ORIGINAL ARTICLE

Implication of CDKAL1 single-nucleotide polymorphism rs 9465871 in obese and non-obese Egyptian children

Nagwa Abdallah Ismail, MD[1], ShadiaRagab, MD[2], Soha M. Abd El Dayem, MD[1], Abeer M Nour Eldin Abd El Baky, MD[1], Mona Hamed, MD[2], Solaf Ahmed Kamel, MD[2], Dalia Adel Abd El Halim, MD[2]

[1]Pediatric Department, Medical Division, National Research Centre, Cairo, Egypt, [2]Clinical & Chemical Pathology Department, Medical Division, National Research Centre, Cairo Egypt

ABSTRACT

Introduction:CDKAL1 single-nucleotide polymorphism rs 9465871variant is a risk locus for Type 2 Diabetes (T2DM).The study evaluated the associations of CDKAL1-rs9465871 with glycosylated hemoglobin A1C Level (HbA1c), fasting insulin level, insulin resistance and metabolic syndrome among obese and non- obese Egyptian children.

Materials and Methods: The study included 43 obese children and 40 normal weight children. Anthropometric body measurements, bio-specimen and biochemistry assays were done. Genotyping of rs9465871 (CDKAL1) was conducted.

Results:The percentages of the CC, CT, and TT genotypes of rs9465871in the lean children were 15%, 42.5%, and 42.5%, respectively. Regarding obese children, the frequencies were 18.6%, 58.1% and 23.3% respectively with no significant statistical difference. Comparison between the CDKAL1 rs 9465871 polymorphism showed that the highest value of fasting insulin was recorded in CC genotype (22.80± 15.18 [uIU/mL] P<.014). Levels of HOMA-IR, FBS and HBA1C were highest in CC group with no statistical significant differences. However, fasting insulin level was higher in the CC group than in the TT+ CT group (P<.01). A higher level of HbA1c was found among CC group at CDKAL1-rs9465871 (5.9%) than TT+CT genotype group (5.6%), with no statistical significant difference. There was increase in the risk of type 2 diabetes the percentages were 78.6% and 46.4% respectively when comparing CC with TT+CT genotype groups (P<.039). CC group was not associated with significant increase in metabolic syndrome.

Conclusion: There is a significant risk association between CDKAL1-rs9465871polymorphism and development of T2DM in a subset of the Egyptian children.

KEY WORDS:
CDKAL1-rs9465871; genotype; obesity; HbA1c; metabolicsyndrome

INTRODUCTION

The incidence of obesity is affected by both genetic and environmental factors. The genetic factors cause about 50% of average variations in obesity.[1, 2] The great progress of research to identify the genetic variations of obesity were done by Genome-wide association studies (GWAS).[3, 4] In Africa, the underlying genetic background remains unknown. There has been recent progress in describing the genetic variations of obesity among populations within Africa. This effort needs to be supported by GWAS studies.[5]

Obesity is the most common finding in children with type 2 diabetes.[6] The genetic activities during childhood may have a role for the later development of type 2 diabetes. Type 2 diabetes occurs when the body cannot efficiently use the insulin that is produced because the cells have become resistant. Over time, insulin resistance can lead to prediabetes and type 2 diabetes because the beta cells fail to keep up with the body's increased need for insulin. Without enough insulin, glucose increases in the bloodstream, leading to prediabetes, diabetes. Signs of insulin resistance or conditions associated with insulin resistance (hypertension [systolic or diastolic blood pressure >95th percentile for age and sex], dyslipidemia, polycystic ovary syndrome, acanthosis nigricans, or small for gestational age at birth). Type 2 diabetes has been increasing sharply among children and teenagers.[7] Application of genetic knowledge on clinical sitting as prediction, prevention, or treatment strategies is unfortunately still far from reality. So, further research is warranted to solve health problems in modern society.[8]

For screening of prediabetes, the American Diabetes Association (ADA) recommended the use of HbA1c levels in both children and adolescents.HbA1c of 5.7 to 6.4 percent indicates prediabetes. Also, the screening include fasting plasma glucose (FPG), levels of 100 to 125 mg/dL indicate prediabetes.[9, 10]

The highest prevalence of metabolic syndrome (Met S) is among obese children. MetS is diagnosed by the occurrence of three or more of the risk factors according to the 2007 International Diabetes Federation (IDF).[11] Park et al, examined the usefulness of HbA1c as a diagnostic marker for Met S and determined that the cut-off value of HbA1c as a marker for MetS was 5.54% in all subjects with no significant difference between genders.[12]

Till now the role of these polygenic effects in the mechanism of obesity remains unclear. The insights into the biology of polygenic effects on obesity are needed. For better understanding the potential functions of CDKAL1-rs9465871 in obesity, the study evaluated the association ofCDKAL1-

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Corresponding Author: Nagwa Abdallah Ismail
Email: nagwa_abdallah@yahoo.com

rs9465871 with glycosylated hemoglobin A1CLevel, fasting insulin level, insulin resistance and metabolic syndrome among obese and non- obese Egyptian children.

