Original Article

Lack of Association between Genetic Polymorphism of *CYP11B2* and Hypertension in Japanese: The Suita Study

Yasuyuki TSUJITA^{*1,2}, Naoharu IWAI^{*1,3}, Tomohiro KATSUYA^{*4}, Jitsuo HIGAKI^{*4}, Toshio OGIHARA^{*4}, Shinji TAMAKI^{*2}, Masahiko KINOSHITA^{*2}, Toshifumi MANNAMI^{*5}, Jun OGATA^{*5}, and Shunroku BABA^{*5}

Aldosterone synthase (*CYP11B2*) is a key enzyme in the biosynthesis of aldosterone. Recently, a polymorphism in the 5'-flanking region of the *CYP11B2* gene [T(−344)C] has been reported to be associated with blood pressure and plasma aldosterone levels. We investigated the association between this polymorphism and hypertension in a large population-based sample of 4,000 Japanese. The genotype distribution in hypertensive subjects ($n=1,535$) was compared to that in normotensive subjects ($n=2,514$). In subjects not receiving antihypertensive medication, the influence of the genotype on blood pressure values adjusted for clinical covariates was analyzed. All analyses were performed separately for men and women. The genotype distribution did not differ between hypertensive and normotensive subjects in either men (frequency of C allele: 30.3% vs. 31.4%, $p=0.48$) or women (31.5% vs. 31.7%, $p=0.93$). There were no significant differences in systolic blood pressure, diastolic blood pressure, or pulse pressure among the three genotypes in either men or women who had not received hypertensive medication. Our data suggest that the T(−344)C polymorphism of *CYP11B2* is unlikely to influence blood pressure status in the Japanese population. (*Hypertens Res* 2001; 24: 105-109)

Key Words: aldosterone, blood pressure, cytochrome P-450 *CYP11B2*, genetics, hypertension

Introduction

Aldosterone synthase (*CYP11B2*), a cytochrome P450 enzyme mainly located in the zona glomerulosa of the adrenal cortex, is a key enzyme in aldosterone synthesis (1, 2). Aldosterone controls the sodium balance and thus influences blood pressure regulation. Rare mutations of this gene are associated with either markedly elevated aldosterone levels and arterial hypertension or insufficient aldosterone synthesis and sodium wasting (3-6). There-

fore, genetic variants of the *CYP11B2* gene that affect its expression may contribute to the development of salt-sensitive hypertension.

Several common polymorphisms have recently been described in the *CYP11B2* gene (7, 8). The Lys173Arg polymorphism has been suggested to be linked to low-renin hypertension in Chilean hypertensives (8). The T(−344)C polymorphism, which is at a putative binding site for the steroidogenic transcription factor (SF-1), has recently been reported to be associated with left ventricular mass in young Finnish adults without clinical heart diseases (9).

From the ^{*1}Research Institute, National Cardiovascular Center, Suita, Japan, ^{*2}the First Department of Internal Medicine, Shiga University of Medical Science, Otsu, Japan, ^{*3}the Department of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan, ^{*4}the Department of Geriatric Medicine, Osaka University Medical School, Suita, Japan, and ^{*5}the Department of Preventive Medicine, National Cardiovascular Center, Suita, Japan.

Address for Reprints: Naoharu Iwai, M.D., Research Institute, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita 565-8565, Japan.

Received October 10, 2000; Accepted in revised form December 11, 2000.

We previously reported that the 173Arg allele completely corresponded to the (−344)C allele, and that the (−344)C allele might be a genetic marker for low-renin hypertension in Japanese (10). The (−344)C allele was reported to be associated with higher systolic blood pressure in Finns (11). The (−344)C allele was reported to be associated with elevated levels of plasma aldosterone, but not in French (12).

On the other hand, the (−344)T allele has also been reported to be associated with hypertension in French (13) and Scottish (14), low-renin hypertension in Japanese (15), higher aldosterone level in French (16), higher excretion rate of tetrahydroaldosterone in Scottish (14), and higher urinary aldosterone excretion and higher 11-deoxycortisol responses to adrenocorticotropin stimulation in Finns (11).

