Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 410–415
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

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

Taku Hamada[a], Kazuhiko Kotani[a,b], Shinji Fujiwara[c,d], Yoshiko Sano[a], Masayuki Domichi[a], Kokoro Tsuzaki[a], Naoki Sakane[a,*]

[a]*Department of Preventive Medicine and Diabetes Education, Clinical Research Institute for Endocrinology and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan*
[b]*Division of Health Administration and Promotion, Faculty of Medicine, Tottori University, Tottori 683-8503, Japan*
[c]*Mima City National Health Insurance Koyadaira Clinic, Tokushima 777-0302, Japan*
[d]*Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, Tochigi 829 0498, Japan*

Received 25 June 2007; accepted 30 October 2007

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

* Corresponding author. Tel.: +81 75 641 9161; fax: +81 75 645 2781.
E-mail address: nsakane@kyotolan.hosp.go.jp (N. Sakane).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.10.019

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

GGT CTT A-3'). The reaction mixture (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 3.5 mmol/L OF $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol was an initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 68°C for 30 seconds; and a final extension at 68°C for 2 minutes. The amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was then placed on a magnetic stand; and the supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/L NaOH and measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

2.4. Statistical analysis

All of the statistical analyses were performed with the Statistical Package of Social Science (SPSS for Windows, version 11.0; SPSS, Chicago, IL). Data are expressed as means ± SD. The allele frequency was determined by gene counting. Deviations in genotype distribution from Hardy-Weinberg equilibrium were analyzed. A 1-way analysis of variance was used to analyze the difference in continuous variables, and the Tukey post hoc test was used to determine the source of significant variance among the genotypes. Differences in sex and smoking between the genotypes or obesity were assessed with the χ^2 test. A multiple regression analysis was used to evaluate the impact of the variant of the UCP3 −55 C/T SNP, age, sex, and lifestyle factors such as BMI, exercise habits, drinking, and smoking on serum HDL-C levels. A P value less than .05 was accepted as statistically significant.

3. Results

The frequency of the UCP3 genotype is shown in Table 1. The C/C genotype had an overall frequency of 49.3%, whereas the C/T genotype and the T/T genotype frequencies were 40.8% and 9.9%, respectively. There was no notable difference in genotype distribution between sexes. The genotype frequency did not differ from those expected for Hardy-Weinberg equilibrium, and the frequency of the −55 T allele (30.0%) was similar to those reported in other studies including Japanese subjects [15,17,27]. There was no significant difference in age, BMI, blood pressure, fasting blood glucose, hemoglobin A_{1c}, total cholesterol, LDL-C, HDL-C, and triglyceride between the C/C and C/T genotypes. However, it is notable that subjects with the T/T genotype had a significantly lower BMI than those with the C/C genotype. Furthermore, subjects with the T/T genotype had significantly higher HDL-C levels. With respect to lifestyle-related factors including exercise habits, alcohol intake, smoking, balanced meals, a daily breakfast, snacks between meals, beverages containing caffeine, and medications for hyperlipidemia, there was no difference between genotypes (data not shown).

In Table 2, a multiple regression analysis revealed that the −55 T allele was significantly associated with elevated serum HDL-C levels and a reduced BMI when adjustments were made for age, sex, and lifestyle factors such as BMI, exercise habits, alcohol intake, and smoking. Furthermore, sex, BMI, alcohol drinking, and smoking were significant and independent factors contributing to the variance in elevated HDL-C levels.

In Fig. 1, the prevalence of obesity was assessed according to the presence of UCP3 genotypes (C/C + C/T vs T/T). Of 282 subjects, 35.8% (n = 101) were obese and 64.2% (n = 181) were nonobese. The odds ratio for obesity (95% confidence interval [CI]) according to the presence of homozygosity for UCP3 −55 C/T SNP was 0.358 (0.132-

Table 1
Clinical and biochemical characteristics of Japanese subjects according to the genotype of the UCP3 −55 C/T polymorphism

