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Genetic Association Study of *THRβ* Polymorphisms with Obesity in Korean Population

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Abstract

The growing problem of obesity is associated with numerous medical conditions. Several studies have reported that activation of thyroid hormone receptor beta (*THRβ*) is involved in lipid metabolism and thermogenesis. To identify the relationship between the *THRβ* gene and obesity, we genotyped eighty two single nucleotide polymorphisms (SNPs) in the gene using the Affymetrix array chip in 209 overweight/obese and 155 normal subjects in Korean population. Of the eighty two polymorphisms, the seven SNPs exhibited a significant association with overweight/obesity in three alternative models (codominant, dominant, and recessive models; $P<0.05$ after adjusting for age and sex) were rs826221 (+267878 T>C), rs4858604 (+186399 A>G), rs1158265 (+200152 T>C), rs1868575 (+206031 G>A), rs1700939 (+238467 T>A), rs1505301 (+241933 T>C), and rs1924768 (+126491 T>C). During haplotype analysis using HapAnalyzer software, 2 haplotypes (block 13: TTAT; block 15: CTGC) containing significant polymorphisms (rs1700939 +238467 T>A and rs4858604+186399 A>G) were detected to be significantly different. The results suggest that the *THRβ* gene may be associated with overweight/obesity in Korean population.

Keywords: *THRβ*, Obesity, Overweight, Single nucleotide polymorphism, Haplotype

Obesity is a growing problem and is associated with numerous medical conditions[1]. Obese individuals have increased risk of type 2 diabetes mellitus, heart diseases, metabolic syndrome, hypertension, and stroke[2]. Obesity is commonly assessed by calculating an individual's body mass index (BMI, kg/m^2). According to the classification of Korean Society for the Study of Obesity and World Health Organization guideline, individuals with a BMI$\geq$23 are classified as overweight and those with a BMI$\geq$25 are considered obesity[3]. Based on family studies and twin study, it has been estimated that the heritability of obesity ranges form 30% to 70%[4]. Several studies have demonstrated the association between BMI or obesity and genetic variants[5,6].

Thyroid hormone (TH) plays a key role in metabolic homeostasis, and TH dysfunction affects body weight[7]. TH acts through its nuclear receptors that are encoded by 2 separate genes, thyroid hormone receptor *α* (*THRα*) and *THRβ*[8]. These genes are known for encoding receptors for fatty acids and cholesterol derivatives, retinoids, vitamin D, steroids, and bile acids[9,10]. Also, studies on THR knockout (KO) mice indicate that *THRα* and *THRβ* play specific developmental and physiological roles in target tissues[11,12]. Several researchers reported that *THRβ* is critical in controlling hepatic cholesterol metabolism and thyroid-stimulating hormone (TSH) suppression. Indeed, the THRβ-selective agonist reduces plasma cholesterol levels with cardiac effects in mice and rats[13,14]. Moreover, it has been reported that TH stimulate phosphatidylinositol-3-kinase (PI3K) activity and *THRβ* is found to interact with the regulatory subunit of PI3K. Finally, activation of the PI3K cascade plays a critical role for glucose utilization processes such as glucose uptake[15,16]. Recent study has reported that *THRβ* mediates T3-induced uncoupling protein-1 (UCP1) gene expression[12]. Although *THRβ* is involved in thermo-

genesis and lipolysis as combining with TH, no studies so far have investigated them in the context of their genetic association. To our knowledge, this is first report that *THRβ* polymorphisms are associated with susceptibility to overweight/obesity.

In this study, we investigated the association between the single nucleotide polymorphisms (SNPs) of the *THRβ* gene and overweight/obesity in Korean population.

Table 1. Clinical and biochemical characteristics of control and overweight/obese subjects.

