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Associations of GCKR, TCF7L2, SLC30A8 and IGFB Polymorphisms with Type 2 Diabetes Mellitus in Egyptian Populations

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Abstract

Novel genes have been identified by Genome-wide association studies (GWAS) to be associated with type 2 diabetes mellitus (T2DM) which have been replicated in different ethnic populations and yielded inconsistent results. We aimed to study the possible association between glucokinase regulator gene (GCKR), transcription factor 7 like 2 (TCF7L2), Solute Carrier Family 30 Member 8 (SLC30A8) and insulin like growth factor binding (IGFB) genes polymorphisms (rs 780094, rs 7903146, 12255372, rs 11558471 and rs 2854843) and T2DM in Egyptian people. This case control study was conducted on 228 subjects divided into two groups; the control group which contains 96 healthy individuals and 132 patients with T2DM. Single nucleotide polymorphisms (SNPs) (rs 780094, rs 7903146, 12255372, rs11558471 and rs 2854843) were detected by real-time polymerase chain reaction (Rt- PCR). For GCKR gene (rs 780094) the variant T allele was associated with T2DM (p=0.001) and the frequency of (TT and CT) genotypes vs. CC genotype was significantly higher in T2DM patients than control (p = 0.001). These genotypes showed higher risk for T2DM (OR = 8.4, 95% CI= 3.2 -22.0 and OR= 5.4, 95% CI= 2.9- 10.0 respectively, p= 0.001). IGFB gene (rs 2854843) showed that the risk of T2DM is increased with TT and CT genotypes (OR= 13.3, 95% CI=6.26 - 28.4, OR= 3.0, 95% CI= 1.5 – 6.0, respectively, p= 0.001). With regard to SLC30A8 gene (rs 11558471) the only genotype that showed significant association with type 2 diabetes is AG genotype (p=0.01). Also, we identified strong association between T2DM and TCAGT, TTGTT haplotypes (p= 0.001 and 0.01 respectively). Conclusion: Our study revealed a significant association between T2DM and TCAGT, TTGTT, TTATT and TCATT haplotypes.

Keywords: type 2 diabetes mellitus, single nucleotide polymorphisms,haplotype, Polymerase Chain Reaction.

1. Introduction

Diabetes mellitus (DM) is a chronic life-threatening disease, and it is the second leading cause of death over the world among adults between age of 35 and 64 years; 1 of every 10 deaths is caused by DM (El-Lebedy *et al.*,2014). DM is one of metabolic diseases caused by impaired insulin action, insulin secretion or defect in both mechanisms. The underlining cause of type 2 diabetes mellitus (T2DM) can be attributed to combination of increased production of insulin hormone as a compensatory response to resistance to its action and inadequate secretory response (Sturgeon *et al.*, 2016); meanwhile, expansion of low physical activity life style and obesity increased prevalence of diabetes (Hasan *et al.*, 2017). On the other hand, genetic factors have an important role in the development of the underlining pathology of diabetes (Murea *et al.*, 2012). The rising prevalence of diabetes highlights the urgent need for aggressive strategies aimed at the prevention and control of diabetes (Liu *et al.*, 2015; Guo *et al.*, 2012). Establishment of single-nucleotide polymorphism (SNP) databases, development and improvement of cost-effective high-throughput genotyping technology, and multi-center consortium large-scale genome-wide association studies (GWAS) are effective methods to investigate genetic susceptibility to T2DM (Xiao *et al.*, 2016). Glucokinase (GCK), expressed in beta cells of pancreas and in the liver, is the key enzyme that encodes rate-limiting step of glycolysis, glucose stimulated insulin secretion and regulating glucose balance (Wang *et al.*, 2018). The activity of GCK is controlled by the glucokinase regulatory protein (GCRP), which binds to GCK. Its inhibitory effect is antagonized by fructose 1-phosphate and enhanced by fructose 6-phosphate. The glucokinase regulator gene (GCKR) encodes GKRP. GCKR(rs780094) is the most commonly reported SNP that has been shown by GWAS studies to be associated with insulin levels, triglycerides (TG), fasting glucose (Wang *et al.*, 2018), and susceptibility to T2DM (Zhou *et al.*, 2018; Ma *et al.*, 2016). The transcription factor 7 like 2 (TCF7L2) gene is considered as an important gene of susceptibility for diabetes in European populations.

