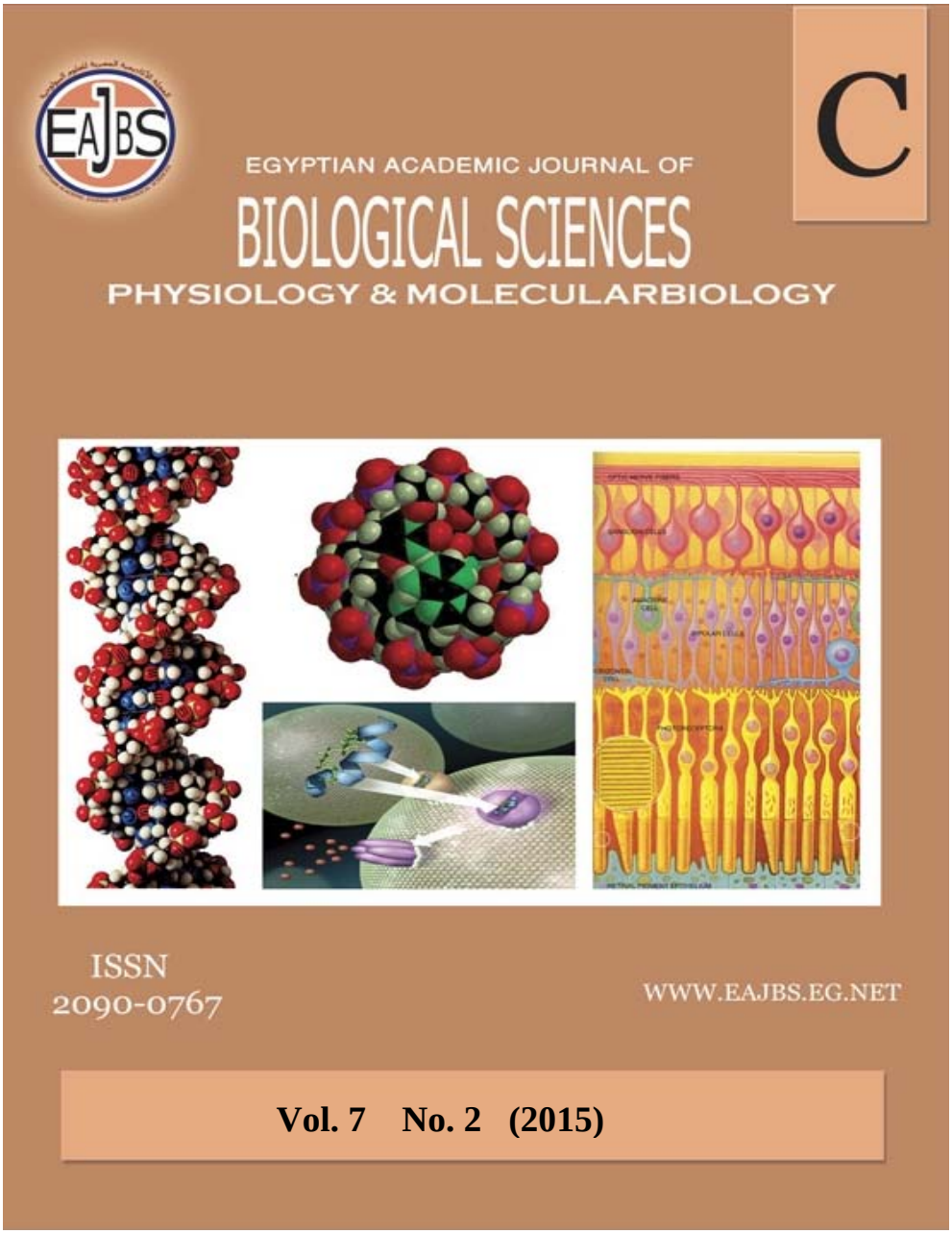

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidateimportant biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 7(2): 69 -79(2015)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbs.eg.net

Association Analysis of Chronic Kidney Disease and Its Risk Factors for Hail Region Population

Abdelbaset Mohamed Elasbali
Clinical Laboratory Science Department, College of Applied Medical Sciences, University of Hail, Kingdom of Saudi Arabia.

ARTICLE INFO
Article History
Received:18/11/2015
Accepted:4/12/2015

***Keywords*:**
Hail
Genetics
Polymorphism
Chronic kidney disease
Diabetes mellitus
Hypertension

ABSTRACT

Hypertension and diabetes mellitus are important risk factors for chronic kidney disease (CKD). The purpose of the present study was to identify genetic variants that confer susceptibility to CKD in individuals with or without hypertension or diabetes mellitus, thereby contributing to the personalized prevention of CKD in such individuals separately. The study population comprised 299 unrelated individuals, including 176 subjects with CKD and 172 controls. The 75 polymorphisms were selected by genome-wide association studies of chronic kidney disease and hypertension with the use of the Gene Chip Human Mapping 500K Array Set (Affymetrix). The genotypes for these polymorphisms were determined by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. The χ^2 test, multivariable logistic regression analysis with adjustment for covariates, as well as a stepwise forward selection procedure revealed that two different polymorphisms were significantly ($P<0.005$) associated with the prevalence of CKD in individuals with or without hypertension or diabetes mellitus: the A→G (Lys625Arg) polymorphism of *CDH4* (rs6142884) in individuals without diabetes mellitus, and the C→T polymorphism of *PTPRN2* (rs1638021) in individuals with hypertension and diabetes mellitus. No polymorphism was significantly associated with CKD in individuals with or without hypertension, in those with diabetes mellitus, or in those without hypertension or diabetes mellitus. Stratification of subjects based on hypertension or diabetes mellitus may thus be fundamental to achieving the personalized prevention of CKD with the use of genetic information.This preliminary study provided information on the frequency of CKD and its associated risk factors in the Hail region. However, larger population needs to be studied to establish the role of these risk factors in the etiology of CKD in Hail region.

