Relationship of -55C/T Polymorphism of Uncoupling Protein 3 (UCP3) Gene with Metabolic Syndrome by ATP III Classification

Daniel Antonio de Luis,* Rocio Aller, Olatz Izaola, Manuel Gonzalez Sagrado, Rosa Conde, David Primo, Beatriz de la Fuente, Hilda F. Ovalle, and Marta Ruiz Mambrilla
Center of Investigation of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid, Spain

Background and aims: The relation of -55C/T polymorphism of uncoupling protein 3 (UCP3) with metabolic syndrome (MS) has been evaluated only in one previous study with contradictory results. The aim of our study was to investigate the association of -55C/T polymorphism of UCP3 gene with MS. **Design:** A population of 817 obese Caucasian patients was analyzed in a cross-sectional survey. Genotype of UCP3 gene -55C/T was studied. To estimate the prevalence of MS, the definitions of the ATP-III were considered. **Results:** Five hundred and ninety-four patients (72.7%) had the genotype -55CC (wild group), whereas 223 patients (27.3%) had the genotype -55C/T. Genotype -55TT was not detected. Prevalence of mutant UCP genotypes was similar

in patients with MS (75.7% wild genotype and 24.3% mutant genotype) and without MS (69.7% wild genotype and 30.3% mutant genotype). Odds ratio of MS wild vs. mutant genotype was 1.17 CI 95%: 0.99–1.38). Total cholesterol and low density lipoprotein (LDL) cholesterol concentrations were lower in mutant-type group than wild-type group in patients with MS. No differences in other parameters were detected between genotypes in the same group of MS. **Conclusion:** -55C/T UCP polymorphism is not major risk factor for the MS. However, in mutant group of -55CC UCP3 gene in patients with MS, total cholesterol and LDL cholesterol were lower than wild-type patients. J. Clin. Lab. Anal. 26:272–278, 2012. © 2012 Wiley Periodicals, Inc.

Key words: 55C/T polymorphism of UCP3 gene; metabolic syndrome; cardiovascular risk factors

INTRODUCTION

The metabolic syndrome (MS) is characterized by the clustering of several metabolic disorders (1) such as increased body weight, insulin resistance, elevated plasma triglyceride levels, low high density lipoprotein (HDL) cholesterol, high blood pressure, and altered glucose homeostasis. The prevalence of the MS ranges between 20% and 25% (2) and this prevalence increases with ageing (3). Environmental factors such as low physical activity and unhealthy diet are strong determinants of the MS. However, genetic factors may also influence the individual susceptibility to the MS. This is supported by the observation that metabolic disorders of the MS tend to cluster in families. For instance, 45–50% of first-degree relatives of type 2 diabetes patients are insulin resistant compared to 20% of individuals without a family history of diabetes (4). Moreover, it has been reported that heritability also

influences other components of the MS such as hypertension (5), cholesterol and triglyceride concentrations (6).

Some investigations have reported associations between the MS and polymorphisms [single nucleotide polymorphisms (SNPs)] in several different genes such as the interleukin 6 (IL-6) (7), angiotensinogen-1-converting enzyme (ACE) (8), low-density lipoprotein-related protein-

*Correspondence to: D. A. de Luis, Center of Investigation of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, C/Los perales 16, Simancas 47130, Valladolid, Spain. Email: dadluis@yahoo.es

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associated protein 1 (9), fatty acid binding protein (10), leptin receptor (11), and cannabinoid receptor (12).

The goal of the present study was to evaluate the possible association of the MS and SNP located in a gene-regulating key metabolic pathway, such as intramitochondrial energy transport (uncoupling protein UCP3) (promotor [-55C/T])(rs1800849). UCP3 belongs to a family of mitochondrial transporters that could uncouple the oxidative phosphorylation by increasing the proton leak of the inner mitochondrial membrane (13). Decreased expression or function of UCP3 could reduce energy expenditure and increase the storage of energy as fat (14). Some studies have pointed to a role of UCP3 in the regulation of whole-body energy homeostasis (15), diet induced obesity (16), and regulation of lipids as metabolic substrates (17). The C/C genotype of a polymorphism in the UCP3 promotor (-55C/T) is associated with increased expression of UCP3 mRNA in muscle of Pima Indians (18). Other authors have shown that T/T genotype was associated with an atherogenic lipid profile in French Caucasians and with a decreased risk of type 2 diabetes (19). Recently, a study has demonstrated an apparently lower risk of obesity in UCP3 -55C/T carriers (20). However, as far as we know, the relation of this polymorphism with MS has been evaluated only in one previous study with contradictory results (21).

