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Association of Genetic Polymorphism of Hormone Sensitive Lipase C-60G and Abdominal Obesity

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Abstract

Understanding the genetic and environmental parameters that contribute to obesity may lead to earlier identification of subject at risk and therefore more effective interventions to prevent the development or the progression of the different complications associated with obesity. The frequency of different genotype of hormone sensitive lipase among individuals with different waist hip circumference and assess the risk of the genetic polymorphism of hormone sensitive lipase C-60G on abdominal obesity. The Cross-sectional study was conducted in the Biochemistry department of RAKMHSU for 4 months from (November 2017 – February 2018).

Keywords: Genetic polymorphism; Lipase; Abdominal obesity

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Introduction

Obesity is the result of an imbalance between food intake and energy expenditure resulting in the storing of energy as fat, primarily in adipose tissue. Genetic epidemiological studies and multiple genome-wide linkage analyses have established that the causes of common obesity are complex involving a Westernized lifestyle in the presence of genetic predisposition. The obese phenotype results from alterations in numerous metabolic pathways involving several organs, as well as neuronal control of peripheral tissue [1].

Hormone-sensitive lipase (*HSL*) is a key enzyme in the mobilization of fatty acids from the triglyceride stores in adipose tissue. The enzyme is activated by catecholamines through cyclic AMP-dependent phosphorylation, whereas insulin prevents this phosphorylation [2].

The human *HSL* gene is localized on long arm of chromosome 19, designated the LIPE locus (19q13.1–19q13.2) [3]. It consists of nine exons encoding the domains that are common to all known isoforms as well as several upstream exons that are either coding or noncoding and utilized in a tissue-specific manner [4].

Several polymorphisms have been described in the *HSL* gene with evidence of association with obesity phenotypes [5]. Among them an *HSL* promoter variant, C-60G which results in the change of promoter activity and gene expression [6]. It was found that the common C-allele was associated with increased waist circumference and WHR in lean controls, but there was no difference in genotype frequency between obese and non-obese subjects [7].

Research Methodology

Study population

66 students of RAKMHSU with different waist hip ratio were enrolled. Individuals with hypertension, DM, renal or metabolic disease were excluded from the study. Informed consent was obtained from all subjects.

Data collection procedure

All participants completed a questionnaire to gather personal, family medical history and dietary record. The average calories intake per day for each subject was calculated from a food sheet filled by the participating individuals for one week.

Anthropometric measurements

Weight, height, waist and hip circumferences of all subjects were measured. Body mass index (BMI) and waist hip ratio (WHR) were calculated. Subjects were classified by BMI according to World Health Organization (WHO) criteria as follows: normal range (BMI 18.5–24.9 kg/m^2), overweight (BMI 25.0–29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2) [8]. Based on WHO criteria abdominal obesity was considered positive if waist-to-hip ratios (WHR) was >0.9 and >0.85 in men and women respectively [9].

DNA extraction from saliva

Saliva sample was collected and mixed in the DNA Genotek kit by inversion and gentle shaking for a few seconds. The sample was incubated at 50°C in a water bath for 1 hour. 500 uL of the sample was transferred into microcentifuge tube and 20 uL of prepIT.L2P (PT-L2P) was added and mixed by vortexing for a few seconds. The sample was incubated on ice for 10 minutes and centrifuged at room temperature (RT) for 5 minutes at 15,000 xg. The clear supernatant was carefully transferred with a pipette to a fresh microcentrifuge tube and 600 uL of room temperature (RT) 100% ethanol was added and mixed gently by inversion 10 times. The sample was left to stand at RT for 10 minutes to allow the DNA to fully precipitate and then centrifuged for 2 minutes at 15,000 xg. The supernatant was discarded and 250 uL of 70% ethanol was added to the DNA pellet. The tube was left to stand at RT for 1 minute. Ethanol was removed without disturbing the pellet and 100 uL of TE solution (10 mM Tris-HCL, 1 mM EDTA, pH 8.0) was added and vortexed for 5 seconds. The sample was incubated overnight at RT and then stored in -20°C. The DNA purity and concentration was determined by spectrophotometer measurement of absorbance at 260 and 280 nm after adding RNase to digest contaminating RNA [10].

