No Association Between α-Adducin 460 Polymorphism and Essential Hypertension in a Japanese Population

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Many unknown genetic factors are involved in the pathogenesis of hypertension. Recently, the reverse genetic approach revealed that some genetic variants, such as angiotensinogen, lipoprotein lipase, and α-adducin gene polymorphisms, increase the risk for hypertension. Both in rat and human, the genetic predisposition to hypertension was confirmed only for angiotensinogen and α-adducin genes. Adducin is a membrane cytoskeletal protein, which is thought to regulate sodium transport. Abnormalities of membrane sodium transport in the kidney play an important role in hypertension. A recent report by Cusi et al showed that the Trp allele of α-adducin polymorphism (Gly 460 Trp) is associated with an increased risk of hypertension in whites, which led us to carry out a case-control study to examine whether the same association is observed in the Japanese population. We recruited 170 hypertensive and 194 normotensive Japanese subjects and compared the genotype distribution of α-adducin 460 polymorphism between cases and controls and between whites and Japanese. Trp allele frequency of controls in the Japanese subjects was twice as high as in the whites. However, no association was observed between α-adducin polymorphism and hypertension. Furthermore, α-adducin 460 polymorphism was not associated with any clinical characteristics. Accordingly, we concluded that α-adducin 460 polymorphism is not a major genetic risk for hypertension in Japanese people. Am J Hypertens 1998;11:502–506 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: **Hypertension, α-adducin, genetics, sodium, polymorphism, cytoskeleton.**

Essential hypertension is known to be caused by polygenes, and its phenotypic expression is modulated by various environmental factors. Recent advances in molecular biology have allowed investigation of the role of candidate genes for hypertension. The affected sib-pair approach revealed that angiotensinogen, lipoprotein lipase, and α-adducin gene polymorphisms are genetic risk factors for human essential hypertension.[1–3] Among them, it was observed that angiotensinogen and α-adducin genes predispose to hypertension both in human and rat.[4,5]

Abnormalities of membrane ion transport in the kidney may play a central role in the pathogenesis of hypertension.[6,7] An excess of circulating volume due to increased sodium reabsorption is a cause of hypertension. The Milan hypertensive strain of rat (MHS) is a genetic model of renal hypertension.[8] Sodium trans-

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port across the cell membrane in MHS is faster than that in the Milan normotensive strain (MNS) at intracellular and molecular levels, which results in sodium retention in MHS.[9–12] Adducin is a heterodimeric protein of the membrane skeleton with α- and β-subunits, and it promotes the spectrin-actin interaction.[13] Even though the precise role of adducin is unknown, recent genetic investigations suggest that its polymorphism may be involved in hypertension. Genetic analysis of MHS revealed that a mutation at position 316 of rat α-adducin gene accounts for up to 50% of the BP difference between MHS and MNS, and that a polymorphism of β-adducin only modulates the effect of the α-unit.[14]

There is approximately 94% homology between rat and human α-adducin genes.[14] A linkage study showed a positive association between hypertension and microsatellite markers mapped close to the α-adducin locus, which suggests that there is a functional mutation in the α-adducin gene.[15] Recently, Cusi et al identified an amino-acid substitution (Gly 460 Trp) using Italian and French populations.[3] They found a significant association between the 460 Trp mutation and hypertension ($P = .0003$). Interestingly, subjects with the Trp allele showed a greater decrease of arterial pressure in the acute salt-sensitivity test and on chronic diuretic treatment. Because they studied only a white population, we carried out a case-control study in the Japanese population to examine the association between α-adducin 460 polymorphism and essential hypertension.

