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Association of *UCP-3*-rs1626521 with Obesity and Stomach Functions in Humans

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Abstract

Objective—To examine the association of gene variants of *uncoupling proteins (UCP)-2* and *-3* with obesity and gastrointestinal (GI) traits.

Methods—In 255 overweight or obese adults, we studied the associations of gene variants in *UCP-2* (−3474, rs659366) and *UCP-3* (rs1626521, rs2075577, rs15763) with body weight (BW) and GI traits. Gene variants were genotyped by TaqMan® assay. We assessed the associations of genotypes with BW and GI traits (gastric emptying, gastric volume, satiety by buffet meal,

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CONFLICTS OF INTEREST

All authors have no financial or personal relationships that could present a potential conflict of interest.

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A. Acosta: fellow co-investigator; analysis and interpretation of data; conduct of the study; drafting and writing and critical revision of the manuscript

M. Camilleri: study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript; funding from NIH

A. Shin: fellow co-investigator; conduct of the study; critical revision of the manuscript

M. Vazquez-Roque: fellow co-investigator; conduct of the study; critical revision of the manuscript

J. Iturrino: fellow co-investigator; conduct of the study; critical revision of the manuscript

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satiation by nutrient drink test and GI hormones) using ANCOVA, corrected for false detection rate (FDR).

Results—We identified a novel *UCP-3* gene variant, rs1626521; it was associated with BW (p=0.039), waist circumference (p=0.035), and with significantly higher postprandial gastric volume (p=0.003) and calories ingested at buffet meal (p=0.006, both significant with FDR). In a subgroup of 11 participants, rs1626521 was also associated with reduced mitochondrial bioenergetics efficiency in skeletal muscle (p=0.051). In an *in vitro* study in HEK293 cells, rs1626521 reduced UCP-3 protein expression (p=0.049). Associations detected between other genotypes and GI traits were non-significant with FDR.

Conclusions—A newly identified functional variant (rs1626521) in *UCP-3* affects postprandial gastric functions and satiety and may contribute to weight gain and alter human mitochondrial function.

Keywords

mitochondria; gastric emptying; accommodation; volume; satiation; satiety; GLP-1; PYY

INTRODUCTION

Obesity is a complex disease, with interactions between genetic predisposition, regulation of food intake and energy expenditure, food-seeking behavior, and the environment [1, 2]. Obesity has a strong association with type 2 diabetes mellitus (T2DM). The control of food intake, that is the size and frequency of meals, is a major factor in the determination of the individual's weight [3] . Gastrointestinal functions such as gastric emptying and capacity (volume) also influence food intake [4] and may, therefore, influence body weight.

There are standardized approaches to study eating behavior in humans [5], including satiation that results in the within-meal inhibition of eating, and satiety which reflects appetite ratings, appetite-related peptides and measures of calorie intake [3]. Quantitative traits facilitate the studies of the role of genetics in complex diseases [6]. Thus, it is considered that endophenotype-based genetic analysis may be more likely to identify susceptibility genes compared to clinical phenotype-based approaches [7]. In obesity, endophenotypes have been identified; recently, we reported that overweight and obese individuals (when compared to normal weight controls) have significant differences in gastrointestinal quantitative traits. These differences are: lower satiation manifested as higher Ensure® volume intake required to experience fullness; accelerated gastric emptying of liquids and solids [with increase of plasma glucagon-like peptide 1 (GLP-1) which may result from the accelerated gastric emptying]; increased fasting gastric volume; and decreased peak postprandial plasma peptide tyrosine-tyrosine (PYY) [4]. The observation of reduced peak postprandial PYY would not be expected given the acceleration of gastric emptying, and may be responsible for the failure to inhibit intake of food given the facts that peripheral administration of $PYY_{3–36}$ inhibited food intake in rodents and humans and that direct administration of $PYY_{3–36}$ into the arcuate nucleus of the hypothalamus inhibited food intake in rodents [8]. In addition, we noted that a higher caloric intake was required to experience satiety in those with abnormal waist circumference [4].

Uncoupling proteins (UCPs), a family of mitochondrial transporters, play a significant role in thermogenesis and energy utilization [9, 10]. UCP-2 is widely expressed in human tissues including the stomach [11, 12]. Subtypes of UCP mRNA and protein are regulated in a tissue- and subtype-specific fashion by leptin and food restriction [13]. The orexigenic hormone ghrelin changes hypothalamic mitochondrial function through UCP-2, activating neuropeptide Y (NPY)/ Agouti-related protein (AgRP) neurons [14], triggering synaptic plasticity of pro-opiomelanocortin (POMC)-expressing neurons, and inducing food intake.

UCP-3 is highly specific for skeletal muscle and brown adipose tissue [15] and may affect the processes of adaptive thermogenesis in humans [16], and fatty acid translocation [17]. However, the effect on thermogenesis is relatively minor. Recent studies suggest that the primary role of UCP-3 is in fatty acid metabolism and possibly in protecting the mitochondria against lipid-induced damage [10] by reducing mitochondrial production of reactive oxygen species (ROS) [18] and increasing the capacity to store energy as fat [19]. Activation of UCP-3 is indirectly regulated by norepinephrine and is dependent upon the availability of free fatty acids [FFAs [20]]. Impaired mitochondrial function in adipocytes may be linked directly to the development of metabolic diseases such as diabetes and insulin resistance [21], and mitochondrial genetic variants are associated with BMI in adults [22].

In epidemiological studies, genetic variation in *UCP* (as summarized in Table 1) has been associated with obesity. For example, there are several associations between genetic variations in *UCP*-2 and obesity, energy, and nerve functions. Since effects of ghrelin appear to be linked to UCP-2 [14], and ghrelin affects gastric motor functions, satiation and appetite [23], it is relevant to assess whether genetic variations in *UCP*-2 alter upper gastrointestinal function.

