Original Article

Aldosterone Synthase Gene T−344C Polymorphism, Sodium and Blood Pressure in a Free-Living Population: A Community-Based Study

Kazumasa YAMAGISHI[1], Takeshi TANIGAWA[1], Renzhe CUI[1),2)], Minako TABATA[1], Ai IKEDA[3),4)], Masayuki YAO[1),5)], Takashi SHIMAMOTO[5)], and Hiroyasu ISO[3)]

There have been few epidemiological studies on the gene-environmental interaction between the aldosterone synthase gene (*CYP11B2*) T−344C polymorphism and sodium in relation to blood pressure in a free-living general population. We hypothesized *a priori* that persons with the T allele of *CYP11B2* would have elevated blood pressure levels in response to a higher sodium intake, and thus the association between the T−344C polymorphism and blood pressure would be more evident among persons with a high sodium intake than among those with a low sodium intake. Study subjects were 2,823 men and women aged 30–74 in a Japanese community. We examined the associations between the T−344C polymorphism and blood pressure levels, stratified by sodium variables estimated by 24-h urinary sodium excretion and a dietary questionnaire. There was no significant difference in blood pressure levels among the CC, TC and TT groups for either or both sexes. However, among persons with higher sodium excretion, mean systolic blood pressure levels tended to be higher in those with the TC (+3.0 mmHg, $p=0.06$) and TT (+2.9 mmHg, $p=0.07$) genotypes than in those with the CC genotype, but this tendency was not seen among those with lower sodium excretion (−4.0 mmHg, $p=0.03$ for TC *vs.* CC; −3.0 mmHg, $p=0.11$ for TT *vs.* CC; p for interaction =0.006). In conclusion, we found no association between *CYP11B2* and blood pressure for total subjects or for persons with a higher sodium intake. However, a possible gene–blood pressure association among persons with higher sodium intake needs to be explored further. (*Hypertens Res* 2007; 30: 497–502)

***Key Words*: *CYP11B2*, gene-environment interaction, salt-sensitivity, epidemiology**

Introduction

Aldosterone is synthesized by CYP11B2, a mitochondrial cytochrome P450 enzyme. Recent biological studies suggest that increase in aldosterone and thereby in epithelial Na^+ channel (ENaC) activity may play an important role in the etiology of hypertension (*1*). T−344C polymorphism (thymidine-to-cytosine substitution at position −344 in the regulatory region) of the *CYP11B2* gene was extensively

From the [1)]Department of Public Health Medicine, Graduate School of Comprehensive Human Sciences, and Institute of Community Medicine, University of Tsukuba, Tsukuba, Japan; [2)]Department of Epidemiology and Community Medicine, Medical College of Nankai University, Tianjin, P.R. China; [3)]Public Health, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Suita, Japan; [4)]Department of Society, Human Development and Health, Harvard School of Public Health, Boston, USA; and [5)]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, Japan (No. 11877069 in 1999–2000 and 19659160 in 2007–2009) and a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (No. 17790382 in 2005–2007).

Address for Reprints: Kazumasa Yamagishi, M.D., Ph.D., Department of Public Health Medicine, Graduate School of Comprehensive Human Sciences, and Institute of Community Medicine, University of Tsukuba, 1–1–1 Tennodai, Tsukuba 305–8575, Japan. E-mail: k-yamagishi@umin.net

Received October 6, 2006; Accepted in revised form January 12, 2007.

examined as a candidate gene of hypertension because high levels of aldosterone increase subsequent risk of hypertension (*2*). Some studies (*3–6*), but not all (*7–12*), reported that the T allele was associated with blood pressure/hypertension, especially for low-renin hypertension (*4*, *5*, *13*), which is considered to correspond to salt-sensitive hypertension (*14*). However, no community-based study has been conducted to examine the genetic association between *CYP11B2* and salt-sensitive hypertension, probably due to the difficulty in identifying this type of hypertension in community-based samples.

Therefore, we conducted a large community-based observational study of 2,823 Japanese men and women to examine associations of *CYP11B2* polymorphism with blood pressure levels, stratified by sodium variables. Our *a priori* hypothesis was that persons with the T allele of *CYP11B2* would have elevated blood pressure in response to high sodium intake, and thus the association between T−344C polymorphism and blood pressure levels would be more evident among persons with high sodium intake.

