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ORIGINAL ARTICLE

Association of candidate gene polymorphisms with chronic kidney disease in Japanese individuals with hypertension

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Although hypertension has been recognized as a risk factor for chronic kidney disease (CKD), the genetic factors for predisposition to CKD in individuals with hypertension remain largely unknown. The purpose of this study was to identify the genetic variants that confer susceptibility to CKD among individuals with hypertension. The study population comprised 3696 Japanese individuals with hypertension (2265 men, 1431 women), including 1257 individuals (789 men, 468 women) with CKD (estimated glomerular filtration rate (eGFR) $<60\,ml\,min^{-1}$ per $1.73\,m^2$) and 2439 controls (1476 men, 963 women; eGFR $\geqslant 60\,ml\,min^{-1}$ per $1.73\,m^2$). The genotypes for 30 polymorphisms of 26 candidate genes were determined. An initial screening of allele frequencies by the χ^2-test revealed that eight polymorphisms were significantly (false discovery rate <0.05) associated with the prevalence of CKD in hypertensive individuals. Subsequent multivariable logistic regression analysis with adjustment for covariates as well as a stepwise forward selection procedure revealed that the T→C (Val591Ala) polymorphism of *APOB* (rs679899), the −681C→G polymorphism of *PPARG* (rs10865710), the T→C (Cys1367Arg) polymorphism of *WRN* (rs1346044), the −850C→T polymorphism of *TNF* (rs1799724), the −219G→T polymorphism of *APOE* (rs405509), the C→T polymorphism of *PTGS1* (rs883484) and the 41A→G (Glu14Gly) polymorphism of *ACAT2* (rs9658625) were significantly ($P<0.05$) associated with the prevalence of CKD. Our results suggest that *APOB*, *WRN*, *ACAT2*, *APOE*, *PPARG*, *TNF* and *PTGS1* are susceptibility loci for CKD among Japanese individuals with hypertension. Determination of the genotypes for these polymorphisms may prove informative for the assessment of genetic risk for CKD among such individuals.
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INTRODUCTION

Chronic kidney disease (CKD) has become a global public health problem,[1] being a leading cause of end-stage renal disease, poor cardiovascular outcome and premature death.[2,3] Identification of genetic markers for CKD risk is thus important both for risk prediction and for intervention to avert future end-stage renal disease and cardiovascular events.

Several risk factors for progression of CKD have been proposed,[4] with hypertension having been recognized as an important risk factor not only for CKD[5–10] but also for coronary heart disease and ischemic stroke. Genetic factors for predisposition to CKD in individuals with hypertension have, however, remained largely unknown. Furthermore, given the ethnic differences in lifestyle and environmental factors, as well as in renal function and genetic background, it is important to examine genetic variants related to CKD in individuals with hypertension in each ethnic group.

We have now performed an association study for 30 polymorphisms of 26 candidate genes and of CKD in 3696 Japanese individuals with hypertension. The purpose of this study was to identify genetic variants that confer susceptibility to CKD among individuals with hypertension and thereby provide a basis for the personalized prevention of this condition.

METHODS

Study population

The study population comprised 3696 unrelated Japanese individuals with hypertension (2265 men, 1431 women) who either visited outpatient clinics of, or were admitted to, one of the participating hospitals (Gifu Prefectural

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General Medical Center and Gifu Prefectural Tajimi Hospital in Gifu Prefecture, Japan; and Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital, and Hirosaki Stroke Center in Aomori Prefecture, Japan) between October 2002 and March 2008 because of various symptoms or for an annual health checkup, or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma Prefecture and Tokyo, Japan.

Glomerular filtration rate was estimated using the simplified prediction equation derived from that in the Modification of Diet in Renal Disease Study and proposed by the Japanese Society of Nephrology:[11] estimated glomerular filtration rate (eGFR) (ml min^{-1} per 1.73 m^2)=194×(age (years))$^{-0.287}$×(serum creatinine (mg per 100 ml))$^{-1.094}$ (×0.739 if female). The National Kidney Foundation–Kidney Disease Outcomes Quality Initiative guidelines recommend a diagnosis of CKD if eGFR is $<$60 ml min^{-1} per 1.73 m^2.[1] Nonlinear relations between GFR and the risk of adverse outcomes, such as death, cardiovascular events and hospitalization, have been shown, with an increased risk being associated with an eGFR of $<$60 ml min^{-1} per 1.73 m^2.[12] We thus adopted the criterion of an eGFR of $<$60 ml min^{-1} per 1.73 m^2 for diagnosis of CKD in this study. On the basis of this criterion, 1257 individuals (789 men, 468 women) were diagnosed with CKD. The control group comprised 2439 individuals (1476 men, 963 women) whose eGFR was $\geqslant$60 ml min^{-1} per 1.73 m^2. The diagnosis of hypertension was the basis of a systolic blood pressure of $\geqslant$140 mm Hg, a diastolic blood pressure of $\geqslant$90 mm Hg or the intake of antihypertensive medication. Individuals with CKD and controls either had or did not have other conventional risk factors for CKD, including diabetes mellitus (fasting blood glucose of $\geqslant$6.93 mmol l^{-1}, HbA_{1c} content of $\geqslant$6.5%, or taking of antidiabetes medication) and hypercholesterolemia (serum total cholesterol of $\geqslant$5.72 mmol l^{-1} or taking of lipid-lowering medication). Our study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology and participating hospitals. Written informed consent was obtained from each participant.