	Control (n=155)	Overweight/obese (n=209)	*P*
Age (years)	43.41±6.03	43.61±6.20	0.081
BMI (kg/m^2)	20.14±1.22	24.53±1.09	<0.001
SBP (mmHg)	114.33±14.15	122.11±17.57	<0.001
DBP (mmHg)	71.81±11.12	76.88±10.18	<0.001
Fasting plasma glucose (mg/dL)	89.10±9.62	94.11±12.11	<0.001
HBA1c (%)	5.31±0.42	5.48±0.61	0.037
TG (mg/dL)	98.71±56.88	141.38±110.11	<0.001
TC (mg/dL)	185.11±27.52	196.22±32.65	0.003
LDL-C (mg/dL)	108.08±27.88	118.49±32.44	0.004
HDL-C (mg/dL)	56.91±14.33	48.94±10.36	<0.001

Data are means±S.D. n, number; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HBA1c, hemoglobin A1c; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol

Clinical Characteristics of Study Subjects

Table 1 show the clinical and metabolic characteristics of overweight/obese and control subjects. BMI values were used to categorize 364 subjects into control (BMI<23, n=155) and overweight/obese (BMI ≥23, n=209) subjects. The mean value of BMI in the overweight/obese group was significantly higher than that in the control subjects ($P<0.001$). The levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose, hemoglobin A1c (HbA1c) triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) in the overweight/obese subjects were higher compared to those of the control subjects ($P<0.005$). Also, the level of high-density lipoprotein cholesterol (HDL-C) was relatively lower in the overweight/obese subjects ($P<0.001$).

Associations between *THRβ* Polymorphisms and Overweight/Obese

Our sample size was small, and it is possible that the associations that we observed between *THRβ* SNPs and overweight/obesity could represent false positives. However, in our study, the power of the sample size was calculated using a genetic power calculator. Our sample provides the power more than 85% to

Table 2. Logistic regression analysis of *THRβ* polymorphisms in control and overweight/obese subjects.

Locus	Genotype	Control n=155 (%)	Overweight/obese n=209 (%)	Codominant		Dominant		Recessive	
				OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs826221	T/T	50 (35.2%)	92 (45.5%)						
(+267878 T>C)	C/T	69 (48.6%)	90 (44.5%)	**2.34 (1.15-4.74)**	**0.040**	1.67 (1.06-2.63)	**0.026**	1.86 (0.97-3.58)	0.062
	C/C	23 (16.2%)	20 (9.9%)						
rs4858604	T/T	73 (47.1%)	68 (32.9%)						
(+241933 T>C)	C/T	63 (40.6%)	106 (51.2%)	**0.53 (0.27-1.04)**	**0.031**	**0.56 (0.36-0.86)**	**0.008**	0.72 (0.39-1.34)	0.30
	C/C	19 (12.3%)	33 (15.9%)						
rs1158265	TT	55 (37.4%)	55 (26.7%)						
(+238467 T>A)	A/T	69 (46.9%)	108 (52.4%)	0.65 (0.40-1.05)	0.081	**0.62 (0.39-0.99)**	**0.044**	0.74 (0.42-1.30)	0.28
	A/A	23 (15.7%)	43 (20.9%)						
rs1868575	G/G	57 (37.0%)	45 (21.5%)						
(+206031 G>A)	G/A	68 (44.2%)	115 (55.0%)	**0.49 (0.27-0.91)**	**0.011**	**0.49 (0.30-0.78)**	**0.002**	0.78 (0.46-1.33)	0.36
	A/A	29 (18.8%)	49 (23.4%)						
rs1700939	T/T	77 (50.0%)	80 (38.5%)						
(+200152 T>C)	C/T	59 (38.3%)	102 49.0%)	0.75 (0.38-1.50)	0.124	**0.65 (0.42-1.00)**	**0.048**	0.95 (0.49-1.82)	0.88
	C/C	18 (11.7%)	26 (12.5%)						
rs1505301	A/A	35 (24.3%)	64 (34.6%)						
(+186399 A>G)	A/G	71 (49.3%)	89 (48.1%)	2.11 (1.12-3.99)	0.066	1.58 (0.96-2.59)	0.068	**1.72 (1.01-2.96)**	**0.047**
	G/G	38 (26.4%)	32 (17.3%)						
rs1924768	T/T	57 (37.0%)	92 (44.0%)						
(+12649 T>C)	C/T	76 (49.4%)	102 (48.8%)	2.38 (1.11-5.07)	0.073	1.42 (0.92-2.20)	0.110	**2.07 (1.02-4.23)**	**0.043**
	C/C	21 (13.6%)	15 (7.2%)						

