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ORIGINAL ARTICLE

Association of interactions between dietary salt consumption and hypertension-susceptibility genetic polymorphisms with blood pressure among Japanese male workers

Takahiro Imaizumi[1] · Masahiko Ando[2] · Masahiro Nakatochi[2] · Shoichi Maruyama[1] ·
Yoshinari Yasuda[3] · Hiroyuki Honda[4] · Yachiyo Kuwatsuka[2] · Sawako Kato[1] ·
Takaaki Kondo[5] · Masamitsu Iwata[6] · Toru Nakashima[6] · Hiroshi Yasui[6] ·
Hideki Takamatsu[7] · Hiroshi Okajima[7] · Yasuko Yoshida[8] · Seiichi Matsuo[1]

Abstract

Background Blood pressure is influenced by hereditary factors and dietary habits. The objective of this study was to examine the effect of dietary salt consumption and single-nucleotide polymorphisms (SNPs) on blood pressure (BP).

Methods This was a cross-sectional analysis of 2728 male participants who participated in a health examination in 2009. Average dietary salt consumption was estimated using electronically collected meal purchase data from cafeteria. A multivariate analysis, adjusting for clinically relevant factors, was conducted to examine whether the effect on BP of salt consumption, SNPs, and interaction between salt consumption and each SNP. This study examined the SNPs *AGT* rs699 (Met235Thr), *ADD1* rs4961 (Gly460Trp), *NPPA* rs5063 (Val32Met), *GPX1* rs1050450 (Pro198Leu), and *AGTR1* rs5186 (A1166C) in relation to hypertension and salt sensitivity.

Results BP was not significantly associated with SNPs or salt consumption. The interaction between salt consumption and SNPs with systolic BP showed a significant association in *NPPA* rs5063 (Val32Met) ($P = 0.023$) and a marginal trend toward significance in rs4961 and rs1050450 ($P = 0.060$ and 0.067, respectively).

Conclusion The effect of salt consumption on BP differed by genotype. Dietary salt consumption and genetic variation can predict a high risk of hypertension.

Keywords Single nucleotide polymorphism · Hypertension · Sodium chloride · Dietary · NPPA

✉ Masahiko Ando
mando@med.nagoya-u.ac.jp

1 Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2 Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan

3 Department of CKD Initiatives, Nagoya University Graduate School of Medicine, Nagoya, Japan

4 Department of Biotechnology, Graduate School of Engineering, Nagoya University, Nagoya, Japan

5 Program in Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan

6 Safety and Health Promotion Division, Toyota Motor Corporation, Toyota, Japan

7 R & D management Division, Toyota Motor Corporation, Toyota, Japan

8 Innovative Research Center for Preventative Medical Engineering, Nagoya University, Nagoya, Japan

Introduction

Extensive evidence shows that dietary salt intake is an important factor in regulating blood pressure (BP). Both the Japanese Society of Hypertension and the WHO have outlined recommendations for daily salt intake (<6 and <5 g/day, respectively) [1, 2], however, the current average daily salt intake in Japan is still higher than recommended (10.4 g/day) [3]. Education regarding a low-salt diet plays an important role in preventing hypertension. In addition, the relationship between dietary salt intake and BP depends on salt sensitivity. Given that salt sensitivity increases with age [4] and that hypertension prevalence in the Japanese population starts to increase during middle age [5], it is important to examine these factors among the Japanese population.

BP is also affected by genetic polymorphism. Various types of single-nucleotide polymorphisms (SNPs), some that are linked to salt sensitivity [6–8] and others that are not [9], have been shown to be associated with hypertension. Some SNPs on the genes related to the renin-angiotensin system, insulin resistance, and natriuretic peptides have been shown to be associated with hypertension; however, the link between these SNPs and salt sensitivity has not been established.

To estimate daily salt intake among participants, we examined meal purchase data which are automatically recorded when an employee buys lunch in cafeteria of Toyota Motor Co., Ltd. The meal purchase data include the individual food contents and dietary elements, such as energy, salt, glucose, protein, and fat. Employees can browse their own data for a specified amount of time. The data were available for up to 6 months before the health examination, so the data are considered an unbiased reflection of an individual's salt intake. Previous research has used dietary interviews and measurement of urinary electrolytes to evaluate daily salt intake [10–12].

