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SULFONYLUREA RECEPTOR-1 GENE POLYMORPHISMS IN EGYPTIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: *The sulfonylureas are antidiabetic agents which bind to sulfonylurea receptor-1 (SUR-1) resulting in increase of insulin secretion. (SUR1) gene encodes the SUR1 protein that plays a vital role in glucose-induced insulin secretion. Polymorphisms in this gene have been associated with development of type 2 diabetes mellitus (T2DM) in many populations and may result in response modulation to sulfonylurea therapy.* ***Aim:*** *The purpose of this study was to assess the influence of SUR-1 genetic polymorphisms (SUR 1 exon 16 (-3C/T, cag GCC-tag GCC), SUR-1 exon 31 (Arg1273Arg AGG-AGA), and (SUR-1 exon 33 (S1369A)) on the genetic predisposition to T2DM diabetes mellitus and to investigate whether these genetic variants may modulate the response to sulfonylurea in Egyptian T2DM patients as assessed by glycated hemoglobin levels (Hb_{A1C}).* ***Methods:*** *A total of 86 unrelated patients with T2DM who were receiving sulfonylurea therapy along with 46 healthy control Volunteers were enrolled in the study. Genotyping of SUR-1 was performed by polymerase chain reaction restriction fragment length polymorphism PCR-RFLP method.* ***Results:*** *The present study observed that heterozygote genotype (T/G) of exon 33 was highly expressed in diabetic patients compared to control group (P=0.000). While the wild type of exons 31 (G/G) and exon 33 (T/T) were significantly higher in controls compared to T2DM patients (P=0.003 and 0.000 respectively).* ***Conclusion:*** *On the basis of these data; presence of the wild type in SUR 1 exon 31 and 33 may have protective effect against T2DM, whereas presence of heterozygous condition at the same exons may confer susceptibility.*

KEY WORDS

Genetic polymorphism; glucose concentration; HbA1c; sulfonylurea receptor-1; T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most challenging health issues that is increased at an alarming rate in both developed and developing countries[(1)]. Susceptibility to T2DM is modulated by genetic factors, as evidenced by twin studies[(2)] and familial aggregation [(3)].

Sulfonylureas are hypoglycemic agents used for promotion of insulin secretion in T2DM and have been the main pharmacologic approach for its management for many decades because of their great efficacy in newly diagnosed patients, limited side effects and low cost [(4)]. Sulfonylureas act as insulin secretagogues by binding to the sulfonylurea receptor-1 (SUR-1) which together with the potassium pore-forming inward-rectifier (Kir6.2) subunits make up the pancreatic β-cell ATP-sensitive potassium (K_{ATP}) channel. This interaction closes the K+ channel, which inhibits potassium efflux

and depolarizes the plasma membrane, leading to an opening of voltage-gated calcium channels and hence increasing in intracellular calcium levels cause release of insulin from the β-cells [5].

Gene encoding SUR-1 is located on chromosome 11p15.1. Numerous polymorphisms of this gene are reported to be associated with susceptibility to T2DM and diabetic phenotypes. These polymorphisms may lead to a loss of activity of potassium channel and to uncontrolled oversecretion of insulin, as well as modulated response to sulfonylurea therapy [6][7]. The field of pharmacogenomics has been applied to sulfonylurea clinical studies in order to investigate the genetic background of response variability among diabetic patients [8].

On this basis, the purpose of this study is to investigate the influence of sulfonylurea receptor 1(SUR1) genetic variants (exon 16 (-3C/T, cagGCC-tagGCC), SUR-1 exon 31 (Arg1273Arg AGG-AGA), and SUR-1 exon 33 (S1369A)) on the genetic predisposition of Egyptian patients toward (T2DM) and to investigate whether these genetic variants may modulate the response to sulfonylurea as assessed by glycated hemoglobin levels (Hb_{A1C}) .

