Original Article

Combined Analysis of Polymorphisms in Angiotensinogen and Adducin Genes and Their Effects on Hypertension in a Japanese Sample: The Shigaraki Study

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We examined the interactions between lifestyle and polymorphisms of salt-sensitive genes and their effects on hypertension in a general Japanese sample (The Shigaraki Study). The study group consisted of 2,902 subjects who underwent a medical examination in 1999 in Shigaraki, a suburban area in Shiga. Among 1,647 subjects not receiving antihypertensive medication, in a combined analysis of angiotensinogen (AGT) and adducin (ADD1) polymorphisms, double homozygosity of 235Thr or 460Trp was not found to be associated with hypertension. A multiple logistic regression analysis showed that age (odds ratio [OR]: 1.07, 95% confidence interval [95% CI]: 1.06–1.08), body mass index (BMI) (OR: 1.18, 95% CI: 1.13–1.23), alcohol consumption (OR: 1.39, 95% CI: 1.16–1.66), family history of hypertension (OR: 1.57, 95% CI: 1.18–2.07), and combined AGT M235T Thr/Thr and ADD1 Trp/Trp polymorphisms (OR: 1.37, 95% CI: 1.03–1.82) were associated with hypertension. However, there was no interaction between eating salty food and combined AGT and ADD1 polymorphisms. Furthermore, eating salty food was not associated with hypertension in a multivariate analysis. Therefore, a combination of the AGT and ADD1 polymorphisms appears to be associated with hypertension. However, a simple questionnaire regarding salt intake was not sufficient to confirm the relationship between salt intake and hypertension and/or salt-sensitive genes. (*Hypertens Res* 2005; 28: 645–650)

Key Words: angiotensinogen M235T polymorphism, adducin Gly460Trp polymorphism, hypertension, lifestyle

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Introduction

The pathophysiological mechanisms related to salt-sensitive essential hypertension are not completely understood. Excess salt intake is an important environmental risk factor for the predisposition to essential hypertension. Therefore, polymorphisms that might increase the formation of angiotensin II (such as the angiotensinogen [AGT] polymorphism) are relevant in the context of sodium sensitivity. The AGT M235T (the substitution of threonine [Thr] for methionine [Met] at codon 235) polymorphism is associated with an increased risk of hypertension (1, 2) and has also been evaluated in relation to salt sensitivity, with controversial results (3, 4). The Gly460Trp genotype of adducin (ADD1) (the substitution of tryptophan [Trp] for glycine [Gly] at codon 460) is also associated with erythrocyte sodium transport, increases in tubular sodium reabsorption, and risk for hypertension (5–7). One epidemiologic study showed that the ADD1 Trp/Trp genotype was associated with higher systolic blood pressure (sBP) among men with a high sodium intake (8).

The purpose of this study was to elucidate the relationship between AGT, ADD1, both genotypes combined, and hypertension in a general Japanese sample. Moreover, we examined the effects of salt intake and polymorphisms of salt-sensitive genes on hypertension, and we conducted a statistical analysis of the interactions between these factors after adjusting for other lifestyle factors.

Methods

Study Population

The Shigaraki Study was based on a medical examination undertaken in 1999 at Shigaraki, a farming community near Kyoto, in western Japan (9–11). A total of 2,902 subjects underwent the examination, of whom 2,395 were enrolled in this genetic study after receiving a full explanation and providing informed consent. Of these subjects, 748 were excluded for the following reasons: undetermined genotype, $n=41$; already taking antihypertensive agents, $n=431$; a serum GOT or GPT level of over 100 IU/l, $n=13$; and/or a history of transient ischemic attack, stroke, angina pectoris, myocardial infarction, or diabetes mellitus, $n=263$. Subjects were between the ages of 30 and 79. This study was approved by the Institutional Review Board of Shiga University of Medical Science (Nos. 11–15, 1999).

Blood Pressure (BP) and Biochemical Examinations

sBP and diastolic blood pressure (dBP) were measured twice using a standard sphygmomanometer on the right arm while the subject was seated after having rested for at least 5 min. Korotkov's first and fourth points were regarded as the sBP

and dBP, respectively, and the BP was measured by a well-trained nurse. The mean of the 2 measurements from each subject was used for the data analysis. In this study, participants were considered hypertensive if they had the following BP values: sBP ≥ 140 mmHg or dBP ≥ 90 mmHg. The non-fasting blood glucose level was measured by the hexokinase method. Participants were considered diabetic if they had a blood glucose level of 200 mg/dl or more, or if they were already being treated for diabetes. The body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m).

