Original Article

Associations of Hypertension and Its Complications with Variations in the Xanthine Dehydrogenase Gene

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Hyperuricemia and oxidative stress participate in the pathophysiology of hypertension and its complications. Xanthine dehydrogenase (XDH) produces urate and, in its oxidase isoform, reactive oxygen species. Here we have studied whether or not the genetic variations in *XDH* could be implicated in hypertension and its complications. By sequencing the promoter region and all exons of *XDH* in 48 subjects, we identified three missense mutations (G172R, A932T, N1109T) in a heterozygous state in addition to 34 variations, including 15 common single nucleotide polymorphisms (SNPs). The three missense mutations and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were genotyped in 953 hypertensive Japanese subjects and in 1,818 subjects from a general Japanese population. Four hypertensive patients with rare missense mutations (G172R or N1109T) in homozygous form had severe hypertension. Multivariate logistic regression analysis showed a significant association of three SNPs with hypertension in men: 47686C>T (exon 22, odds ratio [OR]: 1.52, $p=0.047$) and 69901A>C (intron 31, OR: 3.14, $p=0.039$) in the recessive model, and 67873A>C (N1109T) (exon 31, OR: 1.84, $p=0.018$) in the dominant model. After full adjustment for all confounding factors, only one polymorphism (69901A>C) was found to be associated with carotid atherosclerosis in the dominant model ($p=0.028$). Multiple logistic regression analysis showed that one SNP (66292C>G) was significantly associated with chronic kidney disease (CKD: estimated creatinine clearance <60 mL/min) in the recessive model ($p=0.0006$). Our results suggest that genetic variations in *XDH* contribute partly to hypertension and its complications, including atherosclerosis and CKD. (*Hypertens Res* 2008; 31: 931–940)

***Key Words*: xanthine dehydrogenase gene, missense mutation, single nucleotide polymorphism, hypertension, atherosclerosis, chronic kidney disease**

Introduction

Hypertension is one of the most common and important risk factors for stroke, coronary heart diseases (CHD), and chronic kidney disease (CKD). The major contribution to the etiology of this disorder is proposed to come from the combined effects of genes that modify the response of blood pressure to environmental stresses, including diet and environmental susceptibility genes (*1*). This multifactoral trait increases the

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affected individuals' risks of stroke, CHD, and CKD, and is one of the leading causes of morbidity and mortality in adults (*2*). The population-wide application of preventative measures and analyses of candidate genes to predict modifiable risks, in addition to developing new treatments for hypertension and its complications, are thus very worthwhile (*3*, *4*).

Xanthine oxidoreductase (XOR), best known as the rate-limiting enzyme of the purine degradation pathway, converts hypoxanthine to xanthine and xanthine to uric acid (UA) *via* its two interconvertible isoforms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO); in its oxidase isoform, XOR produces reactive oxygen species (ROS) (*5*). Hyperuricemia is commonly seen in hypertensive patients (*6*). Several large epidemiologic studies have identified an association between increased serum UA levels and cardiovascular risk in the general population (*7–10*), among patients with hypertension (*11*, *12*), between increased serum UA levels and renal failure in the general population (*13*, *14*), and among patients with hypertension (*15*). ROS plays critical roles in the pathogenesis of a number of cardiovascular diseases, including atherosclerosis, hypertension, diabetes mellitus, and heart failure (*16*). They have also been implicated as important mediators of the progression of renal injury in different animal models of hypertension (*17–20*). The conversion of XDH to XO and increased XO activity have been reported in some pathological conditions, including hypertension (*21–23*) and atherosclerosis (*24*). Importantly, treatments with XO inhibitors were recently reported to normalize ROS levels in microvessels from rats fed a high-salt diet (*25*) and to promote endothelial-dependent relaxation in arteries from SHR (*26*). These findings suggest that XO is an important source of ROS in patients with hypertension. Therefore, the XDH gene is suspected to be associated with constitutional susceptibility to hypertension and its complications.

So far, there are no reports about the relation between variations in human XDH gene (*XDH)* and hypertension and its complications. Human *XDH*, located on chromosome 2 at p23.1 (*27*), consists of 36 exons that encode a 1,333-amino acid protein. The aim of the present study was to screen for possible genetic variations in the promoter and all exon regions of *XDH* in 48 patients with hypertension. By genotyping the missense mutations and common single nucleotide polymorphisms (SNPs) in a large hypertensive population and the general population, we further assessed the role of these genetic variations in hypertension and clarified the contributions of common SNPs to hypertension and its complications, including atherosclerosis and CKD.

