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Lifestyle intervention might easily improve blood pressure in hypertensive men with the C genotype of angiotensin II type 2 receptor gene

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BACKGROUND/OBJECTIVES: Recent studies have reported an association of the angiotensin II type 2 receptor (*AT2R*) 3123Cytosine/Adenine (3123C/A) polymorphism with essential hypertension and cardiovascular diseases. The purpose of the study was to investigate whether the *AT2R* 3123C/A polymorphism affects blood pressure for free-living hypertensive men during a 5-month intervention period.
SUBJECTS/METHODS: The subjects were free-living hypertensive Japanese men aged 40 to 75 years who agreed to intervention in the period from 2004 to 2011. Detection of the *AT2R* 3123C/A polymorphism was determined by polymerase chain reaction. The dietary intervention was designed to decrease salt level and to increase potassium level through cooking instructions and self-monitoring of the diet. The exercise session consisted of activities such as stretching, resistance training, and walking. Blood pressure, urinary sodium and potassium excretion, dietary and lifestyle data, and non-fasting venous blood sample were collected at baseline and after the intervention period.
RESULTS: Thirty nine subjects were eligible for participation and the follow-up rate was 97.4%. The C allele proportion was 57.9%. *AT2R* 3123C/A polymorphism was X-chromosome-linked, therefore we analyzed the C and A genotypes. At baseline, no significant differences were observed between the genotype groups. After the intervention, there were no significant differences in lifestyle habit between the groups. Nevertheless, the estimated salt excretion (g/day) was significantly decreased only in the C genotype (13.0-10.3, $P = 0.031$). No significant change was observed in systolic blood pressure (SBP) (mmHg) in the A genotype, but a significant decrease was observed in the C genotype (150.0-141.5, $P = 0.024$).
CONCLUSTIONS: In the C genotype, it might be easy to improve SBP through lifestyle intervention in free-living hypertensive Japanese men, however generalization could not be achieved by the small sample size.

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INTRODUCTION

Blood pressure is independently predictive of cardiovascular disease (CVD) and stroke mortality. High blood pressure is regarded as a complex condition to which genetic, environmental, and demographic factors contribute interactively [1]. The environmental factors include a combination of lifestyle, dietary, and psychological exposures; and genetic mechanisms depend on numerous gene-gene and gene-environment interactions. Innumerable studies have indicated a wide variety of factors contributing to blood pressure variations, including, for example, age, sex, diet, weight, obesity, serum lipids, extra-cellular sodium and potassium balance, as well as gene polymorphisms, involved in blood pressure regulation [2-7]. Thus, hypertension is a multifactorial disease, and control of salt intake is also an important preventive method. Particularly in men, a positive relationship of dietary salt intake to blood pressure was observed in a large-scale representative Japanese population [8]. Previous studies have shown that lifestyle modifications and effective and feasible health education programs at the community level can reduce the risk of hypertension [9-13]. We reported that the urine sodium to potassium excretion ratio improved significantly focusing on cooking instructions and self-monitoring of the diet in hypertensive Japanese men [14].

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The renin-angiotensin system (RAS) plays an important role in regulation of blood pressure and cardiovascular remodeling by sodium and water homeostasis [15]. Most of the biologic actions of angiotensin II (AT) are thought to be mediated by the AT type 1 receptor, however evidence is beginning to emerge that the AT type 2 receptor (*AT2R*) plays a significant role in regulation of blood pressure [16]. To date, associations between the 3123Cytosine/Adenine (3123C/A) polymorphism (in the 3' untranslated region of exon 3 of the X-chromosome-located *AT2R* gene) and essential hypertension, myocardial infarction, as well as CVD have been reported [17-19]. However, the genetic effects of RAS polymorphisms on improvement of blood pressure through lifestyle modifications after dietary and exercise intervention have not been clarified in free-living hypertensive men. Therefore, the purpose of the current study was to investigate whether 3123C/A of the *AT2R* gene affects blood pressure and second-morning spot urinary excretion of salt through a dietary and exercise intervention program employing cooking instructions and self-monitoring of diet and exercise in free-living hypertensive Japanese men. It was hypothesized that this polymorphism of the *AT2R* gene may contribute to the individual variation in blood pressure and urinary excretion of salt after the lifestyle intervention.

