

Interaction of -55CT polymorphism of UCP3 gene with Trp64Arg polymorphism of beta3adrenoreceptor gene on insulin resistance in obese patients

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Abstract. – Background: The aim of our study was to investigate the interaction of tryptophan-to-arginine (Trp64Arg) missense mutation in the beta3 adrenoreceptor (Beta3AR) with polymorphism in the UCP3 promotor (-55C->T) on insulin resistance in obese patients.

Design: A population of 212 obese patients was analyzed. A bipolar electrical bioimpedance, a biochemical analysis and concentrations of adipocytokines were assessed.

Results: One hundred and sixty-two patients (76.4%) had the genotype Trp64/Trp64 (wild type group) and 50 patients Trp64/Arg64 (23.6%) (mutant type group). One hundred and seventy five (87.2%) had the genotype -55CC (wild type group) and 27 patients (22.8%) -55CT (mutant type group). Five patients (2.4%) had both polymorphisms Trp64/Arg64 and -55CT. Patients with one or both mutant genotypes had higher BMI, weight, fat mass, systolic blood pressure and waist circumference than wild type patients. Patients with 55CT or 55CT and Trp64Arg genotype had higher BMI, weight, fat mass, waist circumference, waist to hip ratio glucose, insulin, triglycerides and HOMA than wild type or Trp64Arg mutation.

Conclusion: Higher concentrations of insulin, HOMA, triglycerides, glucose, BMI, weight, fat mass, waist to hip ratio and waist circumference were observed in patients with -55CT genotype alone or -55CT plus Trp64Arg genotypes than in patients without mutation or only Trp64Arg mutation.

Key Words:

Adipocytokines, Cardiovascular risk factor, -55CT UCP3, Trp64Arg beta 3 adrenoreceptor, Obesity.

couple the oxidative phosphorylation by increasing the proton leak of the inner mitochondrial membrane¹. Decreased expression or function of UCP3 could reduce energy expenditure (EE) and increase the storage of energy as fat². Some studies have pointed to a role of UCP3 in the regulation of whole body energy homeostasis³, diet induced obesity⁴, and regulation of lipids as metabolic substrates⁵. The C/C genotype of a polymorphism in the UCP3 promotor (-55C->T) is associated with the increased expression of UCP3 mRNA in muscle of Pima Indians⁶. Other Authors have reported that the -55 T/T genotype is associated with increased BMI and interacts with physical activity⁷. It was shown that T/T genotype was associated with an atherogenic lipid profile in French Caucasians and with a decreased risk of type 2 diabetes⁸. Recently, a study realized in our country (North Area) has demonstrated an apparently lower risk of obesity in UCP3 -55 C/T carriers⁹.

Other candidate gene for obesity and related metabolic disorders is beta 3 adrenoreceptor (Beta3AR). A variant of this gene is the tryptophan-to-arginine (Trp64Arg) missense mutation in the beta3 adrenoreceptor. Beta3-AR is the principle mediator of catecholamine-stimulated thermogenesis and lipolysis, which mainly occurs at subcutaneous and visceral sites¹⁰. Trp64Arg variant in this receptor has been reported to be associated with the increased body weight and the insulin resistance¹¹. Only one report has evaluated the interaction between UCP gene and Beta 3 adrenoreceptor gene polymorphisms¹². However, the polymorphism evaluated of UCP gene was a mutation (A to G) in UCP-1 protein, this last protein is less ubiquitous than UCP-3.

Introduction

Uncoupling protein 3 (UCP3) belongs to a family of mitochondrial transporters that could un-

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Subjects and Methods

Subjects

A population of 212 obesity (body mass index >30) non diabetic outpatients was analyzed in a prospective way. These patients were studied in a Nutrition Clinic Unit. This work was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human patients were approved by the local Ethics Committee (HURH2009). Written informed consent was obtained from all patients. Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol >300 mg/dl, triglycerides >400 mg/dl, blood pressure >140/90 mmHg well as the use of sulphonylurea, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit.

Procedure

Weight, basal serum glucose, C-reactive protein (CRP), insulin, HOMA, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood, leptin, adiponectin, resistin, interleukin-6 and TNF alpha levels were measured. Genotypes of beta3 adrenoreceptor (Beta3AR) and UCP3 promoter gene were studied.

