Association of genetic variants with chronic kidney disease in individuals with different lipid profiles

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Abstract. The purpose of the present study was to identify genetic variants that confer susceptibility to chronic kidney disease (CKD) in individuals with low or high serum concentrations of triglycerides (TG), high-density lipoprotein (HDL)-cholesterol, or low-density lipoprotein (LDL)-cholesterol, thereby contributing to the personalized prevention of CKD in such individuals. The study population comprised 5944 Japanese individuals, including 1706 subjects with CKD [estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2] and 4238 controls (eGFR ≥60 ml/min/1.73 m^2). The genotypes for 296 polymorphisms of 202 candidate genes were determined. The Chi-square test, multivariable logistic regression analysis with adjustment for covariates, and a stepwise forward selection procedure revealed that seven different polymorphisms were significantly (P<0.005) associated with the prevalence of CKD in individuals with low or high serum concentrations of TG or HDL- or LDL-cholesterol: the A→G (Glu23Lys) polymorphism of *KCNJ11* and the 125592C→A (Thr431Asn) polymorphism of *ROCK2* in individuals with low serum TG; the 734C→T (Thr254Ile) polymorphism of *ACAT2* and the C→G (Gln27Glu) polymorphism of *ADRB2* in individuals with high serum TG; the -1607/1G→2G polymorphism of *MMP1* in individuals with low serum HDL-cholesterol; the G→A (Val158Met) polymorphism of *COMT* in individuals with low serum LDL-cholesterol; the 584G→A (Gln192Arg) polymorphism of *PON1* in individuals with high serum LDL-cholesterol. No polymorphism was associated with CKD in individuals with high serum HDL-cholesterol. These results suggest that polymorphisms associated with CKD may differ among individuals with different lipid profiles. Stratification of subjects according to lipid profiles may thus be important for personalized prevention of CKD based on genetic information.

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Introduction

Chronic kidney disease (CKD) has been recognized as a global public health problem; individuals with CKD are at an increased risk not only for end-stage renal disease (ESRD), but also for a poor cardiovascular outcome and premature death (1,2). CKD is a multifactorial disorder that is thought to result from an interaction between genetic background and environmental factors. Principal and treatable risk factors for CKD include diabetes mellitus (3), glomerular nephritis (4), hypertension (5,6), and hyperlipidemia or dyslipidemia (7,8). However, the effect of lipid profiles on the development of CKD is controversial given that there are no large studies proving the effect of lipid reduction on the progression of renal disease (9). Disease prevention is an important strategy for reducing the overall burden of CKD and ESRD, and the identification of markers for disease risk is a key both for risk prediction and for potential intervention to reduce the chance of future cardiovascular events (10). It is thus important to identify markers that confer susceptibility to CKD in individuals with different lipid profiles independently.

Although genetic linkage analyses (11,12) and association studies (13,14) have implicated several loci and candidate genes in predisposition to CKD, the genes that contribute to

genetic susceptibility to CKD remain largely unknown. 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 hypothesized that the association of gene polymorphisms with CKD might be influenced by baseline lipid profiles. Here, we performed an association study of 296 polymorphisms of 202 candidate genes and CKD in 5944 Japanese individuals with low or high serum concentrations of triglycerides (TG), high-density lipoprotein (HDL)-cholesterol, or low-density lipoprotein (LDL)-cholesterol. The purpose of the present study was to identify genetic variants that confer susceptibility to CKD in individuals with different lipid profiles independently and thereby to assess the genetic risk of CKD in such individuals separately.

Materials and methods

Study population. The study population comprised 5944 unrelated Japanese individuals 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.

The glomerular filtration rate 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 (15): eGFR (ml min^{-1} 1.73 m^{-2}) = 194 x [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} (10). 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} (16). We thus adopted the criterion of an eGFR of <60 ml min^{-1} 1.73 m^{-2} for diagnosis of CKD in the present study. On the basis of this criterion, 1706 subjects were diagnosed with CKD. The control subjects comprised 4238 individuals whose eGFR was ≥60 ml min^{-1} 1.73 m^{-2}. The control subjects were recruited from community-dwelling healthy individuals or from patients visiting 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), or diabetes mellitus (fasting blood glucose of ≥6.93 mmol/l or hemoglobin A_{1c} of ≥6.5%, or both, or taking antidiabetes medication). Among the total study population, 3946 and 1998 individuals had low (<1.70 mmol/l) or high (≥1.70 mmol/l) serum concentrations of TG, respectively, and 1023 and 4921 individuals had low (<1.03 mmol/l) or high (≥1.03 mmol/l) serum concentrations of HDL-cholesterol, respectively. The values for LDL-cholesterol were calculated by the Friedewald formula: serum concentration of LDL-cholesterol = (serum concentration of total cholesterol) - (serum concentration of HDL-cholesterol) - [0.2 x (serum concentration of TG)]. Among the total study population, 4535 and 1409 individuals had low (<3.63 mmol/l) or high (≥3.63 mmol/l) serum concentrations of LDL-cholesterol, respectively.

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 the participating hospitals. Written informed consent was obtained from each participant.

Selection of polymorphisms. With the use of public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (OMIM), we selected 202 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 296 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 (data not shown).

Genotyping of polymorphisms. Venous blood (7 ml) was collected in tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 296 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). Detailed genotyping methodology was described previously (17).

Statistical analysis. Quantitative data were compared between subjects with CKD and controls using the unpaired Student's t-test. Categorical data were compared with the Chi-square test. Allele frequencies were estimated using the gene counting method. In the initial screen, the genotype distributions (3x2) or allele frequencies (2x2) of each polymorphism were compared between subjects with CKD and the controls with the Chi-square test. Polymorphisms related (P-value for allele frequency <0.05) to CKD were further examined by multivariable logistic regression analysis with adjustment for covariates, with CKD as a dependent variable and independent variables including age, gender (0, woman; 1, man), body mass index (BMI), smoking status (0, nonsmoker; 1, smoker), serum concentrations of TG or HDL- or LDL-cholesterol, history of hypertension or diabetes mellitus (0, no history; 1, positive history), and genotype of each polymorphism; 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

Table I. Characteristics of subjects with low or high serum concentrations of triglycerides (TG).