SUBJECTS AND METHODS

This prospective study was conducted on 86 patients with T2DM recruited from outpatients of clinic of Medical Services unit, National Research Center along with age and sex-matched 46 healthy volunteers. All patients included in the study fulfilled the following criteria: age above 25 years, assigned to sulphonylurea only as antidiabetic agent and having no history of ketoacidosis. Exclusion criteria were any therapeutic or lifestyle modifications within the last year or any recent acute diabetic complication. All patients underwent thorough history and clinical examination.

The study was approved by the ethical committee of The National Research Center Institute. Written informed consent was obtained from all participants in this study.

Laboratory Methods

Sample Collection and Biochemical Analysis

Seven milliters of venous blood sample was withdrawn after 12–14 hours overnight of fasting from each subject enrolled in the study. The sample was divided into three aliquots. The first was of 1.5 ml collected on Sodium fluoride/potassium oxalate and centrifuged to obtain plasma for Fasting blood glucose assessment (**FBG**). The second whole blood collected on K3EDTA was further subdivided into two portions each of 1.5 ml. One portion used for (SUR-1) gene polymorphism and the other for Hb_{A1C} determination. The third aliquot was of 2 ml and centrifuged serum to assess lipid profile measurement . Biochemical parameters were determined using automatic analyzer (AU 2700, Olympus)

DNA Isolation and Determination of SUR-1 Genotypes

Genomic DNA was extracted from peripheral blood leukocytes using commercially available QIAamp DNA Blood Mini Kit (Qiagen). The purity of extracted DNA was checked. All polymorphisms were detected by restriction fragment length polymorphism (**PCR-RFLP**) method using forward and reverse primers as shown in **Table (1)**. Each polymorphic region was amplified by a polymerase chain reaction (PCR) in a final reaction volume of 25µl of polymerase chain reaction contained: 100 ng genomic DNA, 5 pmol of each primer, polymerase chain reaction buffer with 1 mmol/L of $MgCl_2$, 100 µmol/L of each deoxynucleotide triphospate (dNTP), and 0.5U of Taq polymerase (Fermentas) with some

modifications of PCR amplifying conditions and primer sequence[9],[10].[11].Restriction digests containing 10µl of the PCR product and the corresponding restriction enzyme were incubated at 37°C overnight. The products of digestion were analyzed on polyacrylamide gel and 2 % agarose gel according to previously published protocols.

Table 1: Genotyping conditions of *Polymerase chain reaction (PCR)*

Exon	Forward and reverse primers	Restriction enzyme	Ampilified pcr product	Condition	Restriction pattern (bp)
SUR1 exon 16 (-3C/T)	5-GCATCTGTCTGTCTGTCTTTCTGGG-3 5-GGAGCGAGGACTTGCCGC-3	HpyCH4V	134	95 5min 95 45sec 55 45sec 72 45sec 35 cycle 72 9min	C: 80 + 54 T: 134
SUR1 exon 31 (Arg1273Arg)	5-GTAGAACAGGGTCCTGTGGC-3 5-TGTCTCCAGTGACGAAGGTG-3	Bsl I	250	95 5min 95 45sec 60 45sec 72 45sec 30 cycle 72 9min	G: 132 + 65 + 52 + 1 A: 198 + 52
SUR1 exon 33 (S1369A)	5-AGGGAGAGGGGTGGGAAGAGTCCAA-3 5-ATTGGGTTGGGCCCGTGCACTGAC-3	Mwo I	290	94 5min 94 30sec 58 30sec 72 30sec 35 cycle 72 5min	T: 206 + 82 G: 206 + 41

Table (2): General characteristics of T2DM patients and controls.