Methods

Study Population

Subjects were free-living residents of a farming community of Kyowa (census population in 2000: $n=17,145$), which was similar to that examined in our previous studies (*15–17*). Briefly, we recruited subjects aged 30–74 years who had participated in the 2001 cardiovascular risk survey ($n=2,972$). Physicians explained the protocol to all participants, and obtained written informed consent from 95% ($n=2,823$) of them. The data of these 2,823 persons were used in the present study. The study protocol was approved by the Medical Ethics Committee of the University of Tsukuba.

Population Surveys

The population surveys have been performed annually since 1981. Details of the surveys have been described elsewhere (*15–17*). Well-trained blood pressure observers measured arterial systolic blood pressure (SBP) and fifth-phase diastolic blood pressure (DBP) using standard mercury sphygmomanometers (with a cuff 14 cm wide and 51 cm long) on the right arm of quietly seated participants after a period of at least 5-min rest. When the SBP was ≥140 mmHg and/or the DBP was ≥90 mmHg, the measurement was repeated and the average of the two readings was used in the analyses; otherwise the single reading was used. The methods of blood pressure measurement were standardized uniformly across the surveys, and blood pressure measurement training for blood pressure observers was provided before each annual survey. Hypertension was defined as an SBP of ≥140 mmHg and/or DBP of ≥90 mmHg, and/or use of antihypertensive medication. Some participants collected all urine over 24 h between the 1982 and 2005 surveys (*15*). Subjects who provided urine samples of <500 mL and/or for <20 h, or those with incomplete collections based on records were excluded from the analyses. Consequently, 1,920 subjects completed the 24-h urine collection. In addition, all participants were asked to complete a self-administered dietary questionnaire to estimate present and past sodium intake at the 2001 survey, and 99% of the subjects successfully completed the questionnaire; the method was described in detail and validated elsewhere (*17*). We used age, body mass index and alcohol intake as confounding variables, and sodium excretion, sodium intake as effect modifiers.

DNA Genotyping

CYP11B2 T−344C genotypes were determined by the allele-specific primer–polymerase chain reaction (PCR) method using Taq DNA polymerase (rTaq; Toyobo, Osaka, Japan), as described elsewhere (*18*, *19*). The designed allele-specific sense primers were TxR-GTCTATTAAAAGAATCCAAGG XTC for the T allele and FITC-TATTAAAAGAATCCA AGGXCC for the C allele labeled at the 5′ end either with fluorescein isothiocyanate (FITC) or with Texas red (TxR), and a 5′ biotin-end–labeled antisense primer (biotin-GGACTT TATCTTATCGTGAGATGA), in which X represents an artificial mismatch base (*18*).

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, by Tukey's multiple comparison method. The χ^2 test was used to examine whether the genotype distributions differed from those expected from Hardy-Weinberg equilibrium. The relation between genotype and blood pressure levels was adjusted by age, sex, antihypertensive medication use (yes or no), body mass index and alcohol consumption, and examined by the analysis of covariance, using Dunnett's multiple comparison method with the CC group as a reference. Further analysis was performed stratified by the medians of urinary sodium excretion and sodium intake scores to examine whether the associations between genotype and blood pressure levels were modified by sodium intake. We used dummy variables for adjustment of sex and antihypertensive medication use, and continuous variables for other covariates. The interactions between genotype and sex, stratified sodium variables, *i.e.*, polymorphism (TT, TC, CC) × sex (men or women) or polymorphism × sodium intake/excretion (below or beyond the median values) were tested by analysis of covariance. 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; values of $p<0.05$ were regarded as statistically significant, and values of $0.05\leq p<0.10$ as borderline significant.

Table 1. Means and Proportions of Characteristics According to *CYP11B2* T−344C Genotype, Men and Women Aged 30–74 Years

	Men			Women		
	CC	TC	TT	CC	TC	TT
Number	118	465	465	211	769	795
Age (years)	59.2	58.6	59.3	57.2	56.5	56.7
Systolic blood pressure (mmHg)	134.7	134.2	134.9	130.2	130.8	131.2
Diastolic blood pressure (mmHg)	81.3	80.6	81.0	77.2	77.0	76.8
Use of antihypertensive medication (%)	23	24	23	16	22	23*
Hypertension (%)†	45	47	49	33	40	38
Body mass index (kg/m^2)	23.9	23.9	23.9	23.6	23.5	23.5
Alcohol intake (g/day)	18.8	21.2	21.6	1.7	1.7	1.5
24-h urine collection (*n*)	79	324	317	147	522	531
Urine sodium excretion (mmol/day)	226	202*	202*	184	181	175
Present sodium intake score completed (*n*)	117	461	460	208	765	791
Present sodium intake score	5.7	5.7	5.7	4.8	4.6	4.6
Past sodium intake score completed (*n*)	115	460	456	208	765	788
Past sodium intake score	6.8	6.8	6.9	5.8	5.9	5.9

†Hypertension was defined as systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg and/or use of antihypertensive medication. *$p<0.05$ for difference from CC genotype by Tukey's multiple comparison methods.

