Original Article

Genetic Variations of *CYP2C9* in 724 Japanese Individuals and Their Impact on the Antihypertensive Effects of Losartan

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CYP2C9, a drug-metabolizing enzyme, converts the angiotensin II receptor blocker losartan to its active form, which is responsible for its antihypertensive effect. We resequenced *CYP2C9* in 724 Japanese individuals, including 39 hypertensive patients under treatment with losartan. Of two novel missense mutations identified, the Arg132Gln variant showed a fivefold lower intrinsic clearance toward diclofenac when expressed in a baculovirus-insect cell system, while the Arg335Gln variant had no substantial effect. Several known missense variations were also found, and approximately 7% of the Japanese individuals (53 out of 724) carried one of the deleterious alleles (*CYP2C9*3, *13, *14, *30*, and Arg132Gln) as heterozygotes. After 3 months of losartan treatment, systolic blood pressure was not lowered in two patients with *CYP2C9*1/*30*, suggesting that they exhibited impaired *in vivo* CYP2C9 activity. *CYP2C9*30* might be associated with a diminished response to the antihypertensive effects of losartan. (*Hypertens Res* 2008; 31: 1549–1557)

***Key Words*: CYP2C9, single nucleotide polymorphism, hypertension, losartan**

Introduction

CYP2C9, a major isoform of the cytochrome P450 superfamily, accounts for approximately 20% of the total cytochrome P450 protein in liver microsomes and is responsible for the oxidative metabolism of up to 15% of drugs that undergo phase I metabolism (*1*, *2*). About 30 nonsynonymous variations of CYP2C9 have been identified. Of these, the effects of *CYP2C9*2* (Arg144Cys) and *CYP2C9*3* (Ile359Leu) have been well studied for their reduced metabolic activities towards substrates such as warfarin, tolbutamide, and losar-

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tan, both *in vitro* and *in vivo* (*3*, *4*). The allelic frequencies for these deleterious variations differ considerably among different ethnic populations. In Caucasian populations, the frequencies of *CYP2C9*2* and *CYP2C9*3* were 8–14% and 4–16%, respectively (*5*). In contrast, *CYP2C9*2* was not present in Asian populations, and *CYP2C9*3* was present in only 1–4% of Asian populations. Therefore, interethnic variability reported in the pharmacokinetics and pharmacodynamics of drugs, metabolized mainly by CYP2C9, could not be fully explained by the common variants alone. Recently, a number of novel nonsynonymous variations of CYP2C9 have been identified in different Asian populations (*6–11*). Functional analysis of these variations *in vitro* indicated the existence in Asians of new deleterious alleles of CYP2C9 that might have clinical relevance.

Losartan, the first selective angiotensin II receptor antagonist, was reported to significantly reduce the risk of cardiovascular endpoint outcomes compared with atenolol in high-risk hypertensive patients with left ventricular hypertrophy (*12*). Large interindividual variations in the efficacy and toxicity of losartan have been reported, and it has been suggested that they are genetically determined. A relationship was suggested between the polymorphism in the receptor gene, *AGT1R*, and its humoral and renal hemodynamic responses (*13*). However, losartan is oxidized primarily by CYP2C9 to an active carboxylic acid metabolite, E-3174, which has higher potency and a longer half-life than losartan and is therefore responsible for most of the antihypertensive effects (*14*, *15*). The effects of *CYP2C9*2* and *CYP2C9*3* on losartan oxidation have been extensively studied both *in vitro* and *in vivo*, consistently demonstrating the functional defect of the *CYP2C9*3* allele in decreasing the oxidation of losartan (*16–20*). However, the clinical relevance of genotypes of *CYP2C9* to the variable blood pressure–lowering responses to losartan in hypertensive patients has not been fully clarified. Furthermore, it remains unknown whether the other deleterious CYP2C9 alleles in Asians (*6–11*) might lead to the phenotypes of impaired therapeutic responses to this drug.

We studied several genes responsible for essential hypertension and interindividual differences in responses to warfarin and antihypertensive drugs (*21*, *22*). To identify the functional mutations, we resequenced some candidate genes including *WNK4*, *SCNN1B*, *SCNN1G*, *NR3C2*, and *RGS2* for hypertension (*23–26*) and *VKORC1*, *GGCX*, and *CALU* for warfarin (*22*, *27*). In the course of this resequencing, we noticed that the deleterious mutations are present more frequently than we expected, and the rare mutations with deleterious function would increase the total phenotype change.

In the present study, we resequenced the *CYP2C9* in 724 Japanese individuals. Two novel missense mutations were functionally analyzed in the baculovirus/insect cell expression system with diclofenac as a substrate. Furthermore, we assessed the blood pressure–lowering responses to losartan in hypertensive patients with the deleterious mutations in *CYP2C9*.

Methods

Subjects

Seven hundred twenty-four Japanese subjects in this study were enrolled for genetic sequencing of *CYP2C9*. The study subjects consisted of 312 patients with stroke and 412 patients with hypertension. Stroke patients (87 females and 225 males; average age: 65.36±11.87 years; body mass index: 23.28±3.01 kg/m^2) were admitted to the Cerebrovascular Division of the National Cardiovascular Center (*22*, *28*). They had all experienced an ischemic stroke within 7 d prior to admission. Hypertensive patients (196 females and 216 males; average age: 64.83±10.42 years; body mass index: 24.55±3.69 kg/m^2) were recruited from the outpatients clinic in the Division of Hypertension and Nephrology at the National Cardiovascular Center (*23–26*, *29*). Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or the current use of antihypertensive medication. Ninety-three percent of the study subjects (382 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension, including renal hypertension (10 subjects), renovascular hypertension (9 subjects), primary aldosteronism (7 subjects), and others (4 subjects).

Sixty-nine essential hypertensive patients (30 females and 39 males; average age: 64.36±9.34 years; body mass index: 22.65±7.84 kg/m^2) were taking one of three angiotensin II receptor blockers (losartan, candesartan, and valsartan) for treatment of hypertension. Among them, 39 patients had been receiving 50 mg/d of losartan for more than 3 months. We evaluated the patients' average resting blood pressure measured on three consecutive outpatient clinic visits, before and after losartan treatment.

The study was approved by the Ethics Review Committee of the National Cardiovascular Center, and only those subjects who provided written informed consent for genetic analyses were included in the study.

Resequencing of *CYP2C9* in 724 Japanese Subjects

Whole blood was collected from each participant, and genomic DNA was extracted from peripheral blood leukocyte. From each subject, 687 base pairs of the promoter region, all exons and intron-exon junctions, and the 3′-UTR of *CYP2C9* were amplified and sequenced directly on both strands using an ABI 3730 Automated Sequence Analyzer (Applied Biosystems, Foster City, USA), as described previously (*27*, *30*). Primers were designed to be specific to *CYP2C9*, with particular attention being paid to avoid amplification of sequences from homologous genes (*cf.* Online Table 1). The obtained sequences were examined for the presence of variations using Namihei software (Mitsui Knowl-

edge Industry Co., Ltd., Japan) and Sequencher software (Gene Codes Corporation, Ann Arbor, USA), followed by visual inspection. Novel nonsynonymous single nucleotide polymorphisms (SNPs) were confirmed by sequencing of PCR products generated from new genomic DNA amplifications. The genomic and cDNA sequences of *CYP2C9*, obtained from GenBank (NC_000010.8 and NM 000771.2, respectively), were used as reference sequences. The A of ATG of the initiator Met codon was denoted as nucleotide +1, and the initial Met residue was denoted as amino acid +1. The identified missense mutations were mapped in the human CYP2C9 crystal structure bound with warfarin (*31*) by the PyMOL v0.99 molecular visualization system (DeLano Scientific LLC, San Carlos, USA).

Cloning, Site-Directed Mutagenesis and Vector Constructions

A full-length human NADPH-cytochrome P450 oxidoreductase (OR) cDNA was isolated by PCR from human adult normal liver Quick-Clone cDNA (Clontech, Palo Alto, USA) with the forward primer, 5′-CACCAGTTTCATGATCAA CATGGG-3′, and the reverse primer, 5′-GCCCCTAGCTCC ACACGTCC-3′. The underlined sequence was introduced to the directional TOPO cloning system. The PCR products were cloned directly into the pcDNA3.1D/TOPO vector (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions (pcDNA3.1D/OR). Two single *CYP2C9* variations, 3573 G>A (Arg132Gln) and 42543 G>A (Arg335Gln), were introduced into the wild-type plasmid (pcDNA3.1D/ CYP2C9/Wild-type) as a template using a QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, USA). The primer sequences used for the construction of variant plasmids were as follows: 5′-CTCCCTCATGACGCTGC**A** GAATTTTGGGATGG-3′ (sense) and 5′-CCATCCCAA AATTC**T**GCAGCGTCATGAGGGAG-3′ (antisense) for pcDNA3.1D/CYP2C9/ Arg132Gln. 5′-TGATTGGCAGAA ACC**A**GAGCCCCTGCATGCA-3′ (sense) and 5′-TGCATG CAGGGGCTC**T**GGTTTCTGCCAATCA-3′ (antisense) for pcDNA3.1D/CYP2C9/ Arg335Gln.