Some groups have reported negative results: the T (−344)C polymorphism was not associated with blood pressure in Japanese (17), with blood pressure, serum aldosterone level, or cardiac size or function in Germans (18), or with salt sensitivity or increased activity of the renin-angiotensin system in young normotensive German men (19).

To settle these inconsistent results, we investigated the association between the T(−344)C polymorphism of the *CYP11B2* gene and hypertension in a large sample of the general population in Japan.

Methods

Subjects

The selection criteria and study design of the Suita Study have been described previously (20, 21). The sample consisted of 14,200 men and women aged 30 to 79 years stratified by gender and 10-year age groups who were selected randomly from the municipal population registry. The basic sampling of the population started in 1989 with a cohort study base, and 51.7% ($n=7,347$) of the subjects had paid their initial visit to the National Cardiovascular Center by February 1997. The subjects have visited the National Cardiovascular Center every 2 years for regular health checkups. DNA from leukocytes was collected from subjects who visited the National Cardiovascular Center between May 1996 and February 1998. All of the subjects were Japanese, and only those who gave their written informed consent for genetic analysis of the aldosterone synthase gene were enrolled in the present study ($n=4,049$). The committee on genetic diagnosis and genetic therapy of the National Cardiovascular Center approved the present study.

Measurements

Systolic blood pressure and diastolic blood pressure

values were the means of two physician-obtained measurements (recorded 3 min apart) determined by the first and fifth Korotkoff phases, respectively, in the right arm of the seated subject with a mercury column sphygmomanometer. The pulse pressure was the difference between the systolic and diastolic blood pressure. Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg, a diastolic blood pressure of ≥ 90 mmHg, or current use of antihypertensive drugs. Body height and weight were used to calculate body mass index (BMI) (weight (kg)/height² (m²)). Data were collected on clinical variables including age, alcohol consumption, smoking habit, presence of cerebrovascular accident, presence of ischemic heart disease, diabetes mellitus, proteinuria, and use of antihypertensive drugs.

Genotype Determination

DNA was isolated from peripheral leukocytes according to standard procedures (22). The T(−344)C polymorphism of the *CYP11B2* gene was genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis method described previously (10). The designed primers were 5′-CAGGAG GGATGAGCAGGCAGAGCACAG-3′ (sense, from −677 to −651), and 5′-CTCACCCAGGAACCTGCTCT GGAAACATA-3′ (antisense, from −71 to −42). The PCR profile included 34 cycles of denaturing at 94°C for 45 s, annealing at 58°C for 45 s, and polymerization at 72°C for 45 s. The amplified fragments were digested with Hae III restriction enzyme (Takara Shuzou, Otsu, Japan) and subjected to electrophoresis on 2.0% agarose gels. Fragments of 404 bp (T allele) and of 333 bp and 71 bp (C allele) were detected.

Statistical Analysis

All analyses were performed separately for men and women. The difference in genotype or allele distribution between hypertensive and normotensive subjects was examined by a χ^2 analysis. The association between the *CYP11B2* T(−344)C polymorphism and clinical variables was examined by one-way ANOVA. In subjects not receiving antihypertensive medication, multiple linear regression analyses were performed to predict the blood pressure values with other covariates (age, BMI, alcohol consumption, and waist/hip (W/H) ratio). All statistical analyses were performed using the JMP statistical software package (SAS Ins. Inc., Cary, USA).

