Leptin gene tetranucleotide repeat polymorphism in obese individuals in Egypt

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Abstract

Background: Leptin is a peptide hormone secreted by the adipose tissue. Genetic mutations of the leptin gene were reported to cause severe obesity.

Objectives: This study was undertaken to investigate the association of the polymorphic tetranucleotide repeat locus 3' UTR of leptin gene with obesity in Egyptian cases.

Subjects and Methods: This study has included 120 subjects affected with obesity 57 of them were consistent with the diagnosis of metabolic syndrome (MS) while the rest (63) had simple obesity. These cases were compared to 83 normal weight healthy controls. All participants were subjected to an estimation of their body mass index (BMI), waist hip ratio (WHR), serum as well as characterization of leptin gene tetranucleotide repeat $(TTTC)_n$ polymorphism by PCR technique.

Results: Thirteen different alleles were identified in all cases of obesity versus only 5 alleles in normal controls. The most frequent allele was the 154 bp allele (57.5% in all cases of obesity vs. 92.2% in controls). Total cases with obesity showed a significantly higher carriage rate of class II alleles (I/II + II/II genotypes) compared to healthy controls (48.3% vs. 6.0%, OR=14.6, 95% CI=5.5-38.6, p=<0.0001). This was more apparent in the group with simple obesity (52.3% vs. 6.0%, OR=17.2, 95% CI=6.1-48.1, p=<0.0001) than in MS cases (43.9 % vs. 6.0 %, OR =12.19, 95% CI=4.9-30.4, p=< 0.0001). Interestingly, cases with MS did not differ from those with simple obesity regarding their class I or II allele frequencies (p> 0.05). Although serum lipids were significantly higher in obese cases compared to controls, no difference was found among obese cases with different leptin gene class genotypes (p> 0.05).

Conclusions: Tetranucleotide repeat $(TTTC)_n$ polymorphism in the 3' UTR of the human leptin gene was associated with obesity in Egyptian obese cases showing higher class II allele carriage rate. However, the lipoprotein levels were not affected by this polymorphism.

Key words: Leptin gene; Obesity; Metabolic syndrome; Gene polymorphism.

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Introduction

Obesity is considered a medical condition involving an accumulation of excess body fat potentially posing a threat to health and life of an individual. [1] It has been estimated to be the fifth leading cause of mortality worldwide. [2-4] The assessment of obesity in clinical practice is through the measurement of body mass index (BMI) which is calculated as body weight (kg) divided by height squared (m^2). Obesity is defined by a BMI ≥ 30 kg/m^2 and overweight (also termed pre-obesity) by a BMI between 25 and 29.9 kg/m^2. [5] However, the visceral fat component was found to be more correlated to the waist -to- hip ratio (WHR) which is calculated as the waist measurement divided by the hip measurement. [6,7] Obesity is considered a risk factor for many diseases such as hypertension, type II diabetes mellitus, dyslipidemia, metabolic syndrome, coronary heart disease and certain type of cancer. [8-12] Metabolic syndrome (MS) is a set of risk factors that includes obesity (assessed by waist circumference), decreased ability to process glucose (increased blood glucose and/ or insulin resistance), dyslipidemia and hypertension. [13-15]

Obesity -whether complicated by MS or not- was found to be the result of interplay between genetic and environmental factors. [16] Genetic polymorphism plays a role in obesity through the various genes controlling appetite and metabolism. The percentage of obesity that can be attributed to genetics varies, depending on the population examined. [17, 18]

Leptin has a role in the regulation of body weight as it sends signals to the brain about the body's energy status. Its action is mediated through binding to specific receptor with the release of neuropeptides controlling the higher brain control over feeding. [19-21] So, leptin gene was known as “human obesity gene” and it was mapped to 7q31.3. It consists of 3 exons and 2 introns, which spans approximately 18 kb. [22] Leptin is a peptide hormone secreted by adipose tissue. It is made up of 167 amino acids with an amino-terminal secretory signal sequence of 21 amino acids. [23] It regulates food intake and energy expenditure. [24, 25] Mutations in the gene encoding leptin are reported to cause severe obesity in animal models and humans. [26-28] In addition, numerous polymorphisms of the leptin gene have been described. Particularly, the highly polymorphic leptin tetranucleotide locus located in 476 bp 3' of exon 3 on chromosome 7 that had been proven to be associated with obesity and hypertension. [29-32] Other mutations include the 2548 G>A described in Caucasian and Finnish population; [33, 34] the 19 A>G in Italian population, 2549 C>A in Chinese population [35] and Gln25Gln in Japanese population **[36]** with controversial positive or negative association with obesity.

