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Association of gene polymorphisms with chronic kidney disease in Japanese individuals

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Abstract. Chronic kidney disease (CKD) is recognized as a risk factor not only for end-stage renal disease but also for cardiovascular disease. Early detection and treatment of CKD is a likely key factor for prevention of its complications. Although genetic linkage analyses and association studies have implicated several loci and candidate genes in predisposition to CKD, the genes that underlie genetic susceptibility to this condition have remained largely unknown. The purpose of the present study was to identify genetic variants that confer susceptibility to CKD in Japanese individuals. The study population comprised 4,829 Japanese individuals (2,697 men, 2,132 women), including 757 subjects with CKD [464 men, 293 women; estimated glomerular filtration rate (eGFR) <50 ml min 1.73 m^{-2}] and 4,072 controls (2,233 men, 1,839 women; eGFR ≥60 ml min 1.73 m^{-2}). The genotypes for 40 polymorphisms of 39 candidate genes were determined. The chi-square test, multivariable logistic regression analysis with adjustment for covariates, as well as a stepwise forward selection procedure revealed that six polymorphisms of *F10*, *PITRM1*, *PCSK2*, *JPH3*, *MYO7B*, and *AKAP12* were related (P<0.05) to the prevalence of CKD. Among these polymorphisms, the C→T polymorphism of *F10* (rs5962) was most significantly associated with this condition. Determination of genotypes for the C→T polymorphism of *F10* may prove informative for assessment of genetic risk for CKD in Japanese individuals.

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Introduction

A current epidemic of chronic kidney disease (CKD) is a major health problem worldwide. In Japan, the number of new patients with end-stage renal disease (ESRD) has been increasing during the last four decades (1). Individuals with CKD are at increased risk not only for ESRD but also for a poor cardiovascular outcome and premature death (2,3). Disease prevention is an important strategy for reducing the overall burden of CKD and ESRD, and the identification of markers for disease risk is essential both for risk prediction and for potential intervention to reduce the chance of future cardiovascular events (4).

Although genetic linkage analyses (5) and association studies (6-10) have implicated several loci and candidate genes in predisposition to CKD, the genes that confer susceptibility to this condition remain to be identified definitively. In addition, given the ethnic differences in lifestyle and environmental factors as well as in genetic background and renal function, it is important to examine genetic variants related to CKD in each ethnic group. We have now performed an association study for 40 polymorphisms of 39 candidate genes and CKD in 4,829 Japanese individuals. The purpose of the present study was to identify genetic variants that confer susceptibility to CKD and thereby to provide a basis for the personalized prevention of this condition.

Table I. The 40 polymorphisms examined in the present study.