UCP3 genotype	Wild-type C/C	Heterozygous C/T	Homozygous T/T	*P*		
				C/C vs C/T	C/C vs T/T	C/T vs T/T
n	139	115	28	NS	NS	NS
Sex (male/female)	63/76	48/67	13/15	NS	NS	NS
Smoking (never, past/current)	117/22	99/16	25/3	NS	NS	NS
Age (y)	65 ± 13	65 ± 13	66 ± 14	NS	NS	NS
BMI (kg/m^2)	24.3 ± 3.0	24.2 ± 3.0	22.6 ± 3.2	NS	.015	NS
Systolic blood pressure (mm Hg)	136 ± 18	140 ± 21	138 ± 22	NS	NS	NS
Diastolic blood pressure (mm Hg)	76 ± 12	78 ± 11	78 ± 12	NS	NS	NS
Fasting blood glucose (mmol/L)	5.7 ± 1.9	5.5 ± 1.7	5.2 ± 1.1	NS	NS	NS
HbA_{1c} (%)	5.6 ± 1.1	5.6 ± 1.3	5.4 ± 0.7	NS	NS	NS
Total cholesterol (mmol/L)	4.81 ± 0.93	4.86 ± 0.86	4.80 ± 1.09	NS	NS	NS
LDL-C (mmol/L)	3.1 ± 0.8	3.2 ± 0.7	2.9 ± 0.9	NS	NS	NS
HDL-C (mmol/L)	1.36 ± 0.38	1.38 ± 0.32	1.61 ± 0.47	NS	.004	.009
Triglyceride (mmol/L)	2.54 ± 1.14	2.81 ± 1.59	2.20 ± 0.80	NS	NS	NS

Values are the means ± SD. The differences among genotypes were analyzed by the χ^2 test or the Tukey test after one-way analysis of variance. HbA_{1c} indicates hemoglobin A_{1c}; NS, not significant.

Table 2
Multiple regression analysis with HDL-C levels and BMI as the response variable

Explanatory variable	HDL-C levels		BMI	
	Regression coefficient (95% CI)	*P*	Regression coefficient (95% CI)	*P*
Age	−0.124 (−0.248 to 0.000)	.051	−0.045 (−0.074 to −0.016)	.021
Sex	12.004 (8.206 to 15.803)	.004	1.349 (0.422 to 2.275)	.005
Exercise habits	0.638 (−0.360 to 1.636)	.209	0.171 (−0.059 to 0.400)	.145
Alcohol drinking	3.526 (2.316 to 4.735)	.001	0.218 (−0.081 to 0.517)	.153
Smoking	−5.670 (−10.603 to −0.737)	.024	−0.669 (−1.817 to 0.478)	.252
−55 T allele	8.909 (2.578 to 12.662)	.003	−0.528 (−1.052 to −0.003)	.049
BMI	−1.082 (−1.580 to −0.583)	.001		

Variables were scored as follows: habitual exercise—every day = 0, twice a week = 1, once a week = 2, once a month = 3, no exercise = 4; alcohol drinking—every day = 0, 4 to 5 times a week = 1, 2 to 3 times a week = 2, once a week = 3, none = 4; smoking—never/past = 0, current = 1.

0.972, $P = .037$). The T/T genotype had a significantly lower prevalence of obesity than the other genotypes.