Polymorphism detection

Gene was amplified by polymerase chain reaction on 96 well Amp PCR System 9700 Thermocycler (Applied Biosystems). Primer sequences were synthesized by Promega as follows forward 5'- CAGGACTGGGGCTAGGACTC -3', reverse 5'-GCAGCCTGGGGCAATAAACC -3'. The PCR procedure was performed with a 25 µL reaction mixture (100 ng of DNA, 4 nM of each primer, 2.5 mM of each dNTP, 2.5 mM of $MgCl_2$, 0.025 U Taq polymerase, and 1x PCR buffer, Promega) and consisted of an initial melting step of 3 min at 96°C, followed by 30 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C, and a final elongation step of 10 min at 72°C. The PCR product was digested by Rsa I (Promega) at 37°C overnight. Restriction products were resolved by electrophoresis on a 3% agarose gel stained with ethidium bromide 0.01 µg [7].

Statistical analysis

Genotype and allele frequency was calculated by allele counting [11]. Genotype was investigated in relation to Hardy-Weinberg equilibrium. Statistical analysis was done with SPSS software version 24.0 (SPSS, Inc; Chicago IL). Difference in genotype prevalence and association between case and control group was assessed by the Chi-square, Odds Ratio (OR) and 95% confidence interval (CI) was used to describe the strength of association. Mean value of the calories intake, waist circumference, hip circumference, weight and BMI was compared between different allele groups using Student's t-test. P value <0.05 will be considered significant.

Results and Discussion

The levels of HSL in subcutaneous and visceral adipose tissue in our study were correlated to each other, suggesting that the expression of HSL may be co-regulated in these depots. Even in this obesity-related reduced state of HSL mRNA expression the expression levels in subcutaneous adipose tissue were negatively correlated to measures of fasting plasma triglyceride levels and abdominal obesity. This suggests that as obesity develops, and perhaps particularly abdominal obesity, HSL expression decreases. Below are the results obtained as shown in **Tables 1-6** and **Graphs 1-7.**

Conclusion

In conclusion, the HSL C-60G polymorphism is associated with increased waist circumference in non-obese subjects, but not with obesity per se nor with insulin resistance or type-2 diabetes. Although the HSL C-60G polymorphism affects transcriptional

Table 1 Demographic data of the studied group (value represented in mean ± standard deviation.

Parameters/ BMI	Normal n=46	Overweight/Obese n=20	P-value
Age (years)	19.1 ± 1.0	18.7 ± 1.3	>0.05
BMI (kg/m^2)	22.1 ± 1.9	27.8 ± 3.1**	< 0.001
Waist circumference (cm)	79.6 ± 8.0	88 ± 13.3*	< 0.05
Waist/hip ratio	0.8 ± 0.1	0.9 ± 0.1	>0.05
Percent Body Fat (%)	21.0 ± 5.7	26.3 ± 7.2**	< 0.01
Weight (kg)	59.5 ± 8.1	80.7 ± 15.3**	< 0.001
Calorie intake (Kcal/day)	1566.9 ± 475	1641.7 ± 427	>0.05

Table 2 Frequency of different genotypes of *HSL C-60G* among carries of different BMI.

BMI/Genotype	CC n=47	CG n= 19
Normal (46)	30 (65.2%)	16 (34.8%)
Overweight (16)	13 (81.3%)	3 (18.8%)
Obese (4)	4 (100%)	0(0%)

Table 3 Frequency and odds ratios of different genotypes of *HSL C-60G* among carriers of different BMI.

BMI/Genotype	CC n=47	CG N=19	OR	95% CI
Normal (n=46)	30 (65.21%)	16 (34.8%)	0.33	0.1-1.3
Overweight/Obese (n=20)	17 (85%)	3 (15%)		

Table 4 Association of the different alleles of *HSL C-60G* with BMI.

