and sex to see the association of genotype with T2DM. In all cases a *p* value of <0.05 was considered as statistically significant.

Results

The present study analyzed the possible relationship between T2DM and SNPs at rs7903146 (C>T), rs12255372 (G>T) and rs4506565 (A>T) in TCF7L2 gene in T2DM patients and the normoglycemic control group. The distribution of males was 162 (45.12%; n=359) in the T2DM group and 168 (47.86%; n=351) in the control group with no significant difference between the male and female ratio by chi square (χ^2) test (*p*, 0.6120/0.6811) (Table II). The mean age was 50.94 ± 9.82 and 51.29 ± 8.03 for the T2DM group and control group, respectively (*p*, 0.9011; Table II).

Allele and genotype frequency for minor "T" allele for the SNP rs7903146 is presented in Table III. The heterozygous (CT) genotype frequency of the cases was 0.3816 and that of controls was 0.4074. Odds ratio (OR), with CC genotype taken as the reference, showed 0.9654 (95% CI of 0.6894 to 1.3517; *p*, 0.8374) indicating no significant difference between the patient and control groups (Table III). The homozygous (TT) risk genotype frequency of the patient group was 0.2535, which was slightly higher than that of the control group (0.2165). However, though this genotype showed an OR of 1.2065, higher than the heterozygous (CT) genotype, statistically it was not found to be significant (95% CI of 0.8178 to 1.7799; *p*, 0.3440) (Table III).

Similarly, the allele frequency for minor "T" allele for another SNP, rs12255372, was 0.4554 and 0.3830 for the patient and control groups, respectively (Table III) showed no statistically significant difference (OR, 1.3472; 95% CI of 0.0454 to 1.7362; *p*, 0.0213). The heterozygous (GT) genotype frequencies were 0.4067 and 0.3761 for the patient and control groups respectively with an OR of 1.4233 (95% CI of 1.0241 to 1.9781; *p,* 0.0356). However, homozygous (TT) risk allele frequencies were significantly higher in the patient group (0.2340) than in the control group (0.1510) with a substantially higher OR of 2.0395 (95% CI of 1.3485 to 3.0845; *p*, 0.0007) (Table III).

The SNP, rs4506565 (A>T) of TCF7L2, revealed a significantly higher risk allele and genotype (both heterozygous and homozygous) frequency and OR for T2DM patients than control individuals (Table III). The patient group showed a risk allele frequency of 0.4909 compared to the control group, which showed a frequency of 0.4094 (OR, 1.3909; 95% CI of 1.0934 to 1.7694; *p*, 0.0072). ORs for heterozygous (AT)

Table III. Genotype analysis relating to TCF7L2 variants and risk of T2DM.

SNP variants of TCF7L2	Genotype	T2DM Subjects (n = 359)	Control Subjects (n = 351)	O.R (95% CI)*	*p*-value**
rs7903146 (C>T)	C allele (%)	268 (54.03)	275 (55.67)	-	-
	T allele (%)	228 (45.97)	219 (44.33)	1.0683 (0.8317 to 1.3722)	0.6051
	CC (%)	131 (36.49)	132 (37.61)	-	-
	CT (%)	137 (38.16)	143 (40.74)	0.9654 (0.6894 to 1.3517)	0.8374
	TT (%)	91 (25.35)	76 (21.65)	1.2065 (0.8178 to 1.7799)	0.3440
rs12255372 (G>T)	G allele (%)	275 (54.46)	298 (61.70)	-	-
	T allele (%)	230 (45.54)	185 (38.30)	1.3472 (1.0454 to 1.7362)	0.0213
	GG (%)	129 (35.93)	166 (47.29)	-	-
	GT (%)	146 (40.67)	132 (37.61)	1.4233 (1.0241 to 1.9781)	0.0356
	TT (%)	84 (23.40)	53 (15.10)	2.0395 (1.3485 to 3.0845)	0.0007
rs4506565 (A>T)	A allele (%)	280 (50.91)	313 (59.06)	-	-
	T allele (%)	270 (49.09)	217 (40.94)	1.3909 (1.0934 to 1.7694)	0.0072
	AA (%)	89 (24.79)	134 (38.18)	-	-
	AT (%)	191 (53.20)	179 (50.99)	1.6066 (1.1472 to 2.2498)	0.0058
	TT (%)	79 (22.01)	38 (10.83)	3.1301 (1.9550 to 5.0116)	<0.0001

*Age and sex matched Odds ratio

**p value <0.05 was considered significant

and homozygous (TT) risk allele frequencies were 1.6066 (95% CI of 1.1472 to 2.2498; *p,* 0.0058) and 3.1301 (95% CI of 1.9550 to 5.0116; *p,* <0.0001) respectively and revealed a statistically significant association between rs4506565 and T2DM (Table III).

Discussion

Identification of TCF7L2 as one of the T2DM target genes in genome wide association studies (GWAS) and case-control studies[16-20] has lead researchers to study the gene extensively for various SNPs correlating to the manifestation of hyperglycemia. As a result, SNPs in various loci of TCF7L2 have been shown to be associated with T2DM among many populations of various ethnicity and geographical locations[11,12,15,20,21]. However, there has been a contrasting report showing weak or lack of association between TCF7L2 common variants rs7903146 and rs12255372 and T2DM among Saudi Arabs of the Central Province of the Kingdom[13]. Hence, replication studies on the association of TCF7L2 variants to T2DM for the Eastern Province, which is ethnically and geographically different from the other provinces of Saudi Arabia, would help in generalizing the pattern of TCF7L2 SNPs. This is the first study on a Saudi Arabian population to be conducted to elucidate on the three major common variants of TCF7L2 and its association to T2DM. Our results suggest that TCF7L2 variants rs12255372 and rs4506565 do contribute at a greater extent to the risk of development of T2DM among the Saudi population of the Eastern Province of Saudi Arabia. However, a common variant, rs7903146, failed to demonstrate any correlation for the same population group.

