Association between *UCP1*, *UCP2* and *UCP3* gene polymorphisms with markers of adiposity in European adolescents: the HELENA study

Running title: An original article of *UCPs* and adiposity markers

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ABSTRACT

Aims: To examine the association between *UCP1*, *UCP2* and *UCP3* gene polymorphisms with adiposity markers in European adolescents, and to test if there were gene interactions with objectively measured physical activity and adiposity.

Methods: A cross-sectional study that involves 1.057 European adolescents (12-18 years old) from the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. A total of 18 polymorphisms in *UCP1*, *UCP2* and *UCP3* genes were genotyped. We measured weight, height, waist and hip circumferences and triceps and subscapular skinfold thickness. Physical activity was objectively measured by accelerometry during 7 days.

Results: The C allele of the *UCP1* rs6536991 polymorphism was associated with a lower risk of overweight [odds ratio (OR): T/C + C/C vs. T/T) = 0.72; 95% confidence interval (CI): 0.53 to 0.98; P=0.034; false discovery rate (FDR)=0.048)]. There was a significant interaction between *UCP1* rs2071415 polymorphism and physical activity with waist-to-hip ratio (P = 0.006; FDR = 0.026). Adolescents who did not meet the physical activity recommendations (less than 60min/day of moderate to vigorous physical activity) and carrying the C/C genotype had higher waist-to-hip ratio (+ 0.067; 95% CI, 0.028 to 0.106; P = 0.003), while no differences across genotypes were observed in adolescents meeting the recommendations.

Conclusions: Two *UCP1* polymorphisms were associated with adiposity in European adolescents. Meeting the daily physical activity recommendations may overcome the effect of the *UCP1* rs2071415 polymorphism on obesity-related traits.

Keywords: Physical Activity, Brown Adipose Tissue, Genetic Susceptibility, Adolescents, Uncoupling Protein

INTRODUCTION

Obesity is a major public health problem throughout the world. Despite later reports suggest a plateau in children's and adolescent's body mass index (BMI) from high-income countries, there are still little evidences on strategies to stop this pandemic[1].

Obesity is a result of a complex interaction between environmental and genetic factors[2]. Several studies showed that physical activity may overcome the effect of several gene polymorphisms on obesity-related traits. A genome-wide meta-analysis of 200,452 European adults showed that physical activity may attenuate the deleterious effect of the *FTO* (fat mass- and obesity-associated) gene polymorphisms on obesity[3]. In adolescents, we showed that meeting the daily physical activity recommendations (at least 60 minutes/day of moderate to vigorous physical activity) may offset the genetic predisposition to obesity associated with the *FTO* rs9939609 polymorphism in European adolescents[4].

The strong genetic influence in obesity has been described as a non-mendelian way of inheritance. Only few percent of cases are monogenic obesity type. In these cases, monogenic obesity genes are involved in the control of appetite center and satiety like leptin (LEP), leptin receptor (LEPR), pro-opiomelacortin (POMC) or prohormoneconvertase 1 (PCSK1) [5]. However, 95% of the cases of obesity can be explained by genetic variants of multiple genes and complex gene-gene and gene-lifestyle interactions [2,6,7], among which are uncoupling proteins (UCPs) gene polymorphisms [8].

The most known *UCP* genes include *UCP1*, *UCP2* and *UCP3*. *UCP1* is responsible of heat production through non-shivering thermogenesis in brown adipose tissue (BAT) [9]. Less clear is, however, the role of *UCP2* and *UCP3* genetic variants, which have been related with obesity phenotypes through the potential influence on

