

# Influence of *Interleukin-6* (174G/C) Gene Polymorphism on Obesity in Egyptian Children

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## Abstract

**Citation:** Ibrahim OM, Gabre AA, Sallam SF, El-Alameey IR, Sabry RN, Galal EM, Tawfik SM, Zarouk WA, Mosaad RM, Ramadan A. Influence of *Interleukin-6* (174G/C) Gene Polymorphism on Obesity in Egyptian Children. Open Access Maced J Med Sci. <https://doi.org/10.3889/oamjms.2017.175>

**Keywords:** Obesity; Interleukin-6(174 G/C); Children; polymorphism; Egypt.

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**Received:** 24-Jun-2017; **Revised:** 06-Sep-2017; **Accepted:** 07-Sep-2017; **Online first:** 17-Oct-2017

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**Funding:** This research did not receive any financial support.

**Competing Interests:** The authors have declared that no competing interests exist.

**BACKGROUND:** Obesity is a multi-factorial chronic disorder. A considerable number of studies have been performed to figure out whether there is an association between obesity and polymorphisms of gene *IL-6* (174G/C), but the results are equivocal.

**AIM:** This study aimed to find out whether the *IL-6* (174G/C) gene was associated with the risk of developing obesity in Egyptian children.

**SUBJECTS AND METHODS:** The study included 149 children and adolescents with age ranged between 9.5 – 18 years. Eighty-five of them were obese which BMIZ-score is > 2, and sixty-four children with BMIZ-score ≤ 2 served as control group. Serum level of *IL-6* and genetic analysis for *IL-6* (174G/C) gene polymorphism were done.

**RESULTS:** Obese children had significantly higher serum levels of *IL-6* as compared to those of control children ( $P = 0.003$ ). A high percentage of *IL-6* polymorphism GC was found in obese subjects (93.7%), while the control group had a higher percentage of *IL-6* polymorphism GG (70.6 %).

**CONCLUSION:** Our study showed that carriers of the C allele for the *IL-6* (174G/C) polymorphism have higher BMI. As the G174C polymorphism is likely to affect *IL-6* expression and its physiological regulation; consequently this polymorphism may affect adiposity.

## Introduction

Obesity represents one of the major Public Health problems. Obese children had a shorter life expectancy by 20 years if they remain obese till young adulthood [1]. North African Countries and the region of Middle East just like the other developing countries are not spared from the issue of the world [2, 3]. Genetics, metabolic, social, cultural and environmental factors interact as different cofactors for the development of obesity. The prevalence of obesity in Egyptian children ranged from 13.5 % up to 23.7% [4-5].

Advanced knowledge of human genome variations led to identification of genes susceptible to

contribute in obesity and other related diseases; meanwhile few studies focused specifically on the interactions between obesity and genetic polymorphism [6]. Interleukin-6 (*IL-6*) is an immunomodulator pro-inflammatory cytokine involved in the regulation of the acute phase response [7]. High *IL-6* serum level was found in obese patients as well as patients suffering chronic inflammatory conditions and serum lipid concentrations abnormalities [8]. Interleukin-6 production has been shown to be significantly increased by adipose tissue in a state of obesity. The increased *IL-6* levels in obese individuals may result in a state of insulin resistance and increased risk of cardiovascular complication [9].

Plasmatic levels of cytokines and its transcriptional regulation is influenced by the

polymorphisms of IL-6 (especially 174G/C) as proved in many population groups based genetic studies, that have been performed to figure out whether there is an association between obesity and IL-6 (174G/C) polymorphisms, but results were not conclusive with a lot of controversies [10-11].

Based on this; this study aimed to find out whether IL6 (174G/C) polymorphisms designated as rs1800795 as well, is associated with the risk of developing obesity in Egyptian children.

## Subjects and Methods

This is a cross-sectional study that included one hundred and forty-nine children and adolescents, 71 males and 78 females. Their age ranged between 9.5 to 18 years (mean  $11.72 \pm 2.52$  yrs). This study started by one thousand children and adolescents chosen randomly from three governmental schools at Giza. According to their anthropometric measures, it was found that one hundred and thirty were obese (BMI-Z score  $>2$ ). Eighty-five of them (41 males and 44 females) were fulfilling the criteria and gave the consent to share in the study.

