Title page Word count 220 abstract 2,582 text

27 references, 3 tables and no figure

High sodium intake strengthens the association of *ACE* I/D polymorphism with blood pressure in a community

Kazumasa Yamagishi, MD[1], Takeshi Tanigawa, MD[1], Renzhe Cui, MD[1,2], Minako Tabata[1], Ai Ikeda, PhD[3,4], Masayuki Yao, MD[5], Takashi Shimamoto, MD[5], and Hiroyasu Iso, MD[3]

[1]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

[2]Department of Epidemiology and Community Medicine, Medical College of Nankai University, Tianjin, China.

[2]Public Health, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Suita, Japan

[3]Department of Society, Human Development and Health, Harvard School of Public Health, Boston, MA

[4]Osaka Medical Center for Health Science and Promotion, Osaka, Japan

Support: 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 Grant-in-aid for Young Scientists (B) from the Ministry of Education, Science, Sports and Culture, Japan (No. 17790382 in 2005-2007).

To whom all communications should be sent to 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, Ibaraki-ken 305-8575, Japan

Tel: +81-29-853-2696; fax: +81-29-853-2695 Email: k-yamagishi@umin.net

Short title page

Short title: ACE Gene, Salt Intake and Blood Pressure (40 characters)

Background. Limited evidence is available on a gene-environmental interaction of angiotensin-converting enzyme (*ACE*) gene I/D polymorphism and high blood pressure with salt intake among general populations. We hypothesized that persons with the I allele of *ACE* gene have elevated blood pressure levels in response to a higher sodium intake, and thus the association of *ACE* I/D polymorphism with blood pressure levels was stronger among persons with a higher sodium intake than those with a lower sodium intake.

Methods. We conducted a population-based cross-sectional study of 2,823 men and women aged 30-74 years in a Japanese rural community to examine the association of the *ACE* I/D polymorphism with blood pressure levels stratified by salt intake, as estimated by 24-hour urine collection and dietary questionnaire. Polymorphism of the *ACE* I/D was detected by an allele-specific polymerase chain reaction.

Results. There was no significant difference in blood pressure levels among DD, ID and II groups for either sex or total samples. However, mean difference in diastolic blood pressure levels for II vs DD groups was +3.0mmHg (p=0.003) among persons with higher sodium excretion, +1.8mmHg (p=0.04) among those with higher present sodium intake score, and +1.7mmHg (p=0.06) among those with higher past sodium intake score.

Conclusion. A high sodium intake strengthens the association of *ACE* I/D polymorphism with blood pressure levels in community-based samples. (220 words)

Key words: *ACE,* genetics, molecular epidemiology, salt sensitivity, gene-environment interaction, hypertension, sodium excretion, renin-angiotensin system

INTRODUCTION

Angiotensin-converting enzyme (*ACE*) I/D polymorphism is a polymorphism of either presence or absence of 287 base-pair DNA fragment in intron 16 of *ACE* gene.[1] Genetic associations between polymorphism of the *ACE* I/D and high blood pressure (BP) have been examined extensively, but findings on the association have been inconsistent. Some studies[2-4], but not all[5-7], reported that the D allele was responsible for elevation of BP while other clinical studies showed that I allele, but not D allele, was associated with hypertension.[8,9] As for salt-sensitive hypertension, two clinical studies[10-12] found a significant association with I allele; one study[13] showed an association with D allele; and three studies[14-16] showed no association. However, no population-based study was conducted for the association between I/D polymorphism and salt-sensitive hypertension, probably due to difficulty in the identification of salt-sensitive hypertension in community-based samples.

The aim of this study is to clarify the effect of sodium intake on association between *ACE* I/D polymorphism and BP among free-living community population. We hypothesized *a priori* that persons with the I allele of *ACE* gene elevated BP levels in response to higher sodium intake, and thus the association between I/D polymorphism and BP was stronger among persons with a higher sodium intake than those with a lower sodium intake. To examine the potential gene-environmental interaction, we conducted a large community-based observational study of 2,823 Japanese men and women.