INTRODUCTION

It is well known that chronic kidney disease (CKD) and end-stage renal disease (ESRD), which accelerate cardiovascular disease, are associated with high mortality (Foley *et al.*, 2011). Recent studies suggest that the risk of death is increased in individuals who have impaired renal function but do not require dialysis, compared to those who have preserved renal function (Go *et al.*, 2004; Ruilope *et al.*, 2001).

Disease prevention is an important strategy for reducing the overall burden of CKD and ESRD, and the identification of markers for CKD risk is essential both for risk prediction and for potential intervention to reduce the chance of future cardiovascular events related to CKD (National Kidney Foundation 2002).

Early identification of CKD is a legitimate enterprise if it provides meaningful opportunities for effective and safe interventions that reduce the risk of death, end-stage renal disease, or complications of renal dysfunction (Richard and Christopher, 2008). Progression of CKD in the presence of definite disease, particularly in the presence of certain diseases such as micro albuminuria, can be modified by interventions with the use of inhibitors of angiotensin II; however, the evidence that such approaches can alter the progression of stage 3 CKD in the absence of other definitive features of kidney damage has not yet been proven (Richard and Christopher, 2008). Regardless of the underlying etiology of the CKD, the family physician can make a significant impact in slowing the progression of chronic kidney disease through strict blood pressure control, tight glycemic control, reduction in the degree of proteinuria, and smoking cessation. All chronic kidney disease patients are at significantly increased risk of cardiovascular events; therefore, additional cardiovascular risk factors such as hyperlipidemia shall also be managed aggressively (Murphree and Thelen, 2010).

Although genetic linkage analyses (Gharavi *et al.*, 2005) and association studies (Hanson *et al.*, 2007; Wetmore *et.al*, 2005) have implicated several loci and candidate genes in the predisposition to CKD, the genes that confer susceptibility to this condition remain to be identified definitively. In addition, given ethnic differences in lifestyle and environmental factors as well as in genetic background and renal function, it is necessary to examine genetic variants related to CKD in each ethnic group. We previously showed that genetic variants that confer susceptibility to CKD differ between individuals with or without metabolic syndrome (Yoshida *et al.*, 2013), with or without type 2 diabetes mellitus (Yoshida *et al.*, 2012), with or without hypertension (Yoshida *et al.*, 2011), or with different lipid profiles (Yoshida *et al.*, 2010). To further examine whether the association of polymorphisms with CKD is influenced by the absence or presence of hypertension or diabetes mellitus, we performed an association study for 75 polymorphisms of 176 candidate genes and CKD in 299Saudi individuals with or without hypertension or diabetes mellitus. The purpose of the present study was to identify genetic variants that confer susceptibility to CKD in individuals with or without hypertension or diabetes mellitus independently, and thereby to assess the genetic risk of CKD in such individuals separately.

MATERIALS AND METHODS

Study population

The study population comprised 299 unrelated individuals (173 men, 126 women) who either visited outpatient clinics or were admitted to one of the participating hospitals (King Khaled Hospital, General Hail Hospital, Medical Centers at Baqaha and Samira in Hail region) between February till September 2015 and due to various symptoms or for an annual health checkup, or were recruited to a population-based prospective cohort study of aging and age-related diseases in Hail regaion.

Estimated glomerular filtration rate (eGFR) was calculated with the use of the simplified prediction equation derived from a modified version of that described in the Modification of Diet in Renal Disease (MDRD) Study, as proposed by the Japanese Society of Nephrology (Matsuo *et.al*, 2009): 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's Kidney Disease Outcomes Quality Initiative guidelines recommend a diagnosis of CKD if the eGFR is <60 ml min^{-1} 1.73 m^{-2} (National Kidney Foundation 2002). On the basis of this criterion, 299 subjects (173 men, 126 women) were diagnosed with CKD. The control subjects comprised 172 individuals (97 men, 75 women) recruited from among community-dwelling healthy individuals or patients who visited outpatient clinics regularly for treatment of various common

diseases, with an eGFR ≥60 ml min^{-1} 1.73 m^{-2}. 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, diastolic blood pressure of ≥90 mmHg, or taking antihypertensive medication), diabetes mellitus (fasting blood glucose of ≥6.93 mmol/l, hemoglobin A_{1c} of ≥6.5%, or taking antidiabetes medication), or hypercholesterolemia (serum total cholesterol of ≥5.72 mmol/l or taking lipid-lowering medication). On the basis of these criteria, 299 and 176 subjects were diagnosed with or without hypertension, respectively, CKD was identified in 70/299 (24%), of whom 27/70 (38.6%) were males and 43/70 (61.4%) were females, 49/70 (70%, P=0.005) were cases of diabetes, while 45/70 (64.3%, P=0.005) were with hypertension.