Selection of polymorphisms

Our aim was to identify genes associated with CKD in Japanese individuals with hypertension in a case–control association study by examining the relations of one or two polymorphisms of each candidate gene to CKD. Using public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (OMIM), we selected 26 candidate genes that have been characterized and suggested to be associated with CKD. On the basis of published studies or by searching PubMed and single-nucleotide polymorphism (SNP) databases (dbSNP (NCBI) and the Japanese SNP database (JSNP)), we further selected 30 polymorphisms of these genes, most by located in the promoter region or exons, which might be expected to result in changes in the function or expression of the encoded protein (Table 1). Wild-type and variant alleles of the polymorphisms were determined from the original sources.

Genotyping of polymorphisms

Venous blood (7 ml) was collected in tubes containing 50 mmol l^{-1} ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 30 polymorphisms were

Table 1 The 30 polymorphisms of 26 genes examined in this study

Locus	*Gene*	*Symbol*	*Polymorphism*	*dbSNP*[a]
1p35.1	Gap junction protein, α-4	*GJA4*	1019C→T (Pro319Ser)	rs1764391
1p34.1-p32	Proprotein convertase, subtilisin/kexin-type, 9	*PCSK9*	23968A→G (Glu670Gly)	rs505151
1p34	Low-density lipoprotein receptor-related protein 8	*LRP8*	T→G (Asp46Glu)	rs3820198
1q21-q23	C-reactive protein, pentraxin-related	*CRP*	1444C→T	rs1130864
1q31-q32	Interleukin 10	*IL10*	−819T→C	rs1800871
1q31-q32	Interleukin 10	*IL10*	−592A→C	rs1800872
2p24	Apolipoprotein B	*APOB*	T→C (Val591Ala)	rs679899
2p12-p11.2	Vesicle-associated membrane protein 8	*VAMP8*	A→G	rs1010
3p25	Peroxisome proliferator-activated receptor-γ	*PPARG*	−681C→G	rs10865710
3q21-q25	Angiotensin receptor 1	*AGTR1*	G→A (Ala163Thr)	rs12721226
4q28-q31	Fatty acid-binding protein 2	*FABP2*	2445G→A (Ala54Thr)	rs1799883
5q12	Phosphodiesterase 4D, cAMP-specific	*PDE4D*	TAAA→del (3′-UTR)	rs3839219
5q34	Potassium channel, calcium-activated, large conductance, subfamily M, β-member 1	*KCNMB1*	G→A (Glu65Lys)	rs11739136
6p21.3	Tumor necrosis factor	*TNF*	−863C→A	rs1800630
6p21.3	Tumor necrosis factor	*TNF*	−850C→T	rs1799724
6q25.3-q26	Acetyl-CoA acetyltransferase 2	*ACAT2*	41A→G (Glu14Gly)	rs9658625
6q25.3-q26	Acetyl-CoA acetyltransferase 2	*ACAT2*	734C→T (Thr254Ile)	rs2272296
6q27	Thrombospondin II	*THBS2*	3949T→G	rs8089
7p21	Interleukin 6	*IL6*	−572G→C	rs1800796
7q21.3-q22	Plasminogen activator inhibitor 1	*PAI1*	A→G (Tyr243Cys)	rs13306846
7q22.1	Collagen, type I, α-2	*COL1A2*	G→C (Ala459Pro)	rs42524
8p12-p11.2	Werner syndrome	*WRN*	T→C (Cys1367Arg)	rs1346044
9q32-q33.3	Prostaglandin-endoperoxide synthase 1	*PTGS1*	C→T	rs883484
11q22-q23	Matrix metalloproteinase 1	*MMP1*	−519A→G	(AY769434)
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	−629C→A	rs1800775
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	1061A→G (Ile405Val)	rs5882
19q13.2	Apolipoprotein E	*APOE*	−219G→T	rs405509
22q11.2	Catechol-*O*-methyltransferase	*COMT*	G→A (Val158Met)	rs4680
22q12	Heme oxygenase 1	*HMOX1*	−413T→A	rs2071746
Xq28	Interleukin 1 receptor-associated kinase 1	*IRAK1*	C→T	rs7061789

[a]In instances in which rs numbers in dbSNP were not detected, NCBI GenBank accession numbers are shown in parentheses.

