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Relationship between an Angiotensinogen Gene Polymorphism (M235T) and Serum Lipids: A Cross-Sectional Study among Japanese Workers

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Angiotensinogen (AGT) is a component of the renin-angiotensin system, which plays a central role in blood pressure regulation. Although it is controversial, the association between AGT gene polymorphisms and hypertension, and coronary heart disease is suspected. In a case-control study on the metabolic syndrome, an association between AGT M235T polymorphism and serum total cholesterol (TC) level was demonstrated by Thomas et al. (2001). To reconfirm this relationship, a cross-sectional study among Japanese workers with 876 dyslipidemia and 1,158 non-dyslipidemia subjects was carried out. To evaluate the AGT M235T polymorphism, a PCR-mutant allele specific amplification (MASA) method was employed. No significant difference in the distribution of genetic variance was observed between the two groups. Although it was not significant, the T allele correlating to the lower TC of the present study occurred in a reversed manner to the previous report. In our results, no significant association between AGT M235T and TC was observed.

Key words: angiotensinogen; polymorphism (genetics); cholesterol; cross-sectional study; Japanese workers

Essential hypertension, a major part of hypertension, is a multifactorial disease and some candidate genes have been proposed. The renin-angiotensin system (RAS) has a central role in regulating blood pressure and sodium homeostasis. Angiotensinogen (AGT) plays a role in RAS as a substrate of renin and a precursor of angiotensin II. For that reason, it was considered that the AGT gene polymorphism influenced blood pressure, and the relationship between some polymorphisms and hypertension have been investigated since 1992 (Jeunemaitre et al., 1992; Caulfield et al., 1994; Hata et al., 1994; Tiret et al., 1995; Sato et al., 1997; Ishigami et al., 1997, 1999; Ishikawa et al., 2001; Tiago et al., 2002; Vasku et al., 2002). In particular, the AGT gene M235T polymorphism, with an amino acid substitution of methionine (M) to threonine (T) at codon 235, has been demonstrated to be associated not only with hypertension, but also myocardial infarction (Tiret et

Abbreviations: AGT, angiotensinogen; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, high-density lipoprotein cholesterol; MASA, mutant allele specific amplification; MM, genotype M235M; MT, genotype M235T; M235T, mutation to the threonine of the methionine in amino acid codon 235; OR, odds ratio; RAS, renin-angiotensin system; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TT, genotype T235T

al., 1995; Frossard et al., 1998; Fernandez-Arcas et al., 2001; Buraczynska et al., 2003). Although the relationships are controversial (Fornage et al., 1995; Hingorani et al., 1996; Ichihara et al., 1997; Arnett et al., 1998; Fernandez-Llama et al., 1998; Wang et al., 1999; Rodrigues-Perez et al., 2000; Nair et al., 2003), a hypothesis that the T235 allele plays a role as a "thrifty gene" was proposed by comparing the genotype distribution of anthropoid and human ethnicities (Inoue et al., 1997).

In the current clinical situation, patients with both hypertension and dyslipidemia were commonly observed. Moreover, the metabolic syndrome, in which hypertension is clustered together with obesity, dyslipidemia and insulin resistance in an individual (WHO, 1999; NCEP ATP III, 2001), has reached epidemic proportions, even in Japan.

In such situations, the association between the AGT gene M235T polymorphism and serum total cholesterol level (TC) was demonstrated in a hospital-based case-control study on the metabolic syndrome (Thomas et al., 2001). The mechanism by which RAS influences the lipid metabolism directly has not been discovered (Nishimura et al., 1995; Wierzbicki et al., 2000; Singh et al., 2003; Strazzullo et al., 2004). However, this association is remarkable, whether or not AGT plays any role as the candidate gene responsible for the metabolic syndrome.

Therefore, we planned to evaluate the relationship between the AGT gene M235T polymorphism and serum lipids more closely to the general population than the previous report. For this purpose, a relatively large sample-size worksite based cross-sectional study was carried out.

