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Association of a microsomal triglyceride transfer protein gene polymorphism with blood pressure in Japanese women

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Abstract. Genetic variants of the microsomal triglyceride transfer protein (MTP) have been associated with the serum concentration of low density lipoprotein-cholesterol, predisposition to coronary heart disease, or longevity. The relation of a -493G›T polymorphism in the promoter of *MTP* to blood pressure was examined in a population-based study. The subjects (1124 men, 1108 women) were aged 40-79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. Blood pressure was measured at least twice with subjects in the sitting position. The serum lipid profile was determined after the subjects had fasted overnight. The -493G›T genotype of *MTP* was determined with a fluorescence-based allele-specific DNA primer assay system. There was no difference in the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, or triglycerides among *MTP* genotypes for men or for women. Systolic or diastolic blood pressure was not related to the -493G›T polymorphism in men. For women, however, systolic and diastolic blood pressures were significantly related to *MTP* genotype, with the *T* allele of the polymorphism being associated with low blood pressure. The relation between *MTP* genotype and the prevalence of hypertension was almost significant (P=0.055) for all women. Although *MTP* genotype was not associated with the prevalence of hypertension in premenopausal women, the relation between these parameters was significant (P=0.040) in postmenopausal women, with the *TT* genotype protecting against this condition. These results suggest that *MTP* genotype is a determinant of blood pressure in Japanese women.

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Introduction

Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic renal failure, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of and selection of the most appropriate treatment for hypertension is to identify disease susceptibility genes. Although genetic linkage analyses (2-6) and candidate gene association studies (2,7-10) have implicated various loci and genes in predisposition to hypertension, the genes that confer genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms, it is important to examine polymorphisms related to hypertension in each ethnic group.

Microsomal triglyceride transfer protein is a heterodimeric lipid transfer protein that is essential for the assembly of apolipoprotein B-containing lipoproteins and their secretion from the liver and intestine (11). Mutations in the coding region of the gene for this protein (*MTP*) prevent the production of apolipoprotein B-containing lipoproteins, resulting in the rare genetic disorder abetalipoproteinemia (12). *MTP* is polymorphic, with several genetic variants existing in linkage disequilibrium (13). A common polymorphism (-493G›T) has been identified in the promoter region of *MTP* (located 493 base pairs upstream from the transcription start site), with the less prevalent *T* variant having been associated with a reduced plasma concentration of low density lipoprotein-cholesterol (14). The relation between *MTP* genotype and low density lipoprotein phenotype was confirmed in a large cohort of similar ethnic background (13), but conflicting results have been obtained with other cohorts (15,16). One possible explanation for this discrepancy might be that the phenotype associated with the -493G›T polymorphism of *MTP* is modulated by visceral obesity and hyperinsulinemia (17). The -493G›T polymorphism was recently shown to be associated with the prevalence of coronary heart disease (18). Furthermore, a haplotype marker of *MTP* was associated with longevity, suggesting that *MTP* might modify human life-span (19). Functional analysis of the -493G›T polymorphism with the use of promoter constructs revealed that

the promoter activity of the *T* variant was greater than that of the *G* variant (14).

We hypothesized that *MTP* might be a gene that confers susceptibility to hypertension. We have now performed a large-scale association study for the -493G›T polymorphism of *MTP* and both blood pressure and the prevalence of hypertension in community-dwelling Japanese men and women.

Materials and methods

Study population. The National Institute for Longevity Sciences - Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases (20). The subjects are unrelated individuals stratified by both age and gender, and are randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (21,22). The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited are similar and age at the baseline is 40-79 years, with similar numbers of participants in each decade (40s, 50s, 60s, 70s). The subjects will be followed up every 2 years. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with coronary heart disease, valvular heart disease, cardiomyopathies, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the present study. Individuals receiving anti-hypertensive or lipid-lowering medication were also excluded. We examined the relation of the -493G›T polymorphism of *MTP* (NCBI, dbSNP, rs1800591) to blood pressure and the prevalence of hypertension in 1124 men and 1108 women. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of blood pressure and serum lipid profile. Blood pressure was measured at least twice with subjects in the sitting position according to the guidelines of the American Heart Association (23). Normal blood pressure was defined as both a systolic blood pressure of <140 mmHg and a diastolic blood pressure of <90 mmHg. Hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg, or both.

