G-protein β-3 subunit C825T polymorphism, sodium and arterial blood pressure: A community-based study of Japanese men and women

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Running head: *GNB3* gene, sodium, and blood pressure

Summary

Epidemiological evidence on gene-environment effect of G-protein β-3 subunit C825T polymorphism and sodium on blood pressure in free-living general population is limited. We examined the associations between C825T polymorphism and blood pressure levels, stratified by the sodium variables estimated by 24-h urinary sodium excretion and dietary questionnaire among 1,471 men and women aged 30-74 at a community in Japan. Our *a priori* hypothesis was that individuals with 825T allele have elevated blood pressure among subjects with high sodium intake. Among the whole group, systolic blood pressure level was +2.2 mmHg (p=0.10) higher in TT than CC genotype after adjustment for sex, age, antihypertensive medication use, body mass index, and alcohol consumption. Such difference was more evident among individuals with low sodium excretion (+4.5 mmHg, p=0.01), low present sodium intake (+3.2 mmHg, p=0.11), and low past sodium intake (+4.8 mmHg, p=0.02). No associations were observed among those with high sodium variables. Our results indicate that G-protein β-3 subunit C825T polymorphism is associated with higher systolic blood pressure levels in a large free-living Japanese population, more specifically in women with low sodium intake. This finding helps to explain part of the discrepancy of the previously reported genetic association among different ethnic groups.

Key words: G-protein, epidemiology, salt sensitivity, gene-environment interaction, sodium excretion

INTRODUCTION

G-protein activates various transmembrane receptors and intracellular signaling systems, and regulates arterial blood pressure (BP) (Siffert, 1998). Siffert *et al*. were the first group to describe that C825T (C-to-T substitution at nucleotide 825 in exon 10) polymorphism of the G-protein β-3 subunit (*GNB3*) gene was associated with hypertension (Siffert *et al*, 1998). The same study showed that activation of pertussis toxin-sensitive G-proteins significantly enhanced the TC or TT genotypes of *GNB3* compared with the CC genotype.

Although C825T polymorphism is considered a candidate gene for salt-sensitive hypertension (Beeks *et al*, 2004), it is still uncertain whether the C825T polymorphism affects Na^+/H^+ exchanger. Furthermore, the results from subsequent association studies were inconsistent especially in non-Caucasians (Siffert, 2003), and there has been limited evidence on the gene-environmental interaction among *GNB3* C825T polymorphism, sodium and BP.

The aim of this study was to examine the association between *GNB3* polymorphism and BP levels according to sodium intake, estimated by 24-h urine collection and dietary questionnaires, in a free-living population. We thus performed a community-based study of 1,471 Japanese in a general community. Our *a priori* hypothesis was that individuals with 825T allele of the *GNB3* gene have elevated BP levels in response to a high sodium intake, and thus the association between *GNB3* polymorphism and BP is more evident among individuals with a high sodium intake than those with a low sodium intake.

MATERIALS AND METHODS

Subjects

Subjects were free-living residents of a farming community of Kyowa, central Japan (population 17,145 by the 2000 census) aged 30-74 years, who 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. In the present study, we included 1,471 subjects who provided a written informed consent, a satisfactory 24-h urine sample between 1996 and 2004, i.e. from 5 years prior to the 2001 cardiovascular risk surveys, and completed a salt intake questionnaire. The study protocol was approved by the Medical Ethics Committee of the University of Tsukuba.

Population surveys

The population surveys were performed annually since 1981. Details of the surveys have been described elsewhere (Iso *et al*, 1996; Yamagishi *et al*, 2004). Well-trained medical technicians measured arterial systolic blood pressure (SBP) and fifth-phase diastolic blood pressure (DBP) using standard mercury sphygmomanometers (with 14 cm wide and 51 cm long cuff) on the right arm of quietly seated participants after a period of at least 5-min rest. When SBP was ≥140 mmHg and/or DBP was ≥90 mmHg, the measurement was repeated; the average of two readings was used in the analysis, otherwise the first reading was used. The method of BP measurement was standardized uniformly across the surveys, and BP measurement training for our