Methods

Hypertensive Population

The characteristics of the hypertensive population analyzed in the present study are summarized in Table 1. A total of 953 hypertensive subjects (522 men and 431 woman, average age: 65.1±10.5 years old) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center, as reported previously (*28*, *29*). Briefly, 92% of study subjects (880 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension. The hypertension criteria were a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg, or the use of antihypertensive agents. Hyperlipidemia was defined by a total cholesterol level ≥220 mg/dL or the taking of antihyperlipidemia medication. Diabetes mellitus was defined by a fasting plasma glucose level ≥126 mg/dL, nonfasting plasma glucose ≥200 mg/dL, HbA1c ≥6.5%, or the taking of antidiabetic medication. Smoking was defined as current smoking. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol levels were measured as previously described (*30*). Study subjects underwent routine laboratory tests, including examinations of electrolytes, renal function, blood glucose, HbA1c, plasma renin activity (PRA), and plasma aldosterone concentration (PAC) by radioimmunoassay.

Table 1. Characteristics of Patients with Hypertension

Number	953
Age, years	65.1±10.5
Gender (male/female)	522/431
Body mass index, kg/m^2	24.2±3.3
Systolic blood pressure, mmHg	145.5±19.2
Diastolic blood pressure, mmHg	84.8±13.4
Essential hypertension	880
Secondary hypertension	72
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Ischemic heart disease	102
Stroke	145

Values are expressed as mean±SD.

Evaluation of Atherosclerosis and CKD in the Hypertensive Population

Carotid ultrasonography was used to measure mean intima-medial thickness (IMT) using ultrasonography (SSA-390A; Toshiba, Tokyo, Japan) as previously described (*31*). IMT above 1.0 mm in either the left or right common carotid artery defined with the presence of an atherosclerotic lesion. We also assessed arterial stiffness using brachial-ankle–pulse wave velocity (ba-PWV) measured by form ABI (Omron Health Care, Kyoto, Japan) as described in a previous report (*32*). Estimated creatinine clearance (Ccr) determined with the Cockcroft-Gault formula (*33*) was used for the evaluation of CKD. We defined CKD as Ccr <60 mL/min according to

the guidelines of the National Kidney Foundation (*34*).

Screening of Genetic Variations in *XDH*

We sequenced the promoter region and all exons of *XDH* in 48 randomly chosen patients with hypertension. Blood samples were obtained from all hypertensive patients, and genomic DNA was isolated from peripheral blood leukocytes using an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan) (*35*). All exons with their flanking sequences and 1 kb of the promoter region were directly sequenced with an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA) as described previously (*36*) using 38 sets of primers. Information on the primers and PCR conditions is available on request. The sequences obtained were examined for the presence of variations using Sequencher software (Gene Codes, Ann Arbor, USA), followed by visual inspection.

Genotyping of Missense Mutations and Common SNPs in Hypertensive Subjects and the General Population

Three missense mutations and eight common SNPs with a minor allelic frequency of greater than 10% were genotyped in 953 hypertensive patients and in 1,818 subjects (835 men and 983 women) participating in the Suita Study. We chose just one common SNP for genotyping among SNPs that show strong linkage disequilibrium (LD) with an r^2 above 0.5. The sample selection and study design of the Suita Study were described previously (*37*). Briefly, the subjects visited the National Cardiovascular Center every 2 years for general health checkups. In addition to a routine blood examination that included lipid profiles, glucose levels, blood pressure, and anthropometric measurements, a physician or nurse administered questionnaires covering the subject's personal history of cardiovascular diseases, including angina pectoris, myocardial infarction, and/or stroke. Nondrinkers were those who had had no drink in the past month. Current drinkers were those who were drinking at least 30 mL of ethanol per day, and past drinkers were those who used to drink that much in the past but not in the present. Subjects were regarded as having a disease if they were currently taking antihypertensive, antihyperlipidemic, or antidiabetic medication. Seven-hundred and ninety-five subjects were diagnosed as having hypertension. All of the participants were Japanese. The characteristics of the subjects in the Suita Study are summarized in Table 2.