The aim of our study was to investigate the association of -55C/T polymorphism of UCP3 gene with MS.

SUBJECTS AND METHODS

Subjects

A population of 817 obese Caucasian patients (body mass index > 30 kg/m²) was analyzed in a cross-sectional survey. Exclusion criteria included history of cardiovascular disease or stroke during the previous 12 months, malignant tumor or major surgery during the previous 6 months, as well as the use of glucocorticoids, antineoplastic agents, and drinking and/or smoking habit. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the HURH ethics committee. Written informed consent was obtained from all patients and signed.

Procedures

Weight, indirect calorimetry, blood pressure, fasting glucose, insulin, total cholesterol, low density lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides blood, and cytokines (leptin, adiponectine, IL-6, and tumor necrosis factor alpha [TNF-alpha]) levels were measured. To estimate the prevalence of MS, the definitions

of the ATPIII were considered (22). The cutoff points for the criteria used are three or more of the following; central obesity (waist circumference > 88 cm in women and > 102 cm in men), hypertriglyceridemia (triglycerides > 150 mg/dl or specific treatment), hypertension (systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg or specific treatment), or fasting plasma glucose > 110 mg/dl or drug treatment for elevated blood glucose. Genotype of UCP3 gene polymorphism was studied.

Genotyping of UCP3 Gene Polymorphism (rs1800849)

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, Los Angeles, CA). The polymerase chain reaction (PCR) was carried out with 250 ng of genomic DNA, 0.5 µl of each oligonucleotide primer (primer forward: 5'-GAT CTG GAA CTC ACT CAC CTC-3'; primer reverse: 5'-CTG TTG TCT CTG CTG CTT CT-3'), and 0.25 µl of each probes (wild probe: 5'-Fam-TAT ACA CAC GGG CTG ACC TGA-Tamra-3' and mutant probe: 5'-Hex-CTT ATA CAC ACA GGC TGA CCT GA-Tamra-3') in a 25 µl final volume (Termociclador iCycler IQ, Bio-Rad®, Hercules, CA). DNA was denaturated at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3°C for 45 s). The PCR was run in a 25 µl final volume containing 12.5 µl of IQTM Supermix (Bio-Rad®) with hot start Taq DNA polymerase. Hardy-Weinberg equilibrium was assessed.

Dietary Intake and Anthropometric Measurements

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 3 days including a weekend day. Food scales and models to enhance portion size accuracy were used. National composition food tables were used as reference (23). Aerobic exercise was recorded in the same questionnaire.

Body weight was measured to an accuracy of 0.5 kg and the body mass index was calculated according to the equation: body weight (kg) / height (m²). 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. Tetrapolar body electrical bioimpedance was used to determine body composition (Biodynamics Model 310e, Seattle, WA) (24). Blood pressure was measured twice after a 10-min rest with a random zero mercury sphygmomanometer, and averaged.

Biochemical Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyzer 2, Beckman Instruments, Fullerton, CA). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mUI/l (normal range 0.5–30 mUI/l) and the homeostasis model assessment (HOMA) for insulin sensitivity was calculated using these values (25). CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg/dl) and analytical sensitivity 0.5 mg/dl. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY), 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.

IL-6 and TNF- α were measured by ELISA (R&D systems, Inc., Minneapolis, MN) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal value of IL6 was (1.12–12.5 pg/ml) and TNF- α was (0.5–15.6 pg/ml). Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., TX) with a sensitivity of 0.05 ng/ml and a normal range of 10–100 ng/ml. Adiponectin was measured by ELISA (R&D systems, Inc., Minneapolis, MN) with a sensitivity of 0.246 ng/ml and a normal range of 865–21,424 ng/ml.

Indirect Calorimetry

For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12-h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20-min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure, and moisture) fitted with a face mask (MedGem; Health Tech, Golden, CO), coefficient of variation 5%. Resting metabolic rate (kcal/day) and resting metabolic rate corrected by fat-free mass (kcal/kg/day) were calculated (26).

Statistical Analysis

Sample size was calculated to detect differences over 45% of prevalence of MS with 90% power and 5% significance. The results were expressed as mean $\pm$ standard deviation. The distribution of variables was analyzed with Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed Student's *t*-test. Nonparametric variables were analyzed with the Mann–Whitney *U* test. Qualitative variables were an-

alyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. Bonferroni correction for the multiple different comparisons was applied. The statistical analysis was performed for the combined -55C/T and -55TT as a group and wild-type (WT) -55CC as second group, with a dominant model. A *P*-value under 0.05 was considered statistically significant.