The aim of this study was to evaluate the association of the interaction between salt consumption and hypertension or salt-sensitive SNPs with BP among Japanese male workers in a cross-sectional study using data from annual health examinations.

Materials and methods

Study participants

This study is part of an ongoing observational prospective cohort study investigating metabolic syndrome and chronic kidney disease among male Japanese employees of Toyota Motor Co., Ltd. Study participants were eligible for inclusion in this study if meal data were available. Analysis using SNP data was performed among those who consented to DNA sampling. DNA samples were obtained from 2976 volunteers in 2011 or 2012. Finally, the sample for this study was restricted to 2728 individuals who provided consent for the study protocol (including SNP typing) and whose meal purchase data were available.

Lifestyle exposure

Lifestyle exposure was measured using self-administered questionnaires and included items regarding smoking, stress, exercise habits, and drinking habits. Smoking was defined as current smoking status. Stress was measured using a one-item question with a dichotomous yes/no response. Responses for drinking habits were divided into four categories (none, <3 days per week, 3–6 days per

week, and every day). Exercise habits were also divided into four categories (none, 1 day per week, 2–6 days per week, and every day).

The meal data, which were automatically recorded dietary elements, such as energy, salt, glucose, protein, and fat when an employee buys lunch in the cafeteria, were available to estimate the individual eating habits. Average energy, salt, glucose, protein, and fat consumption were calculated using data from 6 months before the health examination. The average salt consumption was calculated as the total salt consumption during the study period divided by the usage count of each individual. The median of average salt consumption was 5.1 g, so the study participants were categorized into two groups: one group comprised 1482 participants whose average salt consumption was >5 g (high salt group), and the other group comprised 1246 participants whose average salt consumption was ≤5 g (low salt group).

Clinical parameters

The health examinations performed in 2009 included physical measurements and serum biochemical measurements. Physical measurements, including height, weight, body mass index (BMI), and waist circumference were measured in the fasting state. BP was measured using an automatic sphygmomanometer (Kentaro HBP-9020; Omron, Tokyo, Japan) in a sitting position. Any BP reading >160/100 mmHg was confirmed by a mercury manometer measurement made by a physician. Hypertension was defined as systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg and/or under antihypertensive treatment and high-normal BP was defined as SBP ≥130 mmHg and/or DBP ≥85 mmHg and/or under antihypertensive treatment, in accordance with the guidelines of the Japanese Society of Hypertension [1].

SNP selection

In a previous study, we developed the SNP measuring system, including 99 candidate SNPs that were reported to be associated with coronary heart disease, hypertension, dyslipidemia, diabetes mellitus, hyperuricemia, renal disease, or obesity [13]. For this analysis, we selected five SNPs that were associated with salt sensitivity or hypertension: *AGT* rs699 (Met235Thr), *ADD1* rs4961 (Gly460Trp), *NPPA* rs5063 (Val32Met), *GPX1* rs1050450 (Pro198Leu), and *AGTR1* rs5186 (A1166C).

Genotyping SNPs

The DNA was anonymously extracted from the blood samples (0.2 mL each) at DNA Chip Research Inc.

