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Genotypes and haplotypes of β_2-adrenergic receptor and parameters of the metabolic syndrome in Korean adolescents

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Abstract

We investigated the association between single nucleotide polymorphisms (SNPs) and haplotype of the β_2-adrenergic receptor (*ADRB2*) gene and parameters of the metabolic syndrome in Korean adolescents. Body mass index, waist circumference, blood pressure, fasting glucose, triglycerides and high-density lipoprotein (HDL) cholesterol, free fatty acid and insulin, and homeostasis model assessment (HOMA) score were measured in 134 volunteer adolescents (69 male and 65 female). All subjects were genotyped for 5 SNPs: A→G rs1042713 (Arg16Gly), C→G rs1042714 (Gln27Glu), G→A rs1042717 (Leu84Leu), C→A rs1042718 (Arg175Arg), and G→C rs1042719 (Gly351Gly). After adjustment for age and sex, we found that the rs1042717 SNP was associated with higher body weight, fasting insulin, and HOMA score. The rs1042714 SNP was associated with higher body fat, waist circumference, and free fatty acids; and the rs1042719 SNP was associated with higher diastolic blood pressure. The C-A-A-C haplotype at SNPs rs1042714, rs1042717, rs1042718, and rs1042719 was associated with higher body weight, fasting insulin, and HOMA score; the G-G-C-G was associated with higher body fat and waist circumference; the C-G-C-G was associated with lower HDL cholesterol; and the C-G-C-C was associated with lower body weight, lower waist circumference, and higher HDL cholesterol. In conclusion, these findings suggest that the genotypes and haplotypes of *ADRB2* may influence parameters of the metabolic syndrome in Korean adolescents.

The metabolic syndrome is associated with an increased risk of total and cardiovascular mortality [1,2]. As juvenile obesity has increased, the metabolic syndrome in adolescents has become an emerging health problem throughout the world. Although the prevalence of the metabolic syndrome has increased significantly among adolescents over the past decade [3-6], this condition is particularly prevalent (>30%) in overweight adolescents in the United States [3,5-7]. Tracking of cardiovascular disease from childhood to adulthood suggests that early identification of individuals at risk may have long-term benefits for the prevention of cardiovascular morbidity and mortality. The metabolic syndrome is present in 3.3% of Korean adolescents [8], a proportion similar to that reported in adolescents in the United States [3]. Worryingly, the prevalence of this condition among children and adolescents has been increasing in Asian countries. As in other chronic diseases, genetic and environmental influences have been implicated in the metabolic syndrome; and it was recently suggested that the metabolic syndrome may originate in utero [9].

Catecholamines control several metabolic pathways of the metabolic syndrome and exert their effects through different β-adrenergic receptors (β_1, β_2, and β_3). The gene encoding the β_2-adrenergic receptor (*ADRB2*) may be a potential candidate gene for a genetic component of the metabolic syndrome. In adults, polymorphisms in the *ADRB2* gene have been associated with obesity [10,11], type 2 diabetes mellitus [12], and cardiovascular risk factors [13]. Moreover, variants in the human *ADRB2* gene may be associated with obesity in children and adolescents [14] and have been linked to metabolic syndrome susceptibility in adult men [15]. To date, however, the association between genetic risk factors and the metabolic syndrome in

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adolescents has not been determined. We therefore investigated the association between *ADRB2* genotype and haplotype and parameters of the metabolic syndrome among Korean adolescents.

1. Methods

1.1. Study subjects

Study participants were recruited from volunteer adolescents who wanted to participate in this study after they or their parents saw an advertisement poster in the Department of Family Medicine of Asan Medical Center located in Seoul, Korea. They were either apparently healthy adolescents for regular health checkups or primary care patients visiting our department. The study was approved by the Institutional Review Board of Asan Medical Center, and informed consent was obtained from the parents of each participant. Exclusion criteria included obesity secondary to hypothyroidism or Cushing disease, or severe debilitating diseases. Subjects were also excluded from participation if they had been treated with any antiobesity agent or if they had experienced weight loss during the previous 6 months. The study population was composed of 134 adolescents (69 male and 65 female).