Genotyping of UCP-3 Promotor and Beta3AR Gene Polymorphisms

Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA, USA). The polymerase chain reaction (PCR) of UCP-3 promoter gene was carried out with 250 ng of genomic DNA, 0.5 μ L 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 μ L 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 μ L final volume (Termociclador iCycler IQ (Bio-Rad®, Hercules, CA, USA). The polymerase chain reaction (PCR) of Beta3AR was carried out with 250 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'-CAA CCT GCT GGT CAT CGT-3'; primer reverse: 5'-AGG TCG GCT GCG GC-3'), and 0.25 μ L of each probes (wild probe: 5'-Fam-CCA TCG CCT GGA CTC CG-BHQ-1-3') and (mutant probe: 5'-Hex-CAT CGC CCG GAC TCC G- BHQ-1-3') in a 25 μ L final volume (Termociclador iCycler IQ (Bio-Rad®, Hercules, CA, USA). 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° for 45 s). 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° for 45 s). The PCR were run in a 25 uL final volume containing 12.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase. Hardy- Weimberger equilibrium was assessed.

Assays

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.

Serum glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Serum insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values¹³. CRP was measured by immunoturbimetry (Roche Diagnostcis GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensivity 0.5 mg/dl.

Adipocytokines

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml. 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. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml.

Anthropometric Measurements

Body weight was measured to an accuracy of 0.5 kg and body mass index computed as body weight/(height²). 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 composition¹⁴. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Indirect Calorimetry

For the measurement of resting EE, subjects were admitted to a metabolic ward. After a 12 h overnight fast, resting metabolic rate (RMR) 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, Los Angeles, CA, USA), coefficient of variation 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated.

Dietary Intake and Habits

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 weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference. Regular aerobic physical activity (walking was allowed, no other exercises) was maintained during the period study (2-3 hours per week).

Statistical Analysis

Sample size was calculated to detect differences over 10% in insulin resistance with 90% power and 5% significance (n=200). The results were expressed as average \pm 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 and ANOVA test (Bonferroni test as post-hoc test). Non-parametric variables were analyzed with the W-Wilcoxon test and K-Kruskal test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A *p*-value under 0.05 was considered statistically significant.

Results

Two hundred and twelve patients gave informed consent and were enrolled in the study. The mean age was 44.8 \pm 16.7 years and the mean BMI 35.8 \pm 4.9, with 58 males (27.3%) and 154 females (72.7%).

All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.23 \pm 0.1 kg). One hundred and sixty-two patients (48 males/114 females) (76.4%) had the genotype *Trp64/Trp64* (wild type group) with an average age of 44.9 \pm 17 years and 50 patients (10 males/40 females) *Trp64/Arg64* (23.6%) (mutant type group) with an average age of 44.1 \pm 16.2 years.

One hundred and seventy five (53 males/132 females) (87.2%) had the genotype *55CC* (wild type group) with an average age of 44.8 \pm 13.2 years and 27 patients (5 male/22 females) (22.8%) *55CT* (mutant type group) with an average age of 44.5 \pm 13.1 years.

Five patients (2.4%) (1 male/4 females) had both polymorphisms *Trp64/Arg64* and *55CT* with an average age of 44.3 \pm 10.8 years. Mutant homozygous were not detected in both polymorphisms (*-55TT* or *Arg64Arg*).

Table I shows the differences in anthropometric variables. Patients with one or both mutant genotypes had higher BMI, weight, fat mass, systolic blood pressure, waist circumference and waist circumference than wild type patients. Patients with *55CT* or *55CT* and *Trp64Arg* genotypes had higher BMI, weight, fat mass, waist circumference and waist to hip ratio than wild type or *Trp64Arg* genotypes.

Table I. Anthropometric variables.

Characteristics	No mutation	Trp64/Arg64	55CT	55ct and trp64/arg64
BMI	34.7 ± 5.7	35.8 ± 4.8*	36.4 ± 6.5* ⁺	36.3 ± 8.5* ⁺
Weight (kg)	89.4 ± 14.8	94.1 ± 14.1*	96.9 ± 24.3* ⁺	96.5 ± 25* ⁺
Fat free mass (kg)	50.1 ± 13.3	51.6 ± 12.3	51.9 ± 19.5	51.7 ± 10.7
Fat mass (kg)	38.2 ± 11.1	40.2 ± 12.5*	42.7 ± 12.7* ⁺	43.8 ± 12.4* ⁺
Waist circumference	107.1 ± 13.4	107.9 ± 12.4*	114.4 ± 19* ⁺	113 ± 19.8* ⁺
Waist to hip ratio	0.90 ± 0.1	0.90 ± 0.09	0.95 ± 0.1* ⁺	0.96 ± 0.09* ⁺
Systolic BP (mmHg)	128.1 ± 11.1	142.4 ± 13.4*	138.7 ± 12.6*	140.2 ± 7.5*
Diastolic BP (mmHg)	81.5 ± 9.8	80.1 ± 16.8	82 ± 8.2	80.5 ± 2.8
RMR(kcal/day)	1820 ± 435	2161 ± 454	2179.5 ± 479	2056 ± 762
VO ₂ c.(ml/min.)	258.6 ± 65	303.1 ± 71.2	302.5 ± 60.2	299.1 ± 81

