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Lys656Asn polymorphism of leptin receptor gene and metabolic syndrome in obese patients

D.A. DE LUIS, M. GONZALEZ SAGRADO, R. ALLER, O. IZAOLA, R. CONDE, M.J. CASTRO

Center of Investigation of Endocrinology and Clinical Nutrition, Medicine School and Unit of Investigation, Hospital Universitario Rio Hortega, University of Valladolid, Valladolid (Spain)

Abstract. – *Background:* **The etiology of common obesity is complex, because many genetic, environmental and metabolic factors might act. Alterations of the normal leptin receptor gene be involved in the development of obesity. The polymorphism on codon 656 produces a change in charge, making this change a possibility to be functional.**

Objective: **The aim of our study was to investigate the relationship between metabolic syndrome and Lys656Asn polymorphism in obese patients.**

Design: **A population of 714 obese patients (body mass index >30) was analyzed in cross-sectional survey. A bioimpedance, blood pressure, a serial assessment of nutritional intake with 3 days written food records and biochemical analysis were performed.**

Results: **Four hundred and seventy eight patients (66.9%) had the genotype Lys656/Lys 656 (wild group), whereas 236 (33.1%) had either the genotype Lys656/Asn656 (212 patients, 29.7%) or the genotype Asn656/Asn656 (24 patients, 3.4%) (mutant group). Prevalence of metabolic syndrome (MS) with ATP III definition was 49.4% (353 patients; 35.1% males and 64.9% females) and 50.6% patients without MS (n=361; 25.2% males and 75.8% females). Prevalence of leptin receptor (LEPR) genotypes was similar in patients with metabolic syndrome (65.5% wild genotype and 34.5% mutant genotype) and without metabolic syndrome (68.3% wild genotype and 31.7% mutant genotype). No differences in anthropometric and biochemical parameters were detected between genotypes in the same group of metabolic syndrome.**

Conclusion: **The finding of our study is the lack of association of the Lys656/Asn656 and Asn656/Asn656 genotypes with metabolic syndrome.**

Key Words:

Lys656Asn Lepr, Metabolic syndrome, Obesity.

Introduction

The factors associated with cardiovascular disease (CVD)[1] include high blood pressure, high triglyceride levels, hyperglycaemia, low high-density lipoprotein (HDL), and obesity. Many of these risk factors are the ones that make up the so-called syndrome X or metabolic syndrome (MS)[2,3]. One of the most accepted classifications for defining the metabolic syndrome is the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III)[4].

The etiology of common obesity is complex, because many genetic, environmental and metabolic factors might act. The role of leptin and its receptor in the development of obesity in the general population is unclear. Leptin is a hormone that is mainly produced by adipose tissue and binds to receptors in the hypothalamus[5]. The discovery of mutations in the leptin gene and its receptor in rodent models of obesity and in rare cases of morbid obesity patients indicates that leptin functions as an afferent signal in a negative feedback that regulates energy balancing[6]. This has been demonstrated in some severely obese humans who are homozygous for a mutation in the leptin receptor, who have very high leptin levels[7]. These mutations are extremely rare and cannot be responsible for obesity in the general population. Different polymorphisms in leptin receptor gene have been studied with unclear results[8]. The polymorphism on codon 656 produces a change in charge, making this change a possibility to be functional in this receptor. Leptin receptors have been identified in hypothalamic regions and in peripheral tissues such as pancreatic beta cells[9], muscle, adipose tissue and hepatocytes. Leptin has been shown to be able to modulate insulin secretion and action through these receptors.

The aim of our study was to investigate the relationship between metabolic syndrome and Lys656Asn polymorphism in obese patients.

