Association of Genetic Variants with Chronic Kidney Disease in Japanese Individuals

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Background and objectives: Although genetic linkage analyses and association studies have implicated several loci and candidate genes in predisposition to chronic kidney disease (CKD), the genes that underlie genetic susceptibility to this condition have remained uncharacterized. The purpose of the present study was to identify genetic variants that confer susceptibility to CKD in Japanese individuals.

Design, setting, participants, & measurements: The study population comprised 5217 Japanese individuals (2955 men, 2262 women), including 778 subjects (480 men, 298 women) with CKD [estimated GFR (eGFR), <50 ml min^{-1} 1.73 m^{-2}] and 4439 controls (2475 men, 1964 women; eGFR, ≥60 ml min^{-1} 1.73 m^{-2}). The genotypes for 40 polymorphisms of 32 candidate genes were determined.

Results: The chi-square test and multivariable logistic regression analysis with adjustment for covariates revealed that the −219G→T polymorphism of *APOE*, the −519A→G of *MMP1*, the −866G→A of *UCP2*, the −1607/1G→2G of *MMP1*, the A→G (Lys45Glu) of *MMP3*, the G→A (Ala163Thr) of *AGTR1*, the G→A (Gly670Arg) of *PECAM1*, and the −55C→T of *UCP3* were significantly (false discovery rate <0.05) associated with CKD. Comparison of allele frequencies of these polymorphisms by the chi-square test between subgroups of CKD and control subjects individually matched for covariates revealed that the −519A→G of *MMP1* and the −866G→A of *UCP2* were significantly ($P < 0.05$) associated with CKD.

Conclusions: *MMP1* and *UCP2* may be susceptibility loci for CKD in Japanese individuals. Determination of genotypes for these polymorphisms may prove informative for prediction of genetic risk for CKD.

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The Japanese population has a large proportion of elderly individuals, whose number will continue to increase for at least the next two decades. The incidence of renal disease, especially that of chronic kidney disease (CKD) and end-stage renal disease (ESRD), will increase as the size of the elderly population increases, given that renal function decreases with age (1). CKD is a global public health problem; 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 key both for risk prediction and for potential intervention to reduce the chance of future cardiovascular events (4).

Although genetic linkage analyses (5,6) and association studies (7–9) have implicated several loci and candidate genes in predisposition to CKD, the genes that contribute to genetic 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, it is important to examine genetic polymorphisms related to CKD in each ethnic group. We have now performed an association study for 40 polymorphisms of 32 candidate genes and CKD in

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5217 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.

Materials and Methods

Study Population

The study population comprised 5217 unrelated Japanese individuals (2955 men, 2262 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 and Tokyo, Japan.

GFR was estimated 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 (10): eGFR (mL min^{-1} 1.73 m^{-2}) = 194 × [age (years)]$^{-0.287}$ × [serum creatinine (mg/dl)]$^{-1.094}$ × [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} 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 markedly when values fall below 45 ml min^{-1} 1.73 m^{-2} (11). In addition, the rate of GFR decline was significantly higher in Japanese individuals younger than 70 yr with an initial value of <50 ml min^{-1} 1.73 m^{-2}, suggestive of a poor outcome for such individuals (12). 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, 778 subjects (480 men, 298 women) were diagnosed with CKD. The control subjects comprised 4439 individuals (2475 men, 1964 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. Although some of these subjects had hypertension, diabetes mellitus, or dyslipidemia, none of them had systemic conditions such as neoplasia, pulmonary disease, or infectious disease. Subjects with CKD and controls thus either had or did not have conventional risk factors for CKD, including hypertension (systolic BP of ≥140 mmHg or diastolic BP 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). Current smokers were defined as those who smoked a total of ≥100 cigarettes or for more than six months and smoked daily recently for one month; former smokers as those who smoked a total of ≥100 or for more than six months but did not smoke recently for one month; and nonsmokers as those who did not smoke a total of ≥100 cigarettes or for more than six months or did not smoke recently for one month. Former smokers were classified into smokers. The diagnosis of myocardial infarction was confirmed by the presence of a wall motion abnormality by left ventriculography and identification of the responsible stenosis in any of the major coronary arteries or in the left main trunk by coronary anigiography. The diagnosis of ischemic stroke was based on the occurrence of a new and abrupt focal neurologic deficit, with neurologic symptoms and signs persisting for >24 h; it was confirmed by positive findings in computed tomography or magnetic resonance imaging (or both) of the head.