Table 1. Characteristics of Men and Women Subjects by *CYP11B2* Genotype

Characteristic	<i>CYP11B2</i> genotype		
	<i>TT</i>	<i>TC</i>	<i>CC</i>
Men (<i>n</i> =1,915)	(<i>n</i> =916)	(<i>n</i> =813)	(<i>n</i> =186)
Age (years)	60.8±12.1	61.1±12.2	61.6±12.3
Body mass index (kg/m ²)	22.9±2.7	23.1±2.9	23.2±3.1
Waist-to-hip circumference ratio	0.90±0.05	0.91±0.06	0.90±0.06
Alcohol consumption (ml/d)	26.3±27.2	24.3±26.4	25.2±24.3
Smoking habit (%)	40.7	38.1	40.3
Ischemic heart disease (%)	4.9	4.1	3.8
Diabetes mellitus (%)	20.3	23.3	23.1
Proteinuria (%)	6.6	7.3	9.2
Use of antihypertensive drugs (%)	18.7	17.7	18.3
Women (<i>n</i> =2,134)	(<i>n</i> =1,017)	(<i>n</i> =885)	(<i>n</i> =232)
Age (years)	58.5±11.8	59.1±11.7	59.5±12.0
Body mass index (kg/m ²)	22.4±3.1	22.2±3.3	22.3±2.9
Waist-to-hip circumference ratio	0.88±0.08	0.88±0.07	0.89±0.08
Alcohol consumption (ml/d)	5.0±11.8	5.2±10.6	4.2±8.7
Smoking habit (%)	9.1	7.7	8.2
Ischemic heart disease (%)	3.3	2.5	2.6
Diabetes mellitus (%)	12.2	14.9	16.8
Proteinuria (%)	3.9	3.7	6.5
Use of antihypertensive drugs (%)	16.6	15.7	14.7

Values are mean±SD. *TT* indicates homozygotes for T allele; *TC*, heterozygotes; *CC*, homozygotes for C allele.

Results

Clinical Characteristics and *CYP11B2* Genotype and Allele Frequencies

The overall frequencies of the genotypes *TT*, *TC*, and *CC* were as follows: in men, 47.8%, 42.5%, and 9.7%, respectively; in women, 47.6%, 41.5%, and 10.9%; and overall, 47.7%, 42.0%, and 10.3%. The allele frequencies for T and C were as follows: in men, 69.1% and 30.9%, respectively; in women, 68.4% and 31.6%; and overall, 68.7% and 31.3%. The observed genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium.

Table 1 summarizes the clinical characteristics of men (*n*=1,915) and women (*n*=2,134) at the index examination according to the *CYP11B2* genotype. There were no significant differences among the three genotypes with regard to any characteristics in either men or women.

Relationship between the *CYP11B2* Genotype and Blood Pressure

The genotype distribution and the allele distribution of the *CYP11B2* T(−344)C polymorphism in hypertensive subjects were compared to those in normotensive subjects for both men and women (Table 2). No significant differ-

ences in the genotype distribution or the allele distribution were observed between hypertensive subjects and normotensive subjects in either men or women.

The influence of the *CYP11B2* genotype on residuals of blood pressure values after adjusting for age, BMI, W/H, and alcohol consumption is shown in Table 3. No significant influence of the genotype on blood pressure was observed.

Discussion

In the present study, we investigated the association between the T(−344)C polymorphism in the promoter region of the human *CYP11B2* gene and blood pressure in a population-based sample (the Suita Study) consisting of 4,049 subjects. Our results indicate that the genotype distribution did not differ between hypertensive and normotensive subjects, and that the genotype had no significant effects on blood pressure values in either men or women.

The association between the T(−344)C polymorphism of the *CYP11B2* gene and hypertension has been investigated by several researchers with conflicting results (10–19). These discrepancies may be due to the low statistical power of relatively small-scale association studies. We have also reported an association between the C (−344) allele and low-renin hypertension (10). However, we did not find such an association between this polymor-

Table 2. Genotype and Allele Distribution of *CYP11B2* T(−344)C Polymorphism in Hypertensive Subjects and Normotensive Subjects

	Men				Women			
	Hypertensive subjects		Normotensive subjects		Hypertensive subjects		Normotensive subjects	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Genotype								
<i>TT</i>	377	49.0	539	47.0	363	47.3	654	47.8
<i>TC</i>	318	41.4	495	43.2	323	42.2	562	41.1
<i>CC</i>	74	9.6	112	9.8	80	10.5	152	11.1
		$\chi^2=0.76, p=0.68$				$\chi^2=0.36, p=0.84$		
Allele								
<i>T</i>	1,072	69.7	1,573	68.6	1,049	68.5	1,870	68.3
<i>C</i>	466	30.3	719	31.4	483	31.5	866	31.7
		$\chi^2=0.49, p=0.48$				$\chi^2=0.0071, p=0.93$		

TT indicates homozygotes for T allele; *TC*, heterozygotes; *CC*, homozygotes for C allele.