Variable (mean+SD)	Patients n=86	Controls n=46	P-Value
Male n(%)	25(29.1)	16 (34.8)	0.419
Female n(%)	61(70.8	30 (65.2)	0.370
Age (Years)	51.49 ±8.41	45.89±8.56	0.329
Duration of diabetes (year)	9.17±6.46	-	-
BMI (kg/m2)	32.26 +5.36	29.90±2.78	0.07
Waist Circumference ratio(cm)	108.69±11.0	97.53±9.29	0.603
Fasting blood glucose (mg/dl)	227.59±104.46	94.27±15.75	0.001 ** <
Hb_{A1C} (%)	9.29±1.58	6.78 ±0.72	0.05 *
Total serum cholesterol (mg/dl)	237.82±62.64	233.88±58.29	0.535
Serum triglycerides (mg/dl)	*165.63±92.86*	*126.72±63.96*	*0.224*
Serum HDL-C(mg/dl)	*59.91±20.48*	*67.07±19.28*	*0.457*
Serum LDL-C(mg/dl)	139.83±60.32	141.86±57.38	0.41
Serum HDL/LDL	0.54±0.37	0.59±0.38	0.403

BMI : body mass index;HDL-C: high density lipoprotein cholesterol;LDL-C: low density lipoprotein cholesterol

HbA1C: glycated hemoglobin;*: p –significant;**: p –highly significant

RESULTS

Diamographic and biochemical data of T2DM patients and controls are shown in Table (2).Family history was found in 72 out of 86 T2DM patients (83.7%). Smoking history was recorded in 14 patients out of 86 (16.27%). **(Table 2).**

Distribution of sulphonylurea receptor-1 polymorphisms among NIDDM patients and controls..

SUR-1 Exon 16 (-3C / T) Genotype and Allele Frequency.

The genotype and allele frequencies for the Sulphonylurea receptor exon 16 (-3c/t) polymorphism are presented in **Table (3), Fig1.** We could not detect statistically significant differences in the distribution of the genotypes between the patients and the controls. Neither C nor T alleles frequency of exon 16 had statistically significant difference between NIDDM patients and control group.

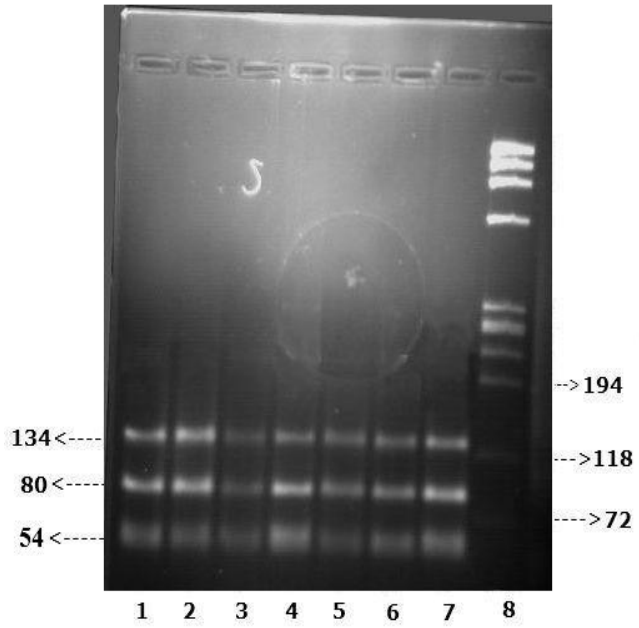

Figure (1): SUR-1 exon 16 -3C/ T genotypes: Lane (8): Øx174 DNA/BSUR1(HaeIII) Marker9, Lane (1, 2, 3, 4, 5, 6, and 7):HPYCH4V enzyme restricted PCR product into heterozygous CT genotype of SUR-1 exon 16 -3C/T as it was restricted into 134, 80, 54bp.

Table (3): Genotypes and alleles distribution of Sur-1 exon 16(-3C/T) among diabetic patients and Control.

