Association between *CNTF* polymorphisms and adiposity markers in European adolescents: the HELENA study

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ABSTRACT

Aims: To examine the association between polymorphisms of the ciliary neurotrophic factor gene (*CNTF*) and total and central adiposity markers in European adolescents.

Methods: This cross-sectional study involved 1057 European adolescents aged 12-18 years, all enrolled in the *Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study*. Five polymorphisms of *CNTF* were genotyped and the weight, height, waist and hip circumference, and triceps and subscapular skinfold thickness of the subjects determined.

Results: The T allele of rs2509914, the C allele of rs2515363, and the G allele of rs2515362, were significantly associated (after Bonferroni correction) with higher values for several adiposity markers under different inheritance models. The *CNTF* CCGGA haplotype (rs2509914, rs17489568, rs2515363 rs1800169 and rs2515362) was also significantly associated with lower BMI, waist circumference, waist/height and waist/hip ratio values compare to the TCCGG haplotype under several inheritance models.

Conclusions: Three polymorphisms - rs2509914, rs2515363 and rs2515362 - and the CCGGA haplotype of *CNTF* were significantly associated with adiposity in European adolescents. These results suggest the potential role of genetics, and CTNF in particular, in the development of obesity-related phenotypes.

Keywords: CNTF, Obesity, Genetic Susceptibility, Adolescents, SNPs

Introduction

Obesity is a worldwide health problem, particularly in young people(1). The obese phenotype probably occurs as a result of complex interactions between an individual's genetic background, and environmental, behavioural and socioeconomic factors. In simple terms, however, it is the result of a chronic excess of energy intake over energy expenditure. Key factors that regulate energy intake include satiety, while energy expenditure is influenced by basal metabolism, physical activity, thermoregulation and digestive processes(2,3).

Many genes have been related to obesity, but those involved in the regulation of the appetite centre in the brain are especially important. In monogenetic obesity(4), hormones and other molecules such as leptin (LEP), leptin receptor (LEPR), pro-opiomelacortin (POMC) and prohormone convertase 1 (PCSK1) play important roles. However, polygenic obesity, the most common form, involves many more complex interactions between multiple genes and factors.

Neurotrophic factors help in the control and development of synaptic function and plasticity(5). These factors can also control body weight, and mutations of brain-derived neurotrophic factor (BDNF) and BDNF receptor (TrkB) have been associated with severe obesity in humans(6,7). Ciliary neurotrophic factor (CNTF) is a neurocytokine belonging to the interleukin 6 family mainly involved in response to injury to the nervous system (brain lesions lead to a significant increase in *CNTF* mRNA and CTNF in the hypothalamus)(2). However, it has also been related to the control of body weight. In a placebo-controlled trial, the subcutaneous administration of recombinant CNTF to people with obesity led to a reduction in food intake and subsequent weight loss via the promotion of satiety(8). The mechanisms underlying the satiety-improving role of CNTF seem to be related to similarities between the signalling cascades of leptin and CNTF,

which overlap both in receptors and mediators. Leptin inhibits orexigenic hormones such as neuropeptide Y (NPY) and agouti-related peptide (AgRP), which increase food intake, and stimulates anorexigenic factors such as α-melanocyte stimulating hormone (αMSH), which inhibits food intake(9). This raises the possibility that CNTF acts on leptin-responsive neurons to regulate satiety and body weight(2). However, while it has been reported that CNTF may reduce body weight and improve insulin action in both rodents and humans,(10) the evidence is poor (especially in humans) that endogenous CNTF signalling is involved in the control of energy balance, certainly in obesity and hyperphagia(11,12). Indeed, some studies have found an association between CNTF signalling and BMI and body weight(13), but others have not(14–16).

Data obtained in the *Healthy Lifestyle in Europe by Nutrition in Adolescence* (HELENA) study provide a means of studying the association between *CNTF* polymorphisms and 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 nine countries (Greece, Germany, Belgium, France, Hungary, Italy, Sweden, Austria and Spain), and includes information on 5 polymorphisms of the *CNTF* gene as well as markers of adiposity. To our knowledge, *CNTF* polymorphisms have not previously been identified in genome-wide association studies of body weight or body composition in people of any age.

The aim of the present study was to examine the association of *CNTF* polymorphisms with total and central adiposity markers in European adolescents.

Material and Methods

Study subjects

This cross-sectional study involved 1057 adolescents aged 12-18 years - all enrolled in the HELENA study, which was undertaken in 2006-2007 - for whom data on *CNTF* polymorphisms (rs2509914, rs17489568, rs2515363, rs1800169 and rs2515362) and adiposity markers are available. After receiving complete information about the aims and methods of the HELENA study, the study subjects and their parents or guardians provided written consent to be involved. All data examined in the present work were collected from subjects who met the general HELENA study inclusion criteria[17]. The study was performed following the guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh 2000) and Good Clinical Practice, and adhering to the legislation regarding clinical research in humans in each of the participating countries. The protocol was approved by the local human research review committees of the centres involved [18].