Table 2 Primers, probes and other PCR conditions for genotyping of polymorphisms significantly (FDR <0.05) associated with chronic kidney disease by the χ^2-test in individuals with hypertension

Gene	*Polymorphism*	*Sense primer*	*Antisense primer*	*Probe 1*	*Probe 2*	*Annealing temperature (°C)*	*Cycles*
APOB	T→C (Val591Ala)	AACATGGTGTGTCAGCTCAAAA	TCCATGACAGTTGGAAGTTGAGA	TTCTTTCAGAGCTTCTTTCACT	TTCTTTCAGAACTTCTTTCACTA	60	50
FABP2	2445G→A (Ala54Thr)	AGCTGACAATTACACAAGAAGGAA	GTTGTAATTAAAGGTGACACCAAG	AATGTTTCGAAAAGCGCTTGATT	TCAAAGAATCAAGCACTTTTCGA	60	50
PPARG	−681C→G	TCGATGTTGGCGCTATTCAAGC	TAGGGTCTTAGGATTCACAACCT	AGCTGTATTTTCCATGAAGACAA	AGCTGTATTTTCCATCAAGACAA	60	50
WRN	T→C (Cys1367Arg)	GTACCTTATCCACATGGCAATTG	GCTTCTCTTAGAACTTGAACAGAT	TTTGTTGACATCACATGAAGGTT	CTTCAACCTTCACGTGATGTC	60	50
TNF	−850C→T	GGAGATGTGACCACAGCAATGG	GGTCCTGGAGGCTCTTTCACT	GGACCCCCACTTAACGAAG GGACCCCCCCTTAACGAAG	GGACCCCCCACTTAATGAAGAC GACCCCCCCTTAATGAAGAC	60	50
APOE	−219G→T	CTGGGCGGCAGCCTCCACAT	GAGGTGGGGCATAGAGGTCTT	TCGCCCAGTAATACAGACA	CTCGCCCAGTAATCCAGACAC	60	50
PTGS1	C→T	CACTCTTGCATGTCCAGAGCCTAG	CAAGAAGTATGGAGAAGAAGCAGT	AGAGGGAGAGAGGTTTGCAAG	AGAGGGAGAGAGGGTTTGCAAG	60	50
ACAT2	41A→G (Glu14Gly)	CGCTGGAGACCGCACCATGG	CACCATCTCCACAGGGTTGG	AGAGGACAGAAGGGCTGGG	AGAGGACAGGAGGGCTGGGA	60	50

Table 3 Characteristics of subjects with chronic kidney disease (CKD) and controls among individuals with hypertension

Characteristic	*CKD*	*Controls*	P-*value*
No. of subjects	1257	2439	
Age (years)	71.0 ± 8.7	66.8 ± 9.5	<0.0001
Sex (male/female, %)	62.8/37.2	60.5/39.5	0.1747
Body mass index (kg m^{-2})	23.5 ± 3.4	23.8 ± 3.3	0.0126
Current or former smoker (%)	20.5	28.0	<0.0001
Systolic blood pressure (mm Hg)	154 ± 26	150 ± 22	0.0001
Diastolic blood pressure (mm Hg)	82 ± 15	83 ± 14	0.2950
Hypercholesterolemia (%)	31.0	29.3	0.3004
Serum total cholesterol (mmol l^{-1})	5.22 ± 1.02	5.18 ± 0.99	0.2597
Serum triglyceride (mmol l^{-1})	1.70 ± 1.09	1.60 ± 1.11	0.0221
Serum HDL-cholesterol (mmol l^{-1})	1.32 ± 0.42	1.38 ± 0.39	0.0004
Diabetes mellitus (%)	41.2	32.8	<0.0001
Fasting plasma glucose (mmol l^{-1})	7.10 ± 3.10	6.99 ± 3.04	0.3627
HbA_{1c} (%)	6.01 ± 1.52	5.92 ± 1.51	0.2538
Serum creatinine (μmol l^{-1})	119.8 ± 135.8	62.3 ± 12.58	<0.0001
eGFR (ml min^{-1} per 1.73 m^2)	47.4 ± 11.8	79.1 ± 15.8	<0.0001
End-stage renal failure (%)	3.6	0	<0.0001

Quantitative data are means ± s.d. HDL, high-density lipoprotein.

determined at G&G Science (Fukushima, Japan) using a method that combines the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes and other PCR conditions for genotyping of polymorphisms found to be significantly (false discovery rate (FDR) <0.05) associated with CKD by the χ^2-test are shown in Table 2. Detailed genotyping methodology was described earlier.[13]

