Original Article

A Novel Missense Mutation, F826Y, in the Mineralocorticoid Receptor Gene in Japanese Hypertensives: Its Implications for Clinical Phenotypes

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A gain-of-function mutation resulting in the S810L amino acid substitution in the hormone-binding domain of the mineralocorticoid receptor (MR, locus symbol *NR3C2*) is responsible for early-onset hypertension that is exacerbated in pregnancy. The objective of this study was to test whether other types of missense mutations in the hormone-binding domain could be implicated in hypertension in Japanese. Here, we screened 942 Japanese patients with hypertension for the S810L mutation in exon 6 in the *MR*. We did not identify the S810L mutation in our hypertensive population, indicating that S810L does not play a major role in the etiology of essential hypertension in Japanese. However, we identified a novel missense mutation, F826Y, in three patients in a heterozygous state, in addition to four single nucleotide polymorphisms, including one synonymous mutation (L809L). The F826Y mutation is present in the *MR* hormone-binding domain and might affect the ligand affinity. The F826Y mutation was also identified in 13 individuals (5 hypertensives and 8 normotensives) in a Japanese general population (*n*=3,655). The allele frequency was 0.00178. The frequencies of the F826Y mutation in the hypertensive population (3/942) and in the hypertensive group (5/1,480) and the normotensive group (8/2,175) in the general population were not significantly different, suggesting that this mutation does not greatly affect hypertension. Although it is unclear at present whether or not the F826Y mutation makes a substantial contribution to the mineralocorticoid receptor activity, this missense mutation may contribute, to some extent, to clinical phenotypes through its effects on MR. (*Hypertens Res* 2005; 28: 703–709)

***Key Words*: mineralocorticoid receptor, *NR3C2*, gene variant, hypertension**

Introduction

Aldosterone binds to mineralocorticoid receptor (MR; locus symbol *NR3C2*), a member of the nuclear receptor family, to stimulate renal sodium reabsorption. In response to aldosterone, *MR* undergoes a conformational change, translocates across the nuclear membrane and regulates gene transcrip-

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Table 1. General Characteristics of Patients with Hypertension

Number	942
Age (years)	65.1±10.5
Gender (M/F)	518/424
Body mass index (kg/m^2)	24.2±3.3
SBP (mmHg)	145.5±19.2
DBP (mmHg)	84.8±13.4
Essential hypertension	870
Secondary hypertension	72
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Renal impairment*	110
Ischemic heart disease	102
Stroke**	145

Values are expressed as mean±SD. *Patients who had serum creatinine ≥1.4 mg/dl. **Silent cerebral infarction was included. M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure.

tion, leading to enhancement of the transport of sodium from the tubular lumen to the basolateral side of the principal cells of the collecting duct (*1*). Therefore, mutation in *MR* changes blood pressure by modifying renal salt reabsorption (*2*, *3*).

Heterozygous loss-of-function mutations in *MR* cause a disease featuring salt wasting and hypotension, called pseudohypoaldosteronism type I (PHA I) (*4–6*). Patients with PHA I have been found to carry various nonsense/missense/frameshift mutations, such as R947X, C436X, insT1354, del8bp537, Cys645X, G633R, Q776R, L979P, and S163X (*7–11*). On the other hand, a heterozygous gain-of-function mutation, S810L, in *MR* has been reported in patients with early onset severe hypertension (*12*). Patients with the S810L mutation showed an increase in renal salt reabsorption, a marked elevation of blood pressure, and a marked suppression of aldosterone secretion, and developed hypertension before they were 20 years old. Three pedigree members with S810L died of heart failure before age 50. The female harboring this mutation experienced a dramatic exacerbation of hypertension during pregnancy. The S810L mutation is present in the hormone-binding domain of *MR*, altering an amino acid that is conserved in all *MR*s from *Xenopus* to humans but not found in other nuclear receptors (*12*).

We considered that carriers with other types of missense mutations in the hormone-binding domain may show a milder phenotype compared to the patients with *MR* S810L, or may show other clinical features in other tissues. The aims of this study were to screen for the *MR* S810L mutation in Japanese hypertensives and test whether other types of missense mutations in the hormone-binding domain could be implicated in hypertension.