Assessment of Lifestyle Factors

The patient history regarding daily alcohol intake and number of cigarettes per day was assessed by face-to-face interview (9, 10). The frequency of consumption during a typical week and the alcohol intake on each occasion were determined and used to calculate the alcohol intake per week, which was then divided by 7 to obtain the average intake per day. Subjects were asked to estimate their alcohol intake based on the "gou," a traditional Japanese drinking unit corresponding to 23 g of ethanol. Drinkers were defined as those consuming more than 0.3 gou a week. The participants who reported that they preferred salty foods in a simple questionnaire were defined as those "eating salty food."

Genetic Analysis

DNA was isolated from peripheral leukocytes and the AGT and ADD1 genotypes were determined as previously reported (12, 13). Both genotypes, determined by the polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method for a total of 75 random samples consisting of 25 PCR products for each genotype, were confirmed by direct sequencing. Briefly, after fractionation of the PCR-RFLP products on 1% agarose gels (Nippon Gene, Tokyo, Japan), the desired DNA bands were excised, and the DNA was purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, USA), amplified with the above 5' primer, and analyzed with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, Wellesley, USA).

Statistical Analysis

The Statistical Package for Social Science (SPSS ver. 11.0J; SPSS Japan, Tokyo, Japan) was used for the statistical analysis. Student's or Welch's *t*-test and the Wilcoxon rank sum test (for alcohol consumption) were used for comparisons of means between two categories. For comparisons of means among three or more categories, a one-way analysis of variance or the Kruskal-Wallis test (for alcohol consumption) was used according to the distributions. The χ^2 test was used to compare proportions. Age-adjusted prevalence was calculated directly. A multiple logistic regression analysis was

Table 1. Characteristics of Study Population by AGT M235T Polymorphism in Men and Women, Shigaraki Study in 1999

Risk characteristics	Men (638)				Women (1,009)			
	Met/Met	Met/Thr	Thr/Thr	<i>p</i> -value	Met/Met	Met/Thr	Thr/Thr	<i>p</i> -value
<i>N</i> (1,647)	29	168	441		40	303	666	
Age (years)	55.9±14.9	56.1±16.3	56.1±15.3	0.997	53.8±16.7	52.7±15.8	53.5±15.2	0.739
BMI (kg/m ²)	22.4±3.7	22.1±3.0	22.6±2.8	0.208	21.9±3.0	22.2±3.0	22.1±3.0	0.890
sBP (mmHg)	128.0±16.9	130.6±16.4	130.2±17.5	0.760	125.0±17.3	123.2±18.5	125.2±19.9	0.324
dBp (mmHg)	77.0±11.3	78.5±12.2	78.3±11.6	0.819	76.0±10.2	73.3±11.1	74.6±11.8	0.194
Alcohol consumption (gou/day)	1.38	0.80	0.80	0.251	0.06	0.07	0.07	0.145
Eating salty food (%)	31.0	25.6	19.5	0.119	15.0	6.9	7.5	0.191

N: number of subjects. Values are means±SD. AGT, angiotensinogen; BMI, body mass index; sBP, systolic blood pressure; dBp, diastolic blood pressure. Alcohol consumption: 1 gou=23 g of ethanol.

Table 2. Characteristics of Study Population by ADD1 Gly460Trp Polymorphism in Men and Women, Shigaraki Study in 1999

Risk characteristics	Men (638)				Women (1,009)			
	Gly/Gly	Gly/Trp	Trp/Trp	<i>p</i> -value	Gly/Gly	Gly/Trp	Trp/Trp	<i>p</i> -value
<i>N</i> (1,647)	123	305	210		201	497	311	
Age (years)	55.4±15.9	55.3±15.7	57.6±15.1	0.240	54.7±16.6	53.3±15.0	52.2±15.5	0.187
BMI (kg/m ²)	22.3±2.5	22.5±3.0	22.5±3.0	0.673	21.6±2.8	22.3±3.0	22.1±3.0	0.031
sBP (mmHg)	130.6±17.0	129.6±17.0	130.8±17.6	0.726	124.7±20.2	124.8±19.6	124.3±18.6	0.951
dBp (mmHg)	78.3±11.8	78.0±11.6	78.6±12.0	0.836	72.8±12.1	75.0±11.6	74.0±11.1	0.061
Alcohol consumption (gou/day)	0.77	0.87	0.79	0.390	0.06	0.08	0.07	0.352
Eating salty food (%)	24.4	22.3	19.0	0.482	8.0	8.7	5.8	0.322