In a preliminary genotype-endophenotype study of 62 participants, we showed that variations in *UCP-2* and *UCP-3* were associated with gastrointestinal traits, especially with satiation measured in a nutrient drink test. In addition, we identified novel polymorphisms that were previously associated with obesity. These novel polymorphisms included *UCP-3* rs1626521, which is in high linkage disequilibrium with *UCP* genes previously associated with obesity [24]. The association of *UCP-3* rs1626521 with obesity (e.g. BMI, waist circumference) remains unproven.

Given the interactions of *UCPs* with obesity, our general objective was to assess whether genetic variations in *UCPs* associated with obesity influence gastric functions (e.g. emptying, volume or accommodation), satiation, satiety and selected gut hormones (ghrelin, cholecystokinin, GLP-1 and PYY).

Our hypothesis was that *UCP* genes (previously associated with obesity in epidemiological studies or obesity endophenotypes) are associated with specific quantitative traits of obesity. To explore this hypothesis, we *a priori* identified the pathophysiological mechanism(s) to be tested in association with each gene, based on the theoretically predicted mechanism targeted by that candidate gene.

Our specific aims were: first, to examine the association of the novel *UCP-3* gene variant, rs1626521, with obesity phenotype; second, to examine, in overweight and obese adults, the

associations of genetic variation at *UCP-2* and *UCP-3* with postprandial satiation, satiety, gastric emptying (GE), fasting gastric volume (GV), postprandial GV accommodation, and selected gut hormones. The selected genetic variants were based on prior literature (detailed in Table 1). Having identified significant genetic associations of these variants with obesity and quantitative gastrointestinal traits, we appraised the functional significance of the candidate gene in human tissues and in a model cell system.

METHODS AND PROCEDURES

Participants

We invited 1114 adults to participate in these studies; 288 fulfilled general criteria for screening (obese, non-bulimic, with no systemic disease that would interfere with the study aims). Among the 288 screened, we recruited 255 adults who fulfilled additional inclusion criteria (see below) and did not withdraw from participation (Appendix Figure 1). They were predominantly Caucasian overweight or obese participants as described elsewhere,[24]. A preliminary report of the results of the first 62 individuals who performed the study was published in a pilot study [24]. The main inclusion criteria were: 18–65 years of age, residence within 150 miles from Rochester, Minnesota, no systemic disease that could affect gastrointestinal motility, and not on current treatment for other diseases or that could potentially alter gastrointestinal functions, appetite, or absorption (e.g., orlistat). Weight stability for at least three months was required at the screening visit with the physician. Permissible medications were multivitamins, birth control pills, estrogen, and thyroxine replacement, all at stable doses for at least 30 days prior to the quantitative GI studies. Women of childbearing potential had a negative pregnancy test within 48 hours before any radioisotopes were administered. Participants completed behavioral and physical activity questionnaires described elsewhere[4].

Experimental Protocol

On three different days, participants presented to the Mayo Clinic Clinical Research Unit at 7:00 a.m. after an 8-hour fasting period and underwent one test on each study day: a dual-isotope (^{99m}Tc-egg and ^{111}In-skim milk, 315kcal meal) gastric emptying scintigraphic study of a mixed solid-liquid meal [4]; a nutrient drink satiation test [25]; and a gastric accommodation study by means of single photon emission computed tomography (SPECT) which involves i.v. injection of pertechnetate, $^{99m}TcO_4$ [26]. These methods are described in greater detail in the appendix. Gastric emptying and SPECT studies were performed at least 72 hours apart to avoid downscatter interference by ^{111}In (which has a half-life of >60 hours and has two emission peaks, one of which overlaps with ^{99m}Tc) from the meal ingested during the gastric emptying study with the measurement of gastric volume by ^{99m}Tc-SPECT. Studies of gastrointestinal traits were performed in the morning after an overnight fast, with exception of the *ad-libitum* buffet meal which was consumed at lunch time after a standard breakfast meal ingested during the SPECT study of gastric volumes.

Gastrointestinal Hormones

The gut hormones measured by radioimmunoassay were active ghrelin, total cholecystokinin (CCK), total GLP-1 and total PYY levels, as described in the appendix [4].

Determination of Genotypes

UCP genes variants were selected from previous preliminary studies [24]; a minor allele frequency of above 0.10 (or 10%) in the local population was used as a requisite for inclusion. Selected genes are described in Table 1.

DNA was extracted from whole blood as previously described [24]. Genotyping of all the SNPs were performed using Taqman® SNP Genotyping Assays (Applied Biosystems Inc., Foster City, CA, catalog # C_32667060_10) in accordance with the manufacturer's instructions (Taqman® SNP Genotyping Assays).

