Original Article

High Sodium Intake Strengthens the Association between Angiotensinogen T174M Polymorphism and Blood Pressure Levels among Lean Men and Women: a Community-Based Study

Kazumasa YAMAGISHI, Hiroyasu ISO, Takeshi TANIGAWA, Renzhe CUI, Minako KUDO, and Takashi SHIMAMOTO*

Evidence on the effect of salt intake on the interaction between angiotensinogen (*AGT*) T174M polymorphism and high blood pressure is sparse. We therefore conducted a large population-based cross-sectional study of 2,823 men and women aged 30−74 in a Japanese farming community to examine associations between *AGT* polymorphism and blood pressure levels stratified by age (30−64 and 65−74), body mass index (BMI; median), and salt intake (median) estimated by 24-h urine collection and dietary questionnaire. Our *a priori* hypothesis is that individuals, particularly younger and non-overweight individuals, with the 174M allele have elevated blood pressure levels in response to higher sodium intake, and thus the association between T174M polymorphism and blood pressure is more evident among individuals with higher sodium intake than those with lower sodium intake. There were no differences in systolic or diastolic blood pressure levels (SBP or DBP) between the TT and TM＋MM genotype groups overall. However, the mean difference in DBP between the TM＋MM and TT groups was ＋1.0 mmHg in subjects of younger age (p＝0.06), ＋1.7 mmHg in non-overweight subjects (BMI＜23.5 kg/m^2, p＝0.01), and ＋2.3 mmHg in younger and non-overweight subjects (p＝0.002). Furthermore, among younger and non-overweight subjects, blood pressure differences were larger for those with higher urinary sodium excretion (＋3.1 mmHg, p＝0.03), those with a higher sodium/potassium excretion ratio (＋4.1 mmHg, p＝0.007), those with higher present sodium intake score (＋3.0 mmHg, p＝0.003), and those with higher past sodium intake score (＋3.4 mmHg, p＜0.001). In conclusion, *AGT* T174M polymorphism was associated with higher DBP levels in younger and non-overweight Japanese. This association was more evident among subjects with higher sodium intake.
(*Hypertens Res* 2004; 27: 53−60)

***Key Words*: angiotensinogen, epidemiology, salt sensitivity, gene-environment interaction, sodium excretion**

Introduction

The associations between T174M polymorphism (threonine-to-methionine substitution) of the angiotensinogen (*AGT*) gene and high blood pressure have been extensively studied, but the findings have been inconsistent. A positive association was initially found for Caucasians in both Utah and Paris (*1*) and subsequently in other Caucasian populations (*2*) and Japanese (*3*), but not for other Caucasians (*4–6*),

From the Department of Public Health Medicine, Institute of Community Medicine, University of Tsukuba, Tsukuba, Japan, and *Osaka Medical Center for Health Science and Promotion, Osaka, Japan.

This study was supported by a Grant-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science (No. 11877069 in 1999−2000).

Address for Reprints: Hiroyasu Iso, M.D., Ph.D., Department of Public Health Medicine, Institute of Community Medicine, University of Tsukuba, 1−1−1 Tennodai, Tsukuba 305−8575, Japan. E-mail: fvgh5640@mb.infoweb.ne.jp

Received June 23, 2003; Accepted in revised form October 21, 2003.

Nigerians (*7*), Taiwanese (*8*), Arabs (*9*), or Chinese (*10*). A study of a Canadian genetic isolate showed a positive association between the *AGT* T174M genotype and hypertension for men, but not for women (*2*). A large community-based study of 9,100 Danish showed no association between T174M polymorphism and high blood pressure, but double homozygosity of 235T and 174T was associated with elevated blood pressure in women (*11*). The ECTIM study, which examined the association stratified by body mass index (BMI), showed that among subjects with BMI $<26.0\,kg/m^2$ there was a 2.4-fold higher prevalence of high blood pressure in those with the 174M allele than in those with the 174T allele, but this trend was not apparent in subjects with BMI $\geq 26.0\,kg/m^2$ (*12*). Previously, we conducted a community-based case-reference study of 229 lean and non-drinking Japanese hypertensives and 229 age-, sex- and community-matched normotensives and showed a two-fold higher prevalence of the 174M allele among hypertensives than normotensives (*13*).