Genotype/ Allele	Patients	controls	P-value	Significance
C/C n(%)	32(37.2)	20(43.5)	0.482	NS
C/T n(%)	51(59.3)	21(45.7)	0.133	NS
T/T n(%)	3 (3.5)	5 (10.8)	0.09	NS
Total no	n=86	n=46		
C n (%)	115 (66.86)	61(66.3)	0.516	NS
T n (%)	57 (33.14)	31(33.6)	0.516	NS
Total no	n=172	n=92		

NS: non-significant

SUR-1 Exon 31 (Arg 1273Arg) Genotype and Allele Frequency.

The heterozygous GA genotype of SUR-1 exon 31 was significantly more frequent in NIDDM patients (73.3%) compared to controls (50.0%), and the homozygous GG genotype was significantly more frequent in controls (34.8 %) than in diabetic patients (12.8%). The A allele was more frequent in diabetic patients compared to controls and the G allele is more frequent in controls than in diabetic patients but it did not achieve statistical significance **(Table 4), Fig2.**

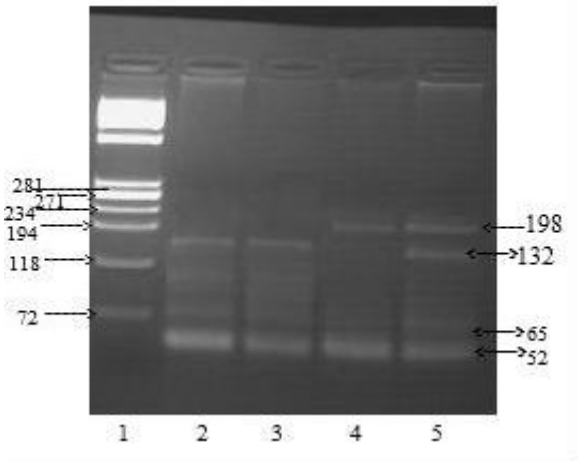

Figure (2): SUR-1 exon 31 (Arg1273 Arg) genotypes:Lane (1): Øx174 DNA/BSUR1(HaeIII) Marker9; Lane (5): Bsl I enzyme restricted PCR product to heterozygous GA genotype of SUR-1 exon 31 (Arg1273 Arg) as it was restricted to 198, 132, 65, 52 bp; Lane (2, 3): Bsl I enzyme restricted PCR product to wild-type GG genotype of SUR-1 exon 31 (Arg1273 Arg) as it was restricted to 132, 65, 52 bp; Lane (4): Bsl I enzyme restricted PCR product to homozygous AA genotype of SUR-1 exon 31 (Arg1273 Arg) as it was restricted to 198, 52 bp.

Table (4): Genotypes and alleles distribution of SUR-1exon31 (Arg1273Arg) among diabetic patients and controls

Genotype/ Allele	Patients	Controls	P-value	Significance
G/G n (%)	11(12.8)	16(34.8)	0.003	S
G/A n (%)	63(73.3)	23(50.0)	0.008	S
A/A n (%)	12(14.0)	7 (15.2)	0.84	NS
Total no	n=86	n=46		
G n (%)	85(49.4)	55(59.8)	0.069	NS
A n (%)	87(50.6)	37(40.2)	0.069	NS
Total no	n=172	n=92		

S : significant
NS: non-significant

SUR-1 Exon 33 (S1369A) Genotype and Allele Frequency.

The heterozygous T/G genotype was found to be more statistically, highly, and significantly prevalent in patients (76.7%) than in controls

(43.5 %) *(P<0.001).* While the homozygous T/T genotype was highly statistically significantly present in controls than in patients (21.7 % and 1.2%, respectively; P<0.001). No statistically significant difference was found for T and G alleles distribution between patients and controls ***(P = 0.32 and 0.31***, respectively) **(Table 5), Fig 3.**

