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The APJ Receptor A445C C Mutant Carrier Improves HDL Metabolism in Korean CVD Patients

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Abstract

Backgrouds: The apelin/APJ receptor system plays a mediator of CVD, but its molecular mechanisms related to APJ receptor polymorphism on HDL metabolism in CVD patients remains unclear.

Methods: 386 CVD outpatients were screened by dyslipidemia (DYS; TG>150 mg/dl and HDLc<40 for men, <50 mg/dl for women) and non-DYS, and biomarkers for HDL metabolism and APJ receptor A445C polymorphisms (SNP rs948847) were examined.

Results: BMI, insulin, HOMA-IR, TC, TG, LDLc, leptin, visfatin, and IL-6 levels were higher as well as adiponectin and HDL peak sizes were lower in DYS than those of non-DYS. High apelin levels were related to lower TC and LDL, and higher adiponectin and leptin levels. Increasing apelin was associated with higher TG and lower HDL in DYS, not in non-DYS. However, APJ A445C C mutant carrier, 5.7% frequencies among CVD, has protective effects in DYS. We found that lower TG/HDL, insulin, HOMA-IR, and visfatin levels and higher adiponectin levels in DYS with C allele compared to DYS with A allele, in spite of high apelin (>231 pg/mL) status. Decreasing TG/HDL in DYS with C allele was strongly improved with HDL metabolism such as increasing HDL with HDL2b and decreasing TG with HDL3 subtypes. Even though there were no changes in the mass of RCT enzymes between the A and C alleles in high apelin group, they were closed to those of non-DYS.

Conclusion: Hyperapelinemia was associated with DYS risks compared to non-DYS, but APJ mutant carrier may have the beneficial effects of the HDL metabolism in the developments of DYS in Korean CVD patients.

Keywords: Dyslipidemia; Apelin; APJ polymorphism; HDL subtypes; Adiponectin; CVD

Abbreviations

BMI: Body Mass Index; CETP: Cholesterylester Transfer Protein; CHO: Carbohydrate; CRP: C-Reactive Protein; CVD: Cardiovascular Diseases; DBP: Diastolic Blood Pressure; DYS :Dyslipidemia; FBS: Fasting Blood Glucose; HDL-c: High-Density Lipoprotein Cholesterol; HOMA-IR: Homeostasis Model Assessment Of Insulin Resistance; IL-6: Interleukin 6LCAT: Lecithin Cholesterol Acyltransferase; LDL-c: Low-Density Lipoprotein Cholesterol; LPL: Lipoprotein Lipase; MCP-1;Monocyte Chemoattractant Protein-1; RCT: Reverse Cholesterol Transport; SBP: Systolic Blood Pressure; TC: Total Cholesterol; TG: Triacylglyceride; TNF-α:Tumor Necrosis Factor Alpha

Introduction

The major causes of mortality in Korea (50% of the total) are cancer, cardiovascular diseases (CVD), suicide, and diabetes in that order [1]. Dyslipidemia (DYS) manifested by elevation of triacylglycerol (TG) and decline of HDLc (TG/HDLc ratio) is an important criteria to evaluate metabolic syndromes and risk factor of CVD [2]. The variable of TG/HDLc ratio is more indicative than the hyperlipidemia, hypertriglyceridemia or hypercholesterolemia, on the development of CVD. However, total HDLc is not always an indicator for CVD because it is continuously modified heterogeneous particle reflected by various biological conditions [3,4]. DYS atients had increased small HDL particles such as HDL3b and HDL3c, and decreased the large HDL particles like HDL2b and HDL2a [5]. This is one of reasons why that the 30% reduction of CVD prevalence can be observed by lipid-lowering medicine in DYS patients, not in the healthy peoples over a 5-year period [6]. The other risk factors of DYS, possible modulators to shift TG/HDL ratio and HDL subtypes, may be suggested by major enzymes of the reverse cholesterol transport (RCT) system such as lecithin cholesterol acyltransferase (LCAT), cholesterylester transfer protein (CETP) and lipoprotein lipase (LPL) [4].

The apelin gene, identified in several tissues, including brain, heart, stomach, skeletal muscle, and the vascular system, encodes a 77-amino-acid-long preproprotein that exerts its function by binding to APJ receptor, a specific G protein-coupled receptor [7,8]. Apelin and APJ are known to play a variety of roles in cardiac function [9,10] drinking behavior [11] and gastric cell proliferation [12,13]. However,