Locus	Gene	Symbol	SNP	dbSNP
1p36.31-p36.11	Acyl-CoA thioesterase 7	*ACOT7*	A→C	rs3789485
2p25.3	Collectin sub-family member 11	*COLEC11*	C→T	rs6739899
2q21.1	Myosin VIIB	*MYO7B*	C→T	rs13015157
2q25	Peroxidasin homolog (*Drosophila*)	*PXDN*	A→G	rs12617409
4p16.3	Phosphatidylinositol glycan anchor biosynthesis, class G	*PIGG*	C→T	rs4499656
4q22.1	Family with sequence similarity 13, member A1	*FAM13A1*	A→G	rs6532094
5q35.1-q35.2	Endoplasmic reticulum-golgi intermediate compartment 1	*ERGC1*	A→G	rs2339745
5q35.3	Collagen, type XXIII, α 1	*COL23A1*	G→T	rs535858
6q24-q25	A kinase (PRKA) anchor protein (gravin) 12	*AKAP12*	A→G	rs756009
6q25.3	Poly (A)-specific ribonuclease (PARN)-like domain containing 1	*PNLDC1*	C→G	rs13209678
7p36	Protein tyrosine phosphatase, receptor type, N polypeptide 2	*PTPRN2*	C→T	rs1638021
8q24.3	Trafficking protein particle complex 9	*TRAPPC9*	C→T	rs12679196
9p13.3	Receptor tyrosine kinase-like orphan receptor 2	*ROR2*	A→G	rs10992119
9q22.3	WNK lysine deficient protein kinase 2	*WNK2*	G→T	rs16936752
9q32	Zinc finger protein 618	*ZNF618*	C→T	rs1330171
10p15.3	WD repeat domain 37	*WDR37*	A→G	rs6560711
10p15.2	Pitrilysin metallopeptidase 1	*PITRM1*	A→G	rs7094698
10p15.2	Pitrilysin metallopeptidase 1	*PITRM1*	C→G	rs7898290
10q11.2	Arachidonate 5-lipoxygenase	*ALOX5*	A→G	rs7913948
10q11.23	Oxoglutarate dehydrogenase-like	*OGDHL*	A→G	rs11101224
11p11.2	Glycosyltransferase-like 1B	*GYLTL1B*	C→G	rs10838532
11q13.4	NAD synthetase 1	*NADSYN1*	A→C	rs3814731
13q12.2	Polymerase (RNA) I polypeptide D, 16k Da	*POLR1D*	C→T	rs14105
13q34	Coagulation factor X	*F10*	C→T	rs5962
14p31	Inositol 1,3,4-triphosphate 5/6 kinase	*ITPK1*	C→T	rs2295394
14q22.1	Atlastin GTPase 1	*ATL1*	C→T	rs4901043
16p13.3	Transducin (ß)-like 3	*TBL3*	A→C	rs8053843
16q23.2	Chromodomain protein, Y-like 2	*CDYL2*	A→G	rs17823453
16q24	COX4 neighbor	*COX4NB*	C→T	rs301164
16q24.3	Junctophilin 3	*JPH3*	C→G	rs2562059
17q21	Wingless-type MMTV integration site family, member 3	*WNT3*	C→G	rs199515
17q23.2	Membrane-associated ring finger (C3HC4) 10	*MARCH10*	C→T	rs2251393
17q25.3	Tubulin folding cofactor D	*TBCD*	A→C	rs8076476
18q11.2	Laminin, α3	*LAMA3*	G→T	rs12373237
20p11.23-p11.21	Acyl-CoA synthetase short-chain family member 1	*ACSS1*	C→T	rs6138473
20p11.2	Proprotein convertase subtilisin/kexin type 2	*PCSK2*	C→G	rs6080699
20q13.3	Cadherin 4, type 1, R-cadherin (retinal)	*CDH4*	A→G	rs6142884
20q13.3	Synovial sarcoma translocation gene on chromosome 18-like 1	*SS18L1*	C→T	rs2427254
21q22.3	Trefoil factor 1	*TFF1*	C→G	rs13051704
22q11.21	Armadillo repeat gene deletes in velocardiofacial syndrome	*ARVCF*	A→G	rs2073746

Materials and methods

Study population. The study population comprised 4,829 unrelated Japanese individuals (2,697 men, 2,132 women) who either visited outpatient clinics of or were admitted to one of the participating hospitals (Gifu Prefectural 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, Japan.

Estimated glomerular filtration rate (eGFR) was calculated with the use of the simplified prediction equation derived from the modified version of that described in the Modification of Diet in Renal Disease (MDRD) Study as proposed by the Japanese Society of Nephrology (11), eGFR (ml min^{-1} 1.73 m^{-2})= 194x[age (years)]$^{-0.287}$x[serum creatinine (mg/dl)]$^{-1.094}$x[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} 1.73 m^{-2} (4). Nonlinear relations between GFR and the risk of adverse events, such as death, cardiovascular events, and hospitalization, have been demonstrated, with an increased risk being associated with an eGFR of <60 ml min^{-1} 1.73 m^{-2} and the risk rising further

Table II. Primers, probes, and other PCR conditions for genotyping of polymorphisms related (P-value for allele frequency <0.05) to chronic kidney disease.

