Original Article

Genetic Variations of *HSD11B2* in Hypertensive Patients and in the General Population, Six Rare Missense/Frameshift Mutations

Kei KAMIDE[1], Yoshihiro KOKUBO[2], Hironori HANADA[3], Junko NAGURA[2], Jin YANG[1], Shin TAKIUCHI[1], Chihiro TANAKA[3], Mariko BANNO[3], Yoshikazu MIWA[1], Masayoshi YOSHII[1], Tetsutaro MATAYOSHI[1], Hisayo YASUDA[1], Takeshi HORIO[1], Akira OKAYAMA[2], Hitonobu TOMOIKE[1),2)], Yuhei KAWANO[1], and Toshiyuki MIYATA[3]

Mutations in the gene encoding 11β-hydroxysteroid dehydrogenase type 2, *HSD11B2*, cause a rare monogenic juvenile hypertensive syndrome called apparent mineralocorticoid excess (AME). In AME, defective HSD11B2 enzyme activity results in overstimulation of the mineralocorticoid receptor (MR) by cortisol, causing sodium retention, hypokalemia, and salt-dependent hypertension. Here, we have studied whether genetic variations in *HDS11B2* are implicated in essential hypertension in Japanese hypertensives and the general population. By sequencing the entire coding region and the promoter region of *HDS11B2* in 953 Japanese hypertensives, we identified five missense mutations in 11 patients (L14F, *n*=5; R74H, *n*=1; R147H, *n*=3; T156I, *n*=1; R335H, *n*=1) and one novel frameshift mutation (4884Gdel, *n*=1) in a heterozygous state, in addition to 19 genetic variations. All genetic variations identified were rare, with minor allele frequencies less than 0.005. Four of 12 patients with the missense/frameshift mutations showed renal failure. Four missense mutations, L14F, R74H, R147H, and R335H, were successfully genotyped in the general population, with a sample size of 3,655 individuals (2,175 normotensives and 1,480 hypertensives). Mutations L14F, R74H, R147H, and R335H were identified in hypertensives (*n*=6, 8, 3, and 0, respectively) and normotensives (*n*=8, 12, 5, and 0, respectively) with a similar frequency, suggesting that these missense mutations may not strongly affect the etiology of essential hypertension. Since the allele frequency of all of the genetic variations identified in this study was rare, an association study was not conducted. Taken together, our results indicate that missense mutations in *HSD11B2* do not substantially contribute to essential hypertension in Japanese. (*Hypertens Res* 2006; 29: 243–252)

***Key Words*: HSD11B2, missense mutation, genetic variation, essential hypertension, salt-sensitivity**

Introduction

In mineralocorticoid target organs, the 11β-hydroxysteroid dehydrogenase (HSD11B) catalyzes the interconversion of the endogenous cortisol and cortisone in humans. Two distinct forms, HSD11B1 and HSD11B2, of HSD11B have been characterized and cloned (*1–3*). HSD11B1 is expressed in most tissues. In contrast, HSD11B2 has been identified in a limited range of tissues, such as the distal tubules of the kid-

From the [1]Division of Hypertension and Nephrology, [2]Preventive Cardiology, and [3]Research Institute, National Cardiovascular Center, Suita, Japan.
This study was supported by the Program for Promotion of Fundamental Studies in Health Science of the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan and by a research grant from the Japanese Ministry of Health, Labor, and Welfare.
Address for Reprints: Kei Kamide, M.D., Ph.D., Division of Hypertension and Nephrology, National Cardiovascular Center, 5–7–1 Fujishirodai, Suita 565–8565, Japan. E-mail: kamide@hsp.ncvc.go.jp
Received September 8, 2005; Accepted in revised form January 13, 2006.

ney (*2*, *4*, *5*). In mineralocorticoid-responsive cells, HSD11B2 converts cortisol to cortisone, which is not a ligand for the mineralocorticoid receptor, permitting aldosterone to occupy the receptor.

Apparent mineralocorticoid excess syndrome (AME) is an autosomal recessive disorder that results in severe low-renin hypertension and other characteristic clinical features (*6–8*). Typical patients present with severe hypertension, hypokalemia, and undetectable aldosterone. Most patients also have low birth weight, polyuria and polydipsia, failure to thrive, and nephrocalcinosis. The syndrome has been associated with sudden fatality. The HSD11B2 deficiency has been demonstrated in patients with AME and explains the pathogenesis of the disease, which results from excess cortisol binding to the mineralocorticoid receptor due to a failure to convert cortisol to cortisone (*9–11*). Over the last two decades, various genetic mutations in the *HSD11B2* gene have been reported (*12–17*). In Japanese patients with AME, two missense mutations (S180F, R208H) and a deletion of 3 nucleotides resulting in R337H and delta Y338 have been identified (*14*, *18*).

In 1998, a mild form of this disease characterized by P227L mutation in the *HSD11B2* gene was reported (*19*). In contrast to the patients with AME, this patient had low-renin hypertension and hypoaldosteronism but no other phenotypic features that would lead to the diagnosis of AME. Afterwards, it was reported that the defective allele frequency in a cohort of Mennonites was 1.7% (*20*). The genetic mutation in the *HSD11B2* gene, which results in a mild HSD11B2 deficiency, may represent an important cause of low-renin hypertension, the diagnostic basis of which is mostly unknown. Together, these findings suggest that, because 40% of patients with essential hypertension have low renin, these patients may have a mild form of AME.