Subjects and Methods

Ethical issues

The present study was approved by the Ethics Committee of the Faculty of Medicine, Tottori University (No. 81, 2000). Written informed consent of gene analysis was obtained from each study subject.

Study population and eligible subjects

The study population was recruited from workers of a company who received annual health checkups in 1998, in Shimane Prefecture in Western Japan. Among the study population of our study, individuals who gave informed consent were chosen as eligible subjects for the present study.

Various measurements

The body mass index (BMI), body weight in kilograms divided by height in meters squared, was used as a measure of body composition. Obesity was defined as a BMI of 25 and over. Blood pressure was measured using a standard mercury sphygmomanometer. Hypertension was defined as a systolic blood pressure (SBP) of 140 mmHg and over and/or a diastolic blood pressure (DBP) of 90 mmHg and over. Blood samples were collected from a peripheral vein. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL), triglyceride (TG) and fasting blood sugar (FBS) were measured using an autoanalyzer (Model 7150 Autoanalyzer, Hitachi). TC, HDL, TG and FBS were determined enzymatically using commercial enzyme kits (cholesterol oxidase method, direct method, free glycerol without blank method, Kyowa Medex, Tokyo, Japan; and the hexokinase glucose-6-phosphatedehydrogenase method, Wako, Tokyo, Japan, respectively). Details of these measurements have been described previously (Kaetsu et al., 2004).

In the present study, dyslipidemia was defined as at least one of the following three criteria: TC 220 mg/dL and over, HDL less than 40 mg/dL, or TG 150 mg and over. Hyperglycemia was defined as FBS 110 mg/dL and over, as a substitute for insulin resistance.

Alcohol drinking and smoking habits were assessed using a questionnaire on lifestyle factors at annual health checkups. Drinkers were defined

as daily drinkers, and occasional and never drinkers were assessed as non-drinkers. Smokers were defined as current smokers, and past and never smokers were assessed as non-smokers.

Identification of the AGT gene M235T polymorphism and genotyping

Genome DNA was extracted using a fully automatic nucleic acid extractor (MagExtractor Genome, Toyobo, Osaka, Japan). The PCR mutant allele specific amplification (MASA) method was employed to analyze the AGT gene M235T polymorphism and genotyping, M235M (MM), M235T (MT) and T235T (TT), respectively. Details of the molecular approach of this study have been described previously (Kishimoto et al., 2001a, 2001b).

Because there was quite a small proportion of the MM genotype (3.8, 30.6 and 65.6% for MM, MT and TT, respectively), genotype specific evaluation was performed in three genotypes, and MM and MT were combined against TT.

Statistical analysis

The software SAS (SAS Institute, Cary, NC) was employed for all statistical analysis. The eligible subjects were divided into two groups according to dyslipidemia to compare the AGT gene M235T genotype distribution using a Mantel-Haenszel chi-square test. The unpaired analysis of results using a Kruskal-Wallis test was performed to assess whether the differences of the quantitative variables between genotypes were significant. These analyses were performed on all the subjects, sex and age specifically. According to the "Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Disease 2002" of the Japan Atherosclerosis Society (Saito, 2004), eligible subjects were divided into younger and older groups with cutoff ages of 45 for males and 55 for females. Moreover, univariate and multivariate unconditional logistic regression analysis was employed to evaluate the relationship between the AGT M235T genotype and the prevalence of abnormalities in each serum lipid.

Results

The total number of eligible subjects was 2,034, with 1,137 male and 897 female subjects, respectively. The descriptive characteristics of the subjects are shown in Table 1. Of the 2,034 subjects, 876 were assigned to the dyslipidemia group and 1,158 to the non-dyslipidemia group (Control group). In the dyslipidemia group, a higher mean age, higher proportion of male subjects, obesity, hypertension, hyperglycemia, alcohol drinkers, and smokers were observed than in the control group.