Gene	Polymorphism	Sense primer (5'→3')	Antisense primer (5'→3')	Probe 1 (5'→3')	Probe 2 (5'→3')	Annealing temperature (˚C)	Cycles
F10	C→T	GTGCCTCTCCTTTGGCAGTCAC	AGAGTTCTGTTCCTCGTGGCAG	AGCCTGGACAACGGGGACT	TCACAGTCCCCATTGTCCAG	60	50
PITRM1	C→G	GGGATTCTCAGGACATGACTGG	TTAACAAGAAAATGTTTGCTGAAGTC	CATCAGGACTCTCTGTGAAGAA	TCCTTCTTCACACAGAGTCCT	60	50
PCSK2	C→G	TGCCAGAAGGATATGGTGTCCA	GGGCAGGGGAGGTCTCCAGC	ACCCAGTCTTTAGCTTCAACTG	TTCAGCAGTTGAACCTAAAGACT	60	50
JPH3	C→G	GGAGCCAGGGCTTTCGTTGTC	AGTGGGGTGCTCAGCCTCAC	CTCTGAAACTCCTCAGAATATC	CTCTGAAACTCCTGAGAATATC	60	50
MYO7B	C→T	AGCCCACGCTGGGCTCCAAG	GGGTTAGCGGCTGTTGCATGC	TCTACGTGCACACGCTGACG	TCTTCTACGTGCACATGCTGA	60	50
MARCH10	C→T	AACTGAAACCAAAGCGTTCACAGT	CCCATAGATGCTCAAGCCAGAC	TAGATTTTCACCTCGTGCCAC	AAGAGAGTGGCACAAGGTGAA	60	50
TRAPPC9	C→T	CCTACGCTTGCTAGCAGCTGG	CTGGCCCGTCCAGTACAATGC	ATGCCCTCTCCGTCTTTTGG	AATGCCCTCTCCATCTTTTGG	60	50
AKAP12	A→G	CGAGGATGACCCAGGCCTCAA	CCACACTTTGCCGGCCTCCAG	AGTGAGGGCAACTTTGCTAA	GAGGGCAACTTCGCTAAATCC	60	50
CDH4	A→G	CTGAGCTGCTGCCCAAGGAG	AGCGTCGGCCGCCGTGATGT	GATCTGCGAGAAGCCCAACC	CAGATCTGCGAGAGGCCCA	60	50
COX4NB	C→T	CTGGAAGCGCTGGAACACTGG	CGCCTGTGTTTTCAGTTAGCGT	CTTGGTATCATGCCTCTGGAG	CTTGGTATCATGCTTCTGGAGA	60	50

Table III. Characteristics of subjects with chronic kidney disease (CKD) and controls.

Characteristic	CKD	Controls	P-value
No. of subjects	757	4072	
Age (years)	71.3±9.2	65.2±10.5	<0.0001
Sex (male/female, %)	61.3/38.7	54.8/45.2	0.0010
Body mass index (kg/m^2)	23.5±4.6	23.5±4.6	0.5619
Smoker (%)	17.6	24.1	<0.0001
Hypertension (%)	75.7	54.4	<0.0001
Systolic blood pressure (mmHg)	149±27	140±23	<0.0001
Diastolic blood pressure (mmHg)	78±16	78±14	0.1973
Hypercholesterolemia (%)	39.6	33.0	0.0004
Serum total cholesterol (mmol/l)	5.23±1.07	5.17±0.99	0.2138
Serum triglyceride (mmol/l)	1.76±1.03	1.59±1.09	<0.0001
Serum HDL-cholesterol (mmol/l)	1.30±0.42	1.40±0.39	<0.0001
Diabetes mellitus (%)	43.3	26.4	<0.0001
Fasting plasma glucose (mmol/l)	7.24±3.48	6.74±2.93	0.0002
Blood glycosylated hemoglobin (%)	6.24±1.66	6.05±8.92	<0.0001
Serum creatinine (μmol/l)	149.9±169.7	61.7±12.6	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	39.3±11.1	79.3±16.9	<0.0001