The study protocol complied with the Declaration of patients and was approved by the Committees on the Ethics of Human Research of College of Applied Medical Sciences, University of Hail and participating hospitals. Written informed consent was obtained from each participant.

Selection of polymorphisms

A total of 75 polymorphisms (data not shown) were selected by genome-wide association studies of CKD and hypertension (P-value for allele frequency $<1.0\times10^{-7}$) with the use of the Gene Chip Human Mapping 500K Array Set (Affymetrix, Santa Clara, CA, USA) (Yamada *et al.*, 2009). The relationship of these polymorphisms to CKD was not previously examined in our studies (Yoshida *et al.*, 2013; Yoshida *et al.*, 2010;Yoshida *et al.*, 2012; Yoshida *et al*; 2009).

Genotyping of polymorphisms

Venous blood (7 ml) was collected into tubes containing 50 mmol/l ethylenediamine-tetraacetic acid (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 75 polymorphisms were determined at G&G Science (Hail, Saudi) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Primers, probes and other conditions for the genotyping of polymorphisms significantly associated with CKD are shown in Table 1. Detailed genotyping methodology was as described previously (Itoh *et al.*, 2013).

Table 1:Primers, probes and other conditions for genotyping of polymorphisms related (P-value for allele frequency of <0.005) to chronic kidney disease.

Gene	Polymorphism	Sense primer (5′→3′)	Antisense primer (5′→3′)	Probe 1 (5′→3′)	Probe 2 (5′→3′)	Annealing (°C)	Cycles
CDH4	A→G (rs6142884)	CTGAGCTGCTGC CCAAGGAG	AGCGTCGGCCGC CGTGATGT	GATCTGCGAGAA GCCCAACC	CAGATCTGCGAG AGGCCCA	60	50
PTPRN2	C→T (rs1638021)	CAGCCCTTCCCA CCTACCAG	CCCAGGTCTCCC AGCCTCAG	AGCGAACCTTTG AGCTTTGC	CCAGCGGCAAAG TTCAAAGG		

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 χ^2 test. Allele frequencies were estimated by the gene counting method, and the χ^2 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 χ^2 test. Polymorphisms with a P-value for allele frequency of <0.005 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, gender (0, woman; 1, man), body mass index (BMI), smoking status (0, non-smoker; 1, smoker), history of hypertension, diabetes mellitus or hypercholesterolemia (0, no history; 1, positive history), and the genotype of each polymorphism. Subsequently, 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 (heterozygotes vs. wild-type homozygotes) and additive 2

(variant homozygotes vs. wild-type homozygotes) models, which were analyzed simultaneously using a single statistical model. We also performed a stepwise forward selection procedure to examine the effects of the 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 from the model were 0.25 and 0.1, respectively. Given the multiple comparisons of genotypes with CKD, we adopted the criterion of a P-value of <0.005 for statistical significance of association. For other clinical background data, a P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed with JMP version 6.0 and JMP Genomics version 3.2 software (SAS Institute, Cary, NC, USA).

RESULTS

Genetic variants related to CKD in individuals with or without hypertension

The characteristics of the subjects with or without hypertension are shown in Table 2. For individuals with hypertension, age, systolic blood pressure, serum concentrations of triglycerides and low density lipoprotein (LDL)-cholesterol, blood glycosylated hemoglobin content and the prevalence of diabetes mellitus were greater, whereas BMI, the percentage of smokers, diastolic blood pressure and serum concentration of high density lipoprotein (HDL)-cholesterol were lower, in subjects with CKD than in controls.

Table 2: Characteristics of subjects with chronic kidney disease and controls among individuals with or without hypertension.

	With hypertension			**Without hypertension**		
No. of subjects	**CKD**	**Controls**	**P-value**	**CKD**	**Controls**	**P-value**
Age (years)	123	97	0.0001	87	77	<0.0001
Gender (male/female, %)	70.9±8.9	66.4±9.8	<0.0001	70.5±9.4	63.8±11.2	0.0001
Body mass index (kg/m^2)	62.2/37.8	59.7/40.3	0.1472	58.4/41.6	49.1/50.9	0.0001
Current or former smoker (%)	23.5±3.4	23.8±3.4	0.0100	23.3±3.3	23.0±3.2	0.1470
No. of subjects	123	97		87	77	<0.0001
Age (years)	70.9±8.9	66.4±9.8	<0.0001	70.5±9.4	63.8±11.2	0.0001
Gender (male/female, %)	62.2/37.8	59.7/40.3	0.1472	58.4/41.6	49.1/50.9	0.0001
Body mass index (kg/m^2)	23.5±3.4	23.8±3.4	0.0100	23.3±3.3	23.0±3.2	0.1470
Current or former smoker (%)	18.5	24.1	0.0002	22.2	24.2	0.3365
Systolic blood pressure (mmHg)	152±27	149±23	0.0079	128±17	127±16	0.6248
Diastolic blood pressure (mmHg)	79±16	81±14	0.0001	73±12	74±11	0.0267
Hypercholesterolemia (%)	31.9	30.5	0.4151	28.9	27.2	0.4310
Serum total cholesterol (mmol/l)	5.21±1.04	5.18±1.02	0.2958	5.21±1.00	5.16±0.95	0.2803
Serum triglyceride (mmol/l)	1.74±1.08	1.69±1.14	0.0062	1.62±0.98	1.48±1.02	<0.0001
Serum HDL-cholesterol (mmol/l)	1.29±0.40	1.34±0.38	<0.0001	1.39±0.39	1.46±0.40	0.0002
Serum LDL-cholesterol (mmol/l)	3.13±0.95	3.05±0.92	0.0110	3.06±0.88	3.00±0.81	0.1952
Diabetes mellitus (%)	42.6	35.1	<0.0001	21.4	16.0	0.0032
Fasting plasma glucose (mmol/l)	7.23±3.27	7.14±3.19	0.4658	6.50±2.96	6.29±2.52	0.1446
Blood glycosylated hemoglobin (%)	6.13±1.62	6.06±1.60	0.0374	5.73±1.50	5.62±1.38	0.2032
Serum creatinine (μmol/l)	120.7±137.9	62.3±12.8	<0.0001	91.9±26.5	61.0±12.3	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	47.3±11.8	79.2±16.2	<0.0001	50.9±8.10	79.4±17.8	<0.0001