The position of the exchanged nucleotide is underlined and in boldface. To ensure that no errors had been introduced during amplification, the entire cDNA regions were confirmed by sequencing the plasmid construct. Both OR and CYP2C9 wild-type or variant cDNAs were subcloned into the baculovirus transfer vector, pFastBac Dual (Invitrogen), 3′ of the P10 promoter, and the polyhedron promoter (polh), respectively (pFastBac Dual/P10.OR/ polh.CYP2C9). Recombinant baculoviruses carrying both CYP2C9 and OR cDNAs were produced according to the Bac-to-Bac Baculovirus Expression system protocol of Invitrogen.

Expression of Recombinant Proteins in Insect Cells and Preparation of Microsomal Fractions

For the expression of recombinant proteins using the baculovirus expression systems, adherent *Spodoptera frugiperda* (*Sf21*) insect cells (3.7×10^8 cells per 225 cm^2 flask) were infected with recombinant baculoviruses at a multiplicity of infection of 4 in supplemented form of Grace's Insect Medium (Invitrogen) with 10% fetal bovine serum and 10 μg/mL gentamycin. At 16–24 h post-infection, the culture media were supplemented with 0.2 mmol/L ferric citrate and 0.3 mmol/L δ-aminolevulinic acid, and the cells were harvested at 72-h post-infection. Microsomal fractions from *Sf21* cells were prepared as described previously (*11*).

Characterization of Protein Expression

The cytochrome P450 content in insect cell microsomes was measured by reduced CO-spectrum using the method of Omura and Sato (*32*). NADPH-cytochrome P450 OR activity in insect cell microsomes was measured using cytochrome C as a substrate as described by Phillips and Langdon (*33*). The molar amount of OR was calculated based on an assumed specific activity of 3.0 μmol cytochrome C reduced/min/nmol purified human OR (*34*). Western blotting of CYP2C9 and OR was performed using 2 μg of microsomal protein from insect cells as described previously (*11*). For immunostaining of OR, goat anti-rat OR antiserum (diluted 1:1,000; Daiichi Pure Chemical Co., Tokyo, Japan) and horseradish peroxidase–conjugated rabbit anti-goat IgG (diluted 1:20,000; Jackson ImmunoResearch Laboratories, West Grove, USA) were used as the first and second antibodies, respectively.

Assay for CYP2C9-Mediated Enzymatic Activity

CYP2C9 activities for the wild-type and two variants were assessed by diclofenac 4′-hydroxylation as described previously (*11*) except that the incubation mixture contained diclofenac (1.0–100 μmol/L), 5 pmol of P450 from insect microsomes, 10 pmol of purified cytochrome b5 (Oxford Biomedical Research, Oxford, UK), and an NADPH regenerating system (1.3 mmol/L $NADP^+$, 3.3 mmol/L glucose 6-phosphate, 3.3 mmol/L $MgCl_2$ and 0.4 unit/mL glucose-6-phosphate dehydrogenase), and the reactions were allowed to proceed for 10 min. The initial mobile phase of high-performance liquid chromatography consisted of 70% of a 30% acetonitrile solution containing 1 mmol/L perchloric acid (A) and 30% of methanol (B) and was delivered for 5 min, after which a 20 min linear gradient from 30% to 100% of B was formed at a flow rate of 1 mL/min. Under these conditions, the retention times of 4′-hydroxydiclofenac, 5-hydroxydiclofenac, and diclofenac were 14.2, 14.7, and 19.6 min, respectively.

Table 1. Genetic Variants in *CYP2C9* Identified in 724 Japanese Individuals

SNP position[a]	SNP position[b]	Location	Nomenclature[c]	Amino acid change	Number of subjects			Minor allele frequency	Flanking sequences (5′ to 3′)	rs ID No.	Reference
					Wild-type	Heterozygote	Homozygote				
−251 C>A[d]	−251	promoter			723	1	0	0.0007	ttattaccaata[C>A]ctaggctccaac		
−162 A>G	−162	promoter			723	1	0	0.0007	cattttatttttt[A>G]tctgtatcagtg		(*27*)
251 T>C	IVS1 + 83	Intron 1			716	7	1	0.0062	cctagaggtaca[T>C]gttacaagaggt	rs9332104	
3136 T>C[d]	IVS1 − 40	Intron 1			722	2	0	0.0014	aaatggacaaaa[T>C]agtaacttcgtt		
3154 T>C	IVS1 − 22	Intron 1			723	1	0	0.0007	cttcgtttgctg[T>C]tatctctgtcta		(*11*)
3235 G>A	228	Exon 2		Val76	706	18	0	0.0124	acccatagtggt[G>A]ctgcatggatat	rs17847036	
3276 T>C	269	Exon 2	*CYP2C9*13*	Leu90Pro	722	2	0	0.0014	ccctgattgatc[T>C]tggagaggagtt		(*6*)
3411 T>C	IVS2 + 73	Intron 2			712	11	1	0.0090	gacttacagagc[T>C]cctcgggcagag	rs9332120	
3451 G>A[d]	IVS2 − 59	Intron 2			723	1	0	0.0007	tggctgcccagt[G>A]tcagcttcctct		
3455 G>C[d]	IVS2 − 55	Intron 2			723	1	0	0.0007	tgcccagtgtca[G>C]cttcctctttct		
3488 G>T[d]	IVS2 − 22	Intron 2			723	1	0	0.0007	atctccctccta[G>T]tttcgtttctctt		
3514 T>C	336	Exon 3		Ile112	721	3	0	0.0021	tgttaggaat[T>C]gttttcagca		(*11*)
3544 G>A[d]	366	Exon 3		Glu122	723	1	0	0.0007	gaaatggaagga[G>A]atccggcgtttc		
3552 G>A	374	Exon 3	*CYP2C9*14*	Arg125His	723	1	0	0.0007	aggagatccggc[G>A]tttctccctcat		(*7*)
3573 G>A[d]	395	Exon 3		Arg132Gln	723	1	0	0.0007	tcatgacgctgc[G>A]gaattttgggat		
3627 G>T	449	Exon 3	*CYP2C9*27*	Arg150Leu	721	3	0	0.0021	aagaggaagccc[G>T]ctgccttgtgga		(*11*)
9032 G>C	IVS3 − 65	Intron 3			592	126	6	0.0953	ctactattatct[G>C]ttaacaaataca	rs9332127	
10411 A>G[d]	IVS4 − 15	Intron 4			723	1	0	0.0007	atttaataaatt[A>G]ttgttttctctt		
33553 A>G[d]	951	Exon 6		Pro317	723	1	0	0.0007	gctgaagcaccc[A>G]gaggtcacaggt		
42543 G>A[d]	1004	Exon 7		Arg335Gln	722	2	0	0.0014	ttggcagaaacc[G>A]gagcccctgcat		
42614 A>C	1075	Exon 7	*CYP2C9*3*	Ile359Leu	677	47	0	0.0325	gtccagagatac[A>C]ttgaccttctcc	rs1057910	
42676 T>C	1137	Exon 7		Tyr379	714	10	0	0.0069	attcagaaacta[T>C]ctcattcccaag		(*11*)
47377 T>C[d]	1176	Exon 8		Thr392	723	1	0	0.0007	aatttccctgac[T>C]tctgtgctacat		
50298 A>T	1425	Exon 9		Gly475	678	46	0	0.0319	agttgtcaatgg[A>T]tttgcctctgtg	rs1057911	
50302 G>A	1429	Exon 9	*CYP2C9*30*	Ala477Thr	722	2	0	0.0014	gtcaatggattt[G>A]cctctgtgccgc		(*11*)
50369 C>T[d]	1496 (*23[e])	3′-UTR			723	1	0	0.0007	atggcctggctg[C>T]tgctgtgcagtc		
50378 A>G[d]	1505 (*32[e])	3′-UTR			722	2	0	0.0014	ctgctgctgtgc[A>G]gtccctgcagct		
50456 C>T[d]	1583 (*110[e])	3′-UTR			721	3	0	0.0021	cctgtcatctca[C>T]attttcccttcc		
50613 T>C[d]	1740 (*267[e])	3′-UTR			722	2	0	0.0014	ttgagttattaa[T>C]atgttattatta		
50614 AT>—	1741_1742 (*268_*269[e])	3′-UTR			721	3	0	0.0021	tgagttattaat[AT>—]gttattattaaa		(*7*)
50742 T>A	1835+34[f] (*396[e])	3′ flanking			686	38	0	0.0263	ttcttttatgca[T>A]aatgtaggtcag	rs9332245	

[a]The A of the ATG of the initiation Met codon is denoted as nucleotide +1. [b]From the translational initiation site or from the end of the nearest exon. [c]Nomenclature for *CYP2C9* allele cited from: http://www.cypalleles.ki.se/cyp2c9.htm [d]Novel mutations identified in this study. [e]The nucleotide following the translation termination codon TGA is numbered *1. [f]The first nucleotide downstream of the 3′-end of exon 9 is numbered +1.

