Association of annexin A5 polymorphisms with obesity

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Abstract. Annexin A5 (ANXA5), which is known as a protein with anticoagulative function, may play a role in triglyceride biosynthesis. Triglycerides are involved in lipid and energy metabolism, which are important in the elucidation of obesity. To investigate the association between single-nucleotide polymorphisms (SNPs) of *ANXA5* and obesity in a Korean population, 372 participants (213 overweight/obese individuals and 159 control subjects) were enrolled from the Kyung Hee University Medical Center and Keimyung University Dongsan Medical Center. The genotypes of five SNPs (rs12510548, rs4240260, rs3756281, rs13136094 and rs6534313) were evaluated in *ANXA5* using the multiple logistic regression analysis with the codominant 1, codominant 2, dominant, recessive and log-additive models. The genotype and allele frequencies of the five investigated SNPs exhibited significant differences between the control and the overweight/obese groups: rs12510548 (P=0.004 in the codominant 2 model, P=0.0019 in the recessive model, P=0.027 in the log-additive model and P=0.026 in allele frequencies); rs4240260 (P=0.002 and Fisher's exact P=0.0006 in the codominant 2 model, P=0.0007 and Fisher's exact P=0.0007 in the recessive model, P=0.020 and Fisher's exact P=0.0019 in the log-additive model and P=0.020 in allele frequencies); rs3756281 (P=0.016 in the codominant 2 model and P=0.0094 in the recessive model); rs13136094 (P=0.0030 and Fisher's exact P=0.0011 in the codominant 2 model, P=0.0012 and Fisher's exact P=0.0013 in the recessive model, P=0.034 and Fisher's exact P=0.0035 in the log-additive model and P=0.024 in allele frequencies); and rs6534313 (P=0.0010 and Fisher's exact P=0.0003 in the codominant 2 model, P=0.0003 and Fisher's exact P=0.0003 in the recessive model, P=0.0075 and Fisher's exact P=0.0010 in the log-additive model and P=0.005 in allele frequencies). Two haplotypes were weakly associated with obesity (GGATG, P=0.037 and CAGCC, P=0.020). Results of the present study suggested that *ANXA5* may be associated with the development of obesity in a Korean population.

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Introduction

Obesity has increased epidemically and is currently an important global health problem, since numerous individuals may be classified as overweight or obese (1). Obesity is commonly assessed by calculating the body mass index (BMI) [weight/(height)^2 in kg/m^2] (2). According to the World Health Organization guidelines regarding BMI revised for Asian populations, individuals with BMI≥23 kg/m^2 are classified as overweight, whereas those with BMI≥25 kg/m^2 are defined as obese (3). Multiple genes, environmental factors and gene-environment interactions play crucial roles in obesity and in the tendency to gain weight. Although the maintenance of body weight is under genetic control, mutations in a single gene rarely result in severe obesity (4). Previous studies reported associations between BMI or obesity and genetic variants, suggesting that polymorphisms in the genes linked to various pathways may contribute to the development of obesity (5-7).

The annexin A5 gene (*ANXA5*), mapped to the chromosome 4q28-q32, is 1.6-kb long and contains 13 exons that code for a polypeptide chain of 320 amino acids (8,9). ANXA5 has also been described as placental anticoagulant protein I, vascular anticoagulant-α, endonexin II, lipocortin V, placental protein 4 and anchorin CII. ANXA5 is a member of the annexin family of calcium-dependent phospholipid-binding proteins (10). In addition, a previous study by Dennis *et al* (11) reported that ANXA5 may affect triglyceride metabolism. These results suggested that an association between ANXA5 and lipid metabolism may be an important aspect of obesity. However, the genetic role of ANXA5 in obesity has not been fully elucidated. To the best of our knowledge, there is only one available association study on *ANXA5* single-nucleotide polymorphism (SNP), which reported that the intronic SNPs (iSNPs) rs4833229 and rs6830321 were associated with

Table I. Demographic and clinical characteristics of study subjects.

Variables	Control (n=159)	Overweight/obese (n=213)	P-value
Age (years)	43.43±6.08	44.79±6.40	0.042
BMI (kg/m^2)	21.15±1.21	25.59±2.03	<0.001
SBP (mmHg)	115.53±16.17	123.89±17.57	<0.001
DBP (mmHg)	71.88±10.37	77.66±11.19	<0.001
Fasting plasma glucose (mg/dl)	90.10±11.62	93.81±14.86	<0.001
HbA_{1C} (%)	5.34±0.41	5.47±0.66	0.037
TG (mg/dl)	97.79±56.88	140.39±118.28	<0.001
TC (70 mg/dl)	186.10±29.92	196.24±33.66	0.003
LDL-C (mg/dl)	109.08±28.92	118.31±31.43	0.004
HDL-C (mg/dl)	56.83±13.25	49.85±11.37	<0.001

Data are presented as mean ± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA_{1C}, glycosylated hemoglobin; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

restenosis in patients who had undergone percutaneous coronary intervention (PCI) (12). Taking into consideration that the role of ANXA5 in coagulation is linked to triglyceride biosynthesis (11), we hypothesized that *ANXA5* SNPs may affect the development of obesity.