SUBJECTS AND METHODS

Subjects

The subjects were free-living men, aged 40-75 years. The eligibility criteria for this study were as follows: systolic blood pressure (SBP) $\geq$ 130 mmHg and < 180 mmHg or diastolic blood pressure (DBP) $\geq$ 85 mmHg and < 110 mmHg. Hypertension is defined SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg by guidelines for management of hypertension 2014 (JSH2014), and persons with high-normal blood pressure were included because of the excess risk for CVD in patients with blood pressure within this range [20,21]. The exclusion criteria were the use of antihypertensive drugs, any previous cardiovascular events, congestive heart failure, or cancer. The study protocol was clearly explained to all participants and written informed consent was obtained from all participants before they were enrolled in the study. Ethical approval for the study was obtained from the Ethical Committee of Kyoto Prefectural University (May 19, 2004. no.1).

Intervention program

The lifestyle intervention program was conducted by the training course for registered dietitians at Kyoto Prefectural University, from 2004 to 2011. The participants attended the sessions, which were held once a month for a total of 5 times, for 4 hours each session. The program consisted of a lecture and cooking instruction conducted by registered dietitians and doctors. The diet featured increased consumption of fruit (2 servings/d), vegetables ($\geq$ 5 servings/d), and dairy products (2 servings/d), as well as moderation in drinking alcohol. Participants cooked lunches themselves three times and took lunch together with staff members five times. We instructed the participants on how to prepare a meal appropriately (energy; 600-700 kcal, vegetables; $\geq$ 2 servings, fruit; = 1 serving, salt; 2-3 g per meal). To decrease salt intake, the participants were taught how to measure seasoning. In particular, the salinity of soup was measured to be 0.6-0.8 %. The exercise session was conducted by exercise instructors, and consisted of activities such as stretching, resistance training, and walking. Participants monitored their walking activity using a pedometer. We set a goal for walking of more than 10,000 steps per day.

Throughout the intervention program, all of the participants kept records of their diets and physical activity diaries. The diet was recorded more than once a week and walking steps were recorded every day. Self-monitoring was used to provide individualized feedback, reinforcement and problem solving.

Methods

Anthropological data were also collected at baseline and after the intervention period from all participants. Each participant's height, weight, and waist circumference were measured. Body mass index (BMI) was calculated using the equation: weight (kg) / height (m^2). Blood pressure was measured by trained doctors using a mercury sphygmomanometer (Yagami Inc., Tokyo, Japan). The participants were asked to sit calmly for 5-10 minutes before the measurements. These blood pressure values were measured twice and the mean value was calculated for each subject. The non-fasting venous blood samples were collected with the participants in a sitting position. The sodium and potassium concentrations were analyzed by flame photometry from the second-morning spot urinary samples. Kawasaki's formula [22] was employed for estimation of salt and potassium excretion from the height, body weight, age, and urinary creatine concentration of the participants.

At baseline and after the intervention period, we evaluated the dietary intake of the participants during the previous 1-2 months using a self-administered food frequency questionnaire (FFQ) [23]. This FFQ included 28 food items, and the participants indicated their frequency of consumption by checking 1 of 6 frequency categories, ranging from "never" to "2 or more times/day." In addition to food frequency, the questionnaire included 22 dietary habit items, 4 drinking habit items, 4 exercise habit items, as well as questions on the physical, lifestyle, smoking state, and current medical treatment for the participants. A regular exerciser was defined as a participant with an exercise frequency of more than 2 times per week.