Genotype distributions are shown as number (%). The first nucleotide of the transcriptional start site is denoted as +1. Genotypes with missing data were omitted for exact analysis. n, number; OR, odds ratio; 95% CI, 95% confidence interval

detect more than 1.6-fold increased risk, assuming an α-level of 0.05. Thus, our case-control study was sufficiently powerful to determine a positive association. Next, we genotyped 209 overweight/obese and 155 control subjects in order to assess whether these polymorphisms were associated with overweight and obesity. Genotype distributions of all the polymorphisms in this study were in Hardy-Weinberg equilibrium at all loci ($P > 0.05$, data not shown). Table 2 shows the genotype distributions of these SNPs in overweight/obese and control subjects and the association between each genotype and the risk of overweight/obesity by logistic regression analysis with adjustment for age and sex. Among eighty two SNPs, seven polymorphisms [rs826221 (+267878 T>C), rs1505301 (+241933 T>C), rs1700939 (+238467 T>A),

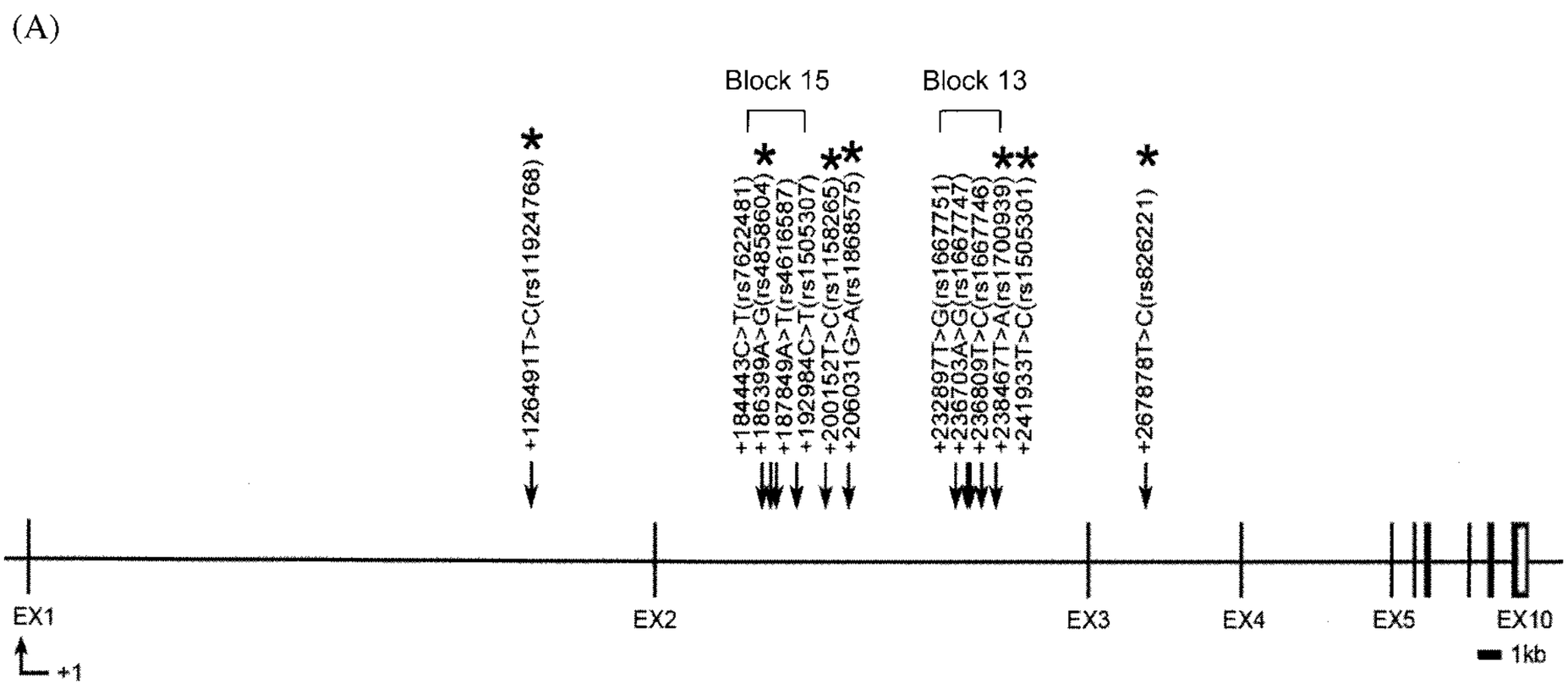

Figure 1. Gene map and linkage disequilibrium (LD) in thyroid hormone receptor beta (*THRβ*) gene. A, Gene map and single nucleotide polymorphisms (SNPs) in the *THRβ* gene on chromosome 3p24. Exons are marked with box. The coding regions are black boxes and untranslation regions are white boxes. The first nucleotide of the transcriptional start site is denoted as +1. Asterisk (*) indicates a significant SNP. Arrow indicates the location of each SNP. EX, exon. B, LD coefficient (|D'|) and LD blocks among *THRβ* SNPs. Block 13 consists of rs1667751, rs1667747, rs1667746, and rs1700939. Block 15 comprises rs7622481, rs4858604, rs4616587, and rs1505307.