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Lyssenko *et al*.2007 found that SNPs in TCF7L2 gene (rs12255372 and rs7903146) have strong association with the risk of diabetes. These gene risk alleles are associated with β-cells dysfunction of pancreases in all subjects (Grant *et al*., 2006).

A number of susceptibility variants for T2DM have been identified by GWAS. The common alleles of SNPs, rs13266634(C/T, Arg276Trp), and rs11558471 (A/G) in the Solute Carrier Family 30 Member 8 (SLC30A8) gene are found to confer the risk susceptibility in T2DM (Abu Seman *et al*., 2015). The genes including SLC30A8 identified by GWAS, however, can only explain approximately 10% of the overall heritable risk of T2DM, which challenges our expectations to translate genetic information into clinical practice (Imamura *et al*., 2011). Insulin-like growth factor 2 mRNA (messenger-ribonucleic acid) binding protein 2 (IGF2BP2) belongs to a family of IGF2 mRNA-binding proteins that is implicated in Insulin growth factor 2 (IGF2) translational regulation, mRNA localization and turnover (Bell *et al*.,2013; Dai *et al*., 2011). IGF-2 plays a role in glucose homeostasis through inhibiting the gluconeogenesis by the liver as well as increasing the uptake of glucose by peripheral tissues (Zachariah *et al*., 2007). Several IGF2BP2 gene variants were identified and investigated for association with T2DM (Gu *et al*., 2012). This study identified 4 novel susceptibility genes associated with T2DM including GCKR, TCF7L2, SLC30A8 and IGFB. We aim to assess the association between GCKR polymorphisms (rs 780094), TCF7L2 polymorphisms (rs 7903146 and 12255372), SLC30A8 polymorphisms (rs 11558471), and IGFB polymorphisms (rs 2854843) and T2DM in Egyptian people.

2. Materials and Methods

2.1. Studied Population

This study is a case-control study that included 228 subjects. They are divided into 96 apparently healthy control subjects who were age and sex matched and 132 patients with T2DM. The cases were recruited from out patients' clinic of the National Research Centre, National Egyptian Institute of Diabetes, and Coronary Care Unit (CCU) of Ain Shams Hospital, Cairo Egypt.

According to American Diabetes Association (ADA) [American Diabetes Association (2018)]; DM is diagnosed when fasting blood glucose (FBG) level ≥126 mg/dl and/or 2 hours post prandial (2HPP) ≥200 mg/dl and/or random blood glucose (RBG) ≥200 mg/dl and/or glycated heamoglobin (HbA1c) ≥ 6.5 %.

All participants answered a questionnaire used for collecting socioeconomic data, as well as family history of diabetes and other diseases. Anthropometric measurements were performed: weight (kg), height (cm), body mass index (BMI) (kg/m^2), waist circumference (cm) (Ong *et al*., 2017).

Hepatic, renal, autoimmune, endocrinal diseases, metabolic disorders and autoimmune diseases were exclusion criteria for diabetic patients.

Ethics Committee's approval and participants' consents were obtained.

