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Association of RS9465871 in CDKAL1GENE Polymorphism With Overweight And Obesity.

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ABSTRACT

We aimed to investigate the possible role of rs9465871in CDKAL1gene polymorphism in the development of obesity**.** We performed a case - control, study on 118 consecutive adults**.** The enrolled subjects were classified into obese group and controls. Obese group includes 49 subjects, 23 males and 26 females with mean age of 37.80 ± 8.72 years. Control group with average values of body mass index includes 35 males and 34 females with mean age of 39.22 ± 7.17 years. All enrolled subjects underwent through clinical examination, biochemical investigations in form of, lipid profile and fasting glucose, fasting insulin, homeostasis model assessment of Insulin resistance, HOMA-IR and homeostasis model assessment of β-cell function, HOMA-B. Rs 9465871 in CDKAL1gene polymorphism was assessed in all enrolled subjects using Real Time PCR Taqman technology. Distribution of rs 9465871 CDKAL1 genotypes C/T polymorphism among the studied obese patients and controls revealed that frequency of distribution of T/T genotype was increased among obese subjects. Frequency of distribution of C/C genotype was decreased among controls. However, these differences did not reach the significant value. It was found that the frequency of C/C genotype was significantly increased among females. Waist circumference and hip circumference were significantly increased among subject with C/T genotype. There was no significant difference between obese and controls as regards C and T alleles distribution. Findings of the current study suggest that CDKAL1 rs9465871 gene polymorphism did not significantly increase susceptibility to obesity development; increased insulin resistance and impaired insulin secretion.

Keywords: Rs9465871CDKAL1-gene polymorphism-Obesity- Insulin resistance.

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INTRODUCTION

Genes enable the body to respond to changes in its environment. Researches of similarities and variations among family members show vicarious scientific proof that a major fraction of the divergence in weight within adults is referred to genetic operators **(1)**.Previous researches have studied the difference between obese and non-obese people for divergence in genes that could affect conductance(like a turn to overeat, or a trend to be sedentary) or metabolism (like a decreased ability to use dietary fats as fuel, or an increased trend to accumulate body fat). These researches have detected variants in several genes that could lead to obesity by increasing food intake and hunger **(2)**.

Scarcely, a pure manner of hereditary obesity among a family is due to a particular type of one gene (monogenic obesity). Moreover, obesity may result from complicated relations between contributing genes and surrounding circumstances that stay poorly understood (multi factorial obesity) **(3)**.

Obesity is due to chronic imbalance of energy in a person who usually takes in more calories from food and drink than are needed to power their body's metabolic and physical functions. The rapidly rising population prevalence of obesity in recent decades has been attributed to an "obesogenic" circumstances, which paves the way for high-calorie diets but restricts chances for physical exercise. The obesity epidemic can be considered a collective response to this environment. Obesity is an important public health problem imposing the risk of developing diabetes, heart diseases, stroke and other serious diseases **(4)**.

The Cyclic-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1 (CDKAL1) gene, mapped to chromosome 6p22.3, encodes a protein that prevents stimulation of CDK5. The action of CDK5 in the control of insulin secretion was assured in pancreatic beta cells efficient in p35, a stimulator of CDK5.These finding suppose that Cdk5/p35 could be used as a drug target for the control of glucose-activated insulin secretion**(5, 6, 7)**.

Moreover, CDKAL1 mRNA was detected in human pancreatic islets and skeletal muscle by reverse transcription-polymerase chain reaction (RT-PCR) **(8).**

In the present study, we investigated the possible role of rs 9465871 in CDKAL1 gene polymorphism in risk development of obesity.