1.2. Anthropometric measurements

Height and weight were measured by an automatic height-weight scale to the nearest 0.1 cm and 0.1 kg, respectively; and body mass index (BMI) was calculated by dividing weight (in kilograms) by height squared (in square meters). Body fat and total body fat mass were determined by bioimpedance analysis (Inbody 3.0; Biospace, Seoul, Korea) [16]. Using a nonelastic tape measure, waist circumference was measured at the end of a normal expiration at the midpoint between the lower border of the rib cage and the iliac crest [17].

1.3. Measurements of parameters of the metabolic syndrome

The components of the metabolic syndrome (systolic and diastolic blood pressure, fasting plasma glucose, triglycerides and high-density lipoprotein [HDL] cholesterol concentrations), as well as other metabolic variables (total cholesterol and fasting insulin concentrations and homeostasis model assessment [HOMA] score), were measured in all subjects.

Systolic and diastolic blood pressures were measured using a mercury sphygmomanometer with the individual in a sitting position and after 10 minutes of rest. Cuff size was selected according to the arm circumference of each participant. Blood samples were obtained from each subject after a 12-hour overnight fast by evacuation from an antecubital vein into vacutainer tubes. Fasting plasma glucose concentration was measured by a glucose oxidase method, and concentrations of total cholesterol and triglycerides were measured by enzymatic procedures using an autoanalyzer (Hitachi-747; Hitachi, Tokyo, Japan). The HDL cholesterol fraction was measured enzymatically after precipitation of apolipoprotein B–containing lipoproteins with $MnCl_2$. Free fatty acids were measured by enzymatic method using an autoanalyzer (Toshiba 200FRr; Toshiba Medical Systems, Tokyo, Japan). Fasting insulin concentration was measured by radioimmunoassay (Dianabott, Tokyo, Japan). Homeostasis model assessment score, an estimate of insulin resistance, was calculated as fasting serum insulin (microunits per milliliter) × fasting plasma glucose (millimoles per liter)/22.5 [18].

1.4. Genotyping of ADRB2 polymorphisms

Five single nucleotide polymorphisms (SNPs), A→G rs1042713 (Arg16Gly), C→G rs1042714 (Gln27Glu), G→A rs1042717 (Leu84Leu), C→A rs1042718 (Arg175Arg), and G→C rs1042719 (Gly351Gly), were genotyped with SNP-IT assays using the SNPstream 25K System (Orchid Biosciences, Princeton, NJ). The latter 4 SNPs were selected for their ability to tag haplotypes of the *ADRB2*. The genomic DNA region spanning the polymorphic site was polymerase chain reaction (PCR) amplified using one phosphothiolated primer and one regular PCR primer, and the amplified PCR products were digested with exonuclease. The 5′ phosphothioates protect one strand of the PCR product from exonuclease digestion, resulting in the generation of a single-stranded PCR template. The single-stranded PCR template was overlaid onto a 384-well plate containing covalently attached SNP-IT primer extension primer designed to hybridize immediately adjacent to the polymorphic site. The SNP-IT primer was extended for a single base with DNA polymerase and a mixture of appropriate acycloterminators, which were labeled with fluorescein isothiocyanate (FITC) or biotin and were complementary to the polymorphic nucleotide. The identity of the incorporated nucleotide was determined using serial colorimetric reactions with anti–alkaline phosphatase–conjugated FITC and streptavidin–horseradish peroxidase, respectively. The yellow and/or blue colors were analyzed with an enzyme-linked immunosorbent assay reader, and the final genotype determinations were made using the QCReview program (Orchid Biosciences).

