Synergistic effects of genetic variants of *APOA5* and *BTN2A1* on dyslipidemia or metabolic syndrome

MIZUHO HIRAMATSU[1], MITSUTOSHI OGURI[2], KIMIHIKO KATO[3,4], HIDEKI HORIBE[5], TETSUO FUJIMAKI[6], SACHIRO WATANABE[7], KEI SATOH[8], YUKITOSHI AOYAGI[9], MASASHI TANAKA[9], DONG-JIK SHIN[10], JONG HO LEE[10,11], YANGSOO JANG[12] and YOSHIJI YAMADA[4]

[1]Department of Cardiology, Nagoya Central Hospital, Nagoya; [2]Department of Cardiology, Japanese Red Cross Nagoya First Hospital, Nagoya; [3]Meitoh Hospital, Nagoya; [4]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu; [5]Department of Cardiovascular Medicine, Gifu Prefectural Tajimi Hospital, Tajimi; [6]Department of Cardiovascular Medicine, Inabe General Hospital, Inabe; [7]Department of Cardiology, Gifu Prefectural General Medical Center, Gifu; [8]Department of Vascular Biology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki; [9]Department of Genomics for Longevity, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; [10]Research Institute of Science for Aging, [11]National Research Laboratory of Clinical Nutrigenetics/Nutrigenomics, College of Human Ecology, and [12]Cardiovascular Center and Cardiovascular Genome Center, College of Medicine, Yonsei University, Seoul, Republic of Korea

Received January 30, 2012; Accepted March 30, 2012

DOI: 10.3892/ijmm.2012.976

Abstract. We previously showed that the -1131T→C polymorphism (rs662799) of the apolipoprotein A-V gene (*APOA5)* and the C→T polymorphism (rs6929846) of the butyrophilin, subfamily 2, member A1 gene (*BTN2A1)* were significantly associated with an increased serum concentration of triglycerides, a decreased serum concentration of high density lipoprotein (HDL)-cholesterol, and the prevalence of metabolic syndrome (MetS) in Japanese individuals. The purpose of the present study was to examine whether these polymorphisms synergistically affect the prevalence of dyslipidemia and MetS in East Asian populations. The study populations comprised 7471 Japanese and 3529 Korean individuals in the dyslipidemia study, and 3474 Japanese and 1671 Korean individuals in the MetS study. Multivariable logistic regression analysis of combined genotypes with adjustment for age, gender and diabetes mellitus revealed that rs662799 and rs6929846 significantly and synergistically affected dyslipidemia. Japanese or Korean individuals with the C allele of *APOA5* and the T allele of *BTN2A1* had a 2.05- or 1.92-fold increased risk for hypertriglyceridemia and a 1.82- or 1.56-fold increased risk for hypo-HDL-cholesterolemia, respectively, compared to those with the TT genotype of *APOA5* and the CC genotype of *BTN2A1*. Similar analysis with adjustment for age and gender revealed that Japanese individuals, but not Korean individuals, with the C allele of *APOA5* and the T allele of *BTN2A1* had a 2.87-fold increased risk for MetS compared to those with the TT genotype of *APOA5* and the CC genotype of *BTN2A1*. Genetic variants of *APOA5* and *BTN2A1* may synergistically affect the prevalence of dyslipidemia in East Asian populations and of MetS in Japanese individuals.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

Key words: polymorphism, genetics, dyslipidemia, metabolic syndrome, *APOA5*, *BTN2A1*

Introduction

The prevalence of dyslipidemia and metabolic syndrome (MetS), which are important risk factors for cardiovascular diseases (1,2), is increasing among Asian populations (3,4). The etiologies of dyslipidemia and MetS are highly complex, with both genetic and environmental factors being thought to play crucial roles. Although recent genome-wide association studies have implicated several loci and candidate genes in predisposition to dyslipidemia (5-7) and MetS (8,9) in Caucasian populations, the genes that confer susceptibility to these conditions in Asian populations remain to be identified definitively.

We previously showed that the -1131T→C polymorphism (rs662799) of the apolipoprotein A-V gene (*APOA5*) and the C→T polymorphism (rs6929846) of the butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) were significantly associated with the prevalence of dyslipidemia (10,11) and MetS (12-14) in Japanese individuals. Given that gene-gene interactions may play important roles in the development of multifactorial complex disorders, we hypothesized that rs662799 of *APOA5* and rs6929846 of *BTN2A1* may synergistically affect the development of dyslipidemia and MetS. We have thus examined the association of rs662799 of *APOA5*, rs6929846 of *BTN2A1*, or combined genotypes for these polymorphisms with the prevalence of dyslipidemia or MetS in Japanese and Korean individuals.