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 genes associated with CKD in the Japanese population in a case-control association study by examining the relations of one to three polymorphisms of each candidate gene to this condition. With the use of public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (OMIM), we selected 32 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 Japanese SNP database (JSNP)], we further selected 40 polymorphisms of these genes, most located in the promoter region or exons, that might be expected to result in changes in the function or expression of the encoded protein (Supplementary 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 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 PCR (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 significantly [false discovery rate (FDR) < 0.05] associated with CKD by the chi-square test are shown in Supplementary Table 2. Detailed genotyping methodology was described previously (13).

Statistical Analysis

Quantitative data were compared between subjects with CKD and controls by the unpaired *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 genotype distributions (3 × 2) or allele frequencies (2 × 2) 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 FDR (14) was calculated from the distribution of *P* values for allele frequency of the 40 polymorphisms. 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 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; the *P* value, FDR, odds ratio, and 95% confidence interval (CI) 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 *versus* wild-type homozygotes) and the additive 2 model (variant homozygotes *versus* wild-type homozygotes), which were analyzed simultaneously with a single statistical model. In multivariable logistic regression analysis, the FDR was calculated from the distribution of 30 *P* values for dominant, recessive, and additive genetic models of selected polymorphisms. Odds ratio of current or former smokers *versus* nonsmokers were also calculated. Allele frequencies of the eight identified polymorphisms were compared by the chi-square test between subgroups of 772 subjects with CKD and 772 controls matched for age, sex, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia individually. With the exception of the initial screen by the chi-square test and multivariable logistic regression analysis (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 5217 study subjects are shown in Table 1. Age, the frequency of male subjects, systolic BP, fasting plasma glucose level, blood glycosylated hemoglobin content, the serum concentration of triglycerides, as well as the prevalence of hypertension, diabetes mellitus, myocardial infarction, and ischemic stroke, were greater, whereas the percentage of smokers and the serum concentration of HDL–cholesterol were lower in subjects with CKD than in controls.

Comparison of allele frequencies with the chi-square test revealed that the −219G→T polymorphism of *APOE* (rs405509), the −519A→G polymorphism of *MMP1* (rs1144393), the −413T→A polymorphism of *HMOX1* (rs2071746), the −866G→A polymorphism of *UCP2* (rs659366), the −1607/1G→2G polymorphism of *MMP1* (rs1799750), the 1429C→T polymorphism of *GNB3* (rs5446), the A→G (Lys45Glu) polymorphism of *MMP3* (rs679620), the G→A (Ala163Thr) polymorphism of *AGTR1* (rs12721226), the G→A (Gly670Arg) polymorphism of *PECAM1* (rs1131012), and the −55C→T polymorphism of *UCP3* (rs1800849) were significantly associated with the prevalence of CKD as defined by an FDR of <0.05 (Table 2). The genotype distributions for these 10 polymorphisms as well as for an additional seven polymorphisms that were related to CKD as defined by a *P* value of <0.05 for allele frequency are also shown in Table 2. In control subjects, the genotype distributions for eight of the initial ten polymorphisms (not those for *HMOX1* and *GNB3*) were in Hardy-Weinberg equilibrium (Supplementary Table 3); the polymorphisms of *HMOX1* and *GNB3* were 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 −219G→T polymorphism of *APOE* (dominant, recessive, and additive 2 models), the −519A→G polymorphism of *MMP1* (dominant and additive 1 and 2 models), the −866G→A polymorphism of *UCP2* (additive 2 model), the −1607/1G→2G polymorphism of *MMP1* (recessive model), the A→G (Lys45Glu) polymorphism of *MMP3* (dominant and additive 1 and 2 models), the G→A (Ala163Thr) polymorphism of *AGTR1* (dominant and additive 1 models), the G→A (Gly670Arg) poly-

Table 1. Characteristics of subjects with chronic kidney disease (CKD) and controls