To the best of our knowledge, the association between *ANXA5* polymorphisms and obesity has not been previously investigated. The aim of this study was to investigate whether *ANXA5* SNPs were associated with obesity in a Korean population.

Subjects and methods

Subjects. A total of 213 overweight/obese (BMI≥23 kg/m^2) and 159 control (18.0<BMI<23 kg/m^2) subjects were recruited from Kyung Hee University Medical Center and Keimyung University Dongsan Medical Center. All the subjects were ethnic Korean. Their demographic and biochemical characteristics are shown in Table I. DNA was isolated from peripheral blood using the G-DEX™ IIb Genomic DNA Extraction kit (iNtRON Biotechnology, Seongnam, Korea). Collection of the subjects was performed according to the Declaration of Helsinki guidelines. All the subjects provided written informed consent prior to enrollment, and informed consent was obtained by legal guardians of the patients if they were of minor age. This study was approved by the the ethics review committee of the Medical Research Institute, School of Medicine, Kyung Hee University and the Institutional Review Board of Kyung Hee University Medical Center, Seoul, Korea.

SNP selection and genotyping. Five iSNPs within the *ANXA5* gene were selected as follows: SNP tagging was performed using the tagging option of the Tagger program (http://www.broad.mit.edu/mpg/tagger/) with known heterozygosity and minor allele frequency >0.05 (http://www.hapmap.org). Genotyping was performed with the Affymetrix Targeted Genotyping Chip array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. Each genotyping was analyzed using GCOS software (Affymetrix).

Statistical analysis. The Hardy-Weinberg equilibrium (HWE) was assessed for each of the five selected SNPs using the SNPStats software (13). The linkage disequilibrium (LD) block of the five selected SNPs was assessed using Haploview software version 4.1 (14). Multiple logistic regression models were performed for the odds ratios (ORs), 95% confidence intervals (CIs) and corresponding P-values, controlling for age and gender as covariables (15). The Student's unpaired t-test was used for assessing statistical differences in age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose, glycosylated hemoglobin (HbA_{1c}), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) between the control and the overweight/obese groups. SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze statistical significance.

Results

Demographic and clinical characteristics of the study subjects. The demographic and clinical characteristics, i.e., age, BMI, SBP, DBP, fasting plasma glucose, HbA_{1c}, TG, TC, LDL-C and HDL-C, were significantly different between the control and overweight/obese subjects (P<0.05, Table I), with some of the clinical characteristics, including BMI, SBP, DBP, fasting plasma glucose, TG and HDL-C, exhibiting more statistically significant differences (P<0.001) (Table I). The genotype distributions of the five selected SNPs were in HWE (P>0.05, data not shown).

Genotype and allele frequencies of ANXA5 SNPs. The genotype and allele frequencies of the investigated SNPs (rs12510548, rs4240260, rs3756281, rs13136094 and rs6534313) are provided in Table II. The genotype frequencies of the five selected SNPs exhibited protective effects on

Table II. Genotype and allele frequencies of *ANXA5* SNPs in the control and overweight/obese groups.

