Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1591 – 1596
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Variation in the 5′-flanking region of the neuropeptide Y2 receptor gene and metabolic parameters

Etsuko Takiguchi, Chika Fukano, Yukari Kimura, Masayo Tanaka, Keiko Tanida, Hidesuke Kaji*

Division of Physiology and Metabolism, University of Hyogo, Akashi 673-8588, Japan

Received 5 October 2009; accepted 12 February 2010

Abstract

A previous report describes that neuropeptide Y (NPY)/NPY2 receptor (NPY2R) is involved in stress-induced visceral obesity. This is a report clarifying the effect on metabolic parameters of single nucleotide polymorphisms in the 5′-flanking region of *NPY2R* gene. Study participants are 317 people (98 men and 219 women, 40-79 years old) undergoing health checkups. The single nucleotide polymorphism typing of rs6857715 and rs6857530 located on the 5′-flanking region of the *NPY2R* gene was performed using allele-specific polymerase chain reaction method. Serum high-density lipoprotein cholesterol (HDL-C) level was significantly lower in men possessing rs6857715 TT genotype compared with CC and in men possessing rs6857530 GG genotype compared with AA. No significant difference was observed between each genotype and other metabolic parameters including body mass index, waist circumference, systolic and diastolic blood pressure, serum low-density lipoprotein cholesterol, triglyceride, and fasting plasma glucose. The variation in the 5′-flanking region of the NPY2R gene was associated with serum HDL-C level in men and was a predictor for serum HDL-C level independent of sex and serum triglyceride level.

1. Introduction

Metabolic syndrome (MetS) is a cluster of visceral obesity, insulin resistance, high blood pressure (BP), and dyslipidemia [1-4]. Its exact criteria are not yet uniform worldwide [5-8].

In fact, MetS is considered to result from combined effects of genetic and environmental factors. Candidate genes of MetS are quite diverse, including those described in our previous report [9-18]. It remains unknown why stress causes obesity, particularly visceral obesity. Kuo et al recently reported that adipose tissue neuropeptide Y (NPY) and NPY2 receptor (NPY2R) are involved in the development of visceral obesity in mice with high-caloric diet under stress, not only by promoting proliferation and differentiation of fat cells but also by angiogenesis in the adipose stromal tissues [19]. Secretion of catecholamines and glucocorticoid is also activated under stress, thereby causing induction of NPY/NPY2R pathway in adipose tissues [19]. In addition to stimulating food intake in the hypothalamus [20-22], this evidence supports the role of adipose tissue NPY or NPY2R in visceral obesity promotion under stress.

Direct and/or indirect action of NPY was therefore characterized as an important candidate to analyze the association between genetic variations of *NPY*/*NPY2R* and MetS. A significant association between a leucine (7) to proline (7) polymorphism in the signal peptide of NPY and birth weight, increased serum low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels, atherosclerosis, and type 2 diabetes mellitus has been reported [23-25]. Because 100% of this single nucleotide polymorphism (SNP) (rs16139) is a type A/A in Asia, the association between this SNP and MetS is unlikely even before analysis.

On the other hand, 5 SNPs, whose minor allele frequency is high in Asia, are recently reported to be associated with obesity in Europe [26-30]. Among them, rs6857715 and rs6857530, located on the 5′-flanking region

Institutional approval: Because the study involved humans, we conformed to the guiding principles of the Declaration of Helsinki; and human subjects gave informed consent to the study that has been approved by the Institutional Committee on Human Research at University of Hyogo.

* Corresponding author. Tel.: +81 78 925 9421; fax: +81 78 925 9421.
E-mail address: hidesuke_kaji@cnas.u-hyogo.ac.jp (H. Kaji).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.02.014

of the *NPY2R* gene, presented the possibility of influencing the regulation of *NPY2R* gene transcription and were considered as good candidates for association study. Therefore, SNP typing was performed to elucidate their association with metabolic parameters.