Quantitative data are the means ± SD. CKD, chronic kidney disease; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

For individuals without hypertension, age, the frequency of male subjects, serum concentration of triglycerides and the prevalence of diabetes mellitus were greater, whereas diastolic blood pressure and serum concentration of HDL-cholesterol were lower, in subjects with CKD than in controls.

Comparison of allele frequencies with the χ^2 test revealed that the C→T polymorphism of *F10* (rs5962) was significantly (P=0.0014) associated with CKD in individuals without hypertension, while no polymorphism was significantly associated with CKD in individuals with hypertension (data not shown). Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status and the prevalence of diabetes mellitus and hypercholesterolemia revealed that no polymorphism was significantly (P<0.005) associated with CKD in individuals without hypertension (data not shown).

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphism associated with CKD by the χ^2 test, as well as the effects of age, gender, BMI, smoking status and the prevalence of diabetes mellitus and hypercholesterolemia on CKD. For individuals without hypertension, age, BMI, gender and the *F10* genotype (dominant model), in descending order of statistical significance, were significant (P<0.005) and independent determinants of CKD (data not shown).

Genetic variants related to CKD in individuals with or without diabetes mellitus

The characteristics of the subjects with or without diabetes mellitus are shown in Table 3. For individuals with diabetes mellitus, age, systolic blood pressure and the prevalence of hypertension were greater, whereas the percentage of smokers and serum concentration of HDL-cholesterol were lower in subjects with CKD than in controls. For individuals without diabetes mellitus, age, the frequency of male subjects, systolic blood pressure, serum concentrations of triglycerides and LDL-cholesterol, and the prevalence of hypertension were greater, whereas the percentage of smokers, diastolic blood pressure and serum concentration of HDL-cholesterol were lower in subjects with CKD than in controls.

Table 3: Characteristics of subjects with chronic kidney disease and controls among individuals with or without diabetes mellitus.

With diabetes mellitus				**Without diabetes mellitus**		
Characteristic	**CKD**	**Controls**	**P-value**	**CKD**	**Controls**	**P-value**
No. of subjects	93	81		87	76	<0.0001
Gender (male/female, %)	62.2/37.8	59.7/40.3	0.1472	58.4/41.6	49.1/50.9	0.0001
Body mass index (kg/m^2)	23.5±3.4	23.8±3.4	0.0100	23.3±3.3	23.0±3.2	0.1470
Current or former smoker (%)	18.5	24.1	0.0002	22.2	24.2	0.3365
Systolic blood pressure (mmHg)	152±27	149±23	0.0079	128±17	127±16	0.6248
Diastolic blood pressure (mmHg)	79±16	81±14	0.0001	73±12	74±11	0.0267
Hypercholesterolemia (%)	32.0	32.0	0.9892	30.4	28.0	0.1213
Serum total cholesterol (mmol/l)	5.22±1.11	5.21±1.13	0.4798	5.20±0.97	5.15±0.94	0.1803
Serum triglyceride (mmol/l)	1.86±1.25	1.80±1.26	0.0681	1.62±0.92	1.52±1.01	<0.0001
Serum HDL-cholesterol (mmol/l)	1.25±0.41	1.27±0.35	0.0315	1.36±0.39	1.44±0.40	<0.0001
Serum LDL-cholesterol (mmol/l)	3.14±1.00	3.10±1.00	0.2081	3.09±0.88	3.00±0.82	0.0085
Fasting plasma glucose (mmol/l)	9.39±4.05	9.57±4.03	0.2702	5.60±1.14	5.70±1.31	0.3255
Blood glycosylated hemoglobin (%)	7.10±1.95	7.27±1.99	0.1052	5.22±0.39	5.21±0.48	0.1372
Serum creatinine (μmol/l)	119.7±114.5	62.3±13.0	<0.0001	107.3±117.1	61.5±12.4	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	46.5±11.8	81.2±17.0	<0.0001	49.5±10.3	78.6±16.9	<0.0001

Quantitative data are the means ± SD. CKD, chronic kidney disease; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

Comparison of allele frequencies with the χ^2 test revealed that two or one polymorphisms were significantly (P<0.005) associated with CKD in individuals with or without diabetes mellitus, respectively (Table 4).

Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status and the prevalence of hypertension and hypercholesterolemia revealed that the A→G polymorphism of *PLA2G3* (rs5753472, additive 2 model) and the C→T polymorphism of *RUVBL2* (rs1062708, additive 2 model) were significantly (P<0.005) associated with CKD in individuals with diabetes mellitus, and that the A→G polymorphism of CDH4 (rs6142884, recessive model) was significantly associated with CKD in individuals without diabetes mellitus (Table 5).