Materials and methods

Study population. The study populations comprised 7471 Japanese and 3529 Korean individuals in the dyslipidemia study, and 3474 Japanese (2744 subjects with MetS, 730 controls) and 1671 Korean (1294 subjects with MetS, 377 controls) individuals in the MetS study. Japanese subjects either visited outpatient clinics of or were admitted to the participating hospitals (Gifu Prefectural Tajimi Hospital, Tajimi; Gifu Prefectural General Medical Center, Gifu; Japanese Red Cross Nagoya First Hospital, Nagoya; Inabe General Hospital, Inabe, and Hirosaki University Hospital and Hirosaki Stroke Center, Hirosaki, Japan) between October 2002 and March 2009 because of various symptoms or for an annual health checkup, or who were recruited to population-based cohort studies of aging and age-related diseases in Nakanojo and Tokyo, Japan. The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committees of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, Tokyo Metropolitan Institute of Gerontology, and participating hospitals. Written informed consent was obtained from each subject.

Korean subjects were drawn from the institutional patient databases of the Cardiovascular Genome Center and Infarction Prognosis Study Registry, Severance Cardiovascular Hospital, Seoul. The study protocol complied with the guidelines for Genome/Genetic Research issued by the Korean government and was approved by the Institutional Review Board of Yonsei University. Written informed consent was obtained from each participant.

Diagnosis of MetS was based on a modified version of the definition proposed by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (3). In this modified version, which was also used in the West of Scotland Coronary Prevention Study (15) and the Women's Health Study (16), body mass index (BMI) replaces waist circumference, given that cut-off values of waist circumferences for men and women are still controversial in Japan. On the basis of the recent recognition of a need to revise BMI criteria for obesity in Japanese and other Asian populations (17), we set the cut-off point for obesity as a BMI of ≥25 kg/m^2. A total of 4038 subjects with MetS had thus three or more of the following five components: i) a BMI of ≥25 kg/m^2; ii) a serum concentration of triglycerides of ≥1.65 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides; iii) a serum concentration of high density lipoprotein (HDL)-cholesterol of <1.04 mmol/l (40 mg/dl) for men or <1.30 mmol/l (50 mg/dl) for women, or drug treatment for reduced HDL-cholesterol; iv) a systolic blood pressure of ≥130 mmHg or diastolic blood pressure of ≥85 mmHg, or drug treatment for hypertension; and v) a fasting plasma glucose level of ≥5.50 mmol/l (100 mg/dl) or drug treatment for elevated glucose. History of obesity, dyslipidemia, hypertension, or diabetes mellitus was evaluated from a detailed questionnaire. Control groups consisted of 1107 individuals who had none of the five components of diagnostic criteria for MetS. Individuals with coronary heart disease, ischemic or hemorrhagic stroke, or other vascular diseases were excluded from the control groups. Individuals with type 1 diabetes mellitus, maturity-onset diabetes of the young, diabetes mellitus associated with mitochondrial diseases or single gene disorders, pancreatic diseases including severe pancreatitis and pancreatic tumor, or other endocrinologic diseases were excluded from the study. Individuals with severe hepatic or renal diseases, chronic inflammatory diseases, or cancers were also excluded.

Measurement of blood pressure, serum lipid profile, and plasma glucose. Blood pressure was measured at least twice with subjects having rested in the sitting position for >5 min; the measurements were taken by a skilled physician according to the guidelines of the American Heart Association (18). Venous blood was collected in the early morning after the subjects had fasted overnight and before they had begun lipid-lowering treatment as appropriate. Blood samples were centrifuged at 1600 x g for 15 min at 4˚C, and serum was separated and stored at -30˚C until analysis. Serum concentrations of triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol (10) and plasma glucose levels (19) were measured as previously described.

Genotyping of polymorphisms. For Japanese individuals, venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells with a DNA extraction kit (Genomix; Talent, Trieste, Italy). Genotypes of rs662799 of *APOA5* and rs6929846 of *BTN2A1* were determined at G&G Science (Fukushima, Japan) by the multiplex bead-based Luminex assay, a method that combines polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Genotyping involved PCR amplification, hybridization, streptavidin-phycoerythrin reaction, and measurement of fluorescence. Detailed genotyping methodology was previously described (12,20,21).

For Korean individuals, genomic DNA was extracted from 5 ml of whole blood with the use of a DNA isolation kit (Wizard Genomic DNA purification kit; Promega, Madison, WI). Genotypes of rs662799 of *APOA5* and rs6929846 of *BTN2A1* were determined with the use of a TaqMan fluorogenic 5' nuclease assay (Applied Biosystems, Foster City, CA). Genotyping methodology was previously described (20,22).

Statistical analysis. Quantitative data were compared between two groups by the unpaired Student's t-test. Categorical data were compared by the χ^2 test. Allele frequency was estimated by the gene counting method, and the χ^2 test was used to identify departures from the Hardy-Weinberg equilibrium. Allele frequencies of rs662799 in *APOA5* and rs6929846 in *BTN2A1* were compared between subjects with MetS and controls by the χ^2 test. Multivariable logistic regression analysis was performed with hypertriglyceridemia (1, serum triglyceride concentration of ≥1.65 mmol/l; 0, that of <1.65 mmol/l), hypo-HDL-cholesterolemia (1, serum HDL-cholesterol concentration of <1.04 mmol/l; 0, that of ≥1.04 mmol/l), or hyper-LDL-cholesterolemia (1, serum LDL-cholesterol concentration of

Table I. Characteristics of the study subjects in the dyslipidemia study.