The minor T allele frequency for the SNP rs7903146 among the Saudi population of the Eastern Province of Saudi Arabia was recorded in this study at 0.4597 for cases and 0.4433 for controls, respectively, with no statistically significant difference (OR of 1.0683; 95% CI of 0.8317 to 1.3722; *p*, 0.6051). The results are similar with earlier reports published for Saudi populations in the Central Province of the Kingdom[13], which showed no significant difference among the distribution of allele frequency for this particular SNP among the two regions. Furthermore, the results of our study are consistent with the earlier reports for the central regions of the Kingdom; rs7903146 variant in TCF7L2 showed no association to T2DM with a statistically insignificant OR of 0.9654 (95% CI of 0.6894 to 1.3517; *p*, 0.8374) for heterozygous (CT) risk allele and an OR of 1.2065 (95% CI of 0.8178 to 1.7799, *p*, 0.3440) for homozygous risk (TT) allele respectively (Table III). Among all the SNPs in TCF7L2, rs7903146 is the most studied and shows the strongest association in various ethnic groups *viz*., Europeans[7,9,18,19,23,28], Asians[5,6,23,26], Africans[8,23], but failed to prove its association among Saudi[13], present study and Emirati[21] populations indicating the possible presence of a dif-

ferent risk allele among these populations.

Many studies have also implicated rs12255372 and rs4506565 or various other combinations reported to be associated with T2DM[11,12,15,20,21]. We also investigated the association of rs12255372 (G>T) polymorphism with T2DM among the Saudi population of the Eastern Province and recorded a significantly high OR of 2.0395 (95% CI of 1.3485 to 3.0845; *p*, 0.0007) for homozygous TT genotype (Table III) indicating a high risk of development of T2DM among the carriers of this minor allele. The results are in contrast to previous data published for the same SNP for the Saudi population of the Central Province[13], suggesting that the population of the Eastern province carries a different risk allele for T2DM than their counterparts in the Central Province. However, the results of the present study are in line with the observations made for an Emirati population[21] in which SNP rs12255372 is associated with T2DM with similar magnitude, but not rs7903146. In addition, an Iranian population also shares the same SNP rs12255372, minor T allele, which is a risk allele in TCF7L2 gene for the development of T2DM[20]. This variant of TCF7L2 had earlier been shown to be associated with T2DM for Caucasian[22], North Europeans[8,9], Africans[23,24], East Asians[25,26] and Indians[6,27], suggesting that rs12255372 affects populations of various ethnic groups.

In addition, the present study also revealed for the first time, for any Saudi population, a moderate association of heterozygous (AT) genotype (OR, 1.6066; 95% CI of 1.1472 to 2.2498; *p,* 0.0058) and a remarkably stronger association of homozygous (TT) genotype (OR, 3.1301; 95% CI of 1.9550 to 5.0116; $p < 0.0001$) in the SNP rs4506565 of TCF7L2 to T2DM (Table III). TCF7L2 SNP rs4506565 had earlier been documented to be associated with T2DM with a varying degree among Lebanese[11] and Tunisian Arabs[12] (Table IV) but no data was available on the Saudi population. Non-Arab populations, such as India[6] and Sweden[29], also documented a significant association of this SNP to T2DM with an OR of 1.48 (1.24–1.77; $p < 0.0001$) and 1.588 (1.251-2.015; $p < 0.0001$), respectively. The present report documents that SNP rs4506565 is strongly associated with T2DM among the Saudi population of the Eastern Province of Saudi Arabia.

Variants of T2DM susceptibility gene, TCF7L2 show varying degree of association to T2DM among various populations. In spite of the inconsistency in replication of the results for several of the variants, common variant rs4506546 has been found to be associated in most of the populations studied, but not among Saudi and Emirati populations. Hence, the previous data, besides the results of present study, further support the hypothesis that Saudi population show strong association with less common variants, such as rs12255372 and rs4506565, than the more common variant rs7903146. However, other than the functional variants of alternative splicing, the precise role of these intronic SNPs in the development of T2DM is yet to be characterized, nevertheless, the TCF7L2 still remains a T2DM susceptibility gene for Saudi populations in Saudi Arabia and ethnically different population groups worldwide.

Conclusions

The present study demonstrates that the less common TCF7L2 variants, rs12255372 and rs4506565, have the strongest association to T2DM in the population of the Eastern Province

Table IV. Comparative analysis of SNPs in TCF7L2 gene for Arab populations

Population	Association with T2DM expressed as O.R*			
	rs7903146	rs12255372	rs4506565	References
Iran	-	Yes	-	21
Tunisia	Yes	Yes	Yes	12
Lebanon	Yes	Yes	Yes	11
United Arab Emirates	No	Yes	-	22
Saudi Arabia (Central Province)	No	No/Weak	-	13
Saudi Arabia (Eastern Province)	No	Yes	Yes	Present study

*Odds ratio

of Saudi Arabia rather than the common variant, rs7903146. The present results, contrary to other studies conducted on populations in the other provinces of the Kingdom, indicate that TCF7L2 is a T2DM susceptibility gene for the population of the Eastern Province of Saudi Arabia. Further association studies are warranted to determine whether other SNPs reported for this gene have a significant association with T2DM in the Eastern Province.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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