Assessment of adiposity

Weight and height were measured following standard methods[17]. Waist and hip circumferences were measured in triplicate using a Seca 200 inelastic anthropometric tape (Seca Deutschland, Hamburg, Germany), and used as surrogate measures of central body fat. Waist/height and waist/hip ratios were then calculated. Body mass index (BMI) was calculated as *weight in kg/(height in m)²*, and the subjects classified as being of normal weight, overweight, or obese according to Cole *et al*[19]. Skinfold thicknesses were measured to the nearest 0.2 mm in triplicate on the left side biceps, triceps, subscapularis, suprailium, thigh, and medial calf, using a Holtain caliper (Holtain Ltd, Crymych, UK). Percentage body fat was calculated from the triceps and subscapular skinfold thickness data using the equations of Slaughter *et al* [20]. Finally, the fat mass index (FMI) was calculated as *fat mass in kg/(height in m)²*.

Genotyping

Blood samples were genotyped using an Illumina System (Illumina Inc., San Diego, CA, USA) and analysed using GoldenGate software (GoldenGate Inc., San Francisco, CA, USA). The genotyping rate was ≥99.9% and all polymorphisms were in Hardy-Weinberg equilibrium (HWE) (P>0.19 in all cases; Table 1; online). All *CNTF* polymorphisms were in linkage disequilibrium (LD) (Figure 1; online).

Statistical analysis

Deviations from Hardy-Weinberg equilibrium (HWE) were determined by means of an exact test considering a p value of 0.05 as a threshold. Associations between polymorphisms and adiposity markers were assessed via linear regression models. Five inheritance models (dominant, recessive, log-additive, codominant and overdominant) were contemplated in all analyses. The adjustment variables were age, sex and centre. The influence of each polymorphism on the phenotypic variables measured was examined using the likelihood ratio test (LRT), employing a model contemplating the polymorphism and a null model without it. Analyses were performed using the SNPassoc R package(21). We considered the associations between all single nucleotide polymorphisms (SNPs) and each phenotype under a given inheritance model as the family test, i.e., the number of tests was equal to the number of SNPs analysed. Therefore, a corrected P value for multiple comparison following the Bonferroni method would be 0.01 (0.05/5). Significant associations were selected to perform further haplotype analysis, i.e. only SNPs and phenotypes significant associated were considered for next analyses. Given these associations were used in further analyses and the linkage disequilibrium existent between gene polymorphisms (see below), which reduces the number of independent tests, we performed a exploratory selection of associations using

a method to control the expected proportion of false positives (False Discovery Rate [FDR])(22,23) instead the Bonferroni correction, which is more stringent(24). Therefore, associations with an FDR of <0.1 were used in haplotype analyses.

LD between polymorphisms and haplotype block structures were assessed using Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview) and haplo.stats R package(25). First, haplotype blocks were generated by the four-gamete rule algorithm(26). For each block, we tested whether the observed frequencies of haplotypes were deviated from those expected under linkage equilibrium. Finally, the association between haplotypes and phenotypes was assessed using a permutation procedure. Only the additive and dominant models were contemplated given the low frequency of some haplotypes. For significant associations, regression analyses were performed between haplotypes and phenotypes with the purpose of testing significant differences between haplotype levels. Given the low number of comparisons (only four haplotypes, disregarding those with very low frequencies), the P values were not corrected for comparisons between haplotype levels.

Results

Table 2 shows the anthropometric characteristics of the study sample.

Association between CNTF *polymorphisms and adiposity markers*

Three (rs2509914, rs2515363 and rs2515362) of the five analysed SNPs showed significant associations with adiposity markers after Bonferroni correction (Figures 2,3,4). Significant associations after less stringent correction (FDR<0.05) are shown in the supplementary appendix (Figures 1-6), along with the P values for all associations (Figures 7-14).

An association was seen between the minor T allele of the rs2509914 polymorphism and increased BMI under the recessive inheritance model (P=0.003), with a larger waist circumference under the codominant, recessive and additive models (P=0.00107, P=0.00034 and P=0.00071, respectively), with a higher waist/height ratio under the codominant, recessive and additive models (P=0.00013, P=5e-05 and P=8e-05, respectively), and with a higher waist/hip ratio the under dominant, codominant, recessive and additive models (P=0.00305, P=0.00262, P=0.00736 and P=0.00056 respectively) (Figure 2).

We also observed an association between the minor C allele of the rs2515363 polymorphism and higher BMI under the codominant and recessive models (P=0.00841 and P=0.00204 respectively), with a higher waist circumference under the codominant, recessive and additive models (P=0.00082, P=0.00023 and P=0.00065 respectively), with a higher waist/height ratio under the codominant, recessive and additive models (P=0.00012, P=4e^{-05} and P=8e^{-05}, respectively), and with a higher waist/hip ratio under the dominant, codominant, recessive and additive models (P=0.0035, P=0.00303, P=0.00775 and P=0.00066 respectively) (Figure 3).

