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A Molecular Variant of the Angiotensinogen Gene and Hypertension in a Case-Control Study in Japanese

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In order to examine the distribution of M235T (the substitution of threonine for methionine at position 235 codon) polymorphism genotypes of the angiotensinogen (AGT) gene and the relationship between M235T polymorphism of the AGT gene and hypertension, a descriptive study and a case-control study were performed among Japanese workers. The subjects were 2042 workers at an occupational site in Shimane Prefecture in Japan. The database was set up for the workers' regular health examination in 1998. The M235T polymorphism of the AGT gene for each worker was defined by the mutant allele specific amplification (MASA) method. The rates of M235M (MM), M235T (MT) and T235T (TT) genotypes were 3.9%, 30.7% and 65.5%, respectively. The odds ratios of MT and TT against MM for hypertension by univariate analysis were 0.77 (95% confidence interval (CI) 0.27–2.18) and 0.77 (95% CI 0.28–2.14), respectively. The odds ratios of MT and TT against MM for hypertension, adjusted for body mass index, fasting blood sugar, drinking habits, cigarette smoking and exercise in a logistic regression model, were 0.90 (95% CI 0.29–2.74) and 0.87 (95% CI 0.30–2.58), respectively. The data from this study suggests that there may be no relationship between the M235T polymorphism of the AGT gene and hypertension. Further prospective studies are needed to resolve this issue.

Key words: angiotensinogen; case-control study; genotype; hypertension; polymorphism

The angiotensinogen (AGT) gene variant, M235T (mutation to the threonine of the methionine in amino acid codon 235), was first reported in the pathogenesis of essential hypertension by Jeunemaitre et al. (1992). Essential hypertension is a multiple factorial disease that is complicated by the interaction of a genetic factor and an environmental factor (Soubrier et al., 1995). There have been many studies investigating the hypothesis that this polymorphism, M235T, is one of the genetic factors of essential hypertension (Hata et al., 1994; Tiila-Riikka et al., 1996; Borecki et al., 1997; Fu-Tien et al., 1997; Frossard et al., 1998); however, despite numerous studies the hypothesis remains controversial.

Most of the reports regarding the relationship between M235T and hypertension implemented a case-control study using hospital-based data that included a relatively small sample size. Thus, we carried out a descriptive study and a case-control study using population-based data with a large sample size in order to clarify the involvement of M235T in hypertension.

Abbreviations: AGT, angiotensinogen; CI, confidence interval; DBP, diastolic blood pressure; MASA, mutant allele specific amplification; MM, genotype M235M; MT, genotype M235T; M235T, mutation to the threonine of the methionine in amino acid codon 235; OD, odds ratio; PCR, polymerase chain reaction; SBP, systolic blood pressure; TT, genotype T235T

Subjects and Methods

Subjects

The research was approved by the ethics committee of Tottori University Faculty of Medicine, and informed consent was obtained from 2042 workers in Shimane Prefecture in Japan who received their regular medical examination in 1998. The case-control study comprised a total of 402 individuals selected from the 2042 workers. The case and control individuals were matched by sex and age (difference within 2 years old). The following selection criteria for hypertensives, borderline and normotensive subjects were applied: (i) hypertensives, borderline and normotensive blood pressure as defined by systolic blood pressure (SBP) ≥ 160 mmHg and/or diastolic blood pressure (DBP) ≥ 95 mmHg, 140 mmHg ≤ SBP < 160 mmHg and/or 90 mmHg ≤ DBP < 95 mmHg, and SBP < 140 mmHg and DBP < 90 mmHg, respectively; (ii) absence of secondary hypertension; and (iii) absence of renal disease or renal insufficiency.

Various measurements and survey of smoking history

Body mass index (BMI) was calculated by the following formula: (weight kg)/(height m)^2. The blood pressure was measured in the right upper arm with a standard sphygmomanometer in a sitting position. The 1st and 5th sound of the Korotkoff sound were used as SBP and DBP, respectively. Measurement of the fasting blood sugar value was carried out using an enzymatic method (Hexokinase, G-6-PDH, Wako, Tokyo, Japan). Drinking habits, cigarette smoking and exercise were assessed using a questionnaire.

