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SHORT COMMUNICATION

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Genetic variation in *UCP2* (uncoupling protein-2) is associated with energy metabolism in Pima Indians

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Abstract *Aims/hypothesis:* Uncoupling protein-2 (UCP2) is thought to play a role in insulin secretion and the development of obesity. In this study, we investigated the effects of genetic variation in *UCP2* on type 2 diabetes and obesity, as well as on metabolic phenotypes related to these diseases, in Pima Indians. *Methods:* The coding and untranslated regions of *UCP2*, and approximately 1 kb of the 5′ upstream region, were sequenced in DNA samples taken from 83 extremely obese Pima Indians who were not first-degree relatives. *Results:* Five variants were identified: (1) a −866G/A in the 5′ upstream region; (2) a G/A in exon 2; (3) a C/T resulting in an Ala55Val substitution in exon 4; and (4, 5) two insertion/deletions (ins/del; 45-bp and 3-bp) in the 3′ untranslated region. Among the 83 subjects whose DNA was sequenced, the −866G/A was in complete genotypic concordance with the Ala55Val and the 3-bp ins/del polymorphism. The G/A polymorphism in exon 2 was extremely rare. To capture the common variation in this gene for association analyses, the −866G/A variant (as a representative of Ala55Val and the 3-bp ins/del polymorphism) and the 45-bp ins/del were also genotyped for 864 full-blooded Pima Indians. Neither of these variants was associated with type 2 diabetes or body mass index. However, in a subgroup of 185 subjects who had undergone detailed metabolic measurements, these variants were associated with 24-h energy expenditure as measured in a human metabolic chamber (p=0.007 for the 45-bp ins/del and p=0.03 for the −866G/A after adjusting for age, sex, family membership, fat-free mass and fat mass). *Conclusions/interpretation:* Our data indicate that variation in *UCP2* may play a role in energy metabolism, but this gene does not contribute significantly to the aetiology of type 2 diabetes and/or obesity in Pima Indians.

Keywords Energy metabolism · Genetics · Obesity · Type 2 diabetes mellitus · Uncoupling protein-2

Abbreviations ins/del: insertion/deletion · SNP: single nucleotide polymorphism · UCP2: uncoupling protein-2 · V_{CO2}: carbon dioxide production · V_{O2}: oxygen consumption

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Introduction

Obesity and type 2 diabetes are conditions of abnormal energy metabolism. Both systemically, and at the cellular level, energy metabolism is controlled by a large variety of enzymatic and non-enzymatic proteins. Uncoupling protein-2 (UCP2) is a member of the mitochondrial transporter superfamily that uncouples ATP production from mitochondrial respiration, thereby possibly dissipating energy as heat and affecting the efficiency of energy metabolism [1]. UCP2 also negatively regulates insulin secretion and may be an important link between obesity, beta cell dysfunction and type 2 diabetes [2]. Therefore, *UCP2* is a plausible candidate gene for susceptibility to obesity and type 2 diabetes. Here, we investigated the role of genetic variation in *UCP2* in the development of type 2 diabetes and obesity in Pima Indians, a genetically isolated population of Native Americans from Arizona, with one of the highest prevalences of obesity and type 2 diabetes in the world.

Subjects and methods

Study participants and clinical measurements

The study participants are part of our ongoing longitudinal study of the aetiology of obesity and type 2 diabetes among

the Gila River Indian Community in central Arizona. We selected 83 extremely obese Pima Indians who were not first-degree relatives (37 men and 46 women; BMI=60.0±6.1 kg/m^2 [mean±SD], ranging from 50.5 to 79.6 kg/m^2; age 35.0±9.5 years [mean±SD]) for sequence analysis of *UCP2*. Genotypically unique variants were further genotyped in DNA from 864 (565 diabetic, 299 non-diabetic) full-blooded Pima Indians from 264 nuclear families where at least one family member had type 2 diabetes. Diabetes status was interpreted according to the criteria of the World Health Organization.