The TaqMan-PCR (Roche Molecular Systems, Pleasanton, USA) method was used for genotyping (*35*). The sequences of PCR primers and probes for the TaqMan-PCR method are available on request. All of the participants in the genetic analysis in the present study gave their written informed consent. All clinical data, as well as the results of sequencing and genotyping, were anonymous. The study protocol was approved by the Ethics Review Committee of the National Cardiovascular Center, Japan.

Table 2. Baseline Characteristics of Subjects in Suita Study

	Women (n=983)	Men (n=835)
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*

*$p<0.05$ *vs*. women or men. HDL, high-density lipoprotein.

Statistical Analysis

Values are expressed as means±SD. The distribution of patient characteristics between men and women in the general population and in the hypertensive population was analyzed with Student's t-test or χ^2 analysis.

The associations of genetic models with blood pressures were tested with a logistic regression analysis considering potential confounding risk variables, including age, body mass index (BMI), present illness (hyperlipidemia and diabetes mellitus), lifestyle (current smoking and drinking), and antihypertensive medication by sex. For multivariate risk predictors, the adjusted odds ratios (ORs) were given with 95% confidence intervals. The relationship between genotype and risk of hypertension was expressed in terms of ORs adjusted for possible confounding factors, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (current smoking and drinking) by sex. The relationship between genotype and risk of atherosclerosis or CKD in hypertensive patients was expressed in terms of ORs adjusted for possible confounding factors, including age, sex, BMI, LDL cholesterol, HbA1c, SBP, and DBP for atherosclerosis; and age, BMI, SBP, DBP, and diabetes mellitus for CKD. For each pair of SNPs, the pairwise LD parameters, D' and r^2, were calculated on the basis of the genotyping data using SNPAlyze version 3.1 Pro (Dynacom, Mobara, Japan). All analyses were performed with SAS statistical software release 8.2 (SAS Institute, Cary, USA) or JMP statistical software version 4.0 (SAS Institute). Statistical significance was established at $p<0.05$.

Table 3. Sequence Variations in the Promoter Region and All Exons in *XDH* Identified in 48 Japanese Patients with Hypertension and/or Renal Failure

SNP (allele 1>allele 2)	LD	Amino acid substitution	Region	Allele frequency		Flanking sequence	Genotyping
				Allele 1	Allele 2		
8787C>T	a		intron2	0.979	0.021	gagtgggagtga[c/t]ggagaaggggggg	
11451G>T	a		intron2	0.968	0.032	gcccacagctct[g/t]cccaggcatttc	
11488C>G	b, c		intron2	0.862	0.138	cagactcctctc[c/g]ctgagttcattc	done
26245G>A			intron6	0.958	0.042	ggcaggcaggat[g/a]cccctgctgttg	
26390G>A	b, c, d	Gly172Arg	exon7	0.906	0.094	ggatgctgtgga[g/a]gagatgggaata	done
26479T>A			intron7	0.958	0.042	gcctgggggtaa[t/a]ctgagacttaga	
26504C>T	e		intron7	0.625	0.375	ggagtcagtgca[c/t]gagctccatgtc	
26832G>A	b, c, d	Glu209Glu	exon8	0.915	0.085	tccaacccagga[g/a]cccatttttccc	
28272G>A			intron9	0.989	0.011	gccagggaggct[g/a]ccctggggctgc	
30863C>T	c, d	Val279Val	exon10	0.936	0.064	tcctatgattgt[c/t]tgcccagcctgg	
31503G>T			intron10	0.989	0.011	gtgattccgaac[g/t]tgcgttcccagg	
34636G>A			intron13	0.917	0.083	tttctccccatg[g/a]ggggttcccagc	
37387A>G	f, g		intron14	0.181	0.819	tttgcagcccct[a/g]cagagcaaggtg	done
39048A>G	h		intron15	0.604	0.396	ccctgggcacac[a/g]gctctacacaaa	
44408A>G	i		intron19	0.875	0.125	tggaaaggttat[a/g]catttgcatgga	done
44426G>A			intron19	0.990	0.010	gcatggattatg[g/a]ccatcatccagt	
46476T>C			intron20	0.979	0.021	acttcaagtctg[t/c]atgtgaagcata	
46748G>C	h		intron21	0.660	0.340	ggggtggccctg[g/c]tttgcaaattaa	
46774G>A	e		intron21	0.638	0.362	ttcaagagatat[g/a]cattgaaccctg	done
47686C>T	h	Ile737Ile	exon22	0.670	0.330	ggagatatacat[c/t]ggtggccaagag	done
47804G>A			intron22	0.989	0.011	acccaggtagat[g/a]ccttttgggtca	
47879A>G	e		intron22	0.638	0.362	catgtgggaaat[a/g]ggaagagggaga	
49096G>A	i		intron23	0.875	0.125	gaaggctcacag[g/a]cttctaacactg	
49245A>T	f, g, j		intron24	0.125	0.875	tggggcgggatg[a/t]gccattttgtga	done
50298C>T	g, j		intron24	0.146	0.854	accttttttca[c/t]gggatgatgtgg	
50391T>C			intron24	0.917	0.083	aaacgggactta[t/c]gataaatccctc	
64606G>A		Ala932Thr	exon26	0.990	0.010	atgagtgaagtt[g/a]cagtgacctgtg	done
65050–65051insC	k		intron27	0.135	0.865	tctgctgacccc[-/c]atataggaagct	
65747T>C	k	Phe1010Phe	exon28	0.135	0.865	tggaataagctt[t/c]acagttccttt	
66292C>G	k		intron28	0.135	0.865	tctggcatcctt[c/g]tctttccctagg	done
67157A>G	k		intron30	0.128	0.872	tgtaaggagccc[a/g]tgggatcccgca	
67873A>C		Asn1109Thr	exon31	0.969	0.031	acaagaagaaga[a/c]tcccagtggctc	done
69901A>C			intron31	0.795	0.205	aaacctcacttc[a/c]cctgcctgatgg	done
73380C>T			intron34	0.938	0.063	agacttggccac[c/t]gatgcaccccat	
74894G>A	l		intron34	0.968	0.032	acattccaggcc[g/a]cgctgcagttgg	
75121G>A		Glu1239Glu	exon35	0.989	0.011	catccccattga[g/a]ttcagggtgtcc	
78750G>C	l	3′UTR	exon37	0.969	0.031	tgctgcctttgg[g/c]cttccatggagc	