medical technicians was provided before each annual survey. Hypertension was defined as SBP of ≥140 mmHg and/or DBP of ≥90 mmHg, and/or use of antihypertensive medication. The participants were asked to collect all urine over a 24-hour period between 1996 and 2004 surveys. Subjects who provided urine samples of <500 ml and/or <20 hours, or those with incomplete collections based on records were excluded from the study. At the 2001 survey, all participants were also asked to complete a self-administered dietary questionnaire to estimate present and past 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 questions. Although this scoring system was validated previously (Iso *et al*, 1996; 2000; Yamagishi *et al*, 2004), it was tested again using the samples of the present study. Age and sex-adjusted mean 24-hour sodium excretion values across quintiles of the present sodium intake score (n=1,471) were 165, 173, 186, 197 and 198 mmol/L (p for trend <0.001), and those of past sodium intake score (n=1,471) were 170, 177, 185, 187 and 202 mmol/L (p for trend <0.001). The Spearman correlation coefficient between past and present sodium intake scores was 0.60 (p<0.001). Repeatability of the present and past sodium intake scores was also tested previously (Iso *et al*, 1996; Yamagishi *et al*, 2004) by repeating the questionnaire one to two years apart in a sub-sample of the population (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) (Yamagishi *et al*, 2004). We used age, body mass index and alcohol intake as confounding variables,

and sodium excretion, sodium intake as effect modifier. In the present analyses, we used the data of age, BP, body mass index, alcohol intake, and use of antihypertensive medication at the same year as 24-h urine was collected.

DNA genotyping

GNB3 C825T genotypes were determined by the allele-specific primer-polymerase chain reaction method as described previously (Takarada *et al*, 2002; Ishiguro *et al*, 2005). The designed allele-specific sense primers were TxR-TCTGCGGCATCACGTXCG for the 825C allele and FITC-TCTGCGGCATCACGTXTG for the 825T 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-GAATAGTAGGCGGCCACTGA), in which X represents an artificial mismatch base. For high sensitivity and specificity, we used polymorphic base at the second site from 3' end, and placed an artificial mismatch base next to the polymorphic base, instead of matched base in each primer (Ishiguro *et al*, 2005).

Statistical analyses

The analysis of covariance and chi-square test were used to compare sex-specific age-adjusted mean values and proportions of risk characteristics, by Tukey's multiple comparison method. The chi-square test was used to examine whether the genotype distributions differed from that expected from Hardy-Weinberg equilibrium. The relation between genotype and BP levels was adjusted by age, sex, antihypertensive

medication use (yes or no), body mass index (BMI) and alcohol consumption, and examined by Dunnett's multiple comparison method, with CC group as reference. Further analysis was performed stratified by the medians of urinary sodium excretion and sodium intake scores. For stratified analyses by the sodium intake/excretion, we further adjusted for the year of urine collection (1996-2000 or 2001-2004). We used dummy variables for adjustment of sex, antihypertensive medication and the year of urine collection, and continuous variables for other covariates. 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 (CC, TC, TT) × sex (M or F), polymorphism × sodium intake/excretion (below or beyond the median value). All statistical analyses were performed using SAS version 8.02 software (SAS Institute Inc., Cary, NC). All probability values for statistical tests were two-tailed, and values of $p<0.05$ were regarded as statistically significant.

RESULTS

The frequency of *GNB3* genotypes was 24.1% for the CC genotype, 49.9% for the CT genotype, and 25.9% for the TT genotype. The sex-specific frequencies were 23.5%, 51.2%, and 25.3% for men, and 24.6%, 49.2%, and 26.3% for women, respectively. The genotype distribution was in Hardy-Weinberg equilibrium for men (p=0.92), women (p=0.94), and total subjects (p=1.00). The mean age of the subjects was 60 years for men and 55 years for women. The prevalence of hypertension was 51% for men and 39% for women, and the respective mean values of 24-h urine sodium excretion were 195 mmol and 175 mmol (data not shown in the tables).

Table 1 provides sex-specific age-adjusted characteristics according to *GNB3* genotype. The mean age of the TT group was lower for women and higher for men than those in other genotypes. The mean SBP and DBP were not different among the genotypes. Similarly, the prevalence of antihypertensive medication use and prevalence of hypertension were not different among the genotypes. The mean present sodium intake score of TT group was higher than that of CC group for women, but not for men. The other variables, i.e. BMI, alcohol intake, urine sodium excretion and past sodium intake score were not statistically different among the genotypes.