METHODS

Study Population

Study population we used was similar to that of our previous reports.[17,18] Briefly, we recruited the subjects from a farming rural community of Kyowa, Ibaraki prefecture, central Japan (census population in 2000: n=17,145) where annual cardiovascular risk surveys have been conducted since 1981. We included in the present study individuals who were aged 30-74 years and participated in the 2001 survey (n=2,972). 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 persons were used in the analysis. The study protocol was approved by the Medical Ethics Committee of the University of Tsukuba.

Well-trained BP observers measured arterial systolic blood pressure (SBP) and fifth-phase diastolic blood pressure (DBP) using standard mercury sphygmomanometers (with 14cm width and 51cm length cuff) on the right arm of quietly seated participants after at least five-minute rest. When the first SBP reading was ≥140 mmHg and/or DBP ≥90mmHg, the measurement was repeated; the average of first and second readings was used in the analyses, and otherwise the first reading was used. We measured several potential confounders: body mass index (BMI), alcohol intake, urinary sodium and potassium excretion estimated by 24-hour urine collection, and past and present sodium intake scores estimated by self-administered questionnaire.

All participants in the survey were asked to complete 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 twice 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[18-20], and again tested in the present study. Age and sex-adjusted mean 24-hour sodium excretion values across quintiles of the present sodium intake score (n = 1,913) were 168, 183, 193, 201 and 202 mmol/L (p for trend < 0.001). Repeatability of the present and past sodium intake scores was also tested previously[17,20] by repeating the questionnaire one to two years apart for 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 ($p<0.001$).[17] In the present study, 99% of the subjects completed sodium intake questionnaire.

To estimate salt intake more accurately, some participants collected one 24-hour urine sample, as previously reported.[17,18] Urine samplings with lower than 500 ml or those with incomplete collections based on the records were excluded from the analyses. We included in the analyses a total of 1,920 subjects who completed the 24-hour urine collection between 1982 and 2005.

DNA genotyping

ACE I/D genotypes were determined by allele-specific primer-polymerase chain reaction (PCR) method using Taq DNA polymerase (rTaq ; Toyobo, Osaka, Japan), as described elsewhere.[21,22] The designed allele-specific sense primers were FITC-CTCGATCTCCTGACCTCGTGATCC for the D allele and TxR-CCTGCTGCCTATACAGTCACTTTTATG for the I 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-GATGTGGCCATCACATTCGTCAGAT).

Statistical analyses

Analysis of covariance and chi-square tests were used to compare sex-specific age-adjusted mean values and proportions of risk characteristics according to the *ACE* I/D genotypes with the Tukey's multiple comparison methods. The chi-square test was used to examine whether the genotype distributions differed from that expected from Hardy-Weinberg equilibrium. The relationship between genotype and BP levels was examined by the analysis of covariance, adjusted for age, sex, antihypertensive medication use, body mass index, and alcohol intake. The statistical testing for BP differences between the genotypes DD and ID or II was conducted using the Dunnett's multiple comparison methods, stratified by the medians of urinary sodium excretion, and present and past sodium intake score. For the analysis stratified by urinary sodium excretion, survey years of 24-hour urine collection (1982-1986, 1989-1993, 1994-1997, 1998-2001, 2002-2005) were also adjusted. The interactions between genotype and sex, stratified sodium variables in relation to BP levels were examined using cross-product terms of these variables, i.e., polymorphism (DD, ID, II) × sex (M or F), polymorphism × sodium intake/excretion (below or beyond the median value). The hereditary mode was tested by multiple regression models, assigning DD=0 and ID or II=1 for dominant mode; DD or ID=0 and II=1 for recessive mode; and DD=0, ID=1 and II=2 for additive mode. All statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc.). All p value for statistical tests were two-tailed, and $p<0.05$ was regarded as statistical significance.