Mitochondrial Function Study in Human Skeletal Muscle

Through a concomitant protocol on mitochondrial function in muscle physiology based on open advertisement, all 255 participants in the GI quantitative trait study were eligible to enroll in the muscle physiology study. Eleven individuals participated in both protocols. Subjects consumed a standardized weight-maintenance diet (50% carbohydrate: 20% protein: 30% fat) for the three days preceding their in-patient study. All meals were provided by the metabolic kitchen of the Clinical Research Unit (CRU). Patients were admitted to the in-patient CRU at ~5:00 p.m. on the third day of their standardized diet and remained fasting from 7:00 p.m. At 7:00 a.m. the following morning, vastus lateralis muscle biopsy samples were obtained under local anesthesia (lidocaine, 2%) using a modified Bergstrom needle[27]. Approximately 100mg of muscle tissue was immediately placed in ice-cold biopsy preservation buffer (BIOPS: 10 mM Ca^{++}-EGTA, 0.1 μM free Ca^{++}, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phophocreatine). Mitochondria were isolated from the fresh muscle by gentle homogenization and differential centrifugation, as described in detail elsewhere [27, 28]. Once isolated, mitochondria were resuspended in mitochondrial respiration buffer (MiR05: 0.5 mM EGTA, 3mM $MgCl_2$*$6H_2O$, 60 mM potassium K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM Sucrose, 1 g/l fatty acid free BSA). Mitochondrial capacity and coupling efficiency were measured by high-resolution respirometry (Oxygraph O2k, Oroboros Instruments, Innsbruck, Austria) using a protocol described previously [27, 28]. Mitochondrial oxidative capacity (State 3) was measured using substrates specific for respiratory chain complex I (10mM glutamate, 2mM malate), complex I and II (10mM glutamate, 2mM Malate, 10mM succinate), and complex II alone (10mM glutamate, 2mM malate, 10mM succinate, 0.5 μM rotenone) in the presence of 2.5mM ADP. Oxygen flux rates (JO_2: pmol/s/ml) were calculated using Datlab Software® (Oroboros Instruments, Innsbruck, Austria) after correcting for background oxygen kinetics [27, 28]. Mitochondrial efficiency was determined from the ratio of state 3 to state 4 respirations (respiratory control ratio, RCR).

Uncoupling Protein-3 Expression *in vitro*

The functional effect of *UCP-3* rs1626521 polymorphism on gene expression was unknown. *UCP-3* rs1626521 polymorphism is located in the 3' UTR of the *UCP-3* gene. NCBI Gene ID 7352 was used as the reference sequence for the 3' un-translated regions (UTR) of *UCP-3*. Thus, we cloned the 3' UTR region of *UCP-3* in a luciferase encoding plasmid. Further details are provided in the appendix.

We then constructed a plasmid with each SNP variant in a CMV/chicken B-actin promoter plasmid with luciferase coding gene. Each plasmid construct was verified by DNA sequence. HEK293 cells were transfected with the *UCP*-3-Luc or *UCP*-3-Renilla plasmid containing the G allele or A allele. Twenty-four hours after the transfection, firefly and *Renilla* luciferase activities were quantitated using the dual luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions. Luciferase was quantified using a luminometer (TD-20/20, Turner Designs, Sunnyvale, CA). Statistical analysis was done using unpaired Student's t-test to compare the effect of G allele to A allele on luciferase expression.

Statistical Analysis

All data are presented as means ± SEM, medians ± IQR, or percentages, as noted. We determined *a priori* the specific *UCP* genes to be examined for association with each gastrointestinal quantitative trait. Thus, based on the reported mechanisms of action of *UCP*, we determined that *UCP* genes have a main effect on satiety; we corrected the results for false detection rate resulting from multiple comparisons based on the actual number of genes analyzed in relation to each specific quantitative trait. The univariate associations of genotype with subject characteristics and response measures (e.g., gastric emptying t½ values) were assessed using Fisher's exact test (e.g., association with categorical variables like gender), ANOVA, or a nonparametric (rank sum or Kruskal-Wallis) test as warranted, based on categorical or continuous data and normality of distribution.

Association of the rs1626521*UCP-3* SNP with the quantitative traits was also assessed using a dominant genetic model (that pools the minor allele homozygote with the heterozygote genotype and compares two groups, e.g., GG vs. Ga and aa) using a chi-square test, 2-sample t-test or rank sum test as warranted.

Statistical Power

A sample size assessment for detecting clinically relevant associations for each genotype was examined by estimating the differences between two groups (i.e., assuming a dominant genetic model) that could be detected given the observed variation in the measured responses and the number of subjects that were anticipated in each genotype group, based on our prior studies that estimated the minor allele frequency in the same community [24]. The differences between genotype groups that could be detected with approximately 80% power (two-sided α level of 0.05) using a two-sample t-test [assuming the listed pooled standard deviation (SD)] [24, 29] are shown in Appendix Table 1. The table illustrates the effect sizes demonstrable based on different sizes of groups for each genotype; this is necessary because the minor allele frequencies vary for the different genotypes studied (Table 2). Except for gastric emptying (two endpoints), each physiologic response corresponds to a distinct null hypothesis (e.g., association of genotype of interest with nutrient drink test maximum tolerated volume, i.e., satiation).

The analyses used the SAS® Statistical Package (Version 9.3, SAS Institute, Cary, NC).

These studies were approved by the Mayo Clinic Institutional Review Board, and written informed consent was received from participants prior to inclusion in the studies.

RESULTS

Demographics and Quantitative Traits in Obese and Overweight Participants

Demographics characteristics and quantitative traits of the cohort studied are outlined in Table 3. We recruited 255 overweight and obese participants: 178 females, 77 males, with a mean (±SEM) age of 37.4±0.7 years, BMI 33±0.3kg/m^2, and waist circumference 101.1±0.73cm. We observed the expected differences in body weight, BMI, waist circumference, hip circumference, systolic and diastolic blood pressure, heart rate in the obese compared to the overweight groups. Additionally, obese individuals had a larger fasting gastric volume and lower fasting plasma ghrelin levels when compared to overweight individuals (Table 3).

Associations of *UCP-3* rs1626521 with Body Weight, BMI, Waist Circumference and Physical Activity

In the dominant genetic model analysis, *UCP-3* rs1626521 homozygous GG genotype is associated with higher body weight (mean Δ 5.1kg, p=0.039) and increased waist circumference (Δ 3.31cm, p=0.035). Differences in BMI (mean Δ 1.2kg/m^2, p=0.085) and physical activity (p=0.063) did not reach statistical significance when compared with the combined GA/AA groups (Table 4).