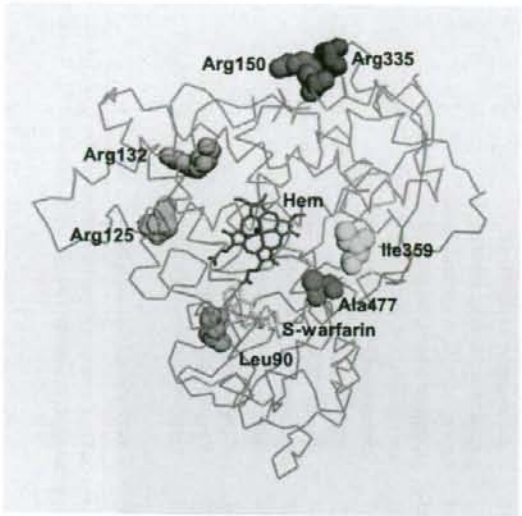

Fig. 1. *Mapping of identified missense variations on the crystal structure of human CYP2C9 protein bound with warfarin (PDB: 10G5). Hem and S-warfarin are shown by red and pink, respectively. The seven missense mutations identified in this study are presented by a space-filling model.*

Statistical Analysis

All SNPs identified were tested for deviations from the Hardy-Weinberg disequilibrium through the use of a χ^2 test. Pairwise linkage disequilibrium (LD) between two SNPs was evaluated by r^2 using SNPAlyze version 4.0 software (DYNACOM Co., Ltd., Mobara, Japan). Kinetic parameters K_m and V_{max} were estimated using a software program designed for non-linear regression analysis of a hyperbolic Michaelis-Menten equation (Prism v.3.0a, GraphPad Software, San Diego, USA). Kinetic data are presented as the mean±SD for three microsomal preparations derived from separate transfections for each variant and analyzed by one-way analysis of variance. Multiple comparisons were made with the Scheffe test.

Results

Resequence of *CYP2C9* in 724 Japanese Subjects

Upon sequencing the *CYP2C9* in 724 Japanese subjects, we identified a total of 31 genetic variations, including 15 novel ones (Table 1). All of the detected variations (except for the SNPs of 251 C>A in intron 1 and 3411 T>C in intron 2) were in Hardy-Weinberg equilibrium for two separate groups ($p \geq 0.81$ in stroke patients and $p \geq 0.82$ in hypertensive patients) and for all subjects ($p \geq 0.66$). Since we did not find any significant differences in frequencies between the stroke patients and the hypertensive patients ($p > 0.05$ by χ^2 test or Fisher's exact test), the data for all subjects were analyzed as one group.

Fourteen variations (seven missense and seven synonymous ones) were identified in the coding regions of CYP2C9. Two out of the seven missense mutations were novel, including Arg132Gln in one hypertensive patient and Arg335Gln in two stroke patients. The other five known missense mutations, Ile359Leu (*CYP2C9*3*), Leu90Pro (*CYP2C9*13*), Arg125His (*CYP2C9*14*), Arg150Leu (*CYP2C9*27*), and Ala447Thr (*CYP2C9*30*), were found in 47, 2, 1, 3, and 2 individuals, respectively. All the missense mutations were heterozygous, and there were no compound heterozygotes. The positions of seven missense mutations on the crystal structure of human CYP2C9 bound with warfarin are shown in Fig. 1.

Seven synonymous variations were identified, of which three novel ones (Glu122Glu; $n=1$, Pro317Pro; $n=1$, and Thr392Thr; $n=1$) were found as single heterozygotes. In the putative promoter region, two variants (−251 C>A and −162 A>G) (*35*) were detected, each in only one individual. A total of 15 variations were found in the intronic, 3′-UTR, and 3′-flanking regions. Five novel variations in introns 1, 2, and 4 and four novel variations in the 3′-UTR were identified with allele frequencies less than 0.01.

LD analysis showed that *CYP2C9*3* was in LD ($r^2 > 0.8$) with two variations, 50298 A>T (Gly475Gly) in exon 9 and 50742 T>A in the 3′-flanking region. LD ($r^2 = 0.7$) was also noted between two intronic variants, 251 T>C in intron 1 and 3411 T>C in intron 2.

Functional Characterization of Two Novel Missense Mutations

To functionally characterize the two novel missense mutations, Arg132Gln and Arg335Gln, the wild-type and two CYP2C9 variants were coexpressed with NADPH-cytochrome P450 OR in *Sf21* insect cells. The holo-CYP2C9 content was not significantly different between the wild-type and variants: 188.6±22.9 pmol/mg microsomal protein for wild-type, 192.3±14.5 pmol/mg microsomal protein for Arg132Gln, and 159.3±5.5 pmol/mg microsomal protein for Arg335Gln, as determined on three lots from independent expression experiments. Quantities of cytochrome P420 were negligible for all preparations (data not shown). Cytochrome C reductase activities varied slightly but were not significantly different among the preparations (632–808 nmol cytochrome C reduced/min/mg protein), and the mean OR/CYP2C9 molar ratios in microsomal fractions were calculated to be 1.2, 1.3, and 1.6 for wild-type, Arg132Gln, and Arg335Gln, respectively.

Immunoblot analyses of CYP2C9 and OR were performed using insect cell microsomes, and representative data from three independent preparations are shown in Fig. 2. Quantitative analysis revealed that neither apo-CYP2C9 nor OR pro-

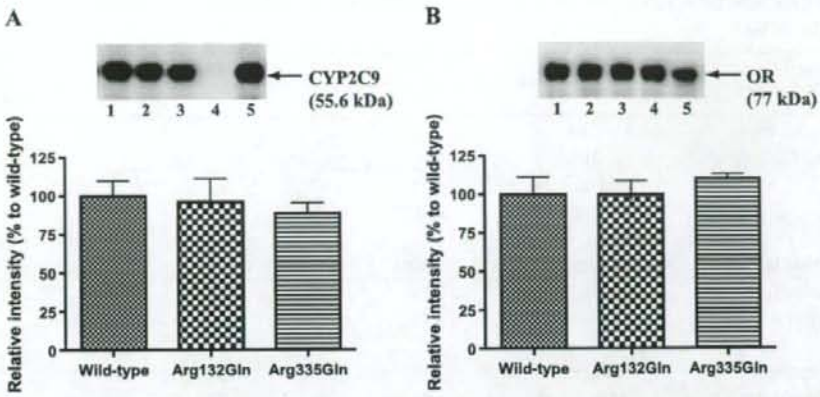

Fig. 2. *Expression of wild-type and two variants of CYP2C9 in insect cell microsomes. Representative Western blots of immunoreactive CYP2C9 (A) and OR (B) proteins (upper) are shown. Lanes 1–3: co-expressed microsomes containing wild-type, Arg132Gln, and Arg335Gln CYP2C9 each with OR; lane 4: microsomes containing solely OR; lane 5: commercially available co-expressed supersomes containing CYP2C9.1 and OR (BD Bioscience, San Jose, USA). Relative intensities of immunoreactive CYP2C9 (A) and OR (B) protein are shown in the lower panels. Each bar represents the mean±SD of three separate experiments.*

Table 2. Kinetic Parameters for Hydroxylation Activities of Wild-Type and Variant CYP2C9 against Diclofenac

Amino acid alteration	K_m (μmol/L)	V_{max} (pmol/min/pmol P450)	Clearance (V_{max}/K_m) (μL/min/pmol P450)
Wild-type	3.4±0.17	79.8±6.6	23.4±0.81
Arg132Gln	1.8±0.05**	7.8±0.4**	4.2±0.31**
Arg335Gln	3.0±0.10*	65.4±2.1*	22.0±0.06*

*$p<0.05$, **$p<0.0001$ *vs.* wild-type. One-way analysis of variance, post-hoc test: Scheffe. Data are represented by means±SD.

tein expression levels were significantly different among the wild-type and two variants ($p=0.77$ for CYP2C9, $p=0.64$ for OR). Catalytic activities of the wild-type and variant (Arg132Gln and Arg335Gln) proteins were assessed using diclofenac as a substrate. Diclofenac 4′-hydroxylation exhibited typical hyperbolic kinetic profiles in both the wild-type and variant proteins (data not shown). The kinetic parameters are summarized in Table 2. The Arg132Gln protein showed a 90% decrease in the V_{max} value and a partial decrease in the K_m value, resulting in fivefold lower intrinsic clearance relative to the wild-type (Table 2). A slight diminution in intrinsic clearance (6%) was observed for the Arg335Gln protein with slightly decreased K_m and V_{max} values (Table 2). The formation of 5-hydroxy diclofenac was observed in neither the wild-type nor variant (Arg132Gln and Arg335Gln) proteins (data not shown), suggesting that these substitutions do not alter the regioselectivity of diclofenac hydroxylation.

