

Association of a polymorphism of *BTN2A1* with dyslipidemia in East Asian populations

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Abstract. We previously identified rs6929846 of the butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) as a susceptibility locus for myocardial infarction in Japanese individuals by a genome-wide association study. The aim of the present study was to examine the relation of the rs6929846 polymorphism of *BTN2A1* to dyslipidemia in Japanese and Korean populations, given that dyslipidemia is an important risk factor for myocardial infarction. A total of 10,953 individuals from three independent subject panels were examined. The relations of the rs6929846 polymorphism of *BTN2A1* to serum concentrations of triglycerides, high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol were examined in each subject panel. The C→T polymorphism (rs6929846) of *BTN2A1* was significantly associated with serum concentrations of triglycerides in Japanese subject panels A (P=0.0004) and B (P=0.0010), and in the Korean population (P=0.0095), with the minor T allele being related to an increased serum concentration of triglycerides. The rs6929846 was associated

with serum concentrations of HDL-cholesterol in Japanese subject panels A (P=0.0047) and B (P=0.0015), with the T allele being related to a decreased serum concentration of HDL-cholesterol, but not in the Korean population. This polymorphism was associated with the serum concentration of LDL-cholesterol only in Japanese subject panel B (P=0.0059), with the T allele being related to an increased serum concentration of LDL-cholesterol. The results suggest that *BTN2A1* may be a susceptibility gene for hypertriglyceridemia in East Asian populations and for low serum HDL-cholesterol in the Japanese population.

Introduction

Dyslipidemia is a multifactorial disorder caused by an interaction between genetic and environmental factors, the latter including a high-fat and high-calorie diet and physical inactivity (1,2). Although recent genome-wide association studies (GWASs) have implicated several loci and candidate genes in predisposition to dyslipidemia in Caucasian populations (3-5), the genes that confer susceptibility to this condition in Asian populations remain to be identified definitively. We previously showed that the C→T polymorphism (rs6929846) of the butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) is significantly associated with the prevalence of myocardial infarction in Japanese individuals by a GWAS (6). Given that dyslipidemia is an important risk factor for myocardial infarction, we hypothesized that the association of rs6929846 with myocardial infarction may be attributable, at least in part, to its effect on susceptibility to dyslipidemia. We thus examined

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the relation of rs6929846 of *BTN2A1* to dyslipidemia in Japanese and Korean populations.

Patients and methods

Study population. A total of 10,953 Japanese or Korean individuals from three independent subject panels was examined. The Japanese subject panel A comprised 3,319 individuals who either visited outpatient clinics of or were admitted to participating hospitals (Gifu Prefectural General Medical Center, Gifu; 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. The Japanese subject panel B comprised 4,297 individuals who either visited outpatient clinics of or were admitted to participating hospitals (Gifu Prefectural Tajimi Hospital, Tajimi; Japanese Red Cross Nagoya First Hospital, Nagoya; and Inabe General Hospital, Inabe, Japan) between October 2002 and March 2009, or who were recruited to population-based cohort studies of aging and age-related diseases in Nakanojo, Kusatsu and Tokyo, Japan. The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology and participating hospitals. Written informed consent was obtained from each subject.

A total of 3,337 Korean subjects was drawn from the institutional patient databases of the Cardiovascular Genome Center and the 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.

Measurement of serum lipid profile. 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 1,600 × g for 15 min at 4°C, and serum was separated and stored at -30°C until analysis. The serum concentrations of triglycerides, high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol were measured as previously described (7).

Genotyping of rs6929846 of *BTN2A1*. For Japanese individuals, venous blood (7 ml) was collected into tubes containing ethylenediaminetetraacetic acid (disodium salt) at a final concentration of 50 mmol/l, and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotype of rs6929846 of *BTN2A1* was determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Genotyping involved PCR amplification, hybridization, streptavidin-phycoerythrin reaction and measurement of fluorescence. Detailed genotyping methodology was described previously (6,8).

For Korean subjects, 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, USA). Genotype of rs6929846 was determined with the use of a TaqMan fluorogenic 5' nuclease assay (Applied Biosystems, Foster City, CA, USA). The PCR mixture (final volume, 5 µl) contained 2 ng of genomic DNA, 2.5 µl of TaqMan Universal PCR Master Mix and 0.125 µl (or 0.25 µl) of 40X (or 20X) Assay Mix. The mixture was incubated first at 50°C for 2 min to activate uracil N-glycosylase and to prevent carryover contamination, and then at 95°C for 10 min to activate DNA polymerase; it was subsequently subjected to 40 cycles of incubation at 92°C for 15 sec and 60°C for 1 min. All reactions were performed in 384-well plates with the use of a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems), and endpoint fluorescence readings were performed with a PRISM 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure accuracy of genotyping (6).

