

# Differences in genotype frequencies of salt-sensitive genes between fishing and nonfishing communities in Japan

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**Purpose:** To identify the differences in genotype frequencies of salt-sensitive genes between residents of fishing communities (FCs) and nonfishing communities (NFCs).

**Methods:** The subjects included 18,156 individuals (8,043 males [44%] and 10,113 females [56%]; average age: 57.2±16.1 years) from the general population who were registered with large-scale genome banks and resided in 30 prefectures and 78 different regions in Japan. The measurement items were age, sex, blood pressure, presence or absence of hypertension, body mass index, alcohol consumption, and smoking habit. Furthermore, to analyze the genotype frequencies of salt-sensitive genes,  $\alpha$ -adducin 1 (*ADD1*), angiotensinogen (*AGT*), angiotensin II receptor type 1 (*AT1*), and guanine nucleotide-binding protein  $\beta$  peptide 3 (*GNB3*) were measured. According to the 2004 government classification of municipalities (cities, towns, and villages), communities existing in areas bordering an ocean and with an ocean port were defined as FCs (28 areas). The others were defined as NFCs (50 areas). A logistic regression model was used for comparison of genotype frequencies between subjects residing in FCs and NFCs.

**Results:** Of the included subjects, 4,916 (27.0%) and 13,240 (73.0%) resided in FCs and NFCs, respectively. In FCs, the mean age was 59.4±16.7 years and men accounted for 41.0% of the cohort (n=2,015). In NFCs, the mean age was 56.4±15.8 years and men accounted for 45.5% of the cohort (n=6,028). The adjusted odds ratios of the AA and AG genotypes compared with the GG genotype for *AGT* were 0.80 (95% confidence interval [CI]: 0.68–0.95) and 0.76 (95% CI: 0.64–0.91), respectively. The adjusted odds ratio of the CC genotype compared with AA for *AT1* was 0.63 (95% CI: 0.40–0.93).

**Conclusion:** The incidence of the salt-sensitive genotypes *AGT* and *AT1* in residents of FCs were significantly lower than in NFCs.

**Keywords:** salt-sensitive, hypertension, single-nucleotide polymorphisms, regional difference, lifestyle, behavior

## Introduction

Hypertension (HT) is a risk factor for cardiovascular disease.<sup>1–4</sup> Regional differences in blood pressure (BP) levels and the prevalence of cardiovascular disease have been reported worldwide, and the mechanisms underlying these differences have been partly explained by sociodemographic and environmental factors.<sup>5–10</sup>

Some previous population-based studies conducted in Japan have demonstrated lower incidences of atherosclerosis and cardiovascular diseases in fishing communities (FCs) compared with nonfishing communities (NFCs). The lower prevalence of atherosclerotic diseases (coronary heart disease and cerebral infarction), lower intima-media thickness, and occurrence of carotid plaques among individuals residing in FCs

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who have higher fish consumption than those living in NFCs suggest that eicosapentaenoic acid and docosahexaenoic acid in fish play a preventive role against atherosclerosis.<sup>11–15</sup>  $\alpha$ -Adducin 1 (*ADD1*), angiotensinogen (*AGT*), angiotensin II receptor type 1 (*ATI*), and guanine nucleotide-binding protein  $\beta$  peptide 3 (*GNB3*) are reportedly salt-sensitive genes associated with HT.<sup>16–18</sup> The Gly460Trp polymorphism of the *ADD-1* gene codes for a cell membrane skeletal protein identified as essential to HT with salt sensitivity.<sup>19</sup> *AGT* and *ATI* are mainly associated with the renin–angiotensin–aldosterone system.<sup>20,21</sup> The G protein is involved in renal Na<sup>+</sup>/H<sup>+</sup> transport, while *GNB3* is its subunit gene.<sup>22</sup>

A recent Japanese study reported a difference in salt-sensitive genotype frequencies among samples divided into four groups by area.<sup>23</sup> However, there has been no comprehensive study comparing genetic factors and the frequencies of salt-sensitive genotypes according to residence in FCs versus NFCs.