A subgroup (n=263) of these study participants, all of whom were non-diabetic, had undergone additional metabolic phenotyping that included measurements of oral glucose tolerance and insulin action. To determine oral glucose tolerance, participants ingested 75 g glucose. Blood for plasma glucose and insulin measurements was drawn before glucose ingestion and at 30, 60, 120 and 180 min after ingestion. The hyperinsulinaemic–euglycaemic clamp technique was used to determine basal glucose appearance and insulin-stimulated glucose disappearance (uptake) rates, as described in detail elsewhere [3]. From this non-diabetic subgroup, 185 subjects had additional measurements taken of body composition as well as of energy expenditure and substrate oxidation in the respiratory chamber. Body composition was estimated by underwater weighing or dual energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI, USA). The measurement of energy expenditure and substrate oxidation in the respiratory chamber has been described previously [3]. Briefly, participants entered the chamber after an overnight fast and remained in the chamber for 23 h. Participants were fed calories to maintain energy balance according to previously determined equations, and the rate of energy expenditure was measured continuously, calculated for each 15-min interval within the chamber, and then extrapolated to 24 h. Carbon dioxide production (V_{CO2}) and oxygen consumption (V_{O2}) were calculated for every 15-min interval. The 24-h respiratory quotient was calculated as the ratio of 24-h V_{CO2} and 24-h V_{O2}. Based upon the 24-h respiratory quotient, 24-h metabolic rate, and 24-h urinary nitrogen excretion, the 24-h oxidation rates of fat, carbohydrate and protein were determined [3].

All studies were approved by the Tribal Council and the Institutional Review Board of the National Institutes of Diabetes and Digestive and Kidney Diseases. All subjects gave written informed consent to participate in the study.

Sequencing of *UCP2* and genotyping of variants

The *UCP2* coding region (930 bp), the 5′ and 3′ UTR regions (381 bp and 336 bp, respectively), and 1 kb upstream of the first exon were sequenced using the Big Dye Terminator (Applied Biosystems, Foster City, CA, USA) on an automated DNA capillary sequencer (model 3730xl; Applied Biosystems). Sequence information for all oligonucleotide primers used for variant screening is available upon request. Genotyping of selected single nucleotide polymorphisms (SNPs) was conducted by TaqMan assay. The genotyping reaction was run on a GeneAmp PCR system 9700 (Applied Biosystems) at 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and 62°C for 1 min, for 38 cycles. The reaction was read on an ABI Prism 7700 sequence detector (Applied Biosystems).

Statistical analyses

Statistical analyses were performed using the statistical analysis system of the SAS Institute (Cary, NC, USA). For continuous variables, the general estimating equation procedure was used to adjust for the relevant covariates, including family membership because some subjects were siblings. The genotype was entered as a class variable. Plasma insulin concentrations and rates of glucose disappearance during the low-dose insulin infusion were log-transformed prior to analyses to approximate a normal distribution. A p value of <0.05 was considered to indicate statistical significance.

Results

Variation in *UCP2*

Five genetic variants were identified by sequencing the coding region and approximately 1 kb of the 5′ upstream region of *UCP2*. One SNP, a −866G/A polymorphism (rs659366), was positioned in the 5′ region. Two SNPs were found in exons, a G/A in the untranslated exon 2 (position 166580 in AC019121) and a C/T in exon 4, predicting an Ala to Val substitution at codon 55 (GCC/GTC; rs660339). Two variants were identified in the 3′ untranslated region, a 45-bp insertion/deletion (ins/del; position 173247 in AC019121) and a 3-bp (AAG) ins/del (position 172329 in AC019121). Among the 83 participants whose DNA was sequenced to identify these variants, the 5′ upstream −866G/A variant was in complete genotypic concordance with the coding Ala55Val variant and the 3′ untranslated AAG ins/del variant. Since these three variants would be predicted to provide identical/nearly identical genotypic information in the group of 864 full-blooded Pima Indians, the Ala55Val and the AAG ins/del were excluded from further genotyping. The untranslated G/A polymorphism in exon 2 was also excluded from further genotyping because it was very rare (A allele frequency 0.006). Therefore, only the 5′ upstream −866G/A variant (G allele frequency 0.46) and the 45-bp ins/del (ins allele frequency 0.42) were genotyped in the 864 full-blooded Pima Indians. Neither variant differed significantly from Hardy–Weinberg equilibrium (p=0.61 for −866G/A and p=0.64 for 45-bp ins/del). The haplotype frequencies were 0.41 for −866A/45-bp ins, 0.13 for −866A/45-bp del, 0.01 for −866G/45-bp ins, and 0.45 for −866G/45-bp del. This corresponds to D'=0.95 and r^2=0.55.

