ORIGINAL ARTICLE

Hypertension-susceptibility gene prevalence in the Pacific Islands and associations with hypertension in Melanesia

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Human essential hypertension is partly caused by genetic factors. Angiotensinogen (*AGT*), G-protein $\beta 3$ -subunit (*GNB3*) and cytochrome P450 3A5 (*CYP3A5*) are candidate hypertension susceptibility genes and risk alleles at these loci have been thought to arise owing to human adaptation to climatic changes following the migration out-of-Africa. This study aimed to reveal the frequencies of hypertension-susceptibility genotypes in Pacific Island populations and associations of these single-nucleotide polymorphisms (SNPs) to hypertension. Genotyping was conducted for 804 individuals from Melanesian, Micronesian and Polynesian populations at SNPs in the genes encoding *AGT* (rs699, rs5049 and rs5051), *GNB3* (rs5443) and *CYP3A5**1/*3 (rs776746). Associations between these SNPs and hypertension were tested for 383 Melanesian Solomon Islanders. We found that the A/A genotype at rs5049 was a risk factor for hypertension ($P=0.025$) in the Melanesian Solomon Islanders; three SNPs for *AGT* were in linkage disequilibrium. The ancestral alleles of rs699, rs5051 and rs776746, and the derived allele of rs5443 were as frequent in the populations surveyed here as in other equatorial populations. Although other polymorphisms associated with hypertension and additional populations remain to be studied, these findings suggest that the Pacific Islanders' susceptibility to hypertension arose because of human migration and adaptation.

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INTRODUCTION

Human essential hypertension, which is a major risk factor for stroke, ischemic heart diseases, myocardial infarction, heart failure, aneurism and chronic renal failure, has become one of the greatest health concerns in the world.¹ Its prevalence is rapidly increasing in developing countries of the Asia Pacific region, associated with drastic changes in lifestyle;^{2,3} candidate environmental factors include high dietary intake of sodium and alcohol, obesity and stress.¹ In addition, several genetic factors have been reported. Angiotensinogen (*AGT*) is a key component of the renin–angiotensin system, which influences all aspects of blood pressure,

including blood vessel contraction, sodium and water balance, and cell development in the heart.⁴ G-protein $\beta 3$ -subunit (*GNB3*) has essential roles in G-protein-coupled receptor-related signal transduction pathways involved in blood pressure regulation. The cytochrome P450 3A5 (*CYP3A5*) enzyme metabolizes cortisol and is related to sodium transport in renal epithelia, increasing sodium and water retention, and blood pressure.⁵ Genes encoding α -adducin, β_2 -adrenergic receptor and angiotensin-converting enzyme inhibitor are also candidates.⁶

It has been hypothesized that susceptibility to hypertension is influenced by ancestral traits, because several known risk factors, such

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as enhanced salt and water avidity and vascular reactivity, are adaptive traits associated with hot and humid environments of the ancestral African climate.⁷ In addition, previous studies have suggested that particular genotypes, associated with susceptibility to elevated blood pressure, are rather prevalent in geographic regions near the equator and/or with high temperatures and precipitation, implicating a role for natural selection during the out-of-Africa migration.⁸ For example, a study of single-nucleotide polymorphisms (SNPs) in the *AGT* gene suggested that ancestral genotypes of rs699 (M235T) and rs5051 (G – 6A), which had also been found in non-human primates, were frequently found even in non-African populations in tropical regions of Asia and America.^{8,9} The frequencies of ancestral genotypes in the *CYP3A5**1/*3 (rs776746) and *AGT* (rs699) genes decrease as the distance from the equator increases.¹⁰ In contrast, the derived allele of C825T (rs5443) in *GNB3*, which is present only in humans and thus seems to be a newly derived allele, has been reported to occur frequently in low-latitude, particularly in Africa;⁸ the derived allele of G – 217A (rs5049) in *AGT*, which is also present only in humans, was not associated with latitude in populations worldwide, but was positively correlated within the African populations. Young *et al.*⁸ also reported that other SNPs or short tandem repeat polymorphisms, which they analyzed, was less strongly associated with latitude than rs5051 or rs5443. These findings suggest that hypertension is a 'thrifty' trait;¹¹ that is, individuals with genotypes associated with improved survival during salt scarcity and low food availability are at a high risk of hypertension in an environment with a high availability of salt and a high-calorie diet. Thus, understanding the underlying genetic variation at loci associated with susceptibility to elevated blood pressure could contribute to our understanding of human evolution under various climatic environments.