1.5. Statistical analysis

All quantitative traits are presented as mean ± SD by sex. Parametric and nonparametric tests were used to compare mean values of quantitative traits across groups according to SNP and haplotypes of *ADRB2* using Student *t* test and analysis of variance for parametric tests and the Mann-Whitney and Kruskal-Wallis tests for nonparametric tests. In a polymorphism with 2 alleles (G and g), G is thought to be associated with a disease; each of the 3 genotypes (gg, Gg, and GG) was obtained. We used codominant (assuming a per-allele effect that places Gg midway between gg and GG), dominant (comparing gg with Gg + GG), and recessive (comparing gg + Gg with GG) genetic models. All analyses

Table 1
Basic characteristics of the study subjects

	Male (n = 69) Mean ± SD	Female (n = 65) Mean ± SD
Age (y)	13.4 ± 3.8	13.5 ± 3.2
Weight (kg)	60.1 ± 19.2	52.1 ± 17.3
BMI (kg/m^2)	22.2 ± 4.7	21.5 ± 5.0
Body fat (%)	22.2 ± 8.3	28.9 ± 7.9
Waist circumference (cm)	77.2 ± 12.4	71.6 ± 12.0
Systolic blood pressure (mm Hg)	114.0 ± 12.7	107.6 ± 11.3
Diastolic blood pressure (mm Hg)	66.3 ± 11.9	65.0 ± 9.7
Fasting glucose (mg/dL)	85.1 ± 6.3	83.4 ± 5.6
Total cholesterol (mg/dL)	171.1 ± 32.1	172.2 ± 30.2
Triglycerides (mg/dL)	100.0 ± 63.0	89.3 ± 43.2
HDL cholesterol (mg/dL)	52.9 ± 12.2	54.8 ± 11.3
Free fatty acid (mg/dL)	563.9 ± 253.8	606.4 ± 274.7
Fasting insulin (μIU/mL)	12.1 ± 7.6	12.4 ± 8.1
HOMA score	2.3 ± 1.5	2.3 ± 1.5

were 2-tailed, and a P value < .05 was considered statistically significant. Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC).

2. Results

The baseline characteristics of the study subjects are presented in Table 1. The mean age was 13.5 years, and the mean BMI was 21.8 kg/m^2. Approximately 35% of our study subjects were at risk for being overweight; and one fifth of the subjects showed abdominal obesity, high triglycerides, low HDL cholesterol, or high insulin levels. High blood pressure and high fasting glucose were detected in 9% and 1.5% of the subjects, respectively. Male subjects had higher waist circumference, blood pressure, fasting glucose, and triglycerides than female subjects, whereas female subjects had higher body fat, HDL cholesterol, and free fatty acid than male subjects.

Table 2 shows the allele, genotype, and haplotype frequencies for *ADRB2* polymorphisms. All tested loci and genotype frequencies were in Hardy-Weinberg equilibrium. Variants were in allelic association, indicating linkage disequilibrium.

Table 3 presents mean values of parameters of the metabolic syndrome relative to *ADRB2* polymorphisms. Individuals homozygous for the G allele at rs1042714 showed significantly higher body fat, waist circumference, and free fatty acids. Carriers of allele A at rs1042717 had significantly higher body weight, fasting insulin, and HOMA score. Individuals homozygous for the C allele at rs1042719 showed significantly higher diastolic blood pressure.

Six haplotypes were derived from the 4 polymorphic sites. Table 4 shows the mean values of parameters of the metabolic syndrome relative to *ADRB2* haplotype. Individuals of haplotype C-G-C-G had significantly lower HDL cholesterol, whereas individuals of haplotype C-A-A-C had significantly higher body weight, fasting insulin, and HOMA score. Individuals of haplotype C-G-C-C had significantly lower body weight, lower waist circumference, and higher HDL cholesterol; and individuals of haplotype G-G-C-G had significantly higher body fat and waist circumference.

3. Discussion

The clustering of obesity, hypertension, and diabetes was estimated to be derived from both genetic (59%) and environmental (41%) determinants [19]. The *ADRB2* gene may be a good candidate for the genetic component because the protein encoded by this gene has been shown to be an important modulator of vascular tone, glucose homeostasis, and adipose tissue lipolyis in humans [20]. We have shown here that certain *ADRB2* variants were significantly correlated with parameters of the metabolic syndrome and insulin resistance in Korean adolescents.