2.2. Laboratory Investigations

2.2.1. Biochemical markers

Venous blood samples after a 10-hours fasting were collected from each subject in a sterile EDTA and plain vacutainer tubes. 2 EDTA tubes blood samples were collected one of them stored at -20 C^o till DNA (double stranded nucleic acid) extraction for genotyping and the other used for measuring HbA1c. Blood on the plain tubes was allowed to clot for 30 minutes, and then centrifuged at 3000g for 10 minutes at- 4 C^o. Sera were stored at -20 C^o till time of analysis. Measurement of serum levels of FBG, lipid profile [total cholesterol (CHO), high density lipoprotein (HDL) cholesterol (HDL-C), TG] and HbA1c were performed on automated clinical chemistry analyzer (OLYMPUS AU400). Low density lipoprotein cholesterol (LDL-C) level was calculated using Friedewald formula (Friedewald *et al.*, 1972).

2.2.2. Genotyping of GCKR, TCF7L2, SLC30A8, and IGFB SNPs

DNA was extracted using QIA amp DNA Blood Mini Kits-50-Catalog no. 51104 (Qiagen ,Hilden, Germany) according to manufacture instruction . DNA integrity was determined by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized through GEL documentation (E-Gel® Imager System with UV Light Base, Thermo scientific). DNA concentration was determined by Nano Drop 2000 Spectrophotometer (Thermo scientific). GCKR rs780094, TCF7L2 rs7903146, TCF7L2 rs12255372, SLC30A8, rs11558471, and IGFB rs2854843 polymorphisms (Applied Biosystems, Foster City, CA, USA) were detected by Rt-PCR using the Rotor Gene Q Rt- PCR (QIAGEN, Germany) (Table1).

Allele discrimination was performed using the TaqMan genotyping protocol (Applied Biosystems, Foster City, CA, USA). PCR reactions were set up in 20 µl reaction volume including 20–30 ng DNA , 10 µl TaqMan genotyping PCR Master Mix and 1 µl TaqMan SNP genotyping assay .The PCR assay was carried out according to manufacturer's instructions including one step of 10 min at 95 °C followed by 40 cycles of DNA denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Final products were analyzed by Rotor Gene software.

Table 1: Genotyping SNPs

Mapped gene	dbSNP	**Sequence [VIC/FAM]**
GCKR	rs780094	CTCAACAAATGTATTGATCAGCAAA[C/T]ATGTGTCAGTCATGGTCTAAAAAA
TCF7L2	rs7903146	TAGAGAGCTAAGCACTTTTTAGATA[C/T]TATATAATTTAATTGCCGTATGAGG
TCF7L2	rs12255372	TGCCCAGGAATATCCAGGCAAGAAT[G/T]ACCATATTCTGATAATTACTCAGGC
SLC30A8	rs11558471	CAGATAATTTAGATATTTACCTGCA[A/G]GAAGGAATAAAGCAGATGCAACCAA
IGFB	rs2854843	AGGAAAGTTATTCAAAATCTAGAAA[C/T]GTCTTCTGCTAAATTCTTAATTAAG.

2.3. Statistical Analysis

The data were analyzed using Microsoft Excel 2010 and statistical package for social science (SPSS version 24.0) for windows (SPSS IBM., Chicago, IL). Continuous normally distributed variables were represented as mean ±SD. with 95% confidence interval, and using the frequencies and percentage for categorical variables, a p value ≤ 0.05 was considered statistically significant. To compare the means of normally distributed variables between groups, the Student's t test was performed. $\chi 2$ test or Fisher's exact test was used to determine the distribution of categorical variables between groups. Spearman's rank correlation coefficient (r) was done to show the correlation between different parameters in this study. Effect modifications were evaluated by stratification, and statistical interaction was assessed by including main effect variables and their product terms in the logistic regression model.

2.3.1. Haplotype Analysis

The data was analyzed using HAPLOTYPE ANALYSIS softwear v1.05 which is a software written in Visual Basic for Applications (VBA) within Excel. This is new software for analysis of data from organelle based on the frequency of haplotypes. Population genetic structure from the population samples (inter-population analysis) was computed, utilizing: Nei's minimum genetic distance and genetic differentiation among the populations and contribution of each of them to the total diversity (Finkeldey and Murillo 1999).