Characteristic	CKD	Controls	*P*
No. of subjects	778	4439	
Age (yrs)	71.4 ± 9.1	65.6 ± 10.3	<0.0001
Sex (female/male, %)	38.3/61.7	44.2/55.8	0.0019
Body mass index (kg/m^2)	23.4 ± 3.6	23.5 ± 4.5	0.5228
Current or former smoker (%)	18.9	26.9	<0.0001
Hypertension (%)	75.6	54.9	<0.0001
Systolic blood pressure (mmHg)	150 ± 26	140 ± 22	<0.0001
Diastolic blood pressure (mmHg)	80 ± 16	79 ± 13	0.2566
Diabetes mellitus (%)	42.5	24.9	<0.0001
Fasting plasma glucose (mmol/L)	7.28 ± 3.36	6.77 ± 2.81	0.0015
Glycosylated hemoglobin (%)	6.16 ± 1.61	5.74 ± 1.39	<0.0001
Hypercholesterolemia (%)	30.0	27.7	0.2340
Serum total cholesterol (mmol/L)	5.24 ± 1.07	5.17 ± 0.95	0.1631
Serum triglycerides (mmol/L)	1.77 ± 1.07	1.57 ± 1.08	<0.0001
Serum HDL-cholesterol (mmol/L)	1.31 ± 0.43	1.42 ± 0.40	<0.0001
Blood urea nitrogen (mmol/L)	8.36 ± 4.28	5.34 ± 1.51	<0.0001
Serum creatinine (μmol/L)	148.6 ± 167.2	61.9 ± 12.4	<0.0001
eGFR (mL min^{-1} 1.73 m^{-2})	39.4 ± 11.0	79.1 ± 16.6	<0.0001
End-stage renal disease (%)	6.0	0	<0.0001
Myocardial infarction (%)	33.2	18.7	<0.0001
Ischemic stroke (%)	16.1	8.8	<0.0001

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

Table 2. Genotype distributions of polymorphisms related (allele frequency, $P < 0.05$) to chronic kidney disease (CKD) as determined by the chi-square test

Gene symbol	Polymorphism	dbSNP	CKD	Controls	*P* (genotype)	*P* (allele frequency)	FDR
APOE	–219G→T	rs405509	–	–	0.0106	0.0028	0.0463
	GG	–	51 (6.5)	398 (9.0)	–	–	–
	GT	–	419 (53.9)	1876 (42.3)	–	–	–
	TT	–	308 (39.6)	2164 (48.7)	–	–	–
MMP1	–519A→G	rs1144393	–	–	0.0100	0.0043	0.0463
	AA	–	602 (77.5)	3612 (81.4)	–	–	–
	AG	–	163 (21.0)	788 (17.8)	–	–	–
	GG	–	12 (1.5)	35 (0.8)	–	–	–
HMOX1	–413T→A	rs2071746			0.0166	0.0048	0.0463
	TT	–	199 (25.6)	1351 (30.4)	–	–	–
	TA	–	381 (49.0)	2085 (47.0)	–	–	–
	AA	–	198 (25.4)	1002 (22.6)	–	–	–
UCP2	–866G→A	rs659366			0.0201	0.0060	0.0463
	GG	–	187 (24.0)	1219 (27.5)	–	–	–
	GA	–	384 (49.4)	2221 (50.0)	–	–	–
	AA	–	207 (26.6)	998 (22.5)	–	–	–
MMP1	–1607/1G→2G	rs1799750			0.0133	0.0074	0.0463
	1G1G	–	94 (12.1)	482 (10.9)	–	–	–
	1G2G	–	368 (47.3)	1901 (42.8)	–	–	–
	2G2G	–	316 (40.6)	2055 (46.3)	–	–	–
GNB3	1429C→T	rs5446	–	–	0.0264	0.0087	0.0463
	CC	–	538 (69.2)	2886 (65.1)	–	–	–
	CT	–	219 (28.1)	1352 (30.5)	–	–	–
	TT	–	21 (2.7)	193 (4.4)	–	–	–
MMP3	A→G (Lys45Glu)	rs679620	–	–	0.0193	0.0091	0.0463
	AA	–	99 (12.7)	431 (9.7)	–	–	–
	AG	–	343 (44.1)	1927 (43.4)	–	–	–
	GG	–	336 (43.2)	2079 (46.9)	–	–	–
AGTR1	G→A (Ala163Thr)	rs12721226	–	–	0.0092	0.0093	0.0463
	GG	–	773 (99.4)	4430 (99.8)	–	–	–
	GA	–	5 (0.6)	7 (0.2)	–	–	–
	AA	–	0 (0)	0 (0)	–	–	–
PECAM1	G→A (Gly670Arg)	rs1131012	–	–	0.0355	0.0114	0.0498
	GG	–	190 (24.4)	919 (20.7)	–	–	–
	GA	–	383 (49.2)	2206 (49.7)	–	–	–
	AA	–	205 (26.4)	1312 (29.6)	–	–	–
UCP3	–55C→T	rs1800849	–	–	0.0238	0.0125	0.0498
	CC	–	360 (46.3)	2208 (49.8)	–	–	–
	CT	–	329 (42.3)	1847 (41.6)	–	–	–
	TT	–	89 (11.4)	383 (8.6)	–	–	–
MMP3	–1171/5A→6A	rs3025058	–	–	0.0796	0.0254	0.0925
	5A5A	–	21 (2.7)	97 (2.2)	–	–	–
	5A6A	–	218 (28.0)	1095 (24.7)	–	–	–
	6A6A	–	539 (69.3)	3245 (73.1)	–	–	–
PECAM1	1454C→G (Leu125Val)	rs668	–	–	0.0810	0.0303	0.0940
	CC	–	166 (21.3)	1102 (24.8)	–	–	–
	CG	–	396 (50.9)	2213 (49.9)	–	–	–
	GG	–	216 (27.8)	1123 (25.3)	–	–	–
TNF	–850C→T	rs1799724			0.10619	0.0317	0.0940
	CC	–	528 (67.9)	3174 (71.5)	–	–	–
	CT	–	221 (28.4)	1129 (25.5)	–	–	–
	TT	–	29 (3.7)	135 (3.0)	–	–	–