4. Discussion

In this study, the most important finding was that UCP3 −55 C/T SNP may itself elevate HDL-C levels, independent of potential confounding factors such as age, sex, BMI, habitual exercise, alcohol drinking, and smoking. In addition, the findings that significant positive associations were found between sex, BMI, alcohol drinking, and smoking and serum HDL-C levels are consistent with previous studies [22,23,28]. High-density lipoprotein mediates the transport of cholesterol from nonhepatic tissues to the liver for conversion to bile acids, a process referred to as *reverse cholesterol transport*; and a decreased plasma HDL-C level is a major risk factor for atherosclerosis and vascular disease [21,29]. Although the mechanism of the potential effects of UCP3 on serum cholesterol levels remains unclear, there is evidence to suggest that UCP2 or UCP3 influences cholesterol levels directly. The −55 C/T UCP3 promoter variant was associated with total and LDL-C levels in 1155 people [30]. A study in mice also identified coincident loci for total cholesterol and percentage body fat at a quantitative trait locus that includes the genes for UCP2 and UCP3 [31]. Moreover, UCP3 transgenic mice [7] and UCP1-overexpressing mice (under the control of the myosin light-chain 2 promoter) on a high-fat diet exhibited lower plasma total cholesterol levels but not triglyceride and nonesterified fatty acid levels [32]. In line with these studies, transgenic mice overexpressing UCP2 or UCP3 had reduced fat masses and altered LDL plus very low-density lipoprotein levels on a low-fat chow diet [33]. Furthermore, effects of the UCP2 and UCP3 genotypes on the HDL-C level and atherogenic index have been reported in the Korean population [29]. The linkage disequilibrium coefficients between the −866 G/A polymorphism in the UCP2 gene and Tyr210Tyr in the UCP3 gene were very high, indicating that influence of Tyr210Tyr in the UCP3 gene on HDL-C levels may be an effect of the highly linked −866 G/A promoter polymorphism in UCP2 [29]. These previous findings may provide clues to the association of UCP genes with the risk of atherosclerosis through their effects on HDL-C [29]. Thus, considering the potential effect of UCP3 on cholesterol levels, the mechanism may include alterations of the metabolic rate. Further study is needed to clarify these points.

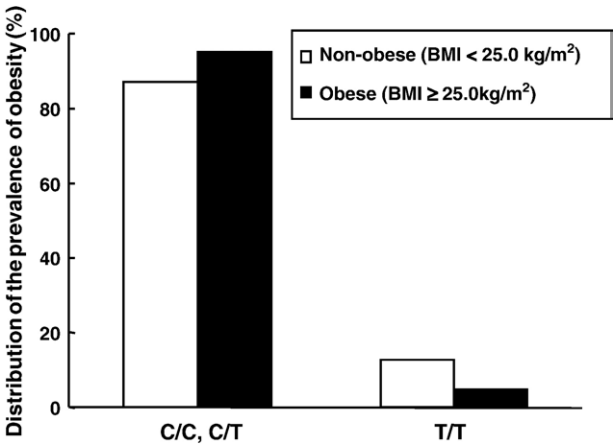

Fig. 1. Prevalence of obesity in the general Japanese population according to UCP3 genotype. Values are percentages of obesity based on BMI levels. A χ^2 analysis was performed for the nonobese (BMI <25.0 kg/m^2, n = 181) vs obese (BMI ≥25.0 kg/m^2, n = 101) subjects. The odds ratio was 0.358 (95% CI, 0.132-0.972; $P = .037$). Among the C/C and C/T genotypes combined, the number of nonobese and obese subjects was 158 and 96, respectively. In the T/T genotype, the number of nonobese and obese subjects was 23 and 5, respectively. The T/T genotype had a lower prevalence of obesity than the C/C and C/T genotypes.

We found that the UCP3 −55 C/T SNP, when expressed in the homozygous T/T genotype, was associated with a reduced prevalence of obesity. The present results suggest the homozygous form to be more important than the heterozygous form to the prevalence of obesity in Japanese. The underlying mechanism is unclear, but there is evidence to suggest that UCP3 plays an important role in protection against obesity. In nondiabetic Pima Indians, the UCP3 −55 C/T SNP contributed to variability in the expression of UCP3 mRNA in skeletal muscle [12], suggesting that the −55 T allele could increase UCP3 mRNA levels compared

with the −55 C allele. In addition, muscle UCP3 mRNA levels were positively related with the sleeping metabolic rate in Pima Indians; and a decrease in muscle UCP3 mRNA levels was associated with an increase in BMI, which is conceived to be due to an observed reduction in fat oxidation rates [8]. Therefore, a possible explanation for the present results is that the homozygous form of the UCP3 −55 C/T SNP may reduce the prevalence of obesity by effect through an increase in UCP3 mRNA levels, which, if similar to the situation in Pima Indians, is associated with increased oxidation of fat and a reduced BMI. Further studies are needed to assess the metabolic rate and substrate oxidation of the homozygous form by examining its direct effect on the UCP3 −55 C/T SNP.