RESULTS

Eight hundred and seventeen patients gave informed consent and were enrolled in the study. The mean age was 43.8 ± 12.8 years and the mean BMI 36.4 ± 6.2 , with 474 females and 343 males.

Five hundred and ninety-four patients (72.7%) had the genotype -55CC (wild group), whereas 223 patients (27.3%) had the genotype -55C/T. Genotype -55TT was not detected. Age was similar in both groups (WT: 43.9 ± 12.3 years vs. mutant group: 43.1 ± 12.2 years: ns). Sex distribution was similar in both groups (WT vs. mutant-type [MT] group), males (41.4% vs. 42.1%) and females (58.6% vs. 57.9%).

Prevalence of MS with ATP III definition was 49.9% (408 patients; 41.5% males and 59.5% females) and 50.1% patients without MS ($n = 409$; 42.0% males and 58.0% females). Prevalence of mutant UCP genotypes was similar in patients with MS (75.7% wild genotype and 24.3% mutant genotype) and without MS (69.7% wild genotype and 30.3% mutant genotype). Odds ratio of MS wild vs. mutant genotype was 1.17 CI 95%: 0.99–1.38). Prevalence of each criteria of MS was calculated in WT and MT genotypes, without statistical differences. Elevated waist circumference was detected in 91.9% patients with WT genotype and 98.6% patients with MT genotype. Elevated levels of triglycerides or specific treatment were detected in 25.1% patients with WT genotype and 26% patients with MT genotype. Elevated levels of blood pressure or specific treatment were detected in 59.9% patients with WT genotype and 55.2% patients with MT genotype. Elevated levels of glucose or specific treatment were detected in 36.1% patients with WT genotype and 38.1% patients with MT genotype.

Table 1 shows the subjects differences in anthropometric and cardiovascular variables with and without MS (MS). Patients with MS had higher weight, BMI, waist circumference, WHR, systolic and diastolic blood pressure, glucose, HOMA, insulin, total cholesterol, LDL cholesterol, and triglycerides than patients without MS. No differences were detected in resting metabolic rate and HDL cholesterol. Table 2 shows the subjects' levels of adipokines (intelerkine-6, TNF- α , leptin, and adiponectin). Patients with MS had lower adiponectin levels than patients without MS.

TABLE 1. Anthropometric and Biochemical Parameters Levels in Patients with Metabolic Syndrome vs. No Metabolic Syndrome

Characteristics	Metabolic syndrome (n = 409)	No metabolic syndrome (n = 408)
BMI	37.3 ± 6.2	35.3 ± 5.4*
Weight (kg)	98.5 ± 19.3	93.9 ± 17.4*
Fat mass (kg)	41.9 ± 14.2	39.4 ± 12.1*
WC (cm)	114.5 ± 14.3	107.7 ± 13.2*
Waist to hip ratio	0.94 ± 0.08	0.90 ± 0.06*
Systolic BP (mmHg)	135.9 ± 15.4	122.8 ± 13.3*
Diastolic BP (mmHg)	86.0 ± 9.7	78.5 ± 9.9*
RMR (kcal/day)	20,710 ± 658	2,105 ± 609
Glucose (mg/dl)	109.5 ± 27.6	91.8 ± 10.8*
Total ch. (mg/dl)	206.5 ± 38.6	196.6 ± 39.9*
LDL ch. (mg/dl)	126.2 ± 38.3	118.6 ± 39.3*
HDL ch. (mg/dl)	53.7 ± 22.9	55.8 ± 18.6
TG (mg/dl)	147.6 ± 79.2	100.9 ± 41.1*
Insulin (mUI/L)	18.6 ± 15.6	13.7 ± 8.1*
HOMA	5.39 ± 5.0	3.10 ± 1.9*

BMI, body mass index; ch, cholesterol; TG, triglycerides; HOMA, homeostasis model assessment; WC, waist circumference; RMR, resting metabolic rate.

*P < 0.05, between groups.

TABLE 2. Serum Adipocytokines Levels in Patients with Metabolic Syndrome vs. No Metabolic Syndrome

Characteristics	Metabolic syndrome (n = 409)	No metabolic syndrome (n = 408)
IL 6 (pg/ml)	2.20 ± 3.10	2.42 ± 9.91
TNF-alpha (pg/ml)	5.47 ± 3.72	6.24 ± 4.12
Adiponectin (ng/ml)	24.3 ± 49.4	33.7 ± 46.8*
Leptin (ng/ml)	71.3 ± 78.1	79.3 ± 68.1

IL-6, interleukin 6; TNF-alpha, tumor necrosis factor alpha.