Characteristic	Japanese individuals	Korean individuals
No. of subjects	7471	3529
Age (years)	66.6±10.6	59.8±10.3
Gender (male/female, %)	56.4/43.6	68.7/31.3
Body mass index (kg/m^2)	23.5±3.3	24.3±3.0
Current of former smoker (%)	22.8	52.0
Hypertension (%)	60.4	66.6
Systolic blood pressure (mmHg)	142±24	126±20
Diastolic blood pressure (mmHg)	78±14	77±12
Diabetes mellitus (%)	32.0	20.6
Fasting plasma glucose (mmol/l)	6.77±3.01	5.85±2.58
Hypercholesterolemia (%)	39.4	54.3
Serum total cholesterol (mmol/l)	5.16±0.99	4.76±1.12
Hypertriglyceridemia (%)	29.6	28.6
Serum triglycerides (mmol/l)	1.62±1.12	1.46±0.88
Low HDL-cholesterol (%)	16.1	32.6
Serum HDL-cholesterol (mmol/l)	1.38±0.40	1.25±0.36
High LDL-cholesterol (%)	19.7	19.2
Serum LDL-cholesterol (mmol/l)	3.06±0.87	2.86±1.03

Quantitative data are means ± SD. Hypertension: systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or taking antihypertensive medication. Diabetes mellitus: fasting plasma glucose level of ≥6.93 mmol/l, blood glycosylated hemoglobin content of ≥6.5%, or taking antidiabetes medication. Hypercholesterolemia: serum concentration of total cholesterol of ≥5.69 mmol/l. Hypertriglyceridemia: serum concentration of triglycerides of ≥1.65 mmol/l. Low HDL-cholesterol: serum concentration of HDL-cholesterol of <1.04 mmol/l. High LDL-cholesterol: serum concentration of LDL-cholesterol of ≥3.64 mmol/l.

≥3.64 mmol/l; 0, that of <3.64 mmol/l) as a dependent variable and independent variables including age, gender (0, woman; 1, man), the prevalence of diabetes mellitus (0, no history of diabetes mellitus; 1, positive history), and genotypes for *APOA5* and/or *BTN2A1* in the dyslipidemia study; or with MetS as a dependent variable and independent variables including age, gender, and genotypes for *APOA5* and/or *BTN2A1*; and P-values, odds ratios, and 95% confidence intervals were calculated. Genotypes for *APOA5* or *BTN2A1* were assessed according to dominant (0, wild-type homozygote; 1, heterozygote and variant homozygote) and recessive (0, wild-type homozygote and heterozygote; 1, variant homozygote) genetic models. To compensate for multiple comparisons of genotypes with dyslipidemia or MetS, we applied Bonferroni's correction for statistical significance of association. The significance level was thus P<0.002 (0.05/27 tests) in Tables II and III; or P<0.006 (0.05/9 tests) in Tables V and VI. In other statistical analyses, a P-value of <0.05 was considered significant. Statistical significance was examined by two-sided tests performed with JMP version 6.0 and JMP Genomics version 3.2 software (SAS Institute, Cary, NC).

Results

Association of polymorphisms of APOA5 and BTN2A1 with serum concentrations of triglycerides, HDL-cholesterol, and LDL-cholesterol. The characteristics of Japanese and Korean subjects are shown in Table I. The rs662799 of *APOA5* was significantly [P<0.004 (0.05/12 tests)] associated with serum concentrations of triglycerides and HDL-cholesterol, but not LDL-cholesterol, in Japanese and Korean individuals (data not shown). In both populations, the serum concentrations of triglycerides were greater, whereas the serum concentrations of HDL-cholesterol were lower, in individuals with the C allele than in those with the TT genotype or in individuals with the CC genotype than in those with the T allele. The rs6929846 of *BTN2A1* was significantly associated with serum concentrations of triglycerides and HDL-cholesterol, but not LDL-cholesterol, in Japanese individuals (data not shown). The serum concentrations of triglycerides were greater, whereas the serum concentrations of HDL-cholesterol were lower, in individuals with the T allele than in those with the CC genotype. There was no association between rs6929846 of *BTN2A1* and serum concentrations of triglycerides, HDL-cholesterol, or LDL-cholesterol in Korean individuals. The genotype distributions for rs662799 and rs6929846 in Japanese and Korean individuals were all in Hardy-Weinberg equilibrium (data not shown).

We examined the relations of combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* to serum concentrations of triglycerides, HDL-cholesterol, and LDL-cholesterol in Japanese and Korean individuals (Table II). The serum concentrations of triglycerides among Japanese and Korean individuals were increased by 41 and 24%, respectively, whereas the serum concentrations of HDL-cholesterols were decreased by 35 and 4%, respectively, in individuals with the C allele of *APOA5* and the T allele of *BTN2A1* compared to those with the TT genotype of *APOA5* and the CC genotype of *BTN2A1*.