The Pacific Islands represent one of the regions of the world that were most recently settled by migrating humans. Linguistic, archeological and genetic studies have suggested that Austronesian (AN)-speaking populations who migrated from continental Asia, passed through the islands of Southeast Asia and Melanesia; arrived in Polynesia <2000 years ago; and now inhabit in Polynesia, Micronesia and parts of Melanesia.^{12–15} Note that the majority of the population of New Guinea and its neighboring islands in Melanesia are Non-AN (NAN)-speaking, and these regions are home to potential descendants of the first human population who settled in this area 50–30 000 years ago; this population, in contrast to AN-speaking populations, did not further migrate from Melanesia to Polynesia or Micronesia likely because they did not develop technologies for long voyages. The AN-speaking population is a candidate population with the thrifty genotype because they have experienced frequent starvation during inter-island voyages^{16,17} while adapting to the climate. Hypertension prevalence is potentially related to both genetic and environmental factors.^{2,3,18,19} In particular, previous studies have suggested that the hypertension was more prevalent in the AN-speaking Melanesians than AN-speaking Micronesians.^{2,18} However, few detailed studies on genotypes associated with hypertension have been conducted in populations of the AN-speaking Melanesians.

This study aimed to reveal the frequencies of hypertension susceptibility genotypes in Pacific Island populations (Melanesians, Micronesians and Polynesians). We targeted SNPs known to be related to latitude in other populations (rs699, rs5049 and rs5051 in *AGT*; rs5443 in *GNB3*; and rs776746 in *CYP3A5*). Special attention was thus paid to associations of these SNPs to hypertension in the Melanesian Solomon Islanders. This is the first investigation of these SNPs and their relationship to hypertension and associated phenotypes conducted in populations of the Pacific Islands.

MATERIALS AND METHODS

Study populations

Study participants were recruited from seven Pacific Islander populations: (1) AN-speaking Melanesians in Balopa, from Manus Province, Papua New Guinea; (2) Kusaghe (note that in our previous papers, named Paradise after their settlement name) in North New Georgia, Western Province of the Solomon Islands; (3) Munda, in the Western Province of the Solomon Islands; (4) AN-speaking Micronesians in Ravaki in the Western Province of the Solomon Islands; (5) AN-speaking Polynesians in Ha'apai Island; (6) Nuku'alofa, the capital of the Kingdom of Tonga; and (7) NAN-speaking Melanesians in Gidra from the Western Province of Papua New Guinea (Figure 1). Sampling was made in the biggest settlement (Kusaghe, Munda, Nuku'alofa) or by visiting two or more settlements (Balopa, Gidra, Ha'apai) in each area so as that participants represented each population. Although Ravaki village was located in the Solomon Islands, the inhabitants had migrated there from overpopulated Gilbert Islands (Kiribati) in the 1960s. All measurements and sampling were performed after obtaining informed consent from each participant. This study was approved by the Health Research Ethics Committee, the Ministry of Health and Medical Services of the Solomon Islands; the Medical Research Advisory Committee of Papua New Guinea; National Health Ethics and Research Committee of Tonga; the Research Ethics Committee of the Faculty of Medicine at the University of Tokyo; and the Graduate School of Comprehensive Human Sciences at the University of Tsukuba, Japan.

Genotyping

Blood was sampled in the field and genomic DNA was extracted using the QIAamp Blood Kit (Qiagen, Hilden, Germany). Genotyping five SNPs (rs699, rs5049, rs5051, rs5443 and rs776746) was performed using TaqMan SNP genotyping assays (Applied Biosystems, Carlsbad, CA, USA). In total, 804 individuals were examined in this study.

Genotype database

Allele frequencies and latitudes of other populations were obtained from the Allele Frequency Database (ALFRED) at Yale University (<http://alfred.med.yale.edu/>). Because latitudes reported in ALFRED ranged from the southernmost to the northernmost latitude inhabited by a given population, the average of this range was used for each population. Populations that were not sampled in their countries of origin (for example, African Americans, European Americans, European-origin mixed ethnicities, European New Zealanders, Japanese in the United States and Canarians) were excluded from analyses, given the possibility that the sampled locations did not reflect their potential adaptation to the climate. Data from only three populations were reported for rs5443 in ALFRED. Therefore, we also used data for rs5443 available in dbSNP through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). The latitudes of the populations shown in dbSNP were identified from the information registered in ALFRED; if complete information for a given population was not available in ALFRED, the latitude was identified from Google Earth (Google Inc., Mountain View, CA, USA) based on the name of the population/location (electronic Supplementary Materials: Supplementary Tables S1–S5). The alleles were classified into ancestral or derived ones on the basis of information registered at the dbSNP (Table 1).