Characteristic	Low serum TG			High serum TG		
	CKD	Controls	P-value	CKD	Controls	P-value
No. of subjects	1073	2873		633	1365	
Age (years)	70.5±8.9	66.3±9.5	<0.0001	69.9±8.7	65.3±9.4	<0.0001
Gender (female/male, %)	40.3/59.7	47.4/52.6	<0.0001	37.0/63.0	38.9/61.1	0.4074
Body mass index (kg/m^2)	23.0±3.4	23.0±3.2	0.7731	24.2±3.2	24.5±6.3	0.2997
Current or former smoker (%)	21.1	25.4	0.0045	21.2	30.5	<0.0001
Hypertension (%)	67.4	51.1	<0.0001	70.5	61.5	<0.0001
Diabetes mellitus (%)	31.2	21.9	<0.0001	37.9	28.7	<0.0001
Serum TG (mmol/l)	1.11±0.32	1.06±0.33	<0.0001	2.44±0.64	2.49±0.68	0.2150
Serum HDL-cholesterol (mmol/l)	1.40±0.41	1.49±0.41	<0.0001	1.21±0.35	1.25±0.32	0.0001
Serum LDL-cholesterol (mmol/l)	3.07±0.85	3.04±0.82	0.1419	3.19±0.93	3.11±0.94	0.0754
Serum creatinine (μmol/l)	108.8±111.3	60.7±12.3	<0.0001	114.3±34.5	120.6±12.3	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	49.0±10.7	79.9±17.5	<0.0001	47.8±11.1	77.0±13.8	<0.0001
End-stage renal disease (%)	2.6	0	<0.0001	2.5	0	<0.0001
Myocardial infarction (%)	26.1	15.7	<0.0001	29.4	23.7	0.0075
Ischemic stroke (%)	14.1	9.3	<0.0001	13.7	8.0	<0.0001

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

(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. Given the multiple comparisons of genotypes with CKD, we adopted the criterion of a P-value of <0.005 for significant association. For other clinical background data, a P-value <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed with JMP version 6.0 software (SAS Institute, Cary, NC).

Results

Polymorphisms related to CKD in individuals with low or high serum concentrations of TG. The characteristics of the subjects with CKD and controls who had low or high serum concentrations of TG are listed in Table I. For individuals with low serum TG, age, the frequency of male subjects, the serum concentration of TG, 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. For individuals with high serum TG, age and 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 nine and seven polymorphisms were related (P<0.05) to CKD in individuals with low or high serum concentrations of TG, respectively (Table II). Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of HDL- and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus revealed that the A→G (Lys45Glu) polymorphism of *MMP3* (additive 2 model), the A→G (Glu23Lys) polymorphism of *KCNJ11* (recessive and additive 2 models), and the 125592C→A (Thr431Asn) polymorphism of *ROCK2* (dominant model) were significantly (P<0.005) associated with CKD in individuals with low serum TG, and that the 1429C→T polymorphism of *GNB3* (dominant model), the C→T polymorphism of *PTGS1* (additive 2 model), the C→G (Gln27Glu) polymorphism of *ADRB2* (dominant model), and the 734C→T (Thr254Ile) polymorphism of *ACAT2* (dominant and additive 1 models) were significantly associated with CKD in individuals with high serum TG (Table III).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms associated with CKD by multivariable logistic regression analysis as well as of age, gender, BMI, smoking status, serum concentrations of HDL- and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus on CKD (Table IV). For individuals with low serum TG, age, hypertension, serum HDL-cholesterol, diabetes mellitus, gender, the *ROCK2* genotype (dominant model), smoking, and the *KCNJ11* genotype (recessive model) were significant (P<0.005) and independent determinants of CKD. For individuals with high serum TG, age, diabetes mellitus, smoking, gender, *ADRB2* (dominant model) and *ACAT2*

Table II. Genotype distributions of polymorphisms related (allele frequency, P<0.05) to chronic kidney disease (CKD) with low or high serum concentrations of triglycerides (TG) as determined by the Chi-square test.

Gene symbol	Polymorphism	dbSNP	CKD	Controls	P-value (genotype)	P-value (allele frequency)
Low serum TG						
MMP3	A→G (Lys45Glu)	rs679620			0.0164	0.0005
	AA		133 (12.4)	276 (9.6)		
	AG		478 (44.5)	1251 (43.6)		
	GG		462 (43.1)	1344 (46.8)		
KCNJ11	A→G (Glu23Lys)	rs5219			0.0120	0.0033
	AA		176 (16.4)	403 (14.0)		
	AG		520 (48.5)	1320 (46.0)		
	GG		377 (35.1)	1149 (40.0)		
APOE	-219G→T	rs405509			0.0135	0.0071
	GG		87 (8.1)	260 (9.0)		
	GT		413 (38.5)	1229 (42.8)		
	TT		573 (53.4)	1383 (48.2)		
TNFRSFBB	C→T (Pro251Leu)	rs34562254			0.0187	0.0074
	CC		474 (44.2)	1128 (39.3)		
	CT		456 (42.6)	1318 (45.9)		
	TT		141 (13.2)	423 (14.8)		
MMP3	-1171/5A→6A	rs3025058			0.0146	0.0076
	5A6A		38 (3.6)	61 (2.1)		
	5A6A		291 (27.1)	719 (25.1)		
	6A6A		744 (69.3)	2091 (72.8)		
ROCK2	125592C→A (Thr431Asn)	rs10011540			0.0380	0.0135
	CC		402 (37.7)	954 (33.5)		
	CA		510 (47.8)	1424 (50.1)		
	AA		154 (14.5)	467 (16.4)		
UCP1	-112A→C	rs10011540			0.0480	0.0163
	AA		931 (86.8)	2407 (83.8)		
	AC		139 (12.9)	449 (15.6)		
	CC		3 (0.3)	16 (0.6)		
ZNF627	A→G	rs4804611			0.0312	0.0209
	AA		867 (80.9)	2238 (78.0)		
	AG		198 (18.5)	590 (20.6)		
	GG		7 (0.6)	41 (1.4)		
THBD	2136C→T (Ala455Val)	rs1042579			0.0246	0.0230
	CC		618 (57.6)	1575 (54.8)		
	CT		402 (37.5)	1091 (38.0)		
	TT		53 (4.9)	206 (7.2)		
High serum TG						
ABCA1	-477C→T	rs2422493			0.0475	<0.0001
	CC		222 (35.1)	35 (2.6)		
	CT		293 (46.3)	395 (29.0)		
	TT		118 (18.6)	934 (68.4)		
GNB3	1429C→T	rs5446			0.0056	0.0012
	CC		454 (71.7)	882 (64.7)		
	CT		162 (25.6)	427 (31.3)		
	TT		17 (2.7)	55 (4.0)		
PTGS1	C→T	rs883484			0.0138	0.0036
	CC		212 (33.5)	537 (39.3)		
	CT		311 (49.1)	643 (47.1)		
	TT		110 (17.4)	185 (13.6)		

Table II. Continued.

