The A>T Polymorphism of the Tribbles Homolog 1 Gene Is Associated with Serum Triglyceride Concentrations in Japanese Community-Dwelling Women

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Recent genome-wide association studies have identified Tribbles homolog 1 (TRIB1) as one of the candidate genes associated with lipid profiles. TRIB1 is known to interact with MAP kinases, thereby regulating their activities. The single nucleotide polymorphism rs2954029 of TRIB1 is located within an intron and is associated with lipid profiles. The aim of the present study is to investigate the TRIB1 rs2954029 (A>T polymorphism) with conventional predictors of coronary artery diseases such as carotid intima-media thickness (CIMT) and cardio-ankle vascular index (CAVI), and with lipid profiles in general population. This study enrolled 2,581 Japanese adults, 942 men and 1,639 women with a median age of 68 years (range 29 to 94 years), who participated in a screening program for the general population living in Goto City, Nagasaki Prefecture, Japan from 2008 to 2010. For the determination of TRIB1 rs2954029 genotypes, the polymerase chain reaction method was used. The differences in each parameter among the TRIB1 rs2954029 genotypes were evaluated using analysis of covariance. Genotype frequencies of TRIB1 rs2954029 in all participants were 25.5% for AA, 50.4% for AT, and 24.0% for TT. In women, the AA genotype showed significantly higher log triglyceride (TG) concentrations than the AT genotype ($P = 0.004$) and the AT + TT genotypes ($P = 0.004$). On the other hand, there were no associations with CIMT and CAVI among the TRIB1 rs2954029 genotypes. In conclusion, the TRIB1 rs2954029 is associated with serum TG concentrations in Japanese community-dwelling women.

Introduction

Dyslipidemia, i.e. abnormal concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), is known to be a risk factor for coronary artery disease (CAD) (Manninen et al. 1992; Gotto and Brinton 2004; Grundy et al. 2004). Recent genome-wide association studies (GWAS) have identified a number of new loci associated with lipid concentrations. Tribbles homolog 1 (TRIB1) is one of the candidate genes associ-

ated with TC, LDL-C, HDL-C, and TG concentrations (Willer et al. 2008; Kathiresan et al. 2008; Mohlke et al. 2008; Teslovich et al. 2010; Kraja et al. 2011). In addition, a large-scale gene-centric analysis has identified TRIB1 as a candidate gene for increased risk of CAD (The IBC 50K CAD Consortium 2011). Therefore, TRIB1 is one of the unique and interesting genes associated with changes in serum levels of various lipids such as TC, LDL-C, HDL-C, and TG, as well as increased risk of CAD.

The tribbles (TRB) protein family consists of three related serine/threonine kinase-like proteins: TRIB1, -2,

and -3. TRIB1 is located on chromosome 8q24 and regulates mitogen-activated protein kinase (MAPK) as a presumed adaptor or scaffolding protein (Kiss-Toth et al. 2004; Hegedus et al. 2006). TRIB1 regulates vascular smooth muscle cell proliferation and chemotaxis via the Jun kinase pathway and is selectively overexpressed in chronically inflamed human atherosclerotic arteries, which suggests that TRIB1 may play an important role in the atherosclerotic process (Sung et al. 2007).

TRIB1 rs2954029, a common single-nucleotide polymorphism (SNP) adjacent to the TRIB1 locus, has been associated with LDL-C, HDL-C, and TG concentrations and with increased risk of CAD (Waterworth et al. 2010; Varbo et al. 2011; Zhang et al. 2011). However, associations between TRIB1 rs2954029 and lipid concentrations, and vascular markers of atherosclerosis progression such as carotid intima-media thickness (CIMT) and cardio-ankle vascular index (CAVI) (Bots and Grobbee 2002; Nakamura et al. 2008) have not been fully investigated in the Japanese general population. The aim of the present study is to investigate the associations between TRIB1 rs2954029 and lipid concentrations, conventional predictors of CAD in general population.

Methods

Prior to the study, the special committee of Nagasaki University (project registration number 0501120073) granted ethical approval. Informed consent was obtained from participants. This study was conducted during a medical screening program for the general population living in Goto City, Nagasaki Prefecture, Japan. Blood samples and medical information were collected by the staff of Nagasaki University, in cooperation with the staff of Goto City. The study initially enrolled 2,581 Japanese adults who participated in the screening program from 2008 to 2010. Of this group, 46 participants receiving the medical screening program more than two times, and 182 participants with incomplete data were excluded; a further 12 participants with marked hypertriglyceridemia (≥ 400 mg/dL) were also excluded from the study, since LDL-C could not be calculated using the Friedewald equation in those cases. Finally, 2,341 participants (858 men, 1,483 women) were included in the analysis.