3. Results

3.1. Characteristics of the Studied Population

This study is a case-control comparative study consisting of 132 patients with T2DM and control group (n = 96). The 2 studied groups showed statistical significant differences as regards height, weight, waist, BMI, FBG, 2HPP, HbA1c, CHO, TG, and LDL among the T2DM group when compared to control group (p of all parameters = 0.001 except height and BMI were p= 0.04 and 0.004, respectively) (Table 2).

Table 2. Demographic and biochemical variables of the studied populations:

	Control N= 96 Mean ±SD	Diabetic N= 132 Mean ±SD	P. value
Weight (kg)	77.6 ±14.2	82.4 ±10.9	0.006*
Height (cm)	164.4 ±12.4	161.4 ±8.4	0.04*
BMI (kg/m^2)	29.2 ±7.6	31.7 ±4.1	0.004*
Waist (cm)	88.5 ±14.3	106.9 ±12.7	0.001**
FBS (mg/dl)	93.0 ±23.1	195.3 ±75.9	0.001**
2H PP (mg/dl)	99.8 ±27.3	222.3 ±73.5	0.001**
HbA1c (%)	5.2 ±0.7	8.6 ±2.0	0.001**
CHO (mg/dl)	162.4 ±34.5	211.4 ±50.6	0.001**
TG (mg/dl)	102.4 ±34.3	199.2 ±87.0	0.001**
HDL (mg/dl)	48.4 ±10.2	47.5 ±16.0	0.625
LDL (mg/dl)	92.3 ±36.2	123.8 ±45.8	0.001**

†Weight, Height, BMI, Waist, FBS, 2H PP, HbA1c, CHO, TG, HDL and LDL are represented as mean ± SD; the data were analyzed by t. test.

*P-value ≤0.05 is significant, **P-value ≤0.001 is highly significant.

3.2. Association Studies for Different SNPs

For the GCKR rs 780094 the T allele showed higher frequency in T2DM patients than in controls (OR =3.99, 95% CI= 2.565-6.215, p = 0.001). The frequency of (CT and TT) genotypes vs. CC genotype was significantly higher in T2DM patients (50% in patients vs., 22.9% in controls and 21.2% in patients vs. 6.3% in controls, respectively) (p = 0.001); these genotypes showed higher risk for T2DM (OR= 5.4, 95% CI =2.9 - 10.0 and OR= 8.4, 95% CI = 3.2 - 22.0 respectively) (p =0.001). Meanwhile, the frequency of (CT + TT) genotypes vs. the CC genotype was significantly higher in T2DM patients than in controls (71.2% in patients vs. 29.2% in controls), with higher risk for T2DM (OR=6, 95% CI=3.4- 10.7) (p=0.001).

Considering the IGFB rs 2854843, the T allele frequency was significantly associated with increased risk of T2DM (OR= 5.8, 95% CI=3.850-8.712, p=0.001). The frequency of TT genotypes vs. CC genotype was significantly higher in T2DM patients when compared to control (57.6% in patients vs. 14.6% in controls) (p = 0.001); it showed increased risk of T2DM (OR= 13.3, 95% CI=6.26 - 28.4). Also, the frequency of (CT + TT) genotypes vs. the CC genotype was significantly higher in T2DM patients than in controls (83.3% in patients vs. 33.8% in control) (OR= 6.4, 95% CI= 3.5 - 11.8, p= 0.001).

As regards the SLC30A8 rs 11558471, the only genotype that showed significant difference between diabetic patients and control group is AG genotype (37.9% in patients vs. 18.8% in control) (p=0.01).

However, the other two SNPs (TCF7L2 rs 7903146 and rs 12255372) were not found to be associated with T2DM. The genetic models are summarized in table 3.