Methods

Hypertensive Subjects

A total of 942 hypertensive subjects (518 male and 424 female; average age: 65.1±10.5 years) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center (*13*, *14*). Ninety-two percent of study subjects (870 subjects) were diagnosed with essential hypertension, including many cases with severe hypertension, early onset and a strong genetic background, and the rest had secondary hypertension, including 36 cases of renal hypertension, 23 of renovascular hypertension, 11 of primary aldosteronism, and 2 of hypothyroid-induced hypertension. The clinical features of the patients in this study are summarized in Table 1.

At the time of the physical examination, blood pressure, body mass index (BMI), and the hematological and biochemical profile were determined. Blood pressure was measured three times with the subject seated after an at least 5-min rest, and these values were averaged. The measurements were performed in the morning after an overnight fast. Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg, diastolic blood pressure (DBP) of ≥90 mmHg, or current use of antihypertensive medication. A majority of patients were treated with antihypertensive drugs. About one-third of hypertensive subjects had hypertensive cardiovascular complications.

All subjects gave their written informed consent to participate in the genetic analysis. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Screening of Mutations in Exon 6 of *MR*

Blood samples were obtained from each subject, and genomic DNA was isolated from peripheral blood leukocytes using an NA-3000 nucleic acid isolation system (KURABO, Osaka, Japan) (*15*). The region of exon 6 was amplified by polymerase chain reaction (PCR) using a pair of specific primers, 5′-aagaagcatcttcctggaatg-3′ and 5′- tggagtcgataccaaaagagac-3′, which flank the 548-bp region containing exon 6. The PCR products were directly sequenced on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA), as described previously (*13*). The obtained sequences were examined for the presence of mutations using Sequencher software (Gene Codes Corporation, Ann Arbor, USA), followed by visual inspection (Fig. 1).

General Population (the Suita Study)

The sample selection and study design of the Japanese general population in the so-called Suita Study have been described previously (*16–18*). Briefly, the subjects visited the

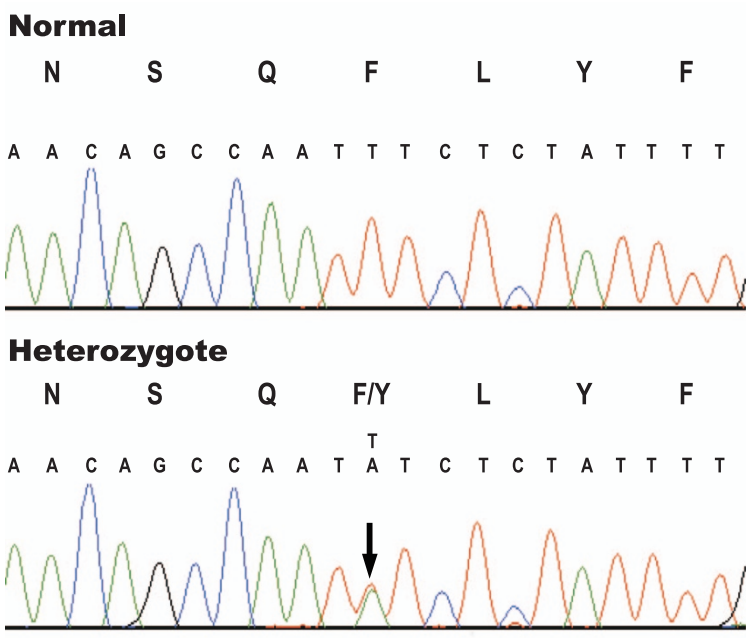

Fig. 1. *The F826Y missense mutation in the mineralocorticoid receptor gene. The upper panel shows the electropherogram for an individual with the normal allele. The lower panel shows the electropherogram for an individual with heterozygous for the F826Y mutation.*

Table 2. Basic Characteristics of Subjects in Japanese General Population (the Suita Study)