The height and weight of each participant were measured, and the body mass index (BMI; kilograms per meter squared) was calculated as an index of obesity. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded at rest.

Blood samples were collected from each participant after an overnight fast. Serum and plasma obtained were separated and stored at −20°C and −80°C, respectively. Serum concentrations of TC, TG, and HDL-C were measured using standard laboratory procedures. LDL-C was calculated using the Friedewald equation. The conversion equation from HbA_{1c} (Japan Diabetes Society: JDS) to HbA_{1c} (National Glycohemoglobin Standardization Program: NGSP) units officially certified by the Japan Diabetes Society was used (Kashiwagi et al. 2012).

CAVI was measured with a VaSera CAVI instrument (Fukuda Denshi, Tokyo, Japan). Cuffs were applied to bilateral upper arms and ankles, with the subject lying supine and the head held in midline position. After the subject rested for a few minutes, the examinations were performed.

Three physicians measured CIMT by ultrasonography of the right and left carotid arteries using a LOGIC Book XP with a 10-MHz linear array transducer (GE Medical Systems, Milwaukee, WI, USA). The far wall of the carotid artery was displayed on a longitudinal two-dimensional ultrasonographic image as two bright white lines separated by a hypoechoic space. The distance from the leading edge of the first bright line (lumen-intima interface) to the leading edge of the second (media-adventitia interface) was defined as the CIMT. Images were analyzed using Intima Scope software (MEDIA CROSS, Tokyo, Japan). The average of the right and left CIMTs was calculated and used in the analysis.

Genomic DNA was automatically extracted from blood cells separated from plasma using a MagExtractor MFX (TOYOBO, Osaka, Japan). TRIB1 rs2954029 was genotyped with Taqman SNP Genotyping assays C_15954645_10 (Applied Biosystems, Foster City, CA, USA) with the 7900HT sequence detection system (Applied Biosystems). We undertook a real-time PCR amplification with sequence-specific primers to amplify the polymorphism and two allele-specific fluorescent reporter probes for detection of TRIB1 rs2954029 alleles. Following PCR, the fluorescence yield for the two different dyes was measured to obtain the allelic discrimination plot and identify individual genotypes (SDS 2.3 software, Applied Biosystems).

The results are expressed as mean ± standard deviation or median (25-75 quartiles). The Shapiro-Wilk test was performed to assess the normality of data. Mann-Whitney's U-test evaluated differences of laboratory values between men and women. Fisher's exact test evaluated differences in smoking status, drinking status, the rate of current treatment for hypertension, dyslipidemia, diabetes mellitus, and past history of CAD. Plasma TG was logarithmically transformed due to a skewed distribution. The Hardy-Weinberg equilibrium was assessed using χ^2 analysis. The differences in each value among the TRIB1 rs2954029 genotypes were evaluated using analysis of covariance (ANCOVA) after adjustment for sex, age, BMI, smoking status, drinking status, current treatment for hypertension, dyslipidemia, diabetes mellitus, and past history of CAD. Probability values (P) less than 0.05 were considered significant in Mann-Whitney's U-test and Fisher's exact test. P values less than 0.0071 were considered significant in ANCOVA because Bonferroni adjustment was used in multiple comparison procedures. All data were statistically analyzed using SPSS ver. 19.0 software (IBM Japan, Tokyo, Japan).

Results

The clinical characteristics of the study participants are shown in Table 1. Most clinical parameters were significantly higher in men than in women, including age, BMI, SBP, DBP, mean CAVI, and mean CIMT. By contrast, TC, HDL-C, and LDL-C were significantly higher in women than in men. The rates of current history of medication for diabetes and past history of ischemic heart diseases were significantly higher in men than in women. The rates of current smokers and current history of medication for dyslipidemia were significantly higher in women than in men.

Genotype frequencies of TRIB1 rs2954029 in all participants were 25.5% for AA, 50.4% for AT, and 24.0% for TT (Table 2). The distribution of TRIB1 rs2954029 was consistent with Hardy-Weinberg equilibrium, and no signif-

Table 1. Characteristics of study participants.