Gene symbol	Polymorphism	dbSNP	CKD	Controls	P-value (genotype)	P-value (allele frequency)
ADRB2	C→G (Gln27Glu)	rs1042714			0.0057	0.0042
	CC		572 (90.4)	1175 (86.1)		
	CG		61 (9.6)	185 (13.5)		
	GG		0 (0.0)	5 (0.4)		
WRN	T→C (Cys1367Arg)	rs1346044			0.0245	0.0063
	TT		550 (86.9)	1123 (82.3)		
	TC		80 (12.6)	229 (16.8)		
	CC		3 (0.5)	13 (0.9)		
COMT	G→A (Val158Met)	rs4680			0.0202	0.0072
	GG		251 (39.6)	630 (46.2)		
	GA		303 (47.9)	594 (43.5)		
	AA		79 (12.5)	141 (10.3)		
ACAT2	734C→T (Thr254Ile)	rs2272296			0.0140	0.0456
	CC		261 (41.2)	474 (34.8)		
	CT		275 (43.5)	679 (49.8)		
	TT		97 (15.3)	211 (15.4)		

genotypes (dominant model) were significant and independent determinants of CKD.

Polymorphisms related to CKD in individuals with low or high serum concentrations of HDL-cholesterol. The characteristics of the subjects with CKD and controls who had low or high serum concentrations of HDL-cholesterol are listed in Table V. For individuals with low serum HDL-cholesterol, age and the prevalence of hypertension were greater, whereas BMI and the percentage of smokers were lower in subjects with CKD than in controls. For individuals with high serum HDL-cholesterol, age, the frequency of male subjects, and the serum concentrations of TG and LDL-cholesterol, 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 seven and five polymorphisms were related ($P<0.05$) to CKD in individuals with low or high serum concentrations of HDL-cholesterol, respectively (Table VI). Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of TG and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus revealed that the 34C→G (Pro12Ala) polymorphism of *PPARG* (additive 1 model) and the -1607/1G→2G polymorphism of *MMP1* (recessive model) were significantly ($P<0.005$) associated with CKD in individuals with low serum HDL-cholesterol, and that the -519A→G polymorphism of *MMP1* (additive 2 model) was significantly associated with CKD in individuals with high serum HDL-cholesterol (Table VII).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms associated with CKD by multivariable logistic regression analysis as well as of age, gender, BMI, smoking status, serum concentrations of TG and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus on CKD (Table VIII). For individuals with low serum HDL-cholesterol, age, hypertension, and the *MMP1* genotype (recessive model) were significant ($P<0.005$) and independent determinants of CKD. For individuals with high serum HDL-cholesterol, age, hypertension, diabetes mellitus, gender, and smoking were significant and independent determinants of CKD.

Polymorphisms related to CKD in individuals with low or high serum concentrations of LDL-cholesterol. The characteristics of the subjects with CKD and controls who had low or high serum concentrations of LDL-cholesterol are listed in Table IX. For individuals with low or high serum LDL-cholesterol, age, the frequency of male subjects, the serum concentration of TG, 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 different sets of four polymorphisms were related ($P<0.05$) to CKD in individuals with low or high serum concentrations of LDL-cholesterol (Table X). Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status, serum concentrations of TG and HDL-cholesterol, and the prevalence of hypertension and diabetes mellitus revealed that the G→A (Val158Met) polymorphism of *COMT* (dominant and additive 1 models) and the A→G (Lys45Glu) polymorphism of *MMP3* (additive 2 model) were significantly ($P<0.005$) associated with CKD in individuals with low serum LDL-cholesterol, and that the 584G→A

Table III. Multivariable logistic regression analysis of polymorphisms related to chronic kidney disease with low or high serum concentrations of triglycerides (TG).

		Dominant		Recessive		Additive 1		Additive 2	
Symbol	Polymorphism	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Low serum TG									
MMP3	A→G (Lys45Glu)	0.0051	0.72 (0.57-0.91)	0.0538		0.0219	0.75 (0.59-0.96)	**0.0026**	0.69 (0.54-0.88)
KCNJ11	A→G (Glu23Lys)	0.0226	0.79 (0.64-0.97)	**0.0023**	0.79 (0.68-0.92)	0.1804		**0.0022**	0.70 (0.56-0.88)
APOE	-219G→T	0.4298		0.0203	1.19 (1.03-1.38)	0.9407		0.1876	→
TNFRSFBB	C→T (Pro251Leu)	0.0066	0.81 (0.70-0.94)	0.3888		0.0102	0.81 (0.69-0.95)	0.0842	
MMP3	-1171/5A→6A	0.0317	0.62 (0.40-0.96)	0.0221	0.83 (0.70-0.97)	0.1131		0.0203	0.59 (0.38-0.92)
ROCK2	125592C→A (Thr431Asn)	**0.0032**	0.79 (0.68-0.92)	0.1057		0.0105	0.81 (0.69-0.96)	0.0111	0.75 (0.60-0.95)
UCP1	-112A→C	0.0347	0.80 (0.64-0.98)	0.4686		0.0432	0.80 (0.64-1.00)	0.4390	
ZNF627	A→G	0.1028		0.3888		0.2222		0.0431	0.43 (0.17-0.92)
THBD	2136C→T (Ala455Val)	0.1843		0.0303	0.70 (0.50-0.96)	0.4704		0.0236	0.68 (0.49-0.94)
High serum TG									
ABCA1	-477C→T	0.3528		0.0068	1.44 (1.10-1.87)	0.9566		0.0136	1.44 (1.08-1.93)
GNB3	1429C→T	**0.0038**	0.73 (0.59-0.90)	0.0629		0.0138	0.76 (0.60-0.94)	0.0334	0.54 (0.29-0.93)
PTGS1	C→T	0.0173	1.28 (1.05-1.58)	0.0145	1.40 (1.07-1.83)	0.0890		**0.0034**	1.56 (1.16-2.10)
ADRB2	C→G (Gln27Glu)	**0.0047**	0.63 (0.46-0.86)	0.6605		0.0083	0.65 (0.47-0.89)	0.6577	
WRN	T→C (Cys1367Arg)	0.0061	0.68 (0.51-0.89)	0.3927		0.0087	0.68 (0.51-0.90)	0.3471	
COMT	G→A (Val158Met)	0.0091	1.31 (1.07-1.59)	0.1529		0.0223	1.28 (1.04-1.58)	0.0356	1.42 (1.02-1.97)
ACAT2	734C→T (Thr254Ile)	**0.0023**	0.73 (0.60-0.89)	0.8462		**0.0016**	0.71 (0.57-0.88)	0.1475	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, BMI, smoking status, serum concentrations of HDL- and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus. P-values <0.005 are shown in bold.