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*Hum Mut* 1998; **11**: 1–3). The nucleotide sequence (GenBank Accession ID: NT_022184.14) was used as a reference sequence. The apparent linkage disequilibrium (LD), defined by r^2 more than 0.5, was indicated by a in the LD column. *XDH*, xanthine dehydrogenase gene; SNP, single nucleotide polymorphism; UTR, untranslated region.

Results

Identification of Genetic Variations in *XDH*

As shown in Table 3, we identified 3 missense mutations in *XDH*. Nine of the 48 individuals had a G-to-A substitution at nucleotide 26390 in exon 7, leading to an amino acid substitution from Gly to Arg at position 172 (G172R). One individual had a G-to-A substitution at nucleotide 64606 in exon 26, leading to a change from Ala to Thr at position 932 (A932T). Three of the 48 individuals had an A-to-C substitution at nucleotide 67873 in exon 31, leading to the substitution of Asn with Thr at position 1109 (N1109T). These missense

Table 4. Clinical Profiles of Four Hypertensive Patients with Two Rare Missense Mutations in Homozygous Form in *XDH*

	Case			
	1	2	3	4
SNP	26390G>A	67873A>C	67873A>C	67873A>C
(Amino acid change)	(G172R)	(N1109T)	(N1109T)	(N1109T)
Age, years old	79	70	74	67
Sex	male	male	female	female
Body mass index, kg/m^2	21.01	23.43	23.68	21.91
Diagnosis	EHT, HL	EHT, HL, HU	EHT	EHT, HL
Hypertension duration, years	5	23	22	2
Hypertension family history	mother	unknown	mother, brother	unknown
CV complications	no	no	stroke	no
Systolic blood pressure, mmHg	144	138	168	170
Diastolic blood pressure, mmHg	70	90	100	96
Medication	ARB, BB, DU	CCB, ARB, HUD	CCB, ACEI	CCB
Na^+, mEq/L	141	141	139	139
K^+, mEq/L	4.8	3.8	3.9	4.1
Cl^-, mEq/L	108	105	104	108
Creatinine, mg/dL	1	0.8	0.5	0.6
Ccr, mL/min	50.8	73.2	84.2	68.9
UA, mg/dL	5.1	4.2	4.8	4.8
Overt proteinuria	yes	no	no	no
PRA, ng/mL/h	0.1	1.3	1.3	0.4
PAC, ng/dL	9.7	35.4	19.8	4.6
FBS, mg/dL	96	115	82	92
HbA1c, %	5	5.9	4.9	5.7
ba-PWV, cm/s	2,189	no data	1,710	1,734
Average IMT, mm	1.0	no data	0.7	1.0