2. Subjects and methods

2.1. Subjects

Among those who came to the city health center for health checkups, 317 people (98 men and 219 women, 40-79 years old) gave their informed consent to participate in this study. Our study was approved by the research ethical committee of the University of Hyogo.

2.2. Diagnosis of MetS

Metabolic syndrome was diagnosed by the Japanese criteria [6], as follows: waist circumference (WC) ≥85 cm (male) or ≥90 cm (female), plus more than 2 of the following: (1) systolic blood pressure (SBP) ≥130 mm Hg or diastolic blood pressure (DBP) ≥85 mm Hg, (2) dyslipidemia: serum TG ≥150 mg/dL or high-density lipoprotein cholesterol (HDL-C) <40 mg/dL, and (3) fasting plasma glucose (FPG) ≥110 mg/dL. Metabolic syndrome was also diagnosed by the Asian criteria of the International Diabetes Federation (IDF) [7], as follows: WC ≥90 cm (male) or ≥80 cm (female), plus more than 2 of the following: (1) SBP ≥130 mm Hg or DBP ≥85 mm Hg; (2) dyslipidemia: serum TG ≥150 mg/dL, male HDL-C <40 mg/dL, or female HDL-C <50 mg/dL; and (3) FPG ≥100 mg/dL. The medical examination result corresponding to the encoded sign was received from the health center of the city.

2.3. SNP typing

The *NPY2R* gene is located on chromosome 4q32.1 and consists of 8.447 kilobases including 2 exons. The coding region is located on the second exon and translated to the protein consisting of 381 amino acids. A recent database (June 4, 2009) listed 88 SNPs of human *NPY2R* gene. The candidate SNPs were selected first by the following conditions: (1) with minor allele frequency more than 10% in Japan or Asia referenced by the National Center of Biological Information SNP database and (2) when linkage disequilibrium between SNPs (r^2) was calculated using Haploview programs, r^2 was more than 0.80 among SNPs located within the 20 kilobases including 5′- and 3′-flanking regions of *NPY2R* gene. In the Japanese population in Tokyo, 2 variants, rs6857715 and rs2234759, each of which was a 5′ variant, fulfilled these 2 conditions. Actually, SNP rs6857715 is reportedly associated with severe obesity in French adults and children [29]. However, no association of the other 5′ variant rs2234759 with metabolic disease has been reported. Another 5′ variant, rs6857530, is reportedly associated with type 2 diabetes mellitus in Swedish people [28] and obesity in Danish white subjects [30]. Therefore, SNPs rs6857715 and rs6857530 were selected for the association study not because they are haplotypes but because they are possibly functional or pathogenic variants. The SNPs rs6857715 and rs6857530 are, respectively, located 598 and 626 base pairs upstream from the transcription initiation site of *NPY2R* gene. The DNA was extracted using the phenol-chloroform method, as described previously [16]. Afterward, SNP typing was performed by allele-specific polymerase chain reaction (PCR) method. Forward primer (GCAGA-CACCTGTTAGGGAAATTGCTG) and reverse primer (TCTAGCTGGGCGG TCCCTGTG) were designed to amplify the 5′-region of the *NPY2R* gene including 2 SNPs by PCR. One probe matches the G allele of rs6857530 (CATGGGC**G***GCAGGATCTG), and the other matches the C allele of rs6857715 (GCTTTACCTTCT**C***GTTTGGAG). Each probe is labeled using a fluorescent dye: Texas red. Subsequently, PCR was undertaken with reagents including each probe using PCR protocol (initial denaturation for 5 minutes at 95°C, followed by 45 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 65°C, and extension for 30 seconds at 72°C, with a final extension of 2 minutes at 72°C). If the probe matches the allele, an intercalator can be inserted into the double-strand but not single-strand DNA, thereby causing fluorescent resonance energy transfer producing fluorescent signals. Full and half signals are, respectively, detectable in homozygotes and heterozygotes of the probe-matching allele.