	Female (n=1,946)	Male (n=1,709)
Age (years)	63.3±11.1	66.1±11.3*
Systolic blood pressure (mmHg)	128.2±19.9	130.8±19.1*
Diastolic blood pressure (mmHg)	76.5±9.8	79.2±10.2*
Body mass index (kg/m^2)	22.4±3.2	23.3±3.0*
Total cholesterol (mg/dl)	216.1±31.3*	198.7±31.4
HDL-cholesterol (mg/dl)	64.9±15.2*	54.9±14.3
Current smokers (%)	6.0	31.1†
Current drinkers (%)	26.4	66.9†
Present illness (%)		
Hypertension	37.3	44.2†
Hyperlipidemia	61.4	60.0
Diabetes mellitus	19.4	32.1†

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 female and male by Student t-test. †p<0.05 between female and male by χ^2 test.

National Cardiovascular Center every 2 years for general health checkups. Lipid profiles, glucose levels, blood pressure, and anthropometry were measured. In addition, a physician or nurse administered a questionnaire to determine any personal history of cardiovascular diseases, including angina pectoris, myocardial infarction, and/or stroke. Blood pressure was measured with the subject seated after an at least 10-min rest. SBP and DBP were taken as the means of two separate measurements by a well-trained doctor using a mercury sphygmomanometer (with an interval of 3 min between measurements). Hyperlipidemia was defined as total cholesterol ≥220 mg/dl or current use of antihyperlipidemia medication. Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or current use of antidiabetic medication. All of the participants were Japanese. 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. Table 2 shows the basic characteristics of the subjects in our population. Age, SBP, DBP, BMI, percentage of current smokers, percentage of current drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in male than in female. Total cholesterol and high-density lipoprotein (HDL)-cholesterol were significantly higher in female than in male. In this population, 1,480 subjects were diagnosed with hypertension.

Genotyping of the *MR* F826Y Mutation in the General Population

The F826Y mutation was genotyped in 3,655 subjects (1,709

Table 3. List of Five Polymorphisms and Their Allele Frequency in Exon 6 and Intron 6 of MR Gene Identified by Direct Sequencing of 942 Japanese with Hypertension

Allele 1/2 SNPs	Amino acid change	Region	Allele 1	Hetero	Allele 2	Total	Allele frequency		Flanking sequence
							Allele 1	Allele 2	
284309A>G	L809L	exon 6	941	1	0	942	0.999	0.001	GGATGTGTCT[A/G]TCATCATTTG
284359T>A	F826Y	exon 6	939	3	0	942	0.998	0.002	AACAGCCAAT[T/A]TCTCTATTTT
284419delA		intron 6	941	1	0	942	0.999	0.001	TAGCCTTCAT[A/–]AAATAAACTG
284457G>A		intron 6	52	349	541	942	0.240	0.760	ATTTCTTTCA[G/A]TAATTTCTAA
284616A>G		intron 6	941	1	0	942	0.999	0.001	TAAATTCCAC[A/G]TAATAATATG

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*22*). The nucleotide sequence (GenBank Accession ID: NT-016606, build 34, version 3) was used as a reference sequence. MR, mineralocorticoid receptor; SNP, single nucleotide polymorphism.

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          810              826
hMR       LSSFALSWRSYKHTNSQFLYFAPDLVFNEEKM
rMR       LSSFALSWRSYKHTNSQLLYFAPDLVFNEEKM
xMR       LSSFALSWRSYKHASSQFLYFAPDLIFNEERM
tMR       LSSFSLSWRSYKHTNGQMLYFAPDLVFNEDRM

hPR       LMVFGLGWRSYKHVSGQMLYFAPDLILNEQRM
          759              778
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Fig. 2. *Sequence alignment of mineralocorticoid receptors from four species and human progesterone receptor. The sequences of mineralocorticoid receptor (*MR*) are from humans (h), the rat* Rattus norvegicus *(r),* Xenopus laevis *(x), and rainbow trout,* Oncorhynchus mykiss *(t). h*PR*, the human progesterone receptor. Ser810 in h*MR*, the corresponding residues in other* MR*s and h*PR*, and F826 in h*MR *are underlined. Phe778 in h*PR *is also underlined (*20*). From the crystallography structure of h*PR*-progesterone, F778 can make a hydrogen bond with bound progesterone. The numbers above the sequence are from h*MR*, and those at the bottom are from human progesterone receptor.*

male and 1,946 female) participating in the Suita Study using the TaqMan-PCR method (*19*). The sequences of the PCR primers were 5′-cttgagctggagatcgtacaaacat-3′ and 5′-ctcat taaagactaggtctggtgcaa-3′, and the probes for the TaqMan-PCR method were 5′-Fam-acagccaatatctct-3′ and 5′-Vic-acagccaatttctct-3′.