SNP	Type	Control [n (%)]	Overweight/ obese [n (%)]	Model	OR (95% CI)	P-value	Fisher's exact P
rs12510548	G/G	71 (44.9)	107 (51.4)	Codominant 1	0.93 (0.60-1.44)	0.74	
intron	C/G	68 (43.0)	94 (45.2)	Codominant 2	0.25 (0.10-0.64)	0.004	
	C/C	19 (12.0)	7 (3.4)	Dominant	0.78 (0.51-1.19)	0.25	
				Recessive	0.26 (0.11-0.64)	0.0019	
				Log-additive	0.68 (0.48-0.96)	0.027	
	G	210 (66.5)	308 (74.0)				
	C	106 (33.5)	108 (26.0)	Allele	0.69 (0.50-0.96)	0.026	
rs4240260	G/G	71 (44.9)	108 (51.7)	Codominant 1	0.92 (0.59-1.43)	0.71	
intron	A/G	69 (43.7)	96 (45.9)	Codominant 2	0.19 (0.07-0.55)	0.002	0.0006
	A/A	18 (11.4)	5 (2.4)	Dominant	0.77 (0.51-1.18)	0.23	
				Recessive	0.20 (0.07-0.56)	0.0007	0.0007
				Log-additive	0.66 (0.47-0.94)	0.020	0.0019
	G	211 (66.8)	312 (74.6)				
	A	105 (33.2)	106 (25.4)	Allele	0.68 (0.50-0.94)	0.020	
rs3756281	A/A	67 (43.2)	90 (46.4)	Codominant 1	1.01 (0.64-1.58)	0.98	
intron	A/G	69 (44.5)	95 (49.0)	Codominant 2	0.35 (0.15-0.82)	0.016	
	G/G	19 (12.3)	9 (4.6)	Dominant	0.86 (0.56-1.33)	0.51	
				Recessive	0.35 (0.15-0.79)	0.0094	
				Log-additive	0.75 (0.53-1.06)	0.098	
	A	203 (65.5)	275 (70.9)				
	G	107 (34.5)	113 (29.1)	Allele	0.78 (0.57-1.07)	0.13	
rs13136094	T/T	79 (50.0)	119 (56.4)	Codominant 1	0.96 (0.61-1.48)	0.84	
intron	C/T	62 (39.2)	87 (41.2)	Codominant 2	0.21 (0.07-0.59)	0.0030	0.0011
	C/C	17 (10.8)	5 (2.4)	Dominant	0.80 (0.52-1.21)	0.29	
				Recessive	0.21 (0.08-0.59)	0.0012	0.0013
				Log-additive	0.69 (0.49-0.97)	0.034	0.0035
	T	220 (69.6)	325 (77.0)				
	C	96 (30.4)	97 (23.0)	Allele	0.68 (0.49-0.95)	0.024	
rs6534313	G/G	74 (49.0)	121 (58.2)	Codominant 1	0.88 (0.56-1.37)	0.57	
intron	C/G	59 (39.1)	82 (39.4)	Codominant 2	0.17 (0.06-0.50)	0.0010	0.0003
	C/C	18 (11.9)	5 (2.4)	Dominant	0.71 (0.46-1.09)	0.12	
				Recessive	0.18 (0.07-0.51)	0.0003	0.0003
				Log-additive	0.62 (0.44-0.88)	0.0075	0.0010
	G	207 (68.5)	324 (77.9)				
	C	95 (31.5)	92 (22.1)	Allele	0.62 (0.44-0.87)	0.005	

ORs, 95% CIs, and P-values were from multiple logistic regression analyses with the codominant 1, codominant 2, dominant and recessive models controlling age and gender as covariates. ANXA5, annexin 5; SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

the development of overweight/obesity in the codominant 2 model (rs12510548; OR=0.25, 95% CI: 0.10-0.64; P=0.004) (rs4240260; OR=0.19, 95% CI: 0.07-0.55; P=0.002; Fisher's exact P=0.0006) (rs3756281; OR=0.35, 95% CI: 0.15-0.82; P=0.016) (rs13136094; OR=0.21, 95% CI: 0.07-0.59; P=0.0030; Fisher's exact P=0.0011) (rs6534313; OR=0.17, 95% CI: 0.06-0.50; P=0.0010; Fisher's exact P=0.0003) and recessive model (rs12510548; OR=0.26, 95% CI: 0.11-0.64; P=0.0019) (rs4240260; OR=0.20, 95% CI: 0.07-0.56; P=0.0007; Fisher's exact P=0.0007) (rs3756281; OR=0.35, 95% CI: 0.15-0.79; P=0.0094) (rs13136094; OR=0.21, 95% CI: 0.08-0.59; P=0.0012; Fisher's exact P=0.0013) (rs6534313; OR=0.18, 95% CI: 0.07-0.51; P=0.0003; Fisher's exact P=0.0003). Four out of the five investigated SNPs (rs12510548, rs4240260, rs13136094 and rs6534313) exhibited protective effects in the log-additive model (rs12510548; OR=0.68, 95% CI: 0.48-0.96; P=0.027) (rs4240260; OR=0.66, 95% CI: 0.47-0.94; P=0.020; Fisher's exact P=0.0019)

Table III. Haplotypes of the *ANXA5* SNPs in the control and overweight/obese groups.

Haplotype	Frequency (%)	Control		Overweight/obese		χ^2	P-value
		-	+	-	+		
GGATG	70.2	107	209	114	312	4.36	**0.037**
CAGCC	25.7	221	95	330	96	5.38	**0.020**
CAGTG	3.6	306	10	409	17	0.35	0.55

Bold numbers indicate statistical significance. ANXA5, annexin 5; SNP, single-nucleotide polymorphism.