N: number of subjects. Values are means±SD. BMI, body mass index; ADD1, adducin; sBP, systolic blood pressure; dBp, diastolic blood pressure. Alcohol consumption: 1 gou=23 g of ethanol.

used to clarify the contribution of each independent variable to hypertension. In this analysis, hypertension was regarded as a dependent variable, and each genotype and other factors were regarded as independent variables. The significance of the interaction of eating salty food with AGT and ADD1 genotypes was also tested using an interaction term in this model. All confidence intervals were estimated at the 95% level.

Results

Table 1 shows the characteristics of the study population according to the AGT M235T polymorphism. The frequencies of AGT genotypes Met/Met, Met/Thr, and Thr/Thr were 4.2%, 28.6%, and 67.2%, respectively. No significant differences were observed among the Met/Met, Met/Thr, and Thr/Thr groups with respect to age, BMI, sBP, dBp, alcohol consumption, and the habit of eating relatively more salty food, in comparison to the reported salt intake of other subjects. Table 2 shows the characteristics of the study population according to the ADD1 Gly460Trp polymorphism. The frequencies of ADD1 genotypes Gly/Gly, Gly/Trp, and Trp/Trp were 19.7%, 48.7%, and 31.6%, respectively. Results similar to those given above were obtained. In all, no significant dif-

ferences were observed among the Gly/Gly, Gly/Trp, and Trp/Trp groups in terms of hypertension.

Table 3 shows the characteristics of the combined AGT and ADD1 polymorphism analysis, AGT M235T Thr/Thr and ADD1 Trp/Trp vs. other polymorphisms. There was a significant association between the combined genotypes AGT Thr/Thr and ADD1 Trp/Trp in men and hypertension ($p=0.035$). However, the statistical significance disappeared when we adjusted for age, although the magnitude of the percentage remained almost the same. After adjustments for age, BMI, alcohol consumption, eating salty food, family history of hypertension, and number of cigarettes per day were made, the multivariate prevalence odds ratio and 95% confidence interval (CI) of combined AGT and ADD1 polymorphisms for hypertension were, respectively, 1.33 and 0.88–2.02 for men, and 1.41 and 0.95–2.01 for women. The combined AGT and ADD1 polymorphisms were positively associated with hypertension in both men and women, with an odds ratio of almost the same magnitude; however, the association did not reach a level of statistical significance.

Table 4 shows the multivariate adjusted odds ratios of combined AGT and ADD1 polymorphisms for hypertension when the data for men and women were combined. Multiple

Table 3. Characteristics of Study Population by Combined AGT and ADD1 Polymorphisms in Men and Women, Shigaraki Study in 1999 (AGT M235T Thr/Thr and ADD1 Trp/Trp vs. Others)

Risk characteristics	Men (638)			Women (1,009)		
	Others	Thr/Thr and Trp/Trp	<i>p</i> -value	Others	Thr/Thr and Trp/Trp	<i>p</i> -value
<i>N</i> (1,647)	488	150		800	209	
Age (years)	55.3±15.8	58.6±14.6	0.026	53.4±15.5	52.7±15.4	0.549
BMI (kg/m ²)	22.4±2.9	22.8±3.0	0.189	22.1±3.0	22.1±2.9	0.971
sBP (mmHg)	129.6±16.9	132.0±18.0	0.140	124.3±19.3	125.8±19.7	0.335
dBp (mmHg)	77.9±11.7	79.6±11.9	0.125	74.2±11.6	74.7±11.5	0.598
Alcohol consumption (gou/day)	0.83	0.81	0.723	0.07	0.07	0.144
Family history of hypertension (%)	24.4	18.0	0.104	29.9	30.6	0.834
Eating salty food (%)	22.7	18.0	0.217	8.1	5.7	0.248
Hypertension (%)	28.9	38.0	0.035	23.4	27.3	0.241
Hypertension (%) [†]	28.9	35.9	0.127	23.4	28.1	0.125

Others: AGT M235T polymorphism, Met/Met and Met/Thr; and ADD1 Gly460Trp polymorphism, Gly/Gly and Gly/Trp. Other abbreviations are listed in Tables 1 and 2. [†]Age-adjusted prevalence was calculated by the direct method using the “others” group as the standard population.