Table II. Serum concentrations of triglycerides, HDL-cholesterol, and LDL-cholesterol according to combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* in Japanese and Korean individuals.

APOA5 genotype	TT			TC + CC			
BTN2A1 genotype	CC[a]	CT + TT	P-value[b]	CC	P-value[b]	CT + TT	P-value[b]
Japanese individuals							
No. of subjects	2734	497		3603		637	
Serum triglycerides (mmol/l)	1.39±0.78	1.51±1.00	0.1057	1.74±1.18	**$7.2x10^{-12}$**	1.96±1.74	**$7.2x10^{-12}$**
Serum HDL-cholesterol (mmol/l)	1.96±1.74	1.41±0.44	0.7250	1.35±0.39	**$1.1x10^{-11}$**	1.27±0.36	**$1.1x10^{-11}$**
Serum LDL-cholesterol (mmol/l)	3.04±0.82	3.14±0.96	0.0614	3.06±0.87	0.7987	3.09±0.95	0.5111
Korean individuals							
No. of subjects	1343	361		1402		384	
Serum triglycerides (mmol/l)	1.32±0.71	1.39±0.90	0.4116	1.56±0.98	**$9.0x10^{-12}$**	1.64±0.91	**$3.7x10^{-10}$**
Serum HDL-cholesterol (mmol/l)	1.28±0.36	1.29±0.38	0.9505	1.21±0.35	**$1.6x10^{-7}$**	1.23±0.39	0.0384
Serum LDL-cholesterol (mmol/l)	2.86±0.89	2.91±0.88	0.8250	2.84±0.98	0.9311	2.90±1.68	0.8606
All individuals							
Serum triglycerides (mmol/l)	1.37±0.75	1.46±0.96	0.0671	1.68±1.12	**$2.7x10^{-11}$**	1.83±1.48	**$2.7x10^{-11}$**
Serum HDL-cholesterol (mmol/l)	1.38±0.40	1.36±0.42	0.5133	1.30±0.38	**$3.8x10^{-11}$**	1.26±0.37	**$3.8x10^{-11}$**
Serum LDL-cholesterol (mmol/l)	2.97±0.85	3.04±0.93	0.2193	1.99±0.91	0.8132	3.01±1.30	0.5781

[a]Reference values. [b]Versus TT (*APOA5*)-CC (*BTN2A1*) genotype. P-values of <0.002 are shown in bold.

Multivariable logistic regression with adjustment for age, gender, and the prevalence of diabetes mellitus revealed that rs662799 of *APOA5* was significantly [P<0.004 (0.05/12 tests)] related to hypertriglyceridemia in Japanese and Korean individuals (dominant and recessive models) and to hypo-HDL-cholesterolemia in Japanese (dominant and recessive models) and Korean (dominant model) individuals, with the C allele representing a risk factor for these conditions (data not shown). Similar analyses revealed no relation of rs6929846 of *BTN2A1* to dyslipidemia in Japanese or Korean individuals (data not shown). Multivariable logistic regression analysis of combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* with adjustment for age, gender, and the prevalence of diabetes mellitus revealed that Japanese or Korean individuals with the C allele of *APOA5* and the T allele of *BTN2A1* had a 2.05- or 1.92-fold increased risk for hypertriglyceridemia, respectively and a 1.82- or 1.56-fold increased risk for hypo-HDL-cholesterolemia, respectively, compared to those with the TT genotype of *APOA5* and the CC genotype of *BTN2A1* (Table III).

Association of polymorphisms of APOA5 and BTN2A1 with MetS. The characteristics of Japanese and Korean subjects with MetS and controls are shown in Table IV. For Japanese individuals, age, the frequency of men, BMI, the prevalence of smoking as well as systolic and diastolic blood pressure, serum concentrations of triglycerides and creatinine, and fasting plasma glucose levels were greater, whereas the serum concentration of HDL-cholesterol was lower, in subjects with MetS than in controls. For the Korean individuals, the frequency of men, BMI, the prevalence of smoking as well as systolic and diastolic blood pressure, serum concentrations of triglycerides and creatinine, and fasting plasma glucose levels were greater, whereas age and the serum concentration of HDL-cholesterol were lower, in subjects with MetS than in controls.

Comparison of allele frequencies between subjects with MetS and controls by the χ^2 test revealed that rs662799 of *APOA5* was significantly [P<0.025 (0.05/2 tests)] associated with MetS in Japanese and Korean individuals (data not shown). Similar analysis revealed that rs6929846 of *BTN2A1* was significantly associated with MetS in Japanese individuals, but not in Korean subjects (data not shown). Genotype distributions of rs662799 and rs6929846 were in Hardy-Weinberg equilibrium among subjects with MetS and controls in Japanese and Korean individuals (data not shown). Combined genotype analysis revealed that the prevalence of MetS was significantly [P<0.006 (0.05/9 tests)] increased in Japanese individuals with the C allele of *APOA5* and any genotype of *BTN2A1* compared to those with the TT genotype of *APOA5* and the CC genotype of *BTN2A1* (Table V). No relation was detected between combined genotypes and MetS in Korean individuals.