RESULTS

The frequency of *ACE* genotypes was 12.3% for DD genotypes, 45.4% for ID genotype, and 42.3% for II genotype. The genotype distribution was in Hardy-Weinberg equilibrium for men (p=1.00), women (p=0.99) and total samples (p=1.00). Table 1 summarizes sex-specific clinical and laboratory characteristics. Mean age was 59 years for men and 57 years for women. The prevalence of hypertension was 48% for men and 38% for women, and mean 24-hour urine sodium excretion was 205mmol and 179mmol, respectively.

Table 2 provides sex-specific age-adjusted characteristics according to the genotype. For each sex and total samples, mean age did not vary among DD, ID and II groups. Either mean SBP or DBP levels was not different among three groups. Also, the prevalence of antihypertensive medication use and prevalence of hypertension did not differ among three groups. The other factors, i.e. BMI, alcohol intake, urine sodium excretion, past and present sodium intake score did not vary among three groups.

Mean values of BP levels adjusting for sex, age, antihypertensive medication use, BMI, and alcohol consumption among *ACE* genotypes are shown in Table 3. There was no significant difference in BP levels between DD, ID and II groups for each sex and total samples. However, among subjects with higher sodium excretion, mean diastolic BP was higher in those with ID genotype (+3.0mmHg, p=0.003) and II genotype (+3.0mmHg, p=0.003) than in those with DD genotype for total subjects, but not among those with lower sodium excretion (p=0.02 for interaction). Among subjects with higher sodium excretion, the hereditary mode was statistically significant for dominant mode (p<0.001) and additive mode (p=0.01), but not for recessive mode (p=0.26).

Among subjects with higher present sodium intake, mean DBP levels was higher in ID and II groups compared with DD group for total subjects (+1.7mmHg, p=0.05 and +1.8mmHg, p=0.04, respectively), but not for those with lower present sodium intake;

however the interaction did not reach statistical significance (p=0.17). Among subjects with higher present sodium intake, the p-values for hereditary mode were 0.02 for dominant, 0.37 for recessive, and 0.07 for additive modes. Correspondingly, among subjects with higher past sodium intake, mean DBP levels tended to be higher in ID and II group compared with DD group for total subjects (+1.6 mmHg, p=0.06, and +1.7mmHg, p=0.06, respectively), but not for those with lower present sodium intake albeit insignificant interaction (p=0.24). Among subjects with higher past sodium intake, the hereditary mode were 0.03 for dominant, 0.46 for recessive, and 0.10 for additive modes. These trends among subgroups of each high sodium variables were similarly observed between men and women (sex interaction p=0.19 for higher sodium excretion, p=0.84 and 0.98 for higher present and past sodium intake, respectively), although these trends for each sex did not reach statistical significance probably due to the small number of DD subjects.

These results did not alter materially when restricting subjects without antihypertensive medication (n=2,196); mean DBP in II groups vs DD groups were 77.7 vs 77.3 mmHg (p=0.79) for total samples, 78.0 vs 75.3 mmHg (p=0.02) for persons with higher sodium excretion, 77.5 vs 76.1 mmHg (p=0.17) for persons with higher present sodium intake score, and 77.5 vs 76.3 mmHg (p=0.24) for persons with higher past sodium intake score (data not shown).

When we used first readings of BP for the analyses, the results did not alter materially. Also, when we used mean values of BP measured in 2001 and 2000 surveys for a subsample (n=2,287), the results did not change materially. Moreover, when we used urine samples collected within five-years from 2001 survey (i.e. 1996-2005, n=1,549), the results were essentially same as shown in Table 3.