Table 4. Multivariate Adjusted Relative Odds Ratios and 95% Confidence Intervals (CIs) of Combined Genetic AGT M235T Thr/Thr (TT) and ADD1 Trp/Trp (TT) Polymorphisms for Hypertension (*N*=1,647)

Risk characteristics	Odds ratio (95% CI)	<i>p</i> -values
AGT T/T and ADD1 T/T (both TT=1, others=0)	1.37 (1.03–1.82)	0.031
Age (years)	1.07 (1.06–1.08)	<0.001
BMI (kg/m ²)	1.18 (1.13–1.23)	<0.001
Alcohol consumption (gou/day)	1.39 (1.16–1.66)	<0.001
Eating salty food (yes=1, no=0)	1.25 (0.88–1.77)	0.218
Family history of hypertension	1.57 (1.18–2.07)	0.002
Smoking (number of cigarettes/day)	1.00 (0.99–1.01)	0.481
Sex (men=0, women=1)	1.09 (0.81–1.47)	0.481

Abbreviations are listed in Tables 1 and 2.

logistic regression analysis adjusting for age, BMI, alcohol consumption, eating salty food, family history of hypertension, number of cigarettes per day and sex showed that age, BMI, alcohol consumption, family history of hypertension, and combined AGT and ADD1 polymorphisms were associated with hypertension. However, there was no correlation between eating salty food and hypertension. In addition, there was no interaction between eating salty food and the AGT M235T Thr/Thr plus ADD1 Trp/Trp polymorphism (*p*=0.829).

Discussion

A number of genes have been tested for an association with hypertension, with controversial results. Salt sensitivity is possibly genetically determined. Salt-sensitive individuals tend to more frequently have a familial history of hypertension than do salt-resistant subjects, and there is a familial

resemblance in the response of BP to sodium restriction (14, 15). Such findings suggest the existence of genetic determinants that influence the sensitivity of BP to salt. Hunt and co-workers speculated that the AGT genotype affects BP in response to sodium and the development of hypertension. A greater reduction in BP following a reduction in sodium has been reported in subjects with the Thr/Thr genotype than in those with the Met/Met genotype (16). Similarly, Beeks and co-workers reported that the 460Trp variant of the ADD1 polymorphism is probably associated with a salt-sensitive form of hypertension (17). However, studies of African Americans, who are believed to have a higher prevalence of salt-sensitive hypertension, have not revealed any association between the ADD1 polymorphism and hypertension (18, 19). These discrepancies may be difficult to reconcile. One possible explanation is that essential hypertension is a complex syndrome determined by both genetic and environmental factors. It is possible that the polymorphism of a single gene

exerts only a small effect on the development of hypertension, and this may be masked by differences in genetic phenotypes or environmental factors such as BMI, salt intake, and alcohol consumption (9–11). In the Ohasama study (13), the Gly460Trp polymorphism of ADD1 was associated with ambulatory BP and home BP, but not casual BP. Casual BP usually does not reflect basal BP, being influenced by physical or psychological stress and environmental factors. However, in the present study, we found an association between the AGT M235T Thr/Thr plus ADD1 Trp/Trp polymorphism and hypertension after adjustment for possible confounding lifestyle factors, which indicates the importance of clarifying the combined effects of certain candidate genes on hypertension. Here, we suggest that a combined genetic analysis for demonstrating the presence of both AGT and ADD1 polymorphisms is a good marker for hypertension, as defined by the casual BP. Therefore, we concluded that the accumulation of genetic risk factors increases the frequency of hypertension, irrespective of exposure to environmental risk factors for hypertension.

There were several limitations to the present study. First, we did not examine other candidate genes that might be associated with hypertension (20, 21). Second, the simple questionnaire regarding salt intake used in the present study did not reflect the actual salt intake of each participant. Instead, it might have been more suitable for the purposes of the present study to use a 24-h urinary sodium excretion test, or some other formula to estimate 24-h urinary sodium excretion based on spot urine samples (22).

In conclusion, as regards heredity, double homozygosity of 235Thr or 460Trp might be associated with essential hypertension. However, in the present study, no interaction between these genotypes and salt intake could be determined based on the results of a simple questionnaire. In addition, further investigation will need to be carried out using a large-scale sample.

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