RMR: resting metabolic rate. O₂ c.: Oxygen consumption. **p* < 0.05, in each group with no mutation group. ⁺*p* < 0.05, in each group with Trp64/Arg64.

Table I shows the differences in cardiovascular risk factors. Patients with -55CT or both mutant genotypes had higher glucose, insulin, triglycerides and HOMA than patients with no mutation or Trp64Arg genotype.

Table III shows nutritional intake with 3 days written food records. No statistical differences were detected in calorie, carbohydrate, fat, and protein intakes.

Table IV shows levels of adipocytokines, without statistical differences.

Discussion

The main findings of this study are that the -55CT promoter polymorphism of the UCP-3

gene (alone or in combination with Trp64Arg) had higher effects on anthropometry and cardiovascular risk factors than Trp64Arg polymorphism of the Beta 3 adrenoreceptor gene. These differences may be related with higher concentrations of insulin, HOMA, triglycerides, glucose, BMI, weight, fat mass, waist to hip ratio and waist circumference in patients with 55CT genotype alone or 55CT plus Trp64Arg genotype than patients without mutation or only Trp64Arg mutation.

A previous meta-analysis assessing quantitative phenotypes in relation to a genetic polymorphism, and the results support the association of Trp64Arg polymorphism with BMI across diverse populations¹⁵, fat mass, waist circumference and other anthropometric parameters did not evaluate in this meta-analysis. The higher

Table II. Cardiovascular risk factors.

Characteristics	No mutation	Trp64/Arg64	55CT	55ct and trp64/arg64
Glucose (mg/dl)	99.7 ± 20.3	101.5 ± 13	106 ± 9.3* ⁺	108.9 ± 13.9* ⁺
Total ch. (mg/dl)	207.2 ± 46	201.9 ± 37	208.5 ± 39	203.7 ± 41
LDL-ch. (mg/dl)	127.2 ± 55	126.8 ± 76	126.6 ± 49	119.6 ± 44
HDL-ch. (mg/dl)	54.7 ± 12.8	55.3 ± 12.9	54.7 ± 33	54.4 ± 20
TG (mg/dl)	120.9 ± 55	123.7 ± 76.1	144.1 ± 44* ⁺	192 ± 55* ⁺
Lp (a) (mg/dl)	32.1 ± 39	38.3 ± 35	32.4 ± 18	34.3 ± 23
Insulin (mUI/L)	15.9 ± 6.9	17.1 ± 8.5	20.2 ± 12.7* ⁺	21.4 ± 12.4* ⁺
HOMA	4.2 ± 1.5	4.8 ± 1.5	5.6 ± 3.6* ⁺	5.3 ± 1.9* ⁺
CRP (mg/dl)	5.1 ± 5.2	6.7 ± 5.4	6.5 ± 4.9	6.6 ± 14

Chol: Cholesterol. Lp (a): lipoprotein a. TG: Triglycerides **p* < 0.05, in each group with no mutation group. ⁺*p* < 0.05, in each group with Trp64/Arg64.

Table III. Dietary intake.

Characteristics	No mutation	Trp64/Arg64	55CT	55ct and trp64/arg64
Energy (kcal/day)	1793 ± 632	1823 ± 379	1832 ± 344	1821 ± 775
CH (g/day)	181.7 ± 71	179.8 ± 53	190.9 ± 41	183 ± 78
Fat (g/day)	77.7 ± 23.3	79.5 ± 18.7	79.8 ± 21	81 ± 48
Protein (g/day)	88.2 ± 23	92.3 ± 21	85 ± 20	79.8 ± 21.3
Exercise (hs./week)	2.75 ± 1.5	2.4 ± 2.7	2.4 ± 2.3	2.5 ± 2.8

CH: Carbohydrate. No statistical differences.

visceral fat obesity detected in our study has been confirmed by other Authors with tomography scan in mutant patients, too^{16,17}. However, other meta analysis did not find evidence of this association¹⁸.