Anthropometric and blood pressure measurements

Anthropometric and blood pressure measurements were performed in Melanesian Solomon Islanders (Kusaghe and Munda). All participants were asked to arrive to the survey center (hospital and/or community hall) before 0900 hours. Body height was measured to the nearest 1 mm using a field anthropometer (GPM, Zurich, Switzerland), and weight was recorded to the nearest 0.1 kg using a portable digital scale (Tanita model BC-518, Tokyo, Japan), following a previously reported protocol.²⁰ The sitting blood pressure was measured twice from the arm using a digital blood pressure monitor (HEM757, Omron, Kyoto, Japan); the average of the two values was used. Hypertension was defined as values ≥ 140 mm Hg for systolic blood pressure (SBP) and/or ≥ 90 mm Hg for diastolic blood pressure (DBP). Of the 399 participants, 16 were pregnant female, or had already been diagnosed with

Figure 1 Locations of the study populations and the genotype frequencies for the SNPs rs699, rs5049 and rs5051 in *AGT*; rs5443 in *GNB3*; and rs776746 in *CYP3A5*. The ancestral alleles for each SNP are as follows: rs699-C, rs5049-G, rs5051-T, rs5443-C and rs776746-A. The Ravaki people, who originate from the Gilbert Islands, were sampled in the Solomon Islands. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 The allele frequencies at SNPs in *AGT*, *GNB3* and *CYP3A5* in the Pacific Island populations

	AGT rs699 (M235T) (ancestral = C (235T))			AGT rs5049 (G – 217A) (ancestral = G (– 217G))			AGT rs5051 (G – 6A) (ancestral = T (– 6G))			GNB3 rs5443 (C825T) (ancestral = C (825C))			CYP3A5 rs776746 (*1/*3) (ancestral = A (*1))		
	C/C	C/T	T/T	G/G	G/A	A/A	T/T	T/C	C/C	C/C	C/T	T/T	A/A	A/G	G/G
Balopa	23	9	0	25	5	2	23	9	0	5	17	10	1	6	25
(AN, Melanesia)	71.9%	28.1%	0%	78.1%	15.6%	6.3%	71.9%	28.1%	0.0%	15.6%	53.1%	31.3%	3.1%	18.8%	78.1%
Kusaghe	196	17	0	185	27	3	194	21	0	31	101	83	28	85	101
(AN, Melanesia)	92.0%	8.0%	0%	86.0%	12.6%	1.4%	90.2%	9.8%	0.0%	14.4%	47.0%	38.6%	13.1%	39.7%	47.2%
Munda	132	41	10	159	20	3	128	44	11	20	92	71	14	85	83
(AN, Melanesia)	72.1%	22.4%	5.5%	87.4%	11.0%	1.6%	69.9%	24.0%	6.0%	10.9%	50.3%	38.8%	7.7%	46.7%	45.6%
Ravaki	104	43	9	92	54	11	104	42	9	20	78	60	31	72	51
(AN, Micronesia)	66.7%	27.6%	5.8%	58.6%	34.4%	7.0%	67.1%	27.1%	5.8%	12.7%	49.4%	38.0%	20.1%	46.8%	33.1%
Ha'apai	25	11	2	17	19	2	25	10	2	5	18	15	9	16	13
(AN, Polynesia)	65.8%	28.9%	5.3%	44.7%	50.0%	5.3%	67.6%	27.0%	5.4%	13.2%	47.4%	39.5%	23.7%	42.1%	34.2%
Nuku'alofa	117	39	4	95	65	0	117	39	4	29	75	56	29	87	44
(AN, Polynesia)	73.1%	24.4%	2.5%	59.4%	40.6%	0.0%	73.1%	24.4%	2.5%	18.1%	46.9%	35.0%	18.1%	54.4%	27.5%
Gidra	32	0	0	29	3	0	32	0	0	3	20	9	1	8	23
(NAN, Melanesia)	100%	0%	0%	90.6%	9.4%	0%	100%	0%	0%	9.4%	62.5%	28.1%	3.1%	25.0%	71.9%

Abbreviations: AN, Austronesian; NAN, Non-Austronesian; SNP, single-nucleotide polymorphism.

hypertension and treated with antihypertensive drugs; these participants were excluded from subsequent analyses. Note that, in addition to our attention on an increase of hypertension in Melanesia, a reason Melanesian Solomon Islanders only was chosen is that there was a difficulty to measure blood pressure of extremely obese participants (that is, those with larger arm circumferences than the cuff length) at a reliable level under the field-survey conditions for Micronesian and Polynesian populations.