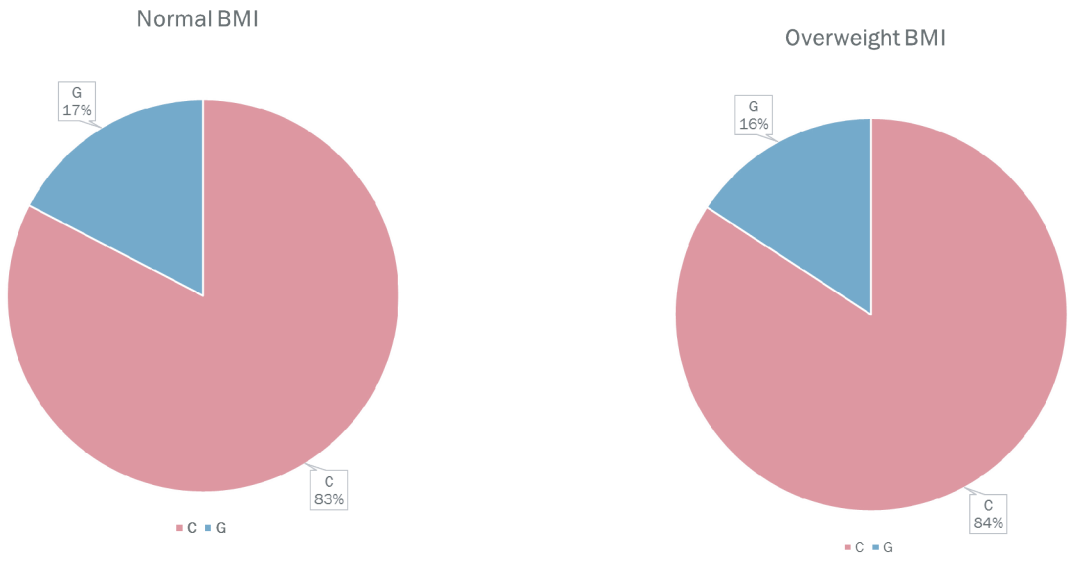
BMI/Allele	C	G	OR	95%CI
Normal	76 [82.6%)	16 (17.4%)	0.39	0.1-1.4
Overweight/Obese	37 (92.5%)	3 (7.5%)		

Table 5 Comparing the mean ± SD value of different parameters of obesity phenotype among carriers of different genotype of *HSL C-60G* gene.

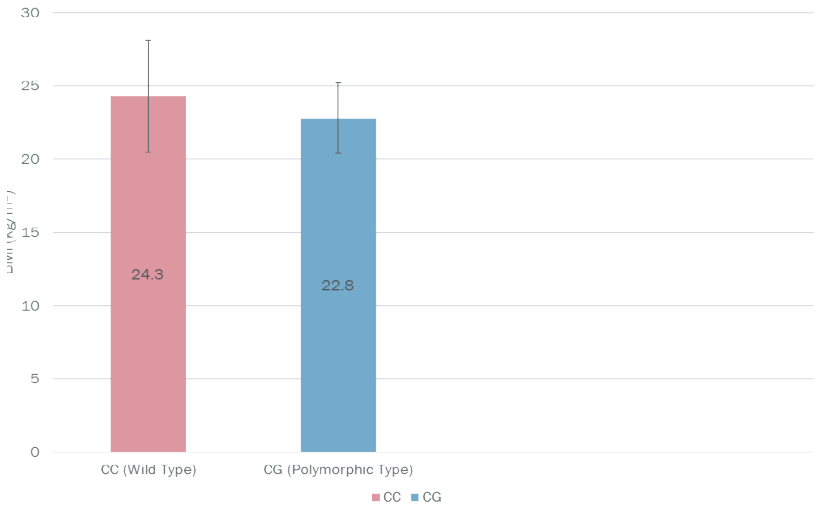
Phenotype/Genotype	CC n=47	CG N=19	P-value
Weight (Kg)	68.1 ± 15.6	60.5 ± 9.6	>0.05
Waist Circumference	83.7 ± 10.8	78.5 ± 8.6	>0.05
Waist/Hip Ratio	0.8 ± 0.1	0.8 ± 0.1	>0.05
BMI (kg/m^2)	24.3 ± 3.8	22.8 ± 2.4	>0.05
Percent Body Fat (%)	24 ± 5.8	23.5 ± 5.8	>0.05
Calories (Kcal)	1667.8 ± 421	1725.7 ± 349	>0.05

Table 6 Nationality.