Statistical Analysis

Values are expressed as the means±SD. The distribution of patient characteristics between male and female in the Japanese general population was analyzed using the Student's *t*-test or χ^2 analysis. The frequencies of the F826Y mutation in the hypertensive population (3/942) and in the normotensive group (5/1,480) and the hypertensive group (8/2,175) in the general population were statistically evaluated by the χ^2-test. Statistical significance was established at $p<0.05$.

Results

We sequenced the region of exon 6 of *MR* in 942 patients with hypertension, including cases of severe, early-onset hypertension with a strong genetic background and cases of secondary hypertension. The results are shown in Table 3. In this study, we were not able to detect S810L, which induced early-onset hypertension exacerbated in pregnancy. However, we identified a novel missense mutation, F826Y, of the *MR*. Three out of 942 patients had a T-to-A substitution at nucleotide 284359 in exon 6 leading to an amino acid substitution from Phe to Tyr at position 826 (F826Y) in a heterozygous form. In addition, we identified one synonymous mutation (284309A>G) encoding for L809 and three additional mutations in intron 6 (Table 3). The F826Y mutation is present in the *MR* hormone-binding domain and is located in a region that is highly conserved among *MR*s from different species, including rat, *Xenopus*, and rainbow trout (Fig. 2). However, the Phe at position 826 is not conserved among different species. At position 826, human *MR* (h*MR*) has a Phe, but the rat, *Xenopus*, and rainbow trout *MR*s have Leu, Phe, and Met, respectively, suggesting that the amino acid residue at position 826 has a less significant function.

The clinical features of the three hypertensive patients with F826Y in *MR* (two females and 1 male) are shown in Table 4. In these patients, electrolyte abnormalities such as hyperkalemia were not remarkable. Patient 1 was a male hypertensive patient with non-insulin-dependent diabetes mellitus (NIDDM), patient 2 a female hypertensive patient with NIDDM and hyperlipidemia, and patient 3 a female hypertensive patient with hyperlipidemia and obesity. Their blood pressures were controlled by a combination of calcium channel blocker, angiotensin II receptor blocker, and β-adrenergic receptor blocker. Serum levels of sodium, potassium, and chloride in these patients were in the normal range. All three patients had serum creatinine levels within the normal limits, although patient 3 had overt proteinuria.

Table 4. Clinical Features of Three Hypertensive Patients with F826Y Mutation in MR Gene

	Case		
	1	2	3
Age (years old)	80	68	64
Gender	male	female	female
BMI (kg/m^2)	20.20	22.52	29.97
Diagnosis	EHT, NIDDM	EHT, NIDDM, HL	EHT, HL, obesity
HT duration (years)	16	27	26
HT family history	unknown	unknown	father
SBP (mmHg)	122	160	140
DBP (mmHg)	72	80	80
Medication	CCB	CCB, ARB, DMD	ARB, BB, HLD
Na^+ (mEq/l)	141	140	140
K^+ (mEq/l)	4.1	3.4	4.1
Cl^- (mEq/l)	105	109	104
Creatinine (mg/dl)	0.8	0.7	0.6
Overt proteinuria	–	–	+
PRA (ng/ml/h)	not measured	0.7	not measured
PAC (ng/dl)	not measured	8.2	not measured
FPG (mg/dl)	124	123	107
HbA1c (%)	6.0	8.4	5.8

MR, mineralocorticoid receptor; BMI, body mass index; EHT, essential hypertension; NIDDM, non-insulin dependent diabetes mellitus; HL, hyperlipidemia; HT, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ARB, angiotensin II recepter blocker; DMD, diabetes mellitus drug; BB, β-adrenegic receptor blocker; HLD, hyperlipidemia drug; PRA, plasma renin activity; PAC, plasma aldosterone concentration; FPG, fasting plasma glucose. Normal values in our institute: Na^+, 136 to 146 mEq/l; K^+, 3.6 to 4.9 mEq/l; Cl^-, 99 to 109 mEq/l; creatinine, 0.6 to 1.1 mg/dl; PRA, 0.2 to 2.7 ng/ml/h; PAC, 2 to 13 ng/dl.