Statistical analyses

Deviation from Hardy–Weinberg equilibrium was tested at each SNP in each population by using Pearson's χ^2 -test. Associations of allele frequencies of pairs

of SNPs were analyzed by using Fisher's exact test. Pearson's correlation coefficients were calculated for allele frequency data and distances of each population from the equator. Inter-population differences in hypertension prevalence were tested by using Fisher's exact test. Multiple logistic regression analysis (homozygote of ancestral allele = 1) and an analyses of covariance (ANCOVA) with a Tukey's *post hoc* test were performed to examine the potential effects of each SNP on hypertension and SBP or DBP; in these models, age, gender (female = 1, male = 0), body mass index (BMI) and population differences (Munda = 1, Kusaghe = 0) were included as explanatory variables to remove confounding effects. Finally, for detecting effects of each SNP independently from other SNPs, in addition to these confounding variables, all SNPs (three in the *AGT* gene and one *GNB3* and one *CYP3A5*)

were included in the logistic regression and general linear models for which significant factors were selected in a stepwise manner; significance levels for both entry and staying were set at 0.15. Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). P -values < 0.05 were considered statistically significant. The haplotype frequencies for three *AGT* SNPs were estimated using expectation maximization algorithms; the estimated haplotype frequencies were used to calculate linkage disequilibrium parameters, $|D'|$ and r^2 , between these polymorphisms.

RESULTS

Table 1 and Figure 1 show the genotype frequencies of *AGT*, *GNB3* and *CYP3A5* polymorphisms for two Polynesian populations (Nuku'alofa and Ha'apai), one Micronesian population (Ravaki), three AN-speaking Melanesian populations (Kusaghe, Munda and Manus) and one NAN-speaking Melanesian population (Gidra). In the NAN-speaking population, ancestral alleles (rs699-C and rs5051-T) only were found for rs699 and rs5051. These ancestral alleles were also the major alleles in all AN-speaking populations. The ancestral allele homozygote at rs5049 (rs5049-G/G) was more frequent in Melanesia AN-speaking populations (78.1%, Balopa; 86.0%, Paradise; 87.4%, Munda) than in non-Melanesian AN-speaking populations (58.6%, Ravaki; 44.7% Ha'apai; 59.4% Nuku'alofa). The derived alleles at rs5443 and rs776746 (rs5443-T and rs776746-G) were more frequent than the ancestral alleles (rs5443-C and rs776746-A) in all populations. No significant deviations from Hardy-Weinberg equilibrium were observed, except for the rs5049

in the Nuku'alofa population. Significant associations were found between rs699 and rs5049 (Fisher's exact test $P < 0.0001$), rs699 and rs5051 ($P < 0.0001$), and rs5049 and rs5051 ($P < 0.0001$); that is, among SNPs in the *AGT* gene.

The relationships between distances from the equator (absolute degrees) and the frequencies of ancestral alleles are shown in Figure 2. In the *AGT* gene, significant negative correlations were found for rs699-C ($r = -0.70$, $P < 0.0001$) and rs5051-T ($r = -0.72$, $P < 0.0001$), whereas no correlation was found for rs5049-G. A positive correlation was found for rs5443-C in the *GNB3* gene ($r = 0.83$, $P < 0.0001$), and a negative correlation was found for rs776746-A in the *CYP3A5* gene ($r = -0.59$, $P < 0.0001$). Within the Pacific Island populations, however, no correlations were observed for ancestral alleles at any of the genotyped SNPs. In addition, the frequencies of ancestral alleles of the Pacific Island populations showed small range variations for rs699 (range: 0.80–1), rs5049 (0.76–0.95), rs5051 (0.81–1) and rs5443 (0.36–0.42); when Gidra (NAN-speaking Melanesian) and Munda (AN-speaking Melanesian) were excluded, the ranges became smaller (0.80–0.96 for rs699, 0.76–0.92 for rs5049, 0.81–0.95 for rs5051 and 0.38–0.42 for rs5443).