Identification of polymorphism

Genome DNA was prepared from white blood cells with the use of a fully automatic nucleic acid extractor (MFX-2000, Toyobo Co., Ltd., Osaka, Japan). The mutant allele specific amplification (MASA) method was used for the analysis of the polymorphism (Takeda et al., 1993). Four primers were designed. The normal sense primer and antisense primer were 5'-AAGACTGGCTGCTCCCTGAT-3' and 5'-GCTGTCCACACTGGCTCCCG-3', respectively. The wild type primer and polymorphism primer including the mutation part were 5'-AAGACTGGCTGCTCCCTGAT-3' and 5'-AAGACTGGCTGCTCCCTGAC-3', respectively. Polymerase chain reaction (PCR) was performed in a TaKaRa PCR Thermal Cycler (Takara Co., Tokyo) with a 10 μL reaction volume containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 25 μmol/L of each dNTP, 1.25 μmol/L sense primer, 1 μmol/L antisense primer, 2 μmol/L wild type primer, 2 μmol/L polymorphism primer, 0.1 U Ampli-Taq DNA polymerase (Perkin Elmer, Foster City, CA) and 1.5 mmol/L $MgCl_2$. The initial denaturation for 3 min at 95˚C was followed by 35 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 65˚C, and extension for 60 s at 72˚C. PCR products were electrophoresed in 6% polyacrylamide gels on a Model Cassette Electrophoresis Unit "DPC" (Daiichi Pure Chemical Co., Ltd., Tokyo) and DNA was visualized by ethidium bromide staining.

Table 1. Subjects related by angiotensinogen codon 235 genotypes by age

Age	Genotype			Total
	MM	MT	TT	
10–19		1(100.0)		1 (100.0)
20–29	13 (3.6)	124 (34.3)	224 (62.0)	361 (100.0)
30–39	18 (4.0)	134 (29.1)	304 (67.0)	454 (100.0)
40–49	21 (3.4)	197 (32.3)	391 (64.2)	609 (100.0)
50–59	21 (4.1)	143 (28.1)	345 (67.8)	509 (100.0)
60–69	4 (4.5)	22 (24.7)	63 (70.8)	89 (100.0)
70–79	2 (10.5)	7 (36.8)	10 (52.6)	19 (100.0)
Total	79 (3.9)	626 (30.7)	1337 (65.5)	2042 (100.0)

The percentage of individual genotypes by age is given in parentheses.

Table 2. Subjects related by angiotensinogen codon 235 genotypes by blood pressure

	SBP (mmHg)	DBP (mmHg)	Genotype			Total
			MM	MT	TT	
Normotensive	< 140	< 90	63 (79.7)	500 (79.9)	1046 (78.2)	1609 (78.8)
Borderline	≥ 140, < 160	≥ 90, < 95	7 (8.9)	61 (9.7)	164 (12.3)	232 (11.4)
Hypertensive	≥ 160	≥ 95	9 (11.4)	65 (10.4)	127 (9.5)	201 (9.8)
Total			79 (100.0)	626 (100.0)	1337 (100.0)	2042 (100.0)

The percentage of individual normotensive, borderline and hypertensive is given in parentheses.
DBP, diastolic blood pressure; MM, M235M; MT, M235T; TT, T235T; SBP, systolic blood pressure.

Statistical analysis

SPSS software (version 8.0, SPSS Japan Inc., Tokyo) was used for all statistical comparisons. The χ^2 statistic was calculated to test the distribution trend of each index by genotype. Relative risks were calculated by the logistic regression analysis method.

Results

The proportions of the genotype found in this study for M235M (MM), M235T (MT) and T235T (TT) were 3.9%, 30.7% and 65.5%, respectively (Table 1). There was no statistically significant relationship between distribution of genotype and age. The allele frequency of 235T was 0.808. As shown in Table 2, there was no significant difference between the normotensive, borderline and hypertensive subjects in terms of the proportions of the genotypes. Table 3 shows the distribution of genotype, drinking habits, cigarette smoking and exercise, and the mean values of BMI and fasting blood sugar related to case and control. The mean values of BMI and fasting blood sugar, and distribution of drinking habits in both case and control groups were statistically different from each other. In regard to the odds ratio (OD) for hypertension, the ODs of MT and TT against MM by univariate analysis were 0.77 [95% confidence interval (CI) 0.27–2.18] and 0.77 (95% CI 0.28–2.14) (Table 4). These ODs showed statistically higher trends in BMI, fasting blood