Statistical analysis

Quantitative data were compared between subjects with CKD and controls using the unpaired Student's *t*-test. Categorical data were compared using the χ^2-test. Allele frequencies were estimated by the gene counting method, and the χ^2-test was used to identify departures from the Hardy–Weinberg equilibrium. In the initial screen, allele frequencies (2×2) of each polymorphism were compared between subjects with CKD and controls using the χ^2-test. Allele frequencies of the identified polymorphisms, using the χ^2-test, were compared between subgroups of CKD and control subjects with hypertension matched for age, sex and smoking status individually. Given the multiple comparisons of genotypes with CKD, the FDR was calculated from the distribution of *P*-values for allele frequencies of the 30 polymorphisms.[14] Polymorphisms with an FDR of <0.05 were further examined by multivariable logistic regression analysis with adjustment for covariates. Multivariable logistic regression analysis was thus performed with CKD as a dependent variable and with independent variables including age, sex (0=woman, 1=man), body mass index, smoking status (0=nonsmoker, 1=smoker), history of diabetes mellitus (0=no history, 1=positive history) and genotype of each polymorphism; *P*-values, odds ratios and 95% confidence intervals were calculated. Each genotype was assessed according to dominant (0=wild-type homozygote, 1=heterozygote =variant homozygote), recessive (0=wild-type homozygote=heterozygote, 1= variant homozygote), and additive ((0, 0)=wild-type homozygote, (1, 0) =heterozygote, (0, 1)=variant homozygote) genetic models. Additive models included the additive 1 model (heterozygotes *vs.* wild-type homozygotes) and the additive 2 model (variant homozygotes *vs.* wild-type homozygotes), which were analyzed simultaneously with a single statistical model. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as those of other covariates on CKD; each genotype was examined according to a dominant or recessive model on the basis of statistical significance in the multivariable logistic regression analysis. The *P*-value levels for inclusion in and exclusion from the model were 0.25 and 0.1, respectively. With the exception of the initial screen by the χ^2-test (FDR <0.05), a *P*-value of <0.05 was considered statistically significant. Statistical significance was

examined by two-sided tests performed with JMP version 6.0 and JMP Genomics version 3.2 software (SAS Institute, Cary, NC, USA).

RESULTS

The characteristics of the 3696 study subjects are shown in Table 3. Age, systolic blood pressure, the serum concentration of triglycerides and the prevalence of diabetes mellitus were greater, whereas body mass index, the percentage of smokers and the serum concentration of high-density lipoprotein-cholesterol were lower in subjects with CKD than in controls.

The success rate of genotyping of 30 polymorphisms in all subjects was 99.9%. Comparisons of allele frequencies with the χ^2-test revealed that 17 polymorphisms were related (*P*-value for allele frequency <0.05) to the prevalence of CKD (Table 4). Among these polymorphisms, T→C (Val591Ala) of *APOB* (rs679899), 2445G→A (Ala54Thr) of *FABP2* (rs1799883), −681C→G of *PPARG* (rs10865710), T→C (Cys1367Arg) of *WRN* (rs1346044), −850C→T of *TNF* (rs1799724), −219G→T of *APOE* (rs405509), C→T of *PTGS1* (rs883484) and 41A→G (Glu14Gly) of *ACAT2* (rs9658625) were significantly (FDR <0.05) associated with the prevalence of CKD in hypertensive individuals. With the exception of the 2445G→A (Ala54Thr) polymorphism of *FABP2* in controls, the genotype distributions of these latter polymorphisms were in Hardy–Weinberg equilibrium in subjects with CKD and in controls (Table 5); the 2445G→A (Ala54Thr)

Table 4 Genotype distributions of polymorphisms related (*P*-value for allele frequency <0.05) to chronic kidney disease (CKD) among individuals with hypertension as determined by the χ^2-test