MATERIALS AND METHODS

A case control study was conducted on 83 subjects. The studied group was classified into 2 groups according to body mass index: normal weight (BMI 18.5 - 24.9 and obesity BMI ≥ 30). The study protocol was approved by the Human Ethics Committee of National Research Center, and written informed consent was obtained from all children and their parents. They were recruited from pediatrics Clinic at the NRC. All non-obese volunteers were age- matched healthy subjects in good health and taking no medications. Exclusion criteria in the study were medical conditions associated with obesity such as, hypothyroidism, Cushing syndrome or Turner syndrome or subjects taking anti- inflammatory drugs.

All included cases and controls were subjected to full medical history taking, and clinical examination.

Anthropometric indices: Body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. Body mass index was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the level midway between the lowest rib margin and the iliac crest. Hip circumference (HIP C) was measured at the widest level over the greater trochanters in a standing position by the same examiner; then waist to hip ratio (WHR) and Waist to height ratio (WHTR) were calculated .[13]

Puberty was classified according to Tanner stages.[14]

Blood pressure was measured according to American Heart Association guidelines; three times for patients and controls after 5-min rest in sitting position with the use of mercury sphygmomanometer. The mean value of 2nd and 3rd measurement was calculated.

MetS is diagnosed by the occurrence of three or more of the following risk factors according to the 2007 International Diabetes Federation (IDF) [11]: obesity (particularly increased waist circumference (WC), TG ≥ 150 mg/dl, HDL < 40 mg/dl, BP≥ 130/85, basal blood glucose ≥ 100 mg/dl.
The Laboratory Measurements
Ten milliliters of venous blood were withdrawn under complete aseptic precautions from fasting subjects (12 - 14 hrs.). Five milliliters were anticoagulated with EDTA whereas rest of blood was labeled and left to clot at room temperature for 15 min then centrifuged; sera were collected and aliquoted for evaluation of the following:

1) Determination of complete lipid profile (serum triglycerides, HDL, LDL cholesterol)and fasting blood glucose levels were done using Olympus AU 400 supplied from Olympus Life and Material Science (Europe GmbH, Wendenstraße, Hamburg, Germany).15
2) Insulin levels were estimated by Enzyme immunoassay (ELISA).
3) Insulin resistance was calculated by the homeostasis model (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (mU/L) × fasting glucose (mmoL/L)/ 22.5.16
4) Glycosylated Hb (HbA1c) was measured using ion exchange HPLC (high purified liquid chromatography) Kit supplied by Crystal Chem, USA.
5) 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) CAT NO.51104 using automated nucleic acid extractor QIA cube (QIAGEN) .DNA yield was measured by Nano-dropper . 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 Bio systems). Oligonucleotides used for allelic discrimination assays for Rs 9465871 as following: 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 Bio systems).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. While the comparison between more than two groups were done by using One Way Analysis of Variance (ANOVA). Odds ratios (ORs) with 95% confidence intervals (CI) were calculated. P values < 0.05 were considered as statistically significant.

RESULTS

A total number of 83 children were recruited from NRC Pediatrics Clinic during the period between September 2014 and December 2016. They were 40 normal weight and 43 obese children. The mean age was 10.825±3.37 (5-17) years in non-obese children, while in obese children it was 12.093±2.59 (5.5–17) years with no statistical significant difference. It included 56.6% females and 43.4%% males with no statistical significant difference between groups.

The clinical and laboratory characteristics of the studied children are shown in table I. Obese children, as we expected, exhibited higher values in all anthropometric measurements. Diastolic and systolic blood pressure were significantly higher (P<0.001) in the obese group compared to the lean group. The obese group exhibited higher concentrations of fasting insulin and higher HOMA-IR values compared to the lean group (P=0.01& P<0.001 respectively). Significantly, lower levels of HDL-C in obese group was recorded (45.89 ±

Table I: Clinical and laboratory characteristics of the studied children

characteristics	Controls(n = 40) Mean (SD)	Obese group (n = 43) Mean(SD)	P
BMI (kg/m^2)	19.14 (5.28)	34.65 (6.36)	<0.001
Waist hip ratio	.48 (.14)	.71 (.11)	<0.001
Systolic BP[mm Hg]	92.37 (18.46)	109.86 (9.93)	<0.001
Diastolic BP [mm Hg]	62.50 (8.98)	71.11 (9.936)	<0.001
Fasting InsulinuIU/mL	12.57 (11.60)	18.25 (8.918)	.015
HOMA-IR	2.17 (1.52)	3.79 (1.82)	<0.001
FBSmg/dl	83.56 (29.12)	89.46 (12.22)	.401.
Cholesterolmg/dl	161.15 (31.26)	167.27 (36.54)	.420
Triglyceridesmg/dl	84.07 (27.85)	103.67 (35.47)	<.007
HDL-Cmg/dl	54.90 (14.82)	45.89 (15.75)	<.009
LDL-Cmg/dl	88.35 (33.94)	101.72 (34.24)	.080
HgA1C%	5.14 (.65)	5.74 (.71)	.234