CYP2C9 Polymorphisms and the Effectiveness of Losartan in 39 Hypertensive Patients

Among 39 patients taking losartan, 34 patients carried the wild genotype of *CYP2C9*1/*1*, and the other 5 patients carried missense mutations, including *CYP2C9*1/*3* in 2 patients, *CYP2C9*1/*30* in 2 patients, and Arg132Gln mutation in one patient. The changes in systolic and diastolic blood pressure with respect to genotypes at 3 months of losartan treatment are presented in Table 3. Losartan obviously lowered systolic blood pressure in 2 patients with *CYP2C9*3* and in a patient with the Arg132Gln mutation. However, losartan was not effective in 2 patients with *CYP2C9*1/*30*.

Discussion

In the present study, the large-scale direct resequencing effort of the *CYP2C9* allowed us to detect 31 genetic variations in 724 Japanese individuals. We also obtained accurate frequencies of the known variations, *CYP2C9*3*, **13*, **14*, **27* and **30*, that are specific to Asians, except for **3*. As for the novel alleles, Arg132Gln and Arg335Gln, their effects on both protein expression levels and enzymatic activity were assessed using a baculovirus expression system.

The most frequently identified missense mutation in the present study was *CYP2C9*3* (Ile359Leu), with a frequency

Table 3. Patient Characteristics and Blood Pressure Response to Losartan with Respect to Genotypes: Essential Hypertensive Patients Taking Losartan

	CYP2C9 genotype					
	**1/*1*	**1/*3*		**1/*30*		Arg132Gln
Case number	34	2		2		1
Sex (male/female)	21/13	0/2		2/0		1/0
Age (years)	65.10±7.04	70	67	77	71	70
BMI (kg/m^2)	25.10±3.07	21.47	24.20	24.33	25.59	20.7
SBP						
At baseline (mmHg)	151.10±14.75	130[a]	156	155	172	157
At 3 month (mmHg)	142.80±16.23	119	141	151	173	128
Change (mmHg)	−8.70±14.35	−11	−15	−4	1	−29
DBP						
At baseline (mmHg)	88.80±9.26	71[a]	104	81	98	82
At 3 month (mmHg)	84.90±9.98	75	96	83	95	70
Change (mmHg)	−4.20±6.91	4	−8	2	−3	−12

Values are mean±SD. BMI, body mass index; SBP, DBP, systolic and diastolic blood pressures. [a]Office blood pressure in this patient with *CYP2C9 *1/*3* was 130/71 mmHg. Losartan was prescribed because this patient had higher home SBP (over 150 mmHg).

of 0.033, which was in good agreement with the previously published results in Japanese populations (*11*, *36*, *37*). The frequency of *CYP2C9*13* (Leu90Pro), 0.0014 in the present study, was comparable to that recently reported in a Japanese population (*11*) but much lower than those in previous studies of other Asian populations (*6*, *9*). *CYP2C9*13* was first identified in a Chinese individual who showed poor metabolizer phenotype for both lornoxicam and tolbutamide (*6*). Functional analysis of the *CYP2C9*13* protein showed decreased enzymatic activity for tolbutamide and diclofenac (*10*). Another recently published allele, *CYP2C9*14* (Arg125His), was detected in an individual in the present study. This allele was first identified in an Indian patient, and the variant protein exhibited 80–90% lower catalytic activity toward tolbutamide (*7*, *8*). *CYP2C9*27* (Arg150Leu) and **30* (Ala477Thr), both detected recently in a Japanese population (*11*), were also identified in 3 and 2 individuals in the present study, respectively. The *in vitro* study revealed that the *CYP2C9*30* protein had a twofold higher K_m value and a threefold lower V_{max} value than the wild-type towards diclofenac, whereas the catalytic activity of the *CYP2C9*27* protein was similar to the wild-type (*11*).

The novel Arg132Gln variant exhibited a 90% decrease in the V_{max} value toward diclofenac 4′-hydroxylation (Table 2). Arg132 is located in a loop region between the C and D helices (Fig. 1) and is highly conserved in the CYP2C family (http://drnelson.utmem.edu/humP450.aln.html). Arg133, the corresponding residue of CYP2B4, is suggested to play a prominent role in binding its redox partners, cytochrome b5 and P450 reductase (*38*). Accordingly, the loss of catalytic activity of the Arg132Gln variant might reflect the altered affinity of variant protein to these redox partners due to electrostatic changes as proposed for **2* (Arg144Cys), **14* (Arg125His), and **26* (Thr130Arg) (*8*, *11*, *39*).

The Arg335Gln variant showed a similar holo-CYP2C9 content to wild-type in insect cell microsomes. Furthermore, the intrinsic clearance of the Arg335Gln variant was only slightly lower than that of the wild-type. In contrast to Arg335Gln, a substitution in the same position, Arg335Trp (**11*), was reported to exhibit a threefold increase in K_m and more than a twofold decrease in the intrinsic clearance for tolbutamide when expressed in a bacterial cDNA expression system (*40*). In addition, catalytically active *CYP2C9*11* holo protein was expressed at a very low level due to its decreased stability in insect cells (*41*). To confirm whether or not the protein stability of the Arg335Gln variant might be influenced by the *in vitro* expression system used, the wild-type and variant proteins were expressed in a mammalian expression system using COS-1 cells. The protein expression level of Arg335Gln variant in COS-1 microsomes was decreased by only 30% compared with that of the wild-type (data not shown), indicating that the protein stability of the Arg335Gln product was not substantially different between mammalian expression systems and baculovirus/insect cell systems. Thus, the substituted residues (Trp *vs.* Gln) at this position might quite differently influence the stability of protein as well as catalytic activities.

Thirty-nine patients were taking losartan, which is known to exhibit considerable inter-individual variation in its antihypertensive effects. Losartan is primarily oxidized by CYP2C9 to an active carboxylic acid metabolite, E-3174 (*14*–*16*). CYP3A4 also plays a limited role in the metabolic activation of losartan *in vitro*; however, its significance *in vivo* has not been demonstrated (*3*, *15*, *16*). We evaluated the impact of *CYP2C9* variations on the antihypertensive effect of losartan based on the patients' average resting blood pressure measured before and three months after losartan treatment.

Two Japanese hypertensive patients carrying the *CYP2C9*3* heterozygous allele showed lowered systolic blood pressure by losartan (Table 3). This is in line with the previous report that no significant differences in the pharmacokinetics of losartan and E-3174 were observed between *CYP2C9*1/*3* and **1/*1* (*42*). Contrary to our result, a Danish prospective study of optimal monotherapy with losartan in type 1 diabetic patients with nephropathy showed that the reduction in systolic 24 h blood pressure was significantly greater in wild-type patients (n=48) than in *CYP2C9*3* carriers (n=12) (*43*). Furthermore, similar changes in diastolic and systolic 12 h blood pressures were also observed between *CYP2C9*1/*1* (n=4) and **1/*3* (n=3) Japanese patients (*20*). The role of heterozygous *CYP2C9*3* in the blood pressure–lowering response to losartan in hypertensive patients should be further studied in a large cohort of patients.

Inconsistent with our *in vitro* study, systolic blood pressure in a patient with Arg132Gln was obviously lowered by losartan (Table 3). For this variation, the substrate-dependent differences between diclofenac and losartan oxidation are unlikely because Arg132 might interact with redox partners but not with substrates as described above. However, the change in enzymatic activity toward losartan should be further analyzed.