(QIAamp® series; QIAGEN K.K., Tokyo, Japan). All SNP genotyping was performed using the DigiTag2 assay [14]. Target fragments, including target SNP sites, were prepared by multiplex PCR from genomic DNA. A multiplexed oligonucleotide ligation assay was performed, and a labeling reaction was achieved with two 5′ query probes and one common probe prepared for a single SNP site. The 5′ query probes had a sequence complementary to the 5′-flanking region of the target SNP, and each of the probes had an allele-specific sequence. Two types of end digit, CCGTGTCCACTCTAGAAAAACCT and ACCACCGCTTGAATACAAAACAT, were attached to each of the 5′ query probes. The 3′ query probes were designed to possess a sequence complementary to the 3′-flanking region of the target SNP, and each of the probes had a first digit (D1) on its 3′ end. Next, a hybridization reaction with D1 probes on a DNA microarray (NGK Insulators, Ltd., Nagoya, Japan) was performed with separated areas. The following primers were used for genotyping: *AGT* rs699 (Met235Thr): GCAAGGCACTTTGTTTCTCCGAGTCTCTATTTCTTCA (forward) and CTGGTGCTAGTCGCTGCAAAACTTGACACCGAAGA (reverse); *ADD1* rs4961 (Gly460Trp): CTTAAGCAGTTTCAAGCAGATAGTATTATCCGAATTTTAGTA (forward) and TACCCTTATCGATACCCTGCTCTGAGAGAGAAGTCTA (reverse); *NPPA* rs5063 (Val32Met): TTTCCATCCCCAGTTCCTCTTACCCGGAAGCTGTTA (forward) and TCCTTCTCCACCACCACCGTGAGCTTCCTCCTTTTA (reverse); *GPX1* rs1050450 (Pro198Leu): TCAGGTGTTCCTCCCTCGTAGGTTTAGAGGAAACA (forward) and CGGTGACTCATAGAAAATCTCCCTTGTTTGTGGTTAGAA (reverse); and *AGTR1* rs5186 (A1166C): ACGACTACTGCTTAGCATATCTTTGTACAAAATGTGCAGTA (forward) and TTTTGAGGTTGAGTGACATGTTCGAAACCTGTCCATAAA (reverse).

Primers, probe sequences, and PCR conditions for the remaining SNPs in the original cohort were reported elsewhere [13]. All laboratory technicians were blinded to the participants' identity, demographic characteristics, and study outcomes.

Statistical analysis

Baseline characteristics were summarized in medians and interquartile ranges (IQR) for quantitative variables (i.e., age, BMI, waist circumference, SBP, and DBP) and in numbers and percentages for qualitative variables (i.e., smoking, stress, exercise habits, and drinking habits). Between-group comparisons of clinical characteristics were performed using the Wilcoxon's rank-sum test for continuous variables and the Chi-squared test for categorical variables.

Allele frequencies were estimated by the gene-counting method, and the Fisher's exact test was used to identify any significant departure from Hardy–Weinberg equilibrium (HWE).

For each SNP, a score of 0, 1, or 2 was assigned depending on the number of effect alleles. Effect alleles were defined as the alleles shown to increase BP. For example, if A is the allele that confers higher risk, the genotype aa corresponds to zero-risk alleles, Aa corresponds to the presence of one risk allele, and AA corresponds to the presence of two risk alleles. The SNP score was treated as a continuous variable.

Linear regression was applied to examine the relationship between the dependent variables (SBP) and the independent variables (SNPs, salt consumption, and interaction between SNP and salt consumption) for each SNP. Finally, we adjusted for age, BMI, waist circumference, smoking, stress, exercise habits, and drinking habits. Considering the effect of antihypertensive therapy, we performed subgroup analysis in those who did not take antihypertensive medication. $P < 0.05$ was considered statistically significant. The data were analyzed using JMP® Pro 11.0.0 and Hardy–Weinberg package in R 3.1.2. [15].

Results

Baseline characteristics

The baseline characteristics of high salt group ($n = 1482$) and low salt group ($n = 1246$) are described in Table 1. High salt group had significantly higher BMI, abdominal circumference, dietary energy, fat, protein, alcohol consumption, and the proportion of current smokers. However, blood pressure and the proportion of hypertension and high normal blood pressure were not significantly different.

SNP characteristics

The SNPs examined in this study are shown in Table 2. Allele frequencies estimated by the gene-counting method did not show any significant departure from HWE.

Effects of salt consumption and SNPs on BP

Average salt consumption was not associated with SBP in the univariate analysis. There was also no association between any of the five SNPs and SBP in both univariate and multivariate analyses (S1 Table).