3.3. Associations of Haplotypes with T2DM

The haplotype block was constructed for the five SNPs (rs780094, rs 7903146 and 12255372, rs 11558471 and rs 2854843). All five SNPs fell into one block. CCAGC haplotype was the most frequent and associated with control group than in cases (p =0.001) followed by CTATT haplotype (p =0.03), while TCAGT was the most frequent haplotype in T2DM patients followed by TTGTT, TTATT and TCATT and they were strongly associated with the disease (P= 0.001, 0.01, 0.04 and 0.05 respectively), meanwhile taking CCAGC haplotype as reference (OR = 28.6, 7.9, 10.7 and 8.9 respectively) (Table 4). Another finding in this study is that subjects having TCGGT and TTGGT haplotypes are not protective against diabetes (p=0.001 and 0.02 respectively) as they were found in cases only, while, CCATC and CTGTC could be considered protective haplotypes as they were found only in control group (p= 0.001 for both) (Table 4) (Figure 1).

Table 3: Association study of different SNPs variants with T2DM under different genetic models

SNPs	Genotype		Groups		P. value	OR	95% CI	P. value
			Control	Diabetic				
GCKR	CC		68(70.8%)	38(28.8%)	0.001**	1(reference)		
	CT		22(22.9%)	66(50.0%)	0.001**	5.4	2.9 - 10.0	0.001**
	TT		6(6.3%)	28(21.2%)	0.001**	8.4	3.2 - 22.0	0.001**
	CT+TT		28(29.2%)	94(71.2%)	0.001**	6.0	3.4- 10.7	0.001**
	Allele	C	158(0.823)	142(0.538)	0.001**	1(reference)		
		T	34(0.177)	122(0.462)	0.001**	3.993	2.565-6.215	0.001**
SLC30A8	AA		63(65.6%)	68(51.5%)	0.1	1(reference)		
	AG		18(18.8%)	50(37.9%)	0.01*	2.6	1.4 - 4.9	0.01*
	GG		15(15.6%)	14(10.6%)	0.29	0.9	0.4 - 1.9	0.7
	AG+GG		33(34.4%)	64(48.5%)	0.1	1.8	1.05 - 3.1	0.03*
	Allele	A	144(0.750)	186(0.705)	0.5	1(reference)		
		G	48(0.250)	78(0.295)	0.3	1.258	0.826 - 1.915	0.2
IGFB	CC		54(56.3%)	22(16.7%)	0.001**	1(reference)		
	CT		28(29.2%)	34(25.8%)	0.6	3.0	1.5 - 6.0	0.001**
	TT		14(14.6%)	76(57.6%)	0.001**	13.3	6.26 - 28.4	0.001**
	CT+TT		42(33.8%)	110(83.3%)	0.001**	6.4	3.5 - 11.8	0.001**
	Allele	C	136(0.708)	78(0.295)	0.001**	1(reference)		
		T	56(0.292)	186(0.705)	0.001**	5.8	3.850-8.712	0.001**

†OR: Odds Ratio; †CI: Confidence Interval.

††Allele frequency was calculated in 2 N.

*P value ≤ 0.05 significant; **P value ≤ 0.01 highly significant.