Health status data for each of the Solomon Islands Melanesian populations are summarized in Table 2. There were no differences observed in body height between the populations, but both body weight and BMI were greater for the male and female individuals in Munda town than for those in Kusaghe. SBP and DBP were higher in Munda (127.0 mm Hg and 77.3 mm Hg on average, respectively) than

Figure 2 Correlations between the prevalences of the ancestral alleles (rs699-C, rs5049-G, rs5051-T, rs5443-C or rs776746-A) and the distances from the equator.

Table 2 Characteristics of the participants in the Melanesian Solomon Islands populations

	Kusaghe	Munda	P
No. of participants (% females)	209 (50.7)	174 (52.9)	
Age, years [s.d.] (min–max)	37.2 [13.7] (18–74)	44.8 [14.5] (18–79)	<0.0001
Height, cm (s.d.)			
Male	163.7 (5.5)	164.9 (5.6)	NS
Female	154.9 (5.0)	154.6 (4.9)	NS
Weight, kg (s.d.)			
Male	61.9 (6.2)	67.7 (13.8)	0.0006
Female	60.0 (7.9)	63.3 (12.5)	0.032
BMI, kg m ⁻² (s.d.)			
Male	23.1 (1.9)	24.9 (4.7)	0.0017
Female	25.0 (2.9)	26.5 (5.1)	0.015
SBP, mmHg (s.d.)			
Male	116.9 (12.4)	127.0 (20.0)	0.0001
Female	122.3 (15.6)	123.6 (18.5)	NS
DBP, mmHg (s.d.)			
Male	66.6 (7.7)	77.3 (11.9)	<0.0001
Female	76.0 (8.6)	79.1 (11.1)	0.034
Prevalence of hypertension			
18–30	2/77 (2.6%)	2/29 (6.9%)	NS
30–50	7/90 (7.8%)	9/74 (12.2%)	NS
50–	9/42 (21.4%)	29/71 (42.3%)	0.041
Total	18 (8.6%)	40 (23.0%)	<0.0001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; NS, not significant; SBP, systolic blood pressure.

in Kusaghe (116.9 mm Hg and 66.6 mm Hg) in the male individuals ($P=0.0001$ and <0.0001); however, in the female individuals, a significant difference was found only in DBP (79.1 vs 76.0 mm Hg, $P=0.034$). Participants' ages were biased between the two populations; the average age was higher in Munda (44.8 years) than in Kusaghe (37.8 years). The prevalence of hypertension was significantly higher in Munda (40/174, 23.0%) than in Kusaghe (18/209, 8.6%) ($P<0.0001$). This prevalence was higher in Munda than Kusaghe for all age groups (6.9% (2/29) vs 2.6% (2/77) in individuals 18–30 years of age; 12.2% (9/74) vs 7.8% (7/90) in individuals 30–50 years of age; 42.3% (29/71) vs 21.4% (9/42) in individuals ≥ 50 years of age), but a significant difference was only found in the elderly.

Table 3 shows the effects of genotypes on hypertension (SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg); effects of well-known confounding factors (that is, age, gender, BMI and population difference)^{1–3} were adjusted in the logistic regression models. The model fitness indicators (Wald-statistics, likelihood ratios, deviance/the degree of freedom, Pearson residual/the degree of freedom, and Hosmer–Lemeshow Goodness of Fitness) suggested good fitness of each model. The rs5049-A/A genotype (derived allele homozygote) significantly increased the risk of hypertension ($P=0.025$); however, none of the other SNPs exerted significant effects on disease risk. As shown in Table 4, the effect of rs5049-AA was only significant under the recessive model (G/G + G/A vs AA) ($P=0.022$), but not under the dominant model. Although not tabulated, the effects of rs5049 was significant only for ≥ 140 mm Hg SBP but not for ≥ 90 mm Hg DBP; other genotypes were neither associated with ≥ 140 mm Hg SBP nor ≥ 90 mm Hg DBP. Figure 3 shows values of SBP and DBP

separately for different genotypes after adjusting for age, gender, BMI and population in analyses of covariance models; P -values were <0.0001 for each model. For rs699, individuals with the C/T genotype had higher SBP values than those with the T/T genotype did (Tukey's *post hoc* test $P=0.034$). For rs5051, carriers of the ancestral T allele had higher SBP values than individuals with the C/C genotype did ($P=0.031$ for T/T vs C/C and $P=0.028$ for T/C vs C/C). Genotypes at rs5049 did not have any effect on either SBP or DBP.