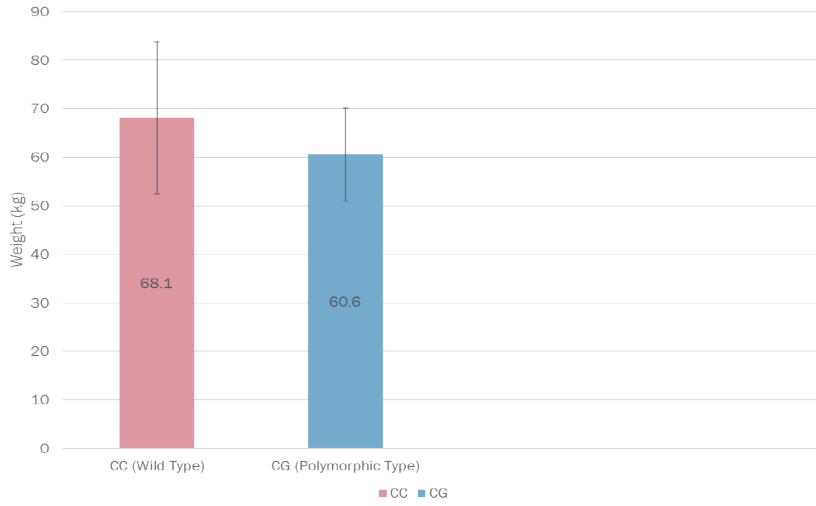
Nationality	Frequency	Percent
Indian	26	39.4
Egypt	6	9.1
Syria	6	9.1
Iraq	5	7.6
Pakistan	4	6.1
UAE	3	4.5
Sudan	2	3.0
Bahrain	2	3.0
Jordan	1	1.5
Somali	1	1.5
Siri Lanka	1	1.5
Tanzania	1	1.5
Yemen	1	1.5
Britain	1	1.5
American	1	1.5
Libyan	1	1.5
Kuwait	1	1.5
Canadian	1	1.5



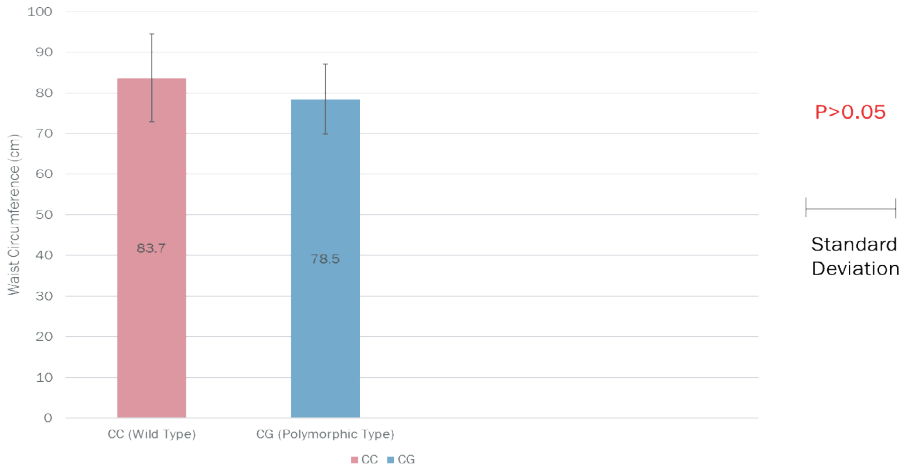
Graph 1 BMI and allele frequency.