MATERIALS AND METHODS

Population Patients with essential hypertension and control subjects were recruited from outpatients at the Department of Geriatric Medicine, Osaka University Medical School. All cases and controls were Japanese and gave informed consent before participating in the research protocol, which was approved by the Hospital Ethics Committee. All cases (n = 170) had a family history of hypertension in first-degree relatives and were diagnosed as having primary hypertension (those with secondary hypertension, diabetes mellitus, or apparent ischemic heart disease were excluded). The criteria for hypertension were defined as systolic blood pressure > 160 mm Hg, diastolic blood pressure > 95 mm Hg, or under antihypertensive therapy. Control subjects (n = 194) without a history of hypertension and without diabetes mellitus were recruited from the same population, and were matched to case patients for gender and age. We also excluded from the control group subjects with first-degree relatives who had hypertension. Participants completed a standard questionnaire on personal medical history and family history of hypertension. Blood pressure was measured twice with the subject seated after 5 min of rest. The characteristics of controls and cases are summarized in Table 1.

TABLE 1. CLINICAL CHARACTERISTICS OF SUBJECTS

	Hypertensive (n = 170)	Control (n = 194)	*P* Value
Age (years)	59.4 ± 0.8	58.8 ± 0.9	NS
Gender (% male)	45.3%	48.5%	NS*
SBP (mm Hg)	180 ± 1.5	119 ± 0.9	.0001
DBP (mm Hg)	105 ± 1.0	75 ± 0.6	.0001
BMI (kg/m^2)	23.9 ± 0.2	22.0 ± 0.2	.0001
T-chol (mg/dL)	210 ± 2.7	209 ± 2.6	NS
TG (mg/dL)	141 ± 6.4	119 ± 5.2	.0005
FPG (mg/dL)	96.4 ± 0.9	92.1 ± 0.7	.0005
Creatinine (mg/dL)	0.85 ± 0.03	0.80 ± 0.02	NS

Data are expressed as mean ± SE.

Significance of differences between hypertensives and controls was examined by ANOVA.

** χ^2 test.*

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; T-chol, total cholesterol; TG, triglyceride; FPG, fasting plasma glucose.

Determination of Genotypes Blood was drawn to obtain the buffy coat. DNA was extracted from 200 mL of buffy coat using QIAamp Kit (QIAGEN Inc., Santa Clarita, CA). Polymerase chain reaction (PCR) was carried out with 100 ng of genomic DNA as a template using a thermal cycler, Omni Gene (Hybaid). To amplify the shorter fragment of the franking region of 460 polymorphism, we newly designed the primer set as follows; sense: 5′-CGACGAAGCTTCCAAGGA-3′ and antisense: 5′-ACAGTAAGGTAGGCACAGA-3.[16] DNA was amplified with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 60 sec, 51°C for 60 sec, and 72°C for 60 sec. PCR products were digested with 1 U of *Pfl*M I (New England Biolabs, Beverly, MA) at 37°C overnight. All digested products were separated on 3.0% MetaPhor agarose gel (FMC BioProducts) and visualized with ethidium bromide staining.

Statistical Analysis All statistical analyses were conducted using StatView 4.5J (Abacus Concepts, Berkeley, CA) and JMP 3.0 (SAS Inc., Cary, NC). The difference in genotype distribution between cases and controls was examined by χ^2 analysis. The association between α-adducin polymorphism and clinical variables was examined by one-way analysis of variance. To assess the quantitative effects of the covariates (gender, age, body mass index, fasting plasma glucose, and triglycerides), we carried out multiple logistic regression analysis using JMP.

TABLE 2. GENOTYPE AND ALLELE DISTRIBUTIONS OF α-ADDUCIN 460 POLYMORPHISM IN CONTROL AND HYPERTENSIVE SUBJECTS

	Hypertensive Subjects		Control Subjects	
Gly 460 Trp	n	%	n	%
Genotype				
Gly/Gly	33	19.4	35	18.0
Gly/Trp	85	50.0	96	49.5
Trp/Trp	52	30.6	63	32.5
	$\chi^2 = 0.043$		NS	
Allele				
Gly	151	44.4	166	42.8
Trp	189	55.6	222	57.2
	$\chi^2 = 0.02$		NS	

Significance of differences between hypertensives and controls was examined by χ^2 test.