As stated by Kato (*14*), large population studies and well-characterized confounding factors will be needed to examine the role of the specific gene polymorphism more thoroughly. We suspected that the lack of association between T174M polymorphism and hypertension in some of the previous studies was due in part to the small number of subjects and the potentially confounding factors of age, BMI, and sodium intake, all of which affect blood pressure levels (*15*). Since some previous studies showed that T174M polymorphism was associated with hypertension primarily among young and/or lean subjects (*12*, *13*), we hypothesized that individuals, in particular young and non-overweight individuals, with the 174M allele of *AGT* have elevated blood pressure levels in response to a higher sodium intake, and thus the association between T174M polymorphism and blood pressure is more evident among individuals with a higher sodium intake than those with a lower sodium intake. Therefore, we conducted a large population-based observational study of 2,823 Japanese men and women to examine the associations between *AGT* polymorphism and blood pressure levels, stratified by these confounding variables.

Methods

Subjects

The subjects were residents of the rural farming community of Kyowa, Ibaraki Prefecture, central Japan (population 17,145 by the 2000 census). In this community, a general stroke prevention program and annual cardiovascular risk surveys have been conducted since 1981 (*16*). We included in the present study individuals who participated in the 2001 survey and who were 30−74 years of age at that time ($n=2,972$). At the time of the survey, physician epidemiologists explained the protocol to all participants, and obtained written informed consent from 95% ($n=2,823$) of them. The data of these 2,823 subjects were used in the analysis. The study protocol was approved by the Medical Ethics Committee of the University of Tsukuba.

Population Surveys

Well-trained blood pressure observers measured arterial systolic blood pressure (SBP) and fifth-phase diastolic blood pressure (DBP) using standard mercury sphygmomanometers on the right arm of quietly seated participants after an at least 5-min rest. When the first SBP reading was ≥ 140 mmHg and/or the first DBP was ≥ 90 mmHg, the measurement was repeated, and the second reading was used in the analysis. We measured several potential confounders (*17*): BMI, alcohol intake, urinary sodium and potassium excretion, and sodium/potassium excretion ratio as estimated by 24-h urine collection, and past and present sodium intake scores as estimated by self-administered questionnaire. Height in stocking feet and weight in light clothing were measured and BMI was calculated as weight in kg divided by the square of the height in m. Interviews were conducted to determine usual weekly ethanol intake in *go*, a traditional Japanese unit of volume corresponding to 23 g ethanol, which was converted to g of ethanol per day.

All participants in the survey were asked to complete a self-administered questionnaire to estimate both present and past habitual sodium intake. Past sodium intake was defined as the intake before the recognition of hypertension for hypertensives, and approximately 10 years before the survey for normotensives. A sodium intake score was calculated by adding one point for each of 10 types of sodium intake: 1) prefer salty-food, 2) use salty seasoning, 3) eat two or more miso soup servings per day, 4) eat pickles two or more times per day, 5) eat salty pickles, 6) put soy sauce on pickles, 7) put soy sauce on meal, 8) eat salt-preserved food one or more times per week, 9) eat salty noodle soup, and 10) do not try to reduce salt intake. This scoring system was previously validated (*13*, *18*), and was tested again in the present study. Sex and age-adjusted mean 24-h sodium excretion values across quintiles of the present sodium intake score ($n=1,674$) were 168, 180, 183, 195, and 203 mmol/l (p for trend <0.0001). The reproducibility of the present and past sodium intake scores was also tested previously (*13*), and was tested again in the present study by repeating the questionnaire after an interval of 1−2 years in a sub-sample ($n=287$); the Spearman correlation coefficient was 0.73 for the present sodium intake score ($p<0.001$), and 0.62 for the past sodium intake score ($p<0.001$). In the present study, 99% of the subjects completed the sodium intake questionnaire.

