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Leptin gene microsatellite polymorphism: Relation to metabolic syndrome

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ABSTRACT

Background: Metabolic syndrome (MS) is a cluster of metabolic disorders. Despite its growing prevalence worldwide, there is a great debate regarding its definition, pathogenesis, and prognosis. Leptin gene (OB) gene polymorphism is a highly informative marker of obesity which is a main component of MS. Objective: to elucidate the degree of association of 3'tetranucleotide microsatellite polymorphism of Leptin (tet-LEP) gene with metabolic syndrome and its related disorders.
Materials and methods: The present study included 106 cases fulfilling all the criteria of MS and 134 age and gender matched control subjects. tet-LEP polymorphic region was amplified by using polymerase chain reaction of DNA extracted from peripheral blood samples. The products of reaction have distinguished two groups of alleles (group I= short alleles, and group II = long alleles). Metabolic parameters were estimated in blood serum.
Results: our data indicated the higher frequency of group I containing genotypes [I/I, I/II] in MS cases as compared to control group. Further analysis of the association of tet-LEP microsatellite polymorphism to each component of metabolic syndrome separately have shown significant association of group I alleles with high body mass index and increased risk of hypertension with odd ratio (5.7, 1.26) respectively but no association was noticed with glucose intolerance and dyslipidemia.
Conclusion: 3'tetranucleotide microsatellite polymorphism of LEP gene was found to be linked to occurrence of metabolic syndrome which could be through the effect on body weight.

1. Introduction

Metabolic syndrome (MS) is a group of metabolic abnormalities. The criteria proposed for the diagnosis of MS is easily applicable, and identifies patients with different combinations of hypertension, atherogenic dyslipidemia, impaired glucose homeostasis, and visceral obesity (Malik et al., 2004). The pathogenesis of MS has been associated with the effect of a genetic predisposition (Ford et al., 2002) in combination with environmental factors. Considering the central role of adipose tissue in MS, an adipocyte related leptin gene has been studied as plausible candidate gene.

Leptin is a hormone mainly produced by adipose tissue. It acts on both energy intake and expenditure to maintain relative stability of body weight and energy storage over long periods of time (Campfield et al., 1996). The human leptin (LEP) gene is located at chromosome 7q31.3 and consists of three exons and two introns spanning ~20 kb in length, encoding a 3.5 kb mRNA (Muy-Rivera et al., 2005).

Mutations in gene encoding leptin are reported to cause severe obesity in both animal models (Zhang et al., 1994) and humans (Montague et al., 1997; Strobel et al., 1998) indicating direct relation of leptin gene and obesity.

Shintani et al. (1996) have identified a highly variable tetranucleotide microsatellite repeat on the LEP 3′ flanking region (*tet-LEP*). Muy-Rivera et al. (2005) characterized the polymorphic alleles by size distribution, short repeats (group I allele 150–166 bp) and long repeats (group II allele 220–252 bp) based on TTTC repeat units. In fact, the effect of the 3′UTR polymorphism of the leptin gene on the expression of leptin was found to be controversial (Elbaz et al., 2015). The *tet-LEP polymorphism* has been also identified and linked to obesity related phenotypes (Ferrannini et al., 1987; Nagy et al., 2009).

The aim of the current study was to investigate the association of tetranucleotide microsatellite polymorphism of LEP gene (tet-LEP) with the incidence of metabolic syndrome in a sample of Egyptian population and to correlate the frequency of tet-LEP two groups of alleles to different components of MS in order to explore if there is any genetic background or interaction between them.

2. Material and Methods

The current study was performed between 2013 and 2015. The patients were selected from the out-patient clinics of the Internal Medicine Department, Faculty of Medicine, Ain Shams University. It

Abbreviations: bp, Base pair; BP, Blood pressure; DNA, Deoxy ribonucleic acid; EDTA, Ethylenediaminetetraacetic acid; HDL, High density lipoprotein; LDL, Low density lipoprotein; LEP, Leptin; MS, Metabolic syndrome; *tet-LEP*, Tetranucleotide microsatellite repeat on the LEP 3′ flanking region.

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Table 1
Demographic and clinicopathological characteristics of the study subjects.

Criteria (Mean ± SD)	Controls $n = 134$ Mean ± SD	Metabolic syndrome $n = 106$ Mean ± SD	Level of significance (p)
Age (years)	46.3 ± 7.4	47.8 ± 7.0	>0.05
Body mass index (kg/m^2)	23.2 ± 2.8	29.9 ± 4.6	<0.001
Waist circumference (cm)	75.1 ± 7.8	115 ± 8.9	<0.001
Fasting blood sugar (mg/dl)	86.4 ± 15.3	139.3 ± 53.0	<0.001
Total cholesterol(mg/dl)	171.8 ± 31.0	208.1 ± 65.3	<0.001
LDL-C (mg/dl)	94.6 ± 24.8	121 ± 45	<0.001
HDL-C (mg/dl)	50.1 ± 13.9	36.2 ± 7.3	<0.001
Triglycerides (mg/dl)	110.0 ± 76	179.8 ± 93	<0.001
Systolic pressure (mm Hg)	122.3 ± 12.5	148.3 ± 10.8	<0.01
Diastolic pressure (mm Hg)	80.3 ± 6.2	89.7 ± 5.6	<0.01
Gender:			
Males = n	76	63	NS
Female = n	58	43	

NS: nonsignificant, SD: standard deviation.

included 240 individuals: 106 cases of metabolic syndrome and 134 age and sex matched control subjects (Table 1).