Table 4: Genotype distributions of SNPs significantly associated with chronic kidney disease among individuals with or without diabetes mellitus as determined by the χ^2 test.

Gene symbol	SNP	dbSNP	CKD (%)	Controls (%)	P-value
With diabetes mellitus					
PLA2G3	A→G	rs5753472			0.0011
	AA		176 (27.5)	123 (22.0)	
	AG		199 (51.0)	183 (50.4)	
	GG		136 (21.5)	172 (27.6)	
RUVBL2	C→T	rs1062708			0.0031
	CC		176 (34.4)	102 (28.5)	
	CT		203 (47.9)	225 (49.4)	
	TT		112 (17.7)	135 (22.1)	
	CT		203 (47.9)	225 (49.4)	
	TT		112 (17.7)	135 (22.1)	
Without diabetes mellitus					
CDH4	A→G	rs6142884			0.0041
	AA		19 (1.7)	51 (1.7)	
	AG		192 (26.1)	133 (21.3)	
	GG		108 (72.2)	192 (77.0)	

Table 5: Multivariable logistic regression analysis of SNPs significantly associated with chronic kidney disease by the χ^2 test among individuals with or without diabetes mellitus.

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)
With diabetes mellitus					
PLA2G3	A→G	0.0093	0.73 (0.58–0.93)	0.0081	0.72 (0.57–0.92)
RUVBL2	C→T	0.0075	0.74 (0.59–0.92)	0.0152	0.72 (0.56–0.94)
Without diabetes mellitus					
CDH4	A→G	0.8411	0.79 (0.39–0.71)	0.0030	0.78 (0.66–0.92)

OR, odds ratio; CI, confidenceinterval. Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status and the prevalence of hypertension and hypercholesterolemia. P-values for allele frequency of <0.005 are shown in bol

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphism associated with CKD by the χ^2 test, as well as the effects of age, gender, BMI, smoking status and the prevalence of hypertension and hypercholesterolemia on CKD (Table 6). For individuals with diabetes mellitus, age and hypertension were a significant (P<0.005) and independent determinant of CKD.

Table 6: Effects of genotypes and other characteristics on chronic kidney disease among individuals with or without diabetes mellitus determined by a stepwise forward selection procedure.

Variable	P-value	R^2
With diabetes mellitus		
Age	<0.0001	0.0433
Hypertension	0.0003	0.0059
Without diabetes mellitus		
Age	<0.0001	0.0603
Hypertension	<0.0001	0.0079
Smoking	<0.0001	0.0041
Gender	0.0002	0.0028
CDH4 (GG vs. AA + AG)	0.0029	0.0019

For individuals without diabetes mellitus, age, hypertension, smoking, gender and the *CDH4* genotype (recessive model), in descending order of statistical significance, were significant and independent determinants of CKD.

Genetic variants related to CKD in individuals with or without hypertension and diabetes mellitus

The characteristics of the subjects with hypertension and diabetes mellitus or without these conditions are shown in Table 7. For individuals with hypertension and diabetes mellitus, age and systolic blood

pressure were greater, whereas BMI, the percentage of smokers and serum concentration of HDL-cholesterol were lower in subjects with CKD than in controls. For individuals without hypertension or diabetes mellitus, age, the frequency of male subjects and serum concentration of triglycerides were greater, whereas the serum concentration of HDL-cholesterol was lower in subjects with CKD than in controls.

Table 7: Characteristics of subjects with chronic kidney disease and controls among individuals with or without hypertension and diabetes mellitus.

With hypertention and diabetes mellitus				**With hypertention or diabetes mellitus**		
Characteristic	**CKD**	**Controls**	**P-value**	**CKD**	**Controls**	**P-value**
No. of subjects	93	81	<0.0001	87	76	<0.0001
Age (years)	70.2±9.0	66.1±9.6	<0.0001	70.6±9.4	63.7±11.3	<0.0001
Gender (male/female, %)	66.0/34.0	66.3/33.7	0.9107	56.2/43.8	46.0/54.0	0.0002
Body mass index (kg/m^2)	23.7±3.5	24.1±3.6	0.0366	23.2±3.2	23.0±3.1	0.2602
Current or former smoker (%)	19.3	24.7	0.0233	22.8	23.6	0.7369
Systolic blood pressure (mmHg)	155±27	150±23	0.0015	129±18	128±17	0.3841
Diastolic blood pressure (mmHg)	80±15	80±15	0.4785	74±12	75±11	0.1781
Hypercholesterolemia (%)	33.2	32.9	0.9105	29.6	26.8	0.2430
Serum total cholesterol (mmol/l)	5.25±1.12	5.23±1.11	0.4068	5.24±0.97	5.16±0.90	0.1413
Serum triglyceride (mmol/l)	1.88±1.26	1.84±1.33	0.0624	1.59±0.91	1.44±1.00	<0.0001
Serum HDL-cholesterol (mmol/l)	1.25±0.42	1.27±0.35	0.0412	1.40±0.39	1.49±0.40	0.0007
Serum LDL-cholesterol (mmol/l)	3.18±1.01	3.12±1.02	0.1437	3.08±0.85	2.99±0.79	0.0819
Fasting plasma glucose (mmol/l)	9.28±3.91	9.58±4.00	0.1190	5.56±1.13	5.66±1.38	0.8941
Blood glycosylated hemoglobin (%)	6.99±1.89	7.19±1.92	0.1035	5.17±0.38	5.18±0.48	0.8077
Serum creatinine (μmol/l)	124.2±125.4	62.3±13.1	<0.0001	89.8±24.2	60.8±12.2	<0.0001
eGFR (ml min^{-1} 1.73 m^{-2})	46.0±12.1	81.1±16.9	<0.0001	49.5±10.3	78.6±16.9	<0.0001
Serum triglyceride (mmol/l)	1.88±1.26	1.84±1.33	0.0624	1.59±0.91	1.44±1.00	<0.0001