To estimate salt intake more accurately, some participants collected one 24-h urine sample. The participants were asked to collect all urine over a period of 24 h using a 3- or 4-l plastic bottle. They were also asked to record the time of each urination, whether they used inappropriate urine collec-

tion methods, and the number of missed urine collections. For each sample, total urine volume was measured, and then the urine was transferred into 2-ml plastic vials and stored at −80 ºC for a month until analysis. Sodium and potassium concentrations were analyzed using an electrolyte analyzer (Inflameter 775, Hitachi, Tokyo, Japan). Urine samples of less than 500 ml or those with incomplete collections based on the records were excluded from the analyses. Urine sodium or potassium excretion was calculated as each concentration (mmol/l) multiplied by the total urine volume (l), and sodium/potassium ratio was calculated as the sodium excretion divided by potassium excretion in mmol. Data of potassium excretion and the sodium/potassium ratio were used in secondary analyses, because potassium excretion is strongly affected by sodium excretion. We included in the analyses a total of 1,681 subjects who completed the 24-h urine collection between 1982 and 2002.

DNA Genotyping

Genomic DNA was extracted from leukocytes in the buffy coat of EDTA anticoagulated blood. *AGT* T174M genotypes were determined by the allele-specific primer-polymerase chain reaction (PCR) method using Taq DNA polymerase (rTaq; Toyobo, Osaka, Japan) as described elsewhere (*19*). The designed allele-specific sense primers were FITC-CAGCTGCTGCTGTCCXCG for the 174T allele and TxR-CAGCTGCTGCTGTCCXTG for the 174M allele labeled at the 5′-end either with Texas red (TxR) or with fluorescein isothiocyanate (FITC), and a 5′-biotin-end-labeled antisense primer (biotin-GCCTGGGGCTGTGAACAC), where X represents an artificial mismatch base. To eliminate the possibility of a false positive result, we used an artificial mismatch base (*i.e.*, either C, G, or T) next to the allelic base, instead of a matched base (A) (*20*). The amplification protocol consisted of initial denaturation at 95 ºC for 5 min; 35 cycles of denaturation at 95 ºC for 30 s, annealing at 60−65 ºC for 30 s, and extension at 72 ºC for 30 s; and a final extension at 72 ºC for 2 min. Fluorescence was measured with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for FITC and of 584 and 612 nm, respectively, for TxR.

Statistical Analyses

The analysis of covariance and χ^2 test were used to compare sex-specific age-adjusted mean values and proportions of risk characteristics. The χ^2 test was used to examine whether the genotype distributions differed from that expected from Hardy-Weinberg equilibrium. The relation between genotype and blood pressure levels was examined by analysis of covariance, stratified by age (30 to 64 and 65 to 74) and median BMI (23.5 kg/m^2). Further analysis was performed stratified by the medians of urinary excretion of sodium, potassium, the sodium/potassium ratio, and present and past sodium intake scores. The interaction between genotype and age, BMI, and urinary variables/sodium intake scores in relation to hypertension were examined using cross-product terms. All statistical analyses were performed using SAS version 8.02 software (SAS Institute Inc., Cary, USA). All probability values for statistical tests were two-tailed, and values of $p<0.05$ were regarded as statistically significant.

Results

The frequency of *AGT* genotypes was 79.6% for the TT genotype, 19.1% for the TM genotype, and 1.3% for the MM genotype. The sex-specific frequencies were 80.3%, 18.5%, and 1.2% for men, and 79.2%, 19.5%, and 1.3% for women, respectively. The genotype distribution was in Hardy-Weinberg equilibrium for men ($p=0.85$), women ($p=0.86$), and total subjects ($p=0.74$). Because of the low prevalence of the MM genotype, we combined the TM group and MM group in the analyses. Table 1 summarizes the sex-specific clinical and laboratory characteristics. The mean age was 59 years for men and 57 years for women. The prevalence of hypertension was 43% for men and 35% for women, and the mean 24-h urine sodium excretions were 199 mmol and 177 mmol, respectively.

Table 2 provides sex-specific age-adjusted characteristics according to genotype. Mean age did not vary between the TT and TM+MM groups. For each sex and total samples, the mean SBP and DBP values were not different between the two groups. The prevalence of antihypertensive medication use and prevalence of hypertension also were not different between the two groups. The other factors—*i.e.*, BMI, alcohol intake, urine sodium excretion, urine potassium excretion, urine sodium/potassium ratio, past and present sodium intake score—did not vary between two groups.