Association of the interaction between salt consumption and SNPs with BP

In the analysis of the interaction between average salt consumption and SNPs, the SNPs rs5063 and rs1050450

Table 1 Baseline characteristics of restricted and overall samples

Characteristics	Average salt consumption >5 g (n = 1482)	Average salt consumption ≤5 g (n = 1246)	P value
Age (years)	46 (41, 50)	46 (41, 52)	0.64
BMI (kg/m^2)	22.7 (20.9, 24.7)	22.3 (20.5, 24.4)	0.005*
Abdominal circumference (cm)	81.5 (76, 86.5)	80 (75, 85.2)	<0.001*
Systolic blood pressure (mmHg)	119 (109, 128)	119 (109, 128)	0.44
Diastolic blood pressure (mmHg)	75 (68, 82)	75 (68, 82)	0.82
Hypertension, n (%)	210 (14.2)	199 (16.0)	0.19
High-normal blood pressure, n (%)	431 (29.1)	379 (30.4)	0.45
Meal purchase data			
No. of meals	104 (88, 110)	105 (86, 111)	0.08
Average energy (kcal/meal)	791 (707, 881)	744 (680, 810)	<0.001*
Average fat (g/meal)	26.1 (21.7, 30.5)	24.8 (21.0, 28.2)	<0.001*
Average protein (g/meal)	28.6 (25.8, 32.0)	26.6 (23.8, 29.3)	<0.001*
Lifestyle			
Stress (yes), n (%)	1212 (81.8)	997 (80.0)	0.24
Exercise habits			0.21
Never	525 (35.4)	401 (32.2)	
1 day/week	346 (23.4)	286 (23.0)	
2–6 days/week	375 (25.3)	334 (26.8)	
Every day	236 (15.9)	225 (18.1)	
Alcohol drinking habits			<0.001*
Never	300 (20.2)	335 (27.0)	
Less than 3 days/week	794 (53.6)	622 (49.9)	
3–6 days/week	319 (21.5)	242 (19.4)	
Every day	69 (4.7)	47 (3.8)	
Current smoker, n (%)	646 (43.6)	380 (30.5)	<0.001*

Continuous data are median (IQR). Categorical data are n values (%)

The abbreviations of the characteristics: *BMI* body mass index

* *P* < 0.05

had a significant interaction with SBP ($P = 0.030$ and 0.029, respectively), and rs4961 had a marginally significant interaction with SBP ($P = 0.075$). After adjusting for age, BMI, abdominal circumference, stress, exercise habits, alcohol drinking, and smoking, rs5063 had a significant interaction with SBP ($P = 0.023$) and rs4961 and rs1050450 had a marginal trend toward significance ($P = 0.060$ and 0.067, respectively). See Table 3 for more information. After we divided the participants according to their salt consumption (>5 g or ≤5 g, which was the approximate mean dietary salt intake), we investigated the contribution of salt consumption to the change in BP for each genotype (Fig. 1). Figure 1 shows that SBP increased as salt consumption increased among those with GG genotype in *NPPA* rs5063.

Because we considered the effect of antihypertensive therapy, we excluded the 251 subjects taking antihypertensive medication. The reexamined results were identical (S2 Table).

In addition, we also examined the effect of SNPs with respect to the mode of inheritance. Because the minor allele frequency of *NPPA* rs5063 was very small, we included only the dominant mode and found the same results. SBP with GG was significantly higher than with GA+AA ($P = 0.015$).

Discussion

This study found a significant association of the interaction between salt consumption and *NPPA* rs5063 (Val32Met) with SBP.

Although there are several limitations, this study is unique that we estimated dietary salt consumption by meal purchase data to investigate the interaction between SNPs and dietary salt consumption. Genome-wide association studies have identified many genetic variations of diseases and traits, but most variants can explain only a small

Table 2 Characteristics and genotypes of SNPs

	Chr	Position	Reported gene	A/a	MAF (%)	Genotype			HWE (P value)
						AA	Aa	aa	
Salt sensitivity									
rs699	1	230,845,794	*AGT* rs699 (Met235Thr)	C/T	19.0	1787 (65.5 %)	844 (30.9 %)	97 (3.6 %)	0.901
rs4961	4	2,906,707	*ADD1* rs4961 (Gly460Trp)	T/G	45.3	816 (29.9 %)	1352 (49.6 %)	560 (20.5 %)	1.000
Hypertension									
rs5063	1	11,907,648	*NPPA* rs5063 (Val32Met)	G/A	9.3	2247 (82.4 %)	451 (16.5 %)	29 (1.1 %)	0.256
rs1050450	3	49,394,834	*GPX1* rs1050450 (Pro198Leu)	C/T	6.9	2365 (86.7 %)	350 (12.8 %)	12 (0.4 %)	1.000
rs5186	3	148,459,988	*AGTR1* rs5186 (A1166C)	A/C	8.1	2305 (84.5 %)	404 (14.8 %)	19 (0.7 %)	0.796

