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Neuropeptide Y gene-by-psychosocial stress interaction effect is associated with obesity in a Korean population

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ABSTRACT

Background: Chronic psychosocial stress is a crucial risk factor in the development of many diseases including obesity. Neuropeptide Y (*NPY*), distributed throughout the peripheral and central nervous system, is believed to pay a role in the pathophysiologic relationship between stress and obesity. Although several animal studies have investigated the impact on obesity of interactions between *NPY* single nucleotide polymorphisms (SNPs) and stress, the same remains to be analyzed in humans.
Methods: To identify *NPY* gene-by-stress interaction effects on human obesity, we analyzed the interaction between four *NPY* SNPs and stress with obesity-related traits, including visceral adipose tissue (VAT). A total of 1468 adult subjects were included for this analysis.
Results: In a SNP-only model without interaction with stress, no significant SNPs were found ($p_{SNP} > 0.05$). However, *NPY* SNPs-by-stress interaction effects were significantly linked to body mass index (BMI), waist circumference, and VAT ($p_{int} < 0.05$), even though a significant interaction effect for rs16135 on BMI was not identified. These significant interaction effects were also detected in interaction results for the binary traits of obesity. Among the obesity traits, mean changes of VAT by increased stress levels in homozygous risk allele carriers were the greatest (range of mean increases for four SNPs (min-max) = 12.57 cm^2 – 29.86 cm^2).
Conclusions: This study suggests that common polymorphisms for *NPY* were associated with human obesity by interacting with psychosocial stress, emphasizing the need for stress management in obesity prevention.

1. Introduction

Psychological stress occurs when there is a mismatch between environmental demands and a person's capacity to cope with these demands (Cohen et al., 2007). During stressful events, the human body responds to the stress by activating the hypothalamic-pituitary-adrenocortical axis (HPA) and the sympathetic-adrenal-medullary system. Prolonged or repeated stress leads to susceptibility to and the development of various pathological conditions, such as vascular disorder, metabolic abnormalities, and depressive symptoms (Charmandari et al., 2005; Cohen et al., 2007).

It has been proposed that chronic stress maybe involved in the etiology of obesity (Bjorntorp, 2001; Torres and Nowson, 2007). Much evidence has demonstrated that stress-induced elevation of glucocorticoids (mainly cortisol in humans), the products of HPA axis activation, stimulate appetite or fat- or sugar-rich food intake or contribute to leptin resistance, resulting in obesity-related phenotypes including weight gain, increased visceral adipose tissue (VAT), and fat storage (Dallman et al., 2006; Sominsky and Spencer, 2014). Within this context, a growing number of studies have indicated a role for *neuropeptide Y* (*NPY*) in mediating the link between chronic stress and obesity (Jeanrenaud and Rohner-Jeanrenaud, 2000; Kuo et al., 2007, 2008; Yeung et al., 2011; Zhang et al., 2014).

NPY is a 36-amino acid peptide derived from the brain and sympathetic nerves that has potent orexigenic/lipogenic properties (Tatemoto et al., 1982; Kuo et al., 2007). *NPY* also play a crucial role in reinforcement of addictive drugs by regulating dopamine levels (Sørensen et al., 2009). Under stressful conditions, glucocorticoids stimulate *NPY* release, leading to an increased appetite and feeding behaviors, and consequently, to fat gain (Jeanrenaud and Rohner-Jeanrenaud, 2000; Yeung et al., 2011; Zhang et al., 2014). Further research was conducted by Kuo et al. (2007) using a rodent model of stress (cold exposure or aggressor stress). This indicated that there might be another potential mechanism by which *NPY*

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and *NPY-Y2* receptors are upregulated by a stressor in fat tissue to induce metabolic syndrome-like symptoms, including visceral obesity, by stimulating angiogenesis and adipogenesis without changes in body weight or food intake (Kuo et al., 2007). Furthermore, *NPY* inhibits lipolysis and promotes lipogenesis in adipose tissue (Park et al., 2014).

To date, effects of *NPY* in stress-induced obesity have mainly been investigated via animal studies (Bernier et al., 2004; Ruohonen et al., 2012; Zhang et al., 2014). Recently, in human model, Aschbacher et al. demonstrated that the combination of chronic stress and highly palatable foods was associated with increased metabolic risk including abdominal adiposity, emphasizing the mechanistic role of peripheral *NPY* levels in this process (Aschbacher et al., 2014). However, there is little published evidence regarding the impact on human obesity of single nucleotide polymorphisms (SNPs) and stress interactions.

In this study, we hypothesized that exposure to psychological stress could be associated with obesity by interacting with *NPY* SNPs. We conducted *NPY* SNPs-by-stress interaction analysis for obesity-related traits including waist circumference (WC), body mass index (BMI), and VAT in a Korean population.