Table 3. Analysis of haplotypes of LD block in overweight/obese and control subjects.

Block	Haplotype	Overweight/obese, n (%)			Control, n (%)			Codominant		Dominant		Recessive	
		H/H	H/−	−/−	H/H	H/−	−/−	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Block13	H1 (TTAT)	52 (25.9%)	107 (53.2%)	42 (20.9%)	54 (37.8%)	66 (46.2%)	23 (16.1%)	0.70 (0.51-0.96)	**0.0288***	0.73 (0.41-1.27)	0.2624	0.58 (0.36-0.91)	**0.0191***
	H2 (ATAT)	6 (3.0%)	67 (33.3%)	128 (63.7%)	2 (1.4%)	40 (28.0%)	101 (70.6%)	1.37 (0.90-2.08)	0.139	1.37 (0.87-2.17)	0.1789	2.17 (0.43-10.91)	0.3473
	H3 (ACGG)	4 (2.0%)	64 (31.8%)	133 (66.2%)	2 (1.4%)	36 (25.2%)	105 (73.4%)	1.37 (0.89-2.12)	0.1563	1.41 (0.88-2.27)	0.1516	1.43 (0.26-7.92)	0.6812
	H4 (ACAT)	0 (0.0%)	24 (11.9%)	177 (88.1%)	1 (0.7%)	10 (7.0%)	132 (92.3%)	1.45 (0.71-2.95)	0.3065	1.63 (0.77-3.44)	0.2023	–	–
block15	H1 (CTGC)	28 (15.2%)	90 (48.9%)	66 (35.9%)	31 (16.9%)	74 (40.2%)	36 (19.6%)	0.70 (0.51-0.96)	**0.0279***	0.61 (0.38-0.99)	**0.0474***	0.64 (0.36-1.12)	0.1184
	H2 (CAAT)	14 (7.6%)	80 (43.5%)	90 (48.9%)	9 (5.0%)	52 (28.3%)	80 (43.5%)	1.27 (0.89-1.81)	0.1959	1.37 (0.88-2.13)	0.1621	1.21 (0.51-2.88)	0.6697
	H3 (TAAC)	11 (6.0%)	66 (35.9%)	107 (58.2%)	5 (2.7%)	46 (25.0%)	90 (48.9%)	1.27 (0.87-1.85)	0.2186	1.27 (0.81-1.99)	0.2995	1.73 (0.59-5.10)	0.3205
	H4 (CAAC)	0 (0.0%)	20 (10.9%)	164 (89.1%)	1 (0.5%)	13 (7.1%)	127 (69.0%)	1.02 (0.51-2.03)	0.9485	1.11 (0.54-2.28)	0.7837	–	–