Studies of the effects of *ADRB2* variants on cardiovascular risk factors in adults have shown inconsistent results. A polymorphism of the *ADRB2* gene was shown to have a significant relationship to central distribution of body fat in Swedish men [13]. Significant associations between *ADRB2* polymorphisms and obesity, hypertriglyceridemia,

Table 2
Allele, genotype, and haplotype frequencies for *ADRB2* polymorphisms among study subjects

	rs1042713 (G16R)		rs1042714 (E27Q)		rs1042717 (L84L)		rs1042718 (R175R)		rs1042719 (G351G)	
Allele	A	0.59	G	0.89	G	0.71	C	0.71	C	0.60
frequency (%)	G	0.41	C	0.11	A	0.29	A	0.29	G	0.40
Genotype	AA	0.33	GG	0.80	GG	0.49	CC	0.50	CC	0.35
frequency (%)	AG	0.52	GC	0.19	GA	0.42	CA	0.42	CG	0.50
	GG	0.15	CC	0.01	AA	0.09	AA	0.08	GG	0.15
Haplotype	rs1042714-rs1042717-rs1042718-rs1042719									
frequency (%)	C-G-C-G	(ht1)	0.48635							
	C-A-A-C	(ht2)	0.29789							
	C-G-C-C	(ht3)	0.11609							
	G-G-C-G	(ht4)	0.09461							
	C-A-C-C	(ht5)	0.00289							
	G-G-C-C	(ht6)	0.00217							

Table 3
Mean values of parameters of the metabolic syndrome according to *ADRB2* polymorphisms among study subjects

Genotype	rs1042713 (G16R)			rs1042714 (E27Q)			rs1042717 (L84L)			rs1042718 (R175R)			rs1042719 (G351G)		
	AA	AG	GG	CC	CG	GG	GG	GA	AA	CC	CA	AA	GG	GC	CC
Model	C	D	R	C	D	R	C	D	R	C	D	R	C	D	R
Weight (kg)															
Mean	56.4	56.0	54.6	57.3	51.7	42.8	54.9	54.6	62.8 *†	55.7	55.1	61.1	57.1	55.7	53.4
P value	.4165	.4255	.6359	.1231	.0968	.9214	.0276	.0407	.1566	.056	.0777	.2251	.9676	.7927	.7877
BMI (kg/m^2)															
Mean	21.6	21.6	22.0	22.1	20.5	24.2	21.3	21.5	24.0	21.5	21.6	23.7	21.9	21.7	21.6
P value	.4264	.5811	.4407	.3467	.2198	.3262	.0855	.1824	.1158	.1419	.2604	.1732	.9111	.9138	.9469
Body fat (%)															
Mean	24.9	25.2	24.8	25.4	23.3	38.8‡	24.9	25.0	27.0	24.9	24.9	27.7	25.1	25.0	26.1
P value	.7122	.9223	.5676	.553	.8697	.0422	.9157	.6819	.3358	.9363	.6477	.2995	.9857	.7784	.6868