Venous blood was collected in the early morning after the subjects had fasted overnight. 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. The serum concentrations of total cholesterol (24), high density lipoprotein-cholesterol (25), low density lipoprotein-cholesterol (26), triglycerides (27) were measured according to the methods previously described. Apolipoproteins A1, A2, B, C2, C3, and E were measured by corresponding turbidimetric immunoassay kits (Eiken Chemical, Tokyo, Japan) with Hitachi 7170 automatic analyzer (Hitachi, Tokyo, Japan).

Determination of genotype. Genotypes for *MTP* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (28). The polymorphic region of *MTP* was amplified by the polymerase chain reaction with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'-ACATTATTTTGAAGTGATTGGXTG-3') or Texas red (5'-ACATTATTTTGAAGTGATTGGXGG-3') and with an antisense primer labeled at the 5' end with biotin (5'-AATT CACACTGAATTTTAGGATTTA-3'). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 4.5 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 sec, annealing at 62.5˚C for 30 sec, and extension at 70˚C for 30 sec; and a final extension at 70˚C for 2 min. The amplified DNA was then incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Statistical analysis. Quantitative data were compared among three groups by one-way analysis of variance and the Tukey-Kramer post hoc test, and between two groups by the unpaired Student's t-test. Blood pressure values were analyzed with adjustment for age and body mass index by the least squares method in a general linear model. Allele frequencies were estimated by the gene-counting method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. The effect of *MTP* genotype on blood pressure was evaluated by multivariable regression analysis; P-values and R^2 were calculated from analysis including age, body mass index, and *MTP* genotype (*GG* = *GT* = 0, *TT* = 1). A P-value of <0.05 was considered statistically significant.

Results

The distribution of -493G›T genotypes of *MTP* was in Hardy-Weinberg equilibrium, and age and body mass index did not differ among genotypes, for men (Table I). Serum concentrations of apolipoprotein A2 were greater in men with the *GT* genotype or in men in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype. Serum concentrations of apolipoprotein C2 were also greater in men with the *GT* genotype than in those with the *GG* genotype. No differences in the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, B, C3, or E were detected among *MTP* genotypes in men. Furthermore, systolic or diastolic blood pressure did not differ among *MTP* genotypes in men either with or without adjustment for age and body mass index.

For women, the distribution of -493G›T genotypes of *MTP* was in Hardy-Weinberg equilibrium, and age and body mass

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lood pressure and other characteristics for all male subjects (n=1124) according to MTP genotype.