Corresponding Author: Daniel A. de Luis, MD; e-mail: dadluis@yahoo.es

Subjects and Methods

Subjects

A population of 714 obese patients (body mass index >30) was analyzed in a cross-sectional survey. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the HURH [Hospital Universitario Rio Hortega]. Written informed consent was obtained from all patients. The recruitment of subjects was a non probabilistic method of sampling among patients send from Primary Care Physicians with obesity from a Nortwest area of Spain (Castilla y León). Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, malignant tumours or major surgery during the previous 6 months as well as the use of glucocorticoids, antineoplastic agents, and drinking and/or smoking habit.

Procedure

Weight, blood pressure, glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and citokines (leptin, adiponectin, resistin, interleukin-6, TNF-alpha) levels were measured at basal time. Genotype of LEPR gene polymorphism was studied. To estimate the prevalence of Metabolic Syndrome, the definitions of the ATPIII was considered[4]. The cutoff points for the criteria used are three or more of the following; central obesity (waist circumference >102 in males and >88 cm in females), hypertriglyceridemia (triglycerides >150 mg/dl or specific treatment), hypertension (systolic BP >130 mmHg or diastolic BP >85 mmHg or specific treatment) or fasting plasma glucose >100 mg/dl or drug treatment for elevated blood glucose.

Genotyping of LEPR Gene Polymorphism

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, Los Angeles, CA, USA). The polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 µL of each oligonucleotide primer (primer forward: 5'-GCA GTT CCT ATG AGA GGA CC-3'; primer reverse: 5'-AAA TTG GGA ATA CCT TCC AAA GT-3'), and 0.25 µL of each probes (wild probe: 5'-Fam-AGT GAC ATT TTT CTC CTT TTT CAT AGT ATC-Tamra-3') and (mutant probe: 5'-Hex-AGT GAC ATT TTT CTC GTT TTT CAT AGT AT- Tamra -3') in a 25 µL final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA, USA). DNA was denaturated at 95ºC for 3 min; this was followed by 50 cycles of denaturation at 95ºC for 15 s, and annealing at 59.3º for 45 s). The PCR were run in a 25 µL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA,USA) with hot start Taq DNA polymerase. Hardy- Weinberger equilibrium was assessed.

Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) with an interassay variation coefficient of less than 5% and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values[10]. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.24 ng/ml and a normal range of 8.65-21.43 ng/ml, interassay coefficients of variation were less than 10%. Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.20 ng/ml with a normal range of 4-12 ng/ml, interassay coefficients of variation were less than 10%. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml, interassay coefficients of variation were less than 15%. Interleukin 6 and TNF alpha were measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNF-alpha (0.5-15.6 pg/ml).

Anthropometric Measurements and Dietary intake

Body weight was measured to an accuracy of 0.05 Kg and body mass index computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Bipolar body electrical bioimpedance was used to determine body composition[11].

Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for three days including a week-end day. Food scales and models to enhance portion size accuracy were used. National composition food tables were used as reference[12]. Aerobic exercise was recorded in the same questionnaire.

Statistical Analysis

Sample size was calculated to detect differences over 45% of prevalence of metabolic syndrome with 90% power and 5% significance. The results were expressed as average ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-t test. Non-parametric variables were analyzed with the U-Mann Whitney test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. The statistical analysis was performed for the combined *Lys656/Asn656* and *Asn656/Asn656* as a mutant group and type *Lys656/Lys 656* as wild group. A p-value under 0.05 was considered statistically significant.

Results

Seven hundred and fourteen patients gave informed consent and were enrolled in the study. The mean age was 42.9±14.8 years and the mean BMI 36.1±6.1, with 215 males and 499 females.

Four hundred and seventy eight patients (66.9%) had the genotype *Lys656/Lys 656* (wild group), whereas 236 (33.1%) had either the genotype *Lys656/Asn656* (212 patients, 29.7%) or the genotype *Asn656/Asn656* (24 patients, 3.4%) (mutant group). Age was similar in both groups (wild type: 42.48±16.3 years vs mutant group: 43.1±16.2 years:ns). Sex distribution was similar in both groups (wild vs mutant type group), males (30.8% vs 28.8%) and females (69.2% vs 71.2%).