Associations of *UCP-3* rs1626521 with GI Quantitative Traits (corrected for false detection)

UCP-3 rs1626521 was associated with less satiety [that is, higher caloric intake (p=0.006)], with higher proportions of carbohydrate and protein calories ingested (p=0.01 and 0.002, respectively) during the ad-libitum buffet meal. *UCP-3* rs1626521 was also associated with larger postprandial gastric volume and accommodation [measured as postprandial volume and change in gastric volume from fasting (ΔGV) respectively; all p<0.003]. These associations with total calorie intake and postprandial gastric volumes were significant when corrected for multiple comparisons (p<0.05 after FDR) (Table 4).

Univariate Associations of *UCP-3* rs1626521 with Other GI Quantitative Traits

There were also univariate associations of this SNP with increased maximal tolerated volume (p=0.049, suggesting reduced satiation), accelerated gastric emptying of liquids [GE $t_{1/2}$ (p=0.033)], and lower fasting plasma ghrelin (p=0.014). There were no nominal associations with age, height, hip circumference, blood pressure, postprandial satiation symptoms, GE of solids, postprandial plasma ghrelin, CCK, GLP-1 and PYY levels (Table 4).

Univariate Associations of Other *UCP* Gene Variants with GI Quantitative Traits

The main findings are summarized in Table 5. There were no associations with *UCP-2* rs659366 and GI quantitative traits.

The ins/ins genotype of *UCP-2* −3474, (45bp ins/del) was associated with decreased volume to fullness during the "satiation" test (p=0.033) and lower fasting gastric volume (p=0.045).

The *UCP-3* (rs2075577) AA genotype was associated with decreased maximum tolerated volume during the "satiation" test (p=0.028) and decreased postprandial gastric volume (p=0.051).

The *UCP-3* (rs15763) GG genotype was associated with increased caloric intake at the ad-libitum buffet meal (p=0.03).

Association of *UCP-3* rs1626521 with Mitochondrial Function in Human Skeletal Muscle

Table 6 shows subgroup demographics and other characteristics in the 11 participants in whom mitochondrial capacity and efficiency were studied in biopsies from skeletal muscle. *UCP-3* rs1626521 genotype was associated overall (p=0.051 by Kruskal-Wallis test) with reduced mitochondrial bioenergetic efficiency assessed by respiratory control ratio. Thus, respiratory control ratio in the AA genotype group differed from that of AG (p=0.038) and from GG (p=0.023) genotypes (Figure 1). There was no difference in state 3 respiration across the *UCP-3* rs1626521 genotypes.

Role of *UCP-3* rs1626521 Polymorphism in Cellular Expression of UCP-3: *in vitro* Assay in HEK293 Cells

To determine the functional effects of the *UCP-3* rs1626521 polymorphism, we transfected HEK-293 cells with a 3'UTR *UCP-3* rs1626521 luciferase reporter gene construct encoding for either the G allele or the A allele. In luciferase activity assay, the 3'UTR *UCP3*-luc encoding the G allele was associated with decreased expression of luciferase by an average 27% when compared to the 3'UTR *UCP3*-luc encoding for the A allele (p=0.049) (Figure 2).

DISCUSSION

We have shown that the novel variant of *UCP-3*, rs1626521, is associated with body weight and waist circumference, as well as with quantitative gastrointestinal functions in obesity. The differences in body weight may be explained by alterations in both domains of energy balance: first, changes in food intake regulation as a result of the genetic variation altering gastric motor function, satiation and satiety; and second, mitochondrial efficiency. These factors may play a role in the development of obesity, insulin resistance and type 2 diabetes.

Genes that have been associated with type 2 diabetes or obesity in epidemiological studies have been preliminarily shown to alter gastric motor functions, satiation or satiety [24, 29, 30]. Previously, other *UCP-3* gene variants have been associated with obesity or abdominal obesity [31–33]. In this paper, we report in a larger patient cohort than in our prior study [24], that *UCP-3* rs1626521 is associated with body weight (an average difference of 5kg in body weight and 1.2kg/m^2 in BMI) and waist circumference (an average difference of 3cm). These differences are clinically relevant and similar to the magnitude of effect of other *UCP-3* genes associated with obesity in Caucasians [33]. However, in addition to the observed association with body mass, our data provide insights on the mechanisms whereby *UCP-3*,

rs1626521 predisposes to obesity, that is, by alterations in food intake, physical activity and mitochondrial efficiency. In addition, whereas the previous associations *UCP-2* and *UCP-3* with obesity were typically observed with the minor allele, the association reported for *UCP-3* rs1626521 polymorphism was with the major GG genotype, which in the local population in the upper Midwest of the United States comprises about 33% of Caucasians. We also noted that, whereas other genetic studies in obesity typically required larger sample sizes (>1000 individuals) to demonstrate genotype-related average differences in BMI of usually less than 1kg/m^2 [34], our study of 255 patients identified an average BMI effect of *UCP-3* rs1626521 of 1.2kg/m^2. It would be of great interest, to validate our findings in other populations, including different ethnicities and a larger cohort of overweight and obese males and compare them to normal weight individuals.

In this study, we have also characterized the functional relevance and potential action of the *UCP-3* rs1626521 polymorphism; our *in vitro* studies in transfected HEK293 cells suggested that rs1626521 is functionally significant by decreasing the expression of UCP-3 protein. This change in expression results in a change in mitochondrial function in human skeletal muscle obtained from 11 participants of the same cohort. The mitochondrial dysfunction may be the result of lipid peroxidation and damage to the mitochondrial machinery in obese people who express less UCP-3. Under normal conditions, UCP-3 is up-regulated in response to lipid overload as a protective mechanism, resulting in reduced production of reactive oxygen species.

The attenuation of this mechanism, observed here in association with the novel polymorphism *UCP-3* rs1626521, may damage the mitochondria and result in decreased energy efficiency and increased lipid accumulation, which could lead to lipotoxicity and insulin resistance [35]. These changes are consistent with the known effects of UCP-3 which increases fatty acid metabolism in muscle and is involved in mitochondrial protection against lipotoxicity [17, 36, 37]. It is interesting to note that the over-expression of UCP-3 in mice causes fat-specific weight loss and improves insulin action [38, 39]. The decreased mitochondrial efficiency in skeletal muscle may also contribute to the decreased physical activity observed in association with *UCP-3* rs1626521 GG genotype.