In the *HSD11B2* gene, the 535G>A polymorphism (synonymous mutation at E178) in exon 3, which can be distinguished by *Alu* I cleavage and the polymorphic microsatellite marker (*21*), have been reported. The minor allele frequency of the 553G>A polymorphism was 0.086 in a healthy Caucasian population and 0.180 in a group of renal transplant patients ($n=61$), indicating association of this polymorphism with end-stage renal disease. This polymorphism was not associated with essential hypertension (*22*). As for the microsatellite marker, a total of 12 alleles were detected. The urinary ratio of cortisol to cortisone metabolites was higher in subjects homozygous for the A7 microsatellite allele than in the corresponding control subjects. Thus, the association of a polymorphic microsatellite marker of the *HSD11B2* gene with reduced HSD11B2 activity suggests that variants of the *HSD11B2* gene contribute to enhanced blood pressure response to salt in humans (*23*). The study demonstrated that a salt-induced blood pressure increase is associated with impaired HSD11B2 activity, as measured by the urinary excretion ratio of cortisol to cortisone metabolites in young Caucasian salt-sensitive men.

The present study was undertaken 1) to identify the genetic variants in the *HSD11B2* gene in Japanese hypertensives, 2) to address whether individuals with heterozygous missense/frameshift mutations show hypertension or renal impairment, and 3) to explain the genetic contribution to a mild form of hypertension including low-renin hypertension and hypoaldosteronism. We sequenced the promoter and exon regions of *HSD11B2* in Japanese hypertensives and genotyped the rare missense/frameshift mutations in the general population. We assessed the role of these genetic variations in hypertension and clarified their contribution to hypertension in Japanese.

Table 1. General Characteristics of Patients with Hypertension

Number	953
Age (years)	65.1±10.5
Gender (M/F)	522/431
Body mass index (kg/m^2)	24.2±3.3
SBP (mmHg)	145.5±19.2
DBP (mmHg)	84.8±13.4
Essential hypertension	880
Secondary hypertension	73
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Renal impairment/failure*	110
Ischemic heart disease	102
Stroke	145

Values are expressed as mean±SD. *Serum creatinine ≥1.4 mg/dl. M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Methods

Hypertensive Patients

A total of 953 hypertensive patients (522 men and 431 woman; average age: 65.0±10.5 years) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center as reported previously (*24–27*). Briefly, 92% of study subjects (880 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension (Table 1). Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg, and/or diastolic blood pressure (DBP) of ≥90 mmHg, or current use of antihypertensive medication. Hyperlipidemia was defined by total cholesterol ≥220 mg/dl or current use of antihyperlipidemia medication. Diabetes mellitus was defined by fasting plasma glucose ≥126 mg/dl or HbA1c ≥6.5% or current use of anti-diabetic medication. Study subjects had routine laboratory tests including electrolytes, renal function, blood glucose, HbA1c, plasma renin activity and plasma aldosterone concentration.

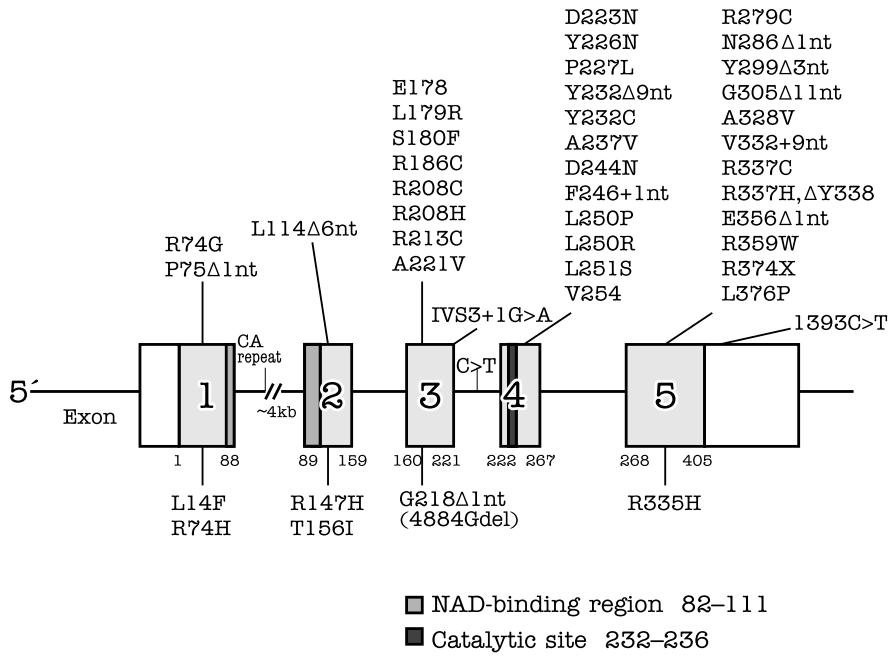

Fig. 1. *Summary of the reported genetic polymorphisms in* HSD11B2*. All polymorphisms in the upper section were reported previously, and the six polymorphisms in the lower section were identified in present study.*

Sequencing of the *HSD11B2* Gene

We sequenced all exons and the promoter region of *HSD11B2* in 953 Japanese hypertensive patients. Blood samples were obtained from hypertensive patients and genomic DNA was isolated from peripheral blood leukocytes. All exons with their flanking sequences and about 1.6 kb of the upstream region were directly sequenced with an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA) using seven sets of primers, as described previously (*28*). Information on the primers and polymerase chain reaction (PCR) conditions is available on request. The obtained sequences were examined for the presence of variations using Sequencher software (Gene Codes Corporation, Ann Arbor, USA), followed by visual inspection. The A of the ATG of the initiator Met codon is denoted as nucleotide +1. The nucleotide sequence (GenBank Accession ID: NT_010498) was used as a reference sequence.