XDH, xanthine dehydrogenase gene; EHT, essential hypertension; HL, hyperlipidemia; HU, hyperuricemia; CV, cardiovascular; ARB, angiotension II receptor blocker; BB, β-adrenergic blocker; DU, diuretics; CCB, calcium channel blocker; HUD, antihyperuricemic drug; ACEI, angiotensin II converting enzyme inhibitor; SNP, single nucleotide polymorphism; Ccr, creatinine clearance; UA, uric acid; PRA, plasma renin activity; PAC, plasma aldosterone conc.; FBS, fasting blood sugar; ba-PWV, brachial-ankle pulse wave velocity; IMT, intima-media thickness. Normal values: body mass index, between >18.5 and <25.0 kg/m^2; SBP, <140 mmHg; DBP, <90 mmHg; 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; Ccr, <60 mL/min; UA, 3.6–7.0 mg/dL; PRA, 0.2 to 2.7 ng/mL/h; PAC, 2 to 13 ng/dL; FBS, <126 mg/dL; HbA1c, <6.5%; ba-PWV, <1,400 cm/s; average IMT, <1.0 mm.

mutations were all found in heterozygous form. In addition, we identified five synonymous variations (26382G>A in exon 8, 80868C>T in exon 10, 47686C>T in exon 22, 65747T>C in exon 28, and 75121G>A in exon 35) encoded for E209 (minor allelic frequency, 0.085), for V279 (0.064), for I787 (0.33), for F1010 (0.135), and for E1239 (0.011), respectively. Twenty-nine additional variations in the introns and a 3′-untranslated region were also detected. Among all the variations, there were 15 common polymorphisms with a minor allelic frequency of over 0.1 (11488C>G, 26504C>T, 37387A>G, 39048A>G, 44408A>G, 46748G>C, 46774G>A, 47879A>G, 49096G>A, 49245A>T, 50298C>T, 65050–65051 ins C, 66292C>G, 67157A>G, and 69901A>C).

Characteristics of Hypertensive Subjects with Missense Mutations in Homozygous Form

After genotyping the three missense mutations in 953 patients with hypertension, including secondary hypertension, we found one subject with G172R and three with N1109T in homozygous form. The characteristics of these four patients with rare missense mutations in the homozygous form are shown in Table 4. All four had resistant hypertension despite antihypertensive drug therapy. One of the patients with N1109T (patient 2) had hyperuricemia and was taking allopurinol. The patient with G172R (patient 1) and the two others with N1109T (patients 2 and 4) had hyperlipidemia. Patients 1 and 4 had low PRA levels (0.1 and 0.4 ng/mL/h, respectively) and high average IMT values (1.0 mm for both). Patient 1 had low Ccr (50.8 mL/min) and overt proteinurea. Three of the four patients had high ba-PWV values: no data

Table 5. Comparison of Hypertension Prevalence by Genotypes of Three Polymorphisms of *XDH* in a Japanese General Population by Sex

SNP	Genotype group	Women		Men	
		Odds ratio (95% CI)	*p**	Odds ratio (95% CI)	*p**
47686C>T	CC	1		1	
[CC/CT/TT=815/819/244]	CT+TT	0.90 (0.68–1.20)	0.469	1.10 (0.83–1.46)	0.521
	CC+CT	1		1	
	TT	1.04 (0.67–1.62)	0.861	1.52 (1.01–2.29)	0.047
67873A>C	AA	1		1	
[AA/AC/CC=1,720/154/5]	AC+CC	0.97 (0.58–1.61)	0.906	1.84 (1.11–3.06)	0.018
	AA+AC	1		1	
	CC	0.62 (0.05–7.33)	0.704	3.98 (0.20–80.72)	0.368
69901A>C	AA	1		1	
[AA/AC/CC=1,372/463/42]	AC+CC	1.30 (0.95–1.78)	0.099	1.11 (0.80–1.53)	0.530
	AA+AC	1		1	
	CC	0.96 (0.40–2.35)	0.936	3.14 (1.06–9.27)	0.039

*Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) for hypertension. *XDH*, xanthine dehydrogenase gene; SNP, single nucleotide polymorphism; CI, confidence interval; [ ], sample numbers of three kinds of genotypes.

on ba-PWV were available for patient 2.