In Egypt, the prevalence of obesity in adults was reported to be very high, particularly among women; with parallel increase in the frequency of diabetes mellitus and of hypertension. [37] So, we were interested to test for the association of an important leptin gene 3’ UTR tetranucleotide repeat $(TTTC)_n$ polymorphism with obesity among Egyptian obese subjects.

Subjects and Methods

This is a case controlled relatively small scaled pilot study involving 120 subjects affected with obesity in addition to 83 healthy normal weight matched controls from the central area of the Nile Delta of Egypt. Obese cases were recruited from the Department of Obesity and Diabetes, Internal Medicine Specialized Hospital, Mansoura University, Egypt. Controls were selected from healthy unrelated blood donors from the same locality. Obese cases were in the form of 21(17.5%) males and 99 (82.5%) females with an age mean ± SD of 31.5 ± 11.2 years. Of them, 21(17.5%) had a positive parental consanguinity whereas 73(60.3%) had positive family history of obesity. According to the definition of metabolic syndrome given by WHO and others, [13, 14, 38] 57(47.5%) were classified as having MS while the rest, 63(52.5%) were not complicated and were characterized as just having simple obesity.

For all participants, the levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein (LDL-C) were determined by enzymatic methods using kits including: Tcho-1, TG-LH (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom, BT29 4QY), Cholestest NHDL., and Cholestest LDL (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan), respectively. In addition, DNA was extracted using DNA extraction and purification Kit

(Gentra Systems, USA) according to manufacturer's instructions and stored at -20 ^{o}C till being processed. The tetranucleotide repeat (TTTC)n polymorphism in the 3' UTR of the human leptin gene was determined through amplification by polymerase chain reaction (PCR) using a forward and reverse oligonucleotide primers : 5'-AGT TCA AAT AGA GGT CCA AAT CA-3' and 5'-TTC TGA GGT TGT GTC ACT GGC A -3'. The protocol of amplification has included an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation 94°C at 30 seconds, annealing at 54°C for 30 sec and extension at 72°C for 1 min with a final extension at 72°C for 10 minutes. [32] Leptin alleles were separated by electrophoresis on agarose gel stained with ethidium bromide and sized comparatively against a standard DNA marker. Alleles size equal or more than 217 bp were termed class II while those less than 217 bp were termed class I alleles.

Statistical analysis

Statistical analysis of data was done using the software statistical package SPSS program version 17. Student t-test was used to compare the numerical values related to lipid profile, body mass index and waist hip ratio, whereas Chi square, Fisher exact and odds ratio with 95% confidence interval were used to compare frequencies of different genotypes and alleles among cases and controls. Moreover, multivariate cluster analysis was used to make an analytical differentiation of frequencies of class I/class II alleles in the Egyptian controls compared to other world populations.

Results

The general characteristics of cases of obesity and healthy controls are shown in Table 1. The mean age (±SD) and gender frequency in cases were non-significantly different from that of the controls, whereas the mean values (±SD) of weight, BMI, WHR, serum levels of TC, TG, HDL-C and LDL-C were significantly higher in cases of obesity than in controls ($p < 0.001$ for each). Allelic analysis of the leptin gene polymorphism showed that 13 different alleles were identified in all cases of obesity (11 alleles in MS cases and 12 in cases of simple obesity) versus only 5 alleles in normal controls (Table 2). The most frequent allele (154 bp) was present in 57.5% of all obesity cases (57.9% of cases with metabolic syndrome, 57.1% of cases with simple obesity) versus 92.2% of controls. In addition, cases with obesity showed a significantly higher carriage rate of class II alleles (I/II + II/II genotypes) compared to healthy controls (48.3% vs. 6.0%, OR=14.6, 95% CI=5.5-38.6, $p = 0.0001$). This was more apparent in the group of simple obesity (52.3% vs.6.0%, OR=17.2, 95% CI=6.1-48.1, p=0.0001) than in cases with MS (43.9% vs. 6.0%, OR=12.9, 95% CI=4.9-30.4, $p < 0.0001$). However, cases with MS did not show any significant difference from cases with simple obesity regarding frequencies of the leptin gene class genotypes ($p > 0.05$) (Table 3).