**Exclusion criteria:** were factors that might lead to the misclassification of a child's weight status, that are: (1) an acute or chronic illness affecting weight; (2) genetic conditions associated with obesity or failure to thrive; and/or (3) use of medications associated with weight gain or loss.

**Inclusion criteria:** apart from obesity; all obese subjects and non-obese controls were in good health. This study was a part of project that approved by the Medical Ethical Committee of the National Research Center with registration number 14046. All participants were informed about the objectives of the study and volunteered to participate and the parents of all subjects provided a written informed consent.

### Anthropometric assessments

Anthropometric assessments were performed according to techniques described in the Anthropometric Standardization Reference Manual [12]. Weight was measured using a calibrated Seca scale to the nearest 0.1 kg (Seca, Hamburg, Germany), whereas height (in cm) was measured using a Seca 225 stadiometer to the nearest 0.1 cm, with the children dressed in minimal clothes and without shoes. Each measurement was taken as the mean of three consecutive readings following the recommendations of the International Biological Program [13]. Weight for height, weight for age, height for age, and BMI for age was recorded according to WHO standards using Anthro Plus software for

personal computers [3]. Measurements were expressed as weight-for-age Z-score (WAZ), height for age Z-score (HAZ), and BMI Z-score (BAZ). The participants were then grouped according to the WHO Global Database on Child Growth and Malnutrition using a Z-score cut-off point [14]: Overweight and obese children with BAZ  $>2$  and control children with BAZ  $\leq 2$ .

### Laboratory Investigations

Fasting blood sample was drawn from each participant into vacutainer tube containing EDTA as an anticoagulant. The samples were immediately transferred to the laboratory, centrifuged immediately for 10 min at 4000 and stored at  $-80^{\circ}\text{C}$ .

- Serum interleukin-6 level measuring using the quantitative Enzyme-Linked Immune-Sorbent Assay (ELISA) with a commercial kit provided by DIA source, Belgium [15].

- DNA extraction: Blood samples were collected on  $\text{Na}_2\text{EDTA}$  as an anticoagulant. Genomic DNA was purified from 200  $\mu\text{L}$  whole blood with the QIAamp® DNA Blood Mini Kit (Qiagen) according to the Blood and Body Fluid Spin Protocol in the accompanying handbook. DNA was eluted in 200  $\mu\text{L}$  elution buffer and stored at  $-20^{\circ}\text{C}$ .

- Detection of IL6 (-174G/C) polymorphism: DNA was amplified with primers specific for -174G/C (rs1800795) in a 25  $\mu\text{L}$  reaction mixture containing ; 50 ng  $\mu\text{L}$  genomic DNA,  $\text{ddH}_2\text{O}$  15.7  $\mu\text{L}$ , 10 x buffer 2.5  $\mu\text{L}$ , dNTP (10 mmol) 0.5  $\mu\text{L}$ , each of primer sequences (10  $\mu\text{mol}$ ) 0.5  $\mu\text{L}$ ,  $\text{MgCl}_2$  (25 mmol) 2  $\mu\text{L}$  and 1U Taq enzyme. The PCR was performed in 30 cycles; (  $95^{\circ}\text{C}$  for 30 sec, annealing at  $55^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 1 min), with an initial denaturation at  $95^{\circ}\text{C}$  for 5 min and a final extension at  $72^{\circ}\text{C}$  for 10 min. Followed by final extension  $72^{\circ}\text{C}$  for 5 min. All PCR products were analyzed by 2% agarose gel electrophoresis.

- Enzyme digestion: Post PCR-RFLP was done by incubating five  $\mu\text{L}$  of SNP-PCR product with the restriction enzyme *Nla III* at  $37^{\circ}\text{C}$  for 30 min. The digested SNP-PCR product was electrophoresed on 3% agarose gel [16].

### Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS) version 16. Results were presented as mean  $\pm$  SD, except where otherwise indicated. Comparisons between groups were done by independent samples t-test. Chi-square test was used to estimate differences in qualitative variables. Pearson correlation test was used to determine the relationship between some numerical variables. For all tests, probability values (P) of less than 0.05 were regarded as statistically significant.