Table 2. Blood Pressure Levels According to *CYP11B2* T−344C Genotype, Men and Women Aged 30–74 Years

	Men				Women			
	CC	TC	TT	*p*-value	CC	TC	TT	*p*-value
Total (*n*)†	118	465	465		211	769	795	
Systolic blood pressure (mmHg)	135.0	134.2	134.9	1.00	130.5	130.8	131.1	0.77
Diastolic blood pressure (mmHg)	81.4	80.6	81.0	0.81	77.2	77.0	76.8	0.74
Stratified by urinary sodium excretion‡								
Below median (*n*)	34	162	164		68	256	276	
Systolic blood pressure (mmHg)	139.9	134.6	136.7	0.39	135.1	132.3	132.5	0.32
Diastolic blood pressure (mmHg)	82.1	80.4	81.5	0.91	78.2	77.7	76.9	0.45
Beyond median (*n*)	45	162	153		79	266	255	
Systolic blood pressure (mmHg)	131.5	134.6	135.0	0.23	127.4	130.4	130.1	0.21
Diastolic blood pressure (mmHg)	80.4	80.6	80.2	0.98	77.6	77.3	77.3	0.95
Stratified by present sodium intake score†								
Below median (*n*)	46	205	198		94	364	377	
Systolic blood pressure (mmHg)	137.1	136.2	137.8	0.92	131.1	132.5	132.1	0.74
Diastolic blood pressure (mmHg)	82.5	81.1	82.9	0.94	76.8	77.6	77.5	0.69
Beyond median (*n*)	71	256	262		114	401	414	
Systolic blood pressure (mmHg)	133.1	132.4	132.7	0.97	129.4	129.4	130.3	0.73
Diastolic blood pressure (mmHg)	80.5	80.3	79.6	0.66	77.4	76.5	76.3	0.34
Stratified by past sodium intake score†								
Below median (*n*)	51	194	177		96	312	313	
Systolic blood pressure (mmHg)	137.7	134.0	136.4	0.76	127.7	130.9	130.5	0.16
Diastolic blood pressure (mmHg)	82.9	80.8	82.9	1.00	75.5	77.3	76.4	0.57
Beyond median (*n*)	64	266	279		112	453	475	
Systolic blood pressure (mmHg)	132.6	134.0	134.0	0.63	132.3	130.7	131.7	0.87
Diastolic blood pressure (mmHg)	80.2	80.5	79.7	0.87	78.5	76.8	77.2	0.25

Blood pressure was †adjusted for age, antihypertensive medication use, body mass index, alcohol consumption, and ‡further adjusted for survey year of 24-h urine collection. Median values are 171.2 mmol/day for urinary sodium excretion in women and 195.7 mmol/day in men, 5 for present sodium intake score in women and 6 in men, and 6 for past sodium intake score in women and 7 in men. *p*-values represents differences between TT and CC genotypes examined by Dunnett's multiple comparison methods.