The present study had a limited number of subjects. Earlier studies on associations between the UCP3 gene and obesity have yielded inconsistent results. For example, in a French cohort of morbidly obese subjects, the −55 T/T genotype was associated with increased BMI [13]. Other investigators found that the −55 T allele was associated with a reduced BMI in general populations [14,15]. However, in Danish white subjects and in a Spanish population, no association was observed between the −55 C/T UCP3 SNP and BMI or long-term body weight change [16,17]. The difference in findings might reflect true variability in the association among different populations, particularly of different ethnic groups [34]. Even the importance of a causal variant might vary among different populations, especially if the variant has low genetic effects, variable penetrance, and variable allele frequencies in different populations. In this respect, confirmation of the association observed herein awaits further studies such as a meta-analysis to assess whether the UCP3 −55 C/T SNP is important in obesity and its comorbidities in a large population. Further in vitro functional analyses are also necessary to define the precise contribution of this variant to human obesity.

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. The present findings suggest the importance of the homozygous form for protection against obesity in the general Japanese population.

Acknowledgment

This study was supported in part by a grant-in-aid from the Foundation for the Development of the Community, Japan.

References

[1] Kopelman PG. Obesity as a medical problem. Nature 2000;404: 635-43.
[2] Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell 2001;104:531-43.
[3] Hesselink MK, Mensink M, Schrauwen P. Human uncoupling protein–3 and obesity: an update. Obes Res 2003;11:1429-43.
[4] Saltzman E, Roberts SB. The role of energy expenditure in energy regulation: findings from a decade of research. Nutr Rev 1995;53: 209-20.
[5] Gong DW, He Y, Karas M, Reitman M. Uncoupling protein–3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonist, and leptin. J Biol Chem 1997;272:24129-32.
[6] Matsuda J, Hosoda K, Itoh H, Son C, Doi K, Tanaka T, et al. Cloning of rat uncoupling protein–3 and uncoupling protein–2 cDNAs: their gene expression in rats fed high-fat diet. FEBS Lett 1997;418:200-4.
[7] Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, et al. Mice overexpressing human uncoupling protein–3 expression in skeletal muscle are hyperphagic and lean. Nature 2000;406:415-8.
[8] Schrauwen P, Xia J, Bogardus C, Pratley RE, Ravussin E. Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. Diabetes 1999;48:146-9.
[9] Boss O, Bobbioni-Harsch E, Assimacopoulos-Jeannet F, Muzzin P, Munger R, Giacobino JP, et al. Uncoupling protein–3 expression in skeletal muscle and free fatty acids in obesity. Lancet 1998;351:1933.
[10] Hoeks J, van Baak MA, Hesselink MK, Hul GB, Vidal H, Saris WH, et al. Effect of beta1- and beta2-adrenergic stimulation on energy expenditure, substrate oxidation, and UCP3 expression in humans. Am J Physiol Endocrinol Metab 2003;285:E775-82.
[11] Weigle DS, Selfridge LE, Schwartz MW, Seeley RJ, Cummings DE, Havel PJ, et al. Elevated free fatty acids induce uncoupling protein 3 expression in muscle: a potential explanation for the effect of fasting. Diabetes 1998;47:298-302.
[12] Schrauwen P, Xia J, Walder K, Snitker S, Ravussin E. A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians. Int J Obes Relat Metab Disord 1999;23:1242-5.
[13] Otabe S, Clement K, Dina C, Pelloux V, Guy-Grand B, Froguel P, et al. A genetic variation in the 5' flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 2000;43:245-9.
[14] Halsall DJ, Luan J, Saker P, Huxtable S, Farooqi IS, Keogh J, et al. 2001 Uncoupling protein 3 genetic variants in human obesity: the c −55t promoter polymorphism is negatively correlated with body mass index in a UK Caucasian population. Int J Obes Relat Metab Disord 2001;25:472-7.
[15] Liu YJ, Liu PY, Long J, Lu Y, Elze L, Recker RR, et al. Linkage and association analyses of the UCP3 gene with obesity phenotypes in Caucasian families. Physiol Genomics 2005;22:197-203.
[16] Alonso A, Marti A, Corbalan MS, Martinez-Gonzalez MA, Forga L, Martinez JA. Association of UCP3 gene −55C>T polymorphism and obesity in a Spanish population. Ann Nutr Metab 2005;49:183-8.
[17] Dalgaard LT, Sorensen TI, Drivsholm T, Borch-Johnsen K, Andersen T, Hansen T, et al. A prevalent polymorphism in the promoter of the UCP3 gene and its relationship to body mass index and long term body weight change in the Danish population. J Clin Endocrinol Metabo 2001;86:1398-402.
[18] Franceschini G, Maderna P, Sirtori CR. Reverse cholesterol transport: physiology and pharmacology. Atherosclerosis 1991;88:99-107.
[19] Morrison JA, Sprecher D, McMahon RP, Simon J, Schreiber GB, Khoury PR. Obesity and high-density lipoprotein cholesterol in black and white 9- and 10-year old girls: the National Heart, Lung, and Blood Institute Growth and Health Study. Metabolism 1996;45: 469-74.
[20] Glueck CJ, Taylor HL, Jacobs D, Morrison JA, Beaglehole R, Williams OD. Plasma high-density lipoprotein cholesterol: association with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62:62-9.
[21] Laskarzewski P, Morrison JA, Mellies MJ, Kelly K, Gartside PS, Khoury P, et al. Relationships of measurements of body mass to plasma lipoproteins in schoolchildren and adults. Am J Epidemiol 1980;111: 395-406.