The distributions of genotype and allele frequency are shown in Table 2. The relative frequencies of MM, MT and TT genotypes were 4%, 31% and 65%, respectively. The allele frequencies were 19% and 81% for M and T alleles, respectively. These results are consistent with the Hardy-Weinberg equilibrium, not only for the total subjects, but also when divided according to the presence of dyslipidemia. Neither the genotype nor the allele frequency showed statistic significance between the dyslipidemia and control groups.

The differences of quantitative variables between genotypes are shown in Table 3. Although it was not significant, the subjects carrying the M allele had higher TC. On the other hand, no such differences were seen in either HDL or TG.

The relationships between genotype and dyslipidemia and its components estimated by unconditional logistic regression analysis are shown in Table 4. No significant relationship was observed, both in univariate and multivariate analysis.

Discussion

The relative frequencies of the AGT gene M235T polymorphism in all eligible subjects were 4, 31

Table 1. Descriptive characteristics

		Control	Dyslipidemia	Total	*P*
Number of subjects		1158	876	2034	
Male subjects	(%)	48.3%	66.0%	55.9%	< 0.0001
Older subjects; age ≥ 45 in males, ≥ 55 in females					
Ratio	(%)	24.9%	42.2%	31.9%	< 0.0001
Age, mean ± SD	(year)	40.3 ± 11.6	45.3 ± 10.3	42.4 ± 11.3	< 0.0001
Obese subjects; BMI > 25					
Ratio	(%)	16.3%	34.7%	23.9%	< 0.0001
BMI, mean ± SD		22.1 ± 3.1	23.8 ± 3.0	22.9 ± 3.2	< 0.0001
Hypertension; SBP ≥ 140 and/or DBP ≥ 90 mmHg					
Occurence ratio	(%)	13.7%	28.5%	19.8%	< 0.0001
SBP ≥ 140 mmHg					
Occurence ratio	(%)	10.2%	20.5%	14.5%	< 0.0001
Mean ± SD	(mmHg)	116.8 ± 16.6	125.2 ± 17.5	120.4 ± 17.5	< 0.0001
DBP ≥ 90 mmHg					
Occurence ratio	(%)	9.8%	21.5%	14.7%	< 0.0001
Mean ± SD	(mmHg)	72.6 ± 11.5	78.8 ± 12.2	75.2 ± 12.2	< 0.0001
Dyslipidemia					
Occurence ratio	(%)	0.0%	100.0%	43.1%	
TC ≥ 220 mg/dL					
Occurence ratio	(%)	–	74.3%	32.0%	
Mean ± SD	(mg/dL)	184.0 ± 22.4	232.5 ± 36.9	204.9 ± 38.1	< 0.0001
HDL < 40 mg/dL					
Occurence ratio	(%)	–	11.2%	4.8%	
Mean ± SD	(mg/dL)	66.7 ± 14.7	62.0 ± 19.3	64.7 ± 17.0	< 0.0001
TG ≥ 150 mg/dL					
Occurence ratio	(%)	–	43.7%	18.8%	
Mean ± SD	(mg/dL)	74.5 ± 29.7	158.7 ± 108.1	110.8 ± 85.3	< 0.0001
Hyperglycemia; FBS ≥ 110 mg/dL					
Occurence ratio	(%)	8.9%	16.7%	12.2%	< 0.0001
Mean ± SD	(mg/dL)	96.5 ± 13.7	102.0 ± 21.8	98.8 ± 17.8	< 0.0001
Alcohol drinking					
Current ratio	(%)	23.9%	34.7%	28.6%	< 0.0001
Smoking					
Current ratio	(%)	30.1%	42.1%	35.3%	< 0.0001

Dyslipidemia: at least one of the following features: TC ≥ 220, HDL < 40 and TG ≥ 150 mg/dL.
BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, high density lipoprotein-colesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.
P: *P* value for difference between dyslipidemia and control subjects.
P values were determined by chi-square (sex, older, obese, hypertension, dyslipidemia, hyperglycemia, alcohol drinking and smoking) and Wilcoxon rank sum test (age, BMI, TC, HDL, TG and FBS).

and 65% for the MM, MT and TT genotypes, respectively. Although it was not significant, the subjects carrying the M allele had higher TCs. No significant relationship was observed, both in univariate and multivariate unconditional logistic regression analysis.