The mean values of BP levels adjusted for sex, age, antihypertensive medication use, BMI, and alcohol consumption are shown for each *GNB3* genotype (Table 2). For total subjects, the SBP levels were +2.2 mmHg higher in the TT group than CC group; this difference showed a trend towards significance (p=0.10).

When we performed further stratification by sodium variables (Table 3), the SBP difference between CC and TT groups was greater among subjects with lower sodium excretion (+4.5 mmHg, p=0.01) than among those with higher sodium excretion (+0.2 mmHg, p=0.99), and the interaction was statistically significant (p=0.01). The interaction for SBP was less evident among men (p=0.58) than women (p=0.01). These associations were less evident for DBP.

The SBP difference between CC and TT genotypes was greater among subjects with lower present sodium intake (+3.2 mmHg, p=0.11) than those with higher sodium intake (+1.3 mmHg, p=0.62), although the interaction did not reach statistical significance (p=0.66). The associations were less evident for DBP.

When we stratified by past sodium intake score, the SBP difference between CC and TT genotypes was more evident among subjects with lower past sodium intake (+4.8 mmHg, p=0.02) than those with higher past sodium intake (+0.2 mmHg, p=0.99) although the interaction did not reach statistical significance (p=0.12). Such a trend was not observed for DBP.

The associations between *GNB3* genotypes and BP levels became weaker when restricting subjects without antihypertensive medication (n=1,146); the mean SBP levels in the TT groups vs CC groups were 129.0 vs 127.5 mmHg (p=0.41) for total subjects, 131.1 vs 127.9 mmHg (p=0.15) for subjects with lower sodium excretion, 130.8 vs 128.7 mmHg (p=0.44) for subjects with lower present sodium intake score, and 130.8 vs 127.2 mmHg (p=0.13) for subjects with lower past sodium intake score (data not shown in the tables).

Since second readings of BP levels were not obtained for all subjects in the present study, we examined data of the 2003 and 2004 surveys where two BP readings were obtained (n=1,347). The results, however, did not alter materially when we used the average of the two readings of BP; the mean SBP levels in the TT groups vs CC groups were 132.8 vs 131.2 mmHg (p=0.37) for total subjects, 136.0 vs 131.8 mmHg (p=0.047) for subjects with lower sodium excretion, 134.3 vs 131.7 mmHg (p=0.29) for subjects with lower present sodium intake score, and 132.7 vs 129.2 mmHg (p=0.15) for subjects with lower past sodium intake score (data not shown in the tables).

The mean BP levels were higher in subjects on medication use than those without medication; age-adjusted mean SBPs were 139.9 mmHg in those with medication vs 129.0 mmHg in those without medication ($p<0.0001$), and respective DBP were 81.9 mmHg vs 77.8 mmHg ($p<0.0001$) (data not shown in the tables). The distribution of genotypes was similar between subjects with and without antihypertensive medication; the distributions of CC, CT and TT genotype were 26%, 51% and 22% for subjects with medication, and 23%, 50%, and 27% for subjects without medication (p for chi-square test = 0.19).

DISCUSSION

In a previous study, we showed that high sodium intake strengthened the gene-blood pressure associations for angiotensinogen (*AGT*) T174M (Yamagishi *et al*, 2004) and α-adducin (*ADD1*) G460W polymorphisms (Yamagishi *et al*, 2004). In contrast, in the present study, the *GNB3*-systolic blood pressure association was more evident among subjects with lower sodium intake/excretion, but not with higher intake/excretion. This result was unexpected, but it is possible that high salt intake masks the potential effect of *GNB3* on blood pressure levels. Our finding helps to explain the inconsistency of the reported genetic associations: positive associations in the majority of studies in whites (Siffert, 2003), but no associations in the majority of studies of Japanese (Kato *et al*, 1998; Kario *et al*, 1999; Ishikawa *et al*, 2000; Tozawa, 2001; Suwazono *et al*, 2004; Shioji *et al*, 2004), African-American (Larson *et al* ,2000), Chinese (Huang *et al* , 2003; Li *et al*, 2005), Taiwanese (Tsai *et al*, 2000), and Kazakh in China (Wang *et al*, 2004). This ethnic difference may be explained in part by differences in salt intake, which is higher in Asian and African-American populations than in whites (Intersalt Cooperative Research Group, 1988).