2.4. Measurement of biochemical parameters

Serum HDL-C was measured by a direct method using a specific detergent and enzyme to quantify the specific color reaction. The normal distribution range by this method was 40 to 86 mg/dL in men and 40 to 96 mg/dL in women. Serum TG level was measured by a method using enzyme including lipoprotein lipase, glycerol kinase, and glycerol-3-phosphate oxidase. The normal distribution range by this method was 50 to 149 mg/dL. Fasting plasma glucose was measured by a hexokinase ultraviolet method by quantifying the final product levels of nicotinamide adenine dinucleotide phosphate. The normal distribution range by this method was 70 to 109 mg/dL. Serum LDL-C was measured by a direct method using a specific detergent and enzyme to quantify the specific color reaction. The normal distribution range by this method was 70 to 139 mg/dL.

2.5. Statistical analysis

Hardy-Weinberg (HW) equilibrium was calculated using χ^2 tests with an HW-*P* value cutoff of .01. The statistical differences of mean clinical as well as biochemical parameters among genotypes were analyzed by analysis of variance (ANOVA) using software (SPSS, Tokyo, Japan). Odds ratio of MetS prevalence among each genotype was statistically analyzed by the method of Mantel-Haenszel (SPSS). Multiple regression analysis was performed using

software (SPSS) to assess whether the genotypes are independent predictor for serum HDL-C level.

3. Results

There were 317 subjects, 98 men and 219 women; they were 40 to 79 years old, with a mid value of 61.3. The mid value and range of clinical and biochemical data are as follows: body mass index (BMI), 22.7 (15.8-36.1) kg/m^2; WC, 80.6 (59.5-110) cm; SBP, 132 (90-190) mm Hg; DBP, 80 (46-110) mm Hg; serum LDL-C, 131 (53.0-236) mg/dL; serum HDL-C, 64.8 (31-115) mg/dL; plasma TG, 105 (25-553) mg/dL; and FPG, 93.4 (64.0-284) mg/dL. The SNPs rs6857715 and rs6857530 were, respectively, genotyped in 99.4% and 97.5% of cases. The frequencies (percentage) of genotypes TT, TC, and CC of rs6857715 were 27.6, 44.8, and 27.6, respectively; the allele T frequency was 50. The frequencies of genotype GG, AG, and AA of rs6857530 were 29.8, 45.3, and 24.9, respectively; the allele G frequency was 52.5. No significant deviation from the HW equilibrium was found for either SNP (rs6857715, HW-P = .07; rs6857530, HW-P = .11>.01).

Table 1 presents the mean (±SD) clinical data among each genotype. Neither SNP rs6857715 nor rs6857530 showed any significant difference of clinical data. This result was not different between subjects with and without MetS (data not shown).

Table 2 represents the number and percentage of MetS defined by Japanese and IDF Asia criteria in each SNP type. Regarding rs6857715, odds ratio of MetS prevalence diagnosed by Japanese criteria was 2.13 (95% confidence interval [CI], 0.697-6.51) in genotype TT vs CC and 2.10 (95% CI, 0.740-5.95) in TC vs CC. Regarding rs6857530, odds ratio of MetS prevalence diagnosed by Japanese criteria was 1.36 (95% CI, 0.500-3.69) in genotype GG vs AA and 1.02 (95% CI, 0.39-2.68) in GA vs AA. These nonsignificant trends were similar when MetS was diagnosed by IDF Asia criteria.

Table 3 shows the mean (± SD) biochemical data among each genotype in total subjects or men and women. Serum HDL-C level was significantly lower in men possessing rs6857715 TT genotype compared with CC and in men possessing rs6857530 GG genotype compared with AA. These trends were also observed in total subjects and women (Table 3) and in subjects without MetS (data not shown). Other metabolic parameters including serum LDL-C, TG, and FPG were not significantly different among each SNP, irrespective of sex (Table 3) and of the presence or absence of MetS (data not shown).

Table 4 shows the independent factor contributing to the serum HDL-C levels. In addition to sex and serum TG, both rs6857715 and rs6857530 were independent predictor for serum HDL-C level.