Table 2.—Continued

Gene symbol	Polymorphism	dbSNP	CKD	Controls	*P* (genotype)	*P* (allele frequency)	FDR
CPB2	G→A (Ala147Thr)	rs37742264	–	–	0.0993	0.0329	0.0940
	GG	–	453 (58.2)	2428 (54.7)	–	–	–
	GA	–	282 (36.3)	1693 (38.2)	–	–	–
	AA	–	43 (5.5)	317 (7.1)	–	–	–
HMOX1	G→C (Asp7His)	rs2071747	–	–	0.0498	0.0404	0.1077
	GG	–	676 (86.9)	3711 (83.6)	–	–	–
	GC	–	94 (12.1)	686 (15.5)	–	–	–
	CC	–	8 (1.0)	40 (0.9)	–	–	–
PTGS1	C→T	rs883484	–	–	0.1118	0.0476	0.1123
	CC	–	275 (35.4)	1688 (38.1)	–	–	–
	CT	–	2116 (47.7)	2116 (47.7)	–	–	–
	TT	–	131 (16.9)	631 (14.2)	–	–	–
ABCA1	2583A→G (Ile823Met)	rs4149313	–	–	0.1408	0.0477	0.1123
	AA	–	117 (15.0)	593 (13.4)	–	–	–
	AG	–	365 (46.9)	1998 (45.0)	–	–	–
	GG	–	296 (38.1)	1846 (41.6)	–	–	–

morphism of *PECAM1* (dominant and additive 1 and 2 models), and the −55C→T polymorphism of *UCP3* (recessive and additive 2 models) were significanty (FDR <0.05) associated with the prevalence of CKD (Table 3). The variant *T* allele of *APOE*, *G* allele of the −519A→G polymorphism of *MMP1*, *A* allele of *UCP2*, *A* allele of *AGTR1*, and *T* allele of *UCP3* were risk factors for CKD, whereas the variant *2G* allele of the −1607/1G→2G polymorphism of *MMP1*, *G* allele of *MMP3*, and *A* allele of *PECAM1* were protective against this condition. Multivariable logistic regression analysis with adjustment for age, sex, BMI, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia revealed that odds ratio of current or former smokers *versus* nonsmokers was 0.72 (95% CI, 0.57–0.89; $P = 0.0034$) or 0.21 (95% CI, 0.12–0.36; $P < 0.0001$). Multivariable logistic regression analysis with adjustment for age, sex, BMI, smoking status, systolic BP, fasting plasma glucose level, and the serum concentration of total cholesterol revealed that the −219G→T polymorphism of *APOE* (dominant, recessive, and additive 1 and 2 models), the −866G→A polymorphism of *UCP2* (recessive model), and the G→A (Gly670Arg) polymorphism of *PECAM1* (additive 2 model) were significantly ($P < 0.05$) associated with the prevalence of CKD (Supplementary Table 4). Comparison of allele frequencies of the eight identified polymorphisms by the chi-square test between subgroups of CKD and control subjects matched for age, sex, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia individually revealed that the −519A→G polymorphism of *MMP1* and the −866G→A polymorphism of *UCP2* were significantly ($P < 0.05$) associated with CKD (Table 4). Comparison of allele frequencies of the eight identified polymorphisms by the chi-square test between subjects with or without diabetes mellitus (Supplementary Table 5) or between subjects with or without hypertension (Supplementary Table 6) revealed that none of these polymorphisms were related to diabetes mellitus or hypertension.