Characteristic	*GG*	*GT*	*TT*	*GG + GT*	*GT + TT*
No. (%)	777 (69.1)	309 (27.5)	38 (3.4)	1086 (96.6)	347 (30.9)
Age (years)	57.7±0.4	56.6±0.7	55.1±2.0	57.4±0.4	56.5±0.6
Body mass index (kg/m^2)	22.7±0.1	23.0±0.2	23.4±0.5	22.8±0.1	23.0±0.2
Serum lipid profile (mg/dl)					
Total cholesterol	211.7±1.2	213.3±1.9	216.0±5.4	212.1±1.0	213.6±1.8
LDL-cholesterol	131.3±1.2	133.1±1.9	134.2±5.3	131.8±1.0	133.3±1.8
HDL-cholesterol	57.1±0.5	57.6±0.8	60.2±2.4	57.2±0.5	57.9±0.8
Triglycerides	135.9±3.5	131.0±5.5	132.1±15.5	134.5±2.9	131.1±5.2
Apolipoprotein A1	141.1±1.0	143.9±1.6	143.5±4.5	141.9±0.9	143.8±1.5
Apolipoprotein A2	37.7±0.2	38.5±0.3[a]	38.4±1.0	37.9±0.2	38.5±0.3[b]
Apolipoprotein B	107.7±0.9	110.0±1.4	110.9±3.9	108.4±0.7	110.1±1.3
Apolipoprotein C2	4.60±0.05	4.78±0.08[c]	4.62±0.22	4.65±0.04	4.76±0.07
Apolipoprotein C3	11.0±0.1	11.3±0.2	11.0±0.5	11.1±0.1	11.2±0.2
Apolipoprotein E	4.66±0.05	4.71±0.07	4.69±0.21	4.67±0.04	4.71±0.07
Blood pressure (mmHg)					
Systolic	120.8±0.7	120.7±1.1	126.0±3.3	120.8±0.6	121.3±1.1
Diastolic	75.2±0.4	75.6±0.7	77.1±2.0	75.3±0.4	75.7±0.6
Adjusted systolic	120.9±0.7	120.6±1.1	125.3±3.2	120.8±0.6	121.1±1.0
Adjusted diastolic	75.3±0.4	75.4±0.6	76.5±1.9	75.3±0.4	75.5±0.6

Data are means ± SE. LDL and HDL denote low and high density lipoprotein, respectively. Adjustment of systolic or diastolic blood pressure refers to correction for age and body mass index. Data in the combined group of the *GG* and *GT* genotypes (*GG + GT*) were compared with those in individuals with the *TT* genotype (recessive genetic model). Data in the combined group of the *GT* and *TT* genotypes (*GT + TT*) were compared with those in individuals with the *GG* genotype (dominant genetic model). [a]P=0.029, [b]P=0.025, [c]P=0.044 versus *GG*.

index did not differ among genotypes (Table II). There were no differences in serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, A2, B, C2, C3, or E among *MTP* genotypes for women. Systolic blood pressure was significantly lower in women with the *TT* genotype than in those with the *GG* genotype or in those in the combined group of *GG* and *GT* genotypes; the difference in systolic blood pressure between individuals with the *GG* genotype and those with the *TT* genotype (expressed as a percentage of the larger value) was 7.4%. Systolic blood pressure was also significantly lower in women in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype. Diastolic blood pressure was significantly lower in women with the *TT* genotype than in those in the combined group of *GG* and *GT* genotypes; the difference in diastolic blood pressure between these two groups was 5.9%. After adjustment for age and body mass index, systolic blood pressure was significantly lower in women with the *TT* genotype than in those in the combined group of *GG* and *GT* genotypes. Adjusted systolic blood pressure was also significantly lower in women in the combined group of *GT* and *TT* genotypes than in those with the *GG* genotype.

The effects of the -493G›T genotype of *MTP* on blood pressure were evaluated by multiple regression analysis including age, body mass index, and *MTP* genotype (Table III). The analysis revealed that *MTP* genotype significantly affected systolic and diastolic blood pressures for women.

To clarify the effect of the -493G›T polymorphism on blood pressure, we compared the distribution of genotypes between individuals with hypertension and those with normal blood pressure. One woman was excluded from this analysis because she was borderline hypertensive (hypertension and normal blood pressure at the first and second measurements, respectively). For men, no difference in the distribution of *MTP* genotypes was detected between hypertensive and normotensive groups (data not shown). There was an almost significant (P=0.055) relation between *MTP* genotype and the prevalence of hypertension for all women (Table IV). To examine the possible influence of menopause on the relation between *MTP* genotype and blood pressure, we analyzed the genotype distribution and prevalence of hypertension for premenopausal and postmenopausal women independently. Because of their small number (n=17), perimenopausal women were excluded from this analysis. There was no significant relation between *MTP* genotype and the prevalence of hypertension for premenopausal women. In postmenopausal women, however, *MTP* genotype was associated with the prevalence of hypertension, with the *TT* genotype protecting against this condition (Table IV).