The *GNB3* C825T polymorphism has been suspected to be a genetic marker of salt-sensitive hypertension (Beeks *et al*, 2004). Siffert *et al*. reported significantly enhanced activation of pertussis toxin-sensitive G proteins in TC and TT genotypes of *GNB3* relative to the CC genotype, which implied that *GNB3* might affect Na^+/H^+ exchanger (Siffert *et al*, 1998). Other studies showed significant relations among *GNB3* genotype and low plasma renin activity (Schunkert *et al*, 1998), high response

to diuretics (Turner *et al*, 2001), left ventricular hypertrophy (Poch *et al*, 2000; Semplicini *et al*, 2001) and renal plasma flow (Zeltner *et al*, 2001), which support the above hypothesis. However, it is still uncertain whether the C825T polymorphism affects Na^+/H^+ exchanger since two experimental studies showed no association between C825T polymorphism and salt-sensitivity (Schorr *et al*, 2000; Pamies-Andreu *et al*, 2003). Also in the present study, sodium did not strengthen the associations of *GNB3* with BP levels, which is in contradiction to our *a priori* hypothesis.

In the present study, approximately 20% of the participating individuals used antihypertensive medication, which may have obscured the genetic effect of BP levels. When we excluded individuals with antihypertensive medication from the analysis, the gene-BP association became weaker. Weakening of the association may be caused by the smaller variation of BP levels after the exclusion of subjects with high BP levels. In fact, the mean BP levels were higher in subjects on medication use than those without medication.

The strength of the present study was that we used a large community-based free-living population. In addition, we obtained 24-h urine collection samples and sodium intake scores, which allowed us to test gene-environmental interactions of the *GNB3* C825T genotype with BP levels in a general population. Previously, two experimental studies with smaller numbers of subjects (Schorr *et al*, 2000; Pamies-Andreu *et al*, 2003) examined the relation between C825T genotype and dietary salt or salt sensitivity, with negative results. However, to our knowledge, the

present study is the first report that evaluated the gene-environmental interaction of *GNB3* polymorphism, sodium, and BP in a large free-living general population.

The limitations of the present study warrant discussion. Firstly, we did not have information about the antihypertensive agents used by the population tested. The use of hydrochlorothiazide or clonidine was reported to reduce SBP levels more in subjects with TT genotype than in those with CC genotype (Turner *et al*, 2001; Nürnberger *et al*, 2003). However, in this community, it is our impression that the majority of hypertensive patients were treated primarily by calcium channel blockers, like in other communities of Japan. Long-term use of calcium blockers is unlikely to change sodium excretion or sodium retention (Leonetti, 1994). Secondly, the gene-BP association was primarily obtained for women only, although the interaction of sex in relation to the genotype with BP levels was not statistically significant (p for sex-interaction = 0.94 for SBP). A probable reason for the relatively weak association in men was the smaller number of men than women. Thirdly, the variation of sodium excretion by one 24-h urine sample may be very large and the possibility of misclassification between low and high sodium excretion groups could be real. However, since the study included a large sample size, the impact of misclassification across the stratification is probably small. In the present study, 66% of persons remained in the same group of higher or lower sodium intake after one-year re-collection of urinary sodium excretion in the sub-sample group. Lastly, in Table 3, blood pressure levels were sometimes lower in groups with higher sodium excretion or intake than lower ones with the same genotypes, which was also observed in Japanese populations according to the Intersalt study (Intersalt Cooperative Research

Group, 1988). A plausible reason for this phenomenon is that most of the hypertensives attempted to reduce salt intake when they recognized their blood pressure levels were high, since salt reduction is well known to reduce blood pressure in the Japanese society.

In conclusion, the *GNB3* C825T polymorphism tends to be associated with SBP levels in Japanese population. This association was more specifically observed among women with a low sodium excretion against our *a priori* hypothesis. This finding, however, helps to explain part of the discrepancy of the reported genetic association among different ethnic groups.

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References

Beeks, E., Kessels, A.G., Kroon, A.A., van der Klauw, M.M. & de Leeuw, P.W. (2004) Genetic predisposition to salt-sensitivity: a systematic review. *J Hypertens* **22**, 1243-1249.