Associations of 11 Variations with Hypertension in the General Population

Three missense mutations (G172R, A932T, and N1109T) and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were used for the association studies in the case-control setting for men and woman separately. Adjusted for age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking), a logistic regression analysis of the case-control study showed that three of the eight SNPs were significantly associated with hypertension in men: TT *vs*. CC+CT for 47686C>T (exon 22, OR: 1.52, $p=0.045$) and CC *vs*. AC+AA for 69901A>C (intron 31, OR: 3.14, $p=0.039$) in the recessive model, and AC+CC *vs*. AA for 67873A>C (N1109T) (exon 31, OR: 1.84, $p=0.018$) in the dominant model (Table 5).

SBP was 2.44 mmHg higher in women with the AC+CC genotype of the positively associated SNP 69901A>C in *XDH* than in women with the AA genotype ($p=0.037$). Although there was no significant difference in SBP or DBP between the AC+CC and AA genotypes of 69901A>C in men, DBP was 4.18 mmHg higher in men with the CC genotype of 69901A>C than in men with the AA+AC genotype ($p=0.088$). DBP was 2.75 mmHg higher in men with the AC+CC genotype of the positively associated SNP 67873A>C than in men with the AA genotype ($p=0.021$) (Table 6).

Regarding the three missense mutations, there were 6 subjects with a homozygote allele in *XDH* G172R and 5 subjects with one in N1109T, but no subjects with one in A932T. The subjects with a homozygote allele in G172 and N1109T did not have any specific clinical characteristics (data not shown).

Association of 11 Variations with Carotid Atherosclerosis in Hypertensive Subjects

Three missense mutations (G172R, A932T, and N1109T) and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were tested for associations with carotid atherosclerosis in patients with essential hypertension. After the full adjustment for all confounding factors (age, BMI, SBP, DBP, current smoking status, alcohol consumption, and presence of diabetes mellitus and dyslipidemia), only one polymorphism (69901A>C) was found to be independently associated with carotid atherosclerosis in the dominant model ($\chi^2=4.82$, $p=0.028$). Other factors—age ($\chi^2=67.70$, $p<0.001$), SBP ($\chi^2=15.11$, $p<0.001$), and DBP ($\chi^2=4.28$, $p=0.039$)—were related to carotid atherosclerosis. We compared IMT and ba-PWV values among the alleles in *XDH* 69901A>C. There were no significant differences between alleles in either IMT or ba-PWV. However, ba-PWV values tended to differ significantly (AA: 1,794, AC: 1,825, CC: 2,024 cm/s, $p=0.075$) in *XDH* 69901A>C. These findings may indicate that hypertensive patients with the CC of *XDH* 69901A>C are more susceptible to atherosclerosis than those with the A allele.

Associations of 11 Variations with Chronic Kidney Disease in Hypertensive Subjects

We divided the essential hypertensive patients into two groups using a cutoff estimate of Ccr 60 mL/min. The CKD group (Ccr <60 mL/min) showed significantly higher age

Table 6. Multivariate-Adjusted Blood Pressure Levels on Genotypes of Three SNPs of *XDH* by Sex

SNP	Genotype group	Women				Men			
		SBP, mmHg	*p**	DBP, mmHg	*p**	SBP, mmHg	*p**	DBP, mmHg	*p**
47686C>T	CC	127.60±0.79	0.752	79.69±0.45	0.630	131.73±0.89	0.976	79.58±0.51	0.707
	CT+TT	127.93±0.69		76.40±0.39		131.76±0.78		79.83±0.45	
	CC+CT	127.84±0.56	0.782	76.69±0.31	0.138	131.68±0.63	0.779	79.60±0.36	0.393
	TT	127.40±1.50		75.34±0.85		132.16±1.57		80.44±0.90	
67873A>C	AA	127.87±0.54	0.538	76.48±0.31	0.546	131.50±0.61	0.178	79.48±0.35	0.021
	AC+CC	126.70±1.83		77.13±1.04		134.30±1.99		82.23±1.14	
	AA+AC	127.82±0.52	0.108	76.55±0.30	0.375	131.77±0.59	0.441	79.73±0.34	0.425
	CC	112.54±9.48		71.76±5.38		122.35±12.19		74.14±7.00	
69901A>C	AA	127.10±0.61	0.037	76.33±0.35	0.290	131.23±0.68	0.136	79.37±0.39	0.079
	AC+CC	129.54±0.99		77.03±0.56		133.23±1.15		80.72±0.66	
	AA+AC	127.87±0.53	0.253	76.58±0.30	0.289	131.72±0.59	0.689	79.64±0.34	0.088
	CC	124.04±3.30		74.57±1.87		133.43±4.23		83.82±2.42	