Gene symbol	*Polymorphism*	*dbSNP*	*CKD*	*Controls*	P-*value*	*FDR*
APOB	T→C (Val591Ala)	rs679899			0.0039	0.0499
	TT		936 (74.5)	1921 (78.8)		
	TC		296 (23.6)	479 (19.7)		
	CC		24 (1.9)	38 (1.6)		
FABP2	2445G→A (Ala54Thr)	rs1799883			0.0055	0.0499
	GG		486 (38.7)	1054 (43.2)		
	GA		580 (46.1)	1063 (43.6)		
	AA		191 (15.2)	322 (13.2)		
PPARG	−681C→G	rs10865710			0.0061	0.0499
	CC		649 (51.6)	1346 (55.2)		
	CG		496 (39.5)	934 (38.3)		
	GG		112 (8.9)	159 (6.5)		
WRN	T→C (Cys1367Arg)	rs1346044			0.0091	0.0499
	TT		1099 (87.4)	2058 (84.4)		
	TC		153 (12.2)	363 (14.9)		
	CC		5 (0.4)	18 (0.7)		
TNF	−850C→T	rs1799724			0.0105	0.0499
	CC		860 (68.4)	1761 (72.2)		
	CT		351 (27.9)	609 (25.0)		
	TT		46 (3.7)	69 (2.8)		
APOE	−219G→T	rs405509			0.0107	0.0499
	GG		97 (7.7)	221 (9.1)		
	GT		483 (38.4)	1010 (41.4)		
	TT		677 (53.9)	1208 (49.5)		
PTGS1	C→T	rs883484			0.0119	0.0499
	CC		440 (35.1)	938 (38.5)		
	CT		602 (48.0)	1151 (47.2)		
	TT		213 (17.0)	350 (14.4)		
ACAT2	41A→G (Glu14Gly)	rs9658625			0.0133	0.0499
	AA		804 (64.0)	1443 (59.2)		
	AG		398 (31.7)	887 (36.4)		
	GG		54 (4.3)	109 (4.5)		
PCSK9	23968A→G (Glu670Gly)	rs505151			0.0262	0.0744
	AA		1142 (90.9)	2259 (92.6)		
	AG		109 (8.7)	178 (7.3)		
	GG		6 (0.5)	2 (0.1)		
MMP1	−519A→G	rs1144393			0.0287	0.0744
	AA		997 (79.4)	1995 (81.8)		
	AG		240 (19.1)	430 (17.6)		
	GG		18 (1.4)	14 (0.6)		
HMOX1	−413T→A	rs2071746			0.0292	0.0744
	TT		329 (26.2)	757 (31.0)		
	TA		637 (50.7)	1129 (46.3)		
	AA		291 (23.2)	553 (22.7)		

Table 4 Continued

Gene symbol	*Polymorphism*	*dbSNP*	*CKD*	*Controls*	P-*value*	*FDR*
CETP	−629C→A	rs1800775			0.0313	0.0744
	CC		275 (21.9)	493 (20.2)		
	CA		634 (50.4)	1183 (48.5)		
	AA		348 (27.7)	763 (31.3)		
IL10	−592A→C	rs1800872			0.0332	0.0744
	AA		604 (48.1)	1072 (44.0)		
	AC		525 (41.8)	1099 (45.1)		
	CC		128 (10.2)	268 (11.0)		
IL10	−819T→C	rs1800871			0.0347	0.0744
	TT		604 (48.1)	1073 (44.0)		
	TC		525 (41.8)	1098 (45.0)		
	CC		128 (10.2)	268 (11.0)		
ACAT2	734C→T (Thr254Ile)	rs2272296			0.0427	0.0826
	CC		491 (39.1)	815 (33.4)		
	CT		544 (43.3)	1214 (49.8)		
	TT		221 (17.6)	410 (16.8)		
GJA4	1019C→T (Pro319Ser)	rs1764391			0.0484	0.0826
	CC		860 (68.4)	1579 (64.7)		
	CT		353 (28.1)	772 (31.7)		
	TT		44 (3.5)	88 (3.6)		
TNF	−863C→A	rs1800630			0.0496	0.0826
	CC		941 (74.9)	1744 (71.5)		
	CA		288 (22.9)	639 (26.2)		
	AA		28 (2.2)	56 (2.3)		

Numbers in parentheses are percentages.

Table 5 Hardy–Weinberg *P*-values for subjects with chronic kidney disease (CKD) and controls

Gene	*Polymorphism*	*CKD*	*Controls*
APOB	T→C (Val591Ala)	0.9152	0.1978
FABP2	2445G→A (Ala54Thr)	0.4072	**0.0379**
PPARG	−681C→G	0.2195	0.8593
WRN	T→C (Cys1367Arg)	0.8948	0.6503
TNF	−850C→T	0.1757	0.0653
APOE	−219G→T	0.4022	0.6353
PTGS1	C→T	0.7717	0.9186
ACAT2	41A→G (Glu14Gly)	0.5942	0.0619

P-value of <0.05 is shown in bold.

polymorphism of *FABP2* was therefore excluded from subsequent analysis. Comparison of allele frequencies of the 17 identified polymorphisms by the χ^2-test between subgroups of 1224 CKD patients and 1224 control subjects with hypertension matched for age, sex and smoking status individually revealed that the T→C (Val591Ala) polymorphism of *APOB*, the −681C→G polymorphism of *PPARG*, the T→C (Cys1367Arg) polymorphism of *WRN*, the C→T polymorphism of *PTGS1*, the 41A→G (Glu14Gly) and 734C→T (Thr254Ile) polymorphisms of *ACAT2*, the 23968A→G (Glu670Gly) polymorphism of *PCSK9*, the −413T→A polymorphism of *HMOX1*, the −629C→A polymorphism of *CETP* and the −592A→C and −819T→C polymorphisms of *IL10* were significantly ($P<0.05$) associated with CKD (Table 6).