Table IV. Effects of genotypes and other characteristics on the prevalence of chronic kidney disease with low or high serum concentrations of triglycerides (TG) as determined by a stepwise forward selection procedure.

Variable	P-value	R^2
Low serum TG		
Age	<0.0001	0.0530
Hypertension	<0.0001	0.0114
Serum HDL-cholesterol	<0.0001	0.0047
Diabetes mellitus	0.0002	0.0030
Gender	0.0013	0.0023
ROCK2 (*AA+CA* versus *CC*)	0.0020	0.0022
Smoking	0.0042	0.0018
KCNJ11 (*GG* versus *AA+AG*)	0.0045	0.0017
High serum TG		
Age	<0.0001	0.0429
Diabetes mellitus	<0.0001	0.0090
Smoking	0.0005	0.0048
Gender	0.0013	0.0042
ADRB2 (*GG+CG* versus *CC*)	0.0022	0.0037
ACAT2 (*TT+CT* versus *CC*)	0.0024	0.0037

R^2, contribution rate.

(Gln192Arg) polymorphism of *PON1* (dominant model) and the 2583A→G (Ile823Met) polymorphism of *ABCA1* (additive 2 model) were significantly associated with CKD in individuals with high serum LDL-cholesterol (Table XI).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphisms associated with CKD by multivariable logistic regression analysis as well as of age, gender, BMI, smoking status, serum concentrations of TG and HDL-cholesterol, and the prevalence of hypertension and diabetes mellitus on CKD (Table XII). For individuals with low serum LDL-cholesterol, age, hypertension, diabetes mellitus, serum HDL-cholesterol, smoking, gender, and the *COMT* genotype (dominant model) were significant (P<0.005) and independent determinants of CKD. For individuals with high serum LDL-cholesterol, age, serum HDL-cholesterol, the *PON1* genotype (dominant model), and smoking were significant and independent determinants of CKD.

Discussion

We examined the possible relations of 296 polymorphisms of 202 candidate genes to the prevalence of CKD in individuals with low or high serum concentrations of TG or HDL- or LDL-cholesterol, given that interactions between gene polymorphisms and lipid profiles may be important in the development of CKD. Our association study with three steps of analysis (Chi-square test, multivariable logistic regression analysis, and stepwise forward selection procedure) revealed that seven different polymorphisms were significantly associated with the prevalence of CKD in individuals with different lipid profiles.

Rho-associated, coiled-coil containing protein kinase (ROCK) 1 and 2 are immediate downstream targets of a small GTP-binding protein RhoA (18). The ROCKs affect several cellular functions, one of which is cellular contraction, by controlling actin cytoskeletal assembly (19). The *Asn* allele of the 125592C→A (Thr431Asn) polymorphism of

Table V. Characteristics of subjects with low or high serum concentrations of HDL-cholesterol.

	Low serum HDL-cholesterol			High serum HDL-cholesterol		
Characteristic	CKD	Controls	P-value	CKD	Controls	P-value
No. of subjects	374	649		1332	3589	
Age (years)	70.4±9.2	64.8±10.4	<0.0001	71.1±8.7	65.9±10.2	<0.0001
Gender (female/male, %)	23.0/77.0	20.3/79.7	0.3196	43.5/56.5	49.1/50.9	0.0005
Body mass index (kg/m^2)	23.8±3.3	24.3±3.1	0.0032	23.4±3.4	23.4±4.7	0.5825
Current or former smoker (%)	22.7	37.4	<0.0001	20.7	25.1	0.0009
Hypertension (%)	74.3	62.4	<0.0001	66.9	53.0	<0.0001
Diabetes mellitus (%)	42.8	36.7	0.0541	31.2	21.8	<0.0001
Serum TG (mmol/l)	1.89±0.87	1.86±0.92	0.3634	1.52±0.75	1.46±0.78	0.0001
Serum HDL-cholesterol (mmol/l)	0.88±0.11	0.88±0.11	0.9646	1.46±0.36	1.51±0.35	<0.0001
Serum LDL-cholesterol (mmol/l)	3.07±0.86	3.02±0.87	0.1361	3.13±0.89	3.07±0.86	0.0362
Serum creatinine (μmol/l)	125.1±144.5	65.5±12.8	<0.0001	106.8±104.7	61.1±12.2	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	46.9±11.8	80.2±17.0	<0.0001	49.0±10.5	78.7±16.3	<0.0001
End-stage renal disease (%)	3.7	0	<0.0001	2.3	0	<0.0001
Myocardial infarction (%)	39.6	36.8	0.3836	23.9	15.0	<0.0001
Ischemic stroke (%)	14.7	16.6	0.4132	13.7	7.5	<0.0001

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

Table VI. Genotype distributions of polymorphisms related (allele frequency, P<0.05) to chronic kidney disease (CKD) with low or high serum concentrations of HDL-cholesterol as determined by the Chi-square test.