Quantitative data are the means ± SD. CKD, chronic kidney disease; LDL, low density lipoprotein; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

Comparison of allele frequencies with the χ^2 test revealed that three or one polymorphisms were significantly (P<0.005) associated with CKD in individuals with hypertension and diabetes mellitus or without these conditions, respectively (Table 8).

Table 8: Genotype distributions of SNPs significantly associated with chronic kidney disease among individuals with or without hypertension and diabetes mellitus as determined by the χ^2 test.

Gene symbol	**SNP**	**dbSNP**	**CKD (%)**	**Controls (%)**	**P-value**
With hypertension and diabetes mellitus					
RUVBL2	C→T	rs1062708			0.0027
	CC		176 (33.7)	108 (26.9)	
	CT		150 (48.5)	186 (50.0)	
	TT		92 (17.8)	178 (23.1)	
ZFP30	A→G	rs1478462			0.0033
	AA		221 (82.1)	184 (75.8)	
	AG		89 (17.3)	173 (22.4)	
	GG		3 (0.6)	14 (1.8)	
PTPRN2	C→T	rs1638021			0.0042
	CC		193 (56.8)	171 (48.1)	
	CT		181 (35.1)	225 (42.1)	
	TT		42 (8.1)	76 (9.8)	
	GG		3 (0.6)	14 (1.8)	
PTPRN2	C→T	rs1638021			0.0042
	CC		193 (56.8)	171 (48.1)	
	CT		181 (35.1)	225 (42.1)	
	TT		42 (8.1)	76 (9.8)	
	GG		3 (0.6)	14 (1.8)	
Without hypertension or diabetes mellitus					
JPH3	C→G	rs2562059			0.0027
	CC		109 (72.7)	102 (66.2)	
	CG		112 (26.4)	170 (30.5)	
	GG		4 (0.9)	51 (3.3)	

Multivariable logistic regression analysis with adjustment for age, gender, BMI, smoking status and the prevalence of hypercholesterolemia revealed that the C→T polymorphism of *RUVBL2* (rs1062708, additive 2 model) and the C→T

polymorphism of *PTPRN2*(rs1638021, dominant model) were significantly (P<0.005) associated with CKD in individuals with hypertension and diabetes mellitus (Table 9). No polymorphism was significantly associated with CKD in individuals without hypertension or diabetes mellitus.

Table 9: Multivariable logistic regression analysis of SNPs significantly associated with chronic kidney disease by theχ^2 test among individuals with or without hypertension and diabetes mellitus.

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)
Without hypertension or diabetes mellitus					
RUVBL2	C→T	0.0107	0.72 (0.56–0.93)	0.0169	0.70 (0.52–0.94)
ZFP30	A→G	0.0120	0.69 (0.52–0.92)	0.0734	
PTPRN2	C→T	**0.0043**	0.71 (0.57–0.90)	0.2242	
RUVBL2	C→T	0.0107	0.72 (0.56–0.93)	0.0169	0.70 (0.52–0.94)
Without hypertension or diabetes mellitus					
JPH3	C→G	0.0247	0.75 (0.59–0.96)	0.0123	0.26 (0.08–0.67)

OR, odds ratio; CI, confidence interval. Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status and the prevalence of hypercholesterolemia. P-values for allele frequency of <0.005 are shown in bold.

A stepwise forward selection procedure was performed to examine the effects of genotypes for the polymorphism associated with CKD by the χ^2 test, as well as the effects of age, gender, BMI, smoking status and the prevalence of hypercholesterolemia on CKD (Table 10).

Table 10: Effects of genotypes and other characteristics on chronic kidney disease among individuals with or without hypertension and diabetes mellitus determined by a stepwise forward selection procedure.

Variable	P-value	R^2
With hypertension and diabetes mellitus		
Age	<0.0001	0.0368
PTPRN2 (CT + TT vs. CC)	0.0044	0.0048
Without hypertension or diabetes mellitus		
Age	<0.0001	0.0676
Body mass index	0.0005	0.0059
Gender	0.0034	0.0042
JPH3 (GG vs. CC + CG)	0.0036	0.0042

R^2, contribution rate. P<0.005

For individuals with hypertension and diabetes mellitus, age and *PTPRN2* genotype (dominant model) were significant (P<0.005) and independent determinants of CKD. For individuals without hypertension or diabetes mellitus, age, BMI, gender and the*JPH3* genotype (recessive model), in descending order of statistical significance, were significant (P<0.005) and independent determinants of CKD.

Finally, we examined whether the genotype distributions for the polymorphisms associated with CKD were in Hardy-Weinberg equilibrium. The genotype distributions for the A→G polymorphism of *CDH4* (subjects with CKD, P=0.2050; controls, P=0.3414) and the C→T polymorphism of *PTPRN2* (subjects with CKD, P=0.0658; controls, P=0.6959) were in Hardy-Weinberg equilibrium both in subjects with CKD and in controls.