4. Discussion

The ratio of genotype and allele frequency of rs6857715 (n = 315) and rs6857530 (n = 309) appeared slightly different but was in fact almost consistent with the National Center of Biological Information SNP database. In the database, the respective frequencies (percentage) of genotypes TT, TC, and CC were 15.6 to 15.9, 56.8 to 57.8, and 26.7 to 27.3; the allele T frequency was 44.3 to 44.4 in the Japanese population (n = 88-90). The respective frequencies of genotypes GG, AG, and AA were 33.3, 54.2, and 12.5; the allele A frequency was 39.6 in the Asian population (n = 48).

The present study demonstrated for the first time that both SNP rs6857715 and rs6857530 were associated with the

Table 1
Variation of the 5′-regions of the *NPY2R* gene and clinical characteristics

	rs6857715 (n = 315)			rs6857530 (n = 309)		
	TT	TC	CC	GG	GA	AA
	n = 87	n = 141	n = 87	n = 92	n = 140	n = 77
Men (%)	28.7	31.2	33.3	27.2	32.1	26.4
P value		.806			.437	
Age (y)	58.7 ± 9.6	58.8 ± 9.8	58.8 ± 8.6	58.1 ± 9.6	58.6 ± 9.8	59.1 ± 8.6
P value	.997	1.000		.912	.892	
BMI (kg/m^2)	22.8 ± 3.7	22.6 ± 3.1	22.2 ± 2.7	22.6 ± 3.6	22.5 ± 3.1	22.7 ± 2.9
P value	.364	.592		.964	.912	
WC (cm)	79.8 ± 9.6	80.5 ± 9.4	79.1 ± 8.1	79.3 ± 9.5	80.3 ± 9.3	79.9 ± 8.5
P value	.801	.414		.861	.928	
SBP (mm Hg)	133 ± 20	134 ± 19	132 ± 20	133 ± 21	134 ± 19	132 ± 19
P value	.936	.584		.950	.626	
DBP (mm Hg)	81.3 ± 11	81.1 ± 12	80.4 ± 11	80.9 ± 12	81.3 ± 12	80.5 ± 10
P value	.801	.856		.980	.830	

Statistical difference of men's ratio among each genotype was analyzed using Pearson χ^2 test. *P* value represents the statistical significance of the difference among genotype. Statistical difference of clinical characteristics (mean ± SD) among each genotype was analyzed using 1-way ANOVA. *P* value represents the statistical significance of the difference of subjects possessing rs6857715 genotype TT or TC compared with CC and those possessing rs6857530 genotype GG or AG compared with AA.

Table 2
Variation of the 5′regions of the *NPY2R* gene and MetS

	rs6857715 (n = 315)			rs6857530 (n = 309)		
	TT	TC	CC	GG	GA	AA
	n = 87	n = 141	n = 87	n = 92	n = 140	n = 77
Japanese criteria						
n (%)	10 (11.5)	16 (11.3)	5 (5.7)	11 (11.2)	13 (9.3)	7 (9.1)
Odds ratio	2.13	2.10	1	1.36	1.02	1
95% CI	0.697-6.51	0.740-5.95		0.500-3.69	0.39-2.68	
P value	.185	.163		.549	.962	
IDF Asia criteria						
n (%)	13 (14.9)	20 (14.2)	6 (6.9)	13 (14.1)	16 (11.4)	9 (11.7)
Odds ratio	2.37	2.23	1	1.03	0.97	1
95% CI	0.857-6.56	0.86-5.80		0.40-2.62	0.41-2.32	
P value	.096	.099		.639	.954	

Mantel-Haenszel method was used to analyze the statistical difference of odds ratio among TT vs CC or TC vs CC in rs6857715, and GG vs AA or GA vs AA in rs6857530.

serum HDL-C level and were its predictors, independent of sex, serum TG, and other various parameters. The mechanism of the association remains to be elucidated. Blumenthal et al [31] have previously reported that *NPY1* and *5* receptor gene variants in the untranslated region are associated with serum TG and HDL-C levels. Because of the stimulatory