General Population (the Suita Study)

The sample selection and study design of the Suita Study have been described previously (*29*, *30*). Briefly, the subjects visited the National Cardiovascular Center every 2 years for general health checkups. In addition to performing a routine blood examination that included lipid profiles, glucose levels, blood pressure, anthropometric measurements, a physician or nurse administered questionnaires covering personal history of cardiovascular diseases, including angina pectoris, myocardial infarction, and/or stroke. Blood pressure was measured after at least 10 min of rest in a sitting position. SBP and DBP were means of two measurements performed by well-trained doctors using a mercury sphygmomanometer (with a 3-min interval). The subjects were classified as current drinkers if they drank at least 30 ml ethanol per day, nondrinkers if they had never drunk, and past drinkers if they previously had drunk above 30 ml ethanol per day.

Genotyping of Genetic Variations in the General Population

Genotyping was attempted for six rare missense/frameshift mutations using the TaqMan-PCR method (*31*). The sequences of PCR primers and probes for the TaqMan-PCR method are available on request. Genotyping for two of the six rare mutations—4582C>T (encoded T156I) and 4884Gdel (a frameshift mutation)—failed. Thus, four genetic variations were successfully genotyped in 3,655 participants (1,709 men and 1,946 women) of the large cohort known as the Suita Study. All of the participants for genetic analysis in the present study gave their written informed consent. All clinical data and sequencing and genotyping results were anonymous. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Table 2. Sequence Variations in the Promoter Region and All Exons in *HSD11β2* Identified in Approximately 953 Japanese Patients with Hypertension and/or Renal Failure

SNP name	Region	Amino acid substitution	Allele 1 freq.	Allele 2 freq.	Flanking sequence	Genotyping
−879C>T	promoter		0.999	0.001	TCCTCTGACA[C/T]CCCACCCTCC	
−687C>A	promoter		0.999	0.001	CAGGGGTGAG[C/A]GCGCCTTAGG	
−596 to −595 CGGCAGins	promoter		0.999	0.001	GCAGCGGCAG[CGGCAG]CGGAGACCGG	
−562G>T	promoter		0.999	0.001	TGGTTCCTCG[G/T]GGTGTTCCTG	
−74C>G	promoter		0.999	0.001	ACTCCGCGCC[C/G]CGGCCTAGAA	
40C>T	exon 1	L14F	0.997	0.003	CGCCTGGCTG[C/T]TCGTGGCTGC	done
42C>A	exon 1	L14L	0.999	0.001	CCTGGCTGCT[C/A]GTGGCTGCCC	
82C>T	exon 1	L28L	0.999	0.001	GCGCTCAGAC[C/T]TGCGTCTGGG	
221G>A	exon 1	R74H	0.999	0.001	CGCCTGGCGC[G/A]CCCGCAGCGC	done
4554G>A	exon 2	R147H	0.999	0.001	GACATTAGCC[G/A]CGTGCTAGAG	done
4582C>T	exon 2	T156I	1.000	0.000	AAGGCCCACA[C/T]CACCAGCACC	failed
4681G>A	intron 2		1.000	0.000	GCTGACCTAA[G/A]GCTTCCCTCC	
4884Gdel	exon 3	frame shift	1.000	0.000	TGACTGTGGG[G]AGCCCAGCGG	failed
4910C>G	intron 3		0.995	0.005	TGCCCCCCCC[C/G]ACTGGAGCAA	
4902insC(8–10)	intron 3		0.998	0.002	GCCCCCCCCC[C]ACTGGAGCAA	
4964C>G	intron 3		0.999	0.001	GAGCCCCTTG[C/G]CAAAGCTGAG	
5017G>A	exon 4	P227P	0.997	0.003	TGCCATATCC[G/A]TGCTTGGGGG	
5205G>A	intron 4		0.999	0.001	TATGGGGGCA[G/A]GTCAGGTTTG	
5267G>A	intron 4		0.999	0.001	CAGACCTGGC[G/A]CGGGTTAAAC	
5334C>T	intron 4		0.999	0.001	GCCACTCCTT[C/T]CCCAGAGTCA	
5422C>T	exon 5	Y295Y	1.000	0.000	TGCAGGCCTA[C/T]GGCAAGGACT	
5541G>A	exon 5	R335H	1.000	0.000	GCTCGGCCCC[G/A]CCGCCGCTAT	done
5698G>A	exon 5	Q387Q	1.000	0.000	CCCCACCACA[G/A]GACGCAGCCC	
5759A>G	3′-UTR		1.000	0.000	TCGGTGAGCC[A/G]TGTGCACCTA	
5784C>T	3′-UTR		0.996	0.004	CCAGCCACTG[C/T]AGCACAGGAG	

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*37*). The nucleotide sequence (GenBank Accession ID: NT_010498) was used as a reference sequence. UTR, untranslated region; freq., frequency. Missense mutations were genotyped for general population except two mutations of which genotypes were not determined.

Results

Identification of Genetic Variations in *HSD11B2* in a Japanese Hypertensive Population

We sequenced the promoter and exon regions of *HSD11B2* in 953 hypertensives. As a result, we did not identify the reported common genetic variations in Caucasians and causative genetic variations of AME in the *HSD11B2* gene. Instead, we identified five novel missense mutations and one frameshift mutation in *HSD11B2* (Fig. 1, Table 2). Five patients had a C-to-T substitution at nucleotide 40 in exon 1, which led to an amino acid substitution from L to F at position 14 (L14F). One patient had a G-to-A substitution at nucleotide 221 in exon 1, resulting in an amino acid substitution from R to H at position 74 (R74H). Three patients had a G-to-A substitution at nucleotide 4554 in exon 2, leading to an amino acid substitution from R to H at position 147 (R147H). One patient had a C-to-T substitution at nucleotide 4582 in exon 2, leading to an amino acid substitution from T to I at position 156 (T156I). One patient had a G-to-A substitution at nucleotide 5541 in exon 5, resulting in an amino acid substitution from R to H at position 335 (R335H). We also found one patient with a frameshift mutation that resulted from a guanine deletion at position 4884 in exon 3 (4884Gdel). These missense/frameshift mutations were all found in the heterozygous form.