Quantitative data are means ±SD. HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

markedly when values fall <45 ml min^{-1} 1.73 m^{-2} (12). In addition, the rate of GFR decline was significantly higher in Japanese individuals younger than 70 years with an initial value of <50 ml min^{-1} 1.73 m^{-2}, suggestive of a poor outcome for such individuals (13). We thus adopted the criterion of an eGFR of <50 ml min^{-1} 1.73 m^{-2} for diagnosis of CKD in the present study. On the basis of this criterion, 757 subjects (464 men, 293 women) were diagnosed with CKD. The control subjects comprised 4,072 individuals (2,233 men, 1,839 women) whose eGFR was ≥60 ml min^{-1} 1.73 m^{-2}. The control subjects were recruited from community-dwelling healthy individuals or the patients who visited outpatient clinics regularly for treatment of various common diseases. Subjects with CKD and controls thus either had or did not have conventional risk factors for CKD, including hypertension (systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg, or both, or taking antihypertensive medication), diabetes mellitus (fasting blood glucose of ≥6.93 mmol/l or hemoglobin A_{1c} of ≥6.5%, or both, or taking antidiabetes medication), or hypercholesterolemia (serum total cholesterol of ≥5.72 mmol/l or taking lipid-lowering medication). The 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 genetic variants associated with CKD in the Japanese population in a case-control association study by examining the relations of candidate gene polymorphisms to this condition. Polymorphisms examined in the present study (Table I) were selected from genome-wide association studies of ischemic stroke (14) and myocardial infarction. Given that atherosclerosis is an important risk factor for CKD (15), genetic variants related to atherosclerotic disease such as ischemic stroke and myocardial infarction might also contribute to the development of CKD. We have not examined the relation of these polymorphisms in the previous studies (7,8,16-19).

Genotyping of polymorphisms. Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 40 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Primers, probes, and other PCR conditions for genotyping polymorphisms found to be related (P-value for allele frequency of <0.05) to CKD by the chi-square test are shown in Table II. Detailed genotyping methodology was described previously (20).

Statistical analysis. Quantitative data were compared between subjects with CKD and controls by the unpaired Student's t test. Categorical data were compared by the chi-square test. Allele frequencies were estimated by the gene counting method, and the chi-square test was used to identify departures from Hardy-Weinberg equilibrium. In the initial screen, the allele frequencies of each polymorphism were compared between subjects with CKD and controls by the chi-square test. Given the multiple comparisons of genotypes with CKD, the false discovery rate (FDR) was calculated from the distribution of P values for allele frequency of the 40 polymorphisms (21). Polymorphisms with a P-value for allele frequency of <0.05

SPANDIDOS PUBLICATIONS. Genotype distributions of polymorphisms related (P-value for allele frequency <0.05) to chronic kidney disease determined by the chi-square test.

Gene symbol	SNP	dbSNP	CKD[a]	Controls[a]	P-value	FDR
F10	C→T	rs5962			0.0011	0.0437
	CC		747 (99.6)	4037 (99.9)		
	CT		3(0.4)	1 (0.1)		
	TT		0 (0)	0 (0)		
PITRM1	C→G	rs7898290			0.0074	0.0815
	CC		298 (5.9)	1430 (6.1)		
	CG		352 (33.9)	1946 (36.7)		
	GG		100 (60.2)	662 (57.2)		
PCSK2	C→G	rs6080699			0.0098	0.0815
	CC		42 (5.6)	338 (8.4)		
	CG		292 (38.9)	1611 (39.9)		
	GG		416 (55.5)	2089 (51.7)		
JPH3	C→G	rs2562059			0.0102	0.0815
	CC		539 (71.9)	2711 (67.2)		
	CG		193 (25.8)	1202 (29.8)		
	GG		17 (2.3)	121 (3.0)		
MYO7B	C→T	rs13015157			0.0116	0.0815
	CC		569 (75.9)	3244 (80.3)		
	CT		171 (22.8)	738 (18.3)		
	TT		10 (1.3)	56 (1.4)		
MARCH10	C→T	rs2251393			0.0122	0.0815
	CC		17 (2.3)	55 (1.4)		
	CT		179 (23.8)	847 (21.0)		
	TT		555 (73.9)	3128 (77.6)		
TRAPPC9	C→T	rs12679196			0.0245	0.1380
	CC		507 (67.5)	2510 (62.3)		
	CT		215 (28.6)	1370 (34.0)		
	TT		29 (3.9)	150 (3.7)		
AKAP12	A→G	rs756009			0.0276	0.1380
	AA		0 (0)	1 (0.1)		
	AG		7 (0.9)	85 (2.1)		
	GG		743 (99.1)	3952 (97.8)		
CDH4	A→G	rs6142884			0.0410	0.1821
	AA		17 (2.3)	65 (1.6)		
	AG		182 (24.3)	878 (21.7)		
	GG		551 (73.4)	3095 (76.7)		
COX4NB	C→T	rs301164			0.0457	0.0744
	CC		55 (7.3)	227 (5.6)		
	CT		286 (38.2)	1484 (36.8)		
	TT		408 (54.5)	2323 (57.6)		