Multivariable logistic regression analysis with adjustment for age and gender revealed that rs662799 of *APOA5* was significantly [P<0.01 (0.05/4 tests)] associated with MetS in Japanese individuals (dominant and recessive models), but not in Korean subjects, with the C allele representing a risk factor for this condition (data not shown). Similar analysis revealed that rs6929846 of *BTN2A1* was significantly associated with MetS in Japanese individuals (dominant model), but not in Korean subjects, with the T allele representing a risk factor for this condition (data not shown). Multivariable logistic regression analysis of combined genotypes with adjustment for age and gender revealed that individuals with the C allele of *APOA5* and the T allele of *BTN2A1* had a 2.87-fold increased risk for MetS compared to those with the TT genotype of *APOA5* and the

Table III. Multivariable logistic regression analysis of dyslipidemia and combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* in Japanese and Korean individuals.

APOA5 genotype	TT			TC + CC			
BTN2A1 genotype	CC[a]	CT + TT		CC		CT + TT	
	OR	P-value[b]	OR (95% CI)	P-value[b]	OR (95% CI)	P-value[b]	OR (95% CI)
Japanese individuals							
Hypertriglyceridemia	1	0.2945		**9.4×10^{-22}**	1.78 (1.58-2.00)	**1.8×10^{-13}**	2.05 (1.69-2.48)
Hypo-HDL-cholesterolemia	1	0.6109		**5.2×10^{-7}**	1.46 (1.26-1.69)	**2.1×10^{-7}**	1.82 (1.45-2.27)
Hyper-LDL-cholesterolemia	1	0.0189	1.33 (1.04-1.68)	0.0820		0.1406	
Korean individuals							
Hypertriglyceridemia	1	0.3897		**1.6×10^{-6}**	1.55 (1.30-1.86)	**5.9×10^{-7}**	1.92 (1.49-2.49)
Hypo-HDL-cholesterolemia	1	0.9502		**0.0013**	1.33 (1.12-1.58)	**0.0006**	1.56 (1.21-2.01)
Hyper-LDL-cholesterolemia	1	0.8658		0.9702		0.9115	
All individuals							
Hypertriglyceridemia	1	0.3568		**5.7×10^{-28}**	1.72 (1.56-1.90)	**1.3×10^{-17}**	1.93 (1.66-2.25)
Hypo-HDL-cholesterolemia	1	0.3042		**1.3×10^{-7}**	1.34 (1.20-1.50)	**6.3×10^{-10}**	1.69 (1.43-2.00)
Hyper-LDL-cholesterolemia	1	0.0962		0.0934		0.3359	

[a]Reference values. [b]Versus TT (*APOA5*)-CC (*BTN2A1*) genotype. Hypertriglyceridemia, serum concentrations of triglycerides of ≥1.65 mmol/l; hypo-HDL-cholesterolemia, serum concentrations of HDL-cholesterol <1.04 mmol/l; hyper-LDL-cholesterolemia, serum concentrations of LDL-cholesterol ≥3.64 mmol/l. Multivariable logistic regression analysis was performed with adjustment for age, gender, and the prevalence of diabetes mellitus. OR, odds ratio; CI, confidence interval. P-values of <0.002 are shown in bold.

Table IV. Characteristics of study subjects in the metabolic syndrome (MetS) study.

	Japanese individuals (n=3474)			Korean individuals (n=1671)		
Characteristic	MetS	Controls	P-value	MetS	Controls	P-value
No. of subjects	2744	730		1294	377	
Age (years)	67.1±9.6	61.3±12.7	<0.0001	58.3±11.2	59.8±8.5	0.0037
Gender (male/female, %)	61.4/38.6	41.2/58.8	<0.0001	81.3/18.7	53.9/46.1	<0.0001
Body mass index (kg/m^2)	25.0±3.3	21.6±2.1	<0.0001	25.7±3.1	22.2±1.8	<0.0001
Current or former smoker (%)	27.0	9.9	<0.0001	66.0	39.9	<0.0001
Systolic blood pressure (mmHg)	147±24	116±9	<0.0001	128±23	114±9	<0.0001
Diastolic blood pressure (mmHg)	80±14	70±8	<0.0001	78±14	71±7	<0.0001
Serum triglycerides (mmol/l)	2.14±1.39	0.94±0.32	<0.0001	1.93±1.13	0.97±0.32	<0.0001
Serum HDL-cholesterol (mmol/l)	1.18±0.33	1.74±0.35	<0.0001	1.00±0.24	1.57±0.35	<0.0001
Fasting plasma glucose (mmol/l)	7.64±3.32	4.57±0.60	<0.0001	7.11±3.45	4.63±0.46	<0.0001
Serum creatinine (μmol/l)	69.1±64.5	54.0±11.4	<0.0001	79.0±53.5	57.2±15.9	<0.0001

Quantitative data are means ± SD.