Prevalence of metabolic syndrome (MS) with ATP III definition was 49.4% (353 patients; 35.1% males and 64.9% females) and 50.6% patients without MS (n=361; 25.2% males and 75.8% females). Prevalence of LEPR genotypes was similar in patients with metabolic syndrome (65.5% wild genotype and 34.5% mutant genotype) and without metabolic syndrome (68.3% wild genotype and 31.7% mutant genotype). Prevalence of each criteria of metabolic syndrome was calculated in wild and mutant type genotypes, without statistical differences. Elevated waist circumference was detected in 91.4% patients with wild type genotype and 89.5% patients with mutant type genotype. Elevated levels of triglycerides or specific treatment were detected in 25.5% patients with wild type genotype and 25.8% patients with mutant type genotype. Elevated levels of blood pressure or specific treatment were detected in 60.0% patients with wild type genotype and 53.4% patients with mutant type genotype. Elevated levels of glucose or specific treatment were detected in 38.6% patients with wild type genotype and 32.4% patients with mutant type genotype.

Table I shows the subjects' differences in anthropometric and cardiovascular variables with and without metabolic syndrome (MS). Patients with MS had higher weight, BMI, waist circumference, waist to hip ratio, systolic and diastolic blood pressure, glucose, HOMA, insulin, total cholesterol, LDL cholesterol and triglycerides than patients without MS. Table II shows the subjects' levels of adipokines. Patients with MS had lower adiponectin levels than patients without MS.

Subject's nutritional intake was similar in both groups (MS vs no MS); calory (1946±668 vs. 1872±568 kcal/day), carbohydrate (199.1±84 vs. 187.1±65 g/day), fat (85.5±36 vs. 83.5±35 g/day), protein (91.7±26 vs. 91.8±28 g/day) and fiber intakes (15.22±6.5 vs.14.84±7.2 g/day). Hours of exercise per week were similar (1.71±3.1 vs. 1.40±2.9 hs./week), too.

Table III shows the subjects' differences in anthropometric and cardiovascular variables secondary to genotype in metabolic and no metabolic syndrome. Patients with MS, in both genotypes, had higher weight, BMI, waist circumference, waist to hip ratio, systolic and diastolic blood pressure, glucose, HOMA, insulin, total cholesterol, LDL cholesterol and triglycerides than patients without MS. No differences in these parameters were detected between genotypes in the same group of metabolic syndrome. No differences in dietary intakes or physical activity were detected in both genotypes in metabolic and no metabolic syndrome groups.

Table IV shows the subjects' levels of adipokines in both genotypes in metabolic and no metabolic syndrome. Patients with MS, in both genotypes had lower adiponectin levels than patients without MS. No differences in adipocytokines levels were detected between genotypes in the same group of metabolic syndrome.

Table I. Anthropometric and biochemical variables, metabolic syndrome vs no metabolic syndrome.

Characteristics	Metabolic syndrome (n = 353)	No metabolic syndrome (n = 361)
BMI	37.1 ± 6.1	35.0 ± 5.4*
Weight (kg)	98.2 ± 20.3	93.2 ± 17.2*
Fat mass (kg)	42.3 ± 14.8	39.3 ± 12.5*
WC (cm)	113.8 ± 14.9	106.9 ± 13.4*
Waist to hip ratio	0.95 ± 0.08	0.90 ± 0.08*
Systolic BP (mmHg)	136.2 ± 15.5	122.9 ± 12.9*
Diastolic BP (mmHg)	86.1 ± 10.3	78.2 ± 9.1 *
Glucose (mg/dl)	110.8 ± 31.5	91.4 ± 10.2*
Total ch. (mg/dl)	207.8 ± 38.5	195.9 ± 40.2*
LDL-ch. (mg/dl)	124.8 ± 38.5	116.5 ± 40.1*
HDL-ch. (mg/dl)	54.3 ± 25.1	56.8 ± 19.7
TG (mg/dl)	151.8 ± 81.6	100.2 ± 41.1*
Insulin (mUI/L)	20.4 ± 17.6	13.9 ± 8.3*
HOMA	5.74 ± 4.6	3.18 ± 2.1*

BMI: body mas index. Ch: Cholesterol. TG: Triglycerides HOMA: Homeostasis model assessment. WC: Waist circumference. *$p < 0.05$, between groups.