Determination of genetic polymorphisms

Detection of the *AT2R* 3123C/A polymorphism was determined by polymerase chain reaction (PCR). PCR was performed to amplify a fragment encompassing the C/A polymorphic site at the 3123 nucleotide position in the 3' untranslated region of the human *AT2R* gene. The design of the primers was as follows: sense, 5'-GGA TTC AGA TTT CTC TTT GAA-3'; antisense, 5'-GCA TAG GAG TAT GAT TTA ATC-3'. The 50 μl reaction volume contained 100 ng genomic DNA, 10 pmol of each primer, 200 μmol / l dNTP, 1.5 mmol / l MgCl2, 50 mmol / l KCl, 10 mmol / l Tris-HCl at pH 8.3, and 1.25 units of Taq polymerase (TAKARA BIO INC., Shiga, Japan). Amplification was performed using a Thermal Cycler (Perkin Elmer INC., Massachusetts, USA). After initial denaturation at 96°C for 2 min, PCR was performed for 5 cycles, each one comprised of denaturation at 96°C for 40 seconds, annealing at 60°C for 50 seconds and extension at

72℃ for 30 seconds followed by 25 cycles of denaturation at 96℃ for 40 seconds, annealing at 55℃ for 50 seconds and extension at 72℃ for 30 seconds. After confirming DNA amplification, 17.5 μl of the PCR product was digested with 5 units of Alu I for 3 hours at 37℃, then electrophoresed on 3% agarose gel with ethidium bromide staining and visualized in a UV transilluminator.

Statistical Analysis

The results were presented as median (Interquartile range) or percent. *AT2R* 3123C/A polymorphism was X-chromosome-linked, thus we analyzed the C and A genotypes [24]. Differences in distributions between the genetic type groups at baseline and after the intervention were determined using Mann-Whitney U test or Fisher's exact test. Differences from the baseline and after the intervention within the groups were determined using Wilcoxon signed rank sum test. All statistical analyses were performed using SPSS for Windows, version 21 (IBM INC., Tokyo, Japan). *P* values < 0.05 were considered statistically significant.

RESULTS

A total of 39 subjects were eligible for participation in the lifestyle intervention program and the follow-up rate was 97.4%. The proportion of the C allele in the *AT2R* gene was 57.9%. Table 1 shows the comparison of baseline CVD risks and lifestyle factors according to the *AT2R* genotype. There were no differences between the two genotype groups.

Changes in lifestyle behaviors

Table 2 shows the comparison of changes in dietary habits between the C genotype and the A genotype. At baseline, there were no differences between the two genotype groups. Salt restriction awareness after the intervention improved significantly in both genotypes. In the C genotype, the habit of drinking

Table 1. Comparison of baseline cardiovascular disease risk factors and lifestyle factors between the C genotype and the A genotype groups for hypertensive Japanese men.

	C genotype (n = 22)	A genotype (n = 16)	*P* value
Age (year)	66.0 (62.8, 69.0)	69.0 (63.0, 71.8)	0.468[1)]
Body weight (kg)	62.1 (58.4, 69.1)	66.7 (58.6, 70.1)	0.344[1)]
BMI (kg/m^2)	23.7 (21.7, 24.5)	24.3 (21.9, 25.8)	0.359[1)]
BMI > 25 (%)	13.6	37.5	0.128[2)]
Body fat (%)	22.0 (19.1, 24.3)	22.5 (16.7, 25.6)	0.976[1)]
Waist circumference (cm)[3)]	84.5 (81.7, 88.0)	90.7 (81.6, 96.8)	0.221[1)]
Systolic blood pressure (mmHg)	150.0 (145.5, 162.0)	147.0 (136.3, 162.8)	0.487[1)]
Diastolic blood pressure (mmHg)	92.0 (89.5, 94.3)	95.0 (93.0, 100.0)	0.203[1)]
Dietary behavior			
Be regular in time	72.7	68.8	0.792[1)]
Preferred strong-taste	13.6	31.3	0.937[1)]
Sodium restriction consciousness (Yes)	36.4	37.5	0.742[1)]
Frequency of convenience foods (> 3 times/week)	18.2	12.5	0.829[1)]
Frequency of eating out (> 3 times/week)	4.5	25.0	0.724[1)]
Frequency of vegetables intake (> 2 times/day)	45.5	25.0	0.257[1)]
Housework (No)	59.1	68.8	0.780[1)]
Health perception			
Good health condition	68.2	50.0	0.244[1)]
Breath-fresher (Yes)	40.9	31.3	0.621[1)]
Life satisfaction (Yes)	36.4	25.0	0.747[1)]
Habitual drinker (> 5 times/week)	45.5	43.8	0.436[1)]
Smoking			
Current smoker	9.1	6.3	0.535[1)]
Former smoker	68.2	62.5	
Never	22.7	31.3	
Physical activity			
Regular exerciser (> 2 times/week)	50.0	31.3	0.113[1)]
Walking (> 30 min/day)	72.7	50.0	0.174[1)]
Medical treatment			
Dyslipidemia	22.7	6.3	0.370[2)]
Diabetes	4.5	6.3	1.000[2)]