Data are mean±SD. *Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) for hypertension. SNP, single nucleotide polymorphism; *XDH*, xanthine dehydrogenase gene; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 7. Comparison of Chronic Kidney Disease Prevalence by Genotypes of 66292 C>G in *XDH* in Hypertensives by Sex

Genotype group	Men [CC/CG/GG=11/123/363]		Women [CC/CG/GG=11/83/315]	
	Odds ratio (95% CI)	*p**	Odds ratio (95% CI)	*p**
CG+GG	1	0.5545	1	0.1093
CC	1.51 (0.369–5.924)		3.48 (0.725–16.412)	
GG	1	0.0006	1	0.5617
CC+CG	2.36 (1.348–3.850)		1.18 (0.663–2.084)	

*Multiple logistic regression analysis, adjusted for age, body mass index, diabetes mellitus, systolic blood pressure, and diastolic blood pressure. *XDH*, xanthine dehydrogenase gene; [ ], sample numbers of three kinds of genotypes; CI, confidence interval.

($p<0.001$), lower BMI ($p<0.001$), and lower DBP ($p<0.001$) than the non-CKD group.

As shown in Table 7, after adjustment for age, BMI, SBP, DBP, and the number of patients that suffer from diabetes mellitus, logistic regression analysis showed that one SNP (66292C>G) of the 11 variations was strongly associated with chronic kidney disease in the recessive model in men (OR=2.36, $p=0.0006$). This significant association was still positive after a Bonferroni correction ($p=0.0006<0.05/11$). However, there was no significant difference in Ccr value between GG and CC+CG in *XDH* 66292C>G in male hypertensive patients (GG: 84.73±39.14 *vs*. CC+CG: 80.32±73.26 mL/min, $p=0.384$).

Discussion

The present study is the first to examine the relationships between genetic variations in *XDH* and hypertension or its complications in human. After the screening for possible genetic variations in the promoter and all exon regions of *XDH* in 48 patients with hypertension, 11 variations, including 3 missense mutations and 8 common SNPs, were genotyped and used to assess the roles of these genetic changes in hypertension in a large population of hypertensive subjects and in a general population. The 4 hypertensive patients with a rare missense mutation (G172R or N1109T) in homozygous form had hypertension. More importantly, 67873A>C (N1109T) also showed a positive association with hypertension in men in a multivariable logistic analysis. In addition, DBP was 2.75 mmHg higher in men with the AC+CC genotype of 67873A>C than in men with the AA genotype ($p=0.021$). This indicates that 67873A>C may be a functional risk factor for hypertension in males. Another two SNPs, 47686C>T in the exon region and 69901A>C in the intron region, were also found to be significantly related to hypertension in men. Furthermore, SBP was 2.44 mmHg higher in women with the AC+CC genotype of 69901A>C than those with the AA genotype ($p=0.037$). Since a significant association was obtained in the multivariable analysis with adjustment for confounding risk factors, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (current smoking and drinking) by sex, these

three SNPs appear to be independent risk factors for hypertension. The C allele of 69901A>C was associated with greater susceptibility in male subjects. In females, there was a significant association between 69901A>C and blood pressure. Although there was no significant difference in SBP or DBP between the AC+CC and AA genotypes of 69901A>C in men, DBP was 4.18 mmHg higher in men with the CC genotype of 69901A>C than in men with the AA+AC genotype ($p=0.088$). Taking these findings together, we speculate that, among males, those with 67873A>C (N1109T) were most susceptible to hypertension.