DISCUSSION

In this large community-based association study, there was no significant difference in BP levels among DD, ID and II groups for either sex or total samples. However, we observed significant associations between I allele and elevated BP levels among persons with higher sodium excretion and higher past or present sodium intake scores, as we hypothesized *a priori*. Two previous studies revealed a positive association between I allele and salt-sensitivity among hypertensives. A study of 66 Japanese hypertensives showed that the prevalence of I allele was 77% among salt-sensitive hypertensives, whereas that of D allele was 55% among salt-resistant hypertensives.[10] Another study of 48 Spanish hypertensives showed that patients with II genotype had elevated 24-hour BP after higher salt diet period compared with after lower salt diet period (+9.8mmHg for SBP and +5.2 for DBP), whereas patients with DD genotype showed smaller changes (+1.2mmHg for SBP and –0.2mmHg for DBP)[11], which was confirmed by their subsequent study of larger samples (n=71).[12] Recently, a cross-sectional study of 284 Japanese male workers showed a positive association between salt intake estimated by food frequency questionnaire and the proportion of hypertension among men with *ACE* I allele (39% for men with the highest tertile (≥10.78 g/day) salt intake vs 19% for those with lowest tertile (≤8.15 g/day)), but not among those without it.[23] Also, the European Project on Genes in Hypertension (EPOGH), a recent large family and population-based collaborative study, showed that the left ventricular mass was higher in II genotype than in ID+DD genotype among Slavic offsprings with higher sodium excretion, but not among those with lower sodium excretion.[24] The present study extended evidence that sodium intake modifies the association of *ACE* I/D polymorphism with BP in a large free-living population.

We used large free-living community-based samples, which was the strength of the present study. Furthermore, we obtained sodium intake scores for 99% of subjects and

24-hour urine collection samples for 68% of subjects, which allowed us to test gene-environmental interactions of *ACE* I/D polymorphism with BP levels. As for I/D polymorphism, no previous association study estimated the relationship between salt intake and BP or hypertension in a community-based samples, and the number of subjects on the previous studies of salt-related hypertension was approximately 30-300[23,25], which was one-sixth or less of the present study.

The present study has the several limitations. First, we used only single BP measurement in the analyses so that 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-BP associations. When we used the first reading of BP, or means of BP data measured in 2000 and 2001 surveys, the results did not alter materially. Second, approximately 20% of subjects used antihypertensive medication which may obscure the genetic effect on BP levels. However, since the prevalence of antihypertensive medication use did not differ among the genotypes, this is unlikely. Third, we did not have the individual information which kind of drugs had been prescribed to the subjects for antihypertensive medication. Some antihypertensive drugs may modify the gene-BP association. However in this community, the majority of hypertensive patients seem to be treated primarily by calcium channel blockade like in other communities of Japan. Furthermore, the exclusion of persons on antihypertensive medication did not materially alter the genetic associations. Fourth, the variation of sodium excretion by one 24-h urine sample may be large and the misclassification between low and high sodium excretion groups is inevitable during a long sampling term. In the present study, however, 66% of persons remained in the same group of higher or lower sodium intake estimated from 24-h urine collection conducted within 5 years. Therefore, since our study had a large sample size, the impact of misclassification across the stratification is probably small. Fifth, a large time distance between BP measurement and

urine collection at most 19 years would make the interpretation for the results difficult. However, when we restricted the urine data of 2001±5 years (i.e. 1996-2005), the results did not alter materially. Last, BP levels were sometimes lower in groups of higher sodium excretion or intake than in those of the lower ones in each genotype. A plausible reason for this phenomenon is that most of the hypertensive persons attempt to reduce sodium intake when they recognized their blood pressure levels were high, since sodium modification has been the most common strategy for prevention and control of hypertension in Japanese society.[26]

The present study showed that differences in mean value of DBP between DD and II groups was 2-3mmHg for persons with higher salt intake, which seemed to be small from a viewpoint of clinical practice. However, meta-analyses of nine prospective observational studies demonstrated that a long-term difference of 5mmHg in mean DBP was associated with 34% less stroke and 21% less coronary heart disease.[27] Therefore, our finding, taken together with the results from the meta-analyses, was consistent with the possibility that salt reduction for persons with *ACE* I allele may have a substantial impact on prevention of cardiovascular disease.