We also identified five synonymous polymorphisms, which encoded for L14 (42C>A in exon 1) with a minor allele frequency of 0.001%, L28 (82C>T in exon 1) with a minor allele frequency of 0.001%, P227 (5017G>A in exon 4) with a minor allele frequency of 0.003%, Y295 (5422C>T in exon 5) with a minor allele frequency of 0.0003% and Q387 (5698G>A in exon 5) with a minor allele frequency of 0.0003%. Fourteen additional genetic variations in the promoter, intronic, and 3′-untranslated regions were also identified. All of the genetic variations were rare, with minor allele frequencies less than 0.005 (Table 2).

Table 3. Clinical Profiles of Twelve Hypertensive Patients with Missense/Frameshift Mutations in *HSD11β2* Gene

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Polymorphism	L14F	L14F	L14F	L14F	L14F	R74H	R147H	R147H	R147H	T156I	4884Gdel	R335H
Age (years old)	73	71	64	51	59	70	76	69	85	78	75	67
Sex	male	female	male	female	male	male	male	male	male	male	female	female
BMI (kg/m^2)	21.39	20.45	20.20	24.09	30.30	27.92	24.03	22.12	26.17	21.69	29.97	21.50
Diagnosis	EHT, HL, HU, CRF, NIDDM, hypothyroidism	Renal HT, HL, CGN	EHT	ETH, HL	EHT, HL, obesity	EHT, HL, obesity	EHT, HU, OCI	EHT, HU, OCH, CRF	EHT, AF, AAA, obesity	EHT	RVHT, NIDDM, HL, obesity	EHT, HL
HT duration (years)	24	21	24	<1	9	15	19	20	21	8	30	41
HT initial onset age (years old)	49	50	40	—	50	55	57	49	64	70	45	26
HT family history	none	none	none	father	none	father, brother	mother, brother	none	none	mother	none	farther, mother, brother
SBP (mmHg)	138	136	152	140	130	140	134	138	154	134	170	148
DBP (mmHg)	70	80	88	68	80	86	72	70	84	68	90	80
Antihypertensive drugs	CCB, ARB	CCB, ACEI	CCB, BB, diuretics	CCB, BB, AB	CCB, ARB, BB	CCB, BB	CCB, AB	CCB, ACEI, AB	CCB	CCB, ACEI, AB	CCB, ACEI	CCB, BB
Na^+ (mEq/l)	141	141	140	142	140	141	141	140	143	143	140	139
K^+ (mEq/l)	4.4	5.2	4.1	4.2	4.2	3.6	4.2	5.2	4.5	4.2	4.6	5.0
Cl^- (mEq/l)	110	109	104	107	102	107	106	108	104	111	104	103
Creatinine (mg/dl)	2.7	0.8	0.6	0.5	0.6	1.1	1.3	2.9	1.2	0.8	0.6	0.8
Overt proteinuria	+	+	–	–	+	+	+	–	–	–	–	–
PRA (ng/ml/h)	3.8	0.9	6.3	0.1	0.5	2.9	1.9	no data	3.4	13.2	19.8	3.2
PAC (ng/dl)	8.8	8.5	no data	27.6	12.4	18.9	43.5	no data	7.7	14.6	7.0	14.1
FBS (mg/dl)	128	92	105	89	113	105	95	91	95	96	137	101
HbA1c (%)	6.0	5.6	5.4	5.2	5.6	6.0	5.1	5.2	5.1	5.0	8.7	5.7

BMI, body mass index; EHT, essential hypertension; HL, hyperlipidemia; HU, hyperuricemia; CRF, chronic renal failure; NIDDM, non-insulin dependent diabetes mellitus; HT, hypertension; CGN, chronic glomerulonephritis; OCI, old cerebral infarction; OCH, old cerebral hemorrhage; AF, atrial fibrillation; AAA, abdominal aortic aneurysma; RVHT, renovascular hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ARB, angiotension II receptor blocker; ACEI, angiotensin converting enzyme inhibitor; BB, β-adrenergic blocker; AB, α-adrenergic blocker; PRA, plasma renin activity; PAC, plasma aldosterone concentration; FBS, fasting blood sugar. Normal values in our institute: Na^+, 136–146 mEq/l; K^+, 3.6–4.9 mEq/l; Cl^-, 99–109 mEq/l; creatinine, 0.6–1.1 mg/dl; PRA, 0.2–2.7 ng/ml/h; PAC, 2–13 ng/dl.

Characteristics of Patients with Rare Missense/Frameshift Mutations in the Hypertensive Population

The characteristics of the 12 hypertensive patients who had missense/frameshift mutations (L14F, $n=5$; R74H, $n=1$; R147H, $n=3$; T156I, $n=1$; 4884Gdel, $n=1$; R335H; $n=1$) are shown in Table 3. Five patients out of the twelve had renal impairment including protein urea. Two (cases 1 and 2) of five patients with the L14F mutation had chronic renal failure (CRF) and chronic glomerulonephritis (CGN), and one (case 8) of three patients with the R147H mutation also had CRF. A patient with 4884Gdel (case 11) was diagnosed with renovascular hypertension caused by atherosclerosis with type 2 diabetes, hyperlipidemia and obesity (body mass index [BMI]: 29.97 kg/m^2). This patient was 75 years old, female, and had never smoked or drunk alcohol. This patient had microalbuminurea (urinary albumin excretion: 30.8 mg/g creatinine) without renal dysfunction (creatinine clearance: 112.5 ml/min) or cardiac hypertrophy (left ventricular mass index: 126.4 g/m^2). The average onset age of hypertension of the 12 patients with these missense mutations was 50.5 years. A patient with the R335H mutation (case 12) showed hypertension at her age of 26. Serum sodium levels of all patients were within normal range. There were no patients with hypokalemia as seen in AME.