RESULTS

Distribution of α-Adducin Genotypes in a Japanese Population In hypertensives, body mass index (BMI), fasting plasma glucose (FPG), and triglyceride levels were higher than those in controls. According to PCR-*Pfl*M I-RFLP (restriction fragment polymorphism), Gly 460 Trp α-adducin gene polymorphism was clearly determined in all subjects. The genotype frequencies were not significantly different from the values of the Hardy-Weinberg expectation. In 194 healthy Japanese, the allele frequency of the T mutation was estimated as 57.2% (Table 2), whereas it was 13.6% or 16.3% in the white population. The distribution of the three genotypes was as follows: GG genotype, 18.0%; GT genotype, 49.5%; and TT genotype, 32.5% (Table 3). The Trp allele frequency (57.2% in controls, 55.6% in hypertensives) of Japanese was significantly higher than that of whites (15.1% in controls, 22.0% in hypertensives).

Association Study The genotype and allele frequencies of Gly 460 Trp polymorphism of the α-adducin gene were not significantly different between cases and controls (Table 2). To identify the confounding factors for α-adducin 460 polymorphism, we examined the association between the polymorphism and clinical values. We analyzed the association between each of the α-adducin 460 genotypes (GG, GT, TT) and variants (systolic blood pressure, diastolic blood pressure, body mass index, plasma renin activity, plasma aldosterone concentration, and serum creatinine), but no significant difference was observed. Even with the effects of BMI, FBS, and TG excluded, α-adducin 460 polymorphism was not associated either with hypertension or with other variables in a multiple logistic regression analysis.

DISCUSSION

To identify the causative gene of essential hypertension, a large number of case-control studies and rat cross experiments have been performed. Using genetically hypertensive rats, many quantitative trait loci responsible for BP have been mapped on the rat genome, but few loci, only on chromosome 1, 4, and 8, were confirmed to cosegregate with hypertension by the affected sib-pair method.[1–3] One of these candidates is α-adducin polymorphism, and a recent report which showed a significant association between a newly identified mutation in human α-adducin em-

TABLE 3. ASSOCIATION BETWEEN α-ADDUCIN 460 GENOTYPE AND CLINICAL CHARACTERISTICS

	Gly/Gly	Gly/Trp	Trp/Trp	*P*
Hypertensive subjects	(n = 33)	(n = 85)	(n = 52)	
SBP (mm Hg)	183 ± 3.7	179 ± 2.3	179 ± 2.2	NS
DBP (mm Hg)	102 ± 2.1	106 ± 1.5	106 ± 1.7	NS
BMI (kg/m^2)	24.3 ± 0.7	23.7 ± 0.4	23.9 ± 0.5	NS
PRA (ng/mL/h)	1.37 ± 0.24	1.20 ± 0.19	1.35 ± 0.25	NS
PAC (ng/dL)	15.1 ± 1.4	14.2 ± 0.8	15.4 ± 1.3	NS
TG (mg/dL)	139 ± 14.2	150 ± 9.4	128 ± 11.0	NS
FPG (mg/dL)	98.8 ± 2.1	96.2 ± 1.4	95.3 ± 1.3	NS
Control subjects	(n = 35)	(n = 96)	(n = 63)	
SBP (mm Hg)	117 ± 2.2	118 ± 1.2	121 ± 1.5	NS
DBP (mm Hg)	74 ± 1.6	75 ± 0.8	76 ± 1.0	NS
BMI (kg/m^2)	22.3 ± 0.7	21.9 ± 0.3	22.1 ± 0.3	NS
TG (mg/dL)	109 ± 8.1	118 ± 7.5	125 ± 10.0	NS
FPG (mg/dL)	89.9 ± 1.6	92.5 ± 1.0	92.6 ± 1.4	NS

Data are expressed as mean ± SE.

