Peptides 45 (2013) 22–27

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Influence of variants in the *NPY* gene on obesity and metabolic syndrome features in Spanish children

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ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 16 April 2013
Accepted 17 April 2013
Available online 25 April 2013

Keywords:
Obesity
NPY variants
Metabolic syndrome features
Children

ABSTRACT

Variants in the neuropeptide Y (*NPY*) gene have been associated with obesity and its traits. The objective of the present study was to evaluate the association of single nucleotide polymorphisms (SNPs) in the *NPY* gene with obesity, metabolic syndrome features, and inflammatory and cardiovascular disease (CVD) risk biomarkers in Spanish children. We recruited 292 obese children and 242 normal-body mass index (BMI) children. Height, weight, BMI, waist circumference, clinical and metabolic markers, adipokines, and inflammatory (PCR, IL-6, IL-8 and TNF-α) and CVD risk biomarkers (MPO, MMP-9, sE-selectin, sVCAM, sICAM, and PAI-1) were analyzed. Seven SNPs in the *NPY* gene were genotyped. The results of our study indicate that anthropometric measurements, clinical and metabolic markers, adipokines (leptin and resistin), and inflammatory and CVD risk biomarkers were generally elevated in the obese group. The exceptions to this finding included cholesterol, HDL-c, and adiponectin, which were lower in the obese group, and glucose, LDL-c, and MMP-9, which did not differ between the groups. Both rs16147 and rs16131 were associated with the risk of obesity, and the latter was also associated with insulin resistance, triacylglycerols, leptin, and HDL-c. Thus, we confirm the association of rs16147 with obesity, and we demonstrate for the first time the association of rs16131 with obesity and its possible impact on the early onset of metabolic syndrome features, mainly triacylglycerols, in children.

1. Introduction

Neuropeptide Y (NPY) is a peptide that acts as a neurotransmitter or neuromodulator. It has been implicated in several human diseases including obesity, alcoholism, schizophrenia, and depression, each of which might be considered to have behavioral or psychiatric components [3]. The role of NPY in the hypothalamic control of energy balance is well established. When this potent orexigenic neuropeptide is chronically administered to the central nervous system, it leads to an increase in food intake, body weight, and adiposity in rats [25,29]. The obesity induced by NPY is due not only to hyperphagia but also to increased accumulation of lipids in white adipose tissue, inhibition of thermogenesis in brown adipose tissue, stimulation of hyperinsulinemia, and hypersecretion of corticosteroids [1,21]. Although there is extensive evidence of the key role of NPY in energy regulation in rats, evidence in humans is limited.

The first studies showing a positive association of an *NPY* gene variant (-880I/D) with obesity were those performed by Bray et al. [2] in Mexican-American families. Additionally, there have been many studies examining the functional Leu7Pro polymorphism (rs16139). This SNP has been associated with a large number of conditions related to obesity and metabolic syndrome traits, including increased body mass index (BMI) in adults [6], development of obesity in young adults [28], risk of hypertension [11], high plasma

Abbreviations: NPY, neuropeptide Y; SNP, single nucleotide polymorphism; TAG, triacylglycerols; LDL-c, low-density lipoprotein-cholesterol; BP, blood pressure; BMI, body mass index; CVD, cardiovascular diseases; IOTF, International Obesity Task Force; PAI, 1 plasminogen activator inhibitor-1; IL, interleukin; TNF-α, tumor necrosis factor alpha; sICAM-1, soluble intercellular adhesion molecule 1; sE-selectin, soluble endothelial selectin; MPO, myeloperoxidase; MMP-9, matrix metalloproteinase 9; CRP, C reactive protein; MAF, minor allele frequency; LD, linkage disequilibrium; HOMA-IR, homeostasis model assessment for insulin resistance; OR, odds ratio; CI, confidence interval; VLDL, very low density lipoproteins.

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0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.04.007

low-density lipoprotein-cholesterol (LDL-c) in children and adults [9,22], and elevated plasma triacylglycerols (TAG) [10]. This variant has been associated with metabolic syndrome in patients with coronary artery disease [15]. This SNP has also been shown to correlate with high birth body weight in preschoolers [10], the risk of an accelerated atherosclerotic process or carotid atherosclerosis in adults [11,17], and the risk of type 2 diabetes mellitus in adults [18,26].

Other studies of SNPs in the *NPY* gene have reported an association of rs16147 and rs16135 with ischemic stroke [12,13,31] and the onset of atherosclerosis [23]. Additionally, rs16147 has been associated with being overweight [8] and dietary fat intake and changes in blood pressure (BP) [32]. However, there are only two studies that have investigated the association of rs16147 with obesity; one study found no association of this variant with obesity in two different cohorts of adults [30], and the other demonstrated a significant effect of this variation on age-dependent body weight and BMI during childhood and adolescence in a German population [7]. No associations so far have been reported for rs16131. Thus, the objective of the present study was to evaluate whether some variants in the *NPY* gene, including rs16131, rs16139, and rs16147, are associated with obesity, metabolic syndrome features, and inflammatory and cardiovascular disease (CVD) risk biomarkers in a cohort of Spanish children.

