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Association of CDKAL1 Genetic Polymorphism with the Glycosylated HemoglobinA1C level among Diabetic Adults.

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ABSTRACT

Multiple single nucleotide polymorphisms (SNPs) have been identified as risk loci for Type 2 Diabetes (T2DM). This study was conducted to increase our understanding of the mechanism through which CDKAL1 rs 9465871 variant affect the risk of T2DM. A case control study was conducted on 128 diabetic patients and 79 controls. Collection of body measurements, bio-specimen and biochemistry assays were performed. Genotyping of rs9465871 at cylclin- dependent kinase 5 regulatory subunit associated protein 1- like (CDKAL1) were conducted. Showed that the mean serum HBA1C in controls was (5.02±1.58), which was significantly lower than that in T2DM (8.49±2.21) ($p<0.001$).The mean level of HOMA-IR in controls was (1.84±1.41), while in T2DM it was significantly higher in T2DM group (8.5±15.44) ($p<0.001$) while, the mean level of FBS in controls was (84.98±11.16 mg/l) it was significantly higher in T2DM group (203.72±101.97 mg/l). Comparison between the CDKAL1 rs 9465871 polymorphisms and studied parameters showed no significantly statistical significance in all parameters except HDL. There was significantly higher serum level HDL in CC than TT genotypes ($P<0.04$). CDKAL1 rs94658971 polymorphisms showed positive relation in T2DM and controls. CC was 18.9% in T2DM and 15.49% in controls showing positive distribution. To better understand the potential functions of these genetic polymorphisms of T_2DM we have to increase our sample size and add more $CDKAL_1$-rs variants.

Keywords: type 2 diabetes – HbA1C- CDKAL1 polymorphism

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INTRODUCTION

More than 60 single-nucleotide polymorphisms (SNPs) have been identified to be associated with type 2 diabetes through genome-wide association studies. Several of these SNPs were also associated with T2D-related traits, such as obesity and fasting plasma glucose (FPG). In addition, associations with multiple common diseases (1).

Robust evidence of disease association in different populations has been obtained for several novel susceptibility gene loci identified by GWA studies; CDKAL1 is among the best-replicated susceptibility loci (2-5) .

The CDKAL1 gene encodes a 65 kDa protein-cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (CDKAL1). A cluster of single nucleotide polymorphisms (SNPs) in intron 5 of the CDKAL1 gene were associated with type 2 diabetes in populations of European and Asian descent (5) .

However, GWA studies of type 2 diabetes and obesity have usually focused on testing additive models. An additive model assumes that the disease risk of heterozygous individuals is exactly halfway between those of the two homozygous groups. Non- additive effects include dominant and recessive effect. These effects are common diseases and traits. For obesity and type 2 diabetes, the strongest evidence of a non – additive effect is at the CDKAL1 locus, where a previous study demonstrated a recessive effect. The GIANT Consortium previously tested 32 BMI-associated variants for deviations from the additive model but, overall, found no evidence of deviation from additivity in 105, 643 individuals (6)

In the present study , we aim to investigate the impact of CDKAL1 on insulin and type 2 diabetes.

MATERIALS AND METHODS

One hundred twenty eight diabetic patients ranging in age between 40-55 years were recruited from out patient clinics (NRC), Kasr El Aeiny university Hospital and diabetes institute. The study also included 79 Non- diabetic controls ranging in age between 18-55 years. Adult obesity was diagnosed with body mass index > 30kg/m^2.

The project was submitted to the Ethical Committee, National Research Center. Informed consent was prepared to be signed by every patient.

Phenotype measurements:-

Height, weight, systolic blood pressure, and diastolic blood pressure were measured for each participant. All subjects fasted for 12 hours before blood collection. Plasma glucose, serum glycosylated hemoglobin A1c (HbAlc), serum triglyceride, total cholesterol , high density lipoprotein cholesterol and low density lipoprotein cholesterol levels measured. Insulin was measured and HOMA-IR was calculated.

HOMA-IR = fasting insulin (mU/L) · plasma glucose (mmol/L)/22.5.

Body mass index was calculated as weight (kg) divided by height squared (m^2)

DNA genotyping

Genomic DNA was extracted from 3 ml whole blood by a commercial DNA extraction kit according to manufacture's protocol (QIAamp DNA BLOOD Mini kit ,QIAGEN ,USA) CAT NO.51104 using automated nucleic acid extractor QIAcube (QIAGEN) .DNA yield was measured by Nanodropper . The purified genomic DNA showed A 260/280 ratio between 1.7 to 1.9

Rs 9465871 polymorphism was determined by a predesigned

Taqman SNP genotyping assay (Applied Biosystems).Oligonucleotides used for allelic discrimination assays for Rs 9465871 as following:

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Context sequences for Rs9465871 ([VIC/FAM])

CAGCTGTGTAAGTGTTGCTGAGAAA[C/T]TGAGTTAGATGAAGACTGAAGATTG

The reaction was performed in 25 ul final volume with real time polymerase chain reaction via Quant Studio 12KFlex Real Time PCR System (Applied Biosystems).For genotyping quality control, duplicate samples and negative controls were included to insure accuracy .