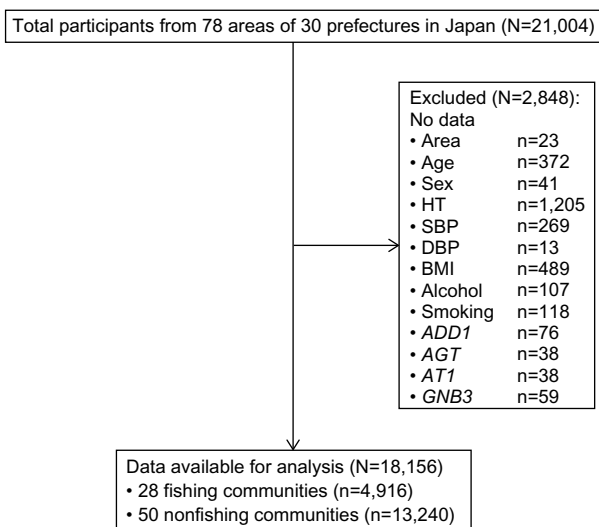
Thus, the purpose of the present study was to compare the frequencies of salt-sensitive genotypes among residents in FCs versus NFCs to identify the correlations between the genetic factors and residence in FCs.

## Materials and methods

### Study design and subjects

This observational study included a total of 21,004 Japanese men and women (age:  $\geq 20$  years) from 78 mostly rural areas of 30 prefectures in Japan. These individuals were enrolled in the Genome Banking Project of the 21st Century COE (Center of Excellence) Program at the Center for Community Medicine, Jichi Medical University (Shimotsuke, Japan) between 2004 and 2008. All subjects provided written informed consent before participation in this study. Of these subjects, 12,361 were residents who underwent annual medical checkups authorized by each municipal government, 5,709 were outpatients who regularly visited clinics and hospitals in their area, 1,846 were inpatients, and 1,088 were patients for whom no information about participation in the project was provided.

Subjects without complete data for all variables were excluded from statistical analysis (Figure 1). The *ADD1*, *AGT*, *ATI*, and *GNB3* genotypes could not be determined for 76, 38, 38, and 59 subjects, respectively. Moreover, data concerning area of residence, age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), HT, hyperlipidemia, diabetes, body mass index (BMI), alcohol consumption, and smoking habits were missing for 23, 372, 41, 1,205, 0, 0, 269, 13, 489, 107, and 118 subjects,



**Figure 1** Flow diagram of recruitment and available data.

**Abbreviations:** *ADD1*,  $\alpha$ -adducin 1; *AGT*, angiotensin II; *ATI*, angiotensin II receptor type 1; alcohol, alcohol consumption; BMI, body mass index; DBP, diastolic blood pressure; *GNB3*, guanine nucleotide-binding protein  $\beta$  peptide 3; HT, hypertension; SBP, systolic blood pressure; smoking, smoking habit.

respectively. Consequently, after excluding these subjects, 18,156 individuals (8,043 males [44%] and 10,113 females [56%]; average age:  $57.2 \pm 16.1$  years) were included in the analysis.

### Definition of FCs and NFCs

Based on residence, the enrolled subjects were divided into two groups, namely FCs and NFCs. FCs were classified on the basis of 2004 government data<sup>24</sup> and required that the community border the ocean and have a fishing port. All other areas that did not meet these criteria were classified as NFCs. Of the total 78 areas, 28 were classified as FCs and 50 as NFCs.

### Measurements

#### Genotyping of single-nucleotide polymorphisms

Blood samples were collected from all subjects and genomic DNA was extracted from the buffy coats of centrifuged blood using the Puregene DNA purification kit (Gentra Systems, Inc., Minneapolis, MN, USA). The database single-nucleotide polymorphism identification numbers for the *ADD1* (Gly460Trp), *AGT* (Gly6Ala), *ATI* (Ala1166Cys), and *GNB3* (Cys825Thr) polymorphisms were rs4961, rs5051, rs5186, and rs6489738, respectively. The TaqMan method was used for genotyping. Probes and primer mixtures were selected from the commercial database of the TaqMan probe system (myScience; Applied Biosystems, Foster City, CA, USA).<sup>25</sup> Genomic DNA (5 ng) was added to the wells of