Our findings also suggest that individuals with *UCP-3* rs1626521 GG genotype reported less frequent physical activity than the AG and AA genotypes and may reflect an intolerance to exercise due to poor energy efficiency and capacity. Prior to our study, UCP-3 had not been associated with energy intake or gastric motor functions. The strength of these associations relies on a strict statistical analysis in which the results were corrected for a false detection rate for multiple comparisons. In summary, these metabolic consequences of altered UCP-3 function may contribute to alterations in body weight. Further studies of the effects of UCP-3 mitochondrial efficiency in organs involved in food intake, satiation and satiety would be of great interest.

In conclusion, a unifying hypothesis of many pathobiological changes in obesity appraised by our current study is that *UCP-3* rs1626521 may explain the association of obesity in part by decreased physical activity (possibly due to decreased mitochondrial efficiency leading to exercise intolerance) and in part by increased postprandial gastric volume and

accommodation, and tolerance of a higher calorie intake at an ad-libitum meal. Overall, these data support the role of mitochondria in the regulation of energy intake, gastrointestinal function, and skeletal muscle mitochondrial efficiency in the development of obesity. *UCP-3* may be an interesting target for potential interventions with pharmacological or intense physical activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known about this subject

-- **G**ene variants of *uncoupling protein (UCP)-2 (rs659366* and +3474 45 bp ins/del) and *UCP-3* (rs2075577 and rs15763) have been associated with obesity.

-- Uncoupling proteins (UCPs), a family of mitochondrial transporters, play a significant role in thermogenesis and energy utilization.

UCP-3 is highly specific for skeletal muscle and brown adipose tissue and may affect the processes of adaptive thermogenesis in humans, and fatty acid translocation.

What this study adds

-- A newly identified functional variant (rs1626521) of *UCP-3* is associated with body weight and waist circumference, as well as with quantitative gastrointestinal functions in obesity.

-- The differences in body weight may be explained by alterations in both domains of energy balance: first, changes in food intake regulation as a result of the genetic variation altering gastric motor function by increased postprandial gastric volume and accommodation, satiation and satiety by higher calorie intake at an ad-libitum meal; and second, mitochondrial efficiency resulting in decreased physical activity.

-- Overall, these data support the role of mitochondria in the regulation of energy intake, gastrointestinal function, and skeletal muscle mitochondrial efficiency in the development of obesity.

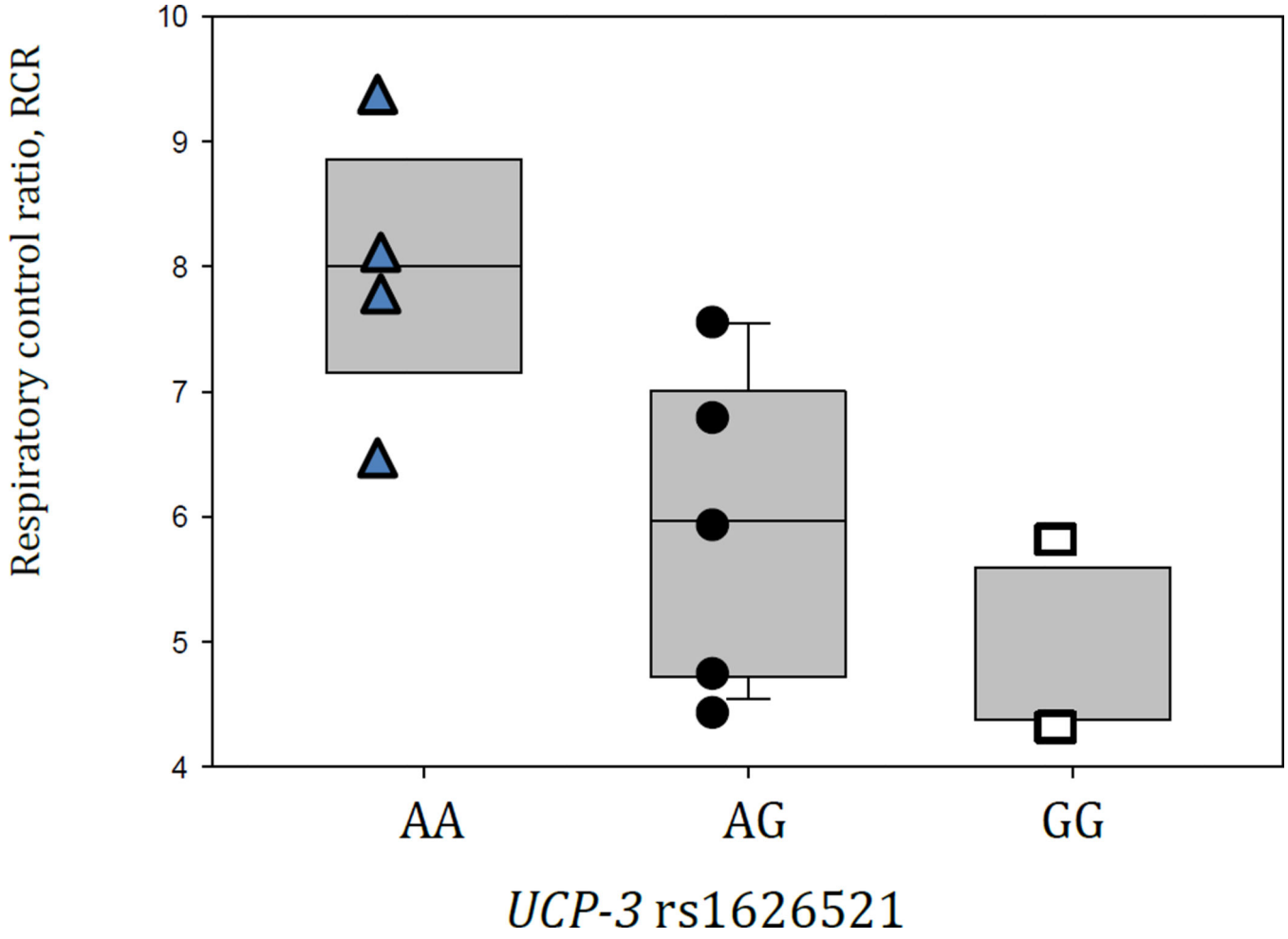

Figure 1.