Table 1: Demographic data of total cases of obesity and healthy controls

	Cases n(%)	**Controls n(%)**	***p***
Total	120(100.0)	83(100.0)	
Age (Years)			
Age range (Years)	13-61	12-65	
Mean age ± SD (Years)	31.5± 11.2	29.62 ± 9.73	0.28
Gender (Sex)			
Male/ Female	21(17.5)/99(82.5)	9(10.9)/74(89.1)	0.19
Consanguinity			
Positive/ Negative	21(17.5)/ 99(82.5)	0(0.00)/ 83.0(100.0)	<0.001**
Family history			
Positive/ Negative	73(60.3) / 47(39.7)	0(0.00)/ 83.0(100.0)	<0.001**
Measurements			
Weight (kg)	104.9±16.5	61.5 ±7.6	<0.001**
Height (cm)	162.0±8.2	168.5±7.0	<0.001**

BMI (kg/m²)	39.9±6.3	21.5±1.6	<0.001**
WHR	0.96 ± 0.14	0.77±0.03	<0.001**
Lipid Profile			
TC (mg/dl)	245.9±60.1	165.0 ±18.3	<0.001**
TG (mg/dl)	128.2±74.2	94.3±28.9	<0.001**
HDL-C (mg/dl)	48.2±15.0	36.9±14.5	<0.001**
LDL-C (mg/dl)	169.1 ±59.7	110.3±17.9	<0.001**

TC= total cholesterol, TG= triglyceride, HDL-C= high-density lipoprotein, Cholesterol, LDL-C = low-density lipoprotein cholesterol, n= number of subjects, (%) = percentage of subjects, BMI= body mass index, WHR=waist-to-hip ratio, Significance using t-test or Chi square test : * p = <0.05 (significant), ** p= <0.001 (extremely significant)

Comparing the frequency of allelic polymorphisms of leptin gene among populations of US African Americans (*n*=600), mixed European Americans (*n*=624), Samoans (*n*=104), Costa Rica (*n*=35), West African (n=82), Central African (n=48), Hong Kong (n=43), Taiwanese (n=73), British (n=35), Cyprus (n=38), Malaysian (*n*=129), Madagascar (*n*=88), Indian (n= 39) and Italian (n=109) [39-41,31], using the cluster multivariate analysis showed that the frequency of the Class I/Class II alleles in our sample (Egyptians n=83) was very near to African and Mediterranean countries but somewhat different from Asian and European countries (Figure 1).

Figure 1: Dendrogram (Cluster analysis) showing the average linkage between groups related to the frequency of Class I/Class II alleles in different populations

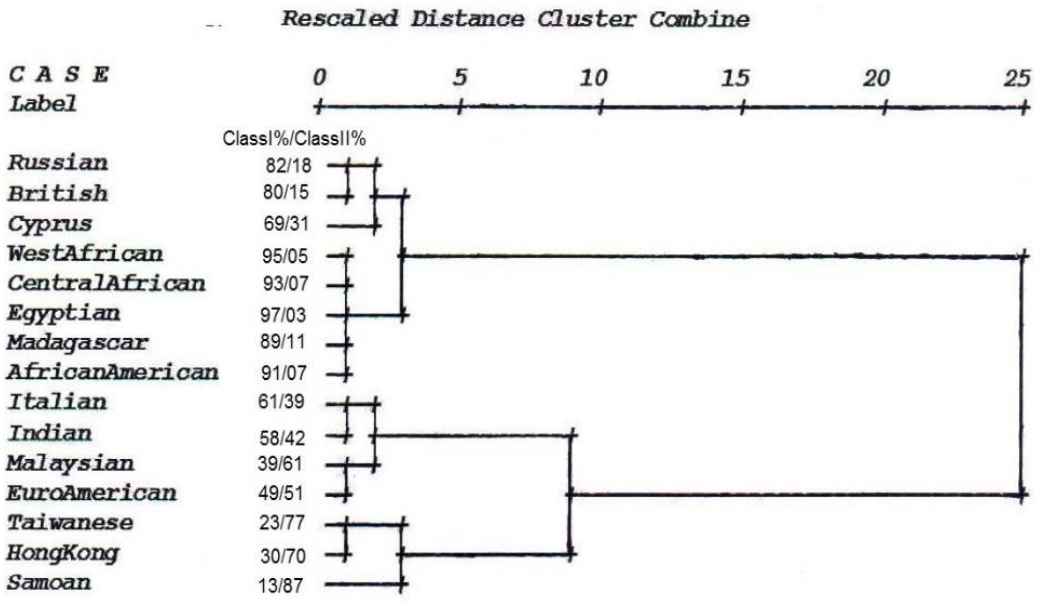

Table 2: Comparison of the allelic frequencies of the leptin gene polymorphism among total cases of obesity, metabolic syndrome, simple obesity and healthy controls