However, losartan was not effective in 2 patients carrying the heterozygous *CYP2C9*30* (Ala477Thr) allele. A serious impact on the pharmacodynamics of losartan was not demonstrated statistically because of the small sample size of individuals with **30*. Ala477 is located in the substrate recognition site-6 region in the β2 sheet, which shows very strong hydrophobic interactions with the substrates (*44*), suggesting the importance of this residue in metabolic activity of CYP2C9 toward various substrates. Therefore, insufficient conversion of losartan to E-3174 by this defective mutation might be responsible for the therapeutic failure of these patients. Pharmacokinetic analysis of *CYP2C9*30* towards losartan would be necessary to further elucidate its clinical relevance.

In conclusion, multiple rare functional variations of *CYP2C9* were detected in a Japanese population. Approximately 7% of the Japanese individuals analyzed (53 of 724) carried one of the functionally deleterious alleles (*CYP2C9*3*, **13*, **14*, **30*, and Arg132Gln). In addition to *CYP2C9*3*, *CYP2C9*30* might also be used for determining inter-individual responses to losartan treatment in Japanese hypertensive patients.

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Original Article

Efficacy and Safety of Long-Term Losartan Therapy Demonstrated by a Prospective Observational Study in Japanese Patients with Hypertension: The Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH) Study

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The Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH) study is a nationwide, prospective, multicentered, observational study that was designed to enroll 30,000 hypertensive Japanese patients from more than 3,000 private practitioners. It is the first large-scale observational study to assess the efficacy and safety of losartan, an angiotensin II receptor antagonist, in Japan. Patients were enrolled between June 2000 and May 2002, and followed up to June 2005. The data from 29,850 patients were used for the analysis of safety and efficacy. These patients were treated with losartan mostly at a daily dose of 25–50 mg. The mean follow-up period was 2.9 years. The patients were aged 62.4±12.1 years (mean±SD) and their mean systolic/diastolic blood pressure was 165.3±17.2/94.3±11.7 mmHg (mean±SD). Mean blood pressure in patients who were evaluated for efficacy decreased from 165.8/94.8 mmHg (n=26,512) at baseline to 145.5/84.4 mmHg after 3 months (n=21,269) and 138.6/80.0 mmHg after 36 months of treatment (n=13,879). Blood pressure was well controlled during the study period by losartan alone or losartan-based combination therapy. In nearly half of the patients, blood pressure was reduced to less than 140/90 mmHg during the study period. In addition to its antihypertensive effect, losartan reduced the uric acid level in patients whose baseline uric acid level was ≥7 mg/dL. Losartan also prevented acceleration of proteinuria. Adverse drug reactions occurred in 1,081 of the 29,850 patients. Long-term losartan therapy was effective and well tolerated in Japanese clinical practice. (*Hypertens Res* 2008; 31: 295–304)

***Key Words*: hypertension, losartan, Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH), efficacy, safety**

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Introduction

The number of persons with hypertension in Japan has been reported to be as high as 30 million (*1*). Therefore, management of hypertension is one of the major public health measures for preventing cardiovascular disease in this country. Although tight blood pressure (BP) control is recommended by the guidelines produced in Western countries and Japan to prevent cardiovascular disease in hypertensive patients (*2–5*), less than 50% of patients actually achieve good BP control (*6–11*).

Losartan potassium (losartan) is a subtype 1 (AT1)–selective angiotensin II (AII) receptor antagonist (ARB) that is widely prescribed as an antihypertensive agent throughout the world. Several large-scale clinical trials have already demonstrated the benefits of antihypertensive therapy with losartan (*12–17*). Losartan not only lowers the BP, but also has a protective effect on target organs. The Losartan Intervention For Endpoint reduction (LIFE) study demonstrated a more favorable effect of this drug on cardiovascular events than atenolol (*12*), while the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study demonstrated a renoprotective effect of losartan (*13*). In this RENAAL study, Japanese patients were included, and a renoprotective effect of losartan was demonstrated (*14*).

Various beneficial effects of losartan have been reported mainly in Western countries (*12*, *13*, *15–17*). However, these results may not be directly applicable to Japanese hypertensive patients, since the hypertensive patients enrolled in these studies usually have more risk factors than ordinary Japanese hypertensive patients. In addition, the percentage of elderly patients in Japan is different from that in Western countries, and genetic and environmental factors may differ between Japanese and Western patients (*18*, *19*).

Several studies in Japanese hypertensive patients have already been conducted, but these studies have mainly assessed small cohorts in specific rural areas. There have been few large-scale studies on the effects of losartan in daily clinical practice, and losartan's therapeutic benefits for Japanese patients have not been well demonstrated.

To investigate the efficacy and safety of losartan-based antihypertensive therapy and to understand the current status of antihypertensive therapy in ordinary clinical practice, a large scale study of losartan-based antihypertensive treatment in Japanese hypertensive patients would be meaningful.

The Japan Hypertension Evaluation with AIIA Losartan Therapy (J-HEALTH) study is a nationwide, prospective, multicentered observational study that was designed to enroll hypertensive Japanese patients (>30,000 subjects) from more than 3,000 private practitioners. This observational study was designed to investigate the efficacy and safety of long-term losartan therapy in ordinary clinical practice as a post-marketing surveillance study.

The present report deals with the efficacy, safety, and other effects of losartan as demonstrated by the J-HEALTH study. The incidence of cardiovascular events and mortality will be discussed in another paper in this series.

Methods

Patient Selection

The design of the J-HEALTH and the patient characteristics were described in detail previously (*20*). Patients were screened between June 2000 and May 2002 in all 47 prefectures of Japan. A total of 31,048 patients were enrolled in the study in proportion to the population of each prefecture. Among the patients thus enrolled, 1,198 patients were excluded from the analysis of safety, mainly due to violations of consent, previous treatment history with losartan, or other regulatory infractions. Consequently, 29,850 patients were eligible for safety evaluation. Among those 29,850 patients, 3,338 patients were ineligible for the analysis of efficacy mainly because of protocol violations or a lack of available BP data. Thus a total of 26,512 patients were eligible for the analysis of efficacy.

Study Design

The J-HEALTH is a nationwide prospective observational study that evaluates the efficacy and safety of long-term losartan therapy in the daily clinical setting. The effects of losartan on serum uric acid, urinary protein, and serum creatinine were also evaluated. The J-HEALTH study was designed to enroll a large number of Japanese hypertensive patients (>30,000 subjects) and was initiated as a post-marketing surveillance study in June 2000. The study period was 5 years in total, including a 2-year enrollment period. Patients were followed up to June 2005. The patients received open-label treatment with losartan for a maximum of 5 years.

The eligible patients were men or women aged ≥20 years with untreated or treated hypertension diagnosed by their personal physicians. Only patients who were not receiving antihypertensive drugs for at least 1 month prior to the study were registered. Patients with a history of losartan treatment at any period were excluded from the study. Each patient was informed of the purpose and methods of the study, as well as the effects and possible risks of losartan therapy, their right to withdraw from the study at any time, and the measures taken for privacy protection before the enrollment. Patients gave verbal informed consent and then underwent a medical history review, physical examination, and laboratory evaluation.

Treatment with losartan was started at a dose of 25–50 mg once daily (usually in the morning), which is the approved dosage in Japan. The dose could be increased up to a maximum of 100 mg once daily, if necessary. Addition of other antihypertensive agents was allowed from 3 months after the start of losartan treatment, if required. No restrictions were placed on the treatment of complications. Patients were fol-

Table 1. Patients' Characteristics at Baseline (n=29,850)

Male, %	44.1	
Age, years*	62.4±12.1	
BMI, kg/m^2 *	24.1±3.6	
Mean clinic SBP, mmHg*	165.3±17.2	
Mean clinic DBP, mmHg*	94.3±11.7	
Grade of HT, %		
<130 and <85 mmHg	0.8	
130–139 or 85–89 mmHg	1.9	
140–159 or 90–99 mmHg	25.1	
160–179 or 100–109 mmHg	45.0	
≥180 or ≥110 mmHg	22.5	
Missing data	4.7	
HT history, months*	33.8±58.0	
HT treatment history, %†	15.4	
Clinic heart rate, bpm*	74.6±10.5	
Smoking habit, %	25.1	
Alcohol consumption, %‡	38.3	
Hyperlipidemia, %	38.8	
Diabetes mellitus, %	13.1	
Hyperuricemia/Gout, %	10.7	
Cardiovascular disease, %	8.0	
Cerebrovascular disease, %	4.4	
Hepatic disease, %	9.6	
Renal disease, %	3.2	
ECG abnormality, %	14.4	
Laboratory test (n)		
Creatinine, mg/dL*	0.9±0.3	(19,031)
Uric acid, mg/dL*	5.3±1.5	(17,202)
Urinary protein >"–", %§	19.1	(15,291)
Potassium, mEq/L*	4.2±0.5	(12,344)

*Mean±SD. †HT treatment history: patients who had a treatment history with antihypertensive drugs 1 month or more before the registration. ‡Alcohol consumption: ≥3 times/week and ≥200 mL/time (1 middle-sized bottle of beer or 2 glasses of diluted whiskey with water). §Urinary protein >"–": result of dipstick test for proteinuria more than negative (–). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension.