Mitochondrial function comparing respiratory control ratio (RCR) with the *UCP-3* rs1626521 genotype. Overall p-value is 0.04 by ANOVA; RCR for AA genotype differed with AG (p=0.038) and GG (p=0.023) genotypes.

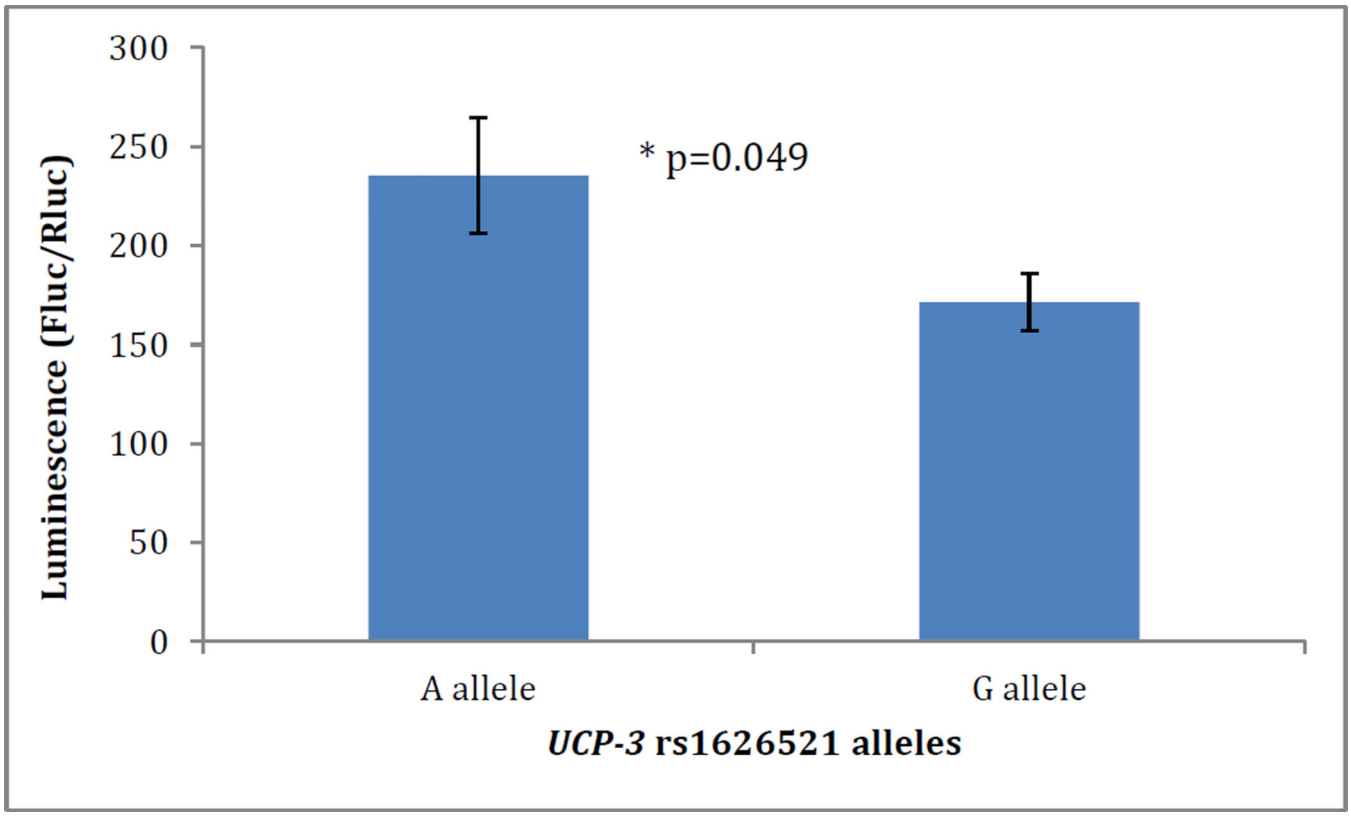

Figure 2.

UCP-3 rs1626521-induced luciferase expression. Hek293 were transfected with a 3'UTR *UCP-3* rs1626521 luciferase reporter gene construct. Data represent mean±SE of three separate experiments. *p=0.049.

Table 1

Candidate genes studied

GENE / Name	*SNP Minor allele*	MAF in locals	Associations with Obesity	Sex	Ethnicity	Study Design	Reference
UCP-2	rs659366 T allele	0.372	Greater BMI by 0.4 or 0.5 kg/m^2 in heterozygotes or homozygous minor vs. homozygous major (p=0.036)	both	Finnish	CS single Center	31
UCP-2	+3474 45 bp ins/del	0.24	Greater BMI by 2 or 3 .kg/m^2 in heterozygotes or homozygous minor vs. homozygous major (p=0.005)	both	German White	CS single center	40
UCP-3	rs2075577 G allele	0.404	Greater BMI by 0.29 or 1. 23 kg/m^2 in heterozygotes or homozygotes minor vs. homozygous major (p=0.019)	Male	Dutch, White	CS single center	33
UCP-3	rs15763 A allele	0.25	+0.38/+1.11 (BMI kg/m^2 Δ of heterozygotes/ homozygotes from WT) (p= 0.033)	Male	Finnish, White	CS single center	31
UCP-3	rs1626521 A allele	0.267	High LD (0.92) with rs15763	Both	White	CS, single center	24

CS=cross-sectional; LD= linkage disequilibrium; MAF= minor allele frequency

Table 2

Minor allele frequencies of selected genes in current study conducted on local population (n=255).