Statistical analysis. Quantitative data were compared between two groups by the unpaired Student's t-test. The Chi-square test was used to identify departures from Hardy-Weinberg equilibrium. A P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided test performed with JMP Genomics version 3.2 software (SAS Institute, Cary, NC, USA).

Results

The characteristics of the 10,953 subjects enrolled in the present study are shown in Table I. The prevalence of hypertriglyceridemia (a serum concentration of triglycerides of ≥1.65 mmol/l), low-serum HDL-cholesterol (a serum concentration of HDL-cholesterol of <1.04 mmol/l) and high-serum LDL-cholesterol (a serum concentration of LDL-cholesterol of ≥3.64 mmol/l) was 32.0, 21.6 and 25.7%, respectively, in Japanese subject panel A; 37.0, 16.2 and 21.9%, respectively, in Japanese subject panel B; and 28.0, 28.4 and 19.5%, respectively, in the Korean population.

The relations of the *BTN2A1* genotype to serum concentrations of triglycerides, HDL-cholesterol and LDL-cholesterol are shown in Table II. The C→T polymorphism (rs6929846) of *BTN2A1* was significantly ($P<0.05$) associated with the serum concentrations of triglycerides in Japanese subject panels A (dominant and recessive models) and B (dominant model), and in the Korean population (recessive model), with the minor T allele being related to an increased serum concentration of triglycerides. The rs6929846 of *BTN2A1* was significantly associated with the serum concentrations of HDL-cholesterol in the Japanese subject panels A and B (dominant model), with the T allele being related to a decreased serum HDL-cholesterol, but not in the Korean population. The rs6929846 of *BTN2A1* was significantly associated with the serum concentrations of LDL-cholesterol in the Japanese subject panel B (recessive model), with the T allele being related to an increased serum concentration of LDL-cholesterol, but not in the Japanese subject panel A or in the Korean population. In all Japanese and Korean individuals, rs6929846 of *BTN2A1* was significantly associated

Table I. Characteristics of the 10,953 study subjects.

Characteristics	Japanese population		Korean population
	Subject panel A	Subject panel B	
No. of subjects	3,319	4,297	3,337
Age (years)	65.1±11.40	68.8±8.90	59.6±10.30
Gender (male/female, %)	57.5/42.5	58.3/41.7	68.1/31.90
Body mass index (kg/m ²)	23.6±3.30	23.5±3.40	24.3±3.00
Current or former smoker (%)	19.8	36.9	53.4
Hypertension (%)	61.5	67.3	65.0
Diabetes mellitus (%)	31.3	38.0	19.6
Serum total cholesterol (mmol/l)	5.15±1.00	5.17±0.98	4.77±1.02
Hypercholesterolemia (%)	27.1	28.7	18.2
Serum triglycerides (mmol/l)	1.58±1.12	1.68±1.12	1.49±0.98
Hypertriglyceridemia (%)	32.0	37.0	28.0
Serum HDL-cholesterol (mmol/l)	1.35±0.41	1.40±0.40	1.26±0.37
Low HDL-cholesterol (%)	21.6	16.2	28.4
Serum LDL-cholesterol (mmol/l)	3.11±0.88	3.01±0.87	2.85±0.91
High LDL-cholesterol (%)	25.7	21.9	19.5

Data for age, body mass index and serum lipid concentrations are the 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: a serum concentration of total cholesterol of ≥5.69 mmol/l. Hypertriglyceridemia: a serum concentration of triglycerides of ≥1.65 mmol/l. Low HDL-cholesterol: a serum concentration of HDL-cholesterol of <1.04 mmol/l. High LDL-cholesterol: a serum concentration of LDL-cholesterol of ≥3.64 mmol/l.

with serum concentrations of triglycerides (dominant and recessive models), HDL-cholesterol (dominant model) and LDL-cholesterol (recessive model). Genotype distributions in the Japanese subject panels A and B and in the Korean population were all in Hardy-Weinberg equilibrium (Table II).

Discussion

Given that genetic factors and interactions between multiple genes and environmental factors are important in common forms of dyslipidemia (9), prediction of the risk for dyslipidemia on the basis of genetic variants would be beneficial for personalized prevention of this condition. We now showed that the *T* allele of rs6929846 in *BTN2A1* was significantly associated with hypertriglyceridemia in Japanese and Korean populations, and with low-serum concentrations of HDL-cholesterol in Japanese individuals.

BTN2A1 is a member of the cluster of butyrophilin genes (*BTNs*) (10). *BTNs* are type I membrane glycoproteins that were initially identified in bovine milk fat globules (11). *BTNs* are located at the extended major histocompatibility complex region in chromosome 6, constitute the immunoglobulin superfamily together with the *B7* gene family and butyrophilin-like gene (*BTNL*) family, and are widely expressed in a variety of human immune cells, suggesting a possible role in immune functions (12). Previous studies have shown significant relations of polymorphisms of *BTNL2* with Kawasaki disease in Taiwanese children (13) and sarcoidosis in German (14) and American (15) populations, both of which are caused by inflammatory autoimmune responses.