METHODS

Study population

We performed a case-control, proof of concept study on 118 consecutive adults from the Out Patient Clinics admitted for routine checkup, Medical Research Center of Excellence, National Research Center. Patients were enrolled in a study about new evidences for obesity. The enrolled subjects were classified into obese group and controls. Obese group includes 49 subjects, 23 males and 26 females with mean age of37.80 ± 8.72years. Control group includes 69 subjects with average values of body mass index (BMI) encompassing35 males and 34 females with mean age of 39.22 ± 7.17years. The study protocol was approved by the Human Ethics Committee of National Research Center, and written informed consent was obtained prior to participation. Patients with any of the following criteria were excluded from the study: diabetes mellitus ,hepatobiliary diseases, chronic liver diseases including viral hepatitis , malignancies, ascites, medications known to cause hepatic steatosis (such as estrogens, corticosteroids, amiodarone, and valproate; at present or within the last 2 years), inflammatory bowel disease and chronic drug or alcohol abuse (more than 20 g/day). Endocrinal diseases

Anthropometric measurements

All patients underwent complete physical examination including measurements of height, body weight, waist circumference (WC)and hip circumference(HC).BMI was calculated according to equation: BMI= weight (kg)/height^2 (m).WC was measured at the level midway between the lowest rib margin and the iliac crest and was plotted on American percentiles for waist circumference**(9).** HC was measured at the widest level over the greater trochanters in a standing position by the same examiner; then calculation of waist /hip

ratio was done. Blood pressure and heart rate were measured in the sitting position after adequate resting time.

Laboratory measurements

Blood samples after 12 h of fasting were collected from all individuals by a sterile venipuncture and are divided as follows: three milliliters blood in an EDTA containing tube for DNA extraction , two milliliters EDTA blood for HbA1C estimation and finally three milliliters blood left in the tubes and allowed to clot for 30 min before centrifugation for 10 min then the sera separated from the clotted samples , part of sera are used immediately for measuring fasting glucose ,lipid profile :total cholesterol , high density lipoprotein(HDL), low density lipoprotein(LDL), very low density lipoprotein(VLDL) and triglycerides by Olympus AU 400 Autoanalyzer and rest of sera were uniquely labeled and stored at -20°c for insulin levels determination.

HBA1c levels were determined by Stanbio kit. Insulin was measured by sandwich Enzyme linked immunosorbent (ELIZA) technique using immunospec kit.

Insulin resistance was calculated by HOMA-IR using the following formula: HOMA-IR = fasting insulin (mU/L) · plasma glucose(mmol/L)/22.5 **(10)**.

HOMA-β index was calculated according to the following formula: (20 × fasting insulin level)/(fasting plasma glucose − 3.5)**(11)**.

DNA Genotyping :

Genomic DNA was extracted from 3 ml whole blood by a commercial DNA extraction kit according to manufacturer's protocol (QIAamp DNA BLOOD Mini kit ,QIAGEN ,USA) 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 . Genotyping of Rs 9465871 was determined using Taqman assay Real Time PCR technique via QuantStudio 12 Kflex Applied Bios stems

Rs 9465871 polymorphism was determined by a predesigned Taqman SNP genotyping assay (Applied Bios stems). Oligonucleotides used for allelic discrimination assays for Rs 9465871as following:

Context sequences for Rs 9465871 ([VIC/FAM])
CAGCTGTGTAAGTGTTGCTGAGAAA**[C/T]**TGAGTTAGATGAAGACTGAAGATTG

The reaction was performed in 25 ul final volume with real time polymerase chain reaction . For genotyping quality control ,duplicate samples and negative controls were included to insure accuracy .

Ultrasound examination

In addition to the routine abdominal ultrasound examination. Transverse scanning was performed to measure the maximum subcutaneous fat thickness(SFT) and visceral fat thickness (VFT). Both measures were obtained 1 cm above umbilicus in the midline of the abdomen. Application of the transducer on the body surface was done without undue pressure that would alter the body layer contour and thickness. SFT was defined as the distance between the external face of the recto abdominal muscle and the internal layer of the skin. VFT was defined as the distance between the anterior wall of the aorta and the internal layer of the recto abdominal muscle perpendicular to the aorta **(12)**.