Table 3. Distribution and mean value of various indices by case and control

		Control	Case	Total	*P* value
		Normotensive	Hypertensive		
Genotype	MM	7 (4)	9 (5)	16 (4)	
	MT	66 (33)	65 (32)	131 (33)	
	TT	128 (64)	127 (63)	255 (63)	
	Total	201 (100)	201 (100)	402 (100)	0.877
BMI	Mean ± SD	23.2 ± 2.9	24.8 ± 3.3	24.0 ± 3.2	0.000
Blood Sugar	Mean ± SD (mg/dL)	102.6 ± 25.8	108.2 ± 22.6	105.4 ± 24.4	0.021
Drinking	Never, sometimes	129 (64)	109 (54)	238 (59)	
	Everyday	72 (36)	92 (46)	164 (41)	
	Total	201 (100)	201 (100)	402 (100)	0.043
Smoking	Never smoked or ex-smoker	141 (70)	129 (64)	270 (67)	
	Active smoker	60 (30)	72 (37)	132 (33)	
	Total	201 (100)	201 (100)	402 (100)	0.19
Exercise	Many times	18 (9)	14 (7)	32 (8)	
	Sometimes, never	183 (91)	187 (93)	370 (92)	
	Total	201 (100)	201 (100)	402 (100)	0.447

The percentages are given in parentheses.
BMI, body mass index; MM, M235M; MT, M235T; TT, T235T.

Table 4. Hypertension risks estimates associated with angiotensinogen M235T gene polymorphism and risk factors based on a case-control study

	Univariate		Multivariate	
	Odds ratio	95% CI	Odds ratio	95% CI
Genotype (1) (MT versus MM)	0.766	0.269–2.179	0.895	0.293–2.735
Genotype (2) (TT versus MM)	0.772	0.279–2.135	0.873	0.295–2.583
BMI	1.191*	1.112–1.274	1.186*	1.107–1.271
Blood sugar	1.011*	1.001–1.021	1.006	0.997–1.015
Drinking	1.512*	1.012–2.260	1.450	0.941–2.234
Smoking	1.322	0.870–2.008	1.242	0.792–1.947
Exercise	1.337	0.631–2.831	1.289	0.589–2.825

* $P < 0.05$.

BMI, body mass index; CI, confidence interval; MM, M235M; MT, M235T; TT, T235T.

sugar and drinking habits. When the multiple risk factors of BMI, fasting blood sugar, drinking habits, cigarette smoking and exercise were adjusted, the ODs of MT and TT against MM were 0.90 (95% CI 0.29–2.74) and 0.87 (95% CI 0.30–2.58), respectively, but these were not significant.

Discussion

In the population examined in this study, the frequency of the wild type MM was the lowest, while that of the mutant type TT was the highest. There were no significant differences in the proportions of the genotypes among normotensive, borderline and hypertensive subjects. The odds ratios of MT and TT against MM for hypertension analyzed by correction using various factors were not significant, and there were no correlations (0.90 and 0.87, respectively).

The allele frequency in the population was higher than that of Caucasians (Jeunemaitre et al., 1992). This agreed with the results reported in several studies on investigation of the allele frequency in Japanese subjects (Iwai et al., 1994; Kamitani et al., 1994; Morise et al., 1995; Nishiuma et al., 1995; Sato et al., 1997). The allele frequency was 0.808 in the present study, and ranged from 0.604 to 0.860 in published studies. This is probably because there are regional differences in the genetic background of the native Japanese.

The relationship between this genetic polymorphism and hypertension has been suggested in studies performed in Japan and in other countries (Jeunemaitre et al., 1992; Hata et al., 1994; Soubrier et al., 1995; Tiila-Riikka et al., 1996; Borecki et al., 1997; Fu-Tien et al., 1997; Frossard et al., 1998). Kato et al. (1999) were the first to cast doubt on this relationship. The different results for the relationship between genotypes and hypertension may be caused by the possibility of bias in choosing controls, differences between races, regional differences in genetic background, differences in the age of subjects and multifactors of the examined disease, because most of the studies were hospital-based case-control studies in a small sample size (Singer et al., 1996).

To improve on the drawbacks of hospital-based case-control studies in a small sample size, we performed a population-based case-control study and obtained no correlations between genotypes and hypertension. This study was an improvement being performed in a population-based pattern, because a case-control study has many kinds of biases. It is very difficult to choose a suitable control group and it was considered necessary to evaluate the relationship by a cohort study which has few information biases compared to a case-control study.

In conclusion, there was no correlation between the M235T polymorphism of the AGT gene and hypertension in a population-based case-control study corrected for various factors in Japanese subjects. We consider it necessary to investigate further by using the method of a cohort study.

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