Huang, X., Ju, Z., Song, Y., Zhang, H., Sun, K., Lu, H., Yang, Z., Jose, P.A., Zhou, G., Wang, M., Wang, W., Feng, S. & Hui, R. (2003) Lack of association between the G protein beta3 subunit gene and essential hypertension in Chinese: a case-control and a family-based study. *J Mol Med* **81**, 729-735.

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

Ishiguro, A., Kubota, T., Soya, Y., Sasaki, H., Yagyu, O., Takarada, Y. & Iga, T. (2005) High-throughput detection of multiple genetic polymorphisms influencing drug metabolism with mismatch primers in allele-specific polymerase chain reaction. *Anal Biochem* **337,** 256-261.

Ishikawa, K., Imai, Y., Katsuya, T., Ohkubo, T., Tsuji, I., Nagai, K., Takami, S., Nakata, Y., Satoh, H., Hisamichi, S., Higaki, J. & Ogihara, T. (2000) Human G-protein β3 subunit variant is associated with serum potassium and total cholesterol levels but not with blood pressure. *Am J Hypertens* **13**, 140-145.

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

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

Kario, K., Fujiwara, M., Sone, Y., Saiki, K., Hoshide, S., Shimada, K., Schwartz, J.E. & Matsuo, M. (1999) G protein β3 subunit gene variant, twenty-four-hour blood pressure, and hypertensive cerebrovascular disease in a Japanese population. *Am J Hypertens* **12**, 1159-1160.

Kato, N., Sugiyama, T., Morita, H., Kurihara, H., Yamori, Y. & Yazaki, Y. (1998) G protein β3 subunit variant and essential hypertension in Japanese. *Hypertension* **32**, 935-938.

Larson, N. Hutchinson, R. & Boerwinkle, E. (2000) Lack of association of 3 functional gene variants with hypertension in African Americans. *Hypertension* **35**, 1297-1300.

Leonetti G. (1994) The effects of calcium antagonists on electrolytes and water balance in hypertensive patients. *J Cardiovasc Pharmacol* **24 Suppl A**, S25-S29.

Nürnberger, J., Dammer, S., Mitchell, A., Siffert, W., Wenzel, R.R., Gössl, M., Philipp, T., Michel, M.C. & Schäfers, R.F. (2003) Effect of the C825 T polymorphism of the G protein β3 subunit on the systolic blood pressure-lowering effect of clonidine in young, healthy male subjects. *Clin Pharmacol Ther* **74**, 53-60.

Pamies-Andreu, E., Ramirez-Lorca, R., Stiefel García-Junco, P., Muñiz-Grijalbo, O., Vallejo-Maroto, I., Garcia Morillo, S., Miranda-Guisado, M.L., Ortíz, J.V. & Carneado de la Fuente, J. (2003) Renin-angiotensin-aldosterone system and G-protein beta-3 subunit gene polymorphisms in salt-sensitive essential hypertension. *J Hum Hypertens* **17**, 187-191.

Poch, E., González, D., Gómez-Angelats, E., Enjuto, M., Paré, J.C., Rivera, F. & de La Sierra, A. (2000) G-protein β_3 subunit gene variant and left ventricular hypertrophy in essential hypertension. *Hypertension* **35[part 2]**, 214-218.

Schorr, U., Blaschke, K., Beige, J., Distler, A. & Sharma, A.M. (2000) G-protein β3 subunit 825T allele and response to dietary salt in normotensive men. *J Hypertens* **18**, 855-859.

Schunkert, H., Hense, H.W., Döring, A., Riegger, G.A.J. & Siffert, W. (1998) Association between a polymorphism in the G protein β3 subunit gene and lower renin and elevated diastolic blood pressure levels. *Hypertension* **32**, 510-513.

Semplicini, A., Siffert, W., Sartori, M., Monari, A., Naber, C., Frigo, G., Santonastaso, M., Cozzutti, E., Winnicki, M. & Palatini, P. (2001) G protein β_3 subunit gene 825T allele is associated with increased left ventricular mass in young subjects with mild hypertension. *Am J Hypertens* **14**, 1191-1195.