The data are expressed as the median (interquartile range) or %. BMI: body mass index
[1)] Comparison between groups were investigated using Mann-Whitney's *U* test.
[2)] Comparison between groups were investigated using Fisher's exact test.
[3)] Waist circumference was measured 10 participants in the C type group and 9 participants in the A type group.

Table 2. Comparison of changes in dietary habits between the C genotype and the A genotype groups for hypertensive Japanese men

	C genotype (n = 22)			A genotype (n = 16)			*P* value[2]	
	Baseline	After	*P* value[1]	Baseline	After	*P* value[1]	Baseline	After
Salt restriction awareness			0.021			0.025	0.742	0.972
Usually	36.4	63.6		37.5	62.5			
Sometimes	50.0	31.8		56.3	37.5			
Rarely	13.6	4.5		6.3	0.0			
Seasoning			0.414			0.655	0.937	0.949
Light	27.3	40.9		43.8	56.3			
Rich	13.6	18.2		31.3	37.5			
No opinion	59.1	40.9		25.0	6.3			
Use soy sauce and salt at the table			0.132			0.257	0.642	0.221
Often	4.5	4.5		12.5	0.0			
Sometimes	40.9	18.2		37.5	43.8			
Rarely	54.5	77.3		50.0	56.3			
Drink Japanese noodle soup			0.034			0.102	0.922	0.867
All	22.7	9.1		31.3	12.5			
Half	59.1	59.1		43.8	56.3			
None	18.2	31.8		25.0	31.3			
Speed of eating			0.157			0.564	0.341	0.281
Fast	36.4	40.9		37.5	37.5			
Normal	54.5	54.5		25.0	31.3			
Slow	9.1	4.5		37.5	31.3			

The data are expressed as the %.
[1] Differences from baseline and after the intervention within the groups were investigated using Wilcoxon's signed rank sum test.
[2] Differences of distributions for the groups at baseline and after the intervention were investigated using Mann-Whitney's *U* test.

Table 3. Comparison of changes in food frequency between the C genotype and the A genotype groups for hypertensive Japanese men.