there have been very few reports as to whether APJ variation influences circulating apelin levels or DYS risk factors. Apelin has been shown to activate endothelial nitric oxide synthase (eNOS) via Akt phosphorylation to stimulate NO production in cultured human umbilical vein endothelial cells [10]. Apelin is also upregulated by insulin, and it inhibits pancreatic insulin secretion [13,14]. Moreover, long-term direct administration of apelin in preclinical animal models showed improved insulin sensitivity, suggesting a potential for therapeutic application [15]. In clinical studies, increased plasma apelin levels were frequently observed in patients with obesity and insulin resistant diseases [14-16]. Furthermore, apelin has also been reported to increase glucose uptake in isolated soleus muscle, which may be mediated by a pathway involving the serine-threonine kinase AMP-activated protein kinase as well as eNOS [10,17]. Therefore, it has been hypothesized that high circulating apelin found in obese people may help to delay the onset of insulin resistance. In addition, adipokines, tumor necrosis factor alpha (TNF-α), leptin, and the plasminogen activator inhibitor type-1 factor, have also been reported to increase the apelin expression in both human and mouse adipose tissues [16,18]. Apelin increases the expression of uncoupling protein-1/-3 and adiponectin to induce the energy expenditure and decreases leptin to be reduction of energy intakes and respiratory quotient or to increase fat oxidation [19]. While the apelin functions on CVD and obesity are still unclear, the apelin level, may be affected by APJ polymorphisms such as APJ A445C and G212A, is strongly associated with insulin resistance, adipokines and inotropic effects. The purposes of this study are to investigate not only the association between serum apelin levels and APJ polymorphism A445C (rs 948847) but also the effects of APJ A445C on the development of DYS risks in Korean CVD patients.

Methods

Study subjects and experimental design

The CVD patients enrolled in this study were 250 men and 136 women between 40 and 80 years of age. These patients, recruited from the Yonsei CVD Medical Center in Seoul from July 2008 through December 2009, were divided into 2 groups: patients with dyslipidemia (DYS) and patients without dyslipidemia (non-DYS; control). DYS patients were defined by 4 serum criteria with a slight modification as follows: (a) TC ≥ 200 mg/dL, (b) LDLc ≥ 130 mg/dL, (c) TG ≥ 150 mg/dL, and (d) HDLc ≤ 40 mg/dL and ≤ 50 mg/dL in male and female, respectively. Accordingly, 108 subjects in the control group and 278 DYS subjects were eligible for further analysis. The institutional review board of Yonsei Severance Hospital of the Yonsei University approved this study protocol, and written informed consent was obtained from all participants.

Anthropometric measurement

Standing heights and weights were measured by experienced medical staff, and the body mass index (BMI) was calculated as the ratio of weight (kg) divided by height (m^2). Systolic and diastolic blood pressure (SBP and DBP) were measured after a 10-min rest by using an automatic BP calculator in a sitting position.

Blood biochemistry

For DNA genotyping, whole blood was collected in EDTA-treated tubes and serum was separated to analyze the other blood profiles. Serum adipokines, c-reactive protein(CRP), leptin, adiponectin, monocyte chemoattractant protein-1(MCP-1), visfatin, interleukin 6(IL-6) and TNF-α were measured using ELISA kits, and plasma apelin concentrations were determined by enzyme immunoassay kit (Human Apelin-12 EIA Kit, EK-057-23; Phoenix pharmaceuticals, Belmont, CA). The levels of TC, TG, HDL-c, and glucose were measured in an autoanalyzer(Hitachi 7080), and LDL-c levels were analyzed using the Friedewald equation [20]. Insulin levels were measured by the ELISA kit and the homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the Matthews equation [21]. The mass of RCT enzymes, LCAT, CETP and LPL, were measured using enzyme immunoassays (Human LCAT, CETP, and LPL ELISA Kits; Daiichi Pure Chemicals, Tokyo, Japan). The resulting color reactions were read at 492 nm using the Thermo Multiscan Spectrometer (Thermo Labsystems, Vantaa, Finland).

Analysis of HDL subfraction

HDL subfraction was obtained by following previously published methods. Fresh serum of 600 μL was dialyzed and HDLs were fractionized by micro-ultracentrifugation (Hitachi CS150GXL, S140AT fixed angle rotor; Japan) [5] HDL (10 μL) was subjected to polyacrylamide gel electrophoresis in 4–30% non-sodium dodecyl sulfate gradient gels (LPE gel 4/30; CSI Scientific, CA) run in the Pore Gradient Lipoprotein Electrophoresis System (PGLE, CSI Scientific Electrophoresis LPE-4003; C.B.S. Scientific non-SDS buffer). Coomassie blue G-250-stained gels were analyzed with Image-Master ID software 4.0 (Amersham Pharmacia Biotech, USA). Using high molecular weight calibration kit standards (relative migration distances: thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 10.4 nm; LDH, 8.2 nm; BSA, 7.1 nm; Amersham, GE Healthcare, UK), the diameters of HDL subtypes were calibrated. The relative proportions of the HDL subclasses were estimated with the following size intervals: HDL3c, 7.2–7.8 nm; HDL3b, 7.8–8.2 nm; HDL3a, 8.2–8.8 nm; HDL2a, 8.8–9.7 nm; and HDL2b, 9.7–12.2 nm. The integrated HDL particle size was calculated by multiplying the size of each HDL subclass by its relative contribution in percent [5].