## Results

The characteristics of the studied groups are shown in Table 1. The mean serum level of IL-6 in obese children was significantly higher as compared to those of control children ( $7.7 \pm 0.46$  pg/mL and  $5.46 \pm 0.40$  pg/mL respectively ( $P = 0.003$ ). Serum IL-6 level had a positive correlation with age ( $r = 0.28$  and  $p = 0.001$ ) and with BAZ ( $r = 0.188$  and  $p = 0.03$ ).

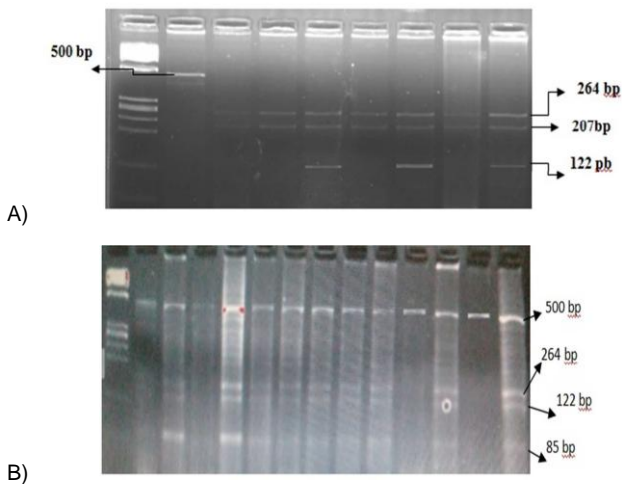
**Table 1: Characteristics of the studied groups**

Parameter	Controls	Obese	P
Number	64	85	
Males: females	30:34	41:44	ns
Mean age (years)	$10.9 \pm 2.3$	$12.3 \pm 2.4$	ns
Z-score BMI ( $\text{kg}/\text{m}^2$ )	$0.13 \pm 0.16$	$2.8 \pm 0.62$	0.00
IL-6 (pg/ml)	$5.46 \pm 0.40$	$7.7 \pm 0.46$	

P value  $\leq 0.05$  is significant.

### Result of genotyping analysis of IL6-174G/C polymorphism

The Nla III restriction enzyme digest the 500 bp applicant of the IL6-174; where G allele yielded three bands (of 264, 207 and 29bp); the -174 C allele yielded four bands (of 264, 122, 85 and 29 bp) (Figure 1A & 1B).



**Figure 1: A)** Agarose gel (3%) photographs of PCR-RFLP products (amplicons digested with restriction enzymes) depicting the genotypes of IL-6 polymorphisms PCR and digestion products run on 3% agarose gel electrophoresis. Lane 1:  $\Phi$ X 174 marker/Hae III digest. Lane 2: The PCR amplified product (500 bp). Lanes 3, 4, 6 and 8: G/G genotype (264, 207 and 29 bp). Lanes 5, 7, and 9: G/C genotype (264, 122 and 29 bp); **B)** Fig (2): PCR and digestion products run on 3% agarose gel electrophoresis. Lane 1:  $\Phi$ X 174 marker / Hae III digest. Lane 2: The PCR amplified product (500 bp). Lanes 3, 4, 6, 7, 8, 9, 10, 12: G/C genotype (500, 264, 122 and 85 bp)

Table 2 shows the distribution of genotype for IL6 (-174G/C) polymorphism: The homozygote genotype (G/G) was 57.05 %, the heterozygote (G/C) 42.95 % and none of our patients was carrying the homozygote genotype (C/C).

**Table 2: The frequency of genotype of IL-6 polymorphism**

	Frequency	Percent
Heterozygous GC	64	42.95
Homozygous GG	85	57.05
Total	149	100

No significant difference in the serum level of IL-6 between subjects with IL-6 (174G/C) polymorphism and those with IL6 (174G/G) polymorphism (Table 3).

**Table 3: The mean serum level of IL-6 in both groups of polymorphism**

Parameter	GC	GG	P
IL-6 (pg/ml) Mean $\pm$ SD	$6.53 \pm 4.97$	$6.99 \pm 4.52$	ns

Table 4 shows that the mean of BMI Z score was significantly higher in IL-6 polymorphism GC as compared to that of polymorphism GG. Our results showed that 95.3 % of IL-6 polymorphism GC was found in obese subjects as compared to 4.7 % in normal weight children.