GENE	*SNP*	MAF	*f*(p)	*f*(q)	q^2	N
UCP-2	rs659366	T allele	0.705	0.295	0.087	255
UCP-2	+3474 ins/del	ins allele	0.601	0.399	0.159	255
UCP-3	rs2075577	G allele	0.571	0.429	0.184	255
UCP-3	rs15763	A allele	0.705	0.295	0.087	255
UCP-3	rs1626521	A allele	0.577	0.423	0.179	255

Table 3

Demographics of study participants (data shown are mean±SEM).

	Total	Overweight	Obese	p value
Participants (n)	255	85	170	
Females, %	69.8	60	74.7	0.02 †
Race (Caucasian, %)	92.3	91.8	92.4	1.0 †
Age, years	37.2±0.7	37.8±1.4	36.9±0.9	0.71
Anthropometrics, Cardiovascular, Fasting Glucose and Physical Activity				
Body Weight, kg	94.3±1.0	80.3±1.1	101.3±1.1	
BMI, kg/m2	33.1±0.3	27.8±0.1	35.7±0.3	
Waist Circumference, cm	101.2±0.8	91.8±1.0	105.8±0.8	
Hip Circumference, cm	116.1±0.7	107.0±0.6	120.6±0.7	
Systolic BP, mmHg	129.9±0.9	127.7±1.7	130.9±1.0	0.050
Diastolic BP, mmHg	78.3±0.7	76.5±1.2	79.2±0.8	0.034
Heart Rate, beats/min	71.4±0.8	67.5±1.5	73.4±0.9	<0.001
Fasting Glucose, mg/dl	100.3±1.1	97.6±1.4	101.7±1.5	0.044
Exercise 1 (%)	68.0	80.5	61.8	0.004 †
Quantitative gastrointestinal traits				
Satiation Volume To Fullness, ml	713±20	690±29	725±26	0.82
Satiation Maximum Tolerated Volume, ml	1278±26	1270±40	1282±34	0.85
Satiation Symptom VAS, mm (scale 0–400)	172.9±4.2	171.9±7.2	173.3±5.2	0.93
Gastric Emptying Solids $T_{1/2}$, min	99.5±1.7	96.6±2.8	100.8±2.1	0.28
Gastric Emptying Liquids $T_{1/2}$, min	19.1±0.7	18.1±0.8	19.6±1.0	0.70
Satiety *ad-libitum* intake at Buffet meal, kcal	963.6±18.3	948.1±31.9	975.6±22.9	0.49
Fasting Gastric Volume, ml	272.2±4.6	261.1±7.1	277.8±5.9	0.049
Postprandial Gastric Volume, ml	754.5±7.8	743.0±13	760.3±9.6	0.31#
Ghrelin fasting (pg/ml)	74.9±3.6	84.7±7.4	70.0±3.9	0.060
Ghrelin peak, pg/ml	67.1±4.2	76.4±7.3	62.6±5.1	0.10
CCK peak (pmol/L)	9.0±0.4	8.2±0.6	9.4±0.5	0.10
GLP-1 peak (pM)	18.1±0.7	17.2±0.9	18.6±0.9	0.86
PYY peak (pg/ml)	161.5±4.7	155.6±6.0	164.4±6.4	0.67

VAS-agg=visual analog score of the aggregate of 4 symptoms (nausea, bloating, fullness, pain)

= 2-sample t-test;

† = Fisher's Exact test; all other analyses were by rank sum test

Table 4

Univariate associations of *UCP-3* rs1626521 with demographics and GI quantitative traits.

Data show mean ± SE	**Genotype**			***p value***	
				General genetic model	**Dominant genetic model**
Genotype	AA	AG	GG		
Participants, n	54	110	91		
Demographics					
Gender, female, %	72	67	71	0.74†	0.78†
Body weight, kg	92.2±2.0	92.9±1.6	97.1±1.8	0.12	**0.039**
Height, cm	167.6±1.0	168.8±0.9	169.3±1.0	0.67	0.51
BMI, kg/m2	32.9±0.7	32.6±0.5	33.9±0.6	0.22	0.085
Waist Circumference, cm	100.2±1.6	99.8±1.1#	103.3±1.3	0.052	**0.035**
Hip Circumference, cm	115.6±1.5	115.5±1.0	117.1±1.1	0.50	0.24
Systolic BP, mmHg	127.2±1.9	130.1±1.4	131.1±1.5	0.38	0.29
Diastolic BP, mmHg	76.2±1.4	79.0±1.1	78.6±1.2	0.27	0.70
Fasting Blood glucose, mg/dl	99.2±1.7	99.4±1.2	102.0±2.6	0.89	0.63
Exercise 1, %	67.3	74.5	60.7	0.12†	**0.063†**
Body Image (scale 9–45)	28.4±1.0	28.7±0.6#	26.7±0.6	**0.04**	**0.016**
Quantitative gastrointestinal traits					
Satiation Volume To Fullness, ml	675±47	746±31	696±32	0.15	0.83
Satiation Maximum Tolerated Volume, ml	1184±57	1358±43	1233±36	**0.049**	0.36
Satiation Symptom VAS, mm (scale 0–400)	164.8±8.3	181.4±6.6	167.1±7.1	0.24	0.25
Gastric Emptying Liquids $T_{1/2}$, min	17.4±1.1	20.1±1.0	18.8±1.4	**0.033**	0.25
Gastric Emptying Solids $T_{1/2}$, min	97.1±3.5	99.1±2.1	101.2±3.4	0.86	0.91
Satiety *ad-libitum* intake at Buffet meal, kcal	879.6±37.7	1035±30.2#	924.8±26.2	**0.006***	0.18
Satiety Carbs, gr	116.3±5.2	136.4±4.1#	122.9±3.6	**0.011***	0.23
Satiety Protein, gr	49.7±2.1	59.2±1.7#	52.1±1.6	**0.002***	0.088