Strong linkage disequilibrium (i.e., $|D'| \approx 1$) was observed between the SNPs rs699 and rs5049 ($|D'| = 1$, $r^2 = 0.009$), rs699 and rs5051 ($|D'| = 0.97$, $r^2 = 0.86$) and rs5049 and rs5051 ($|D'| = 1$, $r^2 = 0.026$), i.e., among SNPs in the *AGT* gene. The stepwise selection of factors by using the general linear models, which included these three SNPs and the other two SNPs (rs5443 and rs776746), as well as age, gender, BMI and population differences revealed a negative effect of the rs5051-C/C genotype ($P=0.034$) on SBP; age and BMI were also significant predictors of SBP (model $R^2 = 0.20$, $P<0.0001$). In contrast, results from the multiple logistic regression model suggested that the rs5049-A/A genotype (odds ratio = 10.31 (1.59: 67.00), $P=0.015$, as well as age and BMI were significant predictors of hypertension.

DISCUSSION

Previous studies had suggested that the SNPs analyzed in this study were related to latitude, because certain genotypes had protective roles in high-temperature environments; in African, European, Asian and American populations, ancestral alleles at rs699 and rs5051 in *AGT*, and rs776746 in *CYP3A5*, were found to be frequent in lower latitudes, whereas those at rs5443 in *GNB3* were found to be more

Table 3 Odds ratios (95% CI) of the effects of *AGT*, *GNB3* or *CYP3A5* polymorphisms and confounding factors on hypertension (SBP $\geq$ 140 mm Hg and/or DBP $\geq$ 90 mm Hg) in the Melanesian Solomon Islands populations^{a,b}

Gene (SNP)	Allele (homozygote of ancestral allele = 1)	Age	Gender (female = 1)	BMI	Population (Munda = 1)	Model Wald likelihood ratio χ^2
<i>AGT</i> (rs699)	C/C	1	1.08 (1.05:1.10)*	1.44 (0.73:2.84)	1.23 (1.13:1.33)*	51.98***
	C/T	1.35 (0.56:3.23)				79.20***
	T/T	0.78 (0.13:4.61)				
<i>AGT</i> (rs5049)	G/G	1	1.08 (1.05:1.11)*	1.37 (0.69:2.71)	1.24 (1.14:1.35)*	52.65***
	G/A	0.44 (0.14:1.40)				85.06***
	A/A	8.89 (1.32:60.05)***				
<i>AGT</i> (rs5051)	T/T	1	1.08 (1.05:1.10)*	1.41 (0.72:2.79)	1.23 (1.13:1.34)*	52.20***
	T/C	1.35 (0.58:3.13)				79.47***
	C/C	0.65 (0.11:3.65)				
<i>GNB3</i> (rs5443)	C/C	1	1.08 (1.05:1.11)*	1.40 (0.71:2.75)	1.24 (1.14:1.35)*	52.40***
	C/T	0.46 (0.17:1.25)				81.16***
	T/T	0.44 (0.16:1.23)				
<i>CYP3A5</i> (rs776746)	A/A	1	1.08 (1.05:1.10)*	1.49 (0.76:2.95)	1.22 (1.13:1.33)*	52.16***
	A/G	1.37 (0.42:4.49)				79.56***
	G/G	1.69 (0.52:5.44)				

Abbreviations: BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

* $P < 0.05$, *** $P < 0.0001$.^aOdds ratios (95% CI) in a model without genotype differences are 1.07 (1.05:1.10)*, 1.43 (0.73:2.81), 1.22 (1.13:1.33)* and 1.47 (0.74:2.93) for age, gender, BMI and population, respectively (model Wald statistic = 51.40 ($P < 0.0001$), likelihood ratio $\chi^2 = 78.63$ ($P < 0.0001$), the number of unique profile = 383, deviance/the degree of freedom = 0.65 (247.06/378), Pearson residual/the degree of freedom = 1.10 (416.30/378), Hosmer–Lemeshow Goodness of Fitness $\chi^2 = 6.48$ ($P = 0.5933$)).^bModel fitness indicators for the SNPs rs699, rs5049, rs5051, rs5443 and rs776746 were as follows, respectively: the number of unique profile = 383 (for all SNPs), deviance/the degree of freedom = 0.66 (246.50/376), 0.64 (240.64/376), 0.65 (246.23/376), 0.65 (244.53/376), 0.65 (246.14/376), Pearson residual/the degree of freedom = 1.06 (399.16/376), 1.13 (426.7/376), 1.07 (400.69/376), 1.02 (384.62/376), 1.08 (404.75/376), Hosmer–Lemeshow Goodness of Fitness $\chi^2 = 2.96$, 6.63, 2.95, 5.96 and 7.28 ($P = 0.9369$, 0.5775, 0.9375, 0.6514 and 0.5072).**Table 4 Genotype distribution of rs5049 in hypertension participants and normal participants**