Table 3
Variation of the 5′-franking region of the *NPY2R* gene and biochemical data

	rs6857715 (n = 315)			Rs6857530 (n = 309)		
	TT	TC	CC	GG	GA	AA
	n = 87 (M: 25)	n = 141 (M: 44)	n = 87 (M: 29)	n = 92 (M: 25)	n = 140 (M: 45)	n = 77 (M: 28)
LDL-C (mg/dL)						
Total	125 ± 35	128 ± 33	133 ± 32	126 ± 35	126 ± 33	135 ± 33
P value	.179	.488		.161	.119	
Men	117 ± 33	126 ± 42	127 ± 29	118 ± 33	125 ± 39	129 ± 34
P value	.537	1.000		.445	.842	
Women	128 ± 36	129 ± 29	136 ± 33	129 ± 36	127 ± 30	139 ± 32
P value	.253	.322		.219	.078	
HDL-C (mg/dL)						
Total	63.1 ± 15	66.4 ± 16	68.1 ± 14	63.9 ± 16	65.9 ± 15	68.3 ± 15
P value	.056	.627		.114	.430	
Men	52.0 ± 13	59.2 ± 12	60.1 ± 10	51.7 ± 13	59.5 ± 12	59.8 ± 10
P value	.027*	.926		.026*	.991	
Women	67.6 ± 13	69.7 ± 16	72.1 ± 14	68.5 ± 15	68.9 ± 15	73.0 ± 15
P value	.169	.519		.171	.190	
TG (mg/dL)						
Total	122 ± 69	125 ± 81	115 ± 70	126 ± 82	119 ± 71	122 ± 73
P value	.728	.471		.904	.956	
Men	162 ± 96	153 ± 99	138 ± 77	181 ± 122	138 ± 77	150 ± 88
P value	.686	.869		.389	.812	
Women	106 ± 47	112 ± 67	100 ± 55	105 ± 47	110 ± 67	105 ± 57
P value	.813	.346		1.000	.847	
FPG (mg/dL)						
Total	104 ± 27	101 ± 28	103 ± 32	102 ± 26	101 ± 28	107 ± 34
P value	.944	.868		.433	.283	
Men	108 ± 30	107 ± 42	110 ± 40	105 ± 28	106 ± 41	122 ± 48
P value	.580	.443		.220	.159	
Women	102 ± 26	98.3 ± 19	95.3 ± 18	100 ± 26	98.8 ± 19	97.7 ± 19
P value	.117	.589		.711	.944	

Statistical difference of biochemical data (mean ± SD) among each genotype was analyzed using ANOVA. M indicates men.
* $P < .05$: TT or TC vs CC; GG or GA vs AA.

Table 4
Independent factors contributing to the serum HDL-C level

	rs6857715 (n = 315)		rs6857530 (n = 309)	
	β	P value	β	P value
Age	−0.083	.128	−0.084	.130
Sex	0.228	<.0001*	0.231	<.0001*
BMI	0.021	.825	0.006	.950
WC	−0.156	.116	−0.156	.134
SBP	0.129	.100	0.138	.079
DBP	−0.057	.456	−0.066	.387
Serum LDL-C	−0.016	.751	−0.006	.905
Serum TG	−0.277	<.0001*	−0.271	<.0001*
FPG	−0.069	.170	−0.076	.134
Genetic variation	0.118	.018*	−0.128	.011*

Statistical analysis was performed using multiple regression analysis. Dependent variable is HDL-C level. Independent variables are various clinical and biochemical parameters and variation of the *NPY2R* gene. Dummy variables included sex (male, 1; female, 2) and genetic variation (rs6857715 TT:1, TC:2, CC:3; rs6857530 AA:1, AG:2, GG:3). β indicates standard coefficient value.

* $P < .05$.

effect of NPY on adipocyte lipoprotein lipase activity and the lack of association of *NPY* gene polymorphism with serum lipid levels [32], they hypothesized that this is a gain in function of *NPY1* and *5* receptor gene polymorphism.