Table 4. Basic Characteristics of Subjects in the General Population

	Women (n=1,946)	Men (n=1,709)
Age (years)	63.3±11.0	66.3±11.1*
Systolic blood pressure (mmHg)	128.0±19.7	131.8±19.4*
Diastolic blood pressure (mmHg)	76.5±9.8	79.7±10.7*
Body mass index (kg/m^2)	22.3±3.2	23.3±2.9*
Total cholesterol (mg/dl)	215.6±30.6*	197.9±30.3
HDL-cholesterol (mg/dl)	64.5±15.3*	55.0±14.1
Current smokers (%)	6.3	30.2†
Current drinkers (%)	29.6	67.2†
Present illness (%)		
Hypertension	38.0	47.3†
Hyperlipidemia	54.4†	27.8
Diabetes mellitus	5.2	12.8†

Values are expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. *$p<0.05$ between women and men by Student t-test. †$p<0.05$ between women and men by χ^2 test. HDL, high-density lipoprotein.

Characteristics of Individuals with Rare Missense/Frameshift Mutations in the General Population

The characteristics of the 3,655 subjects comprising the Japanese general population group (1,709 men, 1,946 women) are summarized in Table 4. Age, SBP, DBP, BMI, percentage of current smokers, percentage of current drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, high-density lipoprotein (HDL)–cholesterol, and percentage of hyperlipidemia were significantly higher in women than in men. In this population, 1,480 subjects were diagnosed with hypertension.

We successfully genotyped four genetic variations in the general population, which had a sample size of 3,655 individuals (2,175 normotensives and 1,480 hypertensives), but the genotyping failed for two of the genetic variations, T156I and 4884Gdel. In the general population, a missense mutation, R335H, was not present. The remaining three mutations, L14F, R74H, and R147H, were found in both hypertensive and normotensive subjects (Table 5). We identified 14 individuals with the L14F mutation. Six individuals with the L14F mutation had hypertension and eight were normotensive. We identified 20 individuals with the R74H mutation. Among them, eight showed hypertension and 12 were normotensive. We identified 8 individuals with the R147H mutation. Among them, three showed hypertension and five were normotensive. There were no statistically significant differences in any clinical characteristics between the subjects with the three missense mutations of *HSD11B2* and the subjects in the general population (Table 5).

Comparison of Missense/Frameshift Mutations in *HSD11B2* between Normotensives and Combined Hypertensives

As seen in Table 6, there was no difference in the prevalence of missense/frameshift mutations of *HSD11B2* between the combined subjects with hypertension and the normotensives.

Discussion

A missense mutation, P227L, in *HSD11B2* was previously identified in a patient with mild low-renin hypertension (*32*). This patient did not demonstrate the typical features of AME. The authors suggested that patients with mild low-renin hypertension may carry the mutations in the *HSD11B2* gene. In our study, we did not identify the P227L mutation in 953 Japanese hypertensives.

Genetic analyses of *HSD11B2* have been reported in two Japanese AME probands (*14*, *18*). In one family, the proband had a compound heterozygous mutation with a missense mutation, R208H, and a deletion of 3 nucleotides in codons 337–338 resulting in a substitution of Arg337 to His and a deletion of Tyr338 (CGCTAT to CAT: R337H and delta Y338) (*18*). Their family members, a father, mother, and elderly sister, who carried the heterozygous mutation were all normotensive and normokalemic, and had normal ratios of urinary [THF plus aTHF]/THE (THF, tetrahydrocortisol; aTHF, allotetrahydrocortisol; THE, tetrahydrocortisone). Another Japanese patient with AME had the homozygous missense mutation, S180F. The enzymatic activity of this mutant was 1.8% compared with the wild-type enzyme when cortisol was used as the substrate and 5.7% when corticosterone was used as the substrate (*14*). Figure 1 summarizes the reported polymorphisms in *HSD11B2*. In our study, none of the three causative genetic defects was identified, indicating that those mutations were not accumulated in the Japanese population.

We identified five novel missense mutations and one frameshift mutation in *HSD11B2* (Fig. 1, Table 2). As shown in Fig. 2A, five of the missense mutations occurred in residues that were highly conserved among the three different species, indicating that these mutations may result in functional changes in *HSD11B2*. However, neither hypertensive patients nor general subjects with these novel missense mutations showed any distinctive clinical characteristics during their health-check-ups.