Genotyping of APJ A445C polymorphism

Genomic DNA was extracted from blood cells using a DNA extraction kit (LaboPass Blood MiniKit; Cosmo Genetech, Korea), as recommended by the manufacturer. DNA quantity/quality was evaluated using a spectrophotometer (Smartspec plus; Bio-Rad Laboratories, Richmond, CA) and by gel analysis (2% agarose gels; Duchefa Biochemie, Haarlem, Netherland). The APJ coding and untranslated regions (SNP, rs 948847; GenBank accession no. NM005161) were PCR amplified using a forward primer (5–CAG CAT GGA GGA AGG TGG–3) and a reverse primer (5–GAC CCG CAG CCT CAG CCG–3), as described by Riccardo et al. [22]. Amplifications were performed in a PCR machine (iCycleriQ PCR system; Bio-Rad), and PCR products amplified by primer pair were digested by Hpy10VI (MBI, Fermentas). Therefore, a wild-type sequence is characterized by 4 fragments of 215, 100, 80, and 46 bp, a hetero-type sequence is characterized by 5 fragments of 215, 135, 100, 80, and 46 bp, and a mutated sequence is characterized by 4 fragments of 135, 100, 80, and 46 bp. The genotype frequencies among these samples were in accordance with the Hardy-Weinberg equilibrium.

Statistical analysis

Statistical analysis was performed with SPSS 18.0 statistical package (SPSS Inc., Chicago, IL). Data were presented as mean ± standard deviation or number with percentage (%) for categorical variables. Genotype frequencies were compared with values predicted by Hardy-Weinberg equilibrium equation by the Chi-Square test. Comparison of mean values across groups was carried out using the t-test for normally distributed continuous variables and the chi-square test for categorical variables. Correlations between HDL subfraction and determinants (continuous variables, including apelin) were determined using Pearson's correlation coefficients (r) and x^2-test. Logistic regression analysis was used to evaluate the association between gene polymorphism and variations (including apelin) according to the presence of dyslipidemia. Analyses were adjusted for factors such as age, sex, and HDL. Comparison of the 4 groups was performed using one way ANOVA analysis. All p-values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of study subjects

Our study subjects were 250 men (average, 58.3 years old) and 136 women (average, 64.7 years old) between 40 and 80 years of age. Their general characteristics by gender or DYS are shown in Table 1. The women had significantly higher BP (SBP and DBP), plasma insulin and FBS, TC, HDLc, LDLc, adiponectin, leptin, HDL2b subtype, and LCAT and CETP mass than the men, whereas the men had higher TG, uric acid. There were no differences in BMI, HOMA-IR, and TG/HDL ratio between the genders. Among CVD patients, the distribution of non-DYS and DYS was 31.2% and 68.8% in men and 22.1% and 77.9% in women. Plasma insulin, HOMA-IR, FBS, TC, TG, LDLc, TG/HDL ratio, leptin, HDL 3 subtypes, LCAT and CETP mass, and BMI were all significantly higher in CVD with DYS, compared to those in non-DYS. In contrast, the levels of adiponectin, HDL particle size, and HDL2b were decreased in DYS. LPL mass was unaffected by gender or DYS. The frequencies of APJ receptor A445C genotype were not significantly different between DYS and non-DYS, but AC+CC genotypes in women (58.6%) were slightly higher than in men (47.3%) in non-DYS, but vice versa in DYS. However, DYS was found to be associated with not only increased BMI, insulin, HOMA-IR, uric acid, lipid profiles (TC, TG, LDLc, and TG/HDL ratio), HDL metabolism (HDL3c and LCAT and CETP mass), and adipokines (leptin, visfatin, and IL-6) but also decreased adiponectin, HDL peak size, HDL2b, and LPL mass. These observations suggested that DYS may explain the development of endothelial dysfunction and early CVD.