In our study, no significant difference of BMI, WC, and MetS prevalence was observed among each SNP. These results are not necessarily consistent with the following previous reports. Siddiq et al [29] reported the association between this SNP and severe obesity in French children and adults. Campbell et al [28] reported that rs6857530 is weakly associated with obesity in men of European descent but not in women. Although the precise reason of the difference remains unknown, it may be due to the difference of ethnic group. They also reported that SNP rs6857715 was associated with the prevalence of type 2 diabetes mellitus in Swedish people but not among all Europeans, requiring further investigations to reach a convincing conclusion. In our study, FPG level tended to be higher, although it was not significant, in women possessing rs6857715 TT genotype compared with CC. The other variations of coding regions in the *NPY2R* gene, L40F, F871, and A172T, are not reportedly associated with obesity in an English population [26]; but genotype T of 585T>C is protective against obesity [27].

The sequence around rs6857715 did not include the consensus sequence possible to bind known transcription factors when assessed using software for DNA analysis (DNASIS-Mac v3.2; Hitachi, Tokyo, Japan). On the other hand, the sequence around rs6857530 included an interferon-γ responsive element. It is interesting to speculate that glucocorticoid-induced interferon-γ is involved in the regulation of *NPY2R* gene transcription in adipose tissues.

Significant associations exist between variations in the 5′-flanking region of the *NPY2R* gene and serum HDL-C level in Japanese men. Further studies are required to understand the underlying mechanism.

Acknowledgment

This study was partially supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Any financial or other relations are disclosed.

References

[1] Björntorp P. Metabolic implication of body fat distribution. Diabetes Care 1991;14:1132-43.
[2] Fryan KN. Visceral fat and insulin resistance: causative or correlative? Br J Nutr 2000;83:71-7.
[3] Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738.
[4] Matuzawa Y, Funakoshi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contributors of adipocytokine, adipocyte-derived bioactive substances. Ann N Y Acad Sci 1999;892:146-54.
[5] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
[6] Examination Committee of Criteria for the Metabolic Syndrome in Japan. Definition and diagnosis criteria of metabolic syndrome. J Jpn Soc Intern Med (in Japanese) 2005;94:794-809.
[7] Alberti KG, Zimmet P, Shaw J, Epidemiology Task Force Consensus Group. The metabolic syndrome—a new world-wide definition. Lancet 2005;366:1059-62.
[8] Kahn R, Ferrannini E, Buse J, Stern M. The metabolic syndrome: time for a critical appraisal. Diabetes Care 2005;28:2289-304.
[9] Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, et al. Quantitative trait loci on chromosome 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A 2000;97:14478-83.
[10] McCarthy JJ, Meyer J, Moliterno DJ, Newby LK, Rogers WJ, Topol EJ. Evidence for substantial effect modification by gender in a large-scale genetic association study of the metabolic syndrome among coronary heart disease patients. Hum Genet 2003;114:87-98.
[11] Lopes C, Diana C, Durand E, Froguel P. PAI-1 polymorphisms modulate phenotypes associated with the metabolic syndrome in obese and diabetic Caucasian population. Diabetologia 2003;46:1284-90.
[12] Shin HD, Park BL, Kim LH, Jung HS, Cho YM, Moon MK, et al. Genetic polymorphisms in peroxisome proliferator-activated receptor γ associated with obesity. Diabetes 2004;53:847-51.
[13] Weng SW, Liou CW, Lin TK, Wei YH, Lee CF, Eng HOL, et al. Association of mitochondrial deoxyribonucleic acid 16189 variant (T→C transition) with metabolic syndrome in Chinese adults. J Clin Endocrinol Metab 2005;90:5037-40.
[14] Sutton BS, Weinert S, Langefeld CD, Williams AH, Campbell JK, Saad MF, et al. Genetic analysis of adiponectin and obesity in Hispanic families: the IRAS family study. Hum Genet 2005;117:107-18.
[15] Baessler A, Hasinoff MJ, Fisher M, Reinhard W, Sonnenberg GE, Olivier M, et al. Genetic linkage and association of the growth hormone secretagogue receptor (ghrelin receptor) gene in human obesity. Diabetes 2005;54:259-67.
[16] Kaji H, Fukano C, Kimura Y, Takiguchi E, Tanida K. Genetic variations at the CCAAT/ enhancer-binding protein δ are associated with metabolic phenotypes in the Japanese population. Metab Synd Relat Disord 2008;6:24-31.
[17] Masuzaki H, Paterson JM, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001;294:2166-70.
[18] Morton NM, Paterson JM, Masuzaki H, Holmes MC, Staels B, Fievet C, et al. Novel adipose tissue-mediated resistance to diet-induced