DISCUSSION

We examined the possible relationship between 75 polymorphisms of 44 genes with the prevalence of CKD in individuals with or without hypertension or diabetes mellitus. Our association study with three steps of analysis (χ^2 test, multivariable logistic regression analysis and stepwise forward selection procedure) revealed that two polymorphisms were significantly associated with CKD: the A→G (Lys625Arg)

polymorphism of *CDH4* (rs6142884) in individuals without diabetes mellitus, and the C→T polymorphism of *PTPRN2* (rs1638021) in individuals with hypertension and diabetes mellitus.

The cadherin 4, type 1, R-cadherin (*CDH4*) gene is a member of a family of cell surface glycoproteins that mediate calcium-dependent cell-cell adhesion and are considered to play an important role in a wide range of cell-cell interactions (Suzuki *et al.*, 1991). Previous reports suggest that *CDH4* may act as a tumor suppressor gene in human gastrointestinal tumors and may potentially be used as a marker for the early diagnosis of gastrointestinal tumorigenesis (Miotto *et al.*, 2014). In addition, *CDH4* has been shown in animal studies to play an important role in neural tract and synaptic development (Obst-Pernberg *et al.*, 2011,Cavodeassi *et al.*, 2015).

In the present study, we demonstrated that the A→G (Lys625Arg) polymorphism of *CDH4* (rs6142884) was significantly associated with CKD in individuals without diabetes mellitus, with the G allele protecting against this condition. The Lys625Arg polymorphism is located in the cadherin repeat domain, which exists as repeats in extracellular regions thought to mediate cell-cell contact when bound to calcium. The association of the A→G (Lys625Arg) polymorphism with CKD might be attributable to effects on cellular adhesion, though the mechanism responsible for this association remains to be elucidated.

The protein tyrosine phosphatase, receptor type, N polypeptide 2 (*PTPRN2*) gene encodes a 1015-amino acid polypeptide with a single transmembrane and one putative tyrosine phosphatase catalytic domain (Wasmeier *et al.*, 1996). *PTPRN2*, which was cloned from a rat insulinoma cDNA library (Wasmeier *et al.*, 1996), is 74% identical to the ICA512/IA-2 autoantigen of type 1 diabetes mellitus in the cytoplasmic domain, but only 29% identical in the luminal domain (Kawasaki *et al.*, 1996). Previous reports suggest that 48 and 61% of sera from patients with new onset type 1 diabetes mellitus are positive for autoantibodies to the full-length and cytoplasmic domain of *PTPRN2*, respectively (Kawasaki *et al.*, 1996). Therefore, *PTPRN2* has been considered a major autoantigen for type 1 diabetes mellitus, and is thus believed to be involved in the pathogenesis of this condition (Kawasaki *et al.*, 1996). We have now shown that the C→T polymorphism of *PTPRN2* (rs1638021) was significantly associated with CKD in individuals with hypertension and diabetes mellitus, with the T allele protecting against this condition, though the underlying mechanism remains unclear.

Studies from Saudi Arabia and other Gulf countries, have only dealt with end stage renal disease (ESRD), as well as, the risk factors associated with CKD, such as, DM, hypertension, and other cardiovascular diseases. A recent review of 44 studies, have described the epidemiology of ESRD in the countries of the Gulf Cooperation Council (GCC; which consist of Saudi Arabia, the United Arab Emirates, Kuwait, Qatar, Bahrain, and Oman), showed that the incidence of ESRD has increased while the prevalence and mortality rate of ESRD in the GCC has not been reported sufficiently (Amal *et al*., 2012). The modification of diet in renal disease study (Hunsicker *et al*., 1997) followed chronic kidney disease patients at all stages for a 2-year period and concluded that 85% of patients had a decline in their GFR, with the average rate of decline (4 ml/min) annually regardless of the baseline GFR.

Vascular disease (primarily hypertension) is the second most common cause of CKD (it causes 21% of adult CKD cases) (Duaine *et al*., 2010). Hypertensive nephrosclerosis is associated with addition signs of hypertensive end-organ damage, because of long periods of poorly controlled hypertension. In the present study, the frequency of hypertension was high approximately 37% and the individuals with hypertension represent 44.3% of cases of CKD. The summarized estimate of hypertension prevalence among ESRD in GCC study was 77.88% (Amal *et al*., 2012)

Our study had several limitations: (i) we used eGFR instead of directly measured GFR to define CKD. (ii) We did not obtain information on the underlying renal disease

in each subject with CKD, though such information could be obtained by detailed clinical examination, including renal biopsy; however, these diagnostic procedures are not considered feasible in a study with subjects recruited from the general population.

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 $P<0.005$ for association to compensate for the multiple comparisons of genotypes with CKD, it is not possible to completely exclude potential statistical errors such as false positives. (vi) Although a previous study showed smoking to be a risk factor for CKD (Orth *et al.*, 2012), the frequency of smoking was lower in subjects with CKD than in controls in the present study. Selection bias thus could not be completely excluded in the present study.

In conclusion, our results suggest that genetic variants that confer susceptibility to CKD differ among individuals with or without hypertension or diabetes mellitus. Stratification of subjects based on hypertension or diabetes mellitus may thus be fundamental to achieving the personalized prevention of CKD with the use of genetic information. Given that our present study may be considered hypothesis generating, validation of our findings will require their replication with independent subject panels.