The mean values of blood pressure levels adjusted for sex, age, antihypertensive medication use, BMI, and alcohol consumption are shown in Table 3 for each *AGT* genotype. For total samples, there was no significant difference in blood pressure levels between the TT group and TM+MM group. Among younger subjects aged 30−64, mean diastolic blood pressure tended to be higher in the TM+MM group than in the TT group (+1.0 mmHg, $p=0.06$), but not among the older subjects. The interaction of genotype with age was not statistically significant ($p=0.24$). When stratified by BMI, mean DBP was 1.7 mmHg higher in TM+MM group than in TT group in subjects with lower BMI (<23.5 kg/m^2, $p=0.01$), but not in those with higher BMI ≥ 23.5 kg/m^2), and the interaction between the two BMI groups was significant ($p=0.04$). These genetic associations among age or BMI subgroup were primarily observed for women. There was no association between the genotype and DBP levels when stratified by urinary sodium, potassium excretion, sodium/potassium excretion ratio, or salt intake scores (data not shown). There was also no association between the genotype

Table 1. Clinical and Laboratory Characteristics (Mean±SD and Proportion), Men and Women Aged 30–74 Years

	Men	Women	Total
Number	1,048	1,775	2,823
Age (years)	59.0±10.6	56.7±10.6	57.5±10.7
Systolic blood pressure (mmHg)	133.9±16.2	130.2±16.4	131.6±16.4
Diastolic blood pressure (mmHg)	80.7±10.2	76.8±10.1	78.3±10.3
Use of antihypertensive medication (%)	23.4	21.5	22.2
Hypertension (%)†	43.1	34.6	37.8
Body mass index (kg/m^2)	23.9±3.0	23.5±3.3	23.6±3.2
Alcohol intake (g/day)	21.1±25.2	1.6±6.6	8.8±18.7
24-h urine collection (*n*)	676	1,005	1,681
Urine sodium excretion (mmol)	199.0±74.4	177.0±65.3	185.9±69.9
Urine potassium excretion (mmol)	54.1±20.1	54.5±20.0	54.4±20.1
Urine sodium/potassium ratio	3.88±1.32	3.47±1.27	3.63±1.31
Present sodium questionnaire completed (*n*)	1,038	1,764	2,802
Present sodium intake score	5.7±1.9	4.6±1.9	5.0±1.9
Past sodium questionnaire completed (*n*)	1,031	1,761	2,792
Past sodium intake score	6.8±1.8	5.9±2.0	6.3±2.0

† Hypertension was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg and/or use of antihypertensive medication.

Table 2. Age-Adjusted Means and Proportions of Characteristics According to Angiotensinogen T174M Genotype in Men and Women Aged 30–74 Years

	Men		Women		Total	
	TT	TM+MM	TT	TM+MM	TT	TM+MM
Number	841	207	1,406	369	2,247	576
Age (years)	59.1	58.4	56.8	56.3	57.7	57.1
Systolic blood pressure (mmHg)	133.9	133.9	130.1	130.5	131.5	131.8
Diastolic blood pressure (mmHg)	80.7	80.9	76.6	77.5	78.1	78.8
Use of antihypertensive medication (%)	23.0	24.9	21.3	22.5	21.9	23.4
Hypertension (%)†	42.1	47.2	34.2	36.2	37.2	40.2
Body mass index (kg/m^2)	23.9	23.8	23.4	23.6	23.6	23.7
Alcohol intake (g/day)	21.2	20.5	1.7	1.3	9.0	8.2
24-h urine collection (*n*)	540	136	793	212	1,333	348
Urine sodium excretion (mmol)	198.0	203.0	175.7	182.1	184.7	190.2
Urine potassium excretion (mmol)	53.7	55.6	54.2	55.9	54.0	55.8
Urine sodium/potassium ratio	3.88	3.88	3.46	3.47	3.63	3.63
Present sodium questionnaire completed (*n*)	832	206	1,401	363	2,233	569
Present sodium intake score	5.7	5.7	4.6	4.7	5.0	5.0
Past sodium questionnaire completed (*n*)	827	204	1,400	361	2,227	565
Past sodium intake score	6.9	6.6	5.9	5.9	6.3	6.2

† Hypertension was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg and/or use of antihypertensive medication. No differences were observed among genotypes for all variables.

and SBP levels for total samples or any stratified subgroups.