muscle metabolism due to the high expression of these genes in skeletal muscle [10], diabetes mellitus and lipid/ lipoprotein-related diseases [11]. UCP2 seems to be involved in the control of reactive oxygen species (ROS) production, a modulator of insulin secretion and a regulator of mitochondrial fatty acid oxidation[12]. More recently, Caron et al.[13] reported that *UCP2* may have a regulating role in thermogenesis of BAT. UCP3 role relates with the coupling regulation of mitochondrial respiration in skeletal muscle mitochondria[14] and as mediator of thermogenesis[15]. The *UCP1* rs6536991 polymorphism[16], *UCP2* rs659366[17], rs660339 polymorphisms[18–21] and *UCP3* rs1800849, rs2075577 polymorphisms[21,22] have been associated with obesity traits, but most of them have been studied in adults and the results are so far controversial. Several studies suggested that the rs659366-A allele (UCP-2) might be protective against obesity in 55 age average subjects[17], whereas others found an association of the rs659366-A allele with a borderline increase of fat body mass index (FBMI) [19] and higher risk of central obesity[20].

Data obtained within the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study provide an excellent opportunity to study the association between *UCP1*, *UCP2* and *UCP3* gene polymorphisms with adiposity makers in European adolescents. The HELENA study was designed to provide reliable data on nutrition and health-related variables in a relatively large sample of European adolescents from 9 different countries and includes information on 18 polymorphisms (SNPs) of the *UCP1*, *UCP2* and *UCP3* genes as well as markers of adiposity. To our knowledge, *UCP* polymorphisms have not been identified in GWAS of body weight or body composition in adults or other age groups.

The aim of this study was therefore to examine the association between 18 *UCP1*, *UCP2* and *UCP3* polymorphisms with total and central adiposity markers in

European adolescents, and to test if there were gene x physical activity interactions on adiposity phenotypes.

Material and methods

Participants

Adolescents were part of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study. A total of 3865 adolescents (12-18 year old) of 10 centers in nine European countries were recruited between 2006 and 2007. Adolescents were randomly selected from schools by using a proportional cluster sampling method, and age was taken into account. One-third of the classes were randomly selected for blood collection, resulting in a total of 1155 blood samples for the subsequent clinical biochemistry assays and genetic analyses. Among these participants, 1057 individuals (552 girls) with data on *UCP1, UCP2 and UCP3* gene polymorphisms, adiposity phenotypes and physical activity were included in this study. After receiving complete information about the aims and methods of the study, all adolescents and their parents or guardians signed an informed written consent. All participants met the general HELENA inclusion criteria[23]. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), Good Clinical Practice, and legislation about clinical research in humans in each of the participating countries. The corresponding local human research review committees of the involved centers approved the protocol.

Assessment of adiposity

Weight and height were measured following standard methods. Waist and hip circumference was measured in triplicate with an anthropometric unelastic tape (SECA 200; Seca Deutschland, Hamburg, Germany) and was used as a surrogate measure of central body fat. We calculated waist to height and waist to hip ratios. BMI was calculated as weight in kilograms divided by height in meters squared. Adolescents

were classified according to BMI (kg/m^2) as normal weight, overweight or obese categories according to Cole et al.[24]. The overweight-obese categories were grouped into one category (hereafter called overweight). Skinfold thickness was measured to the nearest 0.2 mm in triplicate on the left side at the biceps, triceps, subscapularis, suprailium, thigh, and medial calf with a Holtain Caliper (Holtain Ltd, Crymmych, Wales). Body fat percentage was calculated from skinfold thicknesses (triceps and subscapular) using the equations by Slaughter et al.[25]. Finally, fat mass index (FMI) was calculated as fat mass in kilograms divided by height in meters squared.

Assessment of physical activity

Physical activity was assessed during 7 consecutive days with a uniaxial accelerometer (GT1M; ActiGraph, Pensacola, Florida) attached to the lower back[26] . Adolescents were instructed to wear the accelerometer during all time awake and to remove it only during water-based activities. At least 3 days of recording with a minimum of 8 hours registered per day was set as an inclusion criterion[26]. The time-sampling interval (epoch) was set at 15 seconds. We calculated the time engaged in at least moderate physical activity (≥3 metabolic equivalents) based on a standardized cutoff of 2000 counts/min or more. Moderate to vigorous physical activity was dichotomized into less than 60 min/day and 60 min/day or longer[26].