Gene symbol	Polymorphism	dbSNP	CKD	Controls	P-value (genotype)	P-value (allele frequency)
Low serum HDL-cholesterol						
SERPINE1	-668/4G→5G	rs1799768			0.0135	0.0030
	4G4G		134 (35.8)	292 (45.0)		
	4G5G		166 (49.4)	255 (39.3)		
	5G5G		74 (19.8)	102 (15.7)		
PTGS1	C→T	rs883484			0.0147	0.0078
	CC		115 (30.8)	257 (39.6)		
	CT		198 (52.9)	305 (47.1)		
	TT		61 (16.3)	86 (13.3)		
PPARG	34C→G	rs1801282			0.0133	0.0119
	CC		340 (90.9)	618 (95.2)		
	CG		34 (9.1)	30 (4.6)		
	GG		0 (0.0)	1 (0.2)		
MMP1	-1607/1G→2G	rs1799750			0.0003	0.0123
	1G1G		40 (10.7)	76 (11.7)		
	1G2G		185 (49.5)	238 (36.7)		
	2G2G		149 (39.8)	335 (51.6)		
TNFRSF4	2210A→G	rs2298212			0.0443	0.0147
	AA		8 (2.3)	6 (1.0)		
	AG		93 (26.9)	122 (21.4)		
	GG		245 (70.8)	443 (77.6)		
IL10	-592A→C	rs1800872			0.0306	0.0163
	AA		191 (51.1)	276 (42.5)		
	AC		148 (39.6)	301 (46.4)		
	CC		35 (9.3)	72 (11.1)		
IL10	-819T→C	rs1800871			0.0392	0.0197
	TT		191 (51.1)	278 (42.8)		
	TC		148 (39.6)	299 (46.1)		
	CC		35 (9.3)	72 (11.1)		
High serum HDL-cholesterol						
MMP1	-519A→G	rs1144393			0.0051	0.0056
	AA		1048 (78.8)	2927 (81.6)		
	AG		260 (19.5)	631 (17.6)		
	GG		23 (1.7)	28 (0.8)		
WRN	T→C (Cys1367Arg)	rs1346044			0.0303	0.0102
	TT		1158 (86.9)	3022 (84.2)		
	TC		169 (12.7)	539 (15.0)		
	CC		5 (0.4)	27 (0.8)		
KCNJ11	A→G (Glu23Lys)	rs5219			0.0417	0.0117
	AA		212 (15.9)	499 (13.9)		
	AG		639 (48.0)	1666 (46.4)		
	GG		481 (36.1)	1423 (39.7)		
UCP3	-55C→T	rs1800849			0.0477	0.0296
	CC		638 (47.9)	1800 (502)		
	CT		545 (40.9)	1468 (40.9)		
	TT		149 (11.2)	320 (8.9)		
HMOX1	-413T→A	rs2071746			0.0453	0.0355
	TT		359 (26.9)	1097 (30.6)		
	TA		659 (49.5)	1686 (47.0)		
	AA		314 (23.6)	805 (22.4)		

Table VII. Multivariable logistic regression analysis of polymorphisms related to chronic kidney disease with low or high serum concentrations of HDL-cholesterol.

Symbol	Polymorphism	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)
Low serum HDL-cholesterol									
SERPINE1	-668/4G→5G	0.0314	1.36 (1.03-1.79)	0.1827		0.0741		0.0537	
PTGS1	C→T	0.0360	1.36 (1.02-1.81)	0.2837		0.0623		0.0784	
PPARG	34C→G (Pro12Ala)	0.0050	2.18 (1.27-3.78)	0.8402		**0.0043**	2.22 (1.29-3.86)	0.8438	
MMP1	-1607/1G→2G	0.4618		**0.0011**	0.63 (0.48-0.83)	0.0541		0.6659	
TNFRSF4	2210A→G	0.5764		0.0355	0.70 (0.51-0.98)	0.9441		0.4895	
IL10	-592A→C	0.0060	0.68 (0.52-0.90)	0.5488		0.0071	0.67 (0.50-0.90)	0.1755	
IL10	-819T→C	0.0076	0.69 (0.52-0.91)	0.5488		0.0090	0.68 (0.51-0.91)	0.1837	
High serum HDL-cholesterol									
MMP1	-519A→G	0.0170	1.22 (1.04-1.44)	0.0055	2.39 (1.28-4.41)	0.0633		**0.0041**	2.46 (1.32-4.55)
WRN	T→C (Cys1367Arg)	0.0124	0.78 (0.65-0.95)	0.2366		0.0206	0.80 (0.65-0.96)	0.2124	
KCNJ11	A→G (Glu23Lys)	0.0293	0.82 (0.68-0.98)	0.0282	0.86 (0.75-0.98)	0.1247		0.0087	0.77 (0.63-0.93)
UCP3	-55C→T	0.0683		0.0132	1.31 (1.06-1.63)	0.2783		0.0073	1.36 (1.08-1.70)
HMOX1	-413T→A	0.0474	1.16 (1.00-1.34)	0.3707		0.0737		0.0882	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, BMI, smoking status, serum concentrations of TG and LDL-cholesterol, and the prevalence of hypertension and diabetes mellitus. P-values <0.005 are shown in bold.

Table VIII. Effects of genotypes and other characteristics on the prevalence of chronic kidney disease with low or high serum concentrations of HDL-cholesterol as determined by a stepwise forward selection procedure.

Variable	P-value	R^2
Low serum HDL-cholesterol		
Age	<0.0001	0.0566
Hypertension	0.0001	0.0122
MMP1 (*2G2G* versus *1G1G* + *1G2G*)	0.0043	0.0070
High serum HDL-cholesterol		
Age	<0.0001	0.0494
Hypertension	<0.0001	0.0087
Diabetes mellitus	<0.0001	0.0047
Gender	<0.0001	0.0031
Smoking	0.0009	0.0020

R^2, contribution rate.

ROCK2 was associated with greater systolic and diastolic blood pressure and systemic vascular resistance (20). We showed that the 125592C→A (Thr431Asn) polymorphism of *ROCK2* was significantly associated with CKD in individuals with a low serum concentration of TG, with the *A* allele being protective against this condition. Although hypertension has been considered to be a risk factor for newly onset and developing CKD (6), the mechanism of the relation of the *ROCK2* polymorphism to CKD remains to be elucidated.

Potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11) is expressed in pancreatic ß-cells and also in some other tissues, such as skeletal muscle, heart, peripheral nerve, and brain (21). Almost half of the patients diagnosed with diabetes mellitus before six months of age have a mutation in *KCNJ11* (22). The *Lys* allele of the Glu23Lys polymorphism of *KCNJ11* was shown to be related to an increased risk for type 2 diabetes mellitus in Caucasians (23). We showed that the A→G (Glu23Lys) polymorphism of *KCNJ11* was significantly associated with CKD in individuals with a low serum concentration of TG, with the *G* allele being protective against this condition, although the mechanism responsible for the association of the *G* variant with CKD remains unclear.