–		Total	Male	Female	p-value[1)]	CVD/non-DYS (n=108)	CVD/with-DYS	p-value [2)]
		(n=386)	(n=250)	(n=136)			(n=278)	
Anthropometrics								
BMI	kg/m^2	24.9 ± 3.3	24.9 ± 0.1	25.1 ± 0.2	NS	24.1 ± 0.2	25.3 ± 0.1	**
SBP	mmHg	116.4 ± 13.1	115.1 ± 0.6	118.7 ± 0.8	**	116.6 ± 0.9	116.3 ± 0.5	NS
DBP	mmHg	72.8 ± 32.8	70.8 ± 1.5	76.5 ± 2.1	*	71.5 ± 2.3	73.3 ± 1.4	NS
Blood Biochemistry								
TG	mg/dL	174.0 ± 98.8	174.4 ± 4.5	173.2 ± 6.2	*	88.1 ± 5.7	207.3 ± 3.5	**
HDL	mg/dL	41.4 ± 11.0	40.0 ± 0.5	44.1 ± 0.7	**	50.6 ± 0.6	37.8 ± 0.4	**
LDL	mg/dL	100.0 ± 37.9	97.7 ± 1.7	104.2 ± 2.4	*	73.9 ± 2.3	110.1 ± 1.5	**
TG/HDL	_	4.8 ± 3.7	4.9 ± 0.2	4.7 ± 0.2	NS	1.8 ± 0.2	6.0 ± 0.1	*
Insulin	uU/mL	10.9 ± 14.4	11.5 ± 0.7	9.7 ± 0.9	NS	7.8 ± 1.0	12.0 ± 0.6	**
HOMA-IR	_	3.6 ± 5.0	3.7 ± 0.2	3.3 ± 0.3	NS	2.3 ± 0.4	4.0 ± 0.2	**
FBS	mg/dL	127.1 ± 55.1	123.5 ± 2.5	133.6 ± 3.4	*	119.8 ± 3.7	129.9 ± 2.3	*
SGOT	IU/L	27.0 ± 20.1	27.1 ± 0.9	27.0 ± 1.3	NS	26.1 ± 1.4	27.4 ± 0.8	NS
SGPT	IU/L	27.3 ± 17.9	29.2 ± 0.8	23.7 ± 1.1	**	27.3 ± 1.2	27.3 ± 0.7	NS
uric acid	mg/dL	5.4 ± 1.4	5.8 ± 0.1	4.7 ± 0.1	**	4.9 ± 0.1	5.6 ± 0.1	**
adiponectin	ng/mL	5.7 ± 3.8	5.0 ± 0.2	7.0 ± 0.2	**	7.0 ± 0.2	5.3 ± 0.1	**
leptin	ng/mL	30.3 ± 33.4	20.3 ± 1.4	47.9 ± 1.9	<0.001	26.3 ± 2.2	31.7 ± 1.3	*
HDL metabolism								

HDL particle size	nm	9.1 ± 0.6	9.1 ± 0.1	9.1 ± 0.1	NS	9.3 ± 0.1	9.0 ± 0.1	**
HDL_{2b}	%	31.6 ± 3.3	31.3 ± 0.2	32.2 ± 0.2	**	33.6 ± 0.2	30.9 ± 0.1	**
HDL_{3c}	%	14.7 ± 2.4	14.9 ± 0.1	14.3 ± 0.2	**	13.6 ± 0.2	15.1 ± 0.1	**
LCAT mass	ug/mL	8.9 ± 2.8	8.6 ± 0.1	9.3 ± 0.2	**	8.1 ± 0.2	9.2 ± 0.1	**
CETP mass	ug/ml	1.7 ± 0.9	1.6 ± 0.1	2.0 ± 0.1	**	1.5 ± 0.1	1.8 ± 0.1	*
LPL mass	ng/ml	31.1 ± 18.8	30.0 ± 0.9	34.1 ± 1.2	NS	33.2 ± 1.4	30.9 ± 0.9	NS
1) P-values with gender differences (mean ± SE) adjusted by age, sex and plasma TG. NS: no significance. 2) P-vales between with-DYS and non-DYS in CVD (mean ± SE) adjusted by age, sex and plasma TG. (*: p<0.05, **; p<0.01) NS: no significance								

Table 1: General characteristics according to gender and dyslipidemia in CVD patients.

CVD with and without DYS according to plasma apelin

Plasma apelin levels were significantly lower in females than in males, whereas there were not significant differences in apelin adjusted by sex, age, and plasma TG levels. We found that although plasma apelin levels, without adjustment for sex, ages, and TG, were higher in DYS, the pattern was reversed with further adjustments, suggesting that CVD risk factors such as sex, age, and TG may strongly influence plasma apelin levels. The control and DYS groups were subsequently divided into 3 subgroups based on their apelin levels: low (≤ 160 pg/mL), intermediate (161–230 pg/mL), and high (>231 pg/mL). While plasma lipid profiles and HDL subtypes were not affected by apelin levels in CVD patients without DYS, the levels of TC, LDLc, and HDL2b subtype were decreased, and TG/HDL ratio and HDL3a/3b were increased with elevated apelin levels in CVD patients with DYS (Table 2). Moreover, the proportion of HDL2 was negatively correlated with TG/HDL ratio, which was slowly reduced by increasing plasma apelin levels, and the opposite was observed for the proportion of HDL3. There were no differences in the patterns of LCAT, CETP, and LPL mass changed by apelin between CVD patients with or without DYS. It is of interest that LCAT mass was decreased when TG/HDL ratio was below 2.71 and apelin level was below 160 pg/ml (Data not shown). As for plasma cytokines, the levels of CRP, MCP-1, and IL-6 were increased with increasing apelin level in the control group, whereas CRP and leptin levels were increased with increasing apelin levels in DYS patients.