2. Materials and methods

2.1. Subjects

The data used for this study were collected from the Korean Association REsource (KARE) project, which is a part of the Korean Genome Epidemiology Study (KoGES) (Cho et al., 2009). The KoGES is a population-based cohort study that was designed to collect biospecimen and epidemiological data for 40–69 year-old subjects from 2001 to 2012. The KARE project was initiated in 2007 to identify the genetic architecture of various complex or clinical traits in a Korean population. In the initial KARE project, 10,038 healthy participants were recruited from among two large community-based epidemiological cohorts (the Ansan and Ansung cohorts) (2001–2002) included in the KoGES. However, VAT was not measured at baseline for these cohorts, and was included in only the 4th follow-up data collection (2009–2010) from the Ansan cohort. Therefore, a total of 1468 adults from the 4th follow-up data of the Ansan cohort, for whom accurate phenotypic information such as psychosocial stress and VAT data was available, were finally included in this study. The study was approved by the institutional review board of Seoul National University, and written informed consent was obtained from all participants.

2.2. Phenotypes

To assess the psychosocial stress levels of study subjects, we used the Psychosocial Well-being Index short form (PWI-SF) developed by Chang (2000), based on the Goldberg's General Health Questionnaire 60 (Goldberg and Hillier, 1979). This form consists of 18 items (11 positive well-being items and 7 negative feeling items such as pain, discomfort, anxiety, and depression). The stress score of each question ranged from 0 to 3, and total PWI score is the sum of each subscale. A higher PWI-SF score reflected a higher level of psychosocial stress, according to the design of the index. For the 11 well-being items, higher scores indicated higher levels of stress, whereas higher scores for the questions about negative feeling indicated lower levels of stress; therefore, each negative item such as anxiety was reverse calculated. Samples with a score under 9, 9–27, and more than 27 were divided into low, moderate, and high psychosocial stress groups, respectively (Yun et al., 2010). Anthropometric data for obesity-related traits such as weight, height, and WC were obtained, and BMI was calculated by dividing the weight (kg) by the height^2 (m^2). To assess visceral obesity, VAT was included, which was measured by computed tomography (CT). In addition, for analyses of obese group, we transformed obesity-related quantitative such as BMI, WC, and VAT to binary traits. However, to date, there is not a clear consensus on the definition of obesity cut-offs, especially in Asian population. Therefore, the subjects were categorized based on the Asia-Pacific obesity classification as follows (The Asia Pacific Perspective, 2000): the 'overall' obesity group (defined as subjects with a BMI $\geq$ 25 kg/m^2), the 'central' obesity group (defined as male subjects with WC $\geq$ 90 cm or female with WC $\geq$ 80 cm), and the 'visceral' obesity group (defined as subjects with VAT $\geq$ 100 cm^2).

2.3. SNP selection

The SNP genotypes for this study were obtained from the Affymetrix genome-wide human SNP Array 5.0 data of KARE project. The methods used for genotyping quality control (QC) are described in detail in previous GWAS study (Cho et al., 2009). Of the available genome-wide SNPs after quality control, we considered all SNPs within 10 kb up- or downstream of the *NPY* gene position on chromosome 7 to maximize the change of obtaining all potential *NPY* SNPs. Only the four SNP markers (rs16149, rs16135, rs16129, and rs16124) were included in original Affymetrix genome-wide human SNP Array 5.0 data. Therefore, we finally included a total of four SNPs in the final analysis. The three SNPs (rs16149, rs16129, and rs16124) except for rs16135 were in the strong linkage disequilibrium (LD) block (D' = 1.00 and r^2 = 0.89–0.98) (Data not shown).

2.4. Statistical analysis

We first checked the distributions of phenotypes to meet the normality assumptions, and all traits did not follow a normal distribution. All phenotypes were normalized by a square root transformation (Shapiro-Wilk or Kolmogorov-Smirnov $p > 0.05$). To compare the means or frequency of demographic variables among the three psychosocial stress groups, we performed an ANOVA test and a chi-square test using SAS software (version 9.3). The Hardy–Weinberg equilibrium (HWE) for the four SNPs was tested with PLINK software (version 1.9) before analyses (Purcell et al., 2007). We performed multiple linear regression analyses to identify gene-by-stress interactions as well as SNP-only effects for obesity-related traits. In addition, logistic regression analyses were carried out for binary obesity groups such as 'overall', 'central', and 'visceral' obesity. These results were adjusted for sex and age, and were generated under an additive model. To evaluate statistical significance, we considered a significance level of 0.05 with no multiple comparisons adjustment, due to strong LD between SNPs.

3. Results

Table 1 shows the demographic characteristics of the study subjects according to psychosocial stress type. A total of 1468 samples were included in this study. Most subjects were under moderate levels of stress (75.5%), and 212 (14.4%) were experiencing high psychosocial stress. The mean ages were significantly different among the psychosocial stress groups ($p < 0.0001$), and the low stress group had the highest mean age. Compared to the proportion of females in the total number of subjects (38.28%), the proportion of females in the higher stress group was higher (47.64%). Obesity-related traits such as BMI, WC and VAT were not significantly different among stress groups (all $p > 0.05$).

The genetic characteristics of the four SNPs selected for the *NPY* gene are shown in Table 2. Of these, rs16149 and rs16124 SNPs were located in the upstream promoter region and downstream