Table II. Blood pressure and other characteristics for all female subjects (n=1108) according to *MTP* genotype.

Characteristic	*GG*	*GT*	*TT*	*GG + GT*	*GT + TT*
No. (%)	763 (68.9)	313 (28.2)	32 (2.9)	1076 (97.1)	345 (31.1)
Age (years)	57.0±0.4	58.1±0.7	56.4±2.0	57.3±0.4	57.9±0.6
Body mass index (kg/m^2)	22.8±0.1	22.6±0.2	22.2±0.6	22.7±0.1	22.6±0.2
Serum lipid profile (mg/dl)					
Total cholesterol	227.3±1.3	226.8±2.0	224.3±6.2	227.1±1.1	226.6±1.9
LDL-cholesterol	139.7±1.3	139.1±2.0	137.1±6.2	139.6±1.1	138.9±1.9
HDL-cholesterol	66.2±0.6	65.8±0.9	66.3±2.7	66.1±0.5	65.9±0.8
Triglycerides	108.5±2.2	110.3±3.5	110.9±10.8	109.0±1.9	110.3±3.3
Apolipoprotein A1	156.2±1.0	155.1±1.5	154.3±4.7	155.9±0.8	155.0±1.4
Apolipoprotein A2	37.8±0.2	37.9±0.3	36.0±1.0	37.8±0.2	37.7±0.3
Apolipoprotein B	110.3±0.9	110.0±1.4	109.8±4.5	110.2±0.8	110.0±1.4
Apolipoprotein C2	4.56±0.05	4.58±0.07	4.36±0.22	4.56±0.04	4.56±0.07
Apolipoprotein C3	10.8±0.1	10.9±0.1	10.9±0.5	10.8±0.1	10.9±0.1
Apolipoprotein E	5.00±0.04	5.00±0.07	5.05±0.21	5.00±0.04	5.01±0.06
Blood pressure (mmHg)					
Systolic	120.9±0.8	118.7±1.2	112.0±3.6[a,b]	120.3±0.6	118.0±1.1[a]
Diastolic	73.2±0.4	72.4±0.7	68.6±2.1[c]	72.9±0.4	72.0±0.7
Adjusted systolic	120.9±0.7	118.5±1.1	113.3±3.3[d]	120.2±0.6	118.0±1.0[e]
Adjusted diastolic	73.1±0.4	72.4±0.7	69.3±1.9	72.9±0.4	72.1±0.6

Data are means ± SE. LDL and HDL denote low and high density lipoprotein, respectively. Adjustment of systolic or diastolic blood pressure refers to correction for age and body mass index. Data in the combined group of the *GG* and *GT* genotypes (*GG + GT*) were compared with those in individuals with the *TT* genotype (recessive genetic model). Data in the combined group of the *GT* and *TT* genotypes (*GT + TT*) were compared with those in individuals with the *GG* genotype (dominant genetic model). [a]P=0.038 versus *GG*, [b]P=0.022 versus *GG + GT*, [c]P=0.041 versus *GG + GT*, [d]P=0.037 versus *GG + GT*, [e]P=0.020 versus *GG*.

Table III. Effects of *MTP* genotypes on blood pressure.

	Men		Women	
	P-value	R^2	P-value	R^2
Systolic blood pressure				
Age (years)	<0.001	0.060	<0.001	0.066
Body mass index (kg/m^2)	<0.001	0.012	<0.001	0.098
MTP genotype	0.1276		0.0219	0.006
Diastolic blood pressure				
Age (years)	0.598		<0.001	0.026
Body mass index (kg/m^2)	<0.001	0.079	<0.001	0.100
MTP genotype	0.381		0.0405	0.005

Data were analyzed by multiple regression analysis including age, body mass index, and *MTP* genotypes (*GG = GT* = 0, *TT* = 1).

Table IV. Distribution of *MTP* genotype in hypertensive or normotensive women.