The genotype distribution of this polymorphism of the present study was close to the result reported by Thomas et al. (2001). Since the ethnically different genotype distribution of this polymorphism has been reported (Rodriguez-Perez et al., 2000), it may represent the ethnic proximity in the genetic backgrounds between the two study populations. On the other hand, the relationship between the polymorphism and TC occurred in a reversed manner. They reported that the subjects

Table 2. Angiotensinogen M235T genotypes and allele frequencies with dyslipidemia

		Control	Dyslipidemia	Total	*P*
All subjects					
Number		1158	876	2034	
Genotype	T/T	770 (66.5)	564 (64.4)	1334 (65.6)	
	M/T	344 (29.7)	278 (31.7)	622 (30.6)	
	M/M	44 (3.8)	34 (3.9)	78 (3.8)	0.3816
	M/T + M/M	388 (33.5)	312 (35.6)	700 (34.4)	0.3447†
Allele frequency	T allele	1884 (81.3)	1406 (80.3)	3290 (80.9)	
	M allele	432 (18.7)	346 (19.7)	778 (19.1)	0.3788
Male subjects					
Number		559	578	1137	
Genotype	T/T	389 (69.6)	374 (64.7)	763 (67.1)	
	M/T	150 (26.8)	191 (33.1)	341 (30.0)	
	M/M	20 (3.6)	13 (2.2)	33 (2.9)	0.9224
	M/T + M/M	170 (30.4)	204 (35.3)	374 (32.9)	0.0913†
Allele frequency	T allele	928 (83.0)	939 (81.2)	1867 (82.1)	
	M allele	190 (17.0)	217 (18.8)	407 (17.9)	0.2778
Female subjects					
Number		599	298	897	
Genotype	T/T	381 (63.6)	190 (63.8)	571 (63.7)	
	M/T	194 (32.4)	87 (29.2)	281 (31.3)	
	M/M	24 (4.0)	21 (7.0)	45 (5.0)	0.4868
	M/T + M/M	218 (36.4)	108 (36.2)	326 (36.3)	1.0000†
Allele frequency	T allele	956 (79.8)	467 (78.4)	1423 (79.3)	
	M allele	242 (20.2)	129 (21.6)	371 (20.7)	0.4769
Younger subjects; age < 45 in males, < 55 in females					
Number		879	506	1385	
Genotype	T/T	572 (65.1)	326 (64.4)	898 (64.8)	
	M/T	271 (30.8)	161 (31.8)	432 (31.2)	
	M/M	36 (4.1)	19 (3.8)	55 (4.0)	0.9224
	M/T + M/M	307 (34.9)	180 (35.6)	487 (35.2)	0.8537†
Allele frequency	T allele	1415 (80.5)	813 (80.3)	2228 (80.4)	
	M allele	343 (19.5)	199 (19.7)	542 (19.6)	0.9220
Older subjects; age < 45 in males, < 55 in females					
Number		279	370	649	
Genotype	T/T	98 (71.0)	238 (64.3)	436 (67.2)	
	M/T	73 (26.2)	117 (31.6)	190 (29.3)	
	M/M	8 (2.9)	15 (4.1)	23 (3.5)	0.0727
	M/T + M/M	81 (29.0)	132 (35.7)	213 (32.8)	0.0891†
Allele frequency	T allele	469 (84.1)	593 (80.1)	1062 (81.8)	
	M allele	89 (15.9)	147 (19.9)	236 (18.2)	0.0702