HWE *P* values were calculated by Fisher's exact test

SNP single-nucleotide polymorphism, *rs#* rs number, *Chr* chromosome, *A* major allele, *a* minor allele, *HWE* Hardy–Weinberg equilibrium, *MAF* minor allele frequency

Table 3 Association of interaction between each SNP and average salt intake with SBP

	Effect allele	EAF (%)	Model 1[a]		Model 2[b]		
			Coefficient (SE)	P value	Coefficient (SE)	P value	Adjusted P value[c]
Salt intake × rs699	T	19.0	−0.03 (0.35)	0.932	−0.13 (0.32)	0.688	1.00
× rs4961	T	45.3	0.47 (0.27)	0.075	0.45 (0.24)	0.060	0.30
× rs5063	G	90.7	0.98 (0.45)	0.030*	0.92 (0.41)	0.023*	0.12
× rs1050450	T	6.9	1.14 (0.52)	0.029*	0.86 (0.47)	0.067	0.34
× rs5186	C	8.1	−0.22 (0.50)	0.666	−0.17 (0.45)	0.701	1.00

SNP single-nucleotide polymorphism, *DBP* diastolic blood pressure, *EAF* effect allele frequency, *SBP* systolic blood pressure, *SE* standard error

* $P < 0.05$

[a] Adjusted for average dietary salt intake and each SNP

[b] Adjusted for age, body mass index, abdominal circumference, stress, exercise habits, alcohol drinking, and smoking, as well as average dietary salt intake and each SNP

[c] *P* value adjusted with Bonferroni correction for multiple comparisons

proportion of familial clustering [16]. One solution to the remaining "missing" heritability is considering gene-environment interactions, such as the effect of the interaction between SNPs and salt intake with BP.

For example, we found a significant association of the interaction between *NPPA* rs5063 (Val32Met) and salt consumption with BP. *NPPA* is a precursor of atrial natriuretic peptide, which plays a significant role in the regulation of vascular tone and sodium homeostasis [17]. In addition, the A allele of the *NPPA* rs5063 (Val32Met) genotype has shown a protective effect for BP progression and incident hypertension in the previous studies [18, 19]. SNP rs5063 does not affect the plasma level of ANP; however, it might influence the protein structure, since this

polymorphism is within exon 1 of NPPA gene and changes the coding sequence which causes substitution of amino acid. This study, however, is the first to report that *NPPA* rs5063 (Val32Met) polymorphism is associated with salt sensitivity. With the finding of a significant association of the interaction between salt consumption and rs5063 with SBP in Japanese men, this study adds to the evidence regarding gene-environment interactions.

In this study, we found a marginal trend toward significance of the interaction between salt consumption and two SNPs with SBP. One of them is *ADD1* rs4961 (Gly460Trp), reported as a common salt-sensitivity SNP in the Japanese population [20]. Systematic reviews and meta-analyses have shown a significant association
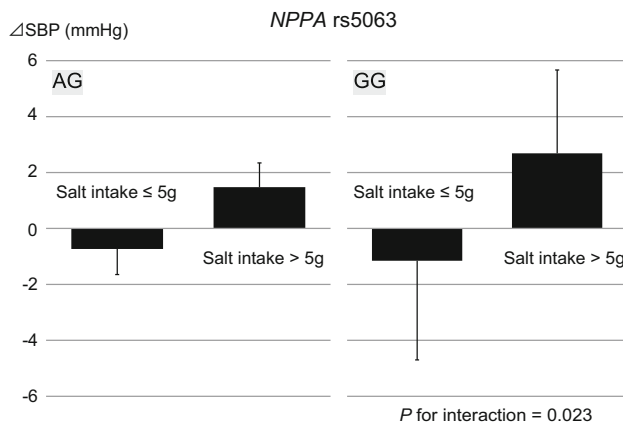
Fig. 1 Effect of interaction between salt consumption and risk alleles of *NPPA* rs5063 on SBP. The bar graphs show the change of SBP according to salt consumption ≤5 g and >5 g among those with AG (*left*) and among those with GG (*right*), respectively. The reference was AA. *Error bars* represent standard errors. *P* for interaction = 0.023