Multivariable logistic regression analysis with adjustment for age, sex, body mass index and the prevalence of smoking and diabetes mellitus revealed that the T→C (Val591Ala) polymorphism of *APOB* (dominant and additive 1 models), the −681C→G polymorphism of *PPARG* (dominant, recessive and additive 2 models), the T→C (Cys1367Arg) polymorphism of *WRN* (dominant and additive 1 models), the −850C→T polymorphism of *TNF* (dominant model), the −219G→T polymorphism of *APOE* (recessive and additive 2 models), the C→T polymorphism of *PTGS1* (recessive and additive 2 models) and the 41A→G (Glu14Gly) polymorphism of *ACAT2* (dominant and additive 1 models) were significantly ($P<0.05$) associated with the prevalence of CKD in hypertensive individuals (Table 7). The variant *C* allele of *APOB*, *G* allele of *PPARG*, *T* allele of *TNF*, *T* allele of *APOE* and *T* allele of *PTGS1* were risk factors for CKD, whereas the variant *C* allele of *WRN* and *G* allele of *ACAT2* were protective against this condition.

Finally, we performed a stepwise forward selection procedure to examine the effects of genotypes for the seven polymorphisms associated with CKD by multivariable logistic regression analysis as well as those of age, sex, body mass index and the prevalence of smoking and diabetes mellitus on CKD (Table 8). Age, diabetes mellitus, sex, smoking, *APOB* genotype (dominant model), *WRN* genotype (dominant model), *ACAT2* genotype (dominant model), *APOE* genotype (recessive model), *PPARG* genotype (recessive model), *TNF* genotype (dominant model) and *PTGS1* genotype (recessive model), in descending order of statistical significance, were significant ($P<0.05$) and independent determinants of CKD.

DISCUSSION

We have examined the possible relations of 30 polymorphisms in 26 candidate genes to the prevalence of CKD in 3696 Japanese individuals with hypertension. Our results show that the T→C (Val591Ala) polymorphism of *APOB* (rs679899), the −681C→G polymorphism of *PPARG* (rs10865710), the T→C (Cys1367Arg) polymorphism of

Table 6 Comparison of allele frequencies of the 17 identified polymorphisms by the χ^2-test between subgroups of chronic kidney disease (CKD) and control subjects with hypertension matched for age, sex and smoking status individually

Gene symbol	*Polymorphism*	*dbSNP*	*CKD*	*Controls*	P-*value*
APOB	T→C (Val591Ala)	rs679899			0.0258
	TT		910 (74.3)	957 (78.2)	
	TC		290 (23.7)	248 (20.3)	
	CC		24 (2.0)	19 (1.5)	
FABP2	2445G→A (Ala54Thr)	rs1799883			0.9764
	GG		498 (40.7)	504 (41.2)	
	GA		550 (44.9)	537 (43.9)	
	AA		176 (14.4)	183 (14.9)	
PPARG	−681C→G	rs10865710			0.0435
	CC		631 (51.6)	657 (53.7)	
	CG		484 (39.5)	495 (40.4)	
	GG		109 (8.9)	72 (5.9)	
WRN	T→C (Cys1367Arg)	rs1346044			<0.0001
	TT		1070 (87.4)	1002 (81.9)	
	TC		149 (12.2)	211 (17.2)	
	CC		5 (0.4)	11 (0.9)	
TNF	−850C→T	rs1799724			0.1276
	CC		835 (68.2)	865 (70.7)	
	CT		343 (28.0)	323 (26.4)	
	TT		46 (3.8)	36 (2.9)	
APOE	−219G→T	rs405509			0.0608
	GG		92 (7.5)	114 (9.3)	
	GT		476 (38.9)	491 (40.1)	
	TT		656 (53.6)	619 (50.6)	
PTGS1	C→T	rs883484			0.0291
	CC		433 (35.4)	470 (38.4)	
	CT		577 (47.2)	580 (47.4)	
	TT		212 (17.4)	174 (14.2)	
ACAT2	41A→G (Glu14Gly)	rs9658625			0.0148
	AA		786 (64.2)	725 (59.2)	
	AG		385 (31.5)	437 (35.7)	
	GG		53 (4.3)	62 (5.1)	
PCSK9	23968A→G (Glu670Gly)	rs505151			0.0284
	AA		1117 (91.3)	1142 (93.3)	
	AG		101 (8.2)	81 (6.6)	
	GG		6 (0.5)	1 (0.1)	
MMP1	−519A→G	rs1144393			0.1683
	AA		970 (79.4)	992 (81.0)	
	AG		235 (19.2)	224 (18.3)	
	GG		17 (1.4)	8 (0.7)	
HMOX1	−413T→A	rs2071746			0.0366
	TT		322 (26.3)	374 (30.6)	
	TA		616 (50.3)	585 (47.8)	
	AA		286 (23.4)	265 (21.6)	
CETP	−629C→A	rs1800775			0.0097
	CC		268 (21.9)	232 (18.9)	
	CA		615 (50.2)	597 (48.8)	
	AA		341 (27.9)	395 (32.3)	
IL10	−592A→C	rs1800872			0.0134
	AA		593 (48.4)	523 (42.7)	
	AC		508 (41.5)	567 (46.3)	
	CC		123 (10.1)	134 (11.0)	
IL10	−819T→C	rs1800871			0.0134
	TT		593 (48.4)	523 (42.7)	
	TC		508 (41.5)	567 (46.3)	
	CC		123 (10.1)	134 (11.0)	
ACAT2	734C→T (Thr254Ile)	rs2272296			0.0417
	CC		480 (39.2)	407 (33.2)	
	CT		528 (43.1)	604 (49.4)	
	TT		216 (17.7)	213 (17.4)	
GJA4	1019C→T (Pro319Ser)	rs1764391			0.0514
	CC		838 (68.5)	793 (64.8)	
	CT		344 (28.1)	381 (31.1)	
	TT		42 (3.4)	50 (4.1)	
TNF	−863C→A	rs1800630			0.5351
	CC		917 (74.9)	899 (73.4)	
	CA		282 (23.0)	303 (24.8)	
	AA		25 (2.1)	22 (1.8)	