a 384-well plate and air-dried for use as templates for the reaction. The polymerase chain reaction mixture contained 2.5  $\mu$ L of probe mix and the same amount of Master Mix solution (Applied Biosystems). The standard thermocycle parameters provided by the manufacturer were used. An ABI 7900HT apparatus (Applied Biosystems) was used for reaction, genotype calling, and data exporting. The *ADD1* T allele, *AGT* A allele, *AT1* C allele, and *GNB3* T allele were previously identified as candidate gene polymorphisms responsible for salt-sensitive HT.<sup>17,18,26</sup>

### Physical examination and assessment of lifestyle

Well-trained public health nurses measured BP using a standard mercury sphygmomanometer on the right arm after at least a 5-minute resting period in the sitting position. Korotkoff's first and fifth sounds were regarded as SBP and DBP, respectively. HT was defined as an SBP of  $\geq 140$  mmHg, DBP of  $\geq 90$  mmHg, or current use of antihypertensive medication. Hyperlipidemia and diabetes were defined as use of antihyperlipidemic and antidiabetic medication, respectively. Height and weight of the subjects were obtained from health checkup records or medical records. BMI was calculated as weight (kg)/height<sup>2</sup> (m<sup>2</sup>).

Information regarding smoking habits and alcohol consumption was obtained through a self-reported questionnaire and then confirmed in an interview by nurses trained for the present study. Smoking habit was categorized as never-smoking, currently smoking, and ex-smoking, and alcohol consumption was categorized as nondrinking, sometimes drinking, and everyday drinking.

### Statistical analysis

All statistical analyses were performed using Stata/SE 12.1 for Mac (Stata Corp LP, College Station, TX, USA). The probability (*P*) values, odds ratios (ORs), and 95% confidence intervals (CIs) were calculated when applicable. A *P*-value of  $>0.05$  was considered statistically significant. Continuous variables (age, SBP, DBP, and BMI) are presented as means  $\pm$  standard deviation, and categorical variables (sex, smoking habit, alcohol consumption, and allele and genotype of the polymorphism) as frequencies (%). The allele frequency was determined by direct counting. Correlations between FCs or NFCs and the other variables were identified using the unpaired *t*-test (continuous variables) or chi-square test (categorical variables). Multiple logistic regression analyses were performed for each genotype (*ADD1*, *AGT*, *AT1*, and *GNB3*) by adjusting for age and sex (Model 1) or by adjusting for age, sex, SBP, DBP, HT, BMI, smoking habit, and alcohol

consumption (Model 2). GG/*ADD1*, GG/*AGT*, AA/*AT1*, and CC/*GNB3* are references of genotype.

### Ethics statement

The study protocol was approved by the Jichi Medical University Epidemiological and Ethical Committee.

### Results

The basic characteristics of the subjects according to the area of residence are shown in Table 1. In comparison with residents from NFCs, those from FCs were, on average, older, had higher BP, and showed lower alcohol consumption. There were more female subjects and more subjects with a history of high BP. Table 2 shows the differences in genotype frequencies of salt-sensitive genes and the results of multivariate analyses performed to assess the relationships between the genotypes of salt-sensitive genes and area of residence (FCs vs NFCs) in Japan. There were significant differences in the AA and AG genotypes of *AGT* and the CC genotype of *AT1* between the two areas. The OR of the AA genotype in *AGT* compared with the GG genotype was 0.79 (95% CI: 0.63–0.89, *P*=0.008) in Model 1 and 0.80 (95% CI: 0.68–0.95, *P*=0.001) in Model 2. The OR of the AG genotype in *AGT* compared with the genotype GG was 0.75 (95% CI: 0.63–0.89, *P*=0.001) in Model 1 and 0.76 (95% CI: 0.64–0.91, *P*=0.002) in Model 2. The OR of the CC genotype in *AT1* compared with the AA genotype was 0.63 (95% CI: 0.40–0.98, *P*=0.04) in Model 1 and 0.63 (95% CI: 0.40–0.99,