	C genotype (n = 22)			A genotype (n = 16)			*P* value[2]	
	Baseline	After	*P* value[1]	Baseline	After	*P* value[1]	Baseline	After
Preserved vegetables			0.037			0.484	0.622	0.819
More than twice a day	4.5	4.5		0.0	0.0			
Almost everyday	22.7	0.0		25.0	25.0			
3 to 5 times a week	40.9	45.5		37.5	25.0			
1 to 2 times a week	13.6	18.2		12.5	18.8			
1 to 2 times a month	13.6	22.7		12.5	6.3			
Rarely	4.5	9.1		12.5	25.0			
Soup			0.164			0.058	0.620	0.747
More than twice a day	0.0	4.5		12.5	6.3			
Almost everyday	50.0	40.9		50.0	37.5			
3 to 5 times a week	36.4	22.7		12.5	12.5			
1 to 2 times a week	13.6	18.2		6.3	25.0			
1 to 2 times a month	0.0	4.5		12.5	12.5			
Rarely	0.0	9.1		6.3	6.3			
Salted fish			0.184			0.931	0.421	0.950
More than twice a day	0.0	0.0		0.0	0.0			
Almost everyday	4.5	9.1		12.5	0.0			
3 to 5 times a week	18.2	18.2		12.5	31.3			
1 to 2 times a week	27.3	36.4		25.0	18.8			
1 to 2 times a month	45.5	36.4		37.5	37.5			
Rarely	4.5	0.0		12.5	12.5			
Alcohol drinking			0.163			1.000	0.436	0.636
More than 5 times a week	45.5	45.5		43.8	50.0			
3 to 4 times a week	4.5	9.1		12.5	0.0			
1 to 2 times a week	13.6	18.2		25.0	31.3			
1 to 2 times a month	9.1	9.1		18.8	18.8			

Table 3. continued

	C genotype (n = 22)			A genotype (n = 16)			*P* value[2]	
	Baseline	After	*P* value[1]	Baseline	After	*P* value[1]	Baseline	After
former	18.2	9.1		0.0	0.0			
Never	9.1	9.1		0.0	0.0			
Green and yellow vegetables			0.593			0.448	0.090	0.404
More than twice a day	13.6	9.1		6.3	12.5			
Almost everyday	50.0	54.5		31.3	31.3			
3 to 5 times a week	27.3	22.7		37.5	37.5			
1 to 2 times a week	9.1	13.6		25.0	18.8			
Other vegetables			0.805			0.070	0.029	0.665
More than twice a day	9.1	13.6		6.3	12.5			
Almost everyday	59.1	50.0		25.0	43.8			
3 to 5 times a week	27.3	31.8		43.8	37.5			
1 to 2 times a week	0.0	4.5		25.0	0.0			
1 to 2 times a month	4.5	0.0		0.0	6.3			
Fruit			0.439			0.124	0.519	0.418
More than twice a day	13.6	9.1		6.3	18.8			
Almost everyday	50.0	59.1		56.3	56.3			
3 to 5 times a week	27.3	13.6		12.5	12.5			
1 to 2 times a week	9.1	13.6		25.0	12.5			
Less than a month	0.0	4.5		0.0	0.0			
Potatoes			0.593			0.861	0.402	0.987
Everyday	9.1	9.1		6.3	6.3			
3 to 5 times a week	36.4	22.7		25.0	31.3			
1 to 2 times a week	40.9	59.1		50.0	50.0			
1 to 2 times a month	13.6	9.1		18.8	0.0			
Rarely	0.0	0.0		0.0	12.5			

The data are expressed as the %.
[1] Differences from baseline and after the intervention within the groups were investigated using Wilcoxon's signed rank sum test.
[2] Differences in the distributions for the groups at baseline and after the intervention were investigated using Mann-Whitney's *U* test.

Table 4. Comparison of the average steps between the C genotype and the A genotype groups for hypertensive Japanese men.

	C genotype (n = 22)	A genotype (n = 16)	*P* value[1]
Period 1	8,875 (7,989, 11,813)	9,472 (6,908, 11,023)	0.642
Period 2	8,233 (7,321, 11,815)	8,260 (7,409, 10,295)	0.686
Period 3	8,937 (8,054, 12,502)	8,989 (7,100, 10,631)	0.439
Period 4	9,069 (7,530, 12,562)	8,803 (7,804, 10,154)	0.688

The data are expressed as the median (interquartile range).
[1] Differences in the distributions for the genotype groups were investigated using Mann-Whitney's *U* test.

Japanese noodle soup showed a significant decrease after the intervention. After the intervention, no differences in dietary habits were observed between the two genotype groups.