Finally, an association was seen between the minor G allele of the rs2515362 polymorphism and a higher BMI under the codominant and recessive models (P=0.00781 and P=0.00188, respectively), with a higher waist circumference under the codominant, recessive and additive models (P=0.00087, P=0.00027 and P=0.00059 respectively), with a higher waist/height ratio under the codominant, recessive and additive models (P=0.00011, P=4e-05 and P=7e-05, respectively), with a higher hip circumference under the recessive model (P=0.00961), and with a higher waist/hip ratio under the dominant, codominant and additive models (P=0.00254, P=0.00291 and P=0.00065 respectively) (Figure 4).

Association between CNTF polymorphism haplotypes and adiposity markers

The *CNTF* block contained the rs2509914, rs17489568, rs2515363 rs1800169 and rs2515362 polymorphisms (Figure 1; online). The *CNTF* CCGGA haplotype was significantly associated with a lower BMI (*under the dominant inheritance model*, global: P=0.0098, difference between groups 0.04, 95%CI =0.01-0.06, P=0.0019; *under the additive inheritance model*, global P=0.04555, difference between haplotype groups 0.02 kg/m² 95%CI=0.00-0.03, P=0.018; differences between groups obtained from models using a response variable log transformed), with lower waist circumference (*under the dominant inheritance model,* global P=0.0049, difference between haplotype groups 0.02, 95%CI=0.01-0.03, P=0.004; *under the additive model*, global P=0.00925, difference between haplotype groups 0.02 cm; 95%CI=0.01-0.03, P=0.0003), with lower waist/height ratio (*under the dominant inheritance model*, global P=$7e^{-04}$, difference between halplotype groups 0.02, 95%CI =0.01-0.03, P=0.0012; *under the additive model,* global P=0.002, difference between haplotype groups 0.02; 95%CI=0.01-0.03, P=0.0001) and with a lower waist/hip ratio (*under the dominant model*, global P=0.01305, difference between haplotype groups 0.01, 95%CI=0.01-0.02, P=0.0005; *under the additive model*, global P=0.01455, difference between haplotype groups 0.01, 95%CI =0.01-0.02, P=0.0002).

Discussion

The present results reveal a solid association between the rs2509914, rs2515363 and rs2515362 *CNTF* polymorphisms and the adiposity markers BMI, waist circumference, waist/height ration and waist/hip ratio in European adolescents. An association was also seen between the *CNTF* CCGGA haplotype (rs2509914, rs17489568, rs2515363,

rs1800169 and rs2515362) and BMI, waist circumference, and the waist/hip ratio and waist/height ratios. Taken together, these findings suggest a role for CNTF in body weight regulation in adolescents.

To our knowledge, only one study has examined the association between *CNTF* polymorphisms and adiposity markers in humans. Heidema *et al*.(27) followed a cohort of stable weight and weight-gaining Dutch adults (N=545), and found a significant association between the A allele of rs1800169 and weight gain (odds ratio=2.15, 95%CI: 1.27–3.64, P=0.004) in women. These findings partially concur with the present results, which showed an association of the G allele of rs1800169 with reduced adiposity marker values. However, this association was observed for both females and males. It may be that the mentioned *CNTF* polymorphisms and haplotype are associated with the altered expression of CNTF proteins, predisposing their possessors to weight gain. *CNTF* plays a role in the regulation of body weight(2), and high levels of *CNTF* due to a neuronal injury-induced inflammatory response and fever(28) leads to weight loss. The administration of recombinant *CNTF*(8) causes the same. Such weight loss is more likely to occur through satiety mechanisms given the similarity between the *CNTF* and leptin signalling cascades: there is a close relationship between the leptin receptor and the *CNTF* receptor complex, an overlap between *CNTF* and leptin activator molecules (e.g., *STAT3*) and *CNTF* and leptin receptors have overlapping distributions in some hypothalamic nuclei involved in feeding control(2). These similarities might explain the underlying mechanism through which these *CNTF* polymorphisms lead to functionally altered CNTF proteins and therefore effect satiety and weight control.

The present results should be taken with caution given the cross-sectional nature of the study and potential variability in the ethnic make-up of the study sample, which was unknown.

In conclusion, an association was seen between the rs2509914, rs2515363 and rs2515362 polymorphisms of *CNTF* and adiposity markers in adolescents from nine European countries. We also observed an association between a *CNTF* haplotype (rs2509914, rs17489568, rs2515363, rs1800169 and rs2515362) and several adiposity markers. These findings suggest that *CNTF* may play an important role in predisposition to the appearance of overweight/obesity in adolescents.

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