Table 2. Antihypertensive Treatment during the Study Period (n=26,512)

Losartan	
Mean dose	47 mg/day
Initial dose	
≤25 mg/day	24%
≤50 mg/day	75%
>50 mg/day	1%
Losartan monotherapy	59%
Combination with other antihypertensive drugs	41%
No. of the drugs (including losartan)	
2-drug	29%
3-drug	9%
≥4-drug	3%
Major antihypertensive drugs	
CCB	32%
Diuretics	7%
β-Blockers	6%
α-Blockers	5%

CCB, calcium channel blockers.

lowed up for a maximum of 5 years.

The demographic data, physical data (height and body weight), history of hypertension, past treatment history of hypertension, BP values, pulse rate, complications, and medical history at enrollment were recorded. To assess complications and the medical history, physicians judged the existence of all disease indicated in the registration form before the start of treatment with losartan at their discretion. In addition, the patients who were on drug treatment for hyperlipidemia or diabetes mellitus (DM) and met the definition of either disease indicated in the relevant guidelines were defined as having hyperlipidemia or diabetes. Laboratory test results, ECG findings, and details of lifestyle modification, such as smoking and/or alcohol cessation/restriction, physical activity and weight loss, were also recorded, if available.

The clinic systolic BP (SBP) and diastolic BP (DBP) were measured by the usual methods at each institution. At each time of measurement, one clinic BP value was reported at the discretion of the physician. The clinic BP data were measured at baseline. During the follow-up period, the clinic BP value was measured every 3 months. The clinic BP data thus obtained were used for analysis of the clinic BP values at baseline and during treatment. Standard laboratory tests were reported every 6 months (if performed) during the study period. The investigators evaluated all adverse events and classified these as definitely related to the test drug, possibly related, definitely not related, or unknown. All losartan-related adverse events were pooled and classified as adverse drug reactions (ADRs). The following patient information was recorded in the case report forms and collected every year: adverse events, clinic BP values, pulse rate, heart rate, body weight, daily dose of losartan, concomitant drugs, laboratory test findings (if performed), and ECG findings (if performed).

Statistical Analysis

Variables were compared using the t-test, the χ^2 test, or analysis of variance (ANOVA), as appropriate. Results were expressed as the means±SD, and differences were considered statistically significant at $p<0.05$. Statistical analysis was conducted with the SAS software package (Version 8.02; SAS Institute Inc., Cary, USA).

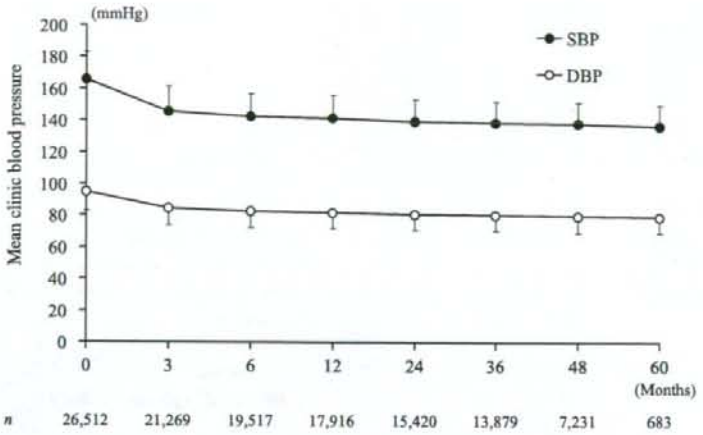


Fig. 1. *Change in mean clinic blood pressure during study period. SBP, systolic blood pressure; DBP, diastolic blood pressure.*

Study Organization

The Monitoring Committee assessed the advisability of continuing the study based on the safety and effectiveness of losartan therapy. The Safety Assessment Committee investigated the causal relationship between each ADR and the drugs administered during the study. The Medical Expert Advisory and Publication Committee were responsible for reviewing the results and writing reports.

Results

Baseline Characteristics

The baseline characteristics of the 29,850 patients (13,163 men [44.1%] and 16,687 women [55.9%]) eligible for analysis of safety are summarized in Table 1. The mean follow-up duration was 2.9 years. The mean age of the patients was 62.4±12.1 years, while the mean SBP and DBP were 165.3±17.2/94.3±11.7 mmHg. The prevalences of hyperlipidemia, DM, hyperuricemia/gout, cardiovascular disease, cerebrovascular disease, and ECG abnormalities were 38.8%, 13.1%, 10.7%, 8.0%, 4.4%, and 14.4%, respectively. Young patients (20–39 years) accounted for 2.9%, middle-aged patients (40–59 years) accounted for 38.4%, and elderly patients (60–79 years) made up 51.3% of the study population. It is worth noting that 2,209 patients (7.4%) were very elderly (≥80 years old). According to the Japanese Society of Hypertension (JSH) 2004/World Health Organization (WHO) classifications of hypertension, Moderate/Grade 2 hypertensive patients were predominant (n=13,429, 45.0%), while the percentages of Mild/Grade 1 and Severe/Grade 3 patients were almost equal (n=7,490, 25.1% *vs.* n=6,721, 22.5%, respectively).

Efficacy of Losartan

Follow-Up of Patients

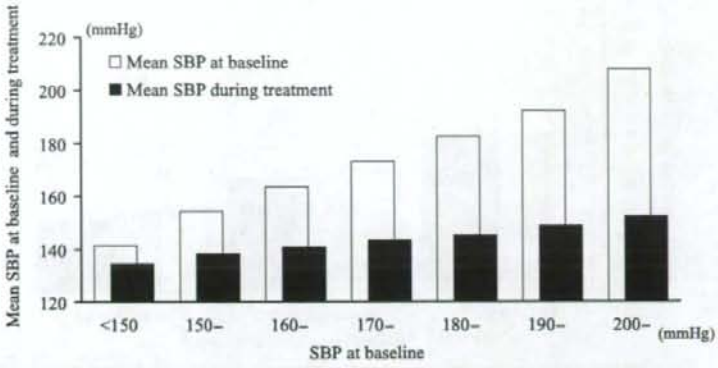
The 26,512 patients eligible for analysis of efficacy were followed up for a maximum of 5 years. However, approximately 45% (11,845 patients) of these patients were not followed up until June 2005, the end of the study period. The main reason for drop-out was failure to visit the clinic (65%).

Clinic Blood Pressure

Table 2 summarizes the antihypertensive therapy provided during the study period. The 26,512 patients were treated with losartan at a mean dose of 47 mg/day, with 59% receiving losartan monotherapy and 41% being treated with losartan-based combination therapy. Calcium channel blockers (CCBs) were the most frequently used concomitant drugs, being combined with losartan in 32% of the patients. Diuretics were prescribed in 7% of the patients. The number of antihypertensive drugs was two in 29%, three in 9%, and four or more in 3% of the patients.

The changes in BP are shown in Fig. 1. The mean BP decreased from 165.8/94.8 mmHg at baseline (n=26,512) to 145.5/84.4 mmHg after 3 months (n=21,269), 138.6/80.0 mmHg after 36 months (n=13,879), and 136.9/79.2 mmHg after 60 months (n=683) of losartan-based treatment. The mean BP during the entire follow-up period was 141.6/82.0 mmHg.

Patients who were treated with losartan alone throughout the study period were defined as the losartan monotherapy group. In the losartan monotherapy group, the mean BP decreased from 163.7/93.9 mmHg at baseline to 143.1/83.4 mmHg after 3 months of treatment and then decreased to 135.9/78.8 mmHg after 60 months of treatment. With combination therapy, the mean BP decreased from 169.0/95.9 mmHg at baseline to 149.1/86.0 mmHg after 3 months and



n	2,259	3,653	4,514	2,866	1,439	544	354
SBP changes							
mmHg	−6.77	−16.03	−22.70	−29.82	−37.41	−43.62	−55.89
%	−5	−10	−14	−17	−20	−23	−27

Fig. 2. *Reduction of mean SBP level by baseline SBP in patients treated with losartan monotherapy. The horizontal axis indicates the range of mean SBP at baseline. The vertical axis indicates the mean SBPs at baseline and during treatment. SBP, systolic blood pressure.*

then decreased to 138.1/79.6 mmHg after 60 months. The mean BP profile was similar between losartan monotherapy and combination therapy. On the whole, the mean BP during treatment was well controlled (<140/90 mmHg) in 46% of all the patients, and the mean follow-up period of these patients was 3.0 years. Furthermore, the BP was well controlled in approximately 50% of the patients receiving losartan monotherapy and 40% of those on combination therapy (data not shown). As shown in Fig. 2, the reduction of clinic BP by losartan monotherapy was more pronounced among the patients with higher baseline BP values than among those with lower baseline values.