Statistical analysis

Data were expressed as mean± SD and percentages .Nonparametric data were expressed as median and range. Mean values between different groups were compared using one way ANOVA test. X2 was used to study the pattern of distribution of different variables. Correlations were performed with Pearson standard linear regression analysis. The SPSS package for windows version 13 was used for the analysis .$p \leq 0.05$ was considered significant, $p \leq 0.001$ was considered highly significant and $p > 0.05$ was considered insignificant.

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RESULTS

The baseline characteristics of the studied subjects are presented in **Table 1**.There was no significant differences between obese subjects and controls as regards age and sex distribution. All components of metabolic syndrome as BMI , anthropometric measures, systolic and diastolic blood pressure , mean values of cholesterol and triglycerides , LDL ,fasting insulin ,HOMA-IR and HOMA-β were significantly increased among obese subjects when compared to controls.

Table 1: Baseline characteristics of the studied subjects

Variables	Obese patients N=49	Controls N=69	p-value
Age (years) mean±SD	37.80 ± 8.72	39.22 ± 7.17	0.613
Sex Males no (%) Females no (%)	 23 (46.90) 26 (53.10)	 35 (50.70) 34 (49.30)	0.685
Weight (kg)mean±SD	84.56 ± 13.08	69.84 ± 5.74	**0.001***
Height (cm)mean±SD	170.50 ± 7.66	172.17 ± 6.25	0.195
BMI (kg/m^2)mean±SD	29.29 ± 5.17	23.75 ± 1.09	**0.001***
WC (cm) mean±SD	96.87 ± 11.88	74.56±12.34	**0.001***
HC (cm) mean±SD	112.2±18.3	85.42±13.34	**0.001***
SCF (cm) median (range)	1.6 (1-3.4)	1.2(0.8-2)	**0.001***
VFT (cm) mean±SD	7.32± 1.35	3.1±1.4	**0.001***
SBP (mmHg)mean±SD	120.56 ± 13.05	102.0±8.2	**0.001***
DBP (mmHg)mean±SD	80.56 ± 11.87	66.8±6.5	**0.001***
FBG (mg/dl) mean±SD	87.17 ± 11.05	84.43 ± 12.36	0.225
Cholesterol (mg/dl) mean±SD	207.52 ± 50.59	166.34 ± 29.66	**0.001***
Triglycerides(mg/dl) mean±SD	117.5± 57.1	98.2±31.3	**0.001***
HDL (mg/dl) mean±SD	46.06 ± 12.11	47.81 ± 8.15	0.391
LDL (mg/dl) mean±SD	135.57 ± 46.33	100.57 ± 30.59	**0.001***
Fasting insulin median (range)	10.1 (0.8-35.9)	5 (4.2-9.3)	**0.001***
HOMA- IR median (range)	2.3 (0.15-7)	1.53 (0.15-12.62)	**0.005***
HOMA -β median (range)	298 (19-808)	138.32 (11.45-2520)	**0.001***
HBA1C (%) mean± SD	5.04± 0.53	4.98± 0.7	**0.6**

* P value is significant

Body mass index(BMI), Waist Circumference (WC), Hip Circumference (HC)

Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Subcutaneous fat thickness (SFT), Visceral fat thickness(VFT), Fasting blood glucose (FBG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Homeostasis model assessment of Insulin resistance (HOMA-IR) and Homeostasis model assessment of β-cell function (HOMA-β)

Results of CDKAL1 genotypes C/T polymorphism:

Distribution of CDKAL1rs9465871genotypes polymorphism among the studied obese patients and controls revealed that frequency of distribution of T/T genotype was increased among obese subjects. Frequency of distribution of C/C genotype was decreased among controls. However, these differences did not reach the significant value as shown in table 2.