Table 4. Association of different SNPs haplotypes and T2DM

Haplotype association	Control		Cases		P. value	OR	95% CI	P. value
	N	%	N	%				
Significant Haplotypes association in control								
CCAGC	68	35.4%	38	14.4%	0.001**	1(reference)		
CTATT	10	5.2%	4	1.5%	0.03*	0.72	0.2 - 2.4	0.6
Significant Haplotypes association in cases								
TCAGT	2	1.0%	32	12.1%	0.001**	28.6	6.5 - 126.12	0.001**
TTGTT	5	2.6%	22	8.3%	0.01*	7.9	2.8 - 22.5	0.001**
TTATT	2	1.0%	12	4.5%	0.04*	10.7	2.3 - 50.5	0.003*
TCATT	2	1.0%	10	3.8%	0.05*	8.9	1.9 - 42.9	0.01*
Specific Haplotypes for control group								
CCATC	12	6.3%	0	0.0%	0.001**			
CTGTC	10	5.2%	0	0.0%	0.001**			
TTATC	2	1.0%	0	0.0%	0.1			
TCGTT	2	1.0%	0	0.0%	0.1			
TCGTC	2	1.0%	0	0.0%	0.1			
CTGGC	2	1.0%	0	0.0%	0.1			
TCATC	2	1.0%	0	0.0%	0.1			
Specific Haplotypes for cases group								
TCGGT	0	0.0%	12	4.5%	0.001**			
TTGGT	0	0.0%	8	3.0%	0.02*			
CTGGT	0	0.0%	4	1.5%	0.09			
TTAGT	0	0.0%	4	1.5%	0.09			
TTAGC	0	0.0%	4	1.5%	0.09			
CCGTT	0	0.0%	2	0.8%	0.23			

*P value ≤ 0.05 is significant; **P value ≤ 0.01 is highly significant.

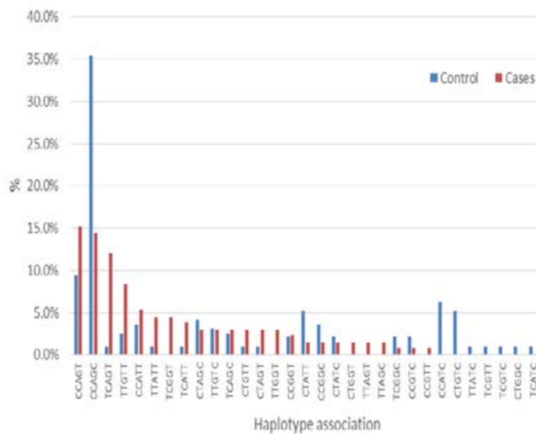

Figure 1. Total haplotype association in control and cases:

4. Discussion

Type 2 diabetes mellitus (T2DM) is a complex disorder resulting from an interaction between environment and genetic factors. Genetic variations can insert major or minor impacts on the disease. For initial prevention of T2DM, it is necessary to recognize genes potentially underlying the disease (Pourahmadi *et al*., 2015). Our study is the first one of GCKR, TCF7L2, SLC30A8 and IGFB risk variants, rs 780094.rs 7903146, rs 12255372, rs 11558471 and rs2854843 in Egyptians.

The association between T2DM and GCKR rs780094 has been replicated in many studies of different ethnic populations since the Diabetes Genetics Initiative GWAS (Ling *et al*., 2011). This association is confirmed again in our study in Egyptian population as TT mutant genotype distributions were significantly different between control and cases (p= 0.001 OR= 8.4 with 95% CI= 3.2 – 22.0), also, the frequency of T allele was different (p= 0.001 and OR = 3.993 with 95% CI= 2.565 – 6.215).

The decrease in β-cell mass and function in pancreatic islets are the underlying mechanisms of the progress of T2DM. *TCF7L2* influences β-cell functions by affecting β-cell survival in pancreatic islets (Huang *et al*., 2018).

An Egyptian study conducted by Alnaggar et al., 2018 showed that there was statistically significant association between type 2 diabetic patients and diabetic nephropathy *and TCF7L2* gene polymorphism rs 12255372, P=0.005, the allelic frequency differed significantly between the studied groups P=0.005, denoting that the G allele was the risky allele for developing T2DM and diabetic nephropathy. Another study on TCF7L2 gene polymorphism (rs12255372) was performed by Hassan *et al*., 2019 in Al Najaf governorate, and it found that minor T allele showed significant association with the risk of T2DM with an OR of 3.52 (95% CI 1,96 – 6.33, p < 0.0001).