Adrenergic, ß-2, receptor, surface (ADRB2) plays an important role in regulating cardiac and vascular functions (24). The *Glu* variant of the C→G (Gln27Glu) polymorphism of *ADRB2* was shown to be related to lower blood pressure in a Japanese population (25). We showed that the C→G (Gln27Glu) polymorphism of *ADRB2* was significantly associated with CKD in individuals with a high serum concentration of TG, with the *G* allele being protective against this condition. Effects of this polymorphism on the regulation of blood pressure may account for its association with CKD.

Acetyl-Coenzyme A acetyltransferase 2 (ACAT2) is an intracellular cholesterol esterification enzyme and uses cholesterol and fatty acid as its enzymatic substrates (26). ACAT2 is mainly found in the liver and intestine (27), the sites where apolipoprotein B-containing lipoproteins are secreted (28). The *Ile* allele of the 734C→T (Thr254Ile) polymorphism of *ACAT2* was shown to be related to an increased risk for coronary heart disease in a Chinese population (26). We showed that the 734C→T (Thr254Ile) polymorphism of *ACAT2* was significantly associated with CKD in individuals with a high serum concentration of TG, with the *T* allele being protective against this condition, although the mechanism

Table IX. Characteristics of subjects with low or high serum concentrations of LDL-cholesterol.

Characteristic	Low serum LDL-cholesterol			High serum LDL-cholesterol		
	CKD	Controls	P-value	CKD	Controls	P-value
No. of subjects	1263	3272		443	966	
Age (years)	71.3±8.7	66.1±10.3	<0.0001	70.0±9.0	64.6±9.9	<0.0001
Gender (female/male, %)	36.6/63.4	42.2/57.8	0.0005	46.1/53.9	53.0/47.0	0.0153
Body mass index (kg/m^2)	23.4±3.3	23.4±4.8	0.8969	23.7±3.5	23.7±3.2	0.7639
Current or former smoker (%)	22.8	28.4	0.0001	16.3	22.5	0.0064
Hypertension (%)	68.6	53.6	<0.0001	68.4	57.3	<0.0001
Diabetes mellitus (%)	33.8	22.9	<0.0001	33.4	28.2	0.0467
Serum TG (mmol/l)	1.60±0.82	1.50±0.83	<0.0001	1.62±0.70	1.58±0.76	0.0431
Serum HDL-cholesterol (mmol/l)	1.34±0.41	1.42±0.41	<0.0001	1.32±0.39	1.38±0.36	0.0002
Serum LDL-cholesterol (mmol/l)	2.72±0.58	2.72±0.56	0.4699	4.23±0.61	4.22±0.64	0.7629
Serum creatinine (μmol/l)	111.2±112.7	62.1±12.2	<0.0001	109.8±120.6	60.5±12.7	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	48.4±11.0	78.9±15.9	<0.0001	49.0±10.5	79.4±18.1	<0.0001
End-stage renal disease (%)	2.5	0.0	<0.0001	2.7	0.0	<0.0001
Myocardial infarction (%)	25.8	16.9	<0.0001	31.6	23.0	0.0007
Ischemic stroke (%)	13.6	8.9	<0.0001	14.9	8.8	0.0008

Quantitative data are the means ± SD. eGFR, estimated glomerular filtration rate.

Table X. Genotype distributions of polymorphisms related (allele frequency, P<0.05) to chronic kidney disease (CKD) with low or high serum concentrations of LDL-cholesterol as determined by the Chi-square test.

Gene symbol	Polymorphism	dbSNP	CKD	Controls	P-value (genotype)	P-value (allele frequency)
Low serum LDL-cholesterol						
COMT	G→A (Val158Met)	rs679620			0.0059	<0.0001
	GG		507 (40.1)	1481 (45.3)		
	GA		620 (49.1)	1447 (442.0)		
	AA		136 (10.8)	343 (10.5)		
MMP3	A→G (Lys45Glu)	rs679620			0.0380	0.0105
	AA		150 (11.9)	326 (10.0)		
	AG		568 (45.0)	1412 (43.2)		
	GG		545 (43.1)	1533 (46.8)		
MMP1	-1607/1G→2G	rs1799750			0.0334	0.0226
	1G1G		149 (11.8)	361 (11.0)		
	1G2G		587 (46.5)	1405 (43.0)		
	2G2G		527 (41.7)	1505 (46.0)		
ALOX5	G→A (Glu254Lys)	rs2228065			0.0160	0.0235
	GG		1216 (96.3)	3187 (97.4)		
	GA		45 (3.6)	84 (2.6)		
	AA		2 (0.1)	0 (0.0)		
High serum LDL-cholesterol						
WRN	T→C (Cys1367Arg)	rs1346044			0.0384	0.0098
	TT		400 (90.3)	826 (85.5)		
	TC		41 (9.3)	132 (13.7)		
	CC		2 (0.4)	8 (0.8)		
PON1	584G→A (Gln192Arg)	rs662			0.0091	0.0102
	GG		409 (42.3)	409 (42.3)		
	GA		444 (46.0)	444 (46.0)		
	AA		113 (11.7)	113 (11.7)		
ABCA1	2583A→G (Ile823Met)	rs4149313			0.0313	0.0124
	AA		73 (16.5)	113 (11.7)		
	AG		204 (46.0)	442 (45.8)		
	GG		166 (37.5)	410 (42.5)		
NOS3	-786T→C	rs2070744			0.0182	0.0298
	TT		374 (84.4)	761 (78.8)		
	TC		64 (14.5)	198 (20.5)		
	CC		5 (1.1)	7 (0.7)		

responsible for the association of the *T* variant with CKD remains unknown.

Matrix metallopeptidase 1 (MMP1) degrades fibrillar collagens, particularly types I and III, which are resistant to most other proteinases (29). The *2G* allele of the -1607/1G→2G polymorphism of *MMP1*, which is located in the promoter region of the gene, was shown to be related to increased transcriptional activity (30). In addition, the -519A→G polymorphism of *MMP1* was shown to be related to the risk of myocardial infarction as part of a haplotype (31) and an increased intima-media thickness of the carotid artery in a German population with hypertension (32). We showed that the -1607/1G→2G polymorphism of *MMP1* was significantly associated with CKD in individuals with a low serum concentration of HDL-cholesterol, with the *2G* allele being protective against this condition. Effects of this polymorphism on the development of atherosclerosis may account for its association with CKD.