P: *P* value for difference between dyslipidemia and control subjects.
P values were determined by chi-square test.
† Compared to T/T.

carrying the M allele of the AGT gene M235T polymorphism (MM/MT) had significantly lower TC than those with the TT genotype. Furthermore, a linear dose-dependent relationship between the genotypes for TC was demonstrated. However, our result showed that the M allele had higher TC, though it was not significant. According to other investigations, which described the relationship between the AGT gene M235T polymorphism and TC, some reported the M allele had lower TC, equivalent to Thomas et al. (Iwai et al., 1995; Nishimura et al., 1995; Batalla

Table 3. Mean value of serum lipids and other variables according to AGT M235T genotype

	TT	MT	MM	MT + MM	*P*	*P*†
All subjects						
Number	1334	622	78	700		
TC (mg/dL)	203.6 ± 36.2	206.6 ± 41.6	213.2 ± 39.3	207.4 ± 41.4	0.1816	0.2627
HDL (mg/dL)	64.7 ± 17.2	64.6 ± 16.6	65.1 ± 16.7	64.7 ± 16.6	0.9998	0.9855
TG (mg/dL)	108.7 ± 79.5	116.1 ± 97.8	102.7 ± 70.9	114.6 ± 95.2	0.5886	0.3853
BMI (kg/m^2)	23.0 ± 3.2	22.6 ± 3.2	22.7 ± 3.1	22.6 ± 3.2	0.0687	0.0219
SBP (mmHg)	120.8 ± 17.3	120.1 ± 17.9	116.0 ± 17.9	119.7 ± 17.9	0.0464	0.0992
DBP (mmHg)	75.5 ± 12.0	72.0 ± 12.6	73.4 ± 11.9	74.8 ± 12.5	0.2767	0.2311
FBS (mg/dL)	99.0 ± 18.1	98.7 ± 17.7	97.7 ± 13.5	98.6 ± 17.2	0.6635	0.3669
Male subjects						
Number	763	341	33	374		
TC (mg/dL)	203.0 ± 36.0	210.6 ± 41.5	204.5 ± 35.5	210.1 ± 41.0	0.0493	0.0199
HDL (mg/dL)	59.6 ± 16.4	60.0 ± 15.6	62.1 ± 17.2	60.2 ± 15.8	0.6789	0.4419
TG (mg/dL)	132.8 ± 91.1	146.3 ± 112.7	117.6 ± 94.9	143.8 ± 111.5	0.0304	0.1651
BMI (kg/m^2)	23.6 ± 3.1	23.3 ± 3.1	23.1 ± 2.6	23.3 ± 3.1	0.4347	0.2227
SBP (mmHg)	124.8 ± 16.5	124.8 ± 16.1	119.4 ± 18.8	124.3 ± 16.4	0.2026	0.6368
DBP (mmHg)	78.6 ± 11.9	78.6 ± 12.1	76.1 ± 13.1	78.3 ± 12.2	0.4510	0.8568