We identified one hypertensive patient having renal artery stenosis with a frameshift mutation (4884Gdel) in *HSD11B2*. This deletion caused the frameshift at S219 with a premature stop codon at position 270 (Fig. 2B). A recent report indicated

Table 5. Accumulated Clinical Profiles of Subjects with Missense Mutations in *HSD11B2* in the General Population

	L14F	R74H	R147H
Number	14	20	8
Age (years old)	67.7±12.3	64.8±13.3	61.5±12.0
Sex (M/F)	7/7	9/11	5/3
Body mass index (kg/m^2)	23.4±4.0	22.4±2.9	23.9±1.7
Systolic blood pressure (mmHg)	125.4±23.0	128.7±23.4	124.9±19.9
Diastolic blood pressure (mmHg)	75.4±11.0	78.2±10.0	75.8±12.9
Total cholesterol (mg/dl)	213.9±34.0	213.8±37.0	199.3±36.4
HDL-cholesterol (mg/dl)	57.9±12.1	63.1±16.9	52.1±18.5
Triglyceride (mg/dl)	93.8±49.3	120.1±93.4	140.7±90.1
Creatinine (mg/dl)	0.8±0.2	0.7±0.2	0.8±0.2
Over proteinuria (yes/no)	1/13	0/20	0/8
FBS (mg/dl)	100.4±20.9	94.5±10.3	99.6±22.3
HbA1c (%)	5.7±0.8	5.4±0.7	5.6±0.9
Current smoker (yes/no)	2/12	4/16	1/7
Current drinker (yes/no)	5/9	9/11	4/4
Hypertension (yes/no)	6/8	8/12	3/5
Hyperlipidemia (yes/no)	10/4	11/9	6/2
Diabetes mellitus (yes/no)	6/8	2/18	2/6
Antihypertensive treatment (yes/no)	4/10	2/18	2/6

Values were expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. M, male; F, female; HDL, high-density lipoprotein; FBS, fasting blood sugar.

Table 6. Number of Subjects with Missense/Frameshift Mutations in the Hypertensive and the General Populations

Mutations	Hypertensive population (n=953)	General population	
		Hypertensive subjects (n=1,480)	Normotensive subjects (n=2,175)
L14F	5	6	8
R74H	1	8	12
R147H	3	3	5
T156I	1	n.d.	n.d.
4884Gdel	1	n.d.	n.d.
R335H	1	0	0
Total	12	17	25

n.d., not determined.

that the heterozygous carriers with the defective allele of the *HSD11B2* gene showed essential hypertension (*16*). It is evident that this frameshift mutation results in the dysfunction of HSD11B2. The allele frequency of this mutation was very low (0.052%, 1 allele/1,906 alleles) in the Japanese hypertensive population. However, it is worth noting that this defective allele might be prevalent in other ethnic populations, because the frequency of some genetic mutations varies with ethnicity. Recently, rare genetic mutations collectively contributing to a quantitative trait variation, such as plasma levels of HDL-cholesterol, have been reported (*33*). We have performed large-scale sequence analyses of five hypertension candidate genes, *WNK4*, *SCNN1B*, *SCNN1G*, *NR3C2* and *RGS2*, to evaluate this hypothesis and found that a low but significant percentage of the hypertensive subjects had missense/frameshift mutations (*24–26*, *34*). Collectively, these rare mutations may make an at least partial contribution to hypertension.

The deduced NAD-binding sites reside in the conserved region from T82 to A111 (*2*), and the deduced catalytic site resides in the conserved region from Y232 to K236 (*35*). So far, more than ten genetic defects in patients with AME, most of whom had a severe deficiency of enzymatic activity confirmed by the expression analysis, have been reported and none of them overlap with the five missense mutations identified in the present study. Therefore, the effects on the HSD11B2 enzymatic activity of the mutations are not clear. In the future, an *in vitro* expression study should be performed to evaluate the activity of mutants and the ratios of urinary cortisol to cortisone metabolites in carriers of the mutations.

In the Caucasian population, a mutation at E178 that is synonymous with 553G>A which can be distinguished by *Alu* I restriction enzyme digestion, has been identified with a prevalence of 8.6% in the control subjects (*21*, *23*). This polymorphism was associated with end-stage renal disease but not with essential hypertension. We did not identify this polymor-