The percentages of patients whose mean BP was controlled to less than 140/90 mmHg during treatment are shown in Fig. 3 after classifying into three age ranges and three grades of hypertension at baseline. The BP was well controlled (BP <140/90 mmHg) in more than 50% of Grade 1 patients, but in less than 50% of Grade 2 or Grade 3 patients. The percentage of patients with a well-controlled BP was similar among age groups for each grade of hypertension.

Proteinuria

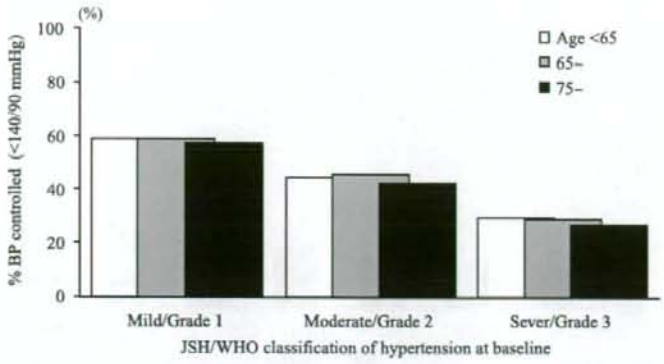
The prevalences of proteinuria in DM and non-DM patients are shown in Fig. 4. The percentage of patients with proteinuria decreased significantly following treatment in both the DM and non-DM groups.

Serum Uric Acid

In patients with baseline serum uric acid ≥7 mg/dL, the mean serum uric acid level decreased without uric acid–lowering drugs from 7.6 mg/dL (baseline) to 6.3 mg/dL (after 60 months), for a mean decrease of 1.3 mg/dL (Fig. 5). In patients whose baseline level was ≥7 mg/dL with uric acid–lowering drugs, there was a decrease from 7.3 mg/dL (baseline) to 6.3 mg/dL (after 60 months), for a mean decrease of 1.0 mg/dL. Thus, the change of uric acid levels was the same whether patients were administered uric acid–lowering drugs or not. The mean serum uric acid level of the patients with a baseline level <7 mg/dL was 4.9–5.0 mg/dL, and showed no clinically significant change throughout the study period.

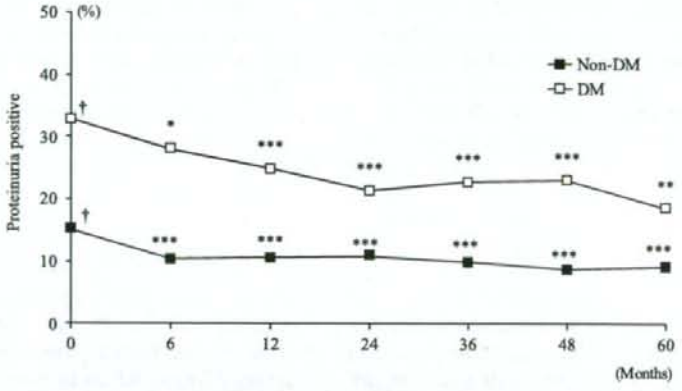
Safety of Losartan

Twenty-nine thousand, eight hundred and fifty subjects were eligible for analysis of ADRs, which were reported in 1,081 of the patients (Table 3). Unfortunately, the incidence of laboratory ADRs could not be determined definitively because laboratory examination was not mandatory for this survey protocol. However, no clinically significant or unknown ADRs were reported by the attending physicians during this study. The most frequent ADRs were dizziness, hepatic dysfunction, headache, anemia, and cough. The most frequently reported ADR of angiotensin converting enzyme inhibitors (ACEIs), cough, was detected in only 46 patients. Although renal dysfunction and hyperkalemia are known to be side effects of ARBs, renal dysfunction, including an increase of serum creatinine levels, was found in 46 patients, and hyperkalemia was found in 26 patients in the present study. Mean creatinine level stratified by sex and the baseline renal function are shown in Fig. 6. Renal dysfunction was defined by baseline serum creatinine ≥1.3 mg/dL for men and 1.2 mg/dL for women according to the JSH 2004 guidelines (*5*). No sus-



Age <65 years	(*n*)	3,849	7,415	4,082
65–	(*n*)	2,458	3,992	1,739
75–	(*n*)	1,318	2,222	995

Fig. 3. *Control rate of BP (< 140/90 mmHg) by grade of hypertension and age at baseline. JSH/WHO classification of hypertension: Mild/Grade 1, SBP 140–159 mmHg or DBP 90–99 mmHg; Moderate/Grade 2, SBP 160–179 mmHg or DBP 100–109 mmHg; Severe/Grade 3, SBP ≥180 mmHg or DBP ≥110 mmHg. BP, blood pressure; JSH, Japanese Society of Hypertension; WHO, World Health Organization; SBP, systolic blood pressure; DBP, diastolic blood pressure.*



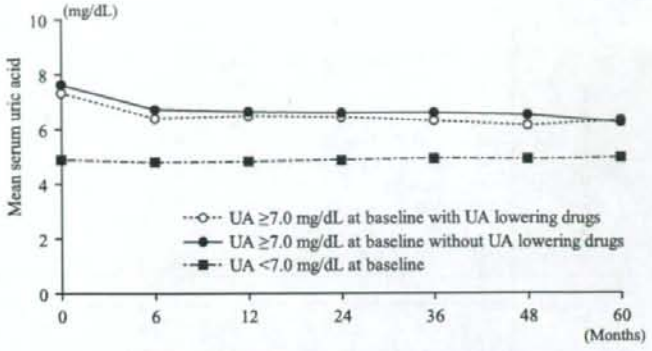
DM	1,576	892	887	730	693	414	102
Non-DM	7,357	3,003	3,720	3,337	3,076	1,977	490

Fig. 4. *Time courses of the proportion of proteinuria-positive in non-DM and DM patients during the study period. Proteinuria positive includes trace ±, +, ++ or +++ on urine dipstick test. DM, diabetes mellitus. **$p < 0.01$, ***$p < 0.001$ vs. †reference category, proportion of proteinuria positive at baseline.*

tained increase in the mean serum creatinine levels was observed in the patients, regardless of their baseline renal function, although the number of the patients decreased with time, especially after 48 months of treatment.

Discussion

The J-HEALTH study is a large scale observational study designed to evaluate the efficacy and safety of losartan-based antihypertensive therapy. A large number of Japanese hypertensive patients (>30,000 subjects) were enrolled in propor-



≥7.0, drugs	854	533	542	465	417	265	62
≥7.0, no drugs	1,310	711	729	581	514	317	95
<7.0	10,524	5,386	6,015	5,107	4,613	2,941	633

Fig. 5. *Time course of the mean serum uric acid levels in patients who had a baseline uric acid level of 7 mg/dL or higher (with and without uric acid–lowering drugs). UA, uric acid.*

Table 3. Summary of Major Drug-Related Adverse Experiences (n=29,850)

	n
Total drug-related adverse experiences*	1,081
Major drug-related adverse experiences	
Dizziness	90
Hepatic function abnormal	61
Headache	48
Anemia	46
Cough	46
Blood pressure decreased	35
Blood creatinine phosphokinase increased	34

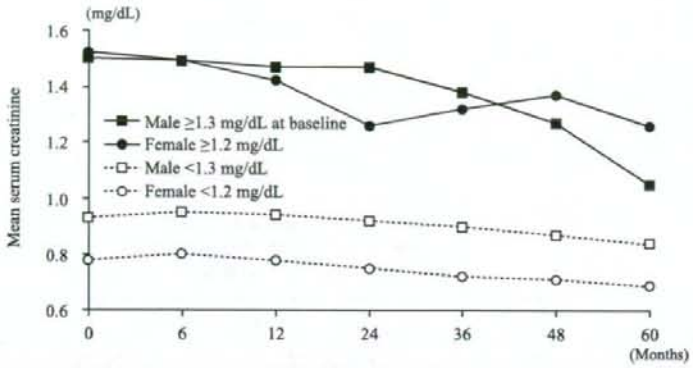
*Determined by the investigator to be possibly, probably or defined drug-related.

tion to the population of each prefecture. There was a broad range of ages and severities of hypertension among the enrolled patients, and thus the data are reasonably representative of the current status of antihypertensive therapy in daily clinical practice in Japan.