**Table 1** Basic characteristics of the subjects (N=18,156)

	FCs (n=4,916)	NFCs (n=13,240)	P-values
Age*	59.4 $\pm$ 16.7	56.4 $\pm$ 15.8	<0.001
Sex			<0.001
Male	2,015 (41.0)	6,028 (45.5)	
Female	2,901 (59.0)	7,212 (54.5)	
Blood pressure*			
Systolic	130.0 $\pm$ 19.6	127.0 $\pm$ 18.1	<0.001
Diastolic	75.4 $\pm$ 11.7	75.3 $\pm$ 11.1	0.81
Hypertension	2,452 (49.9)	5,345 (40.4)	<0.001
Hyperlipidemia	773 (15.7)	1,592 (12.0)	<0.001
Diabetes	673 (13.7)	1,983 (15.0)	0.03
Body mass index*	23.3 $\pm$ 3.5	23.2 $\pm$ 3.4	0.45
Smoking	863 (17.6)	2,306 (17.4)	0.83
Alcohol consumption			<0.001
Everyday	1,098 (22.3)	3,310 (25.0)	
Current	1,147 (23.3)	3,709 (28.0)	
Never	2,671 (54.3)	6,221 (47.0)	

**Notes:** n (%), \*mean  $\pm$  standard deviation. *P*-values were calculated by unpaired *t*-test or chi-square test. Smoking included both current smokers and ex-smokers.  
**Abbreviations:** FCs, fishing communities; NFCs, nonfishing communities.

**Table 2** Results of univariate and multivariate analyses performed to identify associations between salt-sensitive genotypes and FCs versus NFCs in Japan

	Univariate		P-value	Multivariate			
	FCs (n=4,916)	NFC (n=13,240)		Model 1		Model 2	
				OR (95% CI)	P-value	OR (95% CI)	P-value
<i>ADD1</i>			0.64				
	TT	1,478 (30.1)	3,942 (29.8)	0.98 (0.89–1.08)	0.69	0.98 (0.90–1.08)	0.75
	TG	2,404 (48.9)	6,575 (49.7)	0.96 (0.88–1.05)	0.38	0.97 (0.89–1.05)	0.45
	GG	1,034 (21.0)	2,723 (20.6)	Reference		Reference	
<i>AGT</i>			0.004				
	AA	3,294 (67.1)	8,791 (66.4)	0.79 (0.63–0.89)	0.008	0.80 (0.68–0.95)	0.01
	AG	1,412 (28.7)	4,004 (30.2)	0.75 (0.63–0.89)	0.001	0.76 (0.64–0.91)	0.002
	GG	210 (4.3)	445 (3.4)	Reference		Reference	
<i>ATI</i>			0.063				
	CC	24 (0.5)	102 (0.8)	0.63 (0.40–0.98)	0.04	0.63 (0.40–0.99)	0.046
	CA	718 (14.6)	2,021 (15.3)	0.95 (0.87–1.04)	0.3	0.96 (0.87–1.05)	0.35
	AA	4,174 (84.9)	11,117 (84.0)	Reference		Reference	
<i>GNB3</i>			0.37				
	TT	1,243 (25.3)	3,290 (24.8)	1.00 (0.91–1.09)	0.93	0.99 (0.90–1.09)	0.84
	TC	2,418 (49.2)	6,666 (50.3)	0.96 (0.88–1.04)	0.27	0.95 (0.88–1.03)	0.22
	CC	1,255 (25.6)	3,284 (24.8)	Reference		Reference	

**Notes:** n (%). (Model 1) multivariate: age, gender; (Model 2) multivariate: age, gender, systolic blood pressure, diastolic blood pressure, hypertension, body mass index, smoking habit, and alcohol consumption were included as independent variables in the multivariate regression analysis of the other parameters.

**Abbreviations:** *ADD1*,  $\alpha$ -adducin 1; *AGT*, angiotensin II; *ATI*, angiotensin II receptor type 1; CI, confidence interval; FCs, fishing communities; *GNB3*, guanine nucleotide-binding protein  $\beta$  peptide 3; NFCs, nonfishing communities; OR, odds ratio.