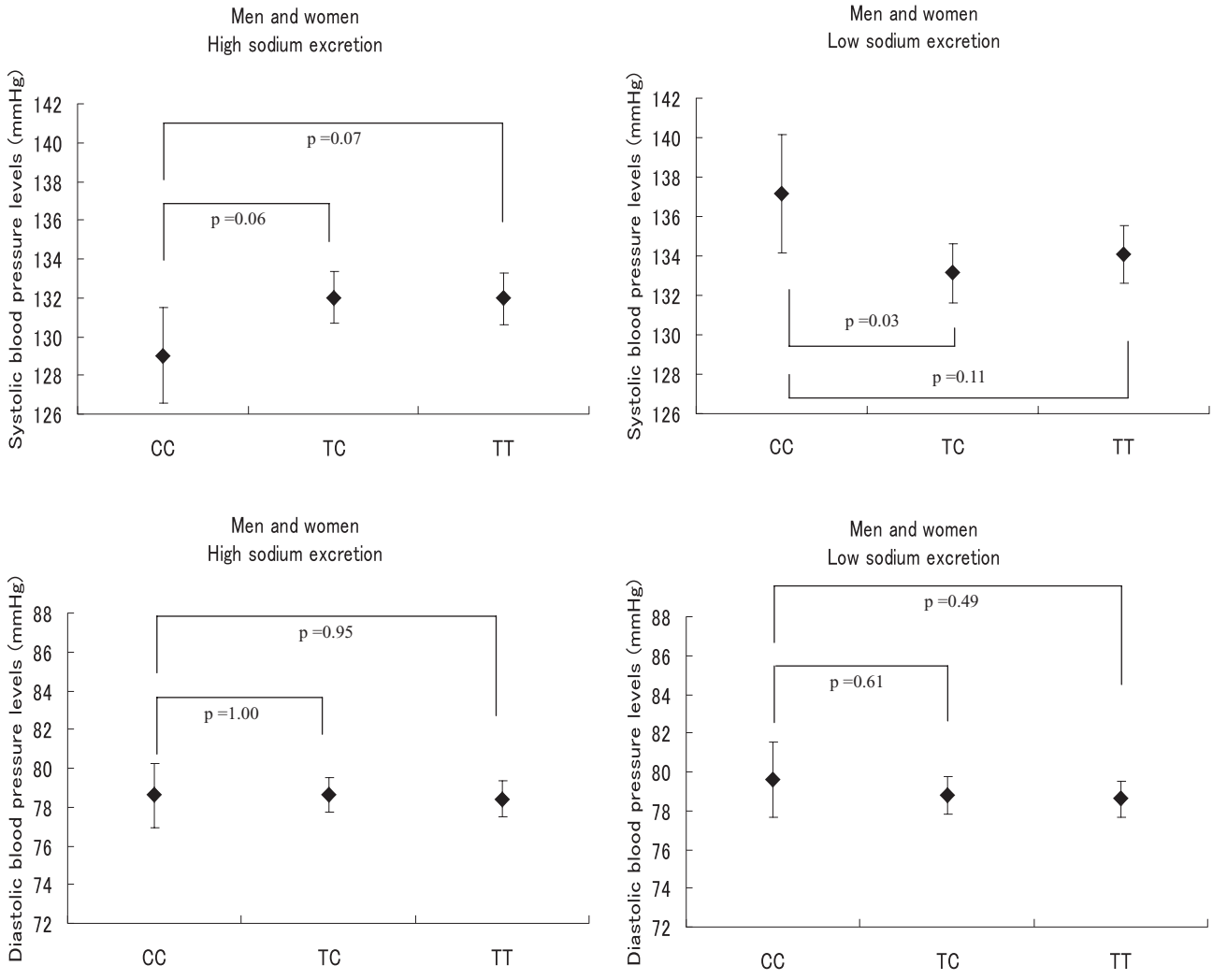

Fig. 1. *Mean systolic and diastolic blood pressure levels and 95% confidence intervals according to CYP11B2 T−344C genotypes by sodium excretion. The median values of urinary sodium excretion were 195.7 mmol/day for men and 171.2 mmol/day for women.*

Results

The sex-specific frequencies of *CYP11B2* genotypes were 11.3%, 44.4%, and 44.4% for men, and 11.9%, 43.3%, and 44.8% for women, respectively. The genotype distribution was in Hardy-Weinberg equilibrium for men ($p=1.00$) and women ($p=0.70$). The mean age of the subjects was 59 years for men and 57 years for women. The prevalence of hypertension was 48% for men and 38% for women, and the respective mean values of 24-h urine sodium excretions were 205 mmol and 179 mmol (data not shown in the tables).

For each sex, mean age did not vary among the CC, TC and TT genotypes (Table 1). Neither mean SBP nor mean DBP was significantly different among the three genotypes. However, the use of antihypertensive medication was more prevalent among women with the TT genotype than those with the CC genotype. Urinary sodium excretion was lower among men with the TC and TT genotypes than those with the CC genotype. The other variables were similar among the genotypes.

The mean values of blood pressure levels adjusted for age, antihypertensive medication use, body mass index and alcohol consumption are shown for each *CYP11B2* genotype (Table 2). Overall, there was no significant difference in blood pressure levels. Also, we found no association between T−344C polymorphism and blood pressure levels when stratified by urinary sodium excretion, present sodium intake, and past sodium intake score (Table 2). However, when men and women were combined (Fig. 1), the SBP levels among persons with the higher urinary sodium excretion tended to be positively associated with the T allele: 129.0 mmHg for the

CC genotype, 132.0 mmHg for the TC genotype ($p=0.06$ *vs*. the CC genotype), and 131.9 mmHg for the TT genotype ($p=0.07$), but not among those with lower sodium excretion: 137.1 mmHg for the CC genotype, 133.1 mmHg for the TC genotype ($p=0.03$) and 134.1 mmHg for the TT genotype ($p=0.11$). The interaction between urinary sodium excretion and genotype in relation to SBP was statistically significant ($p=0.006$).