In the present study, long-term losartan-based antihypertensive therapy was shown to be effective for controlling the BP and well tolerated in Japanese hypertensive patients in routine clinical practice. The patients were followed for a mean of 3.0 years (5 years at maximum). The mean BP during the entire follow-up period was 141.6/82.0 mmHg. Nearly half of the patients had their BP controlled (<140/90 mmHg). Sixty percent of the patients were treated with losartan monotherapy. Long-term use of losartan in daily clinical practice was shown to have beneficial effects on both uric acid and proteinuria.

Proteinuria and albuminuria have been well established as prognostic risk factors for cardiovascular and renal outcomes in both non-hypertensive and hypertensive patients (*21–25*). An antiproteinuric effect of losartan, which was independent of its BP-lowering effect, was demonstrated in long-term trials (*13, 26*). However, few data are available concerning the antiproteinuric effect of losartan when used in daily clinical practice. In our study, losartan was shown to prevent acceleration of proteinuria both in DM and non-DM patients when used in daily clinical practice, although the detailed underlying mechanism remains uncertain because of the study design. Other studies have demonstrated a transient rise in the serum creatinine level soon after initiation of an ARB or ACEI in association with a persistent renal protective effect, particularly in patients with renal insufficiency (*27, 28*). However, a significant transient increase in serum creatinine was not observed in our study.

High serum uric acid is an independent risk factor for cardiovascular events (*29–31*). Losartan has been reported to decrease the serum uric acid level in normal volunteers (*32*) and in hypertensive patients (*33*). Losartan inhibits the uric acid transporter (URAT1) and thus decreases the serum uric acid level, while other ARBs only inhibit URAT1 weakly (*34, 35*). Hoieggen *et al.* (*36*) also indicated that decreased serum uric acid due to losartan was related with the prevention of cardiovascular events. In the present study, losartan was confirmed to decrease the serum uric acid level during the treatment period. Notably, the changes in uric acid were similar in patients with and without concomitant uric acid–lowering drugs, indicating that the serum uric acid level of patients treated with uric acid–lowering drugs was further decreased by losartan therapy. The reduction of uric acid by losartan observed in the present study is also favorable for preventing



n							
Male <1.3 mg/dL	5,748	3,075	3,303	2,812	2,571	1,553	357
Female <1.2 mg/dL	8,124	4,307	4,752	4,089	3,734	2,402	554
Male ≥1.3 mg/dL	437	247	265	220	171	108	19
Female ≥1.2 mg/dL	215	140	129	99	94	48	9

Fig. 6. *Changes in serum creatinine levels by sex and the baseline renal function.*

cardiovascular events in addition to its BP-lowering effect in clinical practice.

The present study demonstrated that losartan was well tolerated in clinical practice. Increases in serum creatinine and hyperkalemia are always a concern when using an ARB or ACEI. A number of studies have reported the necessity of carefully monitoring renal function, such as by measuring creatinine or electrolyte levels, and especially in patients with renal insufficiency (*27*, *37–39*). In the present study, the incidence of renal dysfunction and hyperkalemia associated with losartan therapy were relatively rare. In addition, no sustained increase in mean serum creatinine levels was observed regardless of the patient's renal function. Regular monitoring of renal function is, however, still required when ARBs or ACEIs are administered.

Other studies conducted in Japan and Western countries have reported BP control in less than 50% of the treated hypertensives (*6–11*). Similarly, BP was maintained at less than 140/90 mmHg in nearly half of the patients in the present study. Patients with severe hypertension were less sufficiently controlled than those with mild-to-moderate hypertension. Therefore, we should treat hypertensive patients more strictly to prevent cardiovascular events and increase the rate of BP control, especially in severe hypertensive patients.

This study was not a randomized controlled trial but a prospective observational study supported by general practitioners in clinical practice. Although a large-scale observational study can provide valuable information about the treatment in ordinary clinical practice that is not available in conventional controlled clinical trials, it has some limitations.

First, a long-term observational study based on daily clinical practice permits a considerable number of patients to drop out during the follow-up period. Those who dropped out within the first 3 months of this study, during which period the use of other hypertensive drugs was prohibited, may have had insufficient BP reduction. This kind of bias may influence the rate of losartan monotherapy as well. Therefore, it should be taken into consideration that the rate of losartan monotherapy and the rate of good BP control could have been evaluated only in patients who were followed up for the long-term. However, our data may indicate that good adherence to therapy leads to good BP control in a daily practice setting. Second, in contrast to clinical trials, the post-marketing surveialnce can not regulate items and timing of laboratory examinations. This means that we may have underestimated the incidence of ADRs, including laboratory examination abnormalities, for the whole study period. Despite such limitations, the results of the J-HEALTH study are still valuable as data reflecting hypertensive treatment performed in daily clinical practice in Japan.

In conclusion, long-term losartan-based antihypertensive therapy was effective and well tolerated in a daily clinical practice setting. The BP was maintained at less than 140/90 mmHg with losartan-based antihypertensive treatment in nearly half of the patients over the study period. With losartan monotherapy, the mean BP decreased from 163.7/93.9 mmHg to 135.9/78.8 mmHg. Antiproteinuric and uric acid–lowering effects were also confirmed. However, the BP control rate was still inadequate for treated Japanese hypertensive patients in daily clinical practice, as reported elsewhere. Therefore, stricter treatments, such as multiple antihypertensive treatments, will be needed to improve the BP control rate in hypertensive patients, and especially for those with severe hypertension.

Based on the results of the J-HEALTH, losartan is favorably recommended as an initial therapy for Japanese patients with hypertension, mainly due to its BP-lowering effects and long-term tolerability. However, it is also recommended that multiple agents be considered in order to improve the BP control of Japanese hypertensives, especially in those with severe hypertension.

Acknowledgements

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Appendix

J-HEALTH Committees

Monitoring Committee: Takenori Yamaguchi (Chair), Tanenao Eto, Toshiharu Furukawa, and Katsumi Yoshida.
Event Assessment Committee: Hiroaki Naritomi (Chair), Yoichiro Hashimoto, Uichi Ikeda, Mitsuaki Isobe, Toshio Kushiro, Ken Nagata, Kazuyuki Shimada, and Takemori Yamawaki.
Safety Assessment Committee: Kendo Kiyosawa (Chair), Hiroshi Hirose, Sadayoshi Ito, Akinori Kasahara, Hiroshi Kawabe, Genjiro Kimura, Hirofumi Makino, Mitsuhiko Moriyama, Ikuo Saito, Hiromichi Suzuki, and Eiji Tanaka.
Medical Expert Advisory and Publication Committee: Hiroaki Naritomi (Chair), Toshiro Fujita, Sadayoshi Ito, Toshio Ogihara, Kazuyuki Shimada, Kazuaki Shimamoto, Heizo Tanaka, and Nobuo Yoshiike.
Administrative Office: Post-Marketing Surveillance Department, Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan)

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Original Article

Impact of Blood Pressure Control on Cardiovascular Events in 26,512 Japanese Hypertensive Patients: The Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH) Study, a Prospective Nationwide Observational Study

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The Japan Hypertension Evaluation with Angiotensin II Antagonist Losartan Therapy (J-HEALTH) study was performed to investigate the relationship between blood pressure (BP) and development of stroke or myocardial infarction (MI) in Japanese hypertensive patients. A total of 26,512 hypertensive patients (mean age: 62.2 years, 43.9% men) were analyzed. All patients received open-labelled losartan for a maximum of 5 years. Endpoints were stroke, MI including sudden cardiac death, and all cardiovascular (CV) events (stroke and MI). The mean observation period was 3.0 years. The mean baseline systolic/diastolic BP was 165.8/94.8 mmHg and decreased to 141.6/82.0 mmHg during treatment. The incidences of stroke, MI, and total CV events were 3.90, 1.02, and 4.92 per 1,000 patient-years, respectively. Aging, diabetes, a history of CV disease, and smoking were independent risk factors for CV events. The risk of all CV events was positively related to BP level during treatment, and increased significantly when the BP exceeded 140/90 mmHg. Age was a strong contributor to CV events, but about a half of the very elderly patients (≥85 years, n=692) had a BP below 140/90 mmHg during treatment and significantly fewer events occurred in these patients than in those with a BP of 140/90 mmHg or higher. These results suggest that BP should be below 140/90 mmHg in Japanese patients with hypertension for reducing the risk of CV events. BP was controlled below 140/90 mmHg in a half of the very elderly hypertensive patients in this study, and these patients also had a lower incidence of CV events. (*Hypertens Res* 2008; 31: 469–478)

***Key Words*: losartan, hypertension, blood pressure, cardiovascular disease, observational study**

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