–		CVD/non-DYS				CVD/with-DYS			
		low apelin	intermediate	high apelin[1)]	p-value[2)]	low apelin	intermediate	high apelin[1)]	p-value [2)]
		n=33	n=25	n=22		n=23	n=33	n=22	
Apelin	pg/mL	119.0 ± 4.1[a]	206.5 ± 4.5[b]	290.9 ± 5.2[c]	<0.001	131.6 ± 5.2[a]	204.7 ± 4.3[b]	290.9 ± 5.5c	<0.001
BMI	kg/m^2	24.6 ± 0.4	23.8 ± 0.5	24.8 ± 0.5	NS	24.6 ± 0.5	23.9 ± 0.4	25.2 ± 0.5	NS
Blood Biochemistry									
TC	mg/dL	137.3 ± 3.3	142.4 ± 3.6	136.9 ± 4.1	NS	205.8 ± 6.7[b]	193.8 ± 5.7[a]	174.5 ± 7.0[a]	0.006
TG	mg/dL	84.8 ± 3.6	90.9 ± 4.0	96.7 ± 4.6	NS	169.3 ± 12.6[a]	188.4 ± 10.7[a]	241.5 ± 13.2[b]	<0.001
HDL	mg/dL	49.9 ± 0.9	48.9 ± 1.0	51.5 ± 1.1	NS	39.9 ± 1.4	40.0 ± 1.2	36.3 ± 1.5	NS
LDL	mg/dL	73.3 ± 2.7	73.4 ± 3.0	73.9 ± 3.4	NS	123.7 ± 5.3[b]	116.5 ± 4.5[b]	95.5 ± 5.6[a]	<0.001
TG/HDL		1.8 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	NS	4.5 ± 0.5[a]	5.4 ± 0.4[a]	7.4 ± 0.5[b]	<0.001
insulin	uU/mL	6.5 ± 1.0	8.8 ± 0.9	8.6 ± 1.1	NS	14.3 ± 1.4[b]	8.8 ± 1.2[a]	9.4 ± 1.5[a]	0.009
HOMA-IR		2.0 ± 0.3	2.4 ± 0.3	2.2 ± 0.3	NS	4.8 ± 0.5[b]	2.8 ± 0.5[a]	2.9 ± 0.6[a]	0.018
FBS	mg/dL	124.2 ± 5.6	114.7 ± 6.3	109.3 ± 7.1	NS	121.1 ± 7.3	123.0 ± 6.1	136.2 ± 7.6	NS
adiponectin	ng/mL	7.3 ± 0.6	6.3 ± 0.6	6.6 ± 0.6	NS	5.2 ± 0.4[a]	6.6 ± 0.4[b]	5.0 ± 0.5[a]	0.007
leptin	ng/mL	28.6 ± 4.3	22.8 ± 4.3	28.1 ± 4.9	NS	39.6 ± 5.3[b]	25.9 ± 4.4[a]	41.9 ± 5.5[b]	0.045
HDL subtypes									

Peak size	nm	9.5 ± 0.1	9.1 ± 0.1	9.3 ± 0.1	NS	9.0 ± 0.1^{b}	9.1 ± 0.1^{b}	8.6 ± 0.1^{a}	<0.001
HDL_{2b}	%	33.6 ± 0.5	32.9 ± 0.5	33.4 ± 0.6	NS	32.5 ± 0.5^{b}	32.4 ± 0.4^{b}	28.9 ± 0.5^{a}	<0.001
HDL_{2a}	%	22.5 ± 0.3	22.7 ± 0.3	22.0 ± 0.3	NS	22.2 ± 0.2	22.2 ± 0.2	21.7 ± 0.2	NS
HDL_{3a}	%	18.2 ± 0.3	18.5 ± 0.3	18.3 ± 0.3	NS	18.0 ± 0.3	18.0 ± 0.2	19.3 ± 0.3	<0.001
HDL_{3b}	%	12.1 ± 0.2	12.4 ± 0.2	12.1 ± 0.3	NS	12.6 ± 0.2^{a}	12.6 ± 0.2^{a}	13.9 ± 0.2^{b}	<0.001
HDL_{3c}	%	13.6 ± 0.4	13.5 ± 0.4	13.7 ± 0.4	NS	14.8 ± 0.3^{a}	14.7 ± 0.3^{a}	16.1 ± 0.3^{b}	0.005
RCT enzymes									
LCAT	ug/ml	9.1 ± 0.4^{b}	8.1 ± 0.4^{ab}	7.1 ± 0.5^{a}	0.026	9.8 ± 0.5^{b}	8.6 ± 0.4^{ab}	7.8 ± 0.6^{a}	0.033
CETP	ug/ml	1.3 ± 0.1^{a}	1.6 ± 0.1^{ab}	1.9 ± 0.1^{b}	0.014	1.7 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	NS
LPL	ng/ml	29.2 ± 2.7^{a}	37.9 ± 2.5^{b}	30.2 ± 3.4^{ab}	0.036	22.2 ± 2.4^{a}	44.2 ± 2.1^{b}	26.2 ± 2.8^{a}	<0.001

1) Three groups of plasma apelin levels; low apelin: ≤ 160, intermediate: 161-230, high apelin:>231 pg/ml. 2) P- values for the statistical differences (mean ± SE adjusted by age, sex and plasma TG) according to levels of apelin in CVD with or without DYS. NS: no significance. The same superscripts are not significantly different from each other.