In conclusion, *ACE* I allele was associated with higher DBP levels in persons with a higher sodium intake. The present study supports the hypothesis that *ACE* I allele may play a role of salt-sensitive hypertension.

ACKNOWLEDGMENTS

The authors gratefully appreciate to Toshimi Kohigashi, Toshiko Suzuki, Mizue Fujii, Yoshiko Okano and Akiko Otake for their technical assistances. This study was supported by the grant-in-aid for exploratory research of Japan Society for the Promotion of Science, Japan (No. 11877069 in 1999-2000), and Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Science, Sports and Culture, Japan (No. 17790382 in 2005-2007).

REFERENCES

1 Rigat B, Hubert C, Alhenc-Gelas G, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343-1346,1990.

2 O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 98:1766-1772,1998.

3 Higaki J, Baba S, Katsuya T, Sato N, Ishikawa K, Mannami T, Ogata J, Ogihara T. Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita Study. *Circulation* 101:2060-2065,2000.

4 Uemura K, Nakura J, Kohara K, Miki T. Association of ACE I/D polymorphism with cardiovascular risk factors. *Hum Genet* 107:239-242,2000.

5 Staessen JA, Wang JG, Ginocchio G, Petrov V, Saavedra AP, Soubrier F, Vlietinck R, Fagard R. The deletion/insertion polymorphism of the angiotensiongen converting enzyme gene and cardiovascular-renal risk. *J Hypertens* 15:1579-92,1997.

6 Agerholm-Larsen B, Nordestgaard BG, Tybjærg-Hansen A. ACE gene polymorphism in cardiovascular disease: Meta-analyses of small and large studies in whites. *Arterioscler Thromb Vasc Biol* 20:484-492,2000.

7 Matsubara M, Suzuki M, Fujiwara T, Kikuya M, Metoki H, Michimata M, Araki T, Kazama I, Satoh T, Hashimoto J, Hozawa A, Ohkubo T, Tsuji I, Katsuya T, Higaki J, Ogihara T, Satoh H, Imai Y. Angiotensin-converting enzyme I/D polymorphism and hypertension: the Ohasama study. *J Hypertens* 20:1121-1126,2002.

8 Zee RY, Lou YK, Griffiths LR, Morris BJ. Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. *Biochem Biophys Res*

Commun 184:9-15,1992.

9 Yoshida K, Ishigami T, Nakazawa I, Ohno A, Tamura K, Fukuoka M, Mizushima S, Umemura S. Association of essential hypertension in elderly Japanese with I/D polymorphism of the angiotensin-converting enzyme (ACE) gene. *J Hum Genet* 45:294-298,2000.

10 Hiraga H, Oshima T, Watanabe M, Ishida M, Ishida T, Shingu T, Kambe M, Matsuura H, Kajiyama G. Angiotensin I- converting enzyme gene polymorphism and salt sensitivity in essential hypertension. *Hypertension* 27:569-572,1996.

11 Giner V, Poch E, Bragulat E, Oriola J, González D, Coca A, de la Sierra A. Renin-angiotensin system genetic polymorphisms and salt sensitivity in essential hypertension. *Hypertension* 35:512-517,2000.

12 Poch E, González D, Giner V, Bragulat E, Coca A, de la Sierra A. Molecular basis of salt-sensitivity in human hypertension. Evaluation of renin-angiotensin-aldosterone system gene polymorphisms. *Hypertension* 38:1204-1209,2001.

13 Dengel DR, Brown MD, Ferrell RE, Supiano MA. Role of angiotensin converting enzyme genotype in sodium sensitivity in older hypertensives. *Am J Hypertens* 14:1178-1184,2001.

14 Kojima S, Inenaga T, Matsuoka H, Kuramochi M, Omae T, Nara Y, Yamori Y. The association between salt sensitivity of blood pressure and some polymorphic factors. *J Hypertens* 12:797-801,1994.