Table 2: Distribution of RS 9465871 CDKAL1 genotypes polymorphism among the studied obese patients and controls

RS 9465871CDKAL1 polymorphism	Obese patients N=49	Controls N=69	P-value
T/T no (%)	28 (57.50)	34 (48.50)	0.270
C/T no (%)	13 (25.50)	27 (39.70)	
C/C no (%)	8 (17.00)	8 (11.80)	

As regards impact of CDKAL1 rs 9465871genotypepolymorphism on studied clinical and biochemical parameters among obese and control subjects, it was found that the mean values of waist circumference ,hip circumference were significantly increased among subject with C/T genotype as shown in table 3.

Table 3: The impact of RS 9465871 genotypes C/T polymorphism on studied variables among the obese and control subjects.

Variables	T/T genotype	C/T genotype	C/C genotype	p-value
Age mean±SD	38.92 ± 8.20	38.10 ± 7.86	38.81 ± 6.94	0.878
Sex Males no (%) Females no (%)	 35 (58.30) 25(41.70)	 18 (46.20) 21 (53.80)	 4 (25.00) 12 (75.00)	0.053
Weight kg mean±SD	74.93 ± 9.72	76.83 ± 15.20	75.81 ± 7.90	0.732
Height cm mean±SD	171.68 ± 7.25	170.01 ± 6.96	173.75 ± 4.99	0.175
BMI kg/m^2mean±SD	25.68 ± 3.65	26.74 ± 5.69	25.19 ± 2.34	0.365
WC cm mean±SD	98.67 ± 7.07	105.00 ± 9.43	84.00 ± 14.12	**0.006***
HC cm mean±SD	99.2±15.3	115.42±13.34	103.34±15.36	**0.006***
SCF cm median (range)	1.2(1-3.2)	1.7(1.4-3)	1.1 (1-3.4)	0.605
VF cm mean±SD	7.30 ± 1.25	7.46 ± 1.17	9.50 ± 3.57	0.297
SBP mmHg mean±SD	120.00 ± 16.73	121.11 ± 13.64	120.00 ± 0.00	0.986
DBP mmHg mean±SD	80.00 ± 12.65	78.33 ± 12.50	88.33 ± 7.64	0.473
FBG mg/dl mean±SD	86.08 ± 12.91	84.87 ± 9.32	85.93 ± 14.01	0.882
Cholesterol mg/dl				

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mean±SD	184.64 ± 36.59	179.64 ± 49.78	187.07 ± 57.90	0.810
Triglycerides mg/dl mean±SD	85 (43-364)	90 (40-325)	87 (44-325)	0.884
HDL mg/dl mean±SD	45.77 ± 10.43	47.95 ± 7.66	50.33 ± 12.90	0.234
LDL mg/dl mean±SD	118.80 ± 37.86	109.51 ± 44.46	113.13 ± 48.02	0.551
Fasting insulin median (range)	5.1 (0.8-32.1)	5.5 (4.2-35.9)	5.6 (0.9-35.9)	0.284
HOMA -IR median (range)	1.5 (0.15-7.0)	2.29 (0.15-12.62)	2 (0.16-7.0)	0.159
HOMA-β median (range)	147.16 (12.52-1028.57)	185.56 (11.45-2520)	234 (19.71-808)	0.297

* P value is significant

Body mass index(BMI), Waist Circumference (WC), Hip Circumference (HC)

Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Subcutaneous fat thickness (SFT), Visceral fat thickness (VFT), Fasting blood glucose (FBG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Homeostasis model assessment of Insulin resistance (HOMA-IR) and Homeostasis model assessment of β-cell function (HOMA-β)

Analysis of impact of C and T alleles distribution on studied variables among the obese and control subjects revealed that there was no significant difference between obese and controls as regards C and T alleles distribution. The frequency of C allele distribution was significantly increased among female as shown in table 4.

Table 4: Impact of C and T alleles distribution on studied variables among the obese and control subjects.