Table 2: Changes on the lipid profiles, cytokines, HDL subtypes and RCT enzymes according to plasma apelin concentration.

HDL metabolism in various APJ receptor genotypes carrier with different apelin levels

Biomarkers that are related to HDL metabolism changed by APJ polymorphism and apelin levels are shown in Table 3. Among the subjects with apelin levels above 230 pg/ml, those with AC and CC genotypes had a lower risk of CVD because BMI, TG and TG//HDL were lower, and HDL, HDL2b fraction and adiponectin were higher, compared to those with AA genotype. However, for the subjects with apelin levels between 161 and 230 pg/ml, the risk of CVD was increased in those carrying AC+CC genotypes than those with AA genotype because of increasing TC, LDLc, and the mass of LCAT and LPL (Data not shown) Even with increased apelin levels, poor lipid profiles, and HDL subfraction, the risk pattern for CVD and obesity according to APJ polymorphism was dramatically influenced by apelin levels. The study of the association between the apelin level and DYS risk factors showed that the HDL subfraction, peak size, and HDL2a were significantly lower and HDL3a and HDL3b were significantly higher in the highest tertile of apelin than those in the lowest tertile (Data not shown). Since LCAT activity plays a role in the formation of mature HDL2 particles, it was found to be lower in the highest tertile than in the lowest. DYS or CVD risk factors according to APJ A445C polymorphism in the tertile group of plasma apelin levels, particularly in the DYS group. The TG, TG/HDL ratio and HDL3b in the highest tertile of apelin were significantly lower in C carrier than in A carrier. However, HDL and adiponectin levels in the highest tertile of apelin were higher in the subjects with C carrier than in those with A carrier. Overall, these results suggest that hyperapelinemia is mildly associated with CVD risk factors in Korean DYS patients and that AC+CC genotype in DYS patients may have a protective effect against the development of CVD and atherosclerosis.

Total		1st -Low		2nd -Intermediate		3rd -High[1)]		p-value 2)
		AA	AC+CC	AA	AC+CC	AA	AC+CC	
		(n=32)	(n=24)	(n=25)	(n=33)	(n=21)	(n=23)	
Apelin	pg/mL	125.4 ± 4.9^{a}	119.1 ± 5.7^{a}	204.1 ± 3.9^{b}	206.3 ± 3.3^{b}	296.5 ± 11.7^{c}	289.7 ± 11.1^{c}	‡
BMI	Kg/m^2	24.3 ± 0.6^{a}	24.9 ± 0.5^{ab}	23.4 ± 0.6^{a}	24.2 ± 0.5^{a}	26.4 ± 0.6^{b}	23.9 ± 0.6^{a}	†
Blood biochemistry								
TC	mg/mL	162.4 ± 9.5	170.6 ± 11.1	159.6 ± 7.8	181.5 ± 6.7*	151.1 ± 9.8	157.7 ± 9.4	NS
TG	mg/mL	110.4 ± 12.2^{a}	129.0 ± 14.2^{a}	142.3 ± 16.1^{ab}	146.0 ± 14.0^{ab}	187.4 ± 23.0^{b}	160.3 ± 22.0^{ab}	†
HDL	mg/mL	47.6 ± 1.8	45.0 ± 2.1	44.3 ± 2.1	44.6 ± 1.9	37.7 ± 2.3	46.2 ± 2.2$^{*3)}$	NS
LDL	mg/mL	90.8 ± 7.5	98.5 ± 8.7	87.0 ± 6.7	105.8 ± 5.8*	80.6 ± 6.9	88.9 ± 6.6	NS
TG/HDL	_	2.6 ± 0.4^{a}	3.1 ± 0.4^{a}	3.7 ± 0.6^{a}	3.8 ± 0.5^{a}	5.9 ± 0.9^{b}	4.0 ± 0.9^{ab}	†
Insulin	IU/mL	9.3 ± 2.4	11.2 ± 2.7	8.8 ± 1.3	8.6 ± 1.1	11.1 ± 1.4	7.4 ± 1.4	NS