Table V. Comparison of distributions of combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* in Japanese and Korean individuals by the χ^2 test between subjects with metabolic syndrome (MetS) and controls.

APOA5 genotype	TT			TC + CC			
BTN2A1 genotype	CC[a]	CT + TT[b]	P-value	CC[b]	P-value	CT + TT[b]	P-value
Japanese individuals							
MetS	843 (72.8)	195 (87.4)	**3.6x10^{-6}**	1411 (80.1)	**3.9x10^{-6}**	295 (88.9)	**1.3x10^{-9}**
Controls	315 (27.2)	28 (12.6)		350 (19.9)		37 (11.1)	
Korean individuals							
MetS	437 (74.2)	131 (81.4)	0.0599	553 (78.9)	0.0471	154 (78.6)	0.2184
Controls	152 (25.8)	30 (18.6)		148 (21.1)		42 (21.4)	
All individuals							
MetS	1280 (73.3)	326 (84.9)	**1.7x10^{-6}**	1964 (79.8)	**7.6x10^{-7}**	449 (85.0)	**2.9x10^{-8}**
Controls	467 (26.7)	58 (15.1)		498 (20.2)		79 (15.0)	

[a]Reference values. [b]Numbers in parentheses are percentages. P-values of <0.006 are shown in bold.

CC genotype of *BTN2A1* in Japanese individuals (Table VI). There was no relation between combined genotypes and MetS in Korean individuals.

Discussion

Although genetic epidemiological studies have shown that genetic variants contribute to individual susceptibility to dyslipidemia and MetS in Caucasian populations (5-9), the genetic basis of these conditions in Asian populations remains to be fully identified. We have now shown that rs662799 of *APOA5* and rs6929846 of *BTN2A1* synergistically influenced the prevalence of hypertriglyceridemia and hypo-HDL-cholesterolemia in Japanese and Korean individuals and of MetS in Japanese individuals.

Apolipoprotein A-V plays a pivotal role in the regulation of serum concentrations of triglycerides (23,24). Apolipoprotein A-V was shown to exhibit a high affinity, low elasticity, and slow binding kinetics at hydrophobic interfaces (25), properties that may retard the assembly of triglyceride particles. Apolipoprotein A-V may thus reduce very low density lipoprotein (VLDL)-triglyceride levels by both downregulating hepatic VLDL synthesis and increasing VLDL clearance. Given its location in the promoter region, the -1131T→C polymorphism (rs662799) of *APOA5* may affect the regulation of gene transcription and thereby influence the serum level of apolipoprotein A-V. Peroxisome proliferator-activated receptor-α (PPARα) was shown to regulate the expression of *APOA5* via the PPAR response element at position -271 to -259 in the promoter lesion of *APOA5* (26,27). PPARα agonists induced the expression of *APOA5* in primary liver cells, and caused a dramatic increase in the β-oxidation of the liver and the fatty acid-dependent energy generation. The rs662799 of *APOA5* may affect the affinity of PPARα for the PPAR response

Table VI. Multivariable logistic regression analysis of the metabolic syndrome and combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* in Japanese and Korean individuals.

APOA5 genotype	TT				TC + CC			
BTN2A1 genotype	CC[a]		CT + TT		CC		CT + TT	
	OR	P-value[b]	OR (95% CI)		P-value[b]	OR (95% CI)	P-value[b]	OR (95% CI)
Japanese individuals	1	**5.1x10^{-6}**	2.73 (1.80-4.29)		**1.1x10^{-5}**	1.51 (1.26-1.81)	**5.3x10^{-8}**	2.87 (1.99-4.25)
Korean individuals	1	0.0825			0.0806		0.3356	
All individuals	1	**5.2x10^{-6}**	2.05 (1.51-2.81)		**2.6x10^{-6}**	1.43 (1.23-1.67)	**9.8x10^{-7}**	1.97 (1.51-2.60)

[a]Reference values. [b]P-value vs. the TT (*APOA5*)-CC (*BTN2A1*) genotype. Multivariable logistic regression analysis was performed with adjustment for age and gender. OR, odds ratio; CI, confidence interval. P-values of <0.006 are shown in bold.

element (27,28), thereby influence the diversion of triglycerides from the secretory into the oxidative pathway. We previously showed that rs662799 of *APOA5* was significantly associated with the prevalence of hypertriglyceridemia, hypo-HDL-cholesterolemia (10), and MetS (12) in Japanese individuals. We have now shown that this polymorphism is associated with increased serum concentrations of triglycerides, decreased serum concentrations of HDL-cholesterol, and an increased risk for hypertriglyceridemia and hypo-HDL-cholesterolemia in Korean individuals.