We previously showed that the *T* allele of rs6929846 was associated with an increased risk for myocardial infarction as well as with an increased transcription activity of *BTN2A1* (6). Overexpression of *BTN2A1* decreased the expression of elastin mRNA and increased the mRNA expression of matrix metalloproteinase 3 and interleukin-5. In our preliminary experiment, the serum concentrations of high-sensitivity C-reactive protein were significantly greater in individuals in the combined group of *CT* and *TT* genotypes for rs6929846 of *BTN2A1* than in those with the *CC* genotype in 755 healthy individuals without neoplastic, infectious or inflammatory disease (unpublished data). These observations suggest that the *T* allele of rs6929846 of *BTN2A1* may accelerate inflammatory processes.

Inflammation is a key component of the regulation of lipid metabolism in rodents and primates (16). Inflammatory conditions under bacterial or viral infections were found to result in an increase in the serum concentration of triglycerides and a decrease in the serum HDL-cholesterol in humans (17,18). Multiple cytokines are likely to affect the metabolism of cholesterol or triglycerides through several pathways, including the increased production and decreased clearance of very low-density lipoproteins, impaired reverse cholesterol transport and decreased excretion of bile acids (16,19-21). We now showed that rs6929846 of *BTN2A1* was significantly associated with hypertriglyceridemia in Japanese and Korean populations and with reduced serum concentrations of HDL-cholesterol in Japanese individuals, with the minor *T* allele representing a risk factor for these conditions. Acceleration of the inflammatory process by the *T* allele of

Table II. Serum concentrations of triglycerides, HDL-cholesterol and LDL-cholesterol according to *BTN2A1* genotypes in Japanese subject panels A and B, and in the Korean population.

Study subject	Genotype			P-value		Hardy-Weinberg P-value
	CC	CT	TT	Dominant	Recessive	
Serum concentrations of triglycerides						
Subject panel A	1.55±1.06	1.65±1.03	2.34±3.72	0.0101	0.0004	
No. of subjects	2,649	505	26			0.7210
Subject panel B	1.65±1.03	1.82±1.57	1.69±0.75	0.0010	0.9229	
No. of subjects	3,629	603	31			0.2806
Korean population	1.47±0.98	1.53±0.88	1.94±2.07	0.0579	0.0095	
No. of subjects	2,568	670	32			0.1068
All study subjects	1.57±0.01	1.66±1.20	1.97±2.39	7.8x10⁻⁵	0.0008	
No. of subjects	8,846	1,778	89			0.9737
Serum concentrations of HDL-cholesterol						
Subject panel A	1.35±0.41	1.30±0.38	1.27±0.36	0.0047	0.3644	
No. of subjects	2,675	510	24			0.9544
Subject panel B	1.41±0.39	1.35±0.41	1.34±0.40	0.0015	0.4458	
No. of subjects	3,611	597	30			0.3297
Korean population	1.25±0.36	1.27±0.40	1.20±0.33	0.3611	0.4020	
No. of subjects	2,577	674	32			0.0974
All study subjects	1.35±0.39	1.31±0.40	1.27±0.37	4.3x10⁻⁵	0.1058	
No. of subjects	8,863	1,781	86			0.7379
Serum concentrations of LDL-cholesterol						
Subject panel A	3.10±0.86	3.16±0.97	3.15±0.91	0.1878	0.8209	
No. of subjects	2,580	486	23			0.9828
Subject panel B	3.01±0.86	3.02±0.91	3.44±1.11	0.3909	0.0059	
No. of subjects	3,604	591	31			0.2128
Korean population	2.85±0.91	2.85±0.92	2.97±1.05	0.9288	0.4451	
No. of subjects	2,514	656	31			0.1001
All study subjects	2.99±0.88	2.99±0.94	3.19±1.04	0.5875	0.0372	
No. of subjects	8,698	1,733	85			0.8970

P-values of <0.05 are shown in bold.

rs6929846 may result in alterations in lipid metabolism, although the underlying mechanism remains unknown.

There are limitations to the present study. i) Given that the study subjects were comprised only of Japanese and Korean individuals, validation of our findings is required in other ethnic groups. ii) It is possible that rs6929846 of *BTN2A1* is in linkage disequilibrium with other polymorphisms in *BTN2A1* or in other nearby genes that are actually responsible for the development of dyslipidemia. iii) The functional relevance of rs6929846 of *BTN2A1* to the pathogenesis of dyslipidemia remains unknown. iv) Finally, environmental factors, such as dietary habits, that influence lipid metabolism were not evaluated in the present study.

In conclusion, *BTN2A1* may be a susceptibility gene for hypertriglyceridemia in East Asian populations and for low-serum HDL-cholesterol in the Japanese population. Determination of genotypes for this polymorphism may prove informative for the assessment of the genetic risk for dyslipidemia in East Asian populations.

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