**Table 4: The mean  $\pm$ SD of BMIZ-score (BAZ) in both types of IL-6 polymorphism**

Parameter	GC	GG	P
BMIZ-score, Mean $\pm$ SD	$2.76 \pm 0.68$	$0.86 \pm 1.6$	0.00

P value  $\leq 0.05$  is significant.

On the other hand, 71.8 of IL-6 (174G/G) polymorphism were found in controls as compared to 28.2% in obese children (Table 5). It was noted in the results that, none of our children were carrying C/C genotyping.

**Table 5: The percentage of IL-6 polymorphism in obese and control groups**

IL-6 polymorphism	Controls		Obese		P
	(n)	%	(n)	%	
GG (n=85)	61	71.8	24	28.2	0.00
GC (n=64)	3	4.7	61	95.3	0.00

## Discussion

This study revealed a significantly higher serum level of IL-6 in obese children as compared to those of controls. The Same finding was previously reported in a study of Filippo et al. [11]. Our data showed a positive correlation of IL-6 with both age and BAZ.

These results are by the results of Diego et al. [17] and Paltoglou et al. [18]. In this work, we studied whether the IL6(174G/C) gene is associated with the risk of developing obesity in obese Egyptian children. The analysed polymorphism (174G/C) is localised in the promoter region of the IL-6 gene, where transcription factors frequently exert their functions through its influence on the energy expenditure

processes by different ways. The results revealed that the GG homozygote genotype is predominated in the control group (95.3 %) with a statistically significant difference compared to that of obese children (28.2 %). If we considered the GC genotypes as C carrier; so, the study showed a prevalence 71.8 % (61 of 85), of this C allele among the obese children as compared with 4.7% (3 of 64) in the normal weight children. So, the examined polymorphisms of the pro-inflammatory cytokine *IL-6* (174G/C) could play a role in the regulation of body mass. According to these aspects, the results of this study seem to confirm the hypothetical relation between the *IL6* (174G/C) genotype and the risk of developing obesity among Egyptian children.

Our data match and is replicated with previously published data; as some authors have investigated the relation between polymorphisms in *IL-6* gene and obesity based on experimental evidence on mice suggested an influence on fat mass, fat metabolism, and body mass and on the development of obesity [19]. Zhangbin et al., [20] realized that *IL-6* (174 G/C) polymorphism was also associated with obesity by covering 48 studies with outcome similar to the results in our study.

In a study of Popko et al., [21] the detected C allele in both C/C homozygotes and G/C heterozygotes of *IL6* (174 G/C) gene was related to a significant increase in the sum of 10 skinfold thickness measurements in obese girls; which is consistent with the results of our study. A study enrolling Greek school children had shown an association of the *IL-6* variant rs1800795 with parameters related to obesity [22]. Oana et al. [23] described the G allele at C-174 as being more common in lean subjects and observed the C allele to be associated with indices of obesity. These findings are in concordance with the results of our study, in which the GC heterozygote genotype predominated in the obese group, and the GG genotype was less pronounced in this group than in the normal weight group. In this study, none of our children was carrying C/C genotyping. This is because it is a pilot study and our sample size was small. It was previously reported by Saxena et al. [24] that CC genotype was very rare in their study.

Contrary to our findings, data reported in an Iranian study among 242 persons, sustained more frequent G alleles in the obese but was statistically insignificant [25]. Another meta-analysis didn't find significant associations of *IL6* (174G/C) genotypes with waist-to-hip ratio, waist circumference, or central obesity and did not support a role for this polymorphism in adiposity [26].

In conclusion, our study showed that carriers of the C allele for the *IL-6* polymorphism G174C have higher BMI. As the G174C gene polymorphism is likely to affect *IL-6* expression and its physiological regulation; consequently this polymorphism may affect adiposity and insulin sensitivity. It is recommended in

future studies for the *IL-6* polymorphism G174C to be done in a wider scale of children and adolescents and in different demographic areas as our study enrolled relatively small number of obese and normal children which decreased the power of the group in statistic results.

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