[a]Numbers in parentheses are percentages.

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 independent variables including age, sex (0, woman; 1, man), body mass index (BMI), smoking status (0, nonsmoker; 1, smoker), history of hypertension, diabetes mellitus, or hypercholesterolemia (0, no history; 1, positive history), and genotype of each polymorphism, and the P-value, odds ratio, and 95% confidence interval were calculated. Each genotype was assessed according to dominant, recessive, and additive genetic models. Additive models included the additive 1 model (heterozygotes versus wild-type homozygotes) and the additive 2 model (variant homozygotes versus 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 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-levels for inclusion in and exclusion

Table V. Hardy-Weinberg P-values in subjects with chronic kidney disease (CKD) and controls

Gene	Polymorphism	dbSNP	CKD	Controls
F10	C→T	rs5962	0.9562	0.9937
PITRM1	C→G	rs7898290	0.8055	0.9991
PCSK2	C→G	rs6080699	0.3191	0.2717
JPH3	C→G	rs2562059	0.9548	0.3783
MYO7B	C→T	rs13015157	0.4778	0.0597
MARCH10	C→T	rs2251393	0.5695	0.7847
TRAPPC9	C→T	rs12679196	0.3039	**0.0274**
AKAP12	A→G	rs756009	0.8978	0.4326
CDH4	A→G	rs6142884	0.6680	0.7629
COX4NB	C→T	rs301164	0.6175	0.6191

P-values of <0.05 are shown in bold.

from the model were 0.25 and 0.1, respectively. With the exception of the initial screen by the chi-square 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).

Results

The characteristics of the 4,829 study subjects are shown in Table III. Age, the frequency of male subjects, systolic blood pressure, serum concentrations of triglycerides and creatinine, fasting plasma glucose level, blood glycosylated hemoglobin content, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia were greater, whereas serum concentration of high density lipoprotein (HDL)-cholesterol, eGFR, and the percentage of smokers were lower in subjects with CKD than in controls.

Comparison of allele frequencies with the chi-square test revealed that 10 polymorphisms were related (P-value of <0.05 for allele frequency) to the prevalence of CKD (Table IV). Among these polymorphisms, the C→T polymorphism of *F10* (rs5962) was significantly (FDR <0.05) associated with this condition. The genotype distributions of all 10 polymorphisms, with the exception of the C→T polymorphism of *TRAPPC9* in control subjects, were in Hardy-Weinberg equilibrium both in individuals with CKD and in controls (Table V); the C→T polymorphism of *TRAPPC9* was therefore excluded from subsequent analysis.

Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia revealed that the C→T polymorphism of *F10* (dominant and additive 1 models), the C→G polymorphism of *PITRM1* (dominant and additive 2 models), the C→G polymorphism of *PCSK2* (dominant and additive 2 models), the C→G polymorphism of *JPH3* (dominant and additive 1 models), the C→T polymorphism of *MYO7B* (dominant and additive 1 models), the C→T polymorphisms of *MARCH10* (recessive model), and the A→G polymorphism of *AKAP12* (recessive

Table VI. Multivariable logistic regression analysis of polymorphisms related (allele frequency <0.05) to chronic kidney disease by the chi-square test.