15 Johnson AG, Nguyen TV, Davis D. Blood pressure is linked to salt intake and modulated by the angiotensinogen gene in normotensive and hypertensive elderly subjects. *J Hypertens* 19:1053-1060,2001.

16 Pamies-Andreu E, Ramirez-Lorca R, Stiefel García-Junco P, Muñiz-Grijalbo O, Vallejo-Maroto I, Garcia Morillo S, Miranda-Guisado ML, Ortíz JV, Carneado de la

Fuente J. Renin-angiotension-aldosteron system and G-protein beta-3 subunit gene polymorphism in salt-sensitive essential hypertension. *J Hum Hypertens* 17:187-191,2003.

17 Yamagishi K, IsoH, 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* 17:385-390,2004.

18 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* 27:53-60,2004.

19 Iso H, Shimamoto T, Yokota K, Sankai T, Jacobs DR, Jr, Komachi Y. Community-based education classes for hypertension control. A 1.5-year randomized controlled trial. *Hypertension* 27:968-974,1996.

20 Iso H, Harada S, Shimamoto T, Sato S, Kitamura A, Sankai T, Tanigawa T, Iida M, Komachi Y. Angiotensinogen T174M and M235T variants, sodium intake and hypertension among non-drinking, lean Japanese men and women. *J Hypertens* 18:1197-1206,2000.

21 Takarada Y. Analysis for SNPs using Allele Specific Primer. *Upload* 66:8-9,2002 (in Japanese).

22 Ishiguro A, Kubota T, Soya Y, Sasaki H, Yagyu O, Takarada Y, Iga T: 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.

23 Zhang L, Miyaki K, Araki J, Song Y, Kimura T, Omae K, Muramatsu M. Interaction of angiotensin I-concerting enzyme insertion-deletion polymorphism and daily salt intake influences hypertension in Japanese men. Hypertens Res 2006;29:751-758.

24 Kuznetsova T, Staessen JA, Stolarz K, Ryabikov A, Tikhonoff V, Olszanecka A, Bianchi G, Brand E, Casiglia E, Dominiczak A, Fagard R, Malyutina S, Nikitin Y, Kawecka-Jaszcz K; European Project on Genes in Hypertension (EPOGH) Investigators. Relationship between left ventricular mass and the *ACE* D/I polymorphism varies according to sodium intake. *J Hypertens* 2004;22:287-295.

25 Beeks E, Kessels AGH, Kroon AA, van der Klauw MM, de Leeuw PW. Genetic predisposition to salt-sensitivity: a systematic review. *J Hypertension* 2004;22:1243-1249.

26 Intersalt Cooperative Research Group. Intersalt: an international study of electrolyte excretion and blood pressure. Result for 24 hour urinary sodium and potassium excretion. *Br Med J* 297:319-328,1988.

27 MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A, Stamler J. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 335:765-774,1990.

Table 1. Clinical and laboratory characteristics (mean, standard deviation and proportion), men and women aged 30-74 years.

	Men	Women	Total
Number	1,048	1,775	2,823
Age, year	59.0 ± 10.6	56.7 ± 10.6	57.5 ± 10.7
Systolic blood pressure, mmHg	134.6 ± 16.4	130.9 ± 16.8	132.3 ± 16.8
Diastolic blood pressure, mmHg	80.9 ± 10.2	77.0 ± 10.1	78.4 ± 10.3
Use of antihypertensive medication, %	23	22	22
Hypertension, %†	48	38	42
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
24h urine collection, n	720	1,200	1,920
Urine sodium excretion, mmol	205 ± 75	179 ± 66	189 ± 70
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 (standard errors) and proportions of characteristics according to angiotensin-I converting enzyme I/D genotypes, men and women aged 30-74 years.