The butyrophilin, subfamily 2, member A1 (BTN2A1) belongs to BTN superfamily, and is a cell surface transmembrane glycoprotein that has roles in lipid, fatty acid, and sterol metabolism (Entrez Gene, NCBI). We previously showed that the T allele of rs6929846 of *BTN2A1* was associated with an increased risk for myocardial infarction, an increase in the serum concentration of high sensitivity C-reactive protein as well as with increased transcription activity of *BTN2A1* (20). Overexpression of *BTN2A1* decreased the expression of elastin mRNA and increased the mRNA expression of matrix metallopeptidase 3 and interleukin 5 (20). We also showed that rs6929846 of *BTN2A1* was significantly associated with increased serum concentrations of triglycerides and decreased serum concentrations of HDL-cholesterol (11), and with a increased risk for MetS (14) in Japanese individuals. These observations suggest that the T allele of rs6929846 of *BTN2A1* may accelerate inflammatory, atherosclerotic, and thrombotic processes (20). In the present study, however, rs6929846 alone was not related to serum concentrations of triglycerides, HDL-cholesterol, or LDL-cholesterol, or the prevalence of MetS in Korean individuals.

Previous studies suggested that the effect of rs662799 of *APOA5* on the regulation of serum triglycerides was modulated by other genetic and environmental factors (29-32). Genetic variants of *APOA5* (rs662799) and *APOE* (rs429358, rs7412) synergistically affected serum concentrations of triglycerides (29). In simultaneous analysis of four polymorphisms of LPL (rs320, rs328, rs1801177, rs268), two polymorphisms of *APOA5* (rs662799, rs3135506), and two polymorphisms of *APOE* (rs429358, rs7412), serum concentrations of triglycerides were significantly increased in individuals with two or three triglycerides-raising alleles among these polymorphisms (30). In addition, environmental factors, including a high-fat and high-calorie diet and physical inactivity, may affect gene-gene interactions as well as the development of dyslipidemia (31,32).

We have now shown that polymorphisms of *APOA5* and *BTN2A1* synergistically affected the development of dyslipidemia in East Asian populations and of MetS in Japanese individuals. Systemic inflammation has been shown to play an important role in an increase in insulin resistance, the development of dyslipidemia, and the progression of MetS (33). Acceleration of inflammatory processes by the T allele of rs6929846 in *BTN2A1* might influence the effects of rs662799 of *APOA5* on lipid metabolism, resulting in the synergistic effects of rs662799 and rs6929846 on the development of hypertriglyceridemia, hypo-HDL-cholesterolemia, or MetS.

There are some limitations to our study: i) given that the study subjects comprised of only Japanese and Korean populations, validation of our findings in other ethnic groups is required. ii) the functional relevance of the synergistic effects of *APOA5* and *BTN2A1* on dyslipidemia or MetS has

not been determined; iii) the criteria for diagnosis of MetS proposed by the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity were modified (waist circumference was replaced with BMI), as was also the case in the West of Scotland Coronary Prevention Study (15) and Women's Health Study (16).

In conclusion, our present results suggest that polymorphisms of *APOA5* and *BTN2A1* may synergistically affect the development of dyslipidemia in East Asian populations and of MetS in Japanese individuals. Determination of combined genotypes for rs662799 of *APOA5* and rs6929846 of *BTN2A1* may prove informative for assessment of the genetic risk for dyslipidemia or MetS in the East Asian populations.

Acknowledgements

This study was supported by Grants-in-Aid for the Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (nos. 18209023, 18018021 and 19659149 to Y.Y.) and by a Research Grant from Mie Medical Valley Project (to Y.Y.).