Genotyping

The genotyping was done by an Illumina system (Illumina, Inc, San Diego, California) using the GoldenGate technology (GoldenGate Software, Inc, San Francisco, California). The mean genotyping success rate was 99.84%. All genotype distributions

respected Hardy-Weinberg equilibrium (P > 0.2; Appendix S1). Some polymorphisms exhibited linkage disequilibrium between them (Appendix S2, S3).

Data analysis

Deviations from Hardy-Weinberg equilibrium were assessed by means of an exact test and considering a P value of 0.05 as a threshold. Linkage disequilibrium between polymorphisms was evaluated with "genetics" R package. Associations between SNPs and phenotypes were analyzed by means of general linear models (GLM) using Gaussian and Binomial error distributions for continuous and discrete phenotypes, respectively. Interactions between SNPs of different *UCP* genes were assessed using the same models but including an interaction term for each gene pair. Interaction with physical activity was assessed for SNPs that were significantly associated with obesity phenotypes including an interaction term with both factors. Moreover, we performed the analyses stratified by moderate to vigorous physical activity categories (<60 and ≥60 min/day). Five genetic models (dominant recessive, log-additive, codominant and over dominant and additive) were used for all analyses except in those that rs2071416, rs2735572 and rs17132534 were involved. These polymorphisms were analyzed using only dominant model due to the low number of minor homozygotes (MAF < 0.1; Appendix S1). Previous studies highlighted the association between non-additive models with UCPs, which indicates the importance of perform this five models and compare the additive models with non-additives ones [27]. In all analyses, adjustment variables were age, gender and center. For each polymorphism, P values were computed using the likelihood ratio test (LRT) between a model with the polymorphism or interaction term and a null model without it.

With the purpose of controlling the chance of any false positives, we corrected the significance level of 0.05 by the number of test (polymorphisms) for each genetic model (Bonferroni correction). Therefore, significance threshold was 0.0033 for all models except for dominant model, in which it was 0.0028. Given that some of studied SNPs were in linkage disequilibrium (Appendix S2, S3), the number of independent test would be lower than number of studied polymorphisms and thus Bonferroni correction could be potentially over conservative[28]. Because of this, we also used a less stringent approach, which controls the expected proportion of false positives (False Discovery Rate [FDR]). As in the case of Bonferroni, the family test included all genotyped markers for a given genetic model (18 tests). Significance for the interaction analyses was determined in the same way, i.e. a family test included the interaction between physical activity and the 18 genotyped markers under a given heritage model. All analyses were performed using the "SNPassoc" package in the R environment 3.4.1.

Results

Characteristics of the study sample are shown in Table 1.

Association between UCP polymorphisms and markers of adiposity

Only one of the 18 studied polymorphisms was individually associated with overweight phenotypes after multiple-comparison corrections (Appendix S1 & Fig. 1; Appendix S4:S11). The minor C allele of the *UCP1* rs6536991 polymorphism was associated with a lower risk of overweight [odds ratio (OR): T/C + C/C vs. T/T = 0.72; 95% confidence interval (CI), 0.53 to 0.98; P=0.034; FDR=0.048; Figure 1). The *UCP1* rs6536991 polymorphism was not however nominally associated with BMI (Appendix S5) or body fat percentage (Appendix S10). We found no significant gene-gene interactions, whereas there was an interaction of physical activity with *UCP1* rs2071415 and *UCP3* rs2075577 polymorphisms on waist to hip ratio under codominant model (P<0.0001; FDR=0.004, Appendix S12). However, the number of individuals in the interaction group were rather low.