Haplotype distributions are shown as number (%). Block 13 consists of rs1667751, rs1667747, rs1667746, and rs1700939. Block 15 comprises rs7622481, rs4858604, rs4616587, and rs1505307. H/H, subject with homozygous for common haplotype; H/−, subject with heterozygous for rare haplotype; −/−, subject with homozygous for rare haplotype. LD, linkage disequilibrium; n, number; OR, odds ratio; 95% CI, 95% confidence interval.

Table 4. Analysis of rs1700939 and rs4858604 polymorphisms on lipid profiles in overweight/obese and control subjects.

	rs1700939 (+238467 T>A)					
	TT			AT+AA		
	Overweight/obese (n=55)	Control (n=55)	*P*	Overweight/obese (n=151)	Control (n=92)	*P*
TG (mg/dL)	128.9±74.2	98.3±46.1	0.05	133.8±90.6	97.9±64.1	<0.01*
TC (mg/dL)	193.8±31.9	184.6±26.3	0.06	196.1±34.7	185.3±31.2	0.01*
LDL-C (mg/dL)	115.7±35.2	107.0±24.4	0.13	119.2±30.3	109.4±30.5	0.02*
HDL-C (mg/dL)	51.2±10.5	54.1±12.8	0.04	50.1±11.7	56.3±13.7	<0.01*
	rs4858604 (+186399 A>G)					
	AA			AG+GG		
	Overweight/obese (n=64)	Control (n=35)	*P*	Overweight/obese (n=121)	Control (n=109)	*P*
TG (mg/dL)	131.4±113.9	107.7±52.0	0.25	143.4±127.3	96.5±60.3	<0.01*
TC (mg/dL)	201.0±34.2	192.2±26.9	0.19	193.3±32.4	182.0±29.6	<0.01*
LDL-C (mg/dL)	113.1±26.1	123.5±28.8	0.08	115.5±32.3	105.4±28.1	<0.01*
HDL-C (mg/dL)	51.2±11.1	54.7±12.5	0.17	49.1±12.1	57.2±13.7	<0.01*

Values are mean±S.D. *P* values were calculated by Mann-Whitney U Test (two-tailed). Significant difference between overweight/obese and control subjects (*$P<0.05$). n, number; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol.

rs1868575 (+206031 G>A), rs1158265 (+200152 T>C), rs4858604 (+186399 A>G), and rs1924768 (+126491 T>C)] were found to be associated with overweight/obesity (Table 2). The rare alleles of rs1505301, rs1700939, rs1868575, and rs1158265 increased the risk of overweight/obesity [rs1505301 (+241933 T>C): odds ratio (OR)=0.53, 95% confidence interval (CI) =0.27-1.04, P=0.031 in the codominant model, and OR=0.56, 95% CI=0.36-0.86, P=0.008 in the dominant model; rs1700939 (+238467 T>A): OR=0.62, 95% CI=0.39-0.99, P=0.044 in the dominant model; rs1868575 (+206031 G>A): OR=0.49, 95% CI=0.27-0.91, P=0.011 in the codominant model, OR=0.49, 95% CI=0.30-0.78, P=0.002 in the dominant model; rs1158265 (+200152 T>C): OR=0.65, 95% CI=0.42-1.00, P=0.048 in the dominant model, respectively]. In contrast, the rare alleles of rs826221, rs4858604, and rs1924768 decreased the risk of overweight/obesity [rs826221 (+267878 T>C): OR=2.04, 95% CI=1.02-4.11, P=0.04 in the codominant model, OR=1.67, 95% CI=1.06-2.63, P=0.026 in the dominant model; rs4858604 (+186399 A>G): OR=1.72, 95% CI=1.01-2.96, P=0.047 in the recessive model; rs1924768 (+126491 T>C): OR=2.07, 95% CI=1.02-4.23, P=0.043 in the recessive model, respectively].