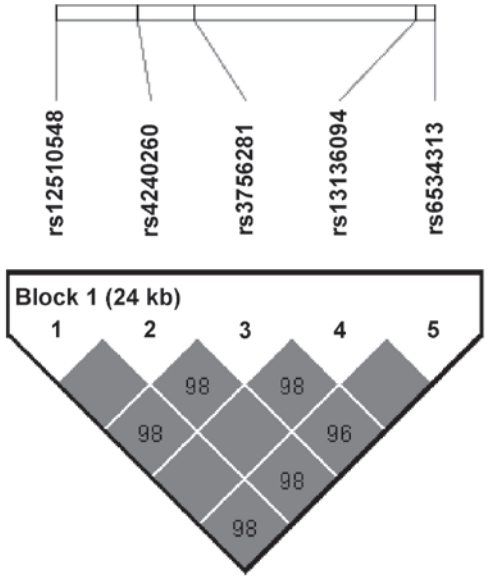

Figure 1. The linkage disequilibrium block consisted of the 5 annexin A5 single-nucleotide polymorphisms (rs12510548, rs4240260, rs3756182, rs13136094 and rs6534313).

(rs13136094; OR=0.69, 95% CI: 0.49-0.97; P=0.034; Fisher's exact P=0.0035) (rs6534313; OR=0.62, 95% CI: 0.44-0.88; P=0.0075; Fisher's exact P=0.0010) (Table II).

The allele frequencies of four SNPs (rs12510548, rs4240260, rs13136094 and rs6534313) were significantly different between the control and overweight/obese subjects (rs12510548; OR=0.69; 95% CI: 0.50-0.96) (rs4240260; OR=0.68, 95% CI: 0.50-0.94) (rs13136094; OR=0.68, 95% CI: 0.49-0.95) (rs6534313; OR=0.62, 95% CI: 0.44-0.87) (Table II). Their minor allele frequencies were ~1.3-1.4-fold lower in the overweight/obese group compared to the control group and they exerted protective effects on the development of overweight/obesity.

Linkage disequilibrium and haplotypes. Fig. 1 shows an LD block consisting of the five consecutive SNPs (rs12510548, rs4240260, rs3756281, rs13136094 and rs6534313). In the LD block, three haplotypes were formed (GGATG, CAGCC and CAGTG). The frequencies of the GGATG, CAGCC and CAGTG haplotypes were 70.2, 25.7 and 3.6%, respectively, and the results indicated that the GGATG (P=0.037) and CAGCC (P=0.020) haplotypes were weakly associated with the development of obesity (Table III).

Discussion

ANXA5 is an intracellular protein that is abundantly present in endothelial cells and platelets and exhibits high affinity for anionic phospholipids in lipid membranes (16). Based on its affinity, fluorescently-labeled ANXA5 is often used in flow cytometric assays to detect cells undergoing apoptosis, during which the lipid consistency of cell membranes rapidly changes (17). ANXA5 is a potent anticoagulant that regulates exocytosis and syncytiotrophoblast membrane fusion (17) and expression of ANXA5 in cancer tissues is an important factor in tumor infiltration, which is associated with cellular energy metabolism and membrane regulating function (18). ANXA5 protects the lipid membrane barrier against damage due to inflammatory mediators (19) and there is an association between inflammatory molecule levels and visceral obesity (20). These results suggested that ANXA5 may play a role in lipid metabolism.

As regards *ANXA5* polymorphisms, there has been only one study on the association of *ANXA5* iSNPs (rs4833229 and rs6830321) and the restenosis rate of PCI (12). It may be considered that the role of ANAX5 in coagulation pathways affected the therapeutic consequences of atherosclerotic disease (12). However, that study demonstrated a significant effect exerted by polymorphisms in the intron region of *ANXA5*, although the two SNPs involved (rs4833229 and rs6830321) were not included in our study.

Our results demonstrated that the five SNPs of *ANXA5* included in this study (rs12510548, rs4240260, rs3756182, rs13136094 and rs6534313) were associated with the risk of obesity. In our haplotype analysis results, GGATG and CAGCC were significantly associated with the risk of overweight/obesity, suggesting that *ANXA5* may be involved in the development of obesity (21). All the significant SNPs were intronic and unlikely to be directly protein-modifying polymorphisms. However, iSNPs may interfere with the mRNA splicing process and gene expression levels (22). The minor allele frequencies of rs12510548, rs4240260, rs3756281, rs13136094 and rs6534313 in our study population were similar to those in Japanese subjects (0.29 vs. 0.21, 0.29 vs. 0.23, 0.32 vs. 0.23, 0.26 vs. 0.22 and 0.26 vs. 0.22, respectively) in the dbSNP Build 137 of the NCBI database (http://www.ncbi.nlm.nih.gov/SNP/).

In summary, our results suggest that there is an association between the five iSNPs (rs12510548, rs4240260, rs3756182, rs13136094 and rs6534313) of *ANXA5* and the development of obesity in a Korean population. To the best of our knowledge,

this is the first study demonstrating the association of *ANXA5* SNPs with the susceptibility to overweight and obesity. Further studies are required to elucidate whether additional *ANXA5* SNPs are associated with obesity and determine the precise role of *ANXA5* in obesity in different populations.

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