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The common −55 C/T polymorphism in the promoter region of the uncoupling protein 3 gene reduces prevalence of obesity and elevates serum high-density lipoprotein cholesterol levels in the general Japanese population

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Abstract

Uncoupling protein 3 (UCP3) is considered to be associated with obesity, given its function in the regulation of energy and lipid metabolism. An increased body mass index (BMI) and a decreased level of high-density lipoprotein cholesterol (HDL-C) are risk factors for cardiovascular disease. The purpose of this study was to investigate whether the UCP3 promoter −55 C/T single nucleotide polymorphism (UCP3 −55 C/T SNP) was associated with obesity according to the criteria for Japanese (BMI ≥25 kg/m^2), BMI, and serum HDL-C levels in the general Japanese population. The subjects, numbering 282 and aged 65 ± 13 years (mean ± SD), were recruited through an annual health checkup of residents of Mima city, Tokushima, in Japan. Body mass index, blood pressure, biochemical indexes including lipid, and lipoprotein profiles were measured. The UCP3 −55 C/T SNP was determined with a fluorescence-based allele-specific DNA primer assay system. The frequency of the −55 T allele was 30.0%. Subjects with the T/T genotype had significantly higher HDL-C levels than those with the C/C genotype or the C/T genotype. Furthermore, subjects with the T/T genotype had a significantly lower BMI than those with the C/C genotype. A multivariate analysis revealed that the −55 T allele was a significant independent variable contributing to the variance in HDL-C levels and BMI. The T/T genotype was associated with a lower prevalence of obesity than the C/C and C/T genotypes, with an odds ratio of 0.358 (95% confidence interval, 0.132-0.972; $P = .037$). In conclusion, the UCP3 −55 C/T SNP was associated with elevated HDL-C levels and a reduced BMI, independent of modifiable factors such as lifestyle. Furthermore, this polymorphism, when expressed in its homozygous form, reduced the prevalence of obesity in Japanese.

1. Introduction

Metabolic disorders such as obesity, diabetes mellitus, and dyslipidemia are the leading causes of atherosclerotic disorders including cardiovascular disease [1]. As a multifactorial disorder, obesity is determined by genetic and environmental factors manifesting in imbalances in energy intake and expenditure [1]. Energy homeostasis is maintained by signals from feedback loops that regulate food intake, energy expenditure, and lipid and energy metabolism. The variation in the genetic factors involved in these pathways may influence the development of obesity [2].

Uncoupling protein 3 (UCP3) is a mitochondrial anion carrier protein with a highly selective expression in skeletal muscle, a major site of thermogenesis in humans, which makes an attractive target for studies into the regulation of body weight [3]. Reduced function or expression of UCP3

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decreases energy expenditure and increases the propensity to store energy as fat [4]. In rodents, UCP3 expression is regulated by thyroid hormone, β_3-adrenergic agonists, and high-fat feeding [5,6]. Transgenic mice overexpressing UCP3 in muscle are lean and resistant to diet-induced obesity [7]. In humans, increased expression of UCP3 messenger ribonucleic acid (mRNA) in muscle is related to an increase in the metabolic rate during sleeping and reduced body mass index (BMI) [8]. Uncoupling protein 3 has also been shown to be involved in the handling of fatty acids to maintain mitochondria oxidative capacity in humans [9-11]. Therefore, UCP3 may be involved in obesity, given its function in the regulation of energy and lipid metabolism.

In addition to some single nucleotide polymorphisms (SNPs) in UCP1 and UCP2 genes, the common C→T SNP at position −55 in the promoter region of the UCP3 gene (UCP3 −55 C/T SNP) is proposed as a candidate determinant of the prevalence of obesity. This polymorphism is associated with increased expression of UCP3 mRNA in the skeletal muscle of Pima Indians [12]. Although it was reported that morbidly obese subjects with the homozygous form of the UCP3 −55 C/T SNP had an increased BMI [13], other investigators found that the −55 T allele was associated with a reduced BMI in general populations [14,15]. In addition, no association between the UCP3 −55 C/T SNP and BMI was observed [16,17]. Furthermore, the genetic association of the UCP3 −55 C/T SNP with lipid levels has not been clarified. A decreased level of high-density lipoprotein cholesterol (HDL-C) in serum is a major risk factor for atherosclerotic disease such as cardiovascular disease [18]. In addition, the role of obesity in the pathogenesis of cardiovascular disease may be mediated through its association with impaired glucose tolerance or HDL-C levels [19-21]. Levels of HDL-C are known to be regulated by environmental variables [22,23], but genetic factors also play a significant role. To the best of our knowledge, it remains unclear whether the UCP3 −55 C/T SNP itself influences HDL-C levels, independent of nonmodifiable factors such as age and sex and modifiable factors such as lifestyle.

Accordingly, the major purpose of this study was to investigate whether the genetic effects of the UCP3 −55 C/T SNP were associated with the prevalence of obesity, BMI, and HDL-C levels in the general Japanese population.

2. Methods

2.1. Subjects

We studied 282 subjects, aged 65 ± 13 years (mean ± SD) and including 124 men and 158 women, recruited through an annual health checkup of residents of Miwa city, Tokushima, in Japan. Related subjects in the same family were not included. The study protocol was approved by the ethics committee of the National Hospital Organization Kyoto Medical Center. All subjects provided written informed consent before being enrolled in the study. To assess lifestyle habits, each of the participants filled out a self-reported questionnaire that included questions regarding the drinking of alcohol, smoking, exercise habits, and other lifestyle-related factors. Drinking was assessed from the frequency of drinking and the amount of alcohol consumed on a weekly basis. With respect to smoking, individuals were classified as a nonsmoker, a past smoker, or a current smoker. Exercise habits were determined from the frequency of physical exercise of more than 3 metabolic equivalents on a weekly basis. Other lifestyle-related factors including the consumption of balanced meals, a daily breakfast, snacks between meals, and beverages containing caffeine were also checked. Eligible subjects had no clinical features of metabolic, kidney, or cardiovascular disease; had no history of diabetes; and were not taking medication known to influence weight loss, blood pressure, and glucose levels. After an overnight fast, body weight was measured using a body fat analyzer (HBS-354-W OMRON, Kyoto, Japan); and BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Obesity was determined as a BMI $\geq$25 kg/m^2 according to the criteria for Japanese [24], and stable BMI levels during a 1-year period before the study recruitment were confirmed in each subject. After the measuring of body weight, blood pressure was measured 3 times at 10-minute intervals using a mercury sphygmomanometer. Venous blood samples were then collected for analysis.

2.2. Serum lipids and lipoprotein cholesterol analyses

Serum total cholesterol (Wako Pure Chemical Industries, Osaka, Japan), HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglyceride levels were determined by enzymatic methods (Daiichi Pure Chemicals, Tokyo, Japan). Blood glucose was measured by the hexokinase method (SHINO-TEST, Tokyo, Japan).

2.3. Genotyping of the −55 C/T polymorphism in the UCP3 gene

A noninvasive genotyping method has been implemented for carefully collecting buccal mucosa cells using cytobrushes without contamination [25]. After the phenol extraction procedure, 0.2 to 2 μg of DNA per subject was obtained. Genotypes were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) [26]. The polymorphic region of UCP3 was amplified using the polymerase chain reaction with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'-AAG GTT TCA GGT CAG CxC G-3') or Texas red (5'-AAG GTT TCA GGT CAG CxT G-3') and with an antisense primer labeled at the 5' end with biotin (5'-TGG CTT GGC ACT