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Data show mean ± SE	**Genotype**			***p value***	
				General genetic model	**Dominant genetic model**
Satiety Fat, gr	23.4±1.1	27.1±0.9	24.5±0.8	**0.044**	0.30
Fasting Gastric Volume, ml	267.5±7.8	262.8±6.3[#]	286.9±9.2	0.058	**0.023**
Postprandial Gastric Volume, ml	710.2±14.8[#]	754.6±11.5	781.9±13.6	**0.0029**[*]	**0.009**
Gastric accom, ratio	2.7±0.1[#]	3.0±0.1	2.9±0.1	**0.032**	0.55
GV delta, ml	442.8±12.1[#]	491.8±9.8	496.2±9.6	**0.0025**[*]	0.065
Gastric Acommodation	98.6±2.4	107.1±2.0	103.1±2.3	**0.032**	0.55
Ghrelin fasting, pg/ml	83.5±5.8	75.3±5.7	69.0±6.6	**0.014**	**0.0017**
Ghrelin peak, pg/ml	68.6±9.3	64.9±6.5	65.9±7.3	0.95	0.11
CCK peak, pmol/L	9.8±1.0	8.9±0.5	8.6±0.5	0.91	0.78
GLP-1 peak, pM	17.5±1.9	18.5±0.9	18.1±1.0	0.17	0.38
PYY peak, pg/ml	161.7±10.3	165.2±7.5	156.6±7.4	0.99	0.87

[#] p value <0.05 when compared to GG genotype on a Dunnett's test or Dunn test in nonparametric comparisons;

[*] significant with adjustment for FDR.

[†] Chi-square test

Table 5

Univariate associations (by general genetic model) of other studied *UCP*-2 and *UCP*-3 genes variants with demographics and GI quantitative traits.

Data show mean ± SE	**General genetic model**			**p**
Gene:	***UCP2* rs659366**			
Genotype	CC	CT	TT	
Participants (n)	86	139	30	
MTV, ml	1266±39	1313±38	1148±66	0.12
Fasting Ghrelin	71.8±6.7	73.8 ±4.8	88.2±8.6	0.080
Gene:	*UCP2* −3474, 45bp ins/del			
Genotype	del/del	del/ins	ins/ins	
Participants (n)	115	114	26	
Volume to fullness, ml	708±27	748±33	573±45	**0.033**
Fasting GV, ml	283.8±7.2	263.4±6.8	260±10.8	**0.045**
Buffet, Kcal	919.9±25.6	1016±29.6	923.9±44.4	0.085
Gene:	*UCP3* rs2075577			
Genotype	AA	AG	GG	
Participants (n)	78	138	45	
Volume to fullness, ml	681±40	749±28	664±34	0.14
MTV, ml	1188±45	1338±37	1257±59	**0.028**
Fasting GV, ml	266.1±8.3	281.2±6.4	256.2±10.6	0.14
Postprandial GV, ml	731.5±13.2	773.1±11.0	738.2±18.1	0.051
Buffet, Kcal	930.1±33.3	988.3±24.4	948.8±48.5	0.15
Gene:	*UCP3* rs15763			
Genotype	AA	AG	GG	
Participants (n)	15	108	132	
LGE $T_{1/2,}$ min	16.4±1.2	19.8±1.0	18.8±1.1	0.23
MTV, ml	1082±98	1319±42	1266±35	0.071
Buffet, Kcal	811.4±42.3	1018±30.0	936.1±24.3	**0.031**

Bolded p values indicate univariate associations; none were significant with FDR correction

Table 6

Demographics of a subgroup of participants in a study of mitochondrial function in skeletal muscle. Note the similarities between obese and overweight, other than BMI and related characteristics.

	Total	Overweight	Obese	p value[§]
Participants (n)	11	3	8	
Females, %	63.6	66.6	63	
Race (Caucasian, %)	90.9	100	87.5	1.0[†]
Age, years	31.7±3.4	30.3±8.8	32.3±3.8	0.82
Anthropometrics				
Body Weight, kg	96.4±3.7	80.1±2.2	102.6±2.7	
BMI, kg/m^2	33.4±1.4	28±0.4	35.5±1.4	
Waist Circumference, cm	99.1±1.8	93.3±2	101.2±1.9	
Hip Circumference, cm	117.6±2.8	108.3±0.8	121.1±3	
Systolic BP, mmHg	126.3±3.2	126.7±9.3	126.1±3.2	0.94
Diastolic BP, mmHg	80±3.3	74±7.9	82.5±3.5	0.29
Heart Rate, beats/min	68.9±3.7	56.3±3.7	73.6±3.7	**0.03**
Fasting Glucose, mg/dl	95.2±2.5	95.5±5.5	97.1±2.5	0.78
Exercise Regularly, %	36.4	66.7	25	0.49 [†]
Mitochondrial function				
State 3 (CI)	255.8±29.2	201±7.9	276.4±38	0.27
State 3 (CI+II)	468.3±36.2	392.7±29.8	496.6±45.3	0.22
State 3 (CII)	314±21.1	270.3±23.6	330.3±25.9	0.22
State 4	74.7±6.8	67.5±6.2	77.4±9.1	0.54
RCR	6.5±0.5	5.9±0.7	6.7±0.6	0.51

[†] Fisher's exact test;

[§] 2-sample t-test unless otherwise noted