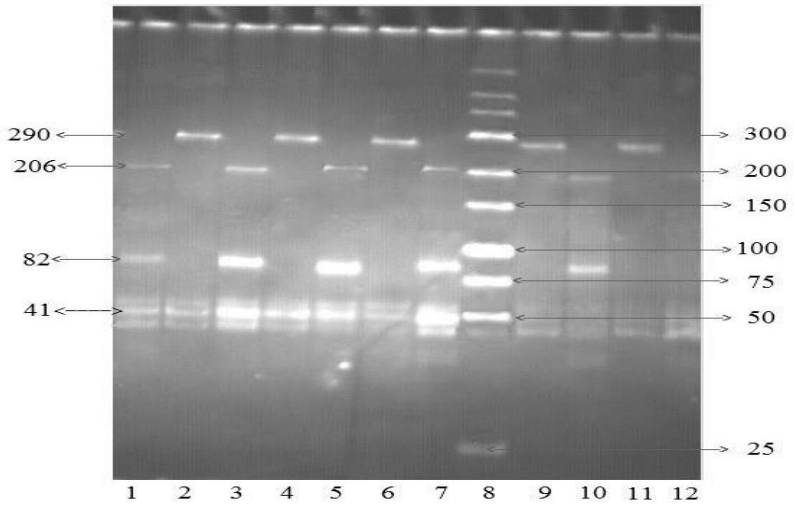

Figure (3): SUR-1 exon 33 (S1369A)genotype: Lane (8): Low range DNA ladder; Lane (2, 4, 6, 9, and 11): PCR product ampilified at 290 bp; Lane (1, 3, 5, 7, and 10): Mwo I enzyme restricted PCR product to heterozygous TG genotype of SUR-1 exon 33 (S1369A) as it was restricted to 206, 82, 41 bp; Lane (12): Mwo I enzyme restricted PCR product to homozygous GG genotype of SUR-1 exon 33 (S1369A) as it was restricted to 206, 41 bp.

Table (5): Genotypes and alleles distribution of SUR-1 exon33 (S1369A) among T2DM patients and controls

Genotype/ Allele	Patient	Controls	P-value	Significance
T/T n (%)	1 (1.2)	10(21.7)	0.000	S
T/G n (%)	66(76.7)	20(43.5)	0.000	S
G/G n (%)	19(22.1)	16(34.8)	0.116	NS
Total no	n=86	n=46		
T n (%)	68 (39.5)	40(43.5)	0.32	NS
G n (%)	104(60.5)	52(56.5)	0.31	NS
Total no	n=172	n=92		

S : significant
NS: non-significant

Difference in Anthropometric Measurements (BMI and Waist Circ.) between Genotypes of SUR1 Gene (Exons 16, 31, and 33) in T2DM Patients.

Statistically significant difference was found in the mean value of both BMI and waist circumference between SUR-1 exon 16 genotypes in T2DM patients ***(P= 0.017 and 0.027,*** respectively).No statistically significant difference was recorded for the other 2 exons of SUR-1 gene (exons 31 and 33) and these

anthropometric parameters in the diabetic patients (**Table 6).**

Table (6): Anthropometric measurements and Genotypes distribution of SUR-1 exon 16, 31 and 33 among T2DM patients.

Genotypes	BMI(kg/m2) Mean ±SD	Waist Circ.(cm) Mean±SD
SUR-1 Exon 16(-3C/T)		
C/C n=32		
C/T n=51	30.52(±4.463)*	105.2(±9.77)*
T/T n=3	33.5 (±5.53)	111.2(±10.8)
	28.9(±6.013)	101.3(±17.6)
P-value		
Significance	0.026	0.027
	S	S
SUR1Exon31 (Arg1237Arg)		
G/G n=11		
G/A n=63	32.4(±6.055)	106.4(±14.18)
A/A n=12	32.7(±5.15)	110.08(±10.68)
	29.4(±5.26)	103.5(±8.11)
P-value		
Significance	0.141	0.128
	NS	NS
SUR1Exon33 (S1369A)		
T/T n=1		
T/G n=66	34.6	101.0
G/G n=19	32.6(±5.4)	108.9(±11.1)
	30.9(±4.9)	108.2(±10.92)
P-value		
Significance	0.446	0.766
	NS	NS

BMI:Body mass index
Waist Circ.: Waist Circumference
*: C /C significant with C/T

Difference in the Biochemical Parameters between Genotypes of SUR-1 Gene (Exons 16, 31, and 33) in T2DM Patients.