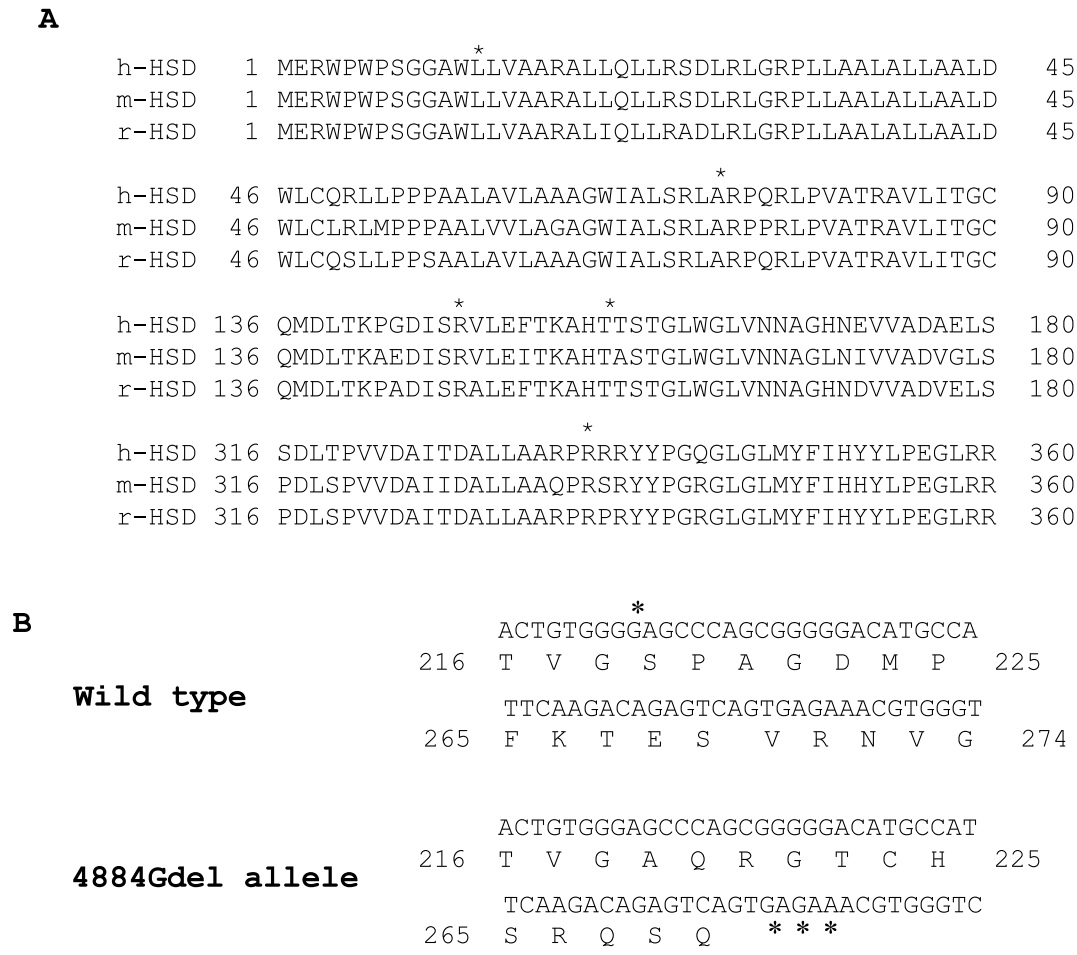

Fig. 2. *Partial amino acid sequence surrounding the mutations in* HSD11B2*. A: Alignment of partial amino acid sequences of HSD11B2 from two species and human HSD11B2. HSD11B2 sequences are from* Homo sapiens *(h),* Mus musculus *(m), and rabbit (r). Numbers indicate the position of amino acid sequence. The asterisks indicate the positions at which missense mutations occur (L14F, R74H, R147H, T156I, R335H) B: Nucleotide and amino acid sequences of wild-type allele and 4884Gdel allele. Numbers indicate the amino acid residues. An asterisk indicates the base deleted in the 4884Gdel allele, which causes a frameshift mutation from S218. This results in a 51–amino-acid extension that is is terminated by a stop codon (indicated by three asterisks).*

phism in our Japanese population.

In the Caucasian population, an intensive genetic study on the *HSD11B2* gene using 587 subjects, including 260 patients with end-stage renal disease, has been conducted, in which one missense mutation, L148V, and three synonymous mutations, T156, E178, and D388, were identified by the combination of single strand conformational polymorphism analysis and DNA sequencing (*36*). The results showed that allele frequencies did not differ significantly between control subjects and end-stage renal disease patients or between patients with hypertension and patients with end-stage renal disease. We did not identify these mutations in our Japanese population. Our results support their findings that the mutations in the *HSD11B2* gene do not affect hypertension.

In summary, we suggest that rare mutations in *HSD11B2*, L14F, R74H, R147H, T156I, R335H, and 4884Gdel may not collectively contribute to the pathogenesis of hypertension, although it was not clear whether abnormalities of electrolytes, renin activity, or aldosterone concentration were present, since our hypertensive patients with these missense/frameshift mutations were taking antihypertensive drugs. Further functional analyses of *HSD11B2* mutants are necessary to clarify the functional defects caused by these genetic variations in Japanese.

Acknowledgements

We would like to express our highest gratitude to Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for his support of the Millennium Genome Project. We would also

like to express our gratitude to Drs. Otosaburo Hishikawa, Yasushi Kotani, and Katsuyuki Kawanishi and Mr. Tadashi Fujikawa for their continuous support of our population survey in Suita City. Finally, we would like to thank the members of the Satsuki-Junyukai and all the staff of the Division of Preventive Cardiology for their help in the medical examinations, and Ms. Y. Tokunaga for her technical assistance.