	Men (n = 858)	Women (n = 1,483)	All (n = 2,341)
Age (years)	67.5 ± 10.5	66.7 ± 10.6*	67.0 ± 10.6
BMI (kg/m^2)	23.5 ± 2.9	22.9 ± 3.4**	23.1 ± 3.2
SBP (mmHg)	143.7 ± 20.4	141.2 ± 21.9**	142.1 ± 21.4
DBP (mmHg)	84.2 ± 11.1	81.6 ± 10.8**	82.6 ± 11.0
TC (mg/dL)	190.6 ± 33.2	206.1 ± 33.4**	200.4 ± 34.1
TG (mg/dL)	83.0 (61.0-122.0)	86.0 (63.0-119.0)	84.0 (62.0-120.0)
HDL-C (mg/dL)	55.5 ± 14.3	61.6 ± 14.5**	59.4 ± 14.7
LDL-C (mg/dL)	115.0 ± 28.8	124.8 ± 29.9**	121.2 ± 29.9
Mean CAVI	8.53 ± 1.52	7.97 ± 1.34**	8.18 ± 1.44
Mean CIMT (mm)	0.75 ± 0.14	0.71 ± 0.14**	0.72 ± 0.14
Smoker, n (%)	35 (4.1)	182 (12.3)**	217 (9.3)
Drinker, n (%)	144 (16.8)	253 (17.1)	397 (17.0)
Hypertension, n (%)	288 (33.6)	511 (34.5)	799 (34.1)
Dyslipidemia, n (%)	58 (6.8)	188 (12.7)**	246 (10.5)
Diabetes, n (%)	59 (6.9)	49 (3.3)**	108 (4.6)
CAD, n (%)	51 (5.9)	57 (3.8)*	108 (4.6)

Values are mean ± standard deviation or median (25th to 75th percentiles).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mean CAVI, mean cardio-ankle vascular index; Mean CIMT, mean carotid intima-media thickness; CAD, coronary artery disease.

*$p < 0.05$ vs. men, **$p < 0.01$ vs. men.

Table 2. Distribution of genotype and Allele frequencies of TRIB1 rs2954029 in study participants.

Genotype	AA	AT	TT
Men (n = 858)	229 (26.7%)	417 (48.6%)	212 (24.7%)
Women (n = 1,483)	368 (24.8%)	764 (51.5%)	351 (23.7%)
All (n = 2,341)	597 (25.5%)	1,181 (50.4%)	563 (24.0%)

Data are number of participants with each genotype (% of each group).

icant differences were observed in the allele frequencies of AA, AT, and TT between men and women.

When adjusted for age, sex, BMI, current smoker, habitual drinker, history of current medication for hypertension, diabetes, and dyslipidemia, no significant differences were observed in any parameters. Since sex is a classical risk factor and is important for development of metabolic traits and/or atherosclerosis, data were then analyzed separately by sex. In men, no significant differences were observed in any parameters. In women, the AA genotype showed significantly higher log TG concentrations than the AT genotype ($P = 0.004$) (Table 3). When comparing the AA genotype with the AT + TT genotypes, the AA genotype showed significantly higher log TG concentrations than the AT + TT genotypes ($P = 0.004$).

Discussion

In this study, the AA genotype of TRIB1 rs2954029 showed significantly higher log TG concentrations than the AT and AT + TT genotypes, only in women. TRIB1 rs2954029 was not associated with TC, LDL-C, HDL-C, mean CAVI, mean CIMT, and past history of ischemic heart disease.

In GWAS findings of > 100,000 individuals, TRIB1 rs2954029 was associated with concentrations of TC, TG, LDL-C, and HDL-C (Teslovich et al. 2010). GWAS in a Chinese population also showed that TRIB1 rs2954029 was associated with TC, TG, and LDL-C (Zhang et al. 2011). Waterworth et al. (2010) showed TRIB1 rs2954029 to be associated with TG and risk of CAD in the analysis of eight studies from GWAS data. The present study indicated that TG was associated with TRIB1 rs2954029 only in women, which was partially consistent with previous studies (Teslovich et al. 2010; Waterworth et al. 2010; Zhang et al. 2011) because TC, LDL-C, and HDL-C were not associated with TRIB1 rs2954029 in this study. This difference might be due to the relatively small sample size compared to previous studies. In addition, population differences in genotype frequencies of TRIB1 rs2954029 might also explain this discrepancy.

Table 3. Comparison of lipid profiles among the TRIB1 rs2954029 genotypes.