There were no differences in FPG, Hb_{A1C} nor lipid profile in neither of genotype subgroups studied **(Table 7).**

Table (7): Clinical characteristics and biochemical parameters of diabetic patients with different SUR-1 polymorphisms

Polymorphism / SUR-1	Total Cholesterol (mg/dl) Mean±SD	Triglyceride (mg/dl) Mean ±SD	HDL-C (mg/dl) Mean ±SD	LDL-C (mg/dl) Mean ±SD	HDL/LDL (mg/dl) Mean ±SD	fasting blood glucose (mg/dl) Mean ±SD	HbA1c (%) Mean ±SD
Exon 16(3C/T)							
C/C n=32	234.1(49.9)	157.1(106.	58.9(17.6)	137.6(58.7)	0.5(0.3)	215.7(108.9)	9.1(1.4)
C/T n=51	243.0(69.3)	3	60.8(22.0)	143.2(62.1)	0.5(0.3)	235.3(104.1)	9.2(1.6)
T/T n=3	188.6(56.0)	169.4(86.1)	55.3(27.0)	105.3(52.2)	0.5(0.2)	222.0(68.2)	10.2(1.6+)
		192.0(56.3)					
P-value	0.318		0.853	0.559	0.801	0.708	0.536
Significance	NS	0.341	NS	NS	NS	NS	NS
		NS					
SUR1exon31							
G/G n=11	250.4(46.8)	150.6(65.5)	60.7(16.1)	156.0(46.9)	0.4(0.2)	180.7(60.0)	8.7(1.4)
G/A n=63	238.8(68.0)	169.7(102.	61.4(22.1)	138.0(66.1)	0.5(0.4)	237.3(104.1)	9.4(1.5)
A/A n=12	221.0(42.1)	2	51.2(11.9)	134.4(33.9)	0.4(0.1)	219.5(130.8)	8.9(1.9)
		157.5(58.6)					
P-value	0.520		0.289	0.629	0.385	0.245	0.267
Significance	NS	0.782	NS	NS	NS	NS	NS
		NS					
SUR1Exon33							
T/T n=1	283.00	103.00	70.000	194.000	0.3	182.0	8.8
T/G n=66	233.2(53.0)	165.8(99.5)	61.6(21.1)	134.5(55.6)	0.5(0.3)	233.8(111.9)	9.3(1.6)
G/G n=19	251.5(89.3)	168.0(68.4)	53.4(17.6)	155.4(73.8)	0.4(0.3)	208.2(74.6)	9.1(1.2)
P-value	0.553	0.577	0.132	0.276	0.130	0.587	0.877
Significance	NS	NS	NS	NS	NS	NS	NS

HDL-C: High density lipoprotein cholesterol
LDL-C: Low density lipoprotein cholesterol
Hb_{A1C} : Glycated haemolglobin
BMI: Body mass index
Waist circ.:waist circumference
S : Significant
NS : non significant

DISCUSSION

Sulfonylurea receptor-1 (SUR-1) gene has been suspected to be involved in the insulin secretion impairment among patients with T2DM. The mechanism of SUR-1 polymorphism in induction of diabetes mellitus is revealed by studies on insulin secretion and insulin sensitivity in different genotypes [12].

In the present study we aimed to address the potential implications of the SUR-1 gene (exon 16, exon 31, and exon 33) polymorphisms on response to sulfonylurea therapy in Egyptian type 2 diabetic patients as assessed by Hb_{A1C}

and to investigate the possible role of these genotypes on susceptibility to T2DM among Egyptien population.