Discussion

The main finding of this study is the lack of association of the Lys656/Asn656 and Asn656/Asn656 genotypes with the metabolic syndrome.

A previous meta-analytic investigation of linkage and association of LEPR polymorphisms with some criteria of metabolic syndrome such as body mass index and waist circumference concluded that, although certain genotypic effects could be population-specific, there was no statistically evidence that any allele (Lys109Arg, Gln223Arg, Lys656Asn) is associated with BMI or waist circumference[13], as our study shows. Nevertheless, other studies have been detected an association between the polymorphism on codon 656 and the isolated metabolic syndrome' criteria. In premenopausal women with impaired glucose tolerance (IGT), associations were found with Lys656Asn for overall glucose response to the glucose load. In postmenopausal women with IGT, associations were found with Lys656Asn for fasting insulin, as well as in response to an oral glucose tolerance test (OGGT)[14]. In other study with nondiabetic obese patients[15], Lys656Asn genotype was associated with high levels of insulin and HOMA. Moreover, in diabetic obese patients, lys656Asn genotype was associated with high levels of HOMA, C reactive protein, leptin and fat mass[16]. Perhaps, these different results could be explained by inclusion criteria and heterogeneity of subjects in the designs of the literature and of our study and an uncontrolled dietary intake could influence on results, too. For example, an interventional study with two hypocaloric diets has shown a different metabolic response secondary to Lys656Asn genotype[17]. Exercise could be an other counfounding

Table II. Circulating adipocytokines, metabolic syndrome vs no metabolic syndrome.

Characteristics	Metabolic syndrome (n = 353)	No metabolic syndrome (n = 361)
IL 6 (pg/ml)	2.07 ± 2.8	2.44 ± 1.3
TNF-α (pg/ml)	5.51 ± 3.8	6.26 ± 4.1
Adiponectin (ng/ml)	27.83 ± 34.4	37.28 ± 41.8*
Resistin (ng/ml)	3.86 ± 1.75	3.95 ± 1.79
Leptin (ng/ml)	77.9 ± 79.1	89.2 ± 61.1

IL-6: interleukin 6. *$p < 0.05$, between groups.

Table III. Anthropometric and biochemical variables.

Characteristics	Metabolic syndrome		No metabolic syndrome	
	WT	MT	WT	MT
BMI	37.6 ± 6.8	36.9 ± 5.4	35.0 ± 5.3^{+}	34.9 ± 5.3^{+}
Weight (kg)	99.9 ± 20.3	97.9 ± 19.4	93.9 ± 17.1^{+}	92.8 ± 17.2^{+}
Fat mass (kg)	42.9 ± 15	42.1 ± 13.2	39.5 ± 12.1^{+}	38.9 ± 11.3^{+}
Waist circumference	114.6 ± 15.4	112.9 ± 13.9	107.4 ± 13^{+}	106.9 ± 12.8^{+}
Waist to hip ratio	0.94 ± 0.1	0.95 ± 0.07	0.90 ± 0.1	0.89 ± 0.1
Systolic BP (mmHg)	135.8 ± 15	136.7 ± 16.1	122.6 ± 32.1	123.3 ± 20.9
Diastolic BP (mmHg)	85.6 ± 10	86.9 ± 10.4	78.1 ± 8.5	78.5 ± 12.3
Glucose (mg/dl)	108.9 ± 28	114.2 ± 36.1	91.9 ± 10.1^{+}	90.5 ± 8.8^{+}
Total ch. (mg/dl)	207.2 ± 38	208.4 ± 46	195.8 ± 41	196.1 ± 41
LDL-ch. (mg/dl)	123.7 ± 38	127.7 ± 38	114.8 ± 40	120.8 ± 35
HDL-ch. (mg/dl)	54.7 ± 26.9	53.3 ± 21.1	56.6 ± 20.4	57.3 ± 17.8
TG (mg/dl)	153.8 ± 87	149.9 ± 68.3	100.8 ± 40.1^{+}	99.5 ± 50^{+}
Insulin (mUI/L)	19.8 ± 16.7	21.7 ± 19.2	13.7 ± 25^{+}	14.2 ± 9.7^{+}
HOMA	5.3 ± 4.6	6.5 ± 7.1	3.2 ± 1.7^{+}	3.1 ± 2.6^{+}