Variables	T allele	C allele	p-value
Age mean±SD	38.72 ± 8.08	38.42 ± 7.37	0.793
Sex Males no (%) Females no (%)	 88 (55.30) 71(44.70)	 26 (36.60) 45 (63.40)	**0.009***
Obese no (%) Controls no (%)	66 (41.50) 93 (58.50)	28 (39.40) 43 (60.60)	0.768
Weight kg mean±SD	75.40 ± 11.26	76.37 ± 12.35	0.557
Height cm mean±SD	171.27 ± 7.17	171.70 ± 6.36	0.669
BMI kg/m^{2}mean±SD	25.94 ± 4.24	26.04 ± 4.53	0.873
WC cm mean±SD	100.44 ± 8.00	92.65 ± 15.70	0.073
HC cm mean±SD	99.2±15.3	115.42±13.34	**0.006***
SCF cm median (range)	1.6(1-3.2)	1.6(1-3.4)	1.000
VFT cm mean±SD	7.38 ± 1.10	8.40 ± 2.46	0.193
SBP mmHg mean±SD	120.48 ± 14.65	120.67 ± 10.33	0.966
DBP mmHg mean±SD	79.29 ± 11.97	82.33 ± 11.47	0.449

FBG mg/dl mean±SD	85.79 ± 12.07	85.33 ± 11.38	0.792
Cholesterol mg/dl mean±SD	183.40 ± 40.05	182.87 ± 52.72	0.940
Triglycerides mg/dl mean±SD	87.0(40-364)	89.0 (40-325)	0.618
HDL mg/dl mean±SD	46.30 ± 9.81	48.99 ± 10.14	0.062
LDL mg/dl mean±SD	116.49 ± 39.54	111.09 ± 45.36	0.367
HBA1C	5.01 ± 0.60	5.02 ± 0.70	0.916
Fasting insulin median (range)	5.2 (0.8-35.9)	5.5 (0.8-35.9)	0.134
HOMA-IR median (range)	1.6 (0.15-12.62)	2 (0.15-12.62)	0.265
HOMA-β median (range)	156.71 (11.45-2520)	209.7 (11.45-2520)	0.205

* P value is significant

Body mass index(BMI), Waist Circumference (WC), Hip Circumference (HC)

Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Subcutaneous fat thickness (SFT), Visceral fat thickness (VFT), Fasting blood glucose (FBG), High density lipoprotein (HDL), Low density lipoprotein (LDL),HBA1C:Glycosylated Hemoglobin . Homeostasis model assessment of Insulin resistance (HOMA-IR) and Homeostasis model assessment of β-cell function (HOMA-β)

DISCUSSION

It was known that obese subjects are at risk of metabolic syndrome (MS) development which includes diabetes mellitus; hypertension and dyslipidemia. The components of MS usually predispose to cardiovascular diseases and diabetic complications which may end the life of obese individuals. So researches revealing the underlying causes for obesity are very important. One of these researches is molecular investigations which can help us to define the predisposing genes that may affect life style of the subjects leading them to be slim or to be obese. In the current study, we investigated the relationship between rs 9465871 in CDKAL1gene polymorphism and obesity. It was found that frequency of T/T genotype was increased among overweight/obese subjects. This increase did not reach the significant value. NG Maggie and his colleagues, support the important but differential contribution of these genetic variants to type 2diabetes and obesity in Asians compared with Europeans. One of these genetic variants was rs 9465871 in CDKAL1gene polymorphism (14) .