Genotype		AA	AT	TT
TC (mg/dL)	All	202.4 ± 1.3	200.2 ± 0.9	198.7 ± 1.4
	Men	190.5 ± 2.1	191.4 ± 1.6	188.9 ± 2.2
	Women	209.6 ± 1.7	205.3 ± 1.2	204.0 ± 1.8
logTG (mg/dL)	All	1.95 ± 0.01	1.94 ± 0.01	1.95 ± 0.01
	Men	1.93 ± 0.01	1.94 ± 0.01	1.96 ± 0.02
	Women	1.97 ± 0.01	1.93 ± 0.01*	1.94 ± 0.01
HDL-C (mg/dL)	All	59.1 ± 0.6	59.7 ± 0.4	59.0 ± 0.6
	Men	55.9 ± 0.9	55.9 ± 0.7	54.3 ± 0.9
	Women	61.0 ± 0.7	61.9 ± 0.5	61.7 ± 0.7
LDL-C (mg/dL)	All	123.0 ± 1.2	121.0 ± 0.8	119.7 ± 1.2
	Men	115.4 ± 1.9	115.4 ± 1.4	113.5 ± 1.9
	Women	127.8 ± 1.5	124.2 ± 1.1	123.0 ± 1.6
Mean CAVI	All	8.16 ± 0.05	8.14 ± 0.03	8.27 ± 0.05
	Men	8.52 ± 0.09	8.47 ± 0.06	8.69 ± 0.09
	Women	7.96 ± 0.06	7.96 ± 0.04	8.02 ± 0.06
Mean CIMT (mm)	All	0.73 ± 0.01	0.72 ± 0.00	0.73 ± 0.01
	Men	0.75 ± 0.01	0.74 ± 0.01	0.75 ± 0.01
	Women	0.71 ± 0.01	0.71 ± 0.00	0.71 ± 0.01
CAD, n (%)	All	28 (4.7)	58 (4.9)	22 (3.9)
	Men	15 (6.6)	25 (6.0)	11 (5.2)
	Women	13 (3.5)	33 (4.3)	11 (3.1)

Data are expressed as mean ± standard error (SE).

TC, total cholesterol; logTG, log triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mean CAVI, mean cardio-ankle vascular index; Mean CIMT, mean carotid intima-media thickness; CAD, coronary artery disease.

*$p < 0.0071$ vs. AA.

Sex-dependent findings were demonstrated in the current study since the association of TRIB1 rs2954029 and TG was found only in women. In general, sex is known to affect the risk of most common diseases, including atherosclerosis and diabetes mellitus, and their preceding risk factors. A previous study which used the same identical sample as the present study, showed an association of the GCKR rs780094 polymorphism with TG only in men (Murata-Mori et al. 2014). Klos et al. (2008) reported that the estrogen receptor 1 polymorphism had a stronger association with TG serum levels in females compared to males. Chiba-Falek et al. (2010) reported that estrogen and SCARB1 polymorphisms acted synergistically to regulate the expression of SCARB1 isoforms and influenced the serum levels of HDL-C and TG. Therefore, sex has been repeatedly shown to be associated with lipid serum levels. Sex hormones do not seem to contribute to the sex-dependent difference of TG associated with of TRIB1 polymorphisms in this study, since the study participants were mainly post-menopausal women. Therefore, the molecular mechanisms underlying the sex-dependent difference remain unknown. The serum levels of TG did not follow the genetic additive model in the present study, possibly since it is affected by many other factors due to the modest effect sizes of the individual genetic variants on lipid traits.

A previous *in vivo* study showed that liver-specific overexpression of TRIB1 reduced levels of plasma TG and cholesterol due to increased very low-density lipoprotein (VLDL) production. In contrast, TRIB1-knock-out mice showed elevated levels of plasma TG and cholesterol due to increased VLDL production (Burkhardt et al. 2010). Satoh et al. (2013) showed that mice selectively lacking TRIB1 in haematopoietic cells developed hypertriglyceridemia and insulin resistance with reduced adipose tissue mass accompanied by increased lipolysis. They also found that supplementation of M2-like macrophages from wild type prevented the development of the lipodystrophic phenotype. M2-like macrophages may be involved in the secretion of IL-10, which is an anti-inflammatory cytokine; therefore, they concluded that TRIB1 was critical for the differentiation of M2-like macrophages, and thus critical for adipose tissue maintenance and suppression of metabolic disorders (Satoh et al. 2013). The functional consequence of the TRIB1 rs2954029 has not been clarified, especially with respect to its influence on lipid traits.

The main limitation to this study was the fact that 77.1% of the participants were older than 60 years old. Therefore, the gene effect for younger people could not be evaluated. Since information regarding lifestyles, such as dietary habits and physical activities, could not be fully col-

lected, possible gene-environmental interactions could not be eliminated.

In conclusion, TRIB1 rs2954029 was associated with TG in Japanese community-dwelling women, but not men, suggesting that the phenotype effect of TRIB1 rs2954029 might be sex-dependent. Further studies are needed in order to clarify the influence of TRIB1 rs2954029 on clinical phenotypes.

Conflict of Interest

The authors declare no conflict of interest.

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