Next, to understand this mutation's frequency and relevance to clinical phenotypes, we genotyped the F826Y mutation in a Japanese general population consisting of 3,655 individuals.

By the TaqMan-PCR genotype method, 13 individuals (7 females and 6 males) were found to be carriers of the F826Y mutation (Table 5). Among them, 3 (individuals 2, 3, and 4) had untreated hypertension, and 2 (individuals 1 and 5) were taking antihypertensive medication. Thus 5 of the 13 were hypertensives. The mean age was not significantly different between the hypertensive and non-hypertensive groups. The creatinine levels of the 5 hypertensives were in the normal range, and none of these patients showed proteinuria or diabetes mellitus. Serum electrolytes were not measured in this population.

Table 6 shows the frequency of the F826Y mutation in the hypertensive population and in the hypertensive group and the normotensive group of the general population. We identified 3 hypertensives with the F826Y mutation in the hypertensive subjects (3/942) and 5 hypertensives (5/1,480) and 8 normotensives (8/2,175) in the general population. Therefore, the frequency of the heterozygous carriers in each group was 0.00318, 0.00338, and 0.00368, respectively, and there was no significant difference in the prevalence of F826Y mutation between hypertensives and normotensives. Unfortunately, the history of pregnancy in each female subject was not fully determined in this study, and thus we could not evaluate the relationship between the F826Y mutation in *MR* and pregnancy-induced hypertension.

Discussion

The S810L mutation in *MR* causes early-onset hypertension that is markedly exacerbated in pregnancy. In our screening for *MR* in 942 Japanese hypertensives, including severe, early-onset cases with a strong genetic background, we did not detect the S810L mutation. Instead, we identified a novel missense mutation, *MR* F826Y, in 3 Japanese hypertensives. This mutation has been identified in 13 out of 3,655 individuals in the Japanese general population of the Suita Study. Therefore, the allele frequency of Y826 in the Japanese general population is 0.00178.

In this study, we did not identify the *MR* S810L mutation in our group of 942 Japanese hypertensives. This indicates that *MR* S810L either may not exist or may be very rare in Japanese, and that it may not be a major factor in essential hypertension in Japanese. In a previous study done in a German/Turkish population, 33 patients with pregnancy-induced hypertension and 5 patients with exacerbation of preexisting hypertension in pregnancy were screened for the S810L missense mutation, but the mutation was not detected, suggesting that it did not play a major role in the etiology of pregnancy-

Table 5. Clinical Profiles of Thirteen Subjects with F826Y Mutation in MR Gene in General Population

	Individual												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Age (years old)	61	67	85	70	65	61	85	72	52	58	43	49	63
Gender	female	male	male	male	female	female	male	female	female	female	female	male	male
BMI (kg/m^2)	22.2	23.5	18.2	23.7	20.6	16.0	16.5	21.0	20.7	19.8	15.7	22.8	21.2
SBP (mmHg)	139	145	155	143	118	111	125	126	119	111	113	125	139
DBP (mmHg)	84	83	83	86	71	82	65	67	84	62	82	82	81
Total cholesterol (mg/dl)	237	205	191	201	230	222	164	214	245	232	127	244	170
HDL-cholesterol (mg/dl)	93	48	56	43	45	78	68	91	67	43	73	57	70
Triglyceride (mg/dl)	82	104	109	88	108	78	47	–	117	–	28	190	43
Creatinine (mg/dl)	0.6	0.7	0.9	1.0	0.7	0.6	0.9	0.6	0.6	0.7	0.6	0.8	0.8
Overt proteinuria	–	–	–	–	–	–	–	2+	–	–	–	–	–
FPG (mg/dl)	88	100	96	106	85	79	84	93	84	96	72	92	87
HbA1c (%)	5.0	5.3	5.8	4.7	5.4	4.4	5.1	5.6	5.0	5.3	4.7	5.3	5.0
Current smoker	no	no	yes	no	no	no	no	no	no	no	no	no	no
Current drinker	no	no	yes	yes	no	no	no	no	no	no	yes	no	yes
Hypertension	yes	yes	yes	yes	yes	no	no	no	no	no	no	no	no
Hyperlipidemia	yes	yes	no	yes	yes	yes	no	no	yes	yes	no	yes	no
Diabetes mellitus	no	no	no	no	no	no	no	yes	no	no	no	no	no
Other diseases	OMI						AP						
Antihypertensive drugs	yes	no	no	no	yes	no	no	no	no	no	no	no	no