Gene symbol	SNP		Dominant		Recessive		Additive 1		Additive 2	
			P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
F10	C→T		**0.0166**	18.5 (2.1-399.1)			**0.0166**	18.5 (2.1-399.1)		
PITRM1	C→G		**0.0300**	0.83 (0.70-0.98)	0.1178		0.0805		**0.0335**	0.76 (0.58-0.98)
PCSK2	C→G		**0.0269**	1.47 (1.06-2.10)	0.1261		0.0606		**0.0188**	1.52 (1.08-2.19)
JPH3	C→G		**0.0255**	0.81 (0.68-0.97)	0.3715		**0.0382**	0.82 (0.68-0.99)	0.2738	
MYO7B	C→T		**0.0100**	1.29 (1.06-1.57)	0.5163		**0.0049**	1.33 (1.09-1.62)	0.6284	
MARCH10	C→T		0.1360		**0.0343**	0.82 (0.68-0.99)	0.3211		0.1042	
AKAP12	A→G		0.8663		**0.0212**	2.56 (1.23-6.24)	0.8989		0.8657	
CDH4	A→G		0.1947		0.0604		0.3972		0.1550	
COX4NB	C→T		0.1934		0.0902		0.4103		0.1201	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, sex, body mass index, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia. P-values of <0.05 are shown in bold.

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I. Effects of genotypes and other characteristics on kidney disease determined by a stepwise forward selection procedure (P<0.05).

Variable	P-value	R^2
Age	<0.0001	0.0581
Diabetes mellitus	<0.0001	0.0215
Hypertension	<0.0001	0.0154
Sex	0.0002	0.0034
Smoking	0.0031	0.0022
F10 (*CT+TT* versus *CC*)	0.0065	0.0018
AKAP12 (*GG* versus *AA+AG*)	0.0095	0.0016
MYO7B (*CT+TT* versus *CC*)	0.0113	0.0016
JPH3 (*CG+GG* versus *CC*)	0.0204	0.0014
PCSK2 (*CG+GG* versus *CC*)	0.0254	0.0013
PITRM1 (*CG+GG* versus *CC*)	0.0286	0.0011
Hypercholesterolemia	0.0459	0.0010

R^2, contribution rate.

model) were significantly (P<0.05) associated with the prevalence of CKD (Table VI). The *T* allele of *F10*, *G* allele of *PCSK2*, *T* allele of *MYO7B*, and *G* allele of *AKAP12* were risk factors for CKD, whereas the *G* allele of *PITRM1*, *G* allele of *JPH3*, and *T* allele of *MARCH10* 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 of age, sex, BMI, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia on CKD (Table VII). Age, diabetes mellitus, hypertension, sex, smoking, *F10* genotype (dominant model), *AKAP12* genotype (recessive model), *MYO7B* genotype (dominant model), *JPH3* genotype (dominant model), *PCSK2* genotype (dominant model), *PITRM1* genotype (dominant model), and hypercholesterolemia, in descending order of statistical significance, were significant (P<0.05) and independent determinants of CKD.

Discussion

We examined the possible relations of 40 gene polymorphisms to the prevalence of CKD in 4,829 Japanese individuals. Our association study with three steps of analysis (chi-square test, multivariable logistic regression analysis, and stepwise forward selection procedure) revealed that the C→T polymorphism of *F10* was significantly (FDR <0.05) associated with CKD in such individuals, with the *T* allele representing a risk factor for this condition.

Coagulation factor X (F10), the zymogen of a vitamin K-dependent serine protease, plays a pivotal role in the coagulation cascade (22). It can be activated through either the contact-activated (intrinsic) pathway or the tissue-factor (extrinsic) pathway (23). F10 is composed of light and heavy chains held together by a disulfide bond (24) as well as synthesized in the liver as a single-chain molecule (25). Activated F10 catalyzes the conversion of prothrombin to thrombin in the presence of phospholipids and calcium ions (26). Thrombin then converts fibrinogen to insoluble fibrin. F10 deficiency results in a serious bleeding disorder which points to an essential role for F10 in hemostasis (22).