References

1. Agarwal AK, Monder C, Eckstein B, *et al*: Cloning and expression of rat cDNA encoding corticosteroid 11 beta-dehydrogenase. *J Biol Chem* 1989; **264**: 18939–18943.
2. Albiston AL, Obeyesekere VR, Smith RE, *et al*: Cloning and tissue distribution of the human 11beta-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 1994; **105**: R11–R17.
3. Tannin GM, Agarwal AK, Monder C, *et al*: The human gene for 11 beta-hydroxysteroid dehydrogenase. Structure, tissue distribution, and chromosomal localization. *J Biol Chem* 1991; **266**: 16653–16658.
4. Agarwal AK, Mune T, Monder C, *et al*: Cloning of cDNA encoding an NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase in sheep kidney. *Endocr Res* 1995; **21**: 389–397.
5. Walker BR, Campbell JC, Williams BC, *et al*: Tissue-specific distribution of the NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase. *Endocrinology* 1992; **131**: 970–972.
6. New MI, Levine LS, Biglieri EG, *et al*: Evidence for an unidentified steroid in a child with apparent mineralocorticoid hypertension. *J Clin Endocrinol Metab* 1977; **44**: 924–933.
7. Geller DS: A mineralocorticoid receptor mutation causing human hypertension. *Curr Opin Nephrol Hypertens* 2001; **10**: 661–665.
8. Ferrari P: Genetics of the mineralocorticoid system in primary hypertension. *Curr Hypertens Rep* 2002; **4**: 18–24.
9. Dave-Sharma S, Wilson RC, Harbison MD, *et al*: Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 1998; **83**: 2244–2254.
10. Ferrari P, Krozowski Z: Role of the 11beta-hydroxysteroid dehydrogenase type 2 in blood pressure regulation. *Kidney Int* 2000; **57**: 1374–1381.
11. Ferrari P, Lovati E, Frey FJ: The role of the 11beta-hydroxysteroid dehydrogenase type 2 in human hypertension. *J Hypertens* 2000; **18**: 241–248.
12. Mune T, Rogerson FM, Nikkila H, *et al*: Human hypertension caused by mutations in the kidney isozyme of 11beta-hydroxysteroid dehydrogenase. *Nat Genet* 1995; **10**: 394–399.
13. Li A, Tedde R, Krozowski ZS, *et al*: Molecular basis for hypertension in the "type II variant" of apparent mineralocorticoid excess. *Am J Hum Genet* 1998; **63**: 370–379.
14. Nunez BS, Rogerson FM, Mune T, *et al*: Mutants of 11beta-hydroxysteroid dehydrogenase (11-HSD2) with partial activity: improved correlations between genotype and biochemical phenotype in apparent mineralocorticoid excess. *Hypertension* 1999; **34**: 638–642.
15. Odermatt A, Dick B, Arnold P, *et al*: A mutation in the cofactor-binding domain of 11beta-hydroxysteroid dehydrogenase type 2 associated with mineralocorticoid hypertension. *J Clin Endocrinol Metab* 2001; **86**: 1247–1252.
16. Lavery GG, Ronconi V, Draper N, *et al*: Late-onset apparent mineralocorticoid excess caused by novel compound heterozygous mutations in the *HSD11B2* gene. *Hypertension* 2003; **42**: 123–129.
17. Quinkler M, Bappal B, Draper N, *et al*: Molecular basis for the apparent mineralocorticoid excess syndrome in the Oman population. *Mol Cell Endocrinol* 2004; **217**: 143–149.
18. Kitanaka S, Katsumata N, Tanae A, *et al*: A new compound heterozygous mutation in the 11beta-hydroxysteroid dehydrogenase type 2 gene in a case of apparent mineralocorticoid excess. *J Clin Endocrinol Metab* 1997; **82**: 4054–4058.
19. Wilson RC, Nimkarn S, New MI: Apparent mineralocorticoid excess. *Trends Endocrinol Metab* 2001; **12**: 104–111.
20. Ugrasbul F, Wiens T, Rubinstein P, *et al*: Prevalence of mild apparent mineralocorticoid excess in Mennonites. *J Clin Endocrinol Metab* 1999; **84**: 4735–4738.
21. Brand E, Chatelain N, Mulatero P, *et al*: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* 1998; **32**: 198–204.
22. Smolenicka Z, Bach E, Schaer A, *et al*: A new polymorphic restriction site in the human 11beta-hydroxysteroid dehydrogenase type 2 gene. *J Clin Endocrinol Metab* 1998; **83**: 1814–1817.
23. Lovati E, Ferrari P, Dick B, *et al*: Molecular basis of human salt sensitivity: the role of the 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Endocrinol Metab* 1999; **84**: 3745–3749.
24. Kamide K, Takiuchi S, Tanaka C, *et al*: Three novel missense mutations of WNK4, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. *Am J Hypertens* 2004; **17**: 446–449.
25. Kamide K, Tanaka C, Takiuchi S, *et al*: Six missense mutations of the epithelial sodium channel beta and gamma subunits in Japanese hypertensives. *Hypertens Res* 2004; **27**: 333–338.
26. Kamide K, Yang J, Kokubo Y, *et al*: A novel missense mutation, F826Y, in the mineralocorticoid receptor gene in Japanese hypertensives: implication of clinical phenotypes. *Hypertens Res* 2005; **28**: 703–709.
27. Matayoshi T, Kamide K, Takiuchi S, *et al*: The thiazide-sensitive Na^+-Cl^- cotransporter gene, *C1784T*, and adrenergic receptor-β3 gene, *T727C*, may be gene polymorphisms susceptible to the antihypertensive effect of thiazide diuretics. *Hypertens Res* 2004; **27**: 821–833.
28. Takiuchi S, Mannami T, Miyata T, *et al*: Identification of 21 single nucleotide polymorphisms in human hepatocyte growth factor gene and association with blood pressure and carotid atherosclerosis in the Japanese population. *Atherosclerosis* 2004; **173**: 301–307.
29. Kokubo Y, Inamoto N, Tomoike H, *et al*: Association of genetic polymorphisms of sodium-calcium exchanger 1 gene, NCX1, with hypertension in a Japanese general population. *Hypertens Res* 2004; **27**: 697–702.
30. Tanaka C, Mannami T, Kamide K, *et al*: Single nucleotide polymorphisms in the interleukin-6 gene associated with

blood pressure and atherosclerosis in a Japanese general population. *Hypertens Res* 2005; **28**: 35–41.
31. Tanaka C, Kamide K, Takiuchi S, *et al*: An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
32. Wilson RC, Dave-Sharma S, Wei JQ, *et al*: A genetic defect resulting in mild low-renin hypertension. *Proc Natl Acad Sci U S A* 1998; **95**: 10200–10205.
33. Cohen JC, Kiss RS, Pertsemlidis A, *et al*: Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004; **305**: 869–872.
34. Yang J, Kamide K, Kokubo Y, *et al*: Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* 2005; **23**: 1497–1505.
35. Agarwal AK, Mune T, Monder C, *et al*: NAD^+-dependent isoform of 11beta-hydroxysteroid dehydrogenase. Cloning and characterization of cDNA from sheep kidney. *J Biol Chem* 1994; **269**: 25959–25962.
36. Zaehner T, Plueshke V, Frey BM, *et al*: Structural analysis of the 11beta-hydroxysteroid dehydrogenase type 2 gene in end-stage renal disease. *Kidney Int* 2000; **58**: 1413–1419.
37. Antonarakis SE, Nomenclature Working Group: Recommendations for a nomenclature system for human gene mutations. *Hum Mut* 1998; **11**: 1–3.