*P < 0.05, between groups.

Subject's nutritional intake was similar in both groups (MS vs. no MS); calories (1,943 ± 650 kcal/day vs. 1,912 ± 762 kcal/day), carbohydrates (199.8 ± 82 g/day vs. 188.2 ± 82 g/day), fats (84.8 ± 37 g/day vs. 84.7 ± 44 g/day), proteins (90.8 ± 26 g/day vs. 91.6 ± 36 g/day), and fiber intakes (15.26 ± 6.5 g/day vs. 15.79 ± 8.9 g/day). Hours of exercise per week were similar (1.78 ± 2.8 h/week vs. 1.53 ± 2.8 h/week), too.

Table 3 shows the subjects differences in anthropometric and cardiovascular variables secondary to genotype in metabolic and no MS groups. Patients with MS, in both genotypes, had higher weight, BMI, fat mass, waist circumference, systolic and diastolic blood pressure, glucose, HOMA, insulin, and triglycerides than patients without MS. Total cholesterol and LDL cholesterol concentrations were lower in MT group than WT group in patients with MS. No differences in other parameters were detected between genotypes in the same group of MS. No

differences in dietary intakes or physical activity were detected in both genotypes in metabolic and no MS groups.

Table 4 shows the subject's levels of adipokines in patients with both genotypes in metabolic and no MS. Patients with MS, in both genotypes, had lower adiponectin levels than patients without MS. No differences in IL-6, leptin, adiponectin, TNF-alpha levels were detected between genotypes in the same group of MS.

DISCUSSION

Numerous investigations have studied the associations between polymorphisms and the different components of MS. However, few have investigated this association with MS as an entity. Our study suggests that the -55C/T UCP polymorphism is not major risk factor for the MS. However, in mutant group of -55C/T polymorphism of UCP3 gene in patients with MS, total cholesterol and LDL cholesterol concentrations were lower than WT patients.

UCP3 is a mitochondrial membrane transporter mainly expressed in skeletal muscle. The ubiquitous expression of UCPs and the expression of UCP3 in skeletal muscle made UCP3 attractive targets for studies on obesity patients and its relation with cardiovascular risk factors, adipocytokines, and anthropometric parameters (27).

Genetic polymorphisms in UCP genes have been variably associated with obesity-related phenotypes. Dalggaard et al. (28) showed that the frequency of the T allele was 26% among obese draftees and 26.9% in the control group. Our group had similar prevalence than this, with 24.3 in patients with MS and 30.3 in patients without MS. In other studies, no difference in genotype frequencies was observed between obese and lean subjects in a French cohort (19), too. However, Liu et al. (29) found statistically association and linkage between -55C/T and BMI. Furthermore, Otabe et al. (30) have been demonstrated that BMI was higher in TT than CC and CT patients. Moreover, other study has demonstrated an apparently lower risk of obesity in UCP3 -55C/T carriers (20), this inverse association may only occur in people with a high level of physical activity. As we can see, it is therefore unclear that the -55C/T variant has an effect on BMI.

In our Caucasian population, total cholesterol and LDL cholesterol levels were lower in mutant group (T carriers) in patients with MS; this is a novel result in the literature without a clear explanation. In the literature, different metabolic phenotype between wild and mutant groups of -55C/T SNPs has been observed in obese patients (31, 32). A hypothesis is that the presence of mutant allele of UCP3 could produce a more beneficial lipid state, for instance in skeletal muscle adipose tissue may modify the rate of consumption of