Table 3. Blood Pressure of Subjects Not Receiving Antihypertensive Medication by *CYP11B2* Genotype

	<i>CYP11B2</i> genotype			ANOVA <i>p</i>
	<i>TT</i>	<i>TC</i>	<i>CC</i>	
Men (<i>n</i> =1,556)	(<i>n</i> =745)	(<i>n</i> =669)	(<i>n</i> =152)	
Systolic blood pressure (mmHg)				
Unadjusted	125.9±18.5	126.1±18.0	125.2±18.3	0.87
Age-adjusted	126.1±17.1	125.9±16.5	125.1±17.5	0.82
Fully adjusted*	126.1±16.6	126.0±16.0	125.0±17.3	0.76
Diastolic blood pressure (mmHg)				
Unadjusted	80.1±10.7	80.2±10.3	80.3±11.0	0.98
Age-adjusted	80.1±10.6	80.2±10.2	80.3±11.1	0.99
Fully adjusted*	80.1±10.1	80.2±9.8	80.2±10.8	0.99
Pulse pressure (mmHg)				
Unadjusted	45.8±12.9	45.9±12.9	44.9±12.9	0.71
Age-adjusted	45.9±11.4	45.8±11.4	44.8±11.2	0.56
Fully adjusted*	45.9±11.3	45.8±11.3	44.8±11.3	0.55
Women (<i>n</i> =1,792)	(<i>n</i> =848)	(<i>n</i> =746)	(<i>n</i> =198)	
Systolic blood pressure (mmHg)				
Unadjusted	124.6±18.9	125.3±19.7	125.8±19.8	0.65
Age-adjusted	125.0±17.1	125.1±18.1	125.0±18.0	0.99
Fully adjusted*	124.9±16.7	125.2±17.9	125.0±17.8	0.96
Diastolic blood pressure (mmHg)				
Unadjusted	77.8±10.0	77.7±10.8	77.2±11.7	0.76
Age-adjusted	77.9±9.8	77.6±10.6	77.0±11.6	0.56
Fully adjusted*	77.8±9.5	77.7±10.2	77.0±11.3	0.63
Pulse pressure (mmHg)				
Unadjusted	46.9±13.2	47.6±13.4	48.6±14.9	0.21
Age-adjusted	47.2±11.7	47.4±12.0	48.0±13.3	0.66
Fully adjusted*	47.2±11.6	47.4±12.0	48.0±13.3	0.66

Values are the mean±SD. *TT* indicates homozygotes for T allele; *TC*, heterozygotes; *CC*, homozygotes for C allele.

*Multiple linear regression, adjusted for age, body mass index, waist-to-hip circumference ratio, and alcohol consumption.

phism and hypertension in the present study. These different results may be due to the different sampling methods: hospital-based sampling vs. population-based sampling. A population of only several hundred subjects may be too small to draw statistically certain conclusions. However, since the present study population was derived from an epidemiological cohort study, certain laboratory tests, such as for plasma renin and aldosterone levels, had not been performed. This is a drawback of the present study population. It is still possible that the *CYP11B2* T(−344)C polymorphism affects aldosterone levels, but not blood pressure levels at large.

Considering the sample size, the *CYP11B2* T(−344)C polymorphism is unlikely to play an important role in the pathogenesis of hypertension in Japanese. However, the present observations do not necessarily exclude the possibility that *CYP11B2* is involved in hypertension. It is possible that some other polymorphism(s) in *CYP11B2* may influence blood pressure. For this reason, it will be necessary to identify a large number of polymorphisms throughout *CYP11B2* and to perform association studies between these polymorphisms and blood pressure in future studies.