	<i>Hypertension participants, n (%)</i>	<i>Normal participants, n (%)</i>	<i>Odds ratio (95% CI)^a</i>	<i>P-value</i>
<i>Genotype</i>				
G/G	52 (89.7)	279 (85.9)	1	NS
G/A	4 (6.9)	42 (12.9)	0.44 (0.14:1.40)	
A/A	2 (3.5)	4 (1.2)	8.89 (1.32:60.05)	
<i>Recessive model</i>				
G/G + G/A	56 (96.5)	321 (98.8)	1	0.022
A/A	2 (3.5)	4 (1.2)	9.40 (1.39:63.46)	
<i>Dominant model</i>				
G/G	52 (89.7)	279 (85.9)	1	NS
G/A + A/A	6 (10.4)	46 (14.1)	1.40 (0.52:3.77)	

Abbreviations: CI, confidence interval; NS, not significant.

^aAge, gender, BMI and population difference were adjusted in the logistic regression models (see Table 3).

frequent in higher latitudes.^{7,10} The present study is the first to reveal that Pacific Island populations, which are characterized by their relatively recent settlement of the Pacific Islands (~2000 years ago), also follow the same allele–latitude relationship gradient. However, Pacific Island populations' adaptation to the climatic changes following 'out-of-Asia' migration remained unclear because the frequencies

were not correlated with the latitude and that the frequencies were at the similar level regardless of large variation in latitude within the Pacific Island populations (1.89°–21.08°). In addition, frequencies of risk alleles (ancestral alleles of rs699 and rs5051 and the derived allele of rs5443) of the Pacific Island populations were similar to those of the Asian populations (Figure 2). The NAN-speaking population Gidra, which has spent tens of thousands of years in Melanesia, showed frequencies of risk alleles (or adaptive alleles in those near the equator) as high as those in the African populations, suggesting that this population reflects adaptation in low-latitude Melanesia. In this regard, the frequencies of risk alleles (rs699-C, rs5049-G and rs5051-T) of Munda, which is an AN-speaking Melanesian population, were similar to those of Gidra. Archeological studies and oral history have suggested that the Munda population descended from the Kazukuru population, which has been identified as an extinct NAN-speaking population that lived in the island.²¹ A previous study hypothesized that the adaptation to latitude occurred relatively recently because Native Americans, who also settled in the Americas only recently, followed the global allele–latitude relations; though the correlation within the Native American population seemed not significant.⁸ In summary, although it remains unclear whether or not adaptation to latitude occurs as recently as 2000 years ago; that is, after the 'out-of-Asia' migration into the Pacific Islands, it is reasonable to consider that the Pacific Island populations also experienced adaptation to the climatic changes after the out-of-Africa migration.

Our results of the associations between these SNPs, hypertension and blood pressure seem controversial. Although rs5049-A contributed to the increased risk of hypertension, this SNP was not associated with elevated SBP or DBP in this study; although 33.3% of the participants with the rs5049-A/A were classified as hypertensive (SBP $\geq$ 140 mm Hg and/or DBP $\geq$ 90 mm Hg), blood pressure of the remaining 66.7% were as low as those of the other genotypes. This discrepancy was thought to be the result of the small number of the

Figure 3 The means and s.e. of SBP and DBP partitioned by *AGT*, *GNB3* and *CYP3A5* genotypes. Comparisons between every pair of genotypes were made using the analysis of covariance by adjusting for age, gender, BMI and population. *P*-values for Tukey's *post hoc* comparison tests are also shown. Note: coefficients (s.e.) for age (years), gender (female=1), BMI and population difference (Munda=1), respectively, in models without genotype variables were 0.39 (0.06) ($P<0.0001$), 0.047 (1.611) (NS), 1.04 (0.21) ($P<0.0001$) and 0.81 (1.66) (NS) for SBP (model $R^2=0.20$, $P<0.0001$) and 0.15 (0.03) ($P<0.0001$), 4.89 (0.99) ($P<0.0001$), 0.70 (0.13) ($P<0.0001$) and 4.41 (1.02) ($P<0.0001$) for DBP (model $R^2=0.28$, $P<0.0001$).