Waist circumference (cm)															
Mean	74.1	73.8	75.7	74.7	72.1	97.0‡	74.1	72.7	80.0	74.6	73.0	79.3	75.6	73.9	72.5
P value	.4265	.5869	.4341	.7782	.4135	.0234	.2025	.4289	.1295	.321	.2562	.1852	.6123	.6993	.6598
Systolic BP (mm Hg)															
Mean	113.7	109.6	108.2	111.3	109.1	109.0	112.5	108.1	113.0	112.7	108.4	111.7	114.1	108.7	110.5
P value	.0899	.1147	.2714	.2197	.1889	.963	.7077	.4525	.6436	.576	.4003	.8078	.2401	.0751	.8947
Diastolic BP (mm Hg)															
Mean	66.2	64.1	65.4	65.3	63.9	72.0	65.7	63.2	70.1	65.7	63.4	69.4	65.4	63.4	69.7‡
P value	.6077	.4125	.9	.7342	.614	.5331	.8309	.5764	.1406	.9332	.5768	.2167	.3756	.8169	.0482
Fasting glucose (mg/dL)															
Mean	83.9	84.1	85.2	84.3	84.6	84.0	83.9	83.9	86.5	84.1	84.2	85.3	85.0	83.5	85.6
P value	.6778	.7633	.6998	.8599	.9095	.7498	.3839	.6601	.2247	.6653	.7885	.6031	.9223	.3921	.3461
Triglycerides (mg/dL)															
Mean	84.9	97.6	105.9	95.0	96.5	68.0	86.8	101.6	108.1	86.5	103.1	100.7	91.0	93.2	109.1
P value	.2182	.2009	.5347	.6276	.73	.4626	.1079	.0945	.466	.1384	.0863	.7583	.3084	.5719	.2452
HDL cholesterol (mg/dL)															
Mean	54.5	52.7	55.1	52.8	56.1	58.0	54.5	53.6	51.3	54.1	53.5	51.4	53.0	52.3	59.8
P value	.7498	.4154	.6247	.1611	.1507	.8276	.2534	.2571	.5395	.3215	.3491	.5529	.1799	.8692	.061
Free fatty acid (mg/dL)															
Mean	564.5	581.9	590.2	540.7	717.3	707.7 *,†	609.6	537.1	569.3	611.9	534.2	588.7	519.6	567.1	563.5
P value	.8988	.977	.839	.0168	.0063	.4266	.1333	.0507	.9898	.1143	.059	.9289	.3526	.3126	.6788
Fasting insulin (μIU/mL)															
Mean	11.4	12.7	14.7	13.1	10.6	20.8	11.2	13.2	16.8 *,†	11.6	13.6	14.9	12.8	11.8	15.2
P value	.0641	.1448	.1201	.4793	.2972	.208	.0151	.0339	.0721	.0713	.0716	.3675	.4116	.9813	.1157
HOMA score															
Mean	2.1	2.4	2.8	2.5	2.0	3.9	2.1	2.5	3.3 *,†,‡	2.2	2.7	2.8	2.4	2.2	2.9
P value	.0637	.1463	.117	.4734	.3028	.2444	.0112	.0328	.044	.0741	.077	.3565	.3986	.9121	.0831