References

1. Kawamoto T, Mitsuuchi Y, Toda K, et al: Role of steroid 11-hydroxylase and steroid 18-hydroxylase in the biosynthesis of glucocorticoids and mineralocorticoids in humans. *Proc Natl Acad Sci USA* 1992; **89**: 1458-1462.
2. Curnow KM, Tusie-Luna MT, Pascoe L, et al: The product of the *CYP11B2* gene is required for aldosterone biosynthesis in the human adrenal cortex. *Mol Endocrinol* 1991; **5**: 1513-1522.
3. Lifton RP, Dluhy RG, Powers M, et al: A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 1992; **355**: 262-265.
4. Pascoe L, Curnow KM, Slutsker L, et al: Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossovers between *CYP11B1* and *CYP11B2*. *Proc Natl Acad Sci USA* 1992; **89**: 8327-8331.
5. Rosler A, Rabinowitz D, Theodor R, Ramirez LC, Ulick S: The nature of the defect in a salt-wasting disorder in Jews of Iran. *J Clin Endocrinol Metab* 1977; **44**: 279-291.
6. Mitsuuchi Y, Kawamoto T, Miyahara K, et al: Congenitally defective aldosterone biosynthesis in humans: inactivation of the P-450C18 gene (*CYP11B2*) due to nucleotide deletion in CMO I-deficient patients. *Biochem Biophys Res Commun* 1993; **190**: 864-869.
7. White PC, Slutsker L: Haplotype analysis of *CYP11B2*. *Endocr Res* 1995; **21**: 437-442.
8. Fardella CE, Rodriguez H, Montero J, et al: Genetic variation in P450c11AS in Chilean patients with low-renin hypertension. *J Clin Endocrinol Metab* 1996; **81**: 4347-4351.
9. Kupari M, Hautanen A, Lankinen L, et al: Associations between human aldosterone synthase (*CYP11B2*) gene polymorphisms and left ventricular size, mass, and function. *Circulation* 1998; **97**: 569-575.
10. Tamaki S, Iwai N, Tsujita Y, Kinoshita M: Genetic polymorphism of *CYP11B2* gene and hypertension in Japanese. *Hypertension* 1999; **33**: 266-270.
11. Hautanen A, Lankinen L, Kupari M, et al: Associations between aldosterone synthase gene polymorphism and the adrenocortical function in males. *J Intern Med* 1998; **244**: 11-18.
12. Pojoga L, Gautier S, Blanc H, et al: Genetic determination of plasma aldosterone levels in essential hypertension. *Am J Hypertens* 1998; **11**: 856-860.
13. Brand E, Chatelain N, Mulatero P, et al: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998; **32**: 198-204.
14. Davies E, Holloway CD, Ingram MC, et al: Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene *CYP11B2*. *Hypertension* 1999; **33**: 703-707.
15. Komiya I, Yamada T, Takara M, et al: Lys(173)Arg and −344T/C variants of *CYP11B2* in Japanese patients with low-renin hypertension. *Hypertension* 2000; **35**: 699-703.
16. Paillard F, Chansel D, Brand E, et al: Genotype-phenotype relationships for the renin-aldosterone system in a normal population. *Hypertension* 1999; **34**: 423-429.
17. Kato N, Sugiyama T, Morita H, et al: Comprehensive analysis of the renin-angiotensin gene polymorphisms with relation to hypertension in the Japanese. *J Hypertens* 2000; **18**: 1025-1032.
18. Schunkert H, Hengstenberg C, Holmer SR, et al: Lack of association between a polymorphism of the aldosterone synthase gene and left ventricular structure. *Circulation* 1999; **99**: 2255-2260.
19. Brand E, Schorr U, Ringel J, Beige J, Distler A, Sharma AM: Aldosterone synthase gene (*CYP11B2*) C−344T polymorphism in Caucasians from the Berlin Salt-Sensitivity Trial (BeSST). *J Hypertens* 1999; **17**: 1563-1567.
20. Mannami T, Konishi M, Baba S, Nishi N, Terao A: Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. *Stroke* 1997; **28**: 518-525.
21. Iwai N, Katsuya T, Ishikawa K, et al: Human prostacyclin synthase gene and hypertension: the Suita Study. *Circulation* 1999; **100**: 2231-2236.
22. Katsuya T, Sato N, Asai T, et al: Association of polymorphism in the promoter region of the apolipoprotein E gene with diastolic blood pressure in normotensive Japanese. *Hypertens Res* 2000; **23**: 271-275.