ACKNOWLEDGMENTS

This work was supported by Scientific Research Deanship, University of Hail, Kingdom of Saudi Arabia (NO. AMS137)

REFERENCE

Amal AH, Fahdah A-S, Eszter P (2012). Vamos, Ghasem Yadegarfar, Azeem Majeed. Epidemiology of end-stage renal disease in the countries of the Gulf Cooperation Council: a systematic review. J. R. Soc. Med. Sh. Rep. 3:38.

Cavodeassi F, Carreira-Barbosa F. and Young RM. (2015). Early stages of zebrafish eye formation require the coordinated activity of Wnt11, Fz5 and the Wnt/beta-catenin pathway. Neuron., 47:43–56.

Doi K, Noiri E., Nakao A., Fujita T., Kobayashi S. and Tokunaga K. (2006). 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.

Foley RN, Parfrey PS. and Sarnak MJ. (2011). Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis., 32:112–119.

Gharavi AG, Yan Y, Scolari F. (2000). IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22–23. Nat Genet., 26:354–357.

Go AS., Chertow GM., Fan D., McCulloch CE. and Hsu CY. (2004). Chronic kidney disease and the risks of death, cardiovascular events and hospitalization. N Engl J Med., 351:1296–1305.

Hanson RL, Craig DW., and Millis MP. (2007). 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.

Hunsicker LG, Adler S., Caggiula A, England BK, Greene T, Kusek JW, Rogers NL, Teschan PE (1997). Predictors in the progression of renal disease in the modification of diet in renal disease study. Kidney Int. 51:1908–19.

Itoh Y, Mizuki N, Shimada T. (2013). High-throughput DNA typing of HLA-A,-B,-C and-DRB1 loci by a PCR-SSOP-Luminex method in the Japanese popul-ation. Immunogenetics., 57:717–729.

Kawasaki E., Hutton JC. and Eisenbarth GS. (1996). Molecular cloning and characterization of the human transmembrane protein tyrosine phosphatase homologue, phogrin, an autoantigen of type 1 diabetes. Biochem Biophys Res Commun., 227:440–447.

Matsuo S, Imai E. and Horio M. (2009). Revised equations for ,estimated GFR from serum creatinine in Japan.Am J Kidney Dis., 53:982–992.

Miotto E, Sabbioni S. and Veronese A. (2014). Frequent aberrant methylation of the CDH4 gene promoter in human colorectal and gastric cancer. Cancer Res., 64:8156–8159.

National Kidney Foundation (2002). K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis., 39:1–266.

Obst-Pernberg K, Medina L. and Redies C. (2011). Expression of R-cadherin and N-cadherin by cell groups and fiber tracts in the developing mouse forebrain: relation to the formation of functional circuits. Neuroscience., 106:505–533.

Orth SR. (2012). Smoking and the kidney. J Am Soc Nephrol., 13:1663–1672.

Richard JG. and Christopher W. (2008). Screening for CKD with eGFR: Doubts and Dangers. CJASN, 3(5):1563-1568.

Ruilope LM., Salvetti A. and Jamerson K. (2001). Renal function and intensive lowering of blood pressure in hypertensive participants of the hypertension optimal treatment (HOT) study. J Am Soc Nephrol., 12:218–225.

Suzuki S., Sano K. and Tanihara H. (1991). Diversity of the cadherin family: evidence for eight new cadherins in nervous tissue. Cell Regul., 2:261–270.

Wasmeier C. and Hutton JC. (1996). Molecular cloning of phogrin, a protein-tyrosine phosphatase homologue localized to insulin secretory granule membranes. J Biol Chem., 271:18161–18170.

Wetmore JB, Hung AM, Lovett DH, Sen S., Quershy O. and Johansen KL. (2005). Interleukin-1 gene cluster polymorphisms predict risk of ESRD. Kidney Int., 68: 278–284.

Yamada Y, Fuku N. and Tanaka M. (2009). Identification of CELSR1 as a susceptibility gene for ischemic stroke in Japanese individuals by a genome-wide association study. Atherosclerosis, 17: 261-269.

Yoshida T, Kato K. and Fujimaki T. (2013). Association of a polymorphism of the apolipoprotein E gene with chronic kidney disease in Japanese individuals with metabolic syndrome. Genomics., 93:221–226.

Yoshida T, Kato K. and Fujimaki T. (2009). Association of genetic variants with chronic kidney disease in Japanese individuals. Clin J. Am Soc Nephrol., 4:883–890.

Yoshida T, Kato K. and Yokoi K. (2011). Association of candidate gene polymorphisms with chronic kidney disease in Japanese individuals with hypertension. Hypertens Res., 32: 411-418.

Yoshida T, Kato K. and Yokoi K.(2009). Association of gene polymorphisms with chronic kidney disease in high-risk or low-risk subjects defined by conventional risk factors. Int J Mol Med., 23:785–792.

Yoshida T, Kato K. and Yokoi K. (2012). Association of genetic variants with chronic kidney disease in Japanese individuals with type 2 diabetes mellitus. Int J Mol Med., 23: 529–537.

Yoshida T, Kato K. and Yokoi K. (2012). Association of genetic variants with chronic kidney disease in individuals with different lipid profiles. Int J Mol Med., 24:233246.