HOMA-IR	_	3.0 ± 0.9	3.6 ± 1.0	3.3 ± 0.4	2.1 ± 0.4	3.2 ± 0.4	2.2 ± 0.4	NS
FBS	mg/mL	122.6 ± 8.3	121.2 ± 9.6	140.8 ± 8.6	104.7 ± 7.4**	124.7 ± 11.8	121.0 ± 11.2	NS
adiponectin	ng/mL	7.5 ± 0.7^{b}	5.6 ± 0.9^{ab}	5.7 ± 0.7^{ab}	7.6 ± 0.6^{b}	3.6 ± 0.7a	6.1 ± 0.6^{b*}	†
leptin	ng/mL	31.0 ± 8.4	43.7 ± 10.3	24.6 ± 5.0	29.4 ± 4.3	28.9 ± 6.9	28.4 ± 6.6	NS
HDL subfraction								
Peak size	nm	9.4 ± 0.2	9.1 ± 0.2	9.1 ± 0.1	9.2 ± 0.1	8.8 ± 0.2	9.1 ± 0.2	NS
HDL_{2b}	%	33.6 ± 0.7^{b}	32.8 ± 0.8^{a*}	32.4 ± 0.7^{a}	32.8 ± 0.6^{a}	29.4 ± 0.9^{a}	32.5 ± 0.9^{a*}	†
HDL_{2a}	%	22.2 ± 0.3	22.5 ± 0.3	22.7 ± 0.4	22.2 ± 0.3	22.1 ± 0.4	21.6 ± 0.4	NS
HDL_{3a}	%	18.1 ± 0.3	17.9 ± 0.4	18.6 ± 0.3	18.0 ± 0.3	19.4 ± 0.5	18.3 ± 0.5	NS
HDL_{3b}	%	12.3 ± 0.3	12.2 ± 0.4	12.7 ± 0.3	12.4 ± 0.2	13.4 ± 0.4	12.6 ± 0.4	NS
HDL_{3c}	%	13.8 ± 0.5	14.6 ± 0.6	13.6 ± 0.5	14.5 ± 0.4	15.6 ± 0.7	14.4 ± 0.7	NS
RCT enzyme								
LCAT mass	ug/ml	9.0 ± 0.9^{ab}	10.3 ± 1.0^{b}	7.7 ± 0.6^{a}	8.9 ± 0.5^{ab}	7.5 ± 0.4^{a}	7.1 ± 0.4^{a}	†
CETP mass	ug/ml	1.6 ± 0.2	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	1.7 ± 0.2	1.8 ± 0.2	NS
LPL mass	ng/ml	29.6 ± 3.1^{a}	24.3 ± 3.5^{a}	40.5 ± 4.1^{b}	42.7 ± 3.4^{c}	22.4 ± 3.8^{a}	28.7 ± 3.9^{a}	‡

1) Plasma apelin levels; low apelin: ≤ 160, intermediate: 161-230, high apelin: >231 pg/ml. 2) P-value for statistic differences among all 6 groups. (Mean ± SE adjusted by age, sex and plasma TG. NS: no significance) The same superscripts are not significantly different from each other. 3) Statistical differences between AA and AC +CC genotypes in three groups of apelin. (t-test; †:p<0.05; ‡:p<0.01.

Table 3: Biomarkers for HDL metabolism according to APJ A445C genotypes with different apelin levels.

Discussion

This current study that the association of APJ polymorphism and CVD risks with the condition of DYS, which is a major risk factor of Korean CVD is the first to report. Studies on the mechanisms underlying accelerated atherosclerosis in DYS indicate the profound influences of insulin resistance and subclinical inflammation, both of which start even years before the diagnosis of the disease [23]. Adipose tissues seem to be an important site for regulating insulin resistance in patients with DYS or other metabolic disorders. Because there have been very few studies on APJ polymorphism, it is hard to compare our results with others. Tasci et al. found a substantial decline of plasma apelin levels in patients with elevated LDLc, which is an established independent risk factor for atherosclerosis-related diseases [24,25]. Apelin displays a "beneficial" role with its inotropic and NO-mediated vasodilatory properties, and it seems likely that along with other classical inotropic G-protein-coupled signaling pathways, the apelin/APJ system is recruited to support the contractility of a failing heart. However, the proposed cardiovascular effect of apelin can only be mediated if APJ is expressed in cardiomyocytes. Tasci et al. reported recently that plasma apelin levels were reduced in patients with DYS as well as in those with high TG and low LDL-c [24,25]. Interestingly, our results, inconsistent with previous studies, showed that DYS patients had higher apelin levels than the control group and CVD risks were exacerbated by high apelin levels in DYS, otherwise, apelin levels did not make any differences to non-DYS.

Based on these data, we tested the hypothesis that the APJ polymorphism may influence the plasma levels and biological activity of the corresponding proteins, lipid profiles, and HDL subfraction, which could have important clinical implications for CVD. According to a previous study, patients with the 212A variant had a significantly lower risk of both heart failure progression and sudden death than those who were homozygous for the G212 variant [22]. In this current study, DYS and non-DYS groups showed no differences in APJ A445C polymorphism frequencies, and plasma apelin levels were not affected by APJ polymorphisms. However, the APJ C carrier showed significantly lower BMI and visfatin and higher HDL level and LCAT activity than those with AA genotype after adjustments for sex and age were made. The development of atherosclerosis may be mildly inhibited in APJ 445C carrier because they showed significantly lower insulin, HOMA-IR, and visfatin levels and significantly higher adiponectin level than those with the A allele. In the contrast of our results, Turkish individuals with APJ A445C CC genotypes had significantly higher weight, SBP and DBP than other genotypes, they concluded CC genotypes may have more risk than the others in developments of hypertension in CVD [26]. However, HDL in control without high BP in CVD was higher in CC than in AA or AC genotypes of Turkish, not in high BP with CVD. BPs in our data was not different between DYS and non-DYS or among the APJ A445C genotypes, but higher HDL levels in CC was similar to Korean DYS.