	Men			Women			Total		
	DD	**ID**	**II**	**DD**	**ID**	**II**	**DD**	**ID**	**II**
Number	123	473	452	225	809	741	348	1,282	1,193
Age, year	59.6	59.0	58.8	57.8	56.4	56.6	58.5	57.4	57.4
Systolic blood pressure, mmHg	135.1 (1.4)	135.4 (0.7)	133.6 (0.7)	130.0 (1.0)	131.3 (0.5)	130.8 (0.6)	131.9 (0.8)	132.8 (0.4)	131.8 (0.5)
Diastolic blood pressure, mmHg	79.6 (0.9)	81.2 (0.5)	80.9 (0.5)	76.5 (0.7)	77.2 (0.4)	76.9 (0.4)	77.6 (0.6)	78.6 (0.3)	78.4 (0.3)
Use of antihypertensive medication, %	27	22	24	21	23	20	23	22	22
Hypertension, %†	48	50	45	37	39	38	41	43	41
Body mass index, kg/m^2	23.8 (0.3)	24.0 (0.1)	23.9 (0.1)	23.6 (0.2)	23.4 (0.1)	23.5 (0.1)	23.7 (0.2)	23.6 (0.1)	23.6 (0.1)
Alcohol intake, g/day	16.7 (2.3)	22.4 (1.2)	21.0 (1.2)	1.9 (0.4)	1.7 (0.2)	1.4 (0.2)	7.1 (1.0)	9.3 (0.5)	8.8 (0.5)
24h urine collection (n)	84	319	317	158	543	499	242	862	816
Urine sodium excretion (mmol)	205 (8)	203 (4)	207 (4)	181 (5)	178 (3)	179 (3)	189 (5)	187 (2)	190 (2)
Present sodium questionnaire completed	123	468	447	223	805	736	346	1,273	1,183
Present sodium intake score	5.8 (0.2)	5.7 (0.1)	5.7 (0.1)	4.4 (0.1)	4.7 (0.1)	4.7 (0.1)	4.9 (0.1)	5.0 (0.1)	5.0 (0.1)
Past sodium questionnaire completed (n)	123	464	444	223	802	736	346	1,266	1,180
Past sodium intake score	6.8 (0.2)	6.8 (0.1)	6.9 (0.1)	5.7 (0.1)	6.0 (0.1)	5.9 (0.1)	6.1 (0.1)	6.3 (0.1)	6.3 (0.1)

†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 statistical significance were observed for age-adjusted means or proportions of characteristics according to genotype using Tukey's multiple comparison methods.

Table 3. Multivariate-adjusted blood pressure levels (standard errors) according to angiotensin-I converting enzyme I/D genotypes, stratified by sodium excretion/intake, men and women ages 30-74 years.