MR, mineralocorticoid receptor; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; FPG, fasting plasma glucose; OMI, old myocardial infarction; AP, angina pectoris. Hypertension: SBP ≥140 mmHg and/or DBP ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: FPG ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl, or HbA1c ≥6.5% or antidiabetic medication. Normal values in our institute: Na^+, 136 to 146 mEq/l; K^+, 3.6 to 4.9 mEq/l; Cl^-, 99 to 109 mEq/l; creatinine, 0.6 to 1.1 mg/dl; PRA, 0.2 to 2.7 ng/ml/h; PAC, 2 to 13 ng/dl.

Table 6. Number of Subjects with F826Y Mutation in *MR* and Its Frequency in Hypertensive and General Populations

	Hypertensive population (n=942)	General population	
		Hypertensive subjects (n=1,480)	Normotensive subjects (n=2,175)
Number	3	5	8
Frequency	0.00318	0.00338	0.00368

induced hypertension in this population (*20*). Our findings were consistent with these previous results in German/Turkish subjects.

If the F826Y mutation leads to a median effect on the function of MR, the phenotype in subjects with this mutation may be different from those with S810L. We identified a total of 8 subjects (8/(942+1,480)) with the F826Y mutation in *MR* among the hypertensive patients (including both our hypertensive population and hypertensive subjects from the general population of the Suita Study), and 8 normotensives (8/2,175) with the F826Y mutation in *MR* in the general population. There was no significant difference in the prevalence of F826Y mutation between the hypertensives and normotensives. Therefore, the F826Y mutation in *MR* does not appear to make a major contribution to hypertension, and its effect on the individual phenotype remains to be clarified.

The crystal structure of human progesterone receptor, which belongs to the steroid/nuclear receptor superfamily, in complex with progesterone has been solved (*21*). This structure indicated that F778 in human progesterone receptor makes a hydrogen-bond with progesterone. The amino acid alignment of h*MR* and human progesterone receptor showed that F778 in human progesterone receptor was very close to F826 in h*MR* (Fig. 2). This suggests that F826 in h*MR* may have an important function in the MR gene. Although the specific clinical features of patients with F826Y were unclear because of its very low allele frequency, our results suggested that this missense mutation plays at least a limited role in the regulation of blood pressure. Furthermore, in a previous report, three pedigree members with S810L with early-onset hypertension died of heart failure before age 50 (*12*). The distal nephron is recognized as the major site of the action of mineralocorticoids. In addition, MR is expressed in the brain, heart, and endothelium. These facts may suggest that *MR* with F826Y is involved in other clinical features in other tissues. Functional analysis for this novel missense mutation would be necessary to clarify its relevance to various clinical fea-

tures, including hypertension or cardiovascular renal impairments.

In summary, we identified one novel missense mutation in the MR gene in three hypertensive patients by sequencing the region of exon 6 in 942 patients with hypertension. This mutation was observed in 13 out of 3,655 individuals in a Japanese general population. Although the functional mechanisms of this mutation—and their relevance to the clinical features associated with the mutation—are unclear, the mutation could affect the MR function to some extent because it is present in the hormone-binding domain. Further accumulation of cases with the F826Y mutation and a follow-up survey may clarify the possible role of this mutation in hypertension or other clinical phenotypes.

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