Metabolic syndrome (MS) cases were diagnosed according to Alberti et al. (2009) with the presence of 3 or more of the following:

- Elevated serum triglycerides level: ≥150 mg/dl (1.7 mmol) or specific treatment for lipid abnormality.
- Reduced HDL cholesterol: <40 mg/dl in men and <50 mg/dl in women, or specific treatment for lipid abnormality.
- Elevated blood pressure: systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension.
- Elevated fasting plasma glucose: ≥100 mg/dl (5.6 mmol/l) or drug treatment of elevated blood sugar.
- Increased waist circumference: country and population specific definition. e.g. Middle East and Mediterranean ≥94 cm in men and ≥80 cm in women.

All participants signed an informed consent and were subjected to thorough history taking regarding age, medical history, current medication use and lifestyle. They were subjected to anthropometric, blood pressure measurements and venous blood withdrawal for further analysis.

2.1. Laboratory Tests

Venous blood samples were collected in fasting state and were divided into two tubes. One tube was with EDTA for DNA extraction. The other was a plain tube to separate serum for spectrophotometric estimation of biochemical parameters including: fasting blood sugars, triacylglycerols, total, low density lipoprotein, and high density lipoprotein cholesterol. Blood sugar and lipid fraction measurements were performed using routine enzymatic tests (Randox and Diasys kits respectively).

2.2. DNA Extraction and Genotyping of Leptin Gene Polymorphism

DNA was extracted from white blood cells using a DNA extraction and purification kit (Promega Corporation, USA) according to manufacturer's instructions and stored at −20 °C until it was processed. The DNA purity and concentration were determined spectrophotometrically at 260 and 280 nm.

According to Shintani et al. (1996) tetranucleotide repeat polymorphism in the 3′-flanking region of the human leptin gene was detected by PCR. Primer sequences were human OB forward (5′-AGTTCA AATA GAGGTCCAAATCA-3′) and human OB reverse (5′-TTCTGAGGTTGTGT CACTGGCA-3′). PCR contained 100 ng genomic DNA templates, 0.2 μmol/l of each primer, 2.0 mmol/l Mg^{2+}, 0.8 mmol/l of each dNTP, 1.5 U *Taq* polymerase (Norgen Biotek Corp., Canada) and reaction buffer. The PCR was performed for 30 cycles of 30 s at 94 °C, 1 min at 54 °C, and 1 min at 72 °C, with initial denaturation for 3 min at 94 °C and final extension for 10 min at 72 °C. The PCR products were run in 2.5% agarose gel. The amplified products were visualized by staining with ethidium bromide (Fig. 1).

2.3. Statistical Analysis

The allele and genotype frequencies were analyzed in relation to MS, biochemical parameters and anthropometric data. Statistical analysis was done with the SPSS software version 19.0 (SPSS, Inc., Chicago, IL). Student t-test was used to compare the numerical values. Difference in genotype prevalence and association between case and control group were assessed by the chi-square, odds ratio (OR) and 95% confidence interval (CI). They were used to describe the strength of association.

3. Results

The current case control study was performed on 106 patients with MS and 134 age and sex matched controls. The demographic and clinicopathological characteristics of cases of MS and controls are shown in Table 1. The mean values (±SD) of BMI, waist circumference, serum levels of TC, TG, LDL, fasting blood sugar and blood pressure measurement were significantly higher in cases of MS than in controls. HDL-C serum levels were significantly lower in cases than in control subjects.

Allelic analysis of the leptin gene polymorphism has shown increased risk of MS in individuals with group I alleles with odd ratio OR (95% CI) 1.4 (0.96–2.04). The risk was increased when in combined homozygous and heterozygous I alleles containing genotypes (I/I+I/II) with OR 1.6 (0.85–2.93), Table 2.

When analyzing the association of tet-LEP with individual components of metabolic syndrome as obesity, dyslipidemia, hypertension, and glucose intolerance level, we found significant association to body mass index (BMI) (Table 3). There was higher frequency of group I alleles in obese cases (85.1%) than those with normal BMI (50%) with

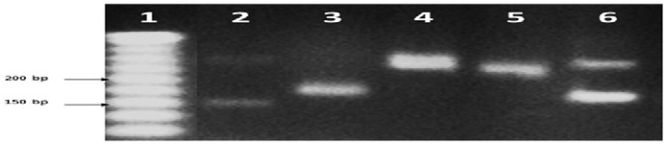

Fig. 1. DNA gel electrophoresis: lane 1: 25 bp DNA ladder marker, lane 3 shows class I/I alleles (150–166 bp). Lanes 4 and 5 show class II/II alleles (220–252 bp). Lanes 2 and 6 show heterozygous group I/II (≤166 bp/≥220 bp).