References

1. Okamura T, Tanaka H, Miyamatsu N, *et al*: The relationship between serum total cholesterol and all-cause or cause-specific mortality in a 17.3-year study of a Japanese cohort. Atherosclerosis 190: 216-223, 2007.
2. Ninomiya T, Kubo M, Doi Y, *et al*: Impact of metabolic syndrome on the development of cardiovascular disease in a general Japanese population: the Hisayama study. Stroke 38: 2063-2069, 2007.
3. Alberti KG, Eckel RH, Grundy SM, *et al*: Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120: 1640-1645, 2009.
4. Pan WH, Yeh WT and Weng LC: Epidemiology of metabolic syndrome in Asia. Asia Pac J Clin Nutr 17 (Suppl 1): S37-S42, 2008.
5. Kooner JS, Chambers JC, Aguilar-Salinas CA, *et al*: Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nature Genet 40: 149-151, 2008.
6. Kathiresan S, Manning AK, Demissie S, *et al*: A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. BMC Med Genet 8 (Suppl 1): S17, 2007.
7. Teslovich TM, Musunuru K, Smith AV, *et al*: Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466: 707-713, 2010.
8. Kraja AT, Vaidya D, Pankow JS, *et al*: A bivariate genome-wide approach to metabolic syndrome. Diabetes 60: 1329-1339, 2011.
9. Park YM, Province MA, Gao X, *et al*: Longitudinal trends in the association of metabolic syndrome with 550 k single-nucleotide polymorphisms in the Framingham Heart study. BMC Proc 3 (Suppl 7): S116, 2009.
10. Yamada Y, Matsuo H, Warita S, *et al*: Prediction of genetic risk for dyslipidemia. Genomics 90: 551-558, 2007.
11. Fujimaki T, Kato K, Oguri M, *et al*: Association of a polymorphism of *BTN2A1* with dyslipidemia in East Asian populations. Exp Ther Med 2: 745-749, 2011.
12. Yamada Y, Kato K, Hibino T, *et al*: Prediction of genetic risk for metabolic syndrome. Atherosclerosis 191: 298-304, 2007.
13. Yamada Y, Ichihara S, Kato K, *et al*: Genetic risk for metabolic syndrome: examination of candidate gene polymorphisms related to lipid metabolism in Japanese people. J Med Genet 45: 22-28, 2008.
14. Oguri M, Kato K, Yoshida T, *et al*: Association of a genetic variant of *BTN2A1* with metabolic syndrome in East Asian populations. J Med Genet 48: 787-792, 2011.
15. Sattar N, Gaw A, Scherbakova O, *et al*: Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. Circulation 108: 414-419, 2003.
16. Ridker PM, Buring JE, Cook NR and Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation 107: 391-397, 2003.
17. Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P and Inoue S: Criteria and classification of obesity in Japan and Asia-Oceania. Asia Pac J Clin Nutr 11 (Suppl 8): S732-S737, 2002.
18. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M and Morgenstern BZ: Human blood pressure determination by sphygmomanometry. Circulation 88: 2460-2470, 1993.
19. Lutz RA and Flückiger J: Kinetic determination of glucose with the GEMSAEC (ENI) centrifugal analyzer by the glucose dehydrogenase reaction, and comparison with two commonly used procedures. Clin Chem 21: 1372-1377, 1975.
20. Yamada Y, Nishida T, Ichihara S, *et al*: Association of a polymorphism of *BTN2A1* with myocardial infarction in East Asian populations. Atherosclerosis 215: 145-152, 2011.
21. Itoh Y, Mizuki N, Shimada T, *et al*: High-throughput DNA typing of HLA-A, -B, -C and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. Immunogenetics 57: 717-729, 2005.
22. Jang Y, Kim JY, Kim OY, Lee JE, Cho H, Ordovas JM and Lee JH: The -1131T→C polymorphism in the apolipoprotein A5 gene is associated with postprandial hypertriacylglycerolemia; elevated small, dense LDL concentrations; and oxidative stress in nonobese Korean men. Am J Clin Nutr 80: 832-840, 2004.
23. Lai CQ, Tai ES, Tan CE, *et al*: The *APOA5* locus is a strong determinant of plasma triglyceride concentrations across ethnic groups in Singapore. J Lipid Res 44: 2365-2373, 2003.
24. Pennacchio LA, Olivier M, Hubacek JA, *et al*: An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 294: 169-173, 2001.
25. Weinberg RB, Cook VR, Beckstead JA, Martin DD, Gallagher JW, Shelness GS and Ryan RO: Structure and interfacial properties of human apolipoprotein A-V. J Biol Chem 278: 34438-34444, 2003.
26. Vu-Dac N, Gervois P, Jakel H, *et al*: Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to peroxisome proliferator-activated receptor alpha activators. J Biol Chem 278: 17982-17985, 2003.
27. Prieur X, Coste H and Rodriguez JC: The human apolipoprotein AV gene is regulated by peroxisome proliferator-activated receptor-alpha and contains a novel farnesoid X-activated receptor response element. J Biol Chem 278: 25468-25480, 2003.
28. Fruchart-Najib J, Bauge E, Niculescu LS, *et al*: Mechanism of triglyceride lowering in mice expressing human apolipoprotein A5. Biochem Biophys Res Commun 319: 397-404, 2004.
29. Sousa MO, Alía P, Pintó X, Corbella E and Navarro MA: Interaction between *APOA5* -1131T→C and APOE polymorphisms and their association with severe hypertriglyceridemia. Clin Chim Acta 395: 68-71, 2008.
30. Ariza MJ, Sánchez-Chaparro MA, Barón FJ, *et al*: Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study. BMC Med Genet 11: 66, 2010.
31. Lai CQ, Corella D, Demissie S, *et al*: Dietary intake of n-6 fatty acids modulates effect of apolipoprotein A5 gene on plasma fasting triglycerides, remnant lipoprotein concentrations, and lipoprotein particle size: the Framingham Heart Study. Circulation 113: 2062-2070, 2006.
32. Kim JY, Kim OY, Koh SJ, Jang Y, Yun SS, Ordovas JM and Lee JH: Comparison of low-fat meal and high-fat meal on postprandial lipemic response in non-obese men according to the -1131T→C polymorphism of the apolipoprotein A5 (APOA5) gene (randomized cross-over design). J Am Coll Nutr 25: 340-347, 2006.
33. Dandona P, Aljada A, Chaudhuri A, Mohanty P and Garg R: Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 111: 1448-1454, 2005.