Shioji, K., Kokubo, Y., Mannami, T., Inamoto, N., Morisaki, H., Mino, Y., Tagoi, N., Yasui, N. & Iwai, N. (2004) Association between hypertension and the α-adducin, β1-adrenoreceptor, and G-protein β3 subunit genes in the Japanese population; the Suita Study. *Hypertens Res* **27**, 31-37.

Siffert, W. (1998) G proteins and hypertension: an alternative candidate gene approach. *Kidney Int* **53**, 1466-1470.

Siffert, W., Rosskopf, D., Siffert, G., Busch, S., Moritz, A., Erbel, R., Sharma, A. M., Ritz, E., Wichmann, H. E., Jakobs, K.H. & Horsthemke, B. (1998) Association of a human G-protein β3 subunit variant with hypertension. *Nat Genet* **18**, 45-48.

Siffert, W. (2003) G-protein β3 subunit 825T allele and hypertension. *Curr Hypertens Rep* **5**, 47-53.

Suwazono Y, Okubo Y, Kobayashi E, Miura K, Morikawa Y, Ishizaki M, Kido T, Nakagawa H & Nogawa K. (2004) Lack of association of human G-protein β3 subunit variant with hypertension in Japanese workers. *J Hypertens* **22**, 493-500.

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

Tozawa, Y. (2001) G protein beta3 subunit variant: tendency of increasing susceptibility to hypertension in Japanese. *Blood Press* **10**, 131-134.

Tsai, C.H., Yeh, H.I., Chou, Y., Liu, H.F., Yang, T.Y., Wang, J.C., Wang, N.M. & Chang, J.G. (2000) G protein beta3 subunit variant and essential hypertension in Taiwan - a case-control study. *Int J Cardiol* **73** ,191-198.

Turner, S.T., Schwartz, G.L., Chapman, A.B. & Boerwinkle, E. (2001) C825T polymorphism of the G protein β_3-subunit and antihypertensive response to a thiazide diuretic. *Hypertension* **37[part 2]**, 739-743.

Wang, X., Wang, S., Lin, R., Jiang, X., Cheng, Z., Turdi, J., Ding, J., Wu, G., Lu, X. & Wen, H. (2004) GNB3 gene C825T and ACE gene I/D polymorphisms in essential hypertension in a Kazakh genetic isolate. *J Hum Hypertens* **18**, 663-668.

Yamagishi, K., Iso, H., Tanigawa, T., Cui, R., Kudo, M. & Shimamoto, T. (2004) 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.

Yamagishi, K., Iso, H., Tanigawa, T., Cui, R., Kudo, M. & Shimamoto, T. (2004) Alpha-adducin G460W polymorphism, urinary sodium excretion and blood pressure in community-based samples. *Am J Hypertens* **17**, 385-390.

Zeltner, R., Delles, C., Schneider, M., Siffert, W. & Schmieder, R.E. (2001) G-protein β_3 subunit gene *(GNB3) 825T* allele is associated with enhanced renal perfusion in early hypertension. *Hypertension* **37**, 882-886.

Table 1. Age-adjusted characteristics according to G-protein β-3 subunit C825T genotype, men and women aged 30-74 years.

	Women			Men			Total		
	CC	CT	TT	CC	CT	TT	CC	CT	TT
Number	218	436	233	137	299	148	355	735	381
Age, year	56.4	55.6	53.6** ‡	59.1	59.9	61.7*	57.4	57.4	56.7
Systolic blood pressure, mmHg	128.6	128.8	130.8	133.8	134.2	136.3	130.8	131.0	132.7
Diastolic blood pressure, mmHg	76.0	77.3	77.7	81.0	80.9	82.1	78.1	78.8	79.3
Use of antihypertensive medication, %	21.9	21.7	17.4	26.2	23.7	23.9	23.9	22.5	19.6
Hypertension, %†	39.0	39.2	37.3	52.5	50.1	50.8	44.7	43.7	42.1
Body mass index, kg/m^2	23.4	23.1	23.5	23.5	23.8	23.7	23.5	23.4	23.5
Alcohol intake, g/day	1.0	1.3	2.2	18.6	20.9	18.9	7.8	9.3	8.7
Urine sodium excretion, mmol	172.3	176.4	178.4	182.0	199.2	199.1	176.1	185.7	186.4
Present sodium intake score	4.3	4.5	4.8*	5.6	5.4	5.4	4.8	4.8	5.0
Past sodium intake score	5.7	5.8	6.0	6.8	6.7	6.6	6.1	6.1	6.2

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

* $p<0.05$ and ** $p<0.01$ compared with CC genotype, ‡ $p<0.05$ compared with CT genotype (Tukey's multiple comparison method).