TABLE 3. Anthropometric and Biochemical Variables

Characteristics	Metabolic syndrome		No metabolic syndrome	
	WT	MT	WT	MT
BMI	37.2 ± 6.3	37.4 ± 6.1	35.3 ± 5.4 ⁺	34.9 ± 5.5 ⁺
Weight (kg)	98.2 ± 19.6	98.4 ± 17.8	93.7 ± 17.4 ⁺	94.2 ± 16.8 ⁺
Fat mass (kg)	41.7 ± 13.9	43.6 ± 14.6	38.5 ± 12.1 ⁺	41.3 ± 14.4 ⁺
Waist circumference	114.9 ± 14.7	113.0 ± 13.8	107.4 ± 13 ⁺	106.9 ± 11.8 ⁺
Waist to hip ratio	0.95 ± 0.1	0.93 ± 0.07	0.90 ± 0.08	0.91 ± 0.1
Systolic BP (mmHg)	135.1 ± 15	137.7 ± 16.0	123.3 ± 14.1 ⁺	122.2 ± 19.9
Diastolic BP (mmHg)	86.5 ± 9.9	84.1 ± 9.7	78.3 ± 10.5 ⁺	78.1 ± 9.3 ⁺
RMR (kcal/day)	2,039 ± 558	2,153 ± 439	2,055 ± 651	2,147 ± 715
Glucose (mg/dl)	111.2 ± 31.0	106.0 ± 18.7	91.4 ± 10.4 ⁺	92.4 ± 10.1 ⁺
Total ch. (mg/dl)	209.2 ± 39	198.5 ± 38.4 [*]	197.2 ± 41.1	194.6 ± 36.9
LDL ch. (mg/dl)	127.9 ± 38.2	119.5 ± 33.1 [*]	116.6 ± 41	121.5 ± 34.8
HDL ch. (mg/dl)	54.1 ± 24.9	53.6 ± 21.9	56.8 ± 18.4	53.6 ± 19.8
TG (mg/dl)	152.5 ± 84	135.1 ± 65.3	98.1 ± 37.4 ⁺	106.4 ± 48.2 ⁺
Insulin (mUI/L)	19.0 ± 16.3	18.1 ± 15.9	13.8 ± 7.6 ⁺	13.7 ± 9.6 ⁺
HOMA	5.5 ± 5.2	5.2 ± 5.4	3.0 ± 1.7 ⁺	3.2 ± 2.7 ⁺

MS, metabolic syndrome; BMI, body mass index; ch, cholesterol; HOMA, homeostasis model assessment; TG, triglycerides; WC, waist circumference; RMR, resting metabolic rate; WT, wild-type genotype -55CC; MT, mutant-type genotype -55C/T

(*). (+) $P < 0.05$, statistical differences between MS and no MS groups in different allele groups (-55CC vs. -55C/T and -55TT).

fatty acids. However, the relationship between this polymorphism and lipid profile is unclear and complex, as Meirhaeghe et al. (32) stated. These authors observed a worse lipid profile in T/T patients than C/C and C/T patients.

These contradictory results from literature could be explained by limitations in these studies. For example, the first limitation of our study and other studies is the cross-sectional design, this type of study is unable to achieve causality or temporality. Second, criteria for recruitment were different in these studies, thus the differences in confounding factors such as age, sex, lifestyle, presence of diabetes mellitus. Third, genetic background with other different genetic single nucleotide polymorphisms in the UCP gene (33) could influence UCP interaction with metabolic parameters. Fourth, dietary intake could influence previous results. In our study, dietary intake did not show statistical differences between

groups, in other studies dietary interventions have shown different responses in both genotypes (34, 35) and in others dietary intake was not controlled (19, 28–30). Finally, the lack of haplotype-based analyses (UCP2-UCP3) of our study cannot exclude the role of these haplotypes in our findings, this hypothesis has been demonstrated by other authors in a study with children and adolescents (36). Finally, the lack of TT genotype and the association with metabolic rate of this polymorphism could be bias factors to influence our results.

In conclusion, our results suggest that the -55C/T UCP polymorphism is not a major risk factor for the MS. However, in mutant group of -55C/T UCP3 gene in patients with MS, total cholesterol and LDL cholesterol concentrations were lower than WT patients. Further studies are needed to study this polymorphism and its clinical implications.

TABLE 4. Circulating Adipocytokines

Characteristics	Metabolic syndrome		No metabolic syndrome	
	WT	MT	WT	MT
IL 6 (pg/ml)	1.84 ± 2.4	2.37 ± 2.1	2.51 ± 1.2	1.97 ± 2.0
TNF-alpha (pg/ml)	5.43 ± 3.5	5.61 ± 4.7	6.16 ± 3.8	6.57 ± 4.8
Adiponectin (ng/ml)	22.6 ± 36.2	25.7 ± 18.6	31.6 ± 50.1 ⁺	35.7 ± 53.1 ⁺
Leptin (ng/ml)	73.1 ± 59.2	70.6 ± 73.3	80.7 ± 69.2	74.4 ± 68.1

MS, metabolic syndrome; BMI, body mass index; ch, cholesterol; HOMA, homeostasis model assessment; TG, triglycerides; WC, waist circumference; WT, wild-type genotype -55CC; MT, mutant-type genotype -55C/T

(*). (+) $P < 0.05$, statistical differences between MS and no MS groups in different allele groups (-55CC vs. -55C/T). No statistical differences between WT and MT in each allele group.

Conflict of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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