Catechol-O-methyltransferase (COMT) is involved in catechol homeostasis and plays a regulatory role (33). In addition, COMT is a key enzyme in the degradation of estrogens, which regulate several biological processes involved in the pathogenesis of myocardial infarction (34). The *Met* allele of the G→A (Val158Met) polymorphism of *COMT* was associated with a decreased activity of COMT (35) and was protective against myocardial infarction among

Table XI. Multivariable logistic regression analysis of polymorphisms related to chronic kidney disease with low or high serum concentrations of LDL-cholesterol.

Symbol	Polymorphism	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)
Low serum LDL-cholesterol									
COMT	G→A (Val158Met)	**0.0008**	1.27 (1.10-1.46)	0.3251		**0.0014**	1.27 (1.10-1.47)	0.0499	1.27 (1.00-1.60)
MMP3	A→G (Lys45Glu)	0.0156	0.76 (0.62-0.95)	0.0085	0.83 (0.72-0.95)	0.0973		**0.0036**	0.71 (0.57-0.90)
MMP1	-1607/1G→2G	0.3897		0.0066	0.83 (0.72-0.95)	0.9941		0.1002	
ALOX5	G→A (Glu254Lys)	0.1097		0.5531		0.1691		0.5526	
High serum LDL-cholesterol									
WRN	T→C (Cys1367Arg)	0.0128	0.62 (0.42-0.90)	0.6093		0.0148	0.62 (0.41-0.90)	0.5705	
PON1	584G→A (Gln192Arg)	**0.0046**	0.71 (0.56-0.90)	0.4050		0.0069	0.70 (0.55-0.91)	0.1067	
ABCA1	2583A→G (Ile823Met)	0.0134	0.65 (0.46-0.92)	0.0183	0.75 (0.58-0.95)	0.0757		**0.0037**	0.58 (0.40-0.84)
NOS3	-786T→C	0.0406	0.72 (0.53-0.98)	0.6704		0.0294	0.70 (0.50-0.96)	0.7421	

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, BMI, smoking status, serum TG and HDL-cholesterol concentrations, and the prevalence of hypertension and diabetes mellitus. P-values <0.005 are shown in bold.

Table XII. Effects of genotypes and other characteristics on the prevalence of chronic kidney disease with low or high serum concentrations of LDL-cholesterol as determined by a stepwise forward selection procedure.

Variable	P-value	R^2
Low serum LDL-cholesterol		
Age	<0.0001	0.0491
Hypertension	<0.0001	0.0113
Diabetes mellitus	<0.0001	0.0074
Serum HDL-cholesterol	<0.0001	0.0036
Smoking	0.0003	0.0024
Gender	0.0009	0.0021
COMT (*AA+GA* versus *GG*)	0.0011	0.0020
High serum LDL-cholesterol		
Age	<0.0001	0.0545
Serum HDL-cholesterol	0.0009	0.0064
PON1 (*AA+GA* versus *GG*)	0.0028	0.0052
Smoking	0.0039	0.0048

R^2, contribution rate.

hypertensive patients (34). We showed that the G→A (Val158Met) polymorphism of *COMT* was significantly associated with CKD in individuals with a low serum concentration of LDL-cholesterol, with the *A* allele representing a risk factor for this condition, although the mechanism responsible for the association of the *A* variant with CKD remains to be elucidated.

Paraoxonase 1 (PON1) is one of the paraoxonase family involved in protecting LDL from lipid oxidation (36). Given that paraoxonases have antioxidant activity and that oxidized LDL is a key mediator of atherosclerosis, the paraoxonases have been considered susceptibility genes for coronary heart disease and ischemic stroke (37,38). The *Arg* allele of the 584G→A (Gln192Arg) polymorphism of *PON1* was shown to be related to an increased risk of stroke (38) and endothelial dysfunction among normotensive diabetic subjects (39). We showed that the 584G→A (Gln192Arg) polymorphism of *PON1* was significantly associated with CKD in individuals with a high serum concentration of LDL-cholesterol, with the *A* allele being protective against this condition. The discordant results might be attributed to the stratification of subjects by lipid profiles in the present study.

Our study has several limitations: (i) We used an estimated glomerular filtration rate (eGFR) instead of a directly measured rate to define CKD. (ii) We were not able to obtain information on the underlying renal disease of CKD in each subject with CKD. (iii) Although a previous study (6,40) showed smoking to be a risk factor for CKD, the frequency of smoking was significantly lower in subjects with CKD than in controls. Selection bias could not be excluded in the present study, given that the subjects were recruited both from patients who visited the hospitals and from community-dwelling individuals. (iv) 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. (v) The functional relevance of the identified polymorphisms to gene transcription or to protein function was not determined in the present study. (vi) Although we adopted the criterion of $P<0.005$ for significant 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.

In conclusion, our present results implicate seven different polymorphisms as being associated with CKD in individuals with low or high serum concentrations of TG or HDL- or LDL-cholesterol. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic factors for CKD and may contribute to the personalized prevention of this condition. Our present study can be considered as hypothesis generating, and validation of our findings will require their replication with independent subject panels.