Alleles (bp)	Healthy controls n=166(%)	Cases of obesity n=240(%)	Metabolic syndrome n=114(%)	Simple obesity n=126(%)
148	3(1.8)	10 (4.2)	6 (5.26)	4 (3.2)
154	153(92.2)	138 (57.5)	66 (57.9)	72(57.1)
160	0(0.0)	4(1.7)	0 (0.0)	4(3.2)
164	0(0.0)	4(1.7)	2 (1.75)	2(1.6)
170	2(1.2)	9(3.8)	8 (7.0)	1(0.8)
200	3(1.8)	10(4.2)	5 (4.3)	5(4.0)
204	0(0.0)	2(0.8)	2 (1.75)	0(0.0)
220	0(0.0)	1(0.4)	0 (0.0)	1(0.8)
228	0(0.0)	4(1.7)	2 (1.75)	2(1.6)
230	0(0.0)	6(2.5)	3 (2.63)	3(2.4)
240	0(0.0)	45(18.8)	16 (14.0)	29(23.0)
250	5(3.1)	5(2.08)	3 (2.63)	2(1.6)
280	0(0.0)	2(0.8)	1 (0.9)	1(0.8)
Class I	161(97)	177 (73.7)	89 (77.96)	88(69.9)
Class II	5 (3.1)	63(26.3)	25 (21.9)	38(30.2)

Class 1*: <217 bp*, Class 2: ≥ *217bp*

Table 3: Comparison of the genotype distribution of the leptin gene polymorphism, homozygote and heterozygote genotypes among total cases of obesity, metabolic syndrome, simple obesity and healthy controls

	Genotype			
	I/I	**I/II**	**II/II**	**I/II +II/II**
	n(%)	n(%)	n(%)	n(%)
Controls	78 (94.0)	5 (6.0)	0 (0.0)	5 (6.0)
Total cases	62 (51.7)	51 (42.5)	7 (5.8)	58 (48.3)
Metabolic syndrome (MS)	32 (56.1)	23 (40.4)	2 (3.5)	25 (43.9)
Simple obesity (SO)	30 (47.6)	28 (44.4)	5 (7.9)	33 (52.3)
Total cases vs Controls				
P	Ref	<0.0001**	0.004*	<0.0001**
OR (95% CI)		12.8(4.8-34.1)	NA	14.6(5.5-38.6)
MS vs Controls				
P	Ref	<0.0001**	0.09	<0.0001**
OR (95% CI)		11.2 (3.9-32.1)	NA	12.9 (4.9-30.4)
SO vs Controls				
P	Ref	<0.0001**	0.002*	<0.0001**
OR (95% CI)		14.6 (5.1-41.2)	NA	17.2 (6.1-48.1)
SO vs MS				
P	Ref	0.6	0.4	0.2
OR (95% CI)		1.3(0.6-2.7)	2.7(0.5-14.8)	0.6 (0.3-1.2)

NA= not applicable, OR (95% CI) = odds ratio & 95% confidence interval, *p=< 0.05 (significant), ** p = <0.001 (extremely significant).

Discussion

This study was done to probe into the genetic background of obesity in Egyptian subjects. Family history of obesity was positive among approximately two thirds of the study sample, in addition to a positive parental consanguinity in about one fifth of obesity cases. These data surely point to the potential impact of genetics in the etiology of obesity among Egyptians. In a plan for a series of studies testing the association of biomarkers with obesity among Egyptians, we started with the highly polymorphic region of leptin gene 3' UTR repeat polymorphism. In fact, the effect of the 3' UTR polymorphism of the leptin gene on the expression of leptin was found to be controversial. So, while Shintani et al., and Porreca *et al*., have reported that there was no statistical significant difference in the leptin level found between hypertensive cases having the class I/I homozygous genotype and other genotypes, [32, 42] Akhter et al. have reported an association of serum leptin levels and hypertension with Class I/I and Class I/ II genotypes. [43]