C indicates codominant model; D, dominant model; R, recessive model.

* $P < .05$ by codominant model, † $P < .05$ by dominant model, and ‡ $P < .05$ by recessive model after adjustment for age and sex.

Table 4
Mean values of parameters of the metabolic syndrome according to *ADRB2* haplotype (rs1042714-rs1042717-rs1042718-rs1042719) among study subjects

Haplotype	C-G-C-G (ht1)			C-A-A-C (ht2)			C-G-C-C (ht3)			G-G-C-G (ht4)		
Model	−/−	ht1/−	ht1/ht1	−/−	ht2/−	ht2/ht2	−/−	ht3/−	ht3/ht3	−/−	ht4/−	ht4/ht4
	C	D	R	C	D	R	C	D	R	C	D	R
Weight (kg)												
Mean	49.9	57.4	58.3	55.4	55.1	61.1‡	57.3	51.7	36.9*	56.9	51.7	42.8
P value	.4334	.4914	.57	.035	.2038	.0478	.0085	.0529	.1151	.1449	.1157	.9168
BMI (kg/m^2)												
Mean	21.1	21.7	22.3	21.4	21.6	23.7	22.1	20.5	18.1	22.0	20.5	24.2
P value	.7228	.9854	.5721	.1113	.2083	.1628	.0573	.0657	.3001	.3825	.2474	.3238
Body fat (%)												
Mean	25.6	24.9	25.1	24.9	24.9	27.7	25.1	25.0	25.4	25.4	23.3	38.8‡
P value	.6929	.4563	.897	.965	.6153	.3073	.9771	.9078	.8309	.5438	.8561	.0439
Waist circumference (cm)												
Mean	72.3	74.4	75.6	74.3	73.0	79.3	75.2	71.7	60.6*	74.4	72.1	97.0‡
P value	.6106	.7312	.6324	.2332	.4485	.1663	.0302	.0622	.0752	.8416	.4617	.0223
Systolic BP (mm Hg)												
Mean	107.1	111.2	114.3	112.7	108.4	111.7	111.3	109.2	106.7	111.2	109.1	109.0
P value	.0611	.1165	.1529	.6284	.4475	.794	.3241	.3212	.6569	.2301	.1987	.9622
Diastolic BP (mm Hg)												
Mean	66.4	64.5	64.9	65.9	63.4	69.4	64.5	67.5	64.3	65.3	63.9	72.0
P value	.5811	.4412	.9188	.9912	.5185	.2275	.3996	.2991	.9086	.7418	.6221	.5364
Fasting glucose (mg/dL)												
Mean	84.5	84.1	84.4	84.1	84.2	85.3	84.7	82.3	86.0	84.2	84.6	84.0
P value	.849	.8889	.8674	.7015	.8377	.6054	.2973	.1734	.7352	.9	.9519	.7519
Triglycerides (mg/dL)												
Mean	109.1	88.4	97.5	87.8	103.1	100.7	98.3	79.4	123.3	95.4	96.5	68.0
P value	.5118	.2224	.8454	.1857	.1231	.7932	.5279	.3038	.4619	.6132	.7132	.4655
HDL cholesterol (mg/dL)												
Mean	59.7	51.5	52.3*,†	54.2	53.5	51.4	52.2	57.1	74.0*,†,‡	53.1	56.1	58.0
P value	.0271	.0028	.5987	.2828	.3044	.536	.0011	.0074	.0029	.1814	.1711	.8303
Free fatty acid (mg/dL)												
Mean	621.0	569.2	523.7	604.2	534.2	588.7	562.5	606.4	650.0	537.7	717.3	487.0
P value	.4205	.6819	.3695	.165	.0637	.8891	.3421	.8233	.3799	.1096	.0874	.7907
Fasting insulin (μIU/mL)												
Mean	13.6	12.0	12.7	11.2	13.6	14.9*,†	13.2	10.4	9.9	12.9	10.6	20.8
P value	.4512	.2123	.9497	.037	.0338	.3304	.1215	.1	.6359	.5022	.3121	.1981
HOMA score												
Mean	2.6	2.3	2.4	2.1	2.6	2.8*,†	2.5	1.9	1.9	2.4	2.0	3.9
P value	.4216	.2067	.9973	.0346	.0346	.315	.1125	.0849	.6958	.4985	.319	.2308

* $P < .05$ by codominant model, †$P < .05$ by dominant model, and ‡$P < .05$ by recessive model after adjustment for age and sex.

and diabetes mellitus have been observed in Japanese subjects [21]; and *ADRB2* polymorphisms have been shown to contribute to body fat and plasma lipid variability in men [11]. Recently, the Arg16Gly and Gln27Glu polymorphisms in the *ADRB2* gene were shown to be associated with the metabolic syndrome in adult men [15]. However, a study reported that genetic variability in the *ADRB2* gene may not be a major determinant for the development of obesity and diabetes [12]. These discrepancies in associations between *ADRB2* variants and obesity and its related risk factors may be due to variations among populations and SNPs of the specific genes.

The *ADRB2* genotype may influence receptor function, receptor density, or efficiency. Reduced expression of the receptor may lead to less efficient stimulation of lipolysis in adipose tissue and excess fat accumulation over time [22]. Variants in the *ADRB2* gene associated with lower receptor function due to reduced expression may enhance the propensity to fat accumulation in abdominal adipose tissue. High waist circumference associated with increased abdominal adipose tissue, a major source of free fatty acids and an important target for catecholamine-mediated lipolysis [23], resulted in higher fluxes of plasma fatty acids to the liver and peripheral tissues. Accordingly, in our study, the variant at rs1042714 (Gln27Glu) had significant associations with higher waist circumference and free fatty acids.

Previous studies among children have shown ambiguous associations between *ADRB2* variations and obesity. For example, a variant of *ADRB2* was associated with the propensity to gain weight from childhood to young adulthood in

male subjects [24]. In other studies, however, polymorphisms in the *ADRB2* gene were not associated with obesity in children and adolescents [25,26]. Less is known, however, about the relationships between *ADRB2* polymorphisms and the metabolic syndrome in adolescents.