As regards TCF7L2 gene polymorphism (rs 7903146) Shah et al. studied 120 individuals, one half of them were homozygous for CC while, the other half were homozygous for TT. In their study, β-cell responsiveness was slightly impaired in the TT genotype group and differed significantly between genotypes, implying that a genetic variant of *TCF7L2* impairs glucose tolerance through effects on insulin secretion and on glucagon (Shah *et al*., 2016).

A study on three TCF7L2 variants (rs7903146, rs12255372, and rs4506565) was performed in India, and an association was detected between these three SNPs and T2DM (Pourahmadi *et al*., 2015). Four TCF7L2 gene SNPs (rs11196205, rs7901695, rs12255372, and rs7903146) were investigated within a Japanese sample, this study showed a significant association between these four variants and T2DM, of all SNPs, rs12255372 showed the strongest association (Hayashi *et al*., 2007). However, studies performed in China (Chang *et al*., 2007), India (Guo *et al*., 2007), and the United Arab Emirates (Saadi *et al*., 2008) found no significant association between this SNP in the TCF7L2 gene and T2DM. Meanwhile, a study in Iran found that genotype distribution of TT mutant of rs7903146 (C/T) and rs12255372 (G/T) were not different between control subjects and T2DM patients (p ≥ 0.05 for both, OR= 0.564, 95% CI= 0.280-1.135 and OR = 0.473 95% CI= 0.170-1.314 respectively) in the sample recruited from Jahrom city (Pourahmadi *et al*., 2015), and this is concordant with our results which showed no significant difference in the frequency of the two studied SNPs (rs 7903146 and rs 12255372 in TCF7L2 gene) (p = 0.2 and 0.6 with OR= 1.9, 95% CI= 0.8- 4.5 and OR= 0.7 95% CI= 0.31-1.6 respectively).

GWAS identified IGF2BP2 gene to be associated with T2DM, and this association has been repeatedly confirmed among different ethnic populations (Grant *et al*., 2006). While many studies confirmed the association (Cauchi *et al*., 2012; Gamboa-Meléndez *et al*., 2012), other studies reported no association (Kommoju *et al*.,2013; Duesing *et al*., 2008). Our results showed a strong association of SNP (rs 2854843) with T2DM as TT and CT+ TT genotypes distribution significantly different between cases and control (p= 0.001, 0.001, OR= 13.3 with 95% CI= 6.26 – 28.4 and OR= 6.4 with 95% CI= 3.5 – 11.8 respectively). The common alleles of SNPs, rs13266634(C/T, Arg276Trp), and rs 11558471(A/G) in the SLC30A8 gene are found to confer the risk susceptibility in T2DM (Abu Seman *et al*., 2015). We studied the risk of SLC30A8 polymorphism (rs 11558471), and we found that AG genotype is the only genotype which showed an increased frequency in T2DM patients than control (p= 0.01 and OR= 2.6 with 95% CI= 1.4 – 4.9).

In our patients, haplotype analysis showed strong association between T2DM and TCAGT, TTGTT, TTATT and TCATT haplotypes (p= 0.001,0.01, 0.04 and 0.05 respectively). Taking CCAGC as a reference showed increased risk of developing T2DM (OR= 28.6, 7.9, 107 and 8.9 respectively), while other haplotypes which include CCAGC and CTATT could be protective against T2DM as they were significantly higher in the control group. Meanwhile, haplotype analysis showed us another finding that there were specific haplotypes for control group (CCATC and CTGTC) and others were specific for

cases group (TCGGT and TTGGT). One of the limitations of our study is that we cannot compare our results with other research works because we did not find another haplotype studies on these five SNPs at the same time.

5. Conclusion

In conclusion, our study identified a significant association of GCKR rs 780094 and IGFB rs 2854843 variants with T2DM in Egyptians. Also, we identified strong association between T2DM and TCAGT, TTGTT, TTATT and TCATT haplotypes. However, CCATC and CTGTC haplotypes were higher in control group than in diabetic patients. Further studies are to be addressed to assess other variants that could modify the risk of T2DM in our population.

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