Table II: Genotype and allele distributions of CDKAL1 rs9465871 polymorphism in lean and obese children

Group	CDKAL1			Total	Sig.
	CC	CT	TT		
Leanchildren	6	17	17	40	
	15%	42.5%	42.5%	100.0%	
Obese children	8	25	10	43	NS
	18.6%	58.1%	23.3%	100.0%	
	14	42	27	83	
	16.9%	51.2%	32.9%	100.0%	

Table III : Comparison between the CDKAL1 rs 9465871 polymorphismsand studied parameters

rs 9465871 polymorphisms	CC Mean Std. Deviation		CT Mean Std. Deviation		TT Mean Std. Deviation		Sig.
Items	No 14		No 27		No 42		
BMI	26.69	7.45	25.85	10.68	28.55	9.07	.490
Waist Height Ratio	5.66	19.67	0.39	0.18	0.42	0.15	.084
Waist Hip Ratio	0.57	0.17	0.60	0.20	0.61	0.16	.746
Systolic BP [mm Hg]	91.86	31.82	102.27	12.79	103.08	13.98	.166
Diastolic BP [mm Hg]	66.36	9.51	66.82	10.41	66.92	10.80	.995
HOMA-IR	3.08	1.63	2.67	2.00	2.99	1.80	.725
Fasting insulinuIU/mL	22.80	15.18	13.21	9.77	14.61	8.15	.014
FBSmg/dl	89.07	45.70	79.00	14.83	80.39	11.14	.349
HgA1C%	5.95	0.68	5.57	0.68	5.69	0.71	.250
Cholesterol mg/dl	167.93	49.85	168.07	33.25	160.71	28.22	.629
Triglyceride mg/dl	89.86	17.67	99.96	35.53	92.20	36.04	.558
HDL-C mg/dl	50.46	16.30	51.06	13.99	49.83	17.68	.940
LDL-C mg/dl	100.36	48.69	97.20	32.29	92.46	29.85	.724

Table IV: Comparison between the CDKAL1 rs 9465871 polymorphisms (CC&CT+TT) and studied parameters

rs 9465871 polymorphisms	CC [no 14]		CT&TT [no 69]		
Items	Mean	Std. Deviation	Mean	Std. Deviation	P
HOMA-IR	3.07	1.62	2.86	1.88	.692
FBS[mg/l]	89.07	45.69	80.01	12.65	.223
HgA1C%	5.94	.68	5.63	.69	.091
Fasting insulin ulU/mL	22.80	15.18	14.20	8.75	.014
Cholesterol mg/dl	167.92	49.84	163.67	30.52	.699
Triglyceride mg/dl	89.85	17.67	95.76	35.82	.423
HDL-C mg/dl	50.45	16.29	50.45	16.29	.739
LDL-C mg/dl	100.35	48.69	94.15	30.88	.504

Table V: Relation between the CDKAL1 rs 9465871 polymorphisms and high HOMA-IR and high Fasting Insulin

CDKAL1	High HOMA-IR		Total	Asymp. Sig. (2-sided	High Fasting Insulin		Total	Asymp. Sig. (2-sided
	no	yes			no	yes		
CC	7	7	14		4	10	14	
	50.0%	50.0%	100.0%		28.6%	71.4%	100.0%	
NON CC	53	16	69	.047	44	25	69	.019
	76.8%	23.2%	100.0%		63.8%	36.2%	100.0%	
Total	60	23	83		48	35	83	
	72.3%	27.7%	100.0%		57.8%	42.2%	100.0%	

Table VI: Relation between the CDKAL1 rs 9465871 polymorphisms and risk of Diabetes and metabolic Syndrome

CDKAL1	risk of Diabetes (HgA1C)		Total	Asymp. Sig. Sig. (2-sided	Metabolic Syndrome based on the International Diabetes Federation (IDF) guidelines		Total	Asymp. Sig. (2-sided
	no	yes			no MS	yes MS		
CC	3	11	14		10	4	14	
	21.4%	78.6%	100.0%		71.4%	28.6%	100.0%	
TT&CT	37	32	69	.039	36	6	42	.25
	53.6%	46.4%	100.0%		85.7%	14.3%	100.0%	
TOTAL	40	43	83		46	10	56	
	48.2%	51.8%	100.0%		85.2%	14.8%	100.0%	