$P=0.046$ ) in Model 2. There were no significant differences in the frequencies of the *ADD1* and *GNB3* genotypes between the two communities.

## Discussion

In the first study of this kind, a large-scale comparison of 21,004 subjects across 78 areas in Japan, divided according to residence into FCs and NFCs, was conducted to identify differences in the genotype frequencies of salt-sensitive genes. Significantly lower frequencies of the responsible genotypes AA/AG (*AGT*) and CC (*ATI*) were found in residents of FCs.

Both *AGT* and *ATI* polymorphisms are significantly associated with essential HT susceptibility (*AGT*; AA vs GG: OR=2.52, 95% CI=1.68–3.78; AA vs GA: OR=2.26, 95% CI=1.48–3.45; *ATI*; (AC+CC) vs AA: OR=1.41, 95% CI=1.19–1.66; (AA+AC) vs CC: OR=0.50, 95% CI=0.38–0.65).<sup>27,28</sup> The pathophysiological mechanisms of these gene polymorphisms related to salt-sensitive essential HT are not completely understood. However, the renin–angiotensin system (RAS) has a pivotal role. A previous study showed that hypertensive patients with the A allele in *AGT* (Gly6Ala) may show altered cell function, specifically regulation of the *AGT* transcription level in *AGT*-producing tissues.<sup>29</sup> Furthermore, *AGT* gene transcription may lead to increased plasma *AGT* levels. Salt-sensitive hypertensive patients tend

to have low renin values.<sup>30</sup> Moreover, the RAS response to high salt intake is blunted in salt-sensitive subjects and is inversely correlated with the BP response.<sup>30</sup> Meanwhile, the *ATI* (Ala1166Cys) polymorphism is in a noncoding region of *AT1R* gene, and therefore the amino acid sequence of the *AT1* receptor is not altered. However, the *ATI* polymorphism might affect messenger RNA stability and transcription, or alternatively be in linkage disequilibrium with other functional polymorphisms.<sup>31</sup> Therefore, it is logical to suppose that salt-sensitive hypertensive patients may differ in genotypes encoding the different steps of the RAS cascade.

Subjects with salt-sensitive genes are suspected to be more susceptible to increases in BP when salt is consumed. It has been reported that Japanese patients, compared with Caucasians, have a higher incidence of polymorphisms in candidate genes.<sup>32</sup> Genetic factors have been reported as a partial explanation for these regional differences.<sup>5–10</sup> The results of the present study showed that, in addition to environmental factors, genetic factors should also be considered when identifying differences in the frequencies of HT. In addition, when performing population approaches such as the public health campaign for prevention of HT in each community, it is necessary to take into account the genetic factors as well as environmental factors.

The strength of the present study lies in the fact that samples were collected from a large national sample of the

population and that precise measurements were conducted by a large organization. There were four main limitations to this study that should be addressed. First, only four genotypes were examined, and other genotypes also considered to influence salt sensitivity were not examined. Second, subjects with missing data were excluded from analysis. However, the excluded cases were not considered to have significant impact according to age or sex. Therefore, the excluded data is not considered to have a significant impact on the overall study results. Third, the amount of fish consumption was not measured in this study. Thus, it is unclear if the patients in FCs have higher rate of fish consumption compared to the patients in NFCs. This issue is study limitation and is necessary to be studied in the future. Fourth, the daily dietary sodium consumption might be one of the potential confounders of higher SBP in FCs group. However, the amount of daily dietary sodium consumption was not measured in this study. Therefore, this study could not discuss a role that *AGT* and *AT1* polymorphisms would play in salt-sensitive HT.

## Conclusion

In this study, the incidence of the salt-sensitive genotypes *AGT* and *AT1* in residents of FCs were significantly lower than NFCs. Future studies evaluating the presence of salt-sensitive genes are required to examine the association between HT and area of residence in FCs.

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## Author contributions

MH, TT, and MO contributed to the conception, design, and acquisition of data; the analysis and interpretation of data; and the writing and revision of the manuscript. EK contributed to the conception and design and the writing and revision of the manuscript.

## Disclosure

The authors report no conflicts of interest in this work.

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