Among the younger and non-overweight subjects, the association between genotype and DBP levels was more evident and reached statistical significance for both sexes and total subjects: the mean difference between the two genotype groups was 3.3 mmHg for men ($p=0.01$), 1.8 mmHg for

Table 3. Multivariate-Adjusted Blood Pressure Levels According to Angiotensinogen T174M Genotype, Stratified by Age and Body Mass Index (BMI), in Men and Women Ages 30−74 Years

	Men			Women			Total		
	TT	TM+MM	*p* for difference	TT	TM+MM	*p* for difference	TT	TM+MM	*p* for difference
Total	841	207		1,406	369		2,247	576	
SBP (mmHg)	133.9	133.9	0.95	130.2	130.3	0.86	131.5	131.7	0.85
DBP (mmHg)	80.7	80.9	0.71	76.6	77.4	0.17	78.1	78.7	0.20
Stratified by age									
30−64-years old	513	135		997	264		1,510	399	
SBP (mmHg)	131.6	131.1	0.75	127.1	127.8	0.45	128.6	128.9	0.67
DBP (mmHg)	81.1	81.7	0.49	76.6	77.9	0.06	78.1	79.1	0.06
65−74-years old	328	72		409	105		737	177	
SBP (mmHg)	137.6	138.6	0.66	137.6	136.8	0.65	137.6	137.6	0.99
DBP (mmHg)	80.0	79.7	0.84	76.5	77.0	0.66	78.0	78.2	0.85
Stratified by BMI									
Below median ($<$23.5 kg/m^2)	372	101		758	178		1,130	279	
SBP (mmHg)	131.0	130.5	0.78	126.1	126.8	0.59	127.7	128.0	0.77
DBP (mmHg)	77.8	79.2	0.18	74.0	75.9	0.02	75.3	77.0	0.01
Median or more ($\geq$23.5 kg/m^2)	469	106		648	191		1,117	297	
SBP (mmHg)	136.2	137.2	0.52	134.7	134.4	0.79	135.3	135.4	0.89
DBP (mmHg)	83.0	82.5	0.66	79.6	79.3	0.75	81.0	80.6	0.56
Restricted to ages 30−64 and BMI $<$23.5 kg/m^2									
	217	62		569	146		786	208	
SBP (mmHg)	127.2	127.4	0.91	123.2	124.9	0.21	124.3	125.6	0.24
DBP (mmHg)	77.3	80.6	0.01	73.8	75.6	0.047	74.8	77.1	0.002

Blood pressures were adjusted for sex, age, antihypertensive medication use, BMI, and alcohol consumption. SBP, systolic blood pressure; DBP, diastolic blood pressure.

women (p=0.047), and 2.3 mmHg for total subjects (p=0.002). Therefore, we used this population for the further stratification analyses according to sodium excretion/intake (Table 4). Mean DBP was 3.1 mmHg higher in the TM+MM group than in the TT group in subjects with higher urinary sodium excretion ($\geq$172.8 mmol/day, p=0.03), but was not significantly different among genotypes in those with lower excretion ($<$172.8 mmol/day). When stratified by urinary potassium excretion, mean DBP was 2.1 mmHg higher in the TM+MM group than in the TT group in subjects with lower urinary potassium excretion ($<$51.3 mmol/day), although this association did not reach the level of statistical significance (p=0.15). Mean DBP was 4.1 mmHg higher in the TM+MM group than in the TT group in subjects with higher sodium/potassium excretion ratios ($\geq$3.46, p=0.007), but there was no significant difference among genotype groups in those with lower ratios ($<$3.46). Mean DBP was higher in the TM+MM group than in the TT group in subjects with higher sodium intake score for both the present (3.0 mmHg, p=0.003) and past (3.4 mmHg, p=0.0004), but this trend was less evident in subjects with lower sodium intake scores. The interaction between the genotype-blood pressure relation and each of urinary sodium excretion, urinary potassium excretion and present sodium intake score did not reach the level of statistical significance, but the interactions with the sodium/potassium excretion ratio and past sodium intake score were of borderline significance (p=0.049 and p=0.051, respectively).