Table VII: Odds Ratio results of CC alleles on studied Items

Results HOMA-IR	Odds ratio	3.3125
	95 % CI:	1.0102 to 10.8614
	Significance level	P = 0.0481
Results HBA1C	Odds ratio	4.2396
	95 % CI:	1.0865 to 16.5428
	Significance level	P = 0.0376
Results MS	Odds ratio	2.6667
	95 % CI:	0.6880 to 10.3358
	Significance level	P = 0.1559
Results high fasting insulin	Odds ratio	4.4000
	95 % CI:	1.2491 to 15.4991
	Significance level	P = 0.0211

15.75and 54.90 ± 14.82 100 mg/dl, P<.009).Higher values of triglycerides in obese group (103.67± 35.47and 84.07 ± 27.85100 mg/dl, P<.007) compared to the lean group.

The percentages of the CC, CT, and TT genotypes of rs9465871in the lean children were 15%, 42.5%, and 42.5%, respectively. Regarding obese children, the frequencies were 18.6%, 58.1% and 23.3% respectively with no significant statistical difference (Table II).

The comparison between the CDKAL1 rs 9465871 polymorphisms (CC, CT and TT) are shown in table III .Regarding to WC/HT, WC/HIP, body mass index, blood pressure, and serum lipid level results showed no significant differences. The highest value of fasting insulin was recorded in CC genotype (22.80± 15.18uIU/mL, P<.014). Levels of HOMA-IR, FBS and HgA1C were highest in CC group with no statistical significant differences between other groups.

Classifying children into 2 groups: CC and TT + CT genotype groups

(Table IV), the results showed no significant differences for the studied parameters when comparing the CC with TT+CT genotype groups, However, fasting insulin level was higher in the CC group than in the TT+ CT group (P<.01). A higher level of HbA1c was found among CC group at CDKAL1-rs9465871 (5.9%) than TT+CT genotype group (5.6%), with no statistical significant difference. Regarding the percentage of children had high HOMA-IR, it was 50.0% and 23.2% respectively when comparing the CC and TT+CT genotype groups, (P<.047).The percentage of high fasting insulin was significantly higher in CC group when comparing with TT+CT genotype group(P<.019) as shown in Table V.

CC group was not associated with significant increase in metabolic syndrome than CT + CC group, CC genotype could increase the risk of type 2 diabetes as the percentages were 78.6% and 46.4% respectively when comparing CC with TT+CT genotype groups (P<.039) as shown in table VI.

The odds ratio of CC alleles on studied factors is shown in table VII

DISCUSSION

The racial differences in risk allele frequencies in most genes lead to variable risks in different populations. Studying the potential role of C Allele of CDKAL1-rs9465871 among obese and non- obese Egyptian children did not show significant association in CC, CT, and TT genotypes of rs9465871 in obese and lean children. So CDKAL1-rs9465871 appears not to predispose to obesity in children. In Africa, 36 polymorphisms in genes not including CDKAL1-rs9465871 were suggested to be associated with various measures of obesity in Africa. CDKAL1-rs9465871 polymorphism was not associated with obesity in black South Africans, Nigerians and Ghanaians.[17]

However, the study observed that C allele of CDKAL1-rs9465871 was associated with high level of HOMA-IR, FPG, HbA1C and significant increase in fasting insulin. This finding reflects the role of the gene in regulating insulin secretion. Similar result was reported by Miyakietal.2010.[18] Jiang etal.2011 reported that HbA1c level was varied significantly by genotypes of CDKAL1-rs9465871, among women.[19] Regarding risk of diabetes the results suggest that C allele of CDKAL1-rs9465871 may have genetic activity during childhood that lead to the later development of type 2 diabetes in adulthood. The result was in concordance with the finding of other authors.[18]

Lastly, there was no significant risk association between C allele of CDKAL1-rs9465871 and the risk of metabolic syndrome based on the International Diabetes Federation (IDF) guidelines 2007.[11] In a large study Sung et al[20] reported that a cut level of 5.5% HbA1chas a maximum sensitivity and specificity to diagnose the Met S. While, in guidelines from the Japanese21 an HbA1c level of 5.2% or less has been recommended to aid in the diagnosis of MetS. Using HbA1c level for the diagnosis of Met S, The results suggested the implication of CDKAL1 single-nucleotide polymorphism rs 9465871 in increasing the risk of MetS.
This study has a limitation that no longitudinal follow up was done, this may be done in another study.

CONCLUSION

The study concluded that there is a significant risk association between CDKAL1-rs9465871 polymorphism and development of T2DM in a subset of the Egyptian children. The effect of CDKAL1-rs9465871 on HbA1c level points to its role as a valuable marker for the increasing risk of T2DM and MetS in our population. To confirm this association, additional studies are needed.

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