In our study, polymorphisms at rs1042717 of the *ADRB2* gene were significantly associated with insulin resistance, measured as fasting insulin concentration or HOMA score. Fasting insulin concentration is a biochemical marker for insulin resistance and may foretell cardiovascular diseases in adolescents. Insulin resistance may begin before the appearance of phenotypes of the metabolic syndrome, such as high blood pressure, high fasting glucose, or dyslipidemia, and may therefore affect the progress of cardiovascular diseases in adolescents. In addition, we observed a significant association between the rs1042719 variant and diastolic blood pressure. Similarly, β_2-adrenergic receptor functional gene variants were related to an increased risk of hypertension in the urban population of Brazil [10], as well as with elevated blood pressure in Swedish men [13]. These findings, together with the results presented here, suggest that *ADRB2* SNPs may contribute to metabolic syndrome susceptibility in Korean adolescents.

In contrast to a previous study, which reported no statistically significant associations between *ADRB2* haplotypes and the metabolic syndrome [15], we found that the C-A-A-C haplotype of *ADRB2* (rs1042714-rs1042717-rs1042718-rs1042719) was significantly associated with higher body weight and insulin resistance. In particular, fasting insulin concentrations and HOMA score were dose-dependently related to this haplotype. In addition, we observed significant associations between the C-G-C-G haplotype and lower HDL cholesterol, and between the G-G-C-G haplotype and higher body fat and waist circumference, suggesting that these *ADRB2* haplotypes may be associated with susceptibility to the metabolic syndrome. The reason for the different results with the previous study might be the differing gene susceptibility according to the subject's ethnic background, environmental factors, and other confounding factors. More studies will be needed to investigate the reasons for the differences.

We found that the C-G-C-C haplotype was associated with lower body weight, lower waist circumference, and higher HDL cholesterol, in agreement with previous findings showing that the Gln27Glu polymorphism in *ADRB2* was associated with a lower probability for low HDL cholesterol concentration [15]. These results suggest that the C-G-C-C haplotype of *ADRB2* may protect against effects of the metabolic syndrome. Because obesity and insulin resistance are major determinants of the metabolic syndrome in adolescents [27], our results showed that the rs1042717 genotype and the C-A-A-C haplotype of *ADRB2* were closely associated with obesity and insulin resistance, which are intermediary factors for the metabolic syndrome. Despite the observation of associations between genotypes or haplotypes and phenotypes, comprehensive answers that would allow the translation of genetic susceptibility into scientifically sound medical practice will require much larger patient populations, well-annotated clinical databases, and sophisticated environmental assessment [28].

This study had several limitations. First, the relatively small number of subjects in this study might have caused some statistically significant findings just by chance. The methods used cannot be sufficient to prove definite associations. The small sample size coupled with multiple comparisons might increase the likelihood of false positives. These findings should be tested in a large sample in a prospective study. It is true that it may not be possible to do the type of wide-scale testing in adolescents necessary to sort out associations between cardiometabolic risk factors in adolescence and various genotypes because of the difficulty in obtaining a sample size sufficient to the task. Second, in adolescents, anthropomorphic measures such as weight, height, BMI, waist circumference, body composition, and lipid measurements are highly influenced by puberty. In the age range studied, there is likely to be great variability among subjects with regard to the stage of puberty. Thus, the stage of puberty would seem to be an important confounder. Third, we used fasting insulin concentration and HOMA score as markers of insulin resistance. The HOMA score has been significantly correlated with the euglycemic-hyperinsulinemic clamp, which is accepted as the criterion standard in defining insulin resistance [29]; and HOMA score has been used in other epidemiologic studies [30]. Last, because our study was conducted in Korean adolescents, we cannot generalize these findings to other ethnic groups.

In conclusion, we have shown that *ADRB2* genotypes and haplotypes may influence parameters of the metabolic syndrome in Korean adolescents. As it seems that variations leading to common disease are diverse, studies on the exact nature of the disease-causing variants in the scope of gene-environmental interaction will be necessary.

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