These results did not alter materially when excluding subjects on antihypertensive medication (n=627); the mean DBP levels in the TM+MM groups *vs.* TT groups were 78.1 *vs.* 77.4 mmHg (p=0.14) for total samples, 77.9 *vs.* 77.3 mmHg (p=0.25) for younger subjects, 76.1 *vs.* 74.7 mmHg (p=0.05) for non-overweight subjects, and 76.0 *vs.* 74.3 mmHg (p=0.02) for younger and non-overweight subjects. Among younger and non-overweight subjects, the respective mean DBP levels were 77.6 *vs.* 74.8 mmHg (p=0.05) for those with higher sodium excretion, 78.0 *vs.* 74.7 mmHg (p=0.02) for those with higher sodium/potassium ratio, 76.5 *vs.* 74.1 mmHg (p=0.02) for those with higher present sodium intake score, and 76.9 *vs.* 74.0 mmHg (p=0.003) for those with higher past sodium intake score (not shown in the tables).

Discussion

The major finding of the present study was that there was a significant association between *AGT* T174M polymorphism

Table 4. Multivariate-Adjusted Blood Pressure Levels According to Angiotensinogen T174M Genotype, Stratified by Urinary Excretion of Sodium, Potassium, the Sodium/Potassium Ratio, and Present and Past Sodium Intake Scores, in Men and Women Aged 30−64 Years with Body Mass Index (BMI)＜23.5 kg/m²

	TT	TM＋MM	*p* for difference
Stratified by urinary sodium excretion			
＜172.8 mmol/day	203	55	
SBP (mmHg)	127.4	126.0	0.56
DBP (mmHg)	76.3	76.8	0.76
≥172.8 mmol/day	211	52	
SBP (mmHg)	124.8	128.0	0.16
DBP (mmHg)	75.5	78.6	0.03
Stratified by urinary potassium excretion			
＜51.3 mmol/day	202	58	
SBP (mmHg)	125.6	129.0	0.12
DBP (mmHg)	76.1	78.2	0.15
≥51.3 mmol/day	212	49	
SBP (mmHg)	126.4	124.9	0.52
DBP (mmHg)	75.6	77.7	0.17
Stratified by sodium/potassium excretion ratio			
＜3.46	209	51	
SBP (mmHg)	127.0	127.2	0.92
DBP (mmHg)	76.3	76.2	0.95
≥3.46	205	56	
SBP (mmHg)	125.0	127.5	0.25
DBP (mmHg)	75.4	79.5	0.007
Stratified by present sodium intake score			
＜5	336	93	
SBP (mmHg)	125.3	125.9	0.70
DBP (mmHg)	75.3	76.8	0.20
≥5	447	113	
SBP (mmHg)	123.6	125.4	0.21
DBP (mmHg)	74.4	77.4	0.003
Stratified by past sodium intake score			
＜6	299	81	
SBP (mmHg)	124.1	124.2	0.94
DBP (mmHg)	75.0	75.5	0.69
≥6	482	123	
SBP (mmHg)	124.5	126.3	0.19
DBP (mmHg)	74.7	78.1	＜0.001

Blood pressures were adjusted for sex, age, antihypertensive medication use, BMI, and alcohol consumption. SBP, systolic blood pressure; DBP, diastolic blood pressure.

and DBP levels among younger non-overweight individuals with high sodium intake, but not among individuals who were older, who were overweight, or who had low sodium intake. Our finding was consistent with a previous smaller study (*13*) which showed a significant association between the *AGT* T174M polymorphism and the prevalence of hypertension among lean and non-drinking Japanese with high sodium intake. The present large study extended the evidence of the association between the 174M allele and high blood pressure levels.