Table 3 shows the comparison of changes in food frequency related to hypertension. In the C genotype, the intake frequency of preserved vegetables decreased significantly after the intervention. In the A genotype, a lowered tendency in the frequency of soup intake was shown after the intervention. In the A genotype, although the frequency of intakes of other vegetables was lower compared with the C genotype at baseline, an increased tendency was shown after the intervention. After the intervention, no differences in food frequency items were observed between the two genotype groups.

Relating to the physical activity, no differences were observed between the two genotype groups at baseline. Table 4 shows a comparison of the average steps between the C genotype and the A genotype. In regard to the recording of the numbers of steps taken, the average number of steps showed no significant difference throughout the period between the two genotype groups.

Changes in CVD risk factors

Table 5 shows a comparison of changes in CVD risk factors between the C genotype and the A genotype groups. No significant change was observed after the intervention in the A genotype group, but BMI (kg/m^2), body fat (%) and low density lipoprotein (LDL)-cholesterol (mg/dl) showed a significant decrease in the C genotype group. In addition, the estimated urinary salt excretion (g/day) was significantly decreased in the C genotype group. Regarding blood pressure, the DBP, a

Table 5. Comparison of changes in cardiovascular disease risk factors between the C genotype and the A genotype groups for hypertensive Japanese men.

	C genotype (n = 22)		*P* value[1]	A genotype (n = 16)		*P* value[1]	Difference in change[2]	*P* value[3]
	Baseline	After		Baseline	After			
Body weight (kg)	62.1 (58.4, 69.1)	61.1 (57.1, 66.7)	0.001	66.7 (58.6, 70.1)	65.5 (58.1, 68.8)	0.205	-0.6 (-1.8, 0.1)	0.225
BMI (kg/m^2)	23.7 (21.7, 24.5)	23.1 (21.3, 24.1)	0.001	24.3 (21.9, 25.8)	23.9 (21.6, 25.4)	0.148	-0.28 (-0.68, -0.03)	0.375
Body fat (%)	22.0 (19.1, 24.3)	21.1 (19.6, 22.6)	0.039	22.5 (16.7, 25.6)	20.9 (18.8, 23.1)	0.485	-0.6 (-1.9, 0.8)	0.723
Waist circumference (cm)[4]	84.5 (81.7, 88.0)	83.4 (80.4, 87.3)	0.113	90.7 (81.6, 96.8)	87.4 (81.1, 97.4)	0.441	-0.9 (-2.4, 0.5)	0.683
Systolic blood pressure (mmHg)	150.0 (145.5, 162.0)	141.5 (134.5, 154.3)	0.024	147.0 (136.3, 162.8)	146.5 (134.8, 165.0)	0.796	-6.5 (-12.5, 7.8)	0.084
Diastolic blood pressure (mmHg)	92.0 (89.5, 94.3)	85.0 (81.5, 92.8)	0.014	95.0 (93.0, 100.0)	89.0 (83.5, 98.8)	0.060	-5.0 (-9.3, 2.0)	1.000
HDL-cholesterol (mg/dl)	59.4 (49.3, 67.7)	60.2 (45.7, 73.0)	0.355	76.2 (52.2, 92.1)	80.6 (51.8, 91.9)	0.518	0.4 (-3.3, 8.9)	0.690
LDL-cholesterol (mg/dl)	126.0 (109.5, 147.3)	125.0 (96.5, 147.8)	0.021	121.0 (107.0, 133.0)	114.0 (104.5, 129.0)	0.178	-5.5 (-15.0, 1.3)	0.657
Estimated salt excretion (g/day)[5]	13.0 (11.0, 15.2)	10.3 (9.2, 14.4)	0.031	12.2 (11.2, 16.7)	12.8 (9.8, 15.1)	0.408	-2.0 (-4.2, 1.3)	0.460
Urine sodium to potassium ratio	2.6 (1.6, 3.8)	1.9 (1.4, 3.1)	0.140	2.5 (1.7, 3.8)	1.9 (1.3, 3.3)	0.121	-0.4 (-1.1, 0.3)	0.767