Numbers in parentheses are percentages.

WRN (rs1346044), the −850C→T polymorphism of *TNF* (rs1799724), the −219G→T polymorphism of *APOE* (rs405509), the C→T polymorphism of *PTGS1* (rs883484) and the 41A→G (Glu14Gly) polymorphism of *ACAT2* (rs9658625) were significantly associated with the prevalence of CKD. Among these polymorphisms, the T→C (Val591Ala) polymorphism of *APOB* was most significantly associated with this condition. Although hypertension has been recognized as a risk factor for CKD, genetic factors for predisposition to CKD in hypertensive individuals have not been determined. Our results showed that determination of the genotypes for these polymorphisms may prove informative for the assessment of genetic risk for CKD among such individuals.

The variant *C* allele of the T→C (Val591Ala) polymorphism of *APOB* was earlier associated with an increased plasma APOB level in hypertriglyceridemic patients.[15] We have now shown that this polymorphism was significantly associated with the prevalence of CKD in individuals with hypertension, with the *C* allele representing a risk factor for this condition. Given that abnormalities in lipoprotein metabolism may play an important role in the acceleration of atherosclerosis and in the development of global glomerulosclerosis,[16,17] the effects of this polymorphism on APOB metabolism may account for its association with CKD, although the underlying mechanism remains to be elucidated.

We found that polymorphisms of *PPARG*, *WRN*, *TNF*, *APOE*, *PTGS1* and of *ACAT2* were also associated with the prevalence of CKD in hypertensive individuals, with none of these polymorphisms having been earlier shown to be associated with this condition. Although the 161C→T polymorphism of *PPARG*[18] was shown to be associated with CKD, the −681C→G polymorphism of *PPARG* has not been related to this condition. The variant *G* allele of the −681C→G polymorphism of *PPARG* was earlier associated with increased height and an increased plasma low-density lipoprotein-cholesterol concentration.[19] *TNF* contributes to the initiation and progression of renal injury.[20,21] Although the −308G→A polymorph-

Table 7 Multivariable logistic regression analysis of polymorphisms significantly (FDR < 0.05) associated with chronic kidney disease by the χ^2-test in individuals with hypertension

		Dominant		*Recessive*		*Additive 1*		*Additive 2*	
Gene symbol	*Polymorphism*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*
APOB	T→C (Val591Ala)	0.0019	1.30 (1.10–1.53)	0.2875		0.0033	1.29 (1.09–1.53)	0.2054	
PPARG	−681C→G	0.0416	1.16 (1.01–1.33)	0.0282	1.34 (1.03–1.74)	0.1512		0.0136	1.40 (1.07–1.83)
WRN	T→C (Cys1367Arg)	0.0073	0.76 (0.61–0.93)	0.4177		0.0100	0.76 (0.62–0.94)	0.3777	
TNF	−850C→T	0.0207	1.20 (1.03–1.40)	0.0828		0.0564		0.0529	
APOE	−219G→T	0.0964		0.0081	1.21 (1.05–1.39)	0.3720		0.0308	1.34 (1.03–1.75)
PTGS1	C→T	0.0996		0.0463	1.21 (1.00–1.47)	0.2907		0.0245	1.27 (1.03–1.57)
ACAT2	41A→G (Glu14Gly)	0.0061	0.82 (0.71–0.94)	0.6556		0.0065	0.81 (0.70–0.94)	0.3904	

Abbreviations: CI, confidence interval; OR, odds ratio.
Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index and the prevalence of smoking and diabetes mellitus.