	Men				Women				Total			
	DD	ID	II	p for difference of DD vs II	DD	ID	II	p for difference of DD vs II	DD	ID	II	p for difference of DD vs II
Total	123	473	452		225	809	741		348	1282	1193	
Systolic blood pressure, mmHg	135.4 (1.4)	135.3 (0.7)	133.6 (0.7)	0.36	129.9 (1.0)	131.2 (0.5)	130.8 (0.5)	0.56	131.9 (0.8)	132.7 (0.4)	131.9 (0.4)	1.00
Diastolic blood pressure, mmHg	79.8 (0.9)	81.1 (0.5)	80.9 (0.5)	0.38	76.3 (0.6)	77.2 (0.3)	76.9 (0.4)	0.64	77.6 (0.5)	78.6 (0.3)	78.4 (0.3)	0.32
Stratified by urinary sodium excretion												
below median	39	169	152		77	270	253		116	439	405	
Systolic blood pressure, mmHg	135.6 (2.6)	137.5 (1.2)	134.6 (1.3)	0.89	132.0 (1.7)	133.4 (0.9)	132.3 (1.0)	0.98	133.2 (1.5)	134.9 (0.7)	133.2 (0.8)	1.00
Diastolic blood pressure, mmHg	81.4 (1.6)	81.5 (0.8)	80.5 (0.8)	0.78	77.5 (1.1)	78.3 (0.6)	76.4 (0.6)	0.56	78.9 (0.9)	79.5 (0.5)	78.0 (0.5)	0.56
beyond median	45	150	165		81	273	246		126	423	411	
Systolic blood pressure, mmHg	136.5 (2.1)	134.5 (1.2)	133.7 (1.1)	0.36	127.4 (1.6)	130.9 (0.8)	129.6 (0.9)	0.32	130.8 (1.2)	132.3 (0.7)	131.1 (0.7)	0.96
Diastolic blood pressure, mmHg	79.0 (1.4)	80.0 (0.8)	81.1 (0.7)	0.25	74.1 (1.1)	78.2 (0.6)	77.5 (0.6)	0.009	75.9 (0.8)	78.9 (0.5)	78.9 (0.5)	0.003
Stratified by present sodium intake score												
below median	53	205	191		114	378	343		167	583	534	
Systolic blood pressure, mmHg	139.7 (2.1)	136.9 (1.0)	136.4 (1.1)	0.24	131.8 (1.4)	132.5 (0.8)	131.9 (0.8)	1.00	134.5 (1.2)	134.0 (0.6)	133.5 (0.6)	0.58
Diastolic blood pressure, mmHg	81.1 (1.4)	82.4 (0.7)	81.9 (0.7)	0.77	78.0 (0.9)	77.7 (0.5)	77.0 (0.5)	0.50	79.2 (0.8)	79.3 (0.4)	78.8 (0.4)	0.82
beyond median	70	263	256		109	427	393		179	690	649	
Systolic blood pressure, mmHg	132.1 (1.8)	134.1 (0.9)	131.3 (0.9)	0.85	128.0 (1.4)	130.0 (0.7)	130.0 (0.7)	0.31	129.7 (1.1)	131.6 (0.6)	130.5 (0.6)	0.65
Diastolic blood pressure, mmHg	79.0 (1.2)	80.0 (0.6)	80.3 (0.6)	0.46	74.7 (0.9)	76.7 (0.4)	76.8 (0.5)	0.054	76.3 (0.7)	78.0 (0.4)	78.1 (0.4)	0.04
Stratified by past sodium intake score												
below median	49	190	183		98	320	303		147	510	486	
Systolic blood pressure, mmHg	137.2 (2.2)	135.2 (1.1)	135.2 (1.1)	0.59	129.9 (1.5)	130.4 (0.8)	130.3 (0.8)	0.94	132.5 (1.2)	132.2 (0.7)	132.1 (0.7)	0.93
Diastolic blood pressure, mmHg	81.3 (1.4)	82.2 (0.7)	81.7 (0.7)	0.94	77.3 (1.0)	76.9 (0.6)	76.3 (0.6)	0.55	78.8 (0.8)	78.8 (0.4)	78.3 (0.4)	0.78
beyond median	74	274	261		125	482	433		199	756	694	
Systolic blood pressure, mmHg	134.0 (1.7)	135.2 (0.9)	132.5 (0.9)	0.58	130.2 (1.3)	131.7 (0.7)	131.2 (0.7)	0.68	131.6 (1.0)	133.0 (0.5)	131.7 (0.6)	1.00
Diastolic blood pressure, mmHg	78.8 (1.2)	80.2 (0.6)	80.4 (0.6)	0.34	75.8 (0.8)	77.3 (0.4)	77.4 (0.5)	0.14	76.8 (0.7)	78.4 (0.4)	78.5 (0.4)	0.06

Blood pressures were adjusted for sex, age, antihypertensive medication use, body mass index and alcohol intake, and for the analysis stratified by urinary sodium excretion, survey years of 24-hour urine collection. Tests for difference from DD genotype were conducted using Dunnett's multiple comparison method. Medians are 195.7 mmol/day for urinary sodium excretion in men and 171.2 mmol/day in women, 5 for present sodium intake score in women and 6 in men, 6 for past sodium intake score in women, and 7 in men..