The associations between *CYP11B2* genotypes and blood pressure levels were not altered materially when subjects were restricted to those without antihypertensive medication ($n=1,112$). For example, the SBP levels in persons with higher sodium excretion were 126.0 mmHg, 129.3 mmHg ($p=0.07$) and 129.2 mmHg ($p=0.08$), respectively, and those in persons with lower sodium excretion were 134.5 mmHg, 130.3 mmHg ($p=0.05$) and 131.1 mmHg ($p=0.13$), respectively.

Discussion

We found no significant difference in blood pressure levels between the CC, TC and TT genotypes for either sex in the total subjects. The lack of association is not surprising, because this finding was consistent with the results of previous studies (*7–12*). However, a positive association was suggested between the *CYP11B2* TT genotype and SBP levels among persons with higher sodium excretion, but not for those with lower sodium excretion.

The results from previous studies examining the association between the T−344C polymorphism and blood pressure/hypertension have been inconsistent. A Japanese population-based study (*10*) showed no association between the T−344C polymorphism and 24-h ambulatory SBP or DBP, but subjects with the CC genotype showed a greater reduction in nocturnal SBP levels than those with the TC or TT genotypes. However, several population-based studies from Japanese (*9*), Indian (*11*), and black and white English (*7*) showed no association between the T−344C polymorphism and blood pressure/hypertension, whereas other population-based studies from Italian (*5*, *12*) and multi-ethnic populations (*6*) showed a positive association with SBP or both SBP and DBP.

Our *a priori* hypothesis was based on the previous reports that showed a positive association between the T−344C polymorphism and low-renin hypertension (*4*, *5*), which is considered to correspond to salt-sensitive hypertension (*13*). Since Japanese populations have a higher sodium intake than Western countries (*20*), we assumed that the high sodium intake may have helped to make the *CYP11B2* gene–blood pressure association more evident. The present study supported our hypothesis. Previous studies (*21–23*), however, showed no association between the T−344C polymorphism and salt sensitivity among relatively young subjects (mean age of 25 to 47 years).

Although our *a priori* hypothesis was not clearly supported, the present study has several strengths. The present study was the first community-based observational study of a gene–sodium intake interaction on blood pressure with a large sample size, which allowed us to conduct stratified analyses. Second, mean sodium intake was higher in the present Japanese subjects than in Western countries, which may have constituted an advantage for examining the gene–sodium intake interaction.

The present study has several limitations. First, we used only a single blood pressure measurement in the analyses, and thus measurement variability may have weakened the genetic associations. However, since the number of the study subjects was large, 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, this possibility is unlikely, since the exclusion of persons on antihypertensive medication did not alter the genetic associations materially. Last, the variation of sodium excretion by one 24-h urine sample may be large, and misclassification between the low and high sodium excretion groups is inevitable. However, since the study had a large sample size, the impact of misclassification across the stratification was probably small. In the present study, 66% of persons remained in the same group of higher or lower sodium intake after 1–5 years re-collection of urinary sodium excretion for the sub-sample.

In conclusion, the *CYP11B2* T allele was not associated with high blood pressure levels for total subjects or for persons with a higher sodium intake. However, a possible gene–blood pressure association among persons with a higher sodium intake needs to be explored in a future study.

Acknowledgements

The authors gratefully acknowledge Ms. Toshimi Kohigashi, Dr. Toshiko Suzuki, Ms. Mizue Fujii, Ms. Yoshiko Okano and Ms. Akiko Ozaki for their technical assistance.