Discussion

We examined the possible relations of 40 polymorphisms in 32 candidate genes to the prevalence of CKD in 5217 Japanese individuals. Our association study with three steps of analysis (chi-square test, multivariable logistic regression analysis, and matched case-control analysis) revealed that the −519A→G polymorphism of *MMP1* (rs1144393) and the −866G→A polymorphism of *UCP2* (rs659366) were significantly associated with the prevalence of CKD.

The −519A→G polymorphism of *MMP1*, which is located in the promoter region of the gene, has been related to the risk of myocardial infarction as part of a haplotype including other polymorphisms of *MMP1* (15). In addition, the *G* allele of this polymorphism was associated with an increased intima-media thickness of the carotid artery in a German population with hypertension (16). We have now shown that the −519A→G polymorphism of *MMP1* was significantly associated with the prevalence of CKD, with the *G* allele representing a risk factor for this condition. Effects of this polymorphism on the development of atherosclerosis may account for its association with CKD.

The *A* allele of the −866G→A polymorphism in the promoter region of *UCP2* was associated with increased transcription of the gene in a human adipocyte cell line PAZ-6 and with a modest but significant reduction in the prevalence of obesity (17). The *A* allele of the −866G→A variant was also associated with reduced risk of coronary heart disease in men with type 2 diabetes mellitus in a 6-yr prospective study (18). The deletion allele of the insertion/deletion polymorphism in the

Table 3. Multivariable logistic regression analysis of polymorphisms related to chronic kidney disease

Symbol	Polymorphism	Dominant			Recessive			Additive 1			Additive 2		
		P	FDR	OR (95% CI)	*P*	FDR	OR (95% CI)	*P*	FDR	OR (95% CI)	*P*	FDR	OR (95% CI)
APOE	–219G→T	**0.0214**	**0.043**	1.44 (1.07–2.00)	**0.0191**	**0.041**	1.21 (1.03–1.42)	0.0858	0.107		**0.0085**	**0.026**	1.54 (1.13–2.15)
MMP1	–519A→G	**0.0059**	**0.022**	1.31 (1.08–1.59)	**0.0351**	0.055	2.17 (1.02–4.35)	**0.0168**	**0.039**	1.27 (1.04–1.55)	**0.0255**	**0.045**	2.27 (1.07–4.57)
UCP2	–866G→A	0.1150	0.133	–	**0.0354**	0.053	1.22 (1.01–1.46)	0.3456	0.358		**0.0242**	**0.045**	1.29 (1.03–1.62)
MMP1	–1607/1G→2G	0.2422	0.269	–	**0.0053**	**0.023**	0.79 (0.68–0.93)	0.7745	0.775		0.0517	0.074	
MMP3	A→G (Lys45Glu)	**0.0011**	**0.017**	0.66 (0.52–0.85)	0.0731	0.095	–	**0.0051**	**0.026**	0.69 (0.53–0.90)	**0.0008**	**0.024**	0.64 (0.50–0.83)
AGTR1	G→A (Ala163Thr)	**0.0040**	**0.040**	6.69 (1.74–24.2)	–	–	–	**0.0040**	**0.040**	6.69 (1.74–24.2)			
PECAM1	G→A (Gly670Arg)	**0.0093**	**0.023**	0.78 (0.65–0.94)	0.1110	0.133	–	**0.0297**	**0.049**	0.80 (0.66–0.98)	**0.0092**	**0.025**	0.74 (0.59–0.93)
UCP3	–55C→T	0.0544	0.074	–	**0.0080**	**0.027**	1.42 (1.09–1.82)	0.2436	0.261		**0.0041**	**0.025**	1.48 (1.13–1.94)

FDR, false discovery rate; OR, odds ratio; CI, confidence interval.
Multivariable logistic regression analysis was performed with adjustment for age, sex, BMI, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia. *P* and FDR values of <0.05 are shown in bold.

3′ region of *UCP2* was related to ESRD in Indian subjects (19). We have now shown that the −866G→A polymorphism of *UCP2* was significantly associated with the prevalence of CKD, with the *A* allele representing a risk factor for this condition, although the underlying molecular mechanism remains elucidated.