Table 1 Metabolic characteristics of non-diabetic Pima Indians grouped by *UCP2* variant genotypes

Genotype	−866 G/A			p_{add}	p_{dom}	p_{rec}	45-bp ins/del			p_{add}	p_{dom}	p_{rec}
	A/A (*n*=80)	A/G (*n*=132)	G/G (*n*=51)				del/del (*n*=81)	ins/del (*n*=135)	ins/ins (*n*=47)			
Age	27±1	26±1	27±1				26±1	26±1	27±1			
Fasting plasma glucose (mmol/l)	5.1±0.1	5.1±0.1	5.0±0.1	0.25	0.22	0.49	5.1±0.1	5.1±0.1	5.1±0.1	0.26	0.48	0.28
2-h plasma glucose (mmol/l)	7.1±0.2	6.9±0.2	7.4±0.2	0.32	0.06	0.94	7.1±0.2	7.0±0.2	7.1±0.3	0.83	0.99	0.74
Fasting plasma insulin (μU/ml)	43±3	40±2	45±3	0.12	0.30	0.18	44±2	42±2	39±3	0.30	0.10	0.99
2-h plasma insulin (μU/ml)	245±22	199±14	229±24	0.76	0.63	0.42	211±21	214±21	246±31	0.25	0.63	0.17
Basal glucose turnover (mg kg $EMBS^{-1}$ min^{-1})	2.0±0.03	1.9±0.02	1.9±0.03	0.17	0.22	0.31	1.9±0.02	1.9±0.02	2.0±0.04	0.31	0.06	0.84
Glucose disposal for low-dose insulin clamp (mg kg $EMBS^{-1}$ min^{-1})	3.39±0.10	3.70±0.10	3.54±0.15	0.39	0.80	0.32	3.64±0.12	3.58±0.10	3.46±0.13	0.13	0.53	0.06
Glucose disposal for high-dose insulin clamp (mg kg $EMBS^{-1}$ min^{-1})	7.8±0.2	8.5±0.2	8.3±0.2	0.15	0.80	0.07	8.5±0.2	8.2±0.2	7.7±0.3	0.01	0.05	0.08

The data are presented as mean±SEM. *p*-values were calculated after adjusting for age and sex for the variable % body fat; and age, sex and % body fat for the variables 2-h plasma glucose, 2-h plasma insulin, and $\log_{10}$ glucose disposal for the low-dose and high-dose insulin clamps
EMBS estimated metabolic body size; $p_{add,\ dom,\ rec}$ *p*-value in additive, dominant and recessive models

Table 2 Metabolic characteristics, as measured in a human respiratory chamber, in non-diabetic Pima Indians grouped by *UCP2* variant genotypes

Genotype	−866 G/A			p_{add}	p_{dom}	p_{rec}	45-bp ins/del			p_{add}	p_{dom}	p_{rec}
	A/A (*n*=54)	A/G (*n*=97)	G/G (*n*=34)				del/del (*n*=59)	ins/del (*n*=93)	ins/ins (*n*=33)			
Age	28±1	27±1	27±1				27±1	27±1	28±1			
% body fat	33±1	32±1	31±1	0.33	0.87	0.18	33±1	32±1	32±2	0.71	0.82	0.75
24-h respiratory quotient	0.848±0.004	0.851±0.002	0.850±0.004	0.94	0.82	0.96	0.851±0.003	0.849±0.002	0.851±0.005	0.72	0.62	0.88
24-h metabolic rate (kcal/day)	2516±66	2337±34	2308±59	0.03	0.25	0.03	2286±46	2426±38	2441±90	0.007	0.15	0.004