Our data did not show metabolic differences between wild and mutant type B 3 adrenoreceptor patients. Mice with knockout of the Beta 3 adrenoreceptor gene showed marked reductions in lipolysis stimulated by Beta 3 agonists¹⁹, and omental adipocyte Beta 3 adrenoreceptor sensitivity was related to waist hip ratio and insulin resistance²⁰. However, other Authors have been detected an inverse relation between visceral obesity in Trp64Arg mutation patients and serum triglycerides²¹. Perhaps, these unclear results in the literature²² may partially explain by differences in ethnic background, baseline BMI, gender distribution, previous weight loss, experimental design (early stage or late stage type 2 diabetes mellitus) and basal adipocytokines levels of participants. Therefore, interaction between gene and ambient could explain these differences with bias in previous studies.

The ubiquitous expression of UCP2, the expression of UCP3 in skeletal muscle, and their homology with UCP1 made UCP3 attractive targets for studies on obesity patients and its rela-

tion with biochemical and anthropometric parameters²³. UCP3 is involved in thermogenesis through the uncoupling of oxidative phosphorylation in skeletal muscle. Genetic polymorphisms in UCP genes have been variably associated with metabolic and obesity-related phenotypes. Dalggaard et al²⁴ tested whether variation of the UCP3 promoter is associated with juvenile or maturity onset obesity or body weight change over a 26 year follow up among Danish subjects. The frequency of C to T variant at -55 of the UCP3 gene was evaluated, frequency of the T allele was 26% among obese draftees and 26.9% in the control group. Our group had lower prevalence than this, with statistical differences in weight and other parameters between wild and mutant 55CT type group. These differences were maintained in patients with both mutant genotypes (-55CT and Trp64Arg).

In other studies, no differences in anthropometric parameters were observed between mutant and wild type genotypes of -55CT UCP-3 gene²⁵. However, Liu et al²⁶ found statistically association and linkage between -55CT and BMI, and subjects carrying the T allele had an average of 3.5% lower BMI than those without it. Furthermore, Otabe et al⁹ have been demonstrated that BMI was higher in TT than CC and CT pa-

Table IV. Circulating adipocytokines.

Characteristics	No mutation	Trp64/Arg64	55CT	55ct and trp64/arg64
Adiponectin (ng/ml)	40.7 ± 17	41.5 ± 11.2	35.9 ± 19	38.5 ± 24
Resistin (ng/ml)	3.8 ± 1.8	3.7 ± 1.5	3.6 ± 0.8	4.4 ± 0.6
TNF-alpha(pg/ml)	6.1 ± 3.6	5.9 ± 5.1	6.8 ± 0.8	6.9 ± 0.6
IL-6 (pg/ml)	2.6 ± 1.5	3.2 ± 3.6	3.2 ± 0.8	3.3 ± 0.6
Leptin (ng/ml)	103.2 ± 90.1	102.7 ± 40	103.2 ± 33	88.8 ± 75

No statistical differences.

tients. In the study by Casell et al²⁷, the waist to hip ratio was higher in females carrying the UCP3 gene -55CT polymorphism, but BMI was no different in both groups. Moreover, a study realized in our country (North Area) has demonstrated an apparently lower risk of obesity in UCP3 -55 C/T carriers⁹. 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, or body fat content.

In our population, insulin, triglyceride, HOMA and glucose levels were higher in mutant group (T carriers) with or without 64Arg carriers than no mutation or 64 Arg carriers alone. This is a novel result in the literature without a clear explanation. Perhaps, the presence of mutant allele of UCP could produce a more proinflammatory state, for instance in skeletal muscle and adipose tissue may modified the insulin resistance. For instance, Meirhaeghe et al²⁸ have detected that TT genotype had a worse lipid profile than subjects bearing wild or heterozygous genotypes.

In conclusion, higher concentrations of insulin, HOMA, triglycerides, glucose, BMI, weight, fat mass, waist to hip ratio and waist circumference in patients with 55CT genotype alone or 55CT genotype plus Trp64Arg genotype than patients without mutation or only Trp64Arg mutation. The results should be interpreted cautiously because of the limited number of subjects with both mutations.

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