FBS (mg/dL)	101.9 ± 20.6	101.8 ± 20.0	97.7 ± 9.9	101.5 ± 19.4	0.5062	0.3140
Femle subjects						
Number	571	281	45	326		
TC (mg/dL)	204.4 ± 36.4	201.8 ± 41.3	219.6 ± 41.0	204.3 ± 41.7	0.0112	0.3879
HDL (mg/dL)	71.4 ± 15.9	70.3 ± 16.1	67.3 ± 16.1	69.9 ± 16.1	0.1077	0.1030
TG (mg/dL)	76.6 ± 43.3	79.4 ± 57.5	91.8 ± 44.2	81.1 ± 56.0	0.0238	0.3963
BMI (kg/m^2)	22.2 ± 3.2	21.8 ± 3.1	22.4 ± 3.5	21.9 ± 3.2	0.1890	0.1328
SBP (mmHg)	115.5 ± 16.9	114.5 ± 18.4	113.5 ± 17.0	114.4 ± 18.2	0.3018	0.1225
DBP (mmHg)	71.2 ± 10.8	70.7 ± 11.9	71.4 ± 10.6	70.8 ± 11.7	0.5097	0.3788
FBS (mg/dL)	95.1 ± 13.2	94.8 ± 13.3	97.8 ± 15.7	95.2 ± 13.7	0.6507	0.5979
Younger subjects; age < 45 in males, < 55 in females						
Number	898	432	55	487		
TC (mg/dL)	200.3 ± 35.9	201.2 ± 40.7	205.5 ± 38.1	201.7 ± 40.4	0.7019	0.8596
HDL (mg/dL)	66.7 ± 17.2	66.1 ± 16.8	64.5 ± 15.8	65.9 ± 16.6	0.5044	0.3683
TG (mg/dL)	98.3 ± 76.4	101.6 ± 83.0	96.8 ± 80.8	101.0 ± 82.7	0.9211	0.7287
BMI (kg/m^2)	22.6 ± 3.2	22.2 ± 3.2	22.2 ± 3.1	22.2 ± 3.2	0.1028	0.0331
SBP (mmHg)	116.5 ± 15.3	115.6 ± 15.7	111.9 ± 15.1	115.2 ± 15.7	0.0843	0.1141
DBP (mmHg)	72.2 ± 10.7	71.5 ± 11.5	70.7 ± 10.7	71.4 ± 11.4	0.2103	0.0869
FBS (mg/dL)	95.9 ± 12.1	95.3 ± 12.6	93.8 ± 8.3	95.1 ± 12.2	0.3450	0.1565
Older subjects; age ≥ 45 in males, ≥ 55 in females						
Number	436	190	23	213		
TC (mg/dL)	210.3 ± 35.8	219.0 ± 40.9	231.7 ± 36.4	220.4 ± 40.6	0.0066	0.0061
HDL (mg/dL)	60.4 ± 16.3	61.4 ± 15.9	66.5 ± 18.8	62.0 ± 16.3	0.3899	0.3278
TG (mg/dL)	130.3 ± 81.5	149.2 ± 118.9	116.9 ± 35.7	145.7 ± 113.3	0.1801	0.0720
BMI (kg/m^2)	23.7 ± 3.0	23.5 ± 3.0	23.9 ± 2.9	23.6 ± 3.0	0.5198	0.5649‡
SBP (mmHg)	129.7 ± 17.7	130.3 ± 18.4	125.7 ± 20.4	129.8 ± 18.6	0.7307	0.9190
DBP (mmHg)	82.1 ± 11.9	83.0 ± 11.3	79.9 ± 12.1	82.7 ± 11.4	0.3635	0.4073
FBS (mg/dL)	105.4 ± 25.4	106.5 ± 24.0	107.2 ± 18.3	106.5 ± 23.4	0.3694	0.2070