[22] Talmud PJ, Hawe E, Robertson K, Miller GJ, Miller NE, Humphries SE. Genetic and environmental determinants of plasma high density lipoprotein cholesterol and apolipoprotein AI concentrations in healthy middle-aged men. Ann Hum Genet 2002;66:111-24.

[23] Kim SM, Han JH, Park HS. Prevalence of low HDL-cholesterol levels and associated factors among Koreans. Circ J 2006;70:820-6.

[24] The Examination Committee of Criteria for Obesity Disease in Japan, Japan Society for the Study of Obesity. New criteria for obesity disease. Japan Cir J 2002;66:987-92.

[25] Nagai N, Sakane N, Ueno LM, Hamada T, Moritani T. The −3826 A→G variant of the uncoupling protein–1 gene diminishes postprandial thermogenesis after a high fat meal in healthy boys. J Clin Endocrinol Metab 2003;88:5661-7.

[26] Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 2002;347:1916-23.

[27] Yamada Y, Metoki N, Yoshida H, Satoh K, Ichihara S, Kato K, et al. Genetic risk for ischemic and hemorrhagic stroke. Arterioscler Thromb Vasc Biol 2006;26:1920-5.

[28] Musha H, Hayashi A, Kida K, Takahashi E, Suzuki K, Kawasaki K, et al. Gender difference in the level of high-density lipoprotein cholesterol in elderly Japanese patients with coronary artery disease. Intern Med 2006;45:241-5.

[29] Cha MH, Kim IC, Kim KS, Kang BK, Choi SM, Yoon Y. Association of UCP2 and UCP3 gene polymorphisms with serum high-density lipoprotein cholesterol among Korean women. Metabolism 2007;56:806-13.

[30] Meirhaeghe A, Amouyel P, Helbecque N, Cottel D, Otabe S, Froguel P, et al. An uncoupling protein 3 gene polymorphism associated with a lower risk of developing type II diabetes and with atherogenic lipid profile in a French cohort. Diabetologia 2000;43:1424-8.

[31] Warden CH, Fisler JS, Shoemaker SM, Wen PZ, Svenson KL, Pace MJ, et al. Identification of four chromosomal loci determining obesity in a multifactorial mouse model. J Clin Invest 1995;95:1545-52.

[32] Li B, Nolte LA, Ju JS, Han DH, Coleman T, Holloszy JO, et al. Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. Nat Med 2000;6:1115-20.

[33] Horvath TL, Diano S, Miyamoto S, Barry S, Gatti S, Alberati D, et al. Uncoupling proteins-2 and 3 influence obesity and inflammation in transgenic mice. Int J Obes Relat Metab Disord 2003;27:433-42.

[34] Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177-82.