Statistical Analysis

The standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 12.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison between groups was made using Student t test for continuous variables and Chi-Square tests for categorical variables. For multiple independent variables, ANOVA test was performed and least significant difference (LSD) method for multiple comparisons. P values < 0.05 were considered as statistically significant.

RESULTS

The mean age of nondiabetic group was 38.87± 7.06 years while it was 47.90 ±11.52 years in T2DM cases. Table 1 showed group statistics of T2DM and controls as regards, BMI, weight, height and waist circumference. A statistically significant difference was present in all studied parameters.

Table 1: Comparison between anthropometry data in T2DM Cases & Controls

	Controls (No 79)		Type 2DM (No 128)		
	Mean	Std. Deviation	Mean	Std. Deviation	Sig. (2-tailed)
BMI	23.64	0.99	31.17	5.03	.000
Weight(Kg)	69.92	5.79	83.69	14.37	.000
HIEGHT(cm)	172.4	6.18	163.32	8.38	.000
Waist Circumference	84.04	10.43	111.81	14.78	.000

Gender percentage in T2DM was 46.9% female and 53.1% male with no statistical significant Table 2 The mean serum HbA1C in controls was (5.02±1.58), which was significantly lower than that in T2DM group (8.49 ±2.21), (p<0.001).

Table 2: Gender percentages in T2 DM Cases and Controls

	GENDER		Total	Sig
	Female	Male		
TYPE 2DM Cases	60	68	128	NS
	46.9%	53.1%	100.0%	
CONTROLS	40	39	79	
	50.6%	49.4%	100.0%	

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The mean level of HOMA-IR in controls was (1.84 ±1.41), while in T_2DM it was significantly higher (8.5±15.44) ($p<0.001$).

While, the mean level of FBS in controls was 84.98±11.16 mg/l it was significantly higher in T2DM group(203.72±101.97 mg/l). Regarding cholesterol the mean serum level in controls was 169.86 mg/l while in T_2DM group was 207.11 mg/l ($p<0.001$).The mean for triglycerides in controls was 91.59 mg/l while in T_2DM it was 180.81 mg/l ($p<0.001$).

Mean for HDL in controls was 48.26 mg/l while in T_2DM it was 45.64 with no significant difference. Mean for LDL in controls was 102.98 mg/l while in T_2DM it was 123.65 mg/l. The mean level of fasting insulin in controls was 3.06 [mU/l] while in T_2DM it was 19.41 [mU/l] ($p<0.001$).Table 3

Table 3: Comparison between laboratory data in T2DM Cases & Controls

	Controls (No 79)		Type 2DM (No 128)		
	Mean	Std. Deviation	Mean	Std. Deviation	Sig. (2-tailed)
HbA1C	5.02	.58	8.49	2.21	.000
HOMA-IR	1.84	1.41	8.50	15.44	.000
FBS[mg/l]	84.98	11.16	203.72	101.97	.000
Cholesterol[mg/l]	169.86	28.21	207.11	51.32	.000
Triglycerides[mg/l]	91.59	37.02	180.81	98.82	.000
HDL [mg/l]	48.26	8.08	45.64	15.19	.111
LDL [mg/l]	102.98	29.13	123.65	42.79	.000
Fasting Insulin [mU/l]	3.06	3.663	19.41	32.02	.000

Table 4: Association of the CDKAL1 rs 9465871 polymorphisms with T2DM Cases and Controls

	CDKAL1			Total	Asymp. Sig. (2-sided
	CC	TT	CT		
T2DM Cases	24	66	37	127	NS
	18.9%	52.0%	29.1%	100.0%	
CONTROLS	12	38	28	78	
	15.4%	48.7%	35.9%	100.0%	

Table (4) showed association of the CDKAL1 rs 9465871 polymorphism with T2DM cases and controls, positive relation was found in distribution but not positively significant.CC was obserned 18.9% in T_2DM and 15.4% in controls showing positive distribution.

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Table 5: Comparison between the CDKAL1 rs 9465871 polymorphisms and studied parameters