P: *P* value for difference between TT versus MT versus MM by Kruskal-Wallis test.
P†: *P* value for differences between TT versus MT + MM by Wilcoxon rank sum test.
‡ *P* value for differences between TT versus MT + MM by Student's *t*-test

Table 4. Odds ratios of AGT M235T genotype for dyslipidemia

		+	–	Univariate			Multivariate†		
				OR	95% CI	*P*	OR	95% CI	*P*
Dyslipidemia									
TT		564	770	1.00			1.00		
MT		278	344	1.10	0.91 – 1.34	0.3140	1.22	0.99 – 1.50	0.0585
MM		34	44	1.06	0.67 – 1.67	0.8200	1.20	0.74 – 1.97	0.4609
P‡	0.3816								
TT		564	770	1.00			1.00		
MT + TT		312	388	1.10	0.91 – 1.32	0.3204	1.22	1.00 – 1.49	0.0508
P‡	0.3447								
TC, ≥ 220 mg/dL									
TT		416	918	1.00			1.00		
MT		205	417	1.09	0.89 – 1.33	0.4326	1.16	0.94 – 1.43	0.1758
MM		30	48	1.38	0.86 – 2.21	0.1806	1.42	0.87 – 2.31	0.1633
P‡	0.1742								
TT		416	918	1.00			1.00		
MT + TT		235	465	1.12	0.92 – 1.36	0.2730	1.18	0.97 – 1.45	0.1017
P‡	0.2730								
HDL, < 40 mg/dL									
TT		68	1266	1.00			1.00		
MT		28	594	0.88	0.56 – 1.38	0.5702	0.95	0.60 – 1.50	0.8192
MM		2	76	0.49	0.12 – 2.04	0.3265	0.66	0.16 – 2.82	0.5781
P‡	0.3101								
TT		68	1266	1.00			1.00		
MT + TT		30	670	0.83	0.54 – 1.29	0.4173	0.92	0.59 – 1.44	0.7191
P‡	0.4168								
TG, ≥ 150 mg/dL									
TT		248	1086	1.00			1.00		
MT		125	497	1.10	0.87 – 1.40	0.4300	1.21	0.93 – 1.57	0.1631
MM		10	68	0.64	0.33 – 1.27	0.2033	0.89	0.43 – 1.82	0.7415
P‡	0.8794								
TT		248	1086	1.00			1.00		
MT + TT		135	565	1.05	0.83 – 1.32	0.7020	1.17	0.91 – 1.52	0.2238
P‡	0.7033								

Dyslipidemia: at least one of the following features: TC ≥ 220, HDL < 40 and TG ≥150 mg/dL.
CI, confidence interval; OR, ddds ratio.
† Adjusted for sex, age, BMI, FBS, alcohol and smoking.
‡ *P* for chi-square.

et al., 2000), and some reported the M allele had higher TC, equivalent to our findings (Matsubara et al., 2003), others still showed no relationship (Iwai et al., 1994; Katsuya et al., 1995; Hingorani et al., 1996; Fujiwara et al., 2002; Robinson et al., 2004). Because the relationship between this polymorphism and TC was not stable among the investigations, and the mechanism by which RAS influences the lipid metabolism directly has not been discovered (Nishimura et al., 1995; Wierzbicki et al., 2000; Singh et al., 2003; Strazzullo et al., 2004), the relationship may in fact be weak or even nonexistent.

Although this work-site cross-sectional study was employed to investigate the relationship between the AGT gene M235T polymorphism and serum lipids more closely to general population than a hospital-based case-control study, some limitations remain in this study. First, the presence of the healthy worker's effect was suspected. The possibility that the exclusion of unhealthy subjects from the study population influenced the

result cannot be denied. Second, the relationships among study subjects were not confirmed. The possibility that relatives and siblings showed similar characteristics and influenced the results cannot be denied. Third, the present and past illnesses of each subject were not evaluated. Because our data originated from the work-site, the history of each subject was not obtained in more detail than the hospital-based data. However, in the current clinical situation of Japanese hypertension therapy, medication is administered subsequent to lifestyle modification (Saruta, 2005). That lifestyle modification improves cardiovascular risk factors, including serum lipids among general population, has been reported (Okazaki et al., 2001). Furthermore, it was reported that the subjects with the TT homozygote of the AGT M235T genotype had a higher prevalence of antihypertension medication among middle-aged Japanese men (Nishimura et al., 1995). By not considering the present and past history of each subject, the possibility that the underestimation of the serum lipids of the subjects carrying the T allele cannot be denied. A prospective cohort study should be performed for a more conclusive evaluation.

Conclusion

The association between the AGT gene M235T polymorphism and serum lipids among Japanese workers was investigated. Although it was not significant, subjects carrying the M allele of the AGT gene M235T polymorphism (MM/MT) had higher TC than those with the TT genotype. The AGT gene M235T polymorphism showed no significant association between serum lipids in our study population.

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