MS: metabolic syndrome. BMI: body mas index. Ch: Cholesterol. HOMA: Homeostasis model assessment. TG: Triglycerides. WC: Waist circumference. WT (wild type genotype Lys656/Lys 656) MT (mutant type genotype Lys656/Asn656 and Asn656/Asn656)*. $^{+}p < 0.05$, statistical differences between MS and no MS groups in different allele groups (Lys656/Lys 656 vs Lys656/Asn656 and Asn656/Asn656).No statistical differences between WT and MT in each allele group.

factor. Lakka et al[18] showed in white patients, that exercise increased sensitivity to insulin and decreased fasting glucose in the LEPR Asn65Asn homozygotes but did not influence glucose homoeostasis in Lys656Asn and Lys656Lys patients. Moreover, our study did not show differences in dietary intakes or physical activity between groups.

The cross sectional design of our study showed the lack of association of this polymorphism with the metabolic syndrome. Therefore, interaction between gene and environmental factors could influence in development metabolic syndrome in the following years of these patients. For example, two polymorphisms (Lys109Arg, Gln223Arg) in the extracellular domain of the leptin receptor predicted the conversion to type 2 diabetes patients from high-risk patients (longitudinal studies)[19].

The differences in anthropometric and metabolic parameters detected between patients with or without metabolic syndrome are the expected[2,3]. The low levels of adiponectin in patients with metabolic syndrome are expected, too. Adiponectin decreases lipid synthesis and glucose production in the liver and causes decreases in glucose and free

Table IV. Circulating adipocytokines.

Characteristics	Metabolic syndrome		No metabolic syndrome	
	WT	MT	WT	MT
IL 6 (pg/ml)	2.21 ± 3.3	1.82 ± 2.1	2.71 ± 1.3	2.07 ± 2.1
TNF-œ (pg/ml)	5.35 ± 3.5	5.77 ± 4.1	6.46 ± 3.9	5.91 ± 4.3
Adiponectin (ng/ml)	32.1 ± 46.2	20.6 ± 19.6	39.8 ± 34^{+}	33.8 ± 28^{+}
Resistin (ng/ml)	3.91 ± 1.8	3.72 ± 1.6	3.78 ± 1.5	4.31 ± 1.7
Leptin (ng/ml	77.1 ± 39	79.6 ± 38	80.5 ± 59	90.8 ± 88

MS: metabolic syndrome.WC: Waist circumference. WT (wild type genotype Lys656/Lys 656)MT (mutant type genotype Lys656/Asn656 and Asn656/Asn656)IL-6: interleukin 6. ^{+}p <0.05, statistical differences between MS and no MS groups in different allele groups (Lys656/Lys 656 vs Lys656/Asn656 and Asn656/Asn656).No statistical differences between WT and MT in each allele group.

fatty acid concentrations in the blood. In offspring of diabetes mellitus type 2 patients[20], adiponectin was associated with energy expenditure and low rats of lipid oxidation. Mantzmoros et al[21] have described a positive significant correlation of adiponectin levels with HDL-cholesterol and a negative with triglycerides concentrations

In conclusion, the finding of our study is the lack of association of the Lys656/Asn656 and Asn656/Asn656 genotypes with the metabolic syndrome.

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