Item	CDKAL1	Mean	Std. Deviation	Minimum	Maximum	Sig
BMI	CC	28.90	5.93	22	43	.617
	TT	28.46	5.49	21	51	
	CT	27.84	5.03	21	44	
WT kg	CC	82.58	15.30	60.00	122.00	.136
	TT	78.02	13.49	55.00	140.00	
	CT	77.15	12.59	55.00	108.00	
HT CM	CC	167.41	8.49	150.00	180.00	.832
	TT	166.39	9.63	145.00	197.00	
	CT	166.78	7.55	150.00	180.00	
WC CM	CC	112.69	19.44	51.00	143.00	.163
	TT	107.97	15.55	73.00	165.00	
	CT	104.72	17.99	56.00	145.00	
HbA1C	CC	7.16	2.37	4.3	14	.669
	TT	7.29	2.45	4.3	17	
	CT	6.94	2.45	4.3	16	
HOMA-IR	CC	5.75	8.52	.03	40.00	.316
	TT	7.31	16.82	.06	152.00	
	CT	4.28	3.78	.10	17.00	
FBS[mg/l]	CC	151.37	76.13	73.00	372.00	.182
	TT	170.64	109.22	60.00	575.00	
	CT	143.05	89.53	63.00	403.00	
Fasting Insulin	CC	13.98	18.81	.10	81.90	.507
	TT	14.91	34.41	.10	306.00	
	CT	10.15	10.94	.10	46.40	
Cholesterol [mg/l]	CC	194.55	43.13	117	327	.644
	TT	194.77	49.41	90	322	
	CT	188.07	47.17	95	299	
Triglyceride [mg/l]	CC	152.08	82.47	46	338	.882
	TT	143.59	95.52	35	528	
	CT	147.91	90.27	45	377	
HDL[mg/l]	CC	51.22	16.64	25	105	.047
	TT	45.00	11.52	17	77	
	CT	46.38	12.57	21	80	
LDL[mg/l]	CC	109.94	33.83	40	203	.264
	TT	119.93	41.47	44	236	
	CT	111.53	38.62	25	204	

LSD shows Significant difference only in HDL between CC and TT

Comparison between the CDKAL1 rs 9465871 polymorphisms and studied parameters were shown in Table (5). No statistical significance was found in all parameters except HDL. There was significantly higher serum level HDL in CC than TT genotypes (P<0.04).

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Table 6: Type of treatment In T2DM cases

Treatment	Frequency	Valid Percent	Cumulative Percent
Insulin	82	64.6	64.5
Oral antidiabetic	39	30.7	95.3
Mixed Treatment	6	4.7	100.0
Total	127	100.0	

Table7: Relation between treatment and the CDKAL1 rs 9465871 polymorphisms

Treatment	CDKAL1			Total	Asymp. Sig. (2-sided)
	CC	TT	CT		
Insulin	19	42	21	82	NS
	23.2%	51.2%	25.6%	100.0%	
Oral Antidiabetic	5	20	14	39	
	12.8%	51.3%	35.9%	100.0%	
Mixed	0	4	2	6	
	.0%	66.6%	33.3%	100.0%	

In Tables 6 and 7 no statistical significance was found between type of treatment and CDKAL1 rs 9465871 polymorphisms.

DISCUSSION

T_2DM is characterized by destruction of pancreatic beta-cells following extended periods of insulin resistance and hyperglycemia (7). In the early stages, insulin resistance is compensated by elevated insulin secretion and as a result many subjects are able to control blood glucose concentrations for extended periods. However, in a sub group of patients an exhaustion of beta-cells occurs more rapidly, resulting in insufficient insulin responses to nutrient influx and further aggravation of hyperglycemia as well as hyperlipidemia, leading to accelerated beta-cell destruction (8) .

Studies of T2D susceptibility genes and loci and their meta-analysis identified a large number of gene variants and confirmed the previously discovered ones. The common intronic variants within the transcription factor 7-like 2 (TCF7L2) gene were reported as the strongest genetic risk factor for T2D (9) . 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 5regulatory subunit-associated protein 1-like 1 (CDKAL1) (10) .

In this study CDKAL1 rs9465871 showed positive relation in distribution between T2DM cases and controls as regards CC genotype which cause increased risk of Diabetes, but no statistical significance.

Jiang et al., 11 found each C allele at CDKAL1 rs9465871 was related with appropriate increase in HbA1c level. The result is consistent with the finding of Miyaki et al., 12 .

Therefore, it is plausible that if a certain variant at CDKAL1 gene can alter the function of beta cells, it will lead to changes of insulin level in blood and ultimately the increase in HBA_1C level. Results suggest that, due to its effect on glycemic phenotype in health adults, $CDKAL_1$-rs9465871 may be a sensitive susceptibility locus of T_2DM in chinese population (11). Comparison between the CDKAL1 rs 9465871 polymorphisms and

studied parameters showed no statistical significance in all parameters except HDL. There was significantly higher serum level HDL in CC than TT genotypes .This was not recorded by other researchers and needs further studies. No significant associations were observed for $CDKAL_1$-rs9465871 with metabolic phenotypes. The same was found in the study done by Jiang et al 11 .

$CDKAL_1$ loci (7756992 and rs7754840) were discovered to be associated with T_2D in Europeans (2), (3) ,(13). A report indicated that another variant in CDKAL1 (rs 10946398) was associated with diabetic nephropathy in Chinese population (14).

The rs7756992 variant has been reported to have a protective effect against diabetic nephropathy in the Tunisian population (15) .

CONCLUSSION

Given the small sample size of our study further researchers are warranted to confirm the results.

To better understand the potential functions of these genetic polymorphisms of T_2DM we have to increase our sample size and add more $CDKAL_1$-rs variants.

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