The present study did not find association between polymorphism of the SUR exon 16 and T2DM. A number of other studies have also failed to show significant association of this polymorphism with T2DM. In a large study of Caucasians from the UK Prospective Diabetes Study (UKPDS), no association was seen between exon 16 variant and T2DM [13]. The same was observed in independent populations from Netherlands [14], Dutch Breda cohort [15], Japanese population [16],[17]and European Caucasians [18]. Unfortunately, nothing is known about the biological consequences of the single base pair substitution at the SUR-1 intron/exon 16 boundary as this variant is located within the 3-splice site, it might impair normal splicing [19]. Contrary to our study, it was found that this variant is associated with diabetes mellitus in Caucasians, and the 16-3c → t allele was then termed risk allele' [20]. Feng and his colleagues observed SUR-1 exon 16 -3c → t polymorphism is associated with T2DM in young Danish Caucasian DM patients[21].

Regarding polymorphisms in exon 31. The present study reported that the polymorphism in exon 31 of the SUR-1 gene is strongly associated with T2DM. We have observed significantly increased frequency of the heterozygote genotype (G/A) of exon 31 in diabetics (73.2%) versus controls (50%). This result was inconsistent with previous studies, that detected that it is a nonfunctional substitution; therefore, it is unlikely that this variant contributes directly to increased risk for diabetes or altered β-cell function [22].On the other hand, have observed that the SNP in exon 31 of the SUR1 gene is strongly associated with T2DM in Turkish patients with concomitant obesity.

Concerning SUR-1 exon 33 polymorphism; we observed that the heterozygote genotype (T/G) of exon 33 was highly significantly expressed in the T2DM patients (76.7) compared to controls (43.5) while the wild-type (homozygous) of the same exon (T/T) was significantly higher in controls (21.7%) compared to T2DM patients (1.2%) .This is in agreement with Holstein results [23]. This observation contradicts with other studies which did not find association between SUR-1 exon 33 polymorphism and T2DM in either Caucasians or Japanese populations [6],[24].

In the present study no association was detected between obesity as measured by BMI and waist circumference and either exon 31 or exon 33 SUR1 polymorphisms On the other hand, we found that this association was detected with SUR1 exon 16 and this indicated that SUR locus may contribute to genetic susceptibility to obesity-induced insulin resistance and T2DM, and this contribution is not only based on alteration in insulin secretion but also based on an extrapancreatic role of the SUR-1 gene. In addition, the hyperinsulinemia might result from an initial defect of the pancreatic beta-cell KATP channels and may contribute to the excess of fat deposition and to the development of insulin resistance [16].

In the present study, no association was found between any of the studied genotypes and glycemic control as assessed by Hb_{A1C} levels. However, Nikolac suggested that diabetics with the wild-type C/C genotype of the SUR-1 exon 16 polymorphism had significantly lower Hb_{A1C} concentration compared to patients with variant T/T genotype[18]. On the other hand Polish investigators failed to confirm association of SUR-1 exon 16 (-3C/T) polymorphism with early failure to sulfonylurea therapy compared to patients treated with sulfonylurea for longer duration. There were

several limitations in our study. The sample size was relatively small. Moreover, the duration of diabetes mellitus was not the same for all studied patients. Moreover, we did not examine other possible contributors that may affect plasma glucose and Hb_{A1C} levels such as diet, physical activity. Further future studies on larger population are warranted to understand the genetic basis of SUR genes with different metabolic parameters in diabetic patients.

CONCLUSION

From the present study , We can conclude that the presence of the homozygous genotype (wild type) in exon 31 and 33 of SUR 1 may have protective effect against T2DM, whereas presence of heterozygous condition at the same exons may confer susceptibility. Larger population studies are recommended to investigate the pharmacokinetics and pharmacodynamics of sulfonylurea drugs that are needed to investigate the influence of key SNPs amidst all potential contributing factors to variability in response to these drugs which in turn will provide information to optimise sulfonylurea use in people with diabetes mellitus.

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