There were differences in the results between multivariable logistic regression analysis and chi-square test individually matched for covariates. Multivariable logistic regression analysis could not fully overcome substantial differences between the CKD and control groups, while the number of control individuals was smaller in the matched case-control analysis than in multivariable logistic regression analysis. Given that the −519A→G polymorphism of *MMP1* and the −866G→A polymorphism of *UCP2* were significantly associated with CKD in both analyses, these genes may be considered as susceptibility loci for this condition.

Although previous studies showed that smoking is a risk factor for CKD (20) and that current smokers are at higher risk of CKD compared with former smokers (21), the frequency of smoking was significantly lower in subjects with CKD than in controls in the present study, even though there was a possibility of more cigarettes and the longer term of smoking in subjects with CKD than in controls. In a case-control study, it is important that controls are selected from the group of individuals who would have been considered for selection as cases, if they had the disease. Furthermore, the exposure history of controls should be representative of the exposure history of the population from which the cases were selected. Given that subjects were recruited by different methods, selection bias could not be excluded in the present study.

Our study has several limitations: (*1*) We used an estimated GFR (eGFR) instead of a directly measured rate to define CKD. (*2*) We were not able to obtain information on proteinuria, the underlying renal disease, or the primary cause of CKD in all subjects 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. (*3*) 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. (*4*) The functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study. (*5*) Given that the subjects with CKD in the present study were survivors of CKD, they were likely not representative of all CKD patients, and there was a possibility of survivor bias. (*6*) 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. The association of gene polymorphisms with CKD in the present study was not confirmed in the databases of other studies. Our present study can thus be considered as hypothesis generating.

In conclusion, our present results suggested that *MMP1*

Table 4. Comparison by the chi-square test of genotype distributions of the eight identified polymorphisms between subgroups of chronic kidney disease (CKD) and control subjects matched for age, sex, smoking status, and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia

Gene symbol	Polymorphism	dbSNP	CKD	Controls	*P* (genotype)	*P* (allele frequency)
APOE	–219G→T	rs405509			0.4027	0.1844
	GG	–	50 (6.5)	59 (7.7)	–	–
	GT	–	311 (40.3)	326 (42.2)	–	–
	TT	–	411 (53.2)	387 (50.1)	–	–
MMP1	–519A→G	rs1144393	–	–	0.0162	0.0078
	AA	–	599 (77.7)	637 (82.5)	–	–
	AG	–	160 (20.7)	131 (17.0)	–	–
	GG	–	12 (1.6)	4 (0.5)	–	–
UCP2	–866G→A	rs659366			0.1173	0.0438
	GG	–	185 (24.0)	209 (27.1)	–	–
	GA	–	384 (49.7)	392 (50.8)	–	–
	AA	–	203 (26.3)	171 (22.1)	–	–
MMP1	–1607/1G→2G	rs1799750			0.1075	0.0526
	1G1G	–	92 (11.9)	82 (10.6)	–	–
	1G2G	–	366 (47.4)	335 (43.4)	–	–
	2G2G	–	314 (40.7)	355 (46.0)	–	–
MMP3	A→G (Lys45Glu)	rs679620	–	–	0.0297	0.0612
	AA	–	98 (12.7)	66 (8.6)	–	–
	AG	–	342 (44.3)	357 (46.2)	–	–
	GG	–	332 (43.0)	349 (45.2)	–	–
AGTR1	G→A (Ala163Thr)	rs12721226	–	–	0.0874	0.0877
	GG	–	767 (99.4)	771 (99.9)	–	–
	GA	–	5 (0.6)	1 (0.1)	–	–
	AA	–	0 (0)	0 (0)	–	–
PECAM1	G→A (Gly670Arg)	rs1131012			0.4451	0.2074
	GG	–	190 (24.6)	171 (22.1)	–	–
	GA	–	379 (49.1)	382 (49.5)	–	–
	AA	–	203 (26.3)	219 (28.4)	–	–
UCP3	–55C→T	rs1800849	–	–	0.4222	0.2610
	CC	–	357 (46.2)	371 (48.1)	–	–
	CT	–	330 (42.8)	331 (42.9	–	–
	TT	–	85 (11.0)	70 (9.0)	–	–

and *UCP2* might be susceptibility loci for CKD in the Japanese population. Determination of genotypes for the studied polymorphisms of these genes may prove informative for prediction of the genetic risk for CKD. 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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Disclosures

None.

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