Furthermore, the relationship between the 174M allele and blood pressure was more obvious among subjects aged ＜65 years than in older-age subjects. This may have been because younger individuals are more likely to be affected by genetic factors rather than by environmental factors. Similarly, this relationship became evident among non-overweight subjects. Among subjects with BMI ＜26 kg/m², the ECTIM study showed a 2.4-fold higher prevalence of high blood pressure in those with the 174M allele than in those with the 174T allele, but this trend was not apparent in subjects with BMI ≥26 kg/m² (*12*). These results suggest that high BMI masks the relationship between the 174M allele and blood pressure levels.

As anticipated, we observed clear associations between *AGT* genotype and blood pressure levels among younger non-overweight persons with higher sodium excretion and higher past or present sodium intake scores. The positive association among persons with higher sodium/potassium excretion ratio, which we determined in secondary analyses, also supported our *a priori* hypothesis. These findings suggest that it is necessary to consider age, BMI, and salt intake when examining the association between *AGT* polymorphism and blood pressure levels. The lack of association between the T174M polymorphism and blood pressure levels or hypertension in many previous studies (*4*–*11*, *21*, *22*) may be related to a lack of adequate control for the influence of these confounding factors.

The mechanism of T174M polymorphism on the elevated blood pressure among individuals with high sodium intake is uncertain. It is unlikely that a blood pressure increase is mediated by increased plasma AGT concentration, because previous studies have shown no association between T174M polymorphism and plasma AGT concentrations (*1*, *5*, *7*, *11*, *23*). However, this does not negate a potential effect of T174M polymorphism on local production of AGT, which may affect blood pressure levels. It is also possible that other functional polymorphisms, in linkage disequilibrium with the T174M polymorphism, may contribute to the development of salt-sensitive hypertension.

In our previous case-control study (*13*), we did not find any association between M235T polymorphism and hypertension even among persons with higher sodium intake, although some previous studies have suggested an association between M235T and salt-sensitive hypertension (*24*, *25*). Thus we did not analyze M235T polymorphism in the present study. Since the prevalence of the 235T allele has been shown to be high (approximately 70−80%) in Japanese (*13*), we considered that the 174M allele, which was 5−15% prevalent in Japanese, may be more effective than 235T for detecting salt-sensitivity among non-Caucasian populations.

The strength of the present study was that we used a large

community-based sample. Furthermore, we obtained 24-h urine collection samples and sodium intake scores, which allowed us to test gene-environmental interactions of the *AGT* T174M genotype with blood pressure levels.

There were several limitations in the present study. First, because we used only single blood pressure measurement in the analyses, measurement variability may have weakened the genetic associations. However, since there were a large number of subjects, we attained enough statistical power to detect gene-blood pressure associations. Second, approximately 20% of subjects used antihypertensive medication, which may have obscured the genetic effect of blood pressure levels. However, since the prevalence of antihypertensive medication use did not differ among the genotypes, the possibility of such an influence is small. The exclusion of subjects on antihypertensive medication did not alter the genetic associations materially.

The public health implications of the present results warrant discussion. Differences in the mean DBP values between subjects with and without the 174M allele were 3−4 mmHg in younger non-overweight subjects whose salt intake was high. These differences would seem to be small from the viewpoint of clinical practice. However, a small difference in the mean blood pressure levels over an entire population translates to substantial effects on the incidence and mortality of cardiovascular disease. A meta-analysis of nine prospective observational studies demonstrated that a long-term difference of 5 mmHg in mean DBP was associated with reductions of 34% and 21% in stroke and coronary heart disease risk, respectively (*26*). In our previous study of Japanese, a difference of 5 mmHg in mean SBP was associated with a 15% reduction in stroke incidence and a 10% reduction in coronary heart disease incidence (*27*). Therefore, salt reduction among members of salt-sensitive populations may have a substantial impact on the prevention of cardiovascular disease.

In conclusion, the *AGT* 174M allele was associated with higher DBP levels in younger and non-overweight Japanese men and women. These associations were more evident among persons with higher sodium intake. The present study also showed the necessity of considering age, BMI, and salt intake when estimating the genetic associations of *AGT* polymorphism with high blood pressure.

Acknowledgements

The authors gratefully acknowledge Professor Aaron R. Folsom of the University of Minnesota, USA for his valuable comments, and Toshimi Kohigashi, Toshiko Suzuki, Mizue Fujii, and Ai Ikeda for their technical assistance.

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