F10 is located at chromosome 13q34 in close proximity to *F7* and spans ~25 kilobases, containing eight exons (27). The overall domain structure of *F10* is highly homologous to that of the other vitamin K-dependent coagulation factors. *F10* is primarily expressed in the liver (28). The -40C→T polymorphism in the promoter region of *F10* was previously associated with coronary heart disease, with the *T* allele representing a risk factor for this condition (29). However, another study did not detect the relation of this polymorphism to plasma F10 level or to thrombosis risk (30). Although the deleterious influence of the *T* allele on coronary heart disease cannot be explained by its effect on the circulating level of F10, the previous observation (29) suggested *F10* as a candidate susceptibility gene for atherothrombotic disease. We have now shown that the C→T polymorphism of *F10* was significantly associated with the prevalence of CKD, with the *T* allele representing a risk factor for CKD. Effects of this polymorphism on the development of atherothrombosis may account for its association with CKD. Given that the *T* allele frequency of this polymorphism was low, validation of our findings is required in large independent subject panels.

Our results suggest that *PITRM1*, *PCSK2*, *JPH3*, *MYO7B*, and *AKAP12* were also candidate genes for CKD in Japanese individuals. The proprotein convertase 2 gene (*PCSK2*) encodes the insulin processing protease. The variant of this gene was shown to be associated with type 2 diabetes mellitus in Japanese individuals, with the *A1* allele (21 CA repeats) representing a risk factor for this condition (31). Although mutations in certain myosins can cause diseases in humans and mice (32), the myosin-VIIb gene (*MYO7B*) has not been related to human disease. An antibody to MYO7B labeled proximal tubule cells of the kidney, specifically the distal tips of apical microvilli on these transporting epithelial cells (33). These observations implicate *MYO7B* as a candidate susceptibility gene for renal dysfunction. The A kinase anchor protein 12 (AKAP12) was originally identified as a cytoplasmic antigen recognized by myasthenia gravis sera (34). The expression of *Akap12* was decreased by hypoxia and upregulated by reoxygenation at the transcriptional level in rats (35). We have now shown that the C→G polymorphism of *PCSK2*, the C→T polymorphism of *MYO7B*, and the A→G polymorphism of *AKAP12* were associated with CKD, with the *G*, *T*, and *G* alleles, respectively, representing risk factors for this condition, although the underlying mechanisms remain to be determined.

The pitrilysin metallopeptidase 1 gene (*PITRM1*) is located at chromosome 10p15.2. The mitochondrial PITRM1 functions as a peptide scavenger in the mitochondrial matrix, clearing mitochondria from toxic peptides and protecting them against pathogenic peptide intruders (36). Polymorphisms of *PITRM1* have not been related to human disease. *JPH3* encodes junctophilin-3, a member of a conserved family of proteins that are components of junctional complexes (37). Holmes *et al* (38) demonstrated a CAG/CTG repeat expansion of ≥40 triplets in an alternatively spliced exon of *JPH3* in affected members of an African American family with Huntington disease-like 2.

We have now shown that the C→G polymorphism of *PITRM1* and the C→G polymorphism of *JPH3* were associated with CKD, with both *G* alleles protecting against this condition, although the molecular mechanisms remain unclear.

Our study has several limitations: (i) we used eGFR instead of directly measured GFR to define CKD, (ii) we have not obtained information on the underlying renal disease or the primary cause of CKD 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 whose subjects are recruited from the general population, (iii) it is possible that one or more of the polymorphisms associated with CKD in the present study are in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of this condition, (iv) the functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study, (v) although we adopted the criterion of FDR <0.05 for association to compensate for the multiple comparisons of genotypes with CKD, it is not possible to exclude completely potential statistical errors such as false positives, (vi) although a previous study (39) showed smoking to be a risk factor for CKD, the frequency of smoking was lower in subjects with CKD than in controls in the present study. Selection bias thus could not be excluded completely in the present study.

In conclusion, our present results suggest that the C→T polymorphism of *F10* is significantly associated with CKD in Japanese individuals. Determination of genotype for this polymorphism may prove informative for assessment of the genetic risk for CKD in such individuals. Validation of our findings will require their replication with independent subject panels as well as long-term follow-up to examine the association of the identified genetic variants with the rate of decline in eGFR.

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