This is also the first report to show a positive relationship between SNPs of *XDH* and CKD in hypertensive patients. It is well reported that age, sex, blood pressure, BMI, and diabetes mellitus are all factors in renal dysfunction (*38–41*). Our results also showed that age, DBP, and BMI differed significantly between hypertensive patients with Ccr <60 mL/min and those with Ccr ≥60 mL/min. But no significant difference in SBP or the number of diabetes mellitus patients was found with or without CKD in these hypertensive subjects. After adjustment for age, sex, BMI, SBP, DBP, and the number of patients having diabetes mellitus, the logistic regression analysis showed that only one SNP (66292C>G) was strongly associated with CKD in hypertensive patients. This indicates that 66292C>G may be an independent risk factor for CKD in hypertensive patients.

SNP 69901A>C was found to be significantly associated with carotid atherosclerosis in hypertensive patients in our study. Although we did not find a significant difference between genotypes in any of the various atherosclerotic variables, hypertensive patients with the A allele of 69901A>C tend to be more susceptible to atherosclerosis than those with the C allele.

How the SNPs of *XDH* influence the pathogenesis of hypertension and its complications, including atherosclerosis and CKD, remains unclear. Among the four SNPs that showed a positive association with hypertension or with atherosclerosis and CKD in hypertensive patients, 67873A>C and 47686C>T are in exon regions, and 69901A>C and 66292C>G are in intron regions. 67873A>C causes a missense mutation in exon 31, leading to an amino acid substitution from Asn to Thr at position 1109. But 47686C>T does not result in a change in amino acids. In addition, the three missense mutations, 26390G>A (G172R), 64606G>A (A932T), and 67873A>C (N1109T), occurred in highly conserved residues among different species, all resulting in a hydrophilic amino acid substitution, which may influence reactive centers of enzymes. The XDH protein consists of three functional subunit domains, each of which binds a different cofactor, from amino acids 1 to 165 for binding $2Fe_2S_2$, from 226 to 531 for binding flavin adenine dinucleotide, and from 590 to 1332 for binding molybdoptern (Mo-Co) (*5*). The missense mutation G172R is not in the predicted functional domain, but A932T and N1109T are in the domain for binding molybdoptern. A932T and N1109T are not in the domain for binding flavin adenine dinucleotide, which is thought to play a major role in the conversion of XDH to XO and which increases ROS production in some pathological conditions, including hypertension and atherosclerosis (*5*). However, it is important to note a recent report that XOR has both inorganic nitrate reductase and nitrite reductase activity at its Mo-Co site (*42*, *43*). This implies that an amino acid mutation at the Mo-Co site may influence nitric oxide production and modulate ROS production. Those four hypertensive patients with A932T and N1109T in the homozygous form all had high blood pressure, N1109T showed significant associations with hypertension and blood pressure, and the Mo-Co–binding site is the most conserved region of *XDH* among human, rat, and mouse (*44*). This strongly indicates that the mutations A932T and N1109T may be functional risk factors for hypertension. Further *in vivo* and *in vitro* studies are needed to clarify this point.

Both 69901A>C and 66292C>G SNPs are in intron regions, while 47686C>T is a synonymous variation and, as such, is probably not functional. These SNPs are considered preferable as genetic markers. Human *XDH* is located on chromosome 2 at p23.1. Recently, Angius *et al*. reported strong evidence that a 0.54-cM region of chromosome 2 (2p 26.5–27.1) harbors a locus-affecting risk of hypertension in an isolated Sardinian population (*45*). In addition, a number of regions of chromosome 2 (57–59, 86, 103, and 96–115 cM) have been found likely to harbor blood-pressure–modifying loci (*45–48*). More importantly, our group recently reported some hypertension-susceptibility genes at 2p24–p25 and a positive relationship between hypertension and SNPs of the Na^+/Ca^{2+} exchanger 1 gene, which is located at 2p22–p23, in a general Japanese population (*49*, *50*). Expanded genotyping and a detailed cross-study of candidate genes are necessary.

In summary, in human *XDH*, we found three SNPs, 47686C>T, 67873A>C, and 69901A>C, that are significantly associated with hypertension. Another SNP, 66292C>G, was significantly associated with CKD, and 69901A>C also showed a positive relation to carotid atherosclerosis in hypertensive patients. These SNPs may be independent risk factors for hypertension or CKD and carotid atherosclerosis in hypertensive patients. There was a limitation in this study owing to its cross-sectional design. Prospective studies investigating the relationships between these SNPs and the development of hypertension, CKD, and atherosclerosis over a long term are necessary. These gene polymorphisms in *XDH* may be useful for predicting and preventing hypertension and its complications in future individualized treatment.

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