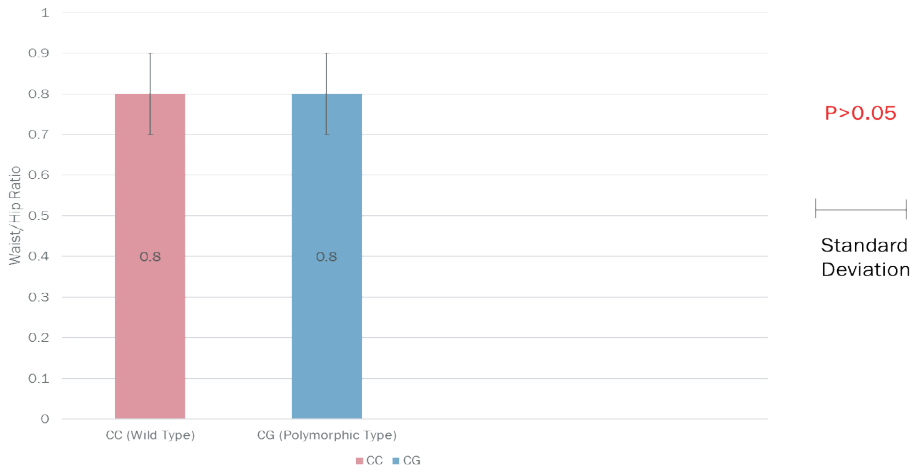
Graph 2 Comparing BMI and genotype.



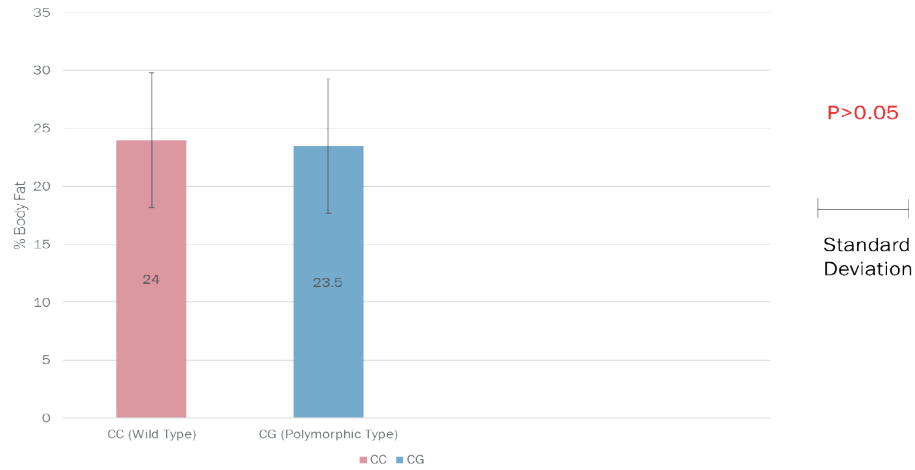
Graph 3 Comparing weight and genotype.



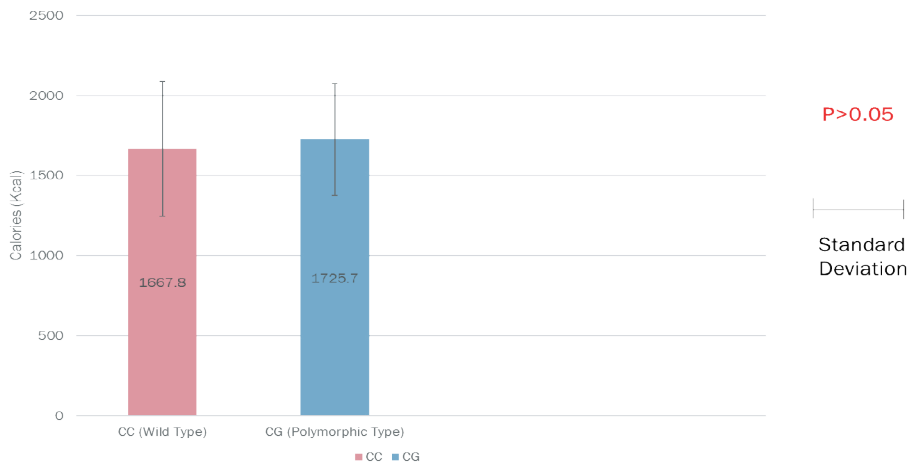
Graph 4 Comparing waist circumference and genotype.



Graph 5 Comparing waist/hip ratio and genotype.



Graph 6 Comparing percent body fat and genotype.



Graph 7 Comparing calories and genotype.

activity *in vitro* these findings remain elusive in the *in vivo* situation. Carrier of *HSL* CG genotype were protected from obesity and carriers of *HSL* CC 60G genotype were 3-folds at risk of obesity.

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