Table 2. Blood pressure levels according to G-protein β-3 subunit C825Tgenotype, men and women aged 30-74 years.

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	CC	CT	TT	p for difference of CC vs TT
Total	355	735	381	
Systolic blood pressure, mmHg	130.7	131.0	132.9	0.10
Diastolic blood pressure, mmHg	78.1	78.8	79.3	0.18
Men	137	299	148	
Systolic blood pressure, mmHg	134.1	134.0	136.5	0.29
Diastolic blood pressure, mmHg	81.2	80.8	82.1	0.62
Women	218	436	233	
Systolic blood pressure, mmHg	128.3	129.0	130.8	0.18
Diastolic blood pressure, mmHg	75.9	77.4	77.6	0.15

Blood pressure values were adjusted for sex, age, antihypertensive medication use, body mass index and alcohol consumption.

Tests for difference from CC genotype were conducted using Dunnett's multiple comparison method.

415 Table 3. Blood pressure levels according to *GNB3* C825T genotype, stratified by sodium excretion/intake, men and women aged 30-74 years.

	Women				Men				Total			
	CC	CT	TT	p for difference of CC vs TT	CC	CT	TT	p for difference of CC vs TT	CC	CT	TT	p for difference of CC vs TT
Stratified by urinary sodium excretion												
Below median	113	218	112		76	144	72		189	362	184	
Systolic blood pressure, mmHg	129.1	129.1	135.0	0.01	134.0	132.6	136.4	0.54	131.0	130.5	135.5	0.01
Diastolic blood pressure, mmHg	76.1	77.1	79.1	0.06	81.8	80.1	81.6	0.99	78.3	78.3	80.1	0.17
Median or more	105	218	121		61	155	76		166	373	197	
Systolic blood pressure, mmHg	127.5	128.8	126.9	0.93	134.3	135.3	136.5	0.55	130.3	131.4	130.5	0.99
Diastolic blood pressure, mmHg	75.7	77.8	76.1	0.95	80.4	81.4	82.6	0.28	77.7	79.2	78.5	0.61
Stratified by present sodium intake score												
Below median	109	229	104		65	146	78		174	375	182	
Systolic blood pressure, mmHg	129.6	130.5	133.0	0.23	135.4	136.8	138.3	0.39	131.9	132.9	135.1	0.11
Diastolic blood pressure, mmHg	75.6	78.1	78.1	0.13	82.0	81.2	83.2	0.67	78.1	79.4	80.0	0.13
Median or more	109	207	129		72	153	70		181	360	199	
Systolic blood pressure, mmHg	126.8	127.6	128.7	0.54	132.5	131.5	134.6	0.60	129.5	129.0	130.8	0.62
Diastolic blood pressure, mmHg	76.2	76.7	77.1	0.73	80.5	80.3	81.0	0.92	78.1	78.1	78.6	0.84
Stratified by past sodium intake score												
Below median	95	188	96		60	129	68		155	317	164	
Systolic blood pressure, mmHg	126.9	128.3	132.2	0.05	133.3	136.0	137.6	0.23	129.5	131.5	134.3	0.02
Diastolic blood pressure, mmHg	75.6	76.8	77.6	0.32	82.7	81.3	83.3	0.91	78.5	78.7	79.8	0.42
Median or more	123	248	137		77	170	80		200	418	217	
Systolic blood pressure, mmHg	129.5	129.3	130.0	0.95	135.0	132.4	135.3	0.99	131.7	130.5	131.9	0.99
Diastolic blood pressure, mmHg	76.2	77.8	77.6	0.41	80.4	80.3	81.2	0.82	77.9	78.8	79.0	0.44

Blood pressure was adjusted for sex, age, antihypertensive medication use, body mass index, alcohol consumption and year of urine collection. Tests for difference from CC genotype were conducted using Dunnett's multiple comparison method. Median values of urinary sodium excretion are 169.9 mmol/day for women and 185.2 mmol/day for 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.