Associations between *THRβ* Haplotypes and Overweight/Obese

Of these 82 variants, the significant 7 polymorphisms (rs826221, rs1505301, rs1700939, rs1868575, rs1158265, rs4858604, and rs1924768) were selected for linkage disequilibrium (LD) and haplotypes analysis. During pair-wise comparisons among the SNPs, the 7 SNPs that exhibited significant association between overweight/obese and controls, revealed weak LD, the haplotypes were meaningless in Korean population using Haploview software version 3. Pairwise comparisons among 82 SNPs displayed 20 LD blocks by the Gabriel method[17] (data not shown). Of 20 LD blocks, haplotypes were constructed in LD block 13 and LD block 15 including significant SNPs (rs1700939 and rs1924768) (Figure 1B). Thus, the haplotypes (frequency>0.1) in LD block 13 and LD block 15 were analyzed using HapAnalyzer software (Table 3). *THRβ* in the LD block 13 and 15 exhibited significant association [OR=0.70, 95% CI=0.51-0.96, P=0.0288 in the codominant model, OR=0.58, 95% CI=0.36-0.91, P=0.0191 in the recessive model; OR=0.70, 95% CI=0.51-0.96, P=0.0279 in the codominant model, OR=0.61, 95% CI=0.38-0.99, P=0.0474 in the dominant model, respectively].

Associations between *THRβ* Polymorphisms and Clinical Characteristics

We also compared the clinical lipid biomarker between the overweight/obese and control groups. Among these 7 polymorphisms, 2 SNPs (rs1700939 and rs4858604) showed significant differences in the clinical lipid biomarker and genotype in overweight/

obese, compared to control subjects (Table 4). The difference in the TG, TC, LDL-C, and HDL-C levels between the genotype with the rare allele of the SNPs (rs1700939 and rs4858604) in the overweight/obese subjects was significant compared to those in the control subjects. Polymorphisms in *THRβ* (rs1700939 and rs4858604) gene were significantly associated with increasing TG, TC, LDL-C, and decreasing HDL -C.

Discussion

TH exerts its effects by interacting with specific nuclear THRs that exist as 3 main isoforms: THRα1, THRβ1, and THRβ2[18]. Several studies indicate that THRs mediate TH-dependent transcriptional control, possibly through association with corepressors and coactivators[19,20]. It is also well known that TH affects body composition and regulates energy expenditure, in part, by the transcriptional control of specific metabolic pathway genes[21,22]. In recent study, it was shown a positive correlation between BMI and TH[23]. Also, it was known that *THRβ* was related to thermogenesis and lipolysis with TH in brown adiposities[24]. Selective *THRβ* activators would be useful therapeutics for improvement of impaired *THRβ*[25,26]. Furthermore, it was reported that *THRβ* participates in stimulating the energy expenditure[11]. This shows that *THRβ* plays important roles on energy metabolism.

Here, we investigated whether the *THRβ* gene polymorphisms are related to overweight/obesity by genotyping the 82 selected SNPs in the Korean population. We found that 7 SNPs among the 82 SNPs were significantly associated with overweight/obesity. The significant SNPs showed weak LD, thus haplotype was not constructed. However, pair-wise comparisons among 82 SNPs displayed 20 LD blocks. Among the 20 LD blocks, block 13 and block 15 that contained the significant SNPs (rs1700939, +238467 T>A and rs4858604, +186399 A>G) exhibited strong LD. A haplotype block is defined as a region displaying a multi-allelic D′>0.95, modified from Zhu *et al.*[27]. Haplotypes of LD block13 (TTAT) and 15 (CTGC) were significantly associated with overweight/obesity, and their attributable risks of overweight/obesity were 20.9% and 35.9%, respectively, in this Korean population.