The data are expressed as the median (interquartile range).
[1] Differences from the baseline and after the intervention within groups were investigated using Wilcoxon's signed rank sum test.
[2] Difference = covariance of the C genotype group-covariance of the A genotype group
[3] Differences in the distributions for the genotype groups were investigated using Mann-Whitney's *U* test.
[4] Waist circumference was measured 10 participants in the C genotype group and 9 participants in the A genotype group.
[5] Values were calculated using Kawasaki's formula.

significant decrease was observed in the C genotype group and a tendency for a decrease was demonstrated in the A genotype group. No significant change in SBP was observed in the A genotype group, but a significant decrease was observed in the C genotype group. The net difference in the change between the two groups was -6.5 ($P = 0.084$).

DISCUSSION

The purpose of the current study was to investigate whether 3123C/A of the *AT2R* gene affects blood pressure and second-morning spot urinary excretion of salt through the lifestyle intervention program employing cooking instructions and self-monitoring of the diet and exercise in free-living hypertensive men during a 5-month intervention program. At baseline, no difference in the dietary and physical status was observed between the C and the A genotype groups. After the intervention, the awareness of the importance of restricting the intake of salt was significantly improved in both genotype groups. In addition, we monitored physical activity levels using a pedometer and confirmed that the numbers of steps were stable from baseline until the end of the intervention in all subjects. Nevertheless, urinary excretion of salt, SBP, BMI, and body fat were significantly improved only in the C genotype group.

Recent studies have reported an association of the 3123C/A polymorphism in the *AT2R* gene with essential hypertension and CVD [17-19]. 3123A allele carriers of the *AT2R* gene showed elevated blood pressure and increased vasopressor response to the injection of angiotensin II in mice [25,26]. It has been shown that expression of the *AT2R* gene can influence the regulation of blood pressure, although the mechanisms of the pathophysiological effects of *AT2R* and related genes have remained unclear, particularly in humans. Hamada *et al*. [27] reported that with subjects in the C genotype group, it was easier to improve SBP and lipid profiles through weight loss intervention in obese women. Thus, it may be more effective for improvement of urinary excretion of salt, SBP, BMI, and body fat for subjects in the C genotype group than those in the A genotype group by employing a lifestyle intervention program for free-living hypertensive men.

Regarding salt sensitivity, it has been reported that several genes or a combination of genes may be identified by salt sensitivity [28]. In this study, estimated urinary salt excretion and blood pressure were significantly improved only in the C genotype group. Thus there is a possibility that the C genotype is a marker of salt sensitivity. And, in the A genotype, because the ability of salt excretion is low, water and sodium might be retained in the body. However we could not clarify the mechanism, therefore further study is desired.

There were several limitations included in the current study. First, the participants were volunteers, suggesting that they were probably more health-conscious than the general population. Second, the evaluation of the blood pressure was performed using clinical blood pressure values. In general, the home blood pressure value shows a better relationship to mortality risk, compared with screening blood pressure values [29]. Third, the evaluations of the estimated salt and potassium excretions were performed using second morning spot urine samples. The method of collecting urine samples for 24 hours is considered to be most reliable, and thus it is used in many clinical and epidemiological studies. However, collection of 24-hour urine sampling is relatively difficult. Measurements performed using the second-morning spot urine sample method have been closely correlated with the values determined in 24-hour pooled urine [30]. In addition, the net difference in the change between the two groups was not statistically significant by the small sample size, thus further studies employing larger sample sizes may be necessary.

The lifestyle intervention may be more effective for improvement of urinary excretion of salt, SBP, BMI, and body fat for subjects in the C genotype group, compared with the A genotype group. The findings suggested that the *AT2R* gene contributes to the individual variation after the intervention in free-living hypertensive Japanese men, however generalization could not be achieved by the small sample size.

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