The data are presented as mean±SEM. *p*-values for variables, 24-h respiratory quotient, carbohydrate and lipid oxidation were calculated after adjusting for age, sex, family membership, % body fat and energy balance. *p*-values for 24-h metabolic rate were calculated after adjusting for age, sex, family membership, fat free mass and fat mass
$p_{add,\ dom,\ rec}$ *p*-value in additive, dominant and recessive models

Association studies

None of the variants that were genotyped in *UCP2* were associated with type 2 diabetes ($OR_{(additive\ model)}$=1.05 [0.81–1.34] 95% CI for −866G/A and 0.99 [0.79–1.26] for 45-bp ins/del) or BMI ($p_{(additive\ model)}$=0.23 for −866G/A and 0.92 for 45-bp ins/del) among 864 full-blooded Pima Indians. Comparable results were obtained in mixed-model analyses using approximately 5,000 observations for the same group of participants (data not shown). In addition, no association was found between these SNPs and detailed measures of insulin action among the 263 non-diabetic study participants who had undergone detailed metabolic phenotyping (Table 1). However, in a subset of these non-diabetic Pima Indians who had undergone measurements of energy expenditure and substrate oxidation rates in a human respiratory chamber (n=185), both variants in *UCP2* showed significant associations with 24-h. metabolic rate ($p_{(additive\ model)}$=0.007 for the 45-bp ins/del and $p_{(additive\ model)}$=0.03 for the −866G/A) (Table 2). However, no association with 24-h respiratory quotient was observed. For the subgroup of individuals with measurements of energy expenditure, the variance in the trait needs to be about 5.7% to give 80% detection power, assuming an additive model. In addition, each of the common haplotypes (excluding the −866G/ 45-bp ins haplotype, which was rare) was analysed for association with metabolic characteristics. However, analyses of these haplotypes did not reveal any strong associations beyond those expected from analysis of individual SNPs.

Discussion

Prior studies examining the −866G/A polymorphism in the *UCP2* promoter have produced controversial results. For example the −866A allele is associated with decreased insulin secretion in Caucasians from Italy [4], while the same allele was associated with increased secretion in Northern European Caucasians, as ascertained in Utah [5]. Another study reported a reduced risk of type 2 diabetes in obese middle-aged humans with a −866G allele [6]. The −866G/A variant has further been shown to be associated with enhanced *UCP2* mRNA expression in adipose tissue in vivo and obesity in middle-aged humans, suggesting that this variant may have an age-dependent effect on body weight [7]. In contrast, no association with obesity could be found in a Danish population [8] or in Mediterranean and central European Caucasian adolescents [9].

In this report we describe an association between a 45-bp ins/del variant in the 3′ untranslated region of *UCP2* and 24-h metabolic rate in Pima Indians, which is consistent with a previous finding among 82 full-blooded Pima Indians reported by Walder et al [10]. Most of the subjects analysed in that study were also part of our current analysis, but the addition of approximately 100 subjects increased the magnitude of the difference of the means of 24-h metabolic rate by genotype (ins/ins compared to del/del) from 30 kcal/day in the previous study [10] to 155 kcal/day in this study (Table 2). In addition, a second variant −866G/A, which is in complete genotypic concordance with an Ala55Val and another 3′ untranslated region ins/del, showed association with the 24-h metabolic rate. Although metabolic rate affects body weight, in our current study no association was found with these variants and obesity in a large cohort of Pima Indians. A difference of 150 kcal/day, which is the approximate difference between the homozygous genotypic groups for the two variants associated with metabolic rate (Table 2), might predict a measurable difference in adult BMI. It is possible that a difference in BMI was not found because other obesity-related genes, that have a much larger effect on BMI in Pima Indians, mask the effect of *UCP2*. A role of the −866G/A variant in fuel partitioning, through its association with increased carbohydrate and decreased lipid oxidation, has been demonstrated in juvenile obese subjects [9]; however, no association with substrate oxidation rates was observed in our study. Therefore, our findings indicate a modest effect of the *UCP2* genotype on metabolic rate, but this effect, by itself, does not explain the variation in body weight in Pima Indians.

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