CDKAL1 is present in human islet cells of pancreas and shares homology with CDK5 regulatory subunit-associated protein 1, an inhibitor of CDK5 (14). CDK5 has been found to control secretion of insulin (15). Moreover, it can keep function under glucotoxic conditions (16). Thus, CDKAL1 may play a role in the control of insulin secretion from pancreatic beta cells, even under glucotoxic circumstances .In the same context, we found that there was associated significant differences of fasting insulin levels; medians of HOMA - IR and HOMA-β withrs9465871 in CDKAL1 gene polymorphism .Insulin resistance is corner stone of obesity development and its complications as diabetes mellitus. There was significant increase of mean values of insulin resistance among obese group. Pories & Dohmin 2012 explained the role of insulin resistance on obesity development. They reported that subjects with obesity who are slightly intolerant to glucose without diabetes, increased fasting insulin happen with normal levels of blood glucose that may activate beta cells to secrete more insulin (17) .This is confirmed by apparently correspondent to increases in blood glucose levels that happen in subjects with hyperinsulinemia after glucose ingestion. Such visible cutting of serum insulin levels from glucose levels is also seen after bariatric surgery in obese subjects. These considerations led to the assumption that hyperinsulinemia is the premier, impact on high fat diet feeding and obesity(17,18), encouraged by the activation of beta cell insulin secretion(19,20) and the inhibition of insulin

degradation(21). So that, primary hyperinsulinemia is what initially causes insulin resistance in target tissues as liver, at least under cases of increased nutrient.

In a previous Japanese study, Miyaki and his colleagues concluded that the combination of the CDKAL1 variant with metabolic syndrome discomposes an interesting issue concerning the mechanism of the evolution and advancement of the disease(22).

To investigate the role of rs 9465871 in CDKAL1gene polymorphism on obesity only, all enrolled subjects were non diabetics as values of glycated hemoglobin (HBA1C) were within normal range among obese and control subjects. In the current study, it was found that, the C allele compared to the T allele was significantly more frequent in subjects with higher values of BMI ,hip circumference, visceral fat thickness ,diastolic blood pressure , fasting insulin, insulin resistance and HOMA-β. These factors share in development of metabolic syndrome. It was evident that insulin secretion and insulin resistance have essential role in obesity develop.

Again Miyaki and his colleagues suggested that Insulin resistance is primarily counterbalanced by a compensative increased insulin secretion to keep normal glucose tolerance (22). In the same study, the authors reported that dysfunction of b-cell may be engaged in pathogenesis of metabolic syndrome, and it is seductive to consider that the dysglycemic part metabolic syndrome might already be affected by increased insulin secretion. Therefore, Miyaki and his colleagues findings suggest that CDKAL1 may not only be a promising molecular marker for the diagnosis of metabolic syndrome , even in healthy subjects, but also may provide descriptive information about lifestyle modification in the prevention of metabolic syndrome(22)

We think it is time to make our researches able to resolve human problems. It is very important to change basic research into applied one. So that, identification of obesity related genes could be used to consider a novel strategy for obesity management. Therapeutic intervention which target the identified genes paves the way for increased survival rate , avoidance of obesity complications as cardiovascular and diabetic complications or even prevent occurrence of obesity from the start. Although obesity is due to interaction of several factors as behavioral and environmental factors ,genes may help people in how to respond to environmental changes. As discussed above,CDKAL1 shares homology with CDK5. Ciudin and his colleagues reported that treatment of diabetes could be achieved by targeting CDK5 through peroxisome proliferator-activated receptors (PPARs) which are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. Targeting CDK5 can ameliorate PPARs activity and metabolic control. A more eye-catching approach is to convene this new consideration of the likely efficacy and selectivity tolerated by the CDK5/p25 inhibitors to confer with fewer side effects of Thiazolidinediones (TZDs) (23). On the basis of these findings, it is clear that CDK5 might be a deductive aim of the cure of diabetes. On the whole, several scientists and Choi and colleagues' work messenger, a new period of drug discovery, in which PPARγ activity could be reasonably aimed to improve diabetes and fudge side effects(24).

Therefore, emerging therapeutic modality for obesity is not impossible but needs more efforts, more researches and successive clinical trials to ameliorate quality of life for our obese patients.

CONCLUSION

Findings of the current study suggest that Rs 9465871CDKAL1 gene polymorphism did not significantly increase susceptibility to obesity development; increased insulin resistance and impaired insulin secretion.

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