The possibilities of reasons why hyperapelinemia may be toxic in DYS developments in CVD could be suggested. Firstly, APJ roles involved in angiogenesis and atherosclerosis-related oxidative stress had been reported. Since the constitutive expression of apelin may primarily accelerate the angiogenic switch and activate tumor neoangiogenesis, blocking apelin action would prevent angiogenic effects in cardiac myocytes [27,28]. This paracrine effect of apelin has not yet been investigated within adipose tissues, but apelin secreted by

adipocytes may stimulate blood vessel growth, leading to increased growth of adipose tissues. Hashimoto et al reported that atherosclerotic lesions were dramatically reduced in APJ and apolipoprotein E double-knockout (*APJ–/–ApoE–/–*) mice fed a high-cholesterol diets compared with *APJ++/ApoE–/–*mice [29]. Since apelin/APJ system is a mediator of oxidative stress in vascular tissue, APJ deficiency is preventative against oxidative stress-linked atherosclerosis. Secondly, hypothesis that high apelin levels lead to apelin resistance or negative side of inflammatory obesity might be suggested. We found that high apelin levels may increase the risk of DYS in CVD patients, even though the mechanism remains unknown as to why increased apelin leads to changes in HDL subfraction, lipid profiles, and other adipokines. Adipokines or adipokine receptors with polymorphic sequences may be potential markers of disease susceptibility. Previous study reported the correlation between apelin and TNF-a expression in adipose tissue of lean and obese humans. The signaling pathways of TNFa for the induction of apelin were dependent of PI3-kinase, c-Jun NH2-terminal kinase, and MAPK but not protein kinase C activation [18,30]. The strong relationship between serum concentration of apelin-12 and adiponectin was found in obese non-diabetic Saudi females [31]. Even though apelin does not directly reflect visceral or subcutaneous fat accumulation, it could be an important determinant in the pathophysiology of obesity-related diseases. Apelin levels were significantly higher in the morbidly obese patients with impaired fasting glucose or diabetes prior to surgery compared to the control, but bariatric surgery resulted in normal levels of apelin [32]. In Chinese women study, C allele of SNP rs3115757 was significantly associated with 2.1 times risk in BMI and 2.3 times risk in WC compared to G allele. They also found that apelin mRNA levels and protein concentrations were higher in cultivated adipocytes treated with high glucose plus insulin than in those with normal glucose [33]. All together, these findings showed that apelin might be a candidate to better understand potential links between obesity and associated disorders such as inflammation and insulin resistance.

However, how those mechanism underlying the development and progression of CVD or DYS according to apelin expression involved in HDL metabolism is still unclear. As for the HDL subfraction, the peak size, HDL2b, and HDL2a were lower in DYS than in the non-DYS; on the contrary, the levels of HDL3a, HDL3b, and HDL3c were higher in the DYS. The more apelin levels, the less LCAT activity in both DYS and non-DYS in CVD, but apelin did not make any differences of CETP. LPL protein was highly expressed in the intermediate apelin levels (161-230 pg/ml). No differences in the HDL subfraction were found between AA and AC+CC genotypes. In spite of no changes in the mass of RCT enzymes between the A and C alleles in high levels of apelin group, their levels were closed to those of non-DYS. Further research is necessary to examine the association of HDL subfraction and RCT enzyme with APJ A445C gene polymorphism. Since the reduction of TG and LPL is related to HDL2 subtypes, the effects of apelin/APJ receptor system on HDL metabolism, HDL particle sizes and RCT enzyme activities, may play a role in the development of DYS in CVD.

In conclusion, the circulating apelin level is associated with DYS risk factors, such as TC, TG/HDL, LDL, insulin, and HOMA-IR. We suggest that the development of atherosclerosis may be inhibited in DYS with APJ C carrier and that the apelin levels above 231 pg/mL lead to other apelin resistance-induced CVD in particular to DYS patients. However, there are several limitations in this study. First, the sample size was not large enough because of the difficulty in recruiting CVD outpatients from one hospital compared to epigenetics. The association between apelin levels and APJ genotypes needs to be proved with a larger sample size. Even though this study is not sufficient for setting up a specific, personalized guideline based on genetic informatics, our results are valuable for future studies on the relationship between APJ polymorphism and other CVD variables.

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Competing Interests

The authors have declared that no competing interests exist.

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