References

1. Pratt JH: Central role for ENaC in development of hypertension. *J Am Soc Nephrol* 2005; **16**: 3154–3159.
2. Vasan RS, Evans JC, Larson MG, *et al*: Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med* 2004; **351**: 33–41.
3. Brand E, Chatelain N, Mulatero P, *et al*: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998; **32**: 198–204.
4. Komiya I, Yamada T, Takara M, *et al*: $Lys^{173}Arg$ and −344T/C variants of *CYP11B2* in Japanese patients with low-renin hypertension. *Hypertension* 2000; **35**: 699–703.
5. Rossi E, Regolisti G, Perazzoli F, *et al*: −344C/T polymorphism of CYP11B2 gene in Italian patients with idiopathic low renin hypertension. *Am J Hypertens* 2001; **14**: 934–941.
6. Barbato A, Russo P, Siani A, *et al*: Aldosterone synthase gene (*CYP11B2*) C−344T polymorphism, plasma aldoste-

rone, renin activity and blood pressure in a multi-ethnic population. *J Hypertens* 2004; **22**: 1895–1901.
7. Zhu H, Sagnella GA, Dong Y, *et al*: Contrasting associations between aldosterone synthase gene polymorphisms and essential hypertension in blacks and in whites. *J Hypertens* 2003; **21**: 87–95.
8. Kato N, Sugiyama T, Morita H, *et al*: Comprehensive analysis of the renin-angiotensin gene polymorphisms with relation to hypertension in the Japanese. *J Hypertens* 2000; **18**: 1025–1032.
9. Tsujita Y, Iwai N, Katsuya T, *et al*: Lack of association between genetic polymorphism of *CYP11B2* and hypertension in Japanese: the Suita Study. *Hypertens Res* 2001; **24**: 105–109.
10. Matsubara M, Kikuya M, Ohkubo T, *et al*: Aldosterone synthase gene (*CYP11B2*) C−344T polymorphism, ambulatory blood pressure and nocturnal decline in blood pressure in the general Japanese population: the Ohasama study. *J Hypertens* 2001; **19**: 2179–2184.
11. Rajput C, Makhijani K, Norboo T, *et al*: *CYP11B2* gene polymorphisms and hypertension in highlanders accustomed to high salt intake. *J Hypertens* 2005; **23**: 79–86.
12. Casiglia E, Tikhonoff V, Mazza A, *et al*: C−344T polymorphism of the aldosterone synthase gene and blood pressure in the elderly: a population-based study. *J Hypertens* 2005; **23**: 1991–1996.
13. Katsuya T, Ishikawa K, Sugimoto K, Rakugi H, Ogihara T: Salt sensitivity of Japanese from the viewpoint of gene polymorphism. *Hypertens Res* 2003; **26**: 521–525.
14. Fisher NDL, Hurwitz S, Ferri C, Jeunemaitre X, Hollenberg NK, Williams GH: Altered adrenal sensitivity to angiotensin II in low-renin essential hypertension. *Hypertension* 1999; **34**: 388–394.
15. Yamagishi K, Iso H, Tanigawa T, Cui R, Kudo M, Shimamoto T: High sodium intake strengthens the association between angiotensinogen T174M polymorphism and blood pressure levels among lean men and women: a community-based study. *Hypertens Res* 2004; **27**: 53–60.
16. Yamagishi K, Iso H, Tanigawa T, Cui R, Kudo M, Shimamoto T: Alpha-adducin G460W polymorphism, urinary sodium excretion, and blood pressure in community-based samples. *Am J Hypertens* 2004; **17**: 385–390.
17. Yamagishi K, Tanigawa T, Cui R, *et al*: High sodium intake strengthens the association of *ACE* I/D polymorphism with blood pressure in a community. *Am J Hypertens* 2007 (in press).
18. Takarada Y: Analysis for SNPs using allele specific primer. *Upload* 2002; **66**: 8–9 (in Japanese).
19. Ishiguro A, Kubota T, Soya Y, *et al*: High-throughput detection of multiple genetic polymorphisms influencing drug metabolism with mismatch primers in allele-specific polymerase chain reaction. *Anal Biochem* 2005; **337**: 256–261.
20. Intersalt Cooperative Research Group: Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* 1988; **297**: 319–328.
21. Poch E, González D, Giner V, *et al*: Molecular basis of salt sensitivity in human hypertension: evaluation of renin-angiotensin-aldosterone system gene polymorphisms. *Hypertension* 2001; **38**: 1204–1209.
22. Pamies-Andreu E, Ramirez-Lorca R, Stiefel Garcia-Junco P, *et al*: Renin-angiotensin-aldosterone system and G-protein beta-3 subunit gene polymorphisms in salt-sensitive essential hypertension. *J Hum Hypertens* 2003; **17**: 187–191.
23. Brand E, Schorr U, Ringel J, *et al*: Aldosterone synthase gene (*CYP11B2*) C−344T polymorphism in Caucasians from the Berlin Salt-Sensitivity Trial (BeSST). *J Hypertens* 1999; **17**: 1563–1567.