2. Materials and methods

2.1. Study design

This is a case–control multicenter study in children. We recruited 292 (149 male and 143 female) obese children and 242 (135 male and 107 female) normal-BMI children, all of them of European-Caucasian heritage and aged 5–15 years between May 2007 and May 2010 in two cities in Spain (Cordoba and Santiago de Compostela). Childhood obesity was defined according to the International Obesity Task Force (IOTF) reference for children [5]. The inclusion criteria were European-Caucasian heritage and the absence of congenital metabolic diseases. The exclusion criteria were non-European-Caucasian heritage, the presence of congenital metabolic diseases (e.g., diabetes or hyperlipidemia), undernutrition, and the use of medication that alters BP, glucose or lipid metabolism. After the initial assessments were completed at the school or primary care center, the children fulfilling the inclusion criteria were invited for a clinical examination at the appropriate participating hospital. The parents or guardians were informed about the purpose and procedures of the study before written consent was obtained, and all children gave their assent. The study was compliant with the Declaration of Helsinki (Edinburgh 2 000 revised) and followed the recommendations of the Good Clinical Practice of the CEE (Document 111/3976/88 July 1990), and the legally enforced Spanish regulation, which regulates the clinical investigation of human beings (RD 223/04 about clinical trials). The Ethics Committee on Human Research of the University of Granada, the Ethics Committee of the Reina Sofía University Hospital of Cordoba, and the Bioethics Committee of the University of Santiago de Compostela approved the study.

2.2. Anthropometric and biochemical measurements

The anthropometric measurements were taken by a single examiner at each hospital. The children were barefoot and in their underwear when the measurements were taken. Body weight (kg) was measured using a standard beam balance. Height (cm) was measured using a precision stadiometer. BMI, defined as weight (kg) divided by the square of height (m^2), was calculated. Waist circumference was measured by applying an inelastic tape horizontally midway between the lowest rib margin and the iliac crest of the standing child at the end of a gentle expiration. BP was measured three times by the same examiner using a mercury sphygmomanometer and following international recommendations [16]. The blood samples were drawn via the antecubital vein after the patient had fasted overnight. The biochemical analyses were performed at the participating University Hospital Laboratories following internationally accepted quality control protocols.

2.3. Adipocytokines, inflammation, and CVD risk biomarkers

The adipocytokines and inflammatory and CVD risk biomarkers were analyzed using three different LINCOplex™ kits with human monoclonal antibodies (Linco Research, MO, USA) on a Luminex® 200™ System (Luminex Corporation, TX). The three kits were the following: (1) Adiponectin (CV: 7.9%), resistin (CV: 6.0%), and active plasminogen activator inhibitor-1 (PAI-1) (CV: 6.6%) (Cat. HADK1-61K-A); (2) interleukin (IL)-6 (CV: 7.8%), IL-8 (CV: 7.9%), leptin (CV: 7.9%), and tumor necrosis factor alpha (TNF-α) (CV: 7.8%) (Cat. HADK2-61K-B); and (3) soluble intercellular adhesion molecule-1 (sICAM-1) (CV: 7.9%), soluble endothelial selectin (sE-selectin) (CV: 11.2%), myeloperoxidase (MPO) (CV: 12.3%), matrix metalloproteinase-9 (MMP-9) (CV: 6.8%), and total PAI-1 (CV: 11.8%) (Cat. HCVD1-67AK). C reactive protein (CRP) (CV: 4%) levels were determined with a particle-enhanced turbidimetric immunoassay (Dade Behring Inc., Deerfield, IL).

2.4. DNA isolation and genotyping

Genomic DNA was extracted from buffy coats using the QIAamp Blood kit (Qiagen, Valencia CA, USA). A total of seven SNPs in different regions of the *NPY* gene were selected from the HapMap and NCBI databases and were among those with a minor allele frequency (MAF) higher than 0.05 and a minimum pair wise linkage disequilibrium (LD) of $r^2 = 0.8$ for the Caucasian population. Genotyping was performed with the Illumina GoldenGate (Illumina, San Diego, CA, USA) protocol on 96-well format Sentrix® arrays. Two hundred and fifty nanograms of DNA sample were used per assay.

The genotyped SNPs had genotype success rates of >95%, with the exception of rs16148 (84.5%), which was excluded from further analyses. The Hardy–Weinberg equilibrium for each SNP was examined; rs16479 and rs16139 were excluded from the study because they did not reach equilibrium in the normal-BMI group. For this reason, we can neither confirm nor refute the association of the widely studied rs16139 with obesity and/or its traits in our population. The allele frequencies of the SNPs examined in the current study were similar to those reported for Caucasians in the HapMap (data not shown).

2.5. Statistical analysis

All continuous variables were expressed as the mean ± SEM. Normality distribution was assessed by the Kolmogorov–Smirnov test. Insulin, homeostasis model assessment for insulin resistance (HOMA-IR), total cholesterol, MMP-9, and total PAI-1 were logarithmically transformed, as they did not follow normality. Homogeneity of variances was estimated using the Levene test. Mean comparisons between obese and normal-BMI children for continuous variables were compared by Student's *t*-test for unpaired samples. The genotypic relative risk was assessed by comparing the obese group with the normal-BMI group and calculating the odds ratio (OR) and the 95% confidence interval (CI) using logistic regression analysis under an additive model adjusted by age and sex. Linear or logistic regressions in the entire population were performed under an additive model to estimate the association