TH exerts widespread effects including a reduction in plasma LDL-C, and TG levels as well as weight loss[9]. In addition, it was reported that *THRβ* also regulated plasma cholesterol and TG[8,25,28]. In order to assess whether the rs1700939 (+238467 T>A) and rs4858604 (+186399 A>G) SNPs of the *THRβ* gene exert an effect on overweight/obese, we compared the clinical features between genotypes of the control and overweight/obese subjects. The result revealed that significant differences in the TG, TC, LDL-C, and HDL-C concentrations were exhibited between subjects with rs1700939 and rs4858604 SNPs i.e. in subjects with the A rare allele and G rare allele, respectively. The presence of A allele of rs1700939 and G allele of rs4858604 was associated with the prevalence of overweight/obesity with increased TG, TC, and LDL-C levels. Furthermore, the HDL-C level in the control population was observed to be higher in the carriers of the A and G genotypes of rs1700939 and rs4858604, compared with those of the overweight/obese group.

In conclusion, we found a significant association between *THRβ* and overweight/obesity in the Korean population. Also, the polymorphisms of *THRβ* may be associated with the increase or decrease of lipid biomarkers in Korean population. All significant SNPs are intronic and unlikely to be direct disease-causing polymorphisms, but the SNPs may be interfering with mRNA splicing process and even the gene expression level. Further work is required to elucidate the exact role of *THRβ* polymorphism in the development of overweight/obesity.

Methods

Subjects

BMI of each subject was calculated from height and weight using the formula: BMI=weight (kg)/[height (m)]^2. As per the classification of Korean Society for the Study of Obesity (underweight, BMI <18; normal, BMI 18 to <23; moderately obese, BMI 23 to <25; obesity I, BMI 25 to <30; obesity II, BMI≥30), normal control (BMI 18 to <23, n=159, 66 men and 89 women) and overweight/obesity (BMI≥23, n=209, 118 men and 91 women) subjects at Kyung Hee University Medical Center were recruited. Subjects with hypertension, diabetes, hyperlipidemia, stoke, and cardiac diseases were excluded. All studies were performed according to the Declaration of Helsinki guidelines. Written informed consent was obtained from all subjects. This study was approved by the Ethics Review Committee of the Medical Research Institute, School of Medicine, Kyung Hee University.

Blood samples were drawn for biochemical measurements: SBP, DBP, fasting plasma glucose, HbA1c, TG, TC, LDL-C, and HDL-C. DNA samples were isolated by using Core One™ Blood Genomic DNA Isolation Kit (CoreBioSystem™, Seoul, Korea).

SNP Selection and Genotyping

We initially selected 82 SNPs within the *THRβ* gene region using the following websites: (1) human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP) (2) HapMap database (http://www.hapmap.org) (3) tag SNPs site (http://broad.mit.edu/mpg/tagger). The SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded. The genotyping was performed using the Affymetrix chip. The chip uses molecular inversion probe (MIP) technology. In brief, a mixture of 2 μg, genomic DNA and MIP was denatured and brought to annealing temperature. After eliminating the linear probes with exonucleases, PCR was performed with common primers. The reactions was then mixed and hybridized onto a tag array. The array was washed and loaded onto the GeneChip Scanner 3000 7G (Affymetrix, San Diego, USA). The images were analyzed using GeneChip operating software (GCOS)[29].

Statistical Analysis

The chi-square (χ^2) test was used to obtain the value of Hardy-Weinberg equilibrium (HWE). SNP analyses were performed using the SNPAnalyzer (ISTECH Inc., Goyang, Korea), SNPStats (http://bioinfo.iconcologia.net/index.php), Helixtree (Golden Helix Inc., MT, USA), and HapAnalyzer programs[30]. The power of sample size was calculated using a genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc). To reduce experimental error, the effective sample size was adjusted (calculated sample size × 100/95). Multiple logistic regression models were used for OR, 95% CI, and *P* value, controlling for age and gender as covariables. The haplotype frequencies were estimated using the expectation-maximization (EM) algorithm of SNPStats and HapAnalyzer softwares. Linkage disequilibrium (LD) blocks were detected using Haploview software (version 3.32). We examined Lewontis's |D′| and r^2 between bi-allelic loci. For the statistical tests, the level of significance was set at 0.05.

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