	GG + GT (%)	*TT* (%)	P-value
All women (n=1107)			
Hypertensive	378 (98.4)	6 (1.6)	0.055
Normotensive	697 (96.4)	26 (3.6)	
Premenopausal women (n=279)			
Hypertensive	45 (97.8)	1 (2.2)	0.874
Normotensive	227 (97.4)	6 (2.6)	
Postmenopausal women (n=811)			
Hypertensive	329 (98.5)	5 (1.5)	0.040
Normotensive	458 (96.0)	19 (4.0)	

Discussion

The regulation of blood pressure involves both the integration of a variety of biological systems that control the structure and tone of the vasculature and the volume and composition of body fluid, as well as the adaptation of these systems to constantly changing physiological needs (29). Microsomal triglyceride transfer protein mediates the transport of triglycerides, cholesteryl esters, and phospholipids between

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lipid surfaces (12). Polymorphisms of *MTP* have ciated with the serum concentration of low density lipoprotein-cholesterol (13,14) as well as with the susceptibility to coronary heart disease (18) and with longevity (19). We have now examined the relation of the -493G›T polymorphism of *MTP* to blood pressure and the prevalence of hypertension in community-dwelling Japanese men and women. Our results show that the *T* allele of this polymorphism is associated with low blood pressure in Japanese women and that the *TT* genotype is protective against hypertension in postmenopausal women.

We failed to detect an association of the -493G›T polymorphism of *MTP* with blood pressure in Japanese men. The reason for this gender difference in the relation of *MTP* genotype to blood pressure remains unclear. It might be attributable, however, at least in part, to the difference in the serum concentration of estrogen between men and women, given that estrogen exerts various favorable effects on vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells (30).

Given that selection bias can influence the results of association studies, it is important that study populations be genetically and ethnically homogeneous. Our study subjects were recruited randomly from individuals resident in the city of Obu and town of Higashiura in central Japan, where the population is thought to share the same ethnic ancestry and to possess a homogeneous genetic background. We also showed that the genotype distribution of the -493G›T polymorphism of *MTP* was in Hardy-Weinberg equilibrium both for men and for women in our study population. We thus appeared to avoid admixture and selection bias.

Although the -493G›T polymorphism of *MTP* has previously been associated with the serum concentration of low density lipoprotein-cholesterol (13,14), another study failed to detect such an association (15). In the present study, among men, serum concentrations of apolipoprotein A2 were greater in individuals with the *T* allele than in those with the *GG* genotype. Serum concentrations of apolipoprotein C2 were also greater in men with the *GT* genotype than in those with the *GG* genotype. Apolipoprotein A2 is a component of high density lipoprotein-cholesterol, and apolipoprotein C2 is a component of triglyceride-rich lipoproteins and high density lipoprotein-cholesterol. Although MTP has important roles in the assembly of apolipoprotein B-containing lipoproteins and their secretion from the liver and intestine (11), the effects of the -493G›T polymorphism of *MTP* on apolipoprotein A2 or C2 metabolism remain unclear. This polymorphism was also not associated with the serum concentrations of total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, or apolipoprotein A1, A2, B, C2, C3, or E in women. It is thus unlikely that the association of this polymorphism with blood pressure for women in the present study was attributable to an effect on serum lipid concentrations. The mechanism by which the -493G›T polymorphism of *MTP* affects blood pressure thus remains unclear.

The *T* variant of the -493G›T polymorphism was previously found to confer an excess risk of coronary heart disease in men that was independent of plasma lipids and eliminated by treatment with pravastatin (18). The effect of the *T* allele on blood pressure in the present study was also independent of serum lipid concentrations. The mechanism responsible for the association of the *T* variant with both an increased risk of coronary heart disease in men (18) and a reduced risk of hypertension in postmenopausal women (our study) remains to be elucidated. It is possible that the -493G›T polymorphism of *MTP* is in linkage disequilibrium with other polymorphisms of nearby genes that are actually responsible for the development of coronary heart disease and hypertension. Our present results, however, suggest that *MTP* genotype is a determinant of blood pressure in Japanese women.

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