visceral obesity in 11β-hydroxysteroid dehydrogenase type 1–deficient mice. Diabetes 2004;53:931-8.
[19] Kuo LE, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, et al. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. Nat Med 2007;13:803-11.
[20] Schwartz MW, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404:661-71.
[21] McMinn JE, Baskin DG, Schwarz MW. Neuroendocrine mechanisms regulating food intake and body weight. Obes Rev 2000;1:37-46.
[22] Broberger C. Brain regulation of food intake and appetite: molecules and networks. J Intern Med 2005;258:301-27.
[23] Karvonen MK, Pesonen U, Koulu M, Niskanen L, Laakso M, Rissanen A, et al. Association of a leucine (7)-to-proline (7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. Nat Med 1998;4:1434-7.
[24] Karvonen MK, Koulu M, Pesonen U, Uusitupa MI, Tammi A, Viikari J, et al. Leucine 7 to proline 7 polymorphism in the preproneuropeptide Y is associated with birth weight and serum triglyceride concentration in preschool aged children. J Clin Endocrinol Metab 2000;85:1455-60.
[25] Niskanen L, Karvonen MK, Valve R, Koulu M, Pesonen U, Mercuri M, et al. Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene is associated with enhanced carotid atherosclerosis in elderly patients with type 2 diabetes and control subjects. J Clin Endocrinol Metab 2000;85:2266-9.
[26] Hung CC, Pirie F, Luan J, Lank E, Motala A, Yeo GS, et al. Studies of the peptide YY and neuropeptide Y2 receptor genes in relation to human obesity and obesity-related traits. Diabetes 2004;53:2461-6.
[27] Lavebratt C, Alpman A, Persson B, Arner P, Hoffstedt J. Common neuropeptide Y2 receptor gene variant is protective against obesity among Swedish men. Int J Obes 2006;30:453-9.
[28] Campbell CD, Lyon HN, Nemesh J, Drake JA, Tuomi T, Gaudet D, et al. Association studies of BMI and type 2 diabetes in the neuropeptide Y pathway: a possible role for NPY2R as a candidate gene for type 2 diabetes in men. Diabetes 2007;56:1460-7.
[29] Siddiq A, Gueorguiev M, Samson C, Hercberg S, Heude B, Levy-Marchal C, et al. Single nucleotide polymorphisms in the neuropeptide Y2 receptor (NPY2R) gene and association with severe obesity in French white subjects. Diabetologia 2007;50:574-84.
[30] Torekov SS, Larsen LH, Andersen G, Albrechtsen A, Glümer C, Borch-Johnsen K, et al. Variants in the 5′ region of the neuropeptide Y receptor Y2 gene (NPY2R) are associated with obesity in 5,971 white subjects. Diabetologia 2006;49:2653-8.
[31] Blumenthal JB, Andersen RE, Mitchell BD, Seibert MJ, Yang H, Herzog H, et al. Novel neuropeptide Y1 and Y5 receptor gene variants: associations with serum triglyceride and high-density lipoprotein cholesterol levels. Clin Genet 2002;62:196-202.
[32] Erkkilä AT, Lindi V, Lehto S, Laakso M, Uusitupa MI. Association of leucine 7 to proline 7 polymorphism in the preproneuropeptide Y with serum lipids in patients with coronary heart disease. Mol Genet Metab 2002;75:260-4.