Interaction between UCP polymorphisms, physical activity and markers of adiposity

There was a significant interaction between physical activity and the *UCP1* rs2071415 polymorphism on waist to hip ratio (P=0.006; FDR=0.026; Figure 2). The C/C genotype was associated with higher waist to hip ratio (+ 0.067; 95%CI, 0.028 to 0.106; P = 0.003) in adolescents who spent less than 60 min/day of moderate to vigorous physical activity (n = 399). On the contrary, the C/C genotype of the *UCP1* rs2071415 polymorphism was not associated with higher waist to hip ratio (– 0.047; 95%CI, -0.099 to 0.004; P = 0.084) in adolescents who spent at least 60 min/day of moderate to vigorous physical activity (n = 290).

Discussion

The results of the present study show that the C allele of the *UCP1* rs6536991 polymorphism was associated with a lower risk of overweight and obesity (OR=0.72) in European adolescents from 9 countries. We also observed an interaction between physical activity and the *UCP1*rs2071415 polymorphism on waist to hip ratio. Adolescents meeting the daily physical activity recommendations may overcome the effect of the *UCP1* rs2071415 polymorphism on waist to hip ratio. Our results of *UCP1* polymorphisms are in agreement with others[16]. Ramos *et al.*[16] showed a significant association between the *UCP1* rs6536991 polymorphism and obesity and BMI in 352 Brazilian adults. They showed that the C allele was associated with a lower risk of obesity (OR=0.69) and a lower BMI in individuals with no obesity.

We did not find significant associations between *UCP2* or *UCP3* polymorphisms and adiposity markers, which is in contrast with the finding by Van Abeelen *et al.*[22] in Dutch men (40-80 years old). They reported a significant association between homozygosity for the minor allele (C) of *UCP3* rs2075577 polymorphism and BMI but not with other adiposity phenotypes such as waist to hip ratio. Another study[21] reported a significant association between the (*UCP3* rs2075577–*UCP2* rs660339) haplotype with BMI. The frequency of the C–T haplotype in patients with obesity was significantly higher than that seen in subjects without it. However, these results were observed in females only. These association discordances between polymorphism and phenotypes may be due to the fact that they are population dependent, with possibly different allele frequencies and penetrance in these populations. Also differences in age, inter-country differences in lifestyle behaviours and sample sizes are important.

A plausible explanation of the observed associations between polymorphisms in the *UCP1* gene and obesity phenotypes could be because this polymorphism may lead to

less functional UCPs proteins. As a result, uncoupling activity could be decreased and therefore reduce heat dissipation as well as lipid oxidation[9], leading to overweight/obesity. Recent studies have described that calcium cycling in the skeletal muscle has a similar role than UCP1 in non-shivering thermogenesis in muscle and BAT of rabbits [29]. Indeed uncoupling ATP hydrolysis in sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) mediated by sarcolipin in mice with overexpression of this small transmembrane proteolipid increased basal metabolic rate and decreased diet-induced obesity risk [30]. In line with this findings, overexpression of UCP2 was associated with an improved fatty acid oxidation [31] suggesting a possible relevant role on the muscle function because *UCP2* and *UCP3* genes are highly expressed in skeletal muscle[10]. More studies are needed to understand if sarcolipin could compensate a functional deficit of UCP1 in overweight individuals such as fatty acid utilization by UCP2.

Findings from our study should be taken with caution owing to its cross-sectional nature. Lifestyle intervention studies in adolescents are needed to determine to what extent the effect of *UCP* genes on obesity-related traits can be modified, especially in genetically predisposed individuals. Results about gene-gene interaction between *UCP1* and *UCP3* polymorphisms must be taken with caution due to low number of individuals in the interaction group with significant differences, and should be confirmed in other studies with larger sample sizes. Unfortunately, we have no information on relatedness patterns among the participants, and we do not know the ethnic/racial make-up of the sample.

In conclusion, we observed an association between the *UCP1* rs6536991 polymorphism and the risk of overweight in adolescents. Our results also suggest that

physical activity could compensate the deleterious effect of the *UCP1* rs2071415 polymorphism on adiposity markers.

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