between the Trp460 allele of *ADD1* and hypertension in Asian people [21–23]. The other one is *GPX1* rs1050450 (Pro198Leu). BP has been shown to be significantly higher for men with the CT (an allele of *GPX1*) or the combined CT and TT genotypes than for those with the CC genotype in prior study [24]. This was also first to show the marginally significant association of the interaction between salt consumption and *GPX1* rs1050450 (Pro198Leu) with BP.

Although the frequency of the T allele of the *AGT* rs699 (Met235Thr) is high in the Japanese population and *AGT* rs699 (Met235Thr) is known as one of the most common salt-sensitivity SNPs in the Japanese population [20], this study did not demonstrate a significant interaction. A similar study by Takeuchi et al. examining the association of selected candidate gene variants and BP traits revealed that *AGT* rs699 (Met235Thr) is a hypertension-susceptibility SNP in the Japanese population; however, the authors were similarly not able to show an association between *ADD1* rs4961 (Gly460Trp) and hypertension or BP traits [25].

While some studies have demonstrated an increased frequency of the C variant of *AGTR1* rs5186 (A1166C) among hypertensive individuals [26–29], no significant association with hypertension or salt sensitivity has been found in the Japanese population [6, 30]. Similarly, this study did not show an interaction with salt consumption, adding further support to the existing evidence. The polymorphism of this SNP may not be responsible for hypertension or salt sensitivity in the Japanese population.

Dietary salt intake has been estimated using urinary chemistry (spot urine test or urinary collection test) and/or dietary interviews [10–12]. However, spot urine tests are simple to administer but unreliable, whereas urinary

collection tests are highly reliable, but so complicated that they are not suitable for a general health examination. Similarly, dietary interviews are convenient but may be susceptible to a recall bias [31, 32]. Participants tend to underestimate their salt intake. It is also possible that the participants may change their lifestyle during the study period, which may not be reflected in either of the previously discussed methods. However, in this study, we used meal purchase data to estimate average the dietary salt consumption for up to 6 months prior the health examination, which, therefore, may better reflect a participant's daily diet. However, there are several limitations in this method. First, we collected data only on meals served for lunch, and the data do not include any kinds of food away from the work place. Second, these data are based on the assumption that the employees finish their meals and do not use condiments, such as additional salt. This could lead to under- or over-reporting of average daily salt intake. In the data, median estimated salt consumption was approximately 5 g, so the data may have overestimated the actual salt intake. Third, all employees do not use the cafeteria on a regular basis. To evaluate the salt consumption which was assumed by meal purchase data, they should have been validated in a different way, such as urine test, but we could not get the permission for gathering urinary samples from the employees. However, the mean number of meals in the cafeteria was over 90 per participant during the study period. Therefore, we can assume that the participants had lunch in the cafeteria on a regular basis.

Limitation of this study is the possibility of a sampling bias and limited sample size. Considering the modest effects of blood pressure susceptibility loci, a larger number of samples would be desirable. Because participation in the study was based on the willingness of individuals, the number of the study participants was limited. New findings have been accumulated, since we started this research, so the selection of SNPs was also limitation. Finally, the cross-sectional nature of this study limits our ability to estimate the longitudinal effects as well as causality of the interaction between salt intake and SNPs.

We demonstrated that individuals who have the high-risk allele SNPs of *NPPA* rs5063 (Val32Met) had high BP under high salt consumption. In conclusion, we recommend that participants with such high-risk genotypes restrict their salt intake. Furthermore, additional longitudinal research is needed to establish the incidence of hypertension and cardiovascular events.

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