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References

1. Weiner DE, Tighiouart H, Amin MG, *et al*: Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol 15: 1307-1315, 2004.
2. Jafar TH, Stark PC, Schmid CH, *et al*: Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. Ann Intern Med 139: 244-252, 2003.
3. Iseki K, Oshiro S, Tozawa M, Ikemiya Y, Fukiyama K and Takishita S: Prevalence and correlates of diabetes mellitus in a screened cohort in Okinawa, Japan. Hypertens Res 25: 185-190, 2002.
4. Moranne O, Watier L, Rossert J, Stengel B and GN-Progress Study Group: Primary glomerulonephritis: an update on renal survival and determinants of progression. Q J Med 101: 215-224, 2008.
5. Haroun MK, Jaar BG, Hoffman SC, Comstock GW, Klag MJ and Coresh J: Risk factors for chronic kidney disease: a prospective study of 23,534 men and women in Washington County, Maryland. J Am Soc Nephrol 14: 2934-2941, 2003.
6. Yamagata K, Ishida K, Sairenchi T, *et al*: Risk factors for chronic kidney disease in a community-based population: a 10-year follow-up study. Kidney Int 71: 159-166, 2007.
7. Muntner P, Coresh J, Smith JC, Eckfeldt J and Klag MJ: Plasma lipids and risk of developing renal dysfunction: the atherosclerosis risk in communities study. Kidney Int 58: 293-301, 2000.
8. Schaeffner ES, Kurth T, Curhan GC, *et al*: Cholesterol and the risk of renal dysfunction in apparently healthy men. J Am Soc Nephrol 14: 2084-2091, 2003.
9. De Jong PE and Brenner BM: From secondary to primary prevention of progressive renal disease: the case for screening for albuminuria. Kidney Int 66: 2109-2118, 2004.
10. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39 (2 Suppl 1): 1-266, 2002.
11. Gharavi AG, Yan Y, Scolari F, *et al*: IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22-23. Nat Genet 26: 354-357, 2000.
12. Hanson RL, Craig DW and Millis MP: Identification of PVT1 as a candidate gene for end-stage renal disease in type 2 diabetes using a pooling-based genome-wide single nucleotide polymorphism association study. Diabetes 56: 975-983, 2007.
13. Doi K, Noiri E, Nakao A, Fujita T, Kobayashi S and Tokunaga K: Functional polymorphisms in the vascular endothelial growth factor gene are associated with development of end-stage renal disease in males. J Am Soc Nephrol 17: 823-830, 2006.
14. Wetmore JB, Hung AM, Lovett DH, Sen S, Quershy O and Johansen KL: Interleukin-1 gene cluster polymorphisms predict risk of ESRD. Kidney Int 68: 278-284, 2005.
15. Imai E, Matsuo S, Makino H, *et al*: Chronic Kidney Disease Japan Cohort (CKD-JAC) study: design and methods. Hypertens Res 31: 1101-1107, 2008.
16. Go AS, Chertow GM, Fan D, McCulloch CE and Hsu CY: Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 351: 1296-1305, 2004.
17. Itoh Y, Mizuki N, Shimada T, *et al*: High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. Immunogenetics 57: 717-729, 2005.
18. Noma K, Oyama N and Liao JK: Physiological role of ROCKs in the cardiovascular system. Am J Physiol Cell Physiol 290: C661-C668, 2006.
19. Rankinen T, Church T, Rice T, Markward N, Blair SN and Bouchard C: A major haplotype block at the rho-associated kinase 2 locus is associated with a lower risk of hypertension in a recessive manner: the HYPGENE study. Hypertens Res 31: 1651-1657, 2008.
20. Seasholtz TM, Wessel J, Rao F, *et al*: Rho kinase polymorphism influences blood pressure and systemic vascular resistance in human twins: role of heredity. Hypertension 47: 937-947, 2006.
21. Hattersley AT and Ashcroft FM: Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. Diabetes 54: 2503-2513, 2005.
22. Gloyn AL, Pearson ER, Antcliff JF, *et al*: Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. N Engl J Med 350: 1838-1849, 2004.
23. Hani EH, Boutin P, Durand E, *et al*: Missense mutations in the pancreatic islet beta cell inwardly rectifying K^+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia 41: 1511-1515, 1998.
24. Barbato E, Piscione F, Bartunek J, *et al*: Role of beta2 adrenergic receptors in human atherosclerotic coronary arteries. Circulation 111: 288-294, 2005.
25. Kato N, Sugiyama T, Morita H, *et al*: Association analysis of beta(2)-adrenergic receptor polymorphisms with hypertension in Japanese. Hypertension 37: 286-292, 2001.
26. He X, Lu Y, Saha N, Yang H and Heng CK: Acyl-CoA: cholesterol acyltransferase-2 gene polymorphisms and their association with plasma lipids and coronary artery disease risks. Hum Genet 118: 393-403, 2005.
27. Anderson RA, Joyce C, Davis M, *et al*: Identification of a form of acyl-CoA:cholesterol acyltransferase specific to liver and intestine in nonhuman primates. J Biol Chem 273: 26747-26754, 1998.
28. Burnett JR, Wilcox LJ, Telford DE, *et al*: Inhibition of ACAT by avasimibe decreases both VLDL and LDL apolipoprotein B production in miniature pigs. J Lipid Res 40: 1317-1327, 1999.
29. Visse R and Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92: 827-839, 2003.
30. Rutter JL, Mitchell TI, Butticè G, *et al*: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 58: 5321-5325, 1998.
31. Pearce E, Tregouet DA, Samnegård A, *et al*: Haplotype effect of the matrix metalloproteinase-1 gene on risk of myocardial infarction. Circ Res 97: 1070-1076, 2005.

32. Armstrong C, Abilleira S, Sitzer M, Markus HS and Bevan S: Polymorphisms in MMP family and TIMP genes and carotid artery intima-media thickness. Stroke 38: 2895-2899, 2007.
33. Männistö PT and Kaakkola S: Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 51: 593-628, 1999.
34. Eriksson AL, Skrtic S, Niklason A, *et al*: Association between the low activity genotype of catechol-O-methyltransferase and myocardial infarction in a hypertensive population. Eur Heart J 25: 386-391, 2004.
35. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL and Weinshilboum RM: Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6: 243-250, 1996.
36. Reddy ST, Wadleigh DJ, Grijalva V, *et al*: Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. Arterioscler Thromb Vasc Biol 21: 542-547, 2001.
37. Wheeler JG, Keavney BD, Watkins H, Collins R and Danesh J: Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. Lancet 363: 689-695, 2004.
38. Ranade K, Kirchgessner TG, Iakoubova OA, *et al*: Evaluation of the paraoxonases as candidate genes for stroke: Gln192Arg polymorphism in the paraoxonase 1 gene is associated with increased risk of stroke. Stroke 36: 2346-2350, 2005.
39. Irace C, Cortese C, Fiaschi E, *et al*: The influence of PON1 192 polymorphism on endothelial function in diabetic subjects with or without hypertension. Hypertens Res 31: 507-513, 2008.
40. Orth SR: Smoking and the kidney. J Am Soc Nephrol 13: 1663-1672, 2002.