Table 8 Effects of genotypes and other characteristics on chronic kidney disease among individuals with hypertension determined by a stepwise forward selection procedure ($P < 0.05$)

Variable	P-*value*	*OR (95% CI)*	R^2
Age	<0.0001	2.12 (1.83–2.46)	0.0353
Diabetes mellitus	<0.0001	1.49 (1.29–1.72)	0.0067
Sex	0.0001	1.31 (1.12–1.53)	0.0034
Smoking	0.0004	0.64 (0.53–0.77)	0.0026
APOB (*TC+CC vs. TT*)	0.0022	1.28 (1.09–1.51)	0.0020
WRN (*TT vs. TC+CC*)	0.0048	1.35 (1.10–1.67)	0.0017
ACAT2 (*AA vs. AG+GG*)	0.0074	1.22 (1.05–1.41)	0.0015
APOE (*TT vs. GG+GT*)	0.0089	1.22 (1.06–1.40)	0.0014
PPARG (*GG vs. CC+CG*)	0.0209	1.38 (1.06–1.79)	0.0012
TNF (*CT+TT vs. CC*)	0.0243	1.21 (1.04–1.41)	0.0010
PTGS1 (*TT vs. CC+CT*)	0.0363	1.23 (1.02–1.50)	0.0010

Abbreviations: CI, confidence interval; OR, odds ratio; R^2, contribution rate.

ism of *TNF*[22,23] was earlier found to be associated with CKD, the −850C→T polymorphism of *TNF* has not been related to this condition. The variant *T* allele of the −850C→T polymorphism of *TNF* was associated with an increased risk of Alzheimer's disease.[24] We have now shown that the variant *G* and *T* alleles of these polymorphisms of *PPARG* and *TNF*, respectively, were risk factors for CKD.

Homozygosity for the *T* (Cys) allele of the T→C (Cys1367Arg) polymorphism of *WRN* was shown to be a risk factor for myocardial infarction in the Japanese population.[25,26] Our results now show that the variant *C* (Arg) allele of this polymorphism of *WRN* was protective against CKD. The −219G→T polymorphism of *APOE* was earlier associated with myocardial infarction in men in France and Northern Ireland, with the *T* allele representing a risk factor for this condition.[27] The *T* allele of this polymorphism was also shown to be a risk factor for coronary heart disease in low-risk Japanese men.[28] Our results now show that the *T* allele of this polymorphism was a risk factor for CKD.

The variant *A* allele of the −1006G→A polymorphism of *PTGS1* was earlier associated with an increased risk of incident ischemic stroke events in Caucasians.[29] In addition, the variant *A* allele of the 10721G→A polymorphism of *PTGS1* was associated with an increased risk of an incident ischemic stroke event or coronary heart disease (myocardial infarction or coronary revascularization) in African Americans.[29] These earlier findings suggest that *PTGS1* is a susceptibility gene for cardiovascular disease. Our results now show that the *T* allele of the C→T polymorphism of *PTGS1* was a risk factor for CKD. The *A* (Glu) allele of the 41A→G (Glu14Gly) polymorphism of *ACAT2* was earlier associated with dyslipidemia in a Chinese population.[30] Our results now show that the *G* (Gly) allele of this polymorphism of *ACAT2* was protective against CKD.

Although earlier studies showed that smoking is a risk factor for CKD,[4,31] the frequency of smoking was significantly lower in subjects with CKD than that in controls in this study. Although there was a possibility of more cigarettes and a longer term of smoking in subjects with CKD than in controls, selection bias could not be excluded in this study, given that most subjects with CKD had cardiovascular disease and controls were recruited from community-dwelling individuals or from patients who visited outpatient clinics.

Our study has several limitations: (i) We used eGFR instead of a directly measured GFR to define CKD. (ii) We were not able to obtain information about the underlying renal disease in each subject with CKD. Such information could be obtained by detailed clinical examination, including renal biopsy, but such diagnostic procedures are not considered feasible for a study in which subjects are recruited from the general population. (iii) It is possible that one or more of the polymorphisms associated with CKD in this study are in linkage disequilibrium with other polymorphisms in the same or nearby genes, which are actually responsible for the development of this condition. (iv) The functional relevance of the identified polymorphisms to gene transcription or to protein function was not examined in this study.

In conclusion, our results suggest that the T→C (Val591Ala) polymorphism of *APOB* (rs679899), the −681C→G polymorphism of *PPARG* (rs10865710), the T→C (Cys1367Arg) polymorphism of *WRN* (rs1346044), the −850C→T polymorphism of *TNF* (rs1799724), the −219G→T polymorphism of *APOE* (rs405509), the C→T polymorphism of *PTGS1* (rs883484) and the 41A→G (Glu14Gly) polymorphism of *ACAT2* (rs9658625) were significantly associated with the prevalence of CKD in Japanese individuals with hypertension. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for CKD in such individuals, and thereby may contribute to the personalized prevention and selection of the most appropriate treatment for this condition. Validation of our findings will require their replication with independent subject panels.

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