participants with the rs5049-A/A genotype. The SNP rs5051, on the other hand, which is in linkage disequilibrium with rs699, was associated with SBP; the derived alleles at rs5051 showed a low frequency but were found to be associated with lower SBP. These findings suggest that the *AGT* gene had significant roles in the hypertensive traits of the Melanesian Solomon Islanders, although we cannot confirm the specific effect of each SNP. Previous studies also yielded controversial results. The rs699-C (235T) allele was significantly associated with hypertension in European²² and Japanese populations,^{23,24} but such an effect was not replicated in an African Caribbean population, in a second European population, or in Malaysians.^{25–27} Although it is possible that the rs5051-T (–6G) allele is associated with hypertension, the effect of this allele has generally been recognized as a result of strong linkage disequilibrium with rs699.²⁸ Unlike rs699 and rs5051, which have been extensively studied, rs5049 has only recently been investigated with respect to hypertension risk; in the present study, we revealed the role of the rs5049-A (–217A) allele in hypertension, which is consistent with previous studies in African American and Taiwanese populations,^{29,30} as well as a meta-analysis of six studies.³¹ On the other hand, in other studies, the rs5443-T (825T) allele in *GNB3* was previously reported to be associated with hypertension in Europeans³² and the male Japanese,³³ whereas no such association between alleles and hypertension was reported for other populations.^{34–36} Carriers of the rs776746-A allele in *CYP3A5* in populations of African origin showed increased blood pressure in previous studies^{37,38} but not in this study. These differences are thought to be caused by other risk factors such as environmental and lifestyle differences, and thus, detailed investigations including not only genetic but also on non-genetic factors are necessary. However, it is noteworthy that all the major alleles in the Pacific Island populations had been previously reported as risk alleles.

It has been suggested that hidden population structure and cryptic relatedness may affect the genetic association studies.^{39,40} As our subjects were recruited from small isolated populations, some subjects might have been close relatives. However, the significant excess of homozygotes was seldom observed at polymorphisms investigated in our study populations,^{16,41–44} although genome-wide analysis using a number of SNPs is necessary to examine the relatedness among our subjects in detail. We therefore judged that cryptic relatedness was only to a limited extent in this study, if any.

The effects of *AGT*, in conjunction with the latitudinal correlation observed here, suggest that genotypes in the Melanesian Solomon Islander populations were positively selected after the out-of-Africa migration (~60 000 years ago) into tropical Asia, though effects of the 'out-of-Asia' migration into the Pacific remained unclear. Our previous studies have suggested that carriers of Q allele of the leptin receptor gene (*LEPR*) polymorphisms Q223R, who were more common in AN-speaking populations than in NAN-speaking populations, displayed lower serum leptin levels. Lower levels of serum leptin are associated with enhanced food intake and suppressed energy expenditure; thus, not surprisingly, the prevalence of obesity was found to be higher in the AN-speaking populations; this phenotype was thought to have developed after the 'out-of-Asia' migration.^{16,17} Increased risks of non-communicable diseases, including cardiovascular diseases,^{3,18} in the NAN-speaking and AN-speaking Melanesians in urbanized lifestyles are thus thought to have genetic components. Although other polymorphisms associated with hypertension and more populations remain to be studied, findings from the present study, as well as our two previous studies suggest that the susceptibility of Pacific Islanders to many non-communicable

diseases is the result of human evolution and migration after the out-of-African migration, through Asia, into the Pacific Islands. It is thus important to consider the understanding genetics in a population when attempting to understand epidemiological patterns.

In conclusion, this study found that (1) ancestral alleles of rs699, rs5051 and rs776746, and the derived allele of rs5443 were as prevalent in Pacific Island populations as in other equatorial populations; (2) rs5049 was not correlated with latitude; (3) the A/A genotype of rs5049 was a risk factor for hypertension in Melanesian Solomon Islanders; and (4) the T/T genotype of rs699 and the C/C genotype of rs5051 were associated with decreased SBP.

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