In Egyptian cases with obesity, we could recognize 13 different allelic variants, of them the 154 bp allele was the most frequent. In cases with MS, 11 alleles were found, of which the 154 bp allele was also the most frequent. The predominant genotype was 154/154 in obese individuals with a frequency of 37% versus 88% in normal individuals. Total cases with obesity showed a significantly higher carriage rate of class II alleles compared to healthy controls. This was more apparent in the group of simple obesity than in cases of MS. However, the frequencies of leptin gene class genotypes did not show any difference between obese cases with MS syndrome and simple obesity. Similarly, serum lipids did not differ in obese cases with different class genotypes. In the study of Das *et al*., among Indian obese and hypertensive individuals the alleles at leptin locus were classified as class I (149-200 bp) and class II (> 217 bp) alleles. They reported that cases of obesity showed 14 different alleles. The predominant allele in obese individuals was the 152 bp allele with a frequency 0.257 compared to the 152 bp and 156 bp alleles in normal subjects with a frequency 0.243 each. The predominant genotype was 152/152 in obese subjects with a frequency of 0.114 compared to the genotype 152/156 in normal subjects with a frequency of 0.135. [41] Agreeing with our results, they have found that in obese individuals, the frequency of genotype II/II was significantly higher as compared to normal and hypertensive individuals, genotype I/II had a significantly higher frequency in obese group as well compared to normal and hypertensive group. [41] In another study done by Moffett *et al*., among African American subjects, they reported a wide heterogeneity of the leptin gene tetranucleotide repeat alleles being classified into three classes; type 1 alleles (146-178 bp); type 2 alleles (165 and 193 bp) and type 3 alleles (210 and 254 bp). They attributed the failure of some population-based studies to detect association between measures of obesity and the leptin gene-(TTTC) locus to be due to this wide heterogeneity. [31] Similarly, Shintani *et al*., in their study on Japanese subjects, recognized class I alleles as being ranged from 149 to 200 bp and class II alleles >217 bp. They found that even among normal controls there was a tendency for greater body weight and higher BMI value with the class I/I genotype. In addition, the frequency of the I/I genotype was significantly higher in patients with hypertension than in normotensive subjects but without a definite association with obesity. [30] On the contrary to our results, the study of Porreca *et al*., from Italy showed that both the distribution of the genotypes in the class I and class II and the frequency of individual alleles did not appear to be associated with BMI or hypertension. [43]

Analysis of the frequencies of leptin gene 3' UTR repeat polymorphism in normal Egyptians showed that it encompasses only 5 alleles. Interestingly, the number of alleles found in normal subjects differed too much in different populations with different ethnicities. For instance, it was 13 alleles in Indians, [41] 11 alleles in Costa Ricans, [39] 22 alleles in normal subjects from Madagascar, 11 in Russians and British, 18 in normal subjects from Hong Kong, 12 in Taiwanese, 12 in Malaysians, 14 in Central Africans, 17 in West Africans, [31] 14 alleles in normal Euro Americans, 9 alleles in Samoans and 19 alleles in African Americans. [40]

In our study, the most frequent allele found among normal Egyptians was the 154 bp allele (92.2%). Similarly, the 154 bp allele was found to be the most frequent one among normal subjects from Madagascar (31%), Russia (36%), Great Britain (36%), Central Africa (29%), West Africa (35%), Euro Americans (19%) and in African Americans (29%). [31-40] A nearly similar size alleles were also reported among normal Indians including the 152 bp and 156 bp alleles (24% each), [41] while in Malaysia it was the 157 bp allele (16%). [31] On the other hand, a bigger size allele (162 bp) was found to be the most frequent one among Costa Ricans (23%). [39] Interestingly, a much bigger size alleles were reported to be most frequent among normal subjects from South East Asia. For instance in normal subjects from Hong Kong, 3 alleles were most frequent : 226 bp, 230 bp and 234 bp alleles (15% each), while in Samoans, the 234 bp allele was the most frequent (27%) and in Taiwanese, the 230 bp allele was the most frequent one. [31,40] Applying the cluster analysis comparing frequencies of the class I and class II alleles in the Egyptian normal subjects to other populations showed that the Egyptian population was very near to African and Mediterranean countries but somewhat different from Asian and European countries.

So, we can come to the conclusion that the Egyptian population has a particular distribution of the allelic tetranucleotide polymorphisms of the leptin gene with a significant increase in the frequency of class II alleles among obese individuals compared to normal controls. Nonetheless, in spite of the presence of a positive association with the class II genoypte of this polymorphism with obesity, we should alert to the potential limitation of the study being just a relatively small pilot study with a limited sample size. Therefore we recommend carrying out another wider scale multicenter study of all relevant leptin and leptin receptor gene polymorphisms together with their expression profile in Egyptian obese subjects.

Conflict of interest

Authors declare absolute freedom from any issue pertinent to conflict of interest related to this work.

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