Significance of differences between hypertensives and controls was examined by ANOVA.

TABLE 4. DIFFERENCES OF ALLELE FREQUENCY OF A-ADDUCIN 460 POLYMORPHISM IN DIFFERENT POPULATIONS

Population	Genotype, %			Allele, %	
	GG	GT	TT	G	T
Normotensives					
Japanese (n = 194)	18.0	49.5	32.5	42.8	57.2
White (n = 332)	73.2	23.5	3.3	84.9	15.1
	$\chi^2 = 66.4$, $P < .0001$			$\chi^2 = 38.3$, $P < .0001$	
Hypertensives					
Japanese (n = 170)	19.4	50.0	30.6	44.4	55.6
White (n = 477)	60.6	34.8	4.6	78.0	22.0
	$\chi^2 = 43.5$, $P < .0001$			$\chi^2 = 24.3$, $P < .0001$	

Significance of differences between Japanese and Whites was examined by χ^2 test.

phasized the importance of this gene.[3] Preliminary results of examining the association between rat adducin gene polymorphism and BP regulation in MHS supported the observation that the adducin gene variant affects renal function by modulating the overall capacity of tubular epithelial cells to transport ions modifying the assembly of the actin cytoskeleton.[17] In humans, Cusi et al revealed that the Trp allele of α-adducin 460 polymorphism is not only associated with an increased risk of hypertension but also increased sensitivity to salt and treatment with hydrochlorothiazide in the white population.[3]

In the present study, however, we could not detect any association between the α-adducin 460 genotype and hypertension in a Japanese population. As shown in Table 4, genotype distribution was quite different between Japanese and whites. The Trp allele frequency of hypertensive patients is 55.6% in Japanese, while it is 20.7% or 23.8% in whites. Comparison of the clinical characteristics between Japanese and whites suggested that more lean and severe hypertensives were recruited in the Japanese cases. Furthermore, all Japanese cases had a family history of hypertension in first-degree relatives, suggesting theoretically that hypertensives in the present study should have a higher genetic risk for hypertension. Considering these results together, we can conclude that α-adducin 460 polymorphism is not a major genetic risk for hypertension in Japanese individuals.

A similar tendency in allele frequency and in the results of an association study across different races were reported in genetic studies of angiotensinogen gene polymorphism. The angiotensinogen M235T polymorphism is well known as a genetic risk for essential hypertension, and its allele frequency has been estimated in various races. For example, T235 allele frequency of the angiotensinogen gene in Japanese is twice as high as in whites, although their hypertension morbidity is similar.[1,18] Moreover, the heterogeneity of its allele frequency was observed even between black Americans and Nigerians.[19] When negative results of association were obtained, authors tended to explain them by a potential role of genetic and environmental heterogeneity.

As for the effect of the angiotensinogen gene, we have already reported that the M235T and C-18T polymorphisms of angiotensinogen were associated with essential hypertension ($\chi^2 = 7.39$, $P < .05$, and $\chi^2 = 10.54$, $P < .005$, respectively) using the same subjects as in the present study.[20] There was no synergistic effect between angiotensinogen polymorphisms and α-adducin 460 polymorphism, suggesting that there is no genetic interaction between them.

Another possible explanation for the inconsistent results among studies is difference of the mean age of populations. To examine the effect of age, we divided the subjects by age into two subgroups, older and younger than 60 years. In each subgroup, we conducted a multiple logistic regression analysis, and obtained the same result, that α-adducin 460 is not associated with hypertension (data not shown). Consequently, it is impossible to explain the inconsistency by age difference.

To conclude, the Trp allele of α-adducin 460 polymorphism is frequent but does not predispose to essential hypertension in Japanese individuals. There still remains the possibility that the adducin gene affects membrane ion transport because we did not investigate the effect on salt-sensitivity or response to diuretic treatment. A large prospective study may be required to confirm the precise role of the adducin gene.

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