Original Article

Evaluation of Single-Nucleotide Polymorphisms of Transcription Factor 7-Like 2 and ATP2B1 Genes as Cardiovascular Risk Predictors in Chronic Kidney Disease

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Abstract

Introduction: Cardiovascular disease (CVD) is the primary cause of morbidity and premature mortality in chronic kidney disease (CKD). The transcription factor 7-like 2 (*TCF7L2)* gene product TCF4 is a transcription factor that acts as a downstream effector in the canonical Wnt signaling pathway and may be important in the development of both type 2 diabetes and renal development and disease. It is, therefore, plausible that mutations in this gene could manifest themselves in reduced kidney function or kidney disease through their effects on hyperglycemia as well as independent of this mechanism. The *ATP2B1* gene encodes the plasma membrane calcium ATPase isoform 1, which removes bivalent calcium ions from eukaryotic cells against very large concentration gradients and is responsible for controlling the contraction and dilation of vascular smooth muscles. **Aim and Objectives:** The aims of this study are (1) to evaluate single-nucleotide polymorphisms (SNPs) of TCF7L2 gene as cardiovascular risk predictors in CKD and (2) to evaluate SNPs of ATP2B1 gene as cardiovascular risk predictors in CKD. **Subjects and Methods:** Fifty clinically diagnosed CKD patients in the age group between 20 and 60 years of both genders were selected as cases and fifty healthy participants from the master health checkup department were selected as controls. Genomic DNA was extracted based on the spin column kit method. The DNA samples were stored at −20°C until analysis. Genotyping for TCF7L2 gene rs7903146 (C/T) and ATP2B1 gene rs11105354 (A/G) was carried out through polymerase chain reaction. **Results:** T allele frequency was observed in 12 controls and 23 cases (odds ratio [OR] = 2.2, 95% confidence interval [CI]: 1.0–4.7). CC genotype was observed in 38 controls and 27 cases and CT genotype in 22 cases and 12 controls. A allele was found in 38 cases and 23 controls (OR = 2, 95% CI: 1.1–3.8). The mean values of cholesterol, low-density lipoprotein, triglycerides, glucose, insulin, urea, and creatinine were high in cases when compared to controls. **Conclusion:** T allele of TCF7L2 gene rs7903146 (C/T) and A allele of ATP2B1 (A/G) gene rs11105354 (A/G) are associated with CVD in CKD patients.

Keywords: *ATP2B1, cardiovascular disease, chronic kidney disease, insulin, single-nucleotide polymorphism, transcription factor 7-like 2*

Introduction

Cardiovascular disease (CVD) is the main cause of morbidity and premature mortality in chronic kidney disease (CKD). It is well-established that patients with kidney failure (CKD Stage 5) are at high risk of CVD morbidity and mortality[1] and patients with earlier stages of CKD also experience a high rate of fatal and nonfatal cardiovascular events.[2] Recent guidelines have, therefore, defined CKD as a cardiovascular risk equivalent, and patients in all stages of CKD are considered in the "highest risk group" for the development of CVD.[3] The high prevalence of CVD in CKD is attributed to the traditional risk factors such as diabetes mellitus (DM), hypertension, hyperlipidemia, and age, but with the advent of genome-wide association scans,[4] numerous risk variants have been identified as candidates for conferring susceptibility to renal and CVDs but most of them with only modest effects.[5]

The *ATP2B1* gene codes the plasma membrane calcium ATPase isoform 1, which plays a critical role in intracellular calcium homeostasis[6] due to its capacity for removing bivalent calcium ions from eukaryotic cells against very large concentration gradients.[7] Although the pathophysiological implications of *ATP2B1*

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gene on the development of hypertension are still unclear, results from *ATP2B1* knockout mouse studies suggested that *ATP2B1* may play an important role in the regulation of blood pressure[8] through alterations of calcium handling and vasoconstriction in vascular smooth muscle cells.[9]

The mammalian plasma membrane calcium ATPase isoforms are encoded by at least four separate genes. The diversity of these enzymes is further increased by alternative splicing of transcripts.[10] The expression of different isoforms and splice variants is regulated in a developmental, tissue-specific, and cell type-specific manner, signifying that these pumps are functionally adapted to the physiological needs of particular cells and tissues.[11]

Transcription factor 7-like 2 (*TCF7L2*) belongs to a family of TCF lymphoid enhancer factor transcription factors and is a key component of the Wnt signaling pathway involved in the regulation of pancreatic beta-cell proliferation, differentiation, and insulin secretion.[12] It is involved in vascular remodeling through the regulation of smooth muscle cell proliferation and endothelial cell growth.[13] TCF7L2, therefore, contributes to CVD. The most significant genetic association with diabetes was detected for two intronic single-nucleotide polymorphisms (SNPs), rs7903146 (intron 3) and rs12255372 (intron 4), located 50 kb from each other. These polymorphisms were also found to be linked to diabetic coronary atherosclerosis.[14]

Hence, the aim of our study was to investigate the rs7903146 (C/T) polymorphism of the TCF7L2 gene and rs11105354 (A/G) of ATP2B1 genes as cardiovascular risk predictors in CKD.

Subjects and Methods

The present cross-sectional study was conducted in a tertiary health-care setup after obtaining the Institutional Human Ethics Committee approval and written informed consent from study participants. Fifty clinically diagnosed CKD patients in the age group between 20 and 50 years of both genders were selected as cases and fifty healthy participants visiting master health checkup were selected as controls. Patients with congenital heart disease (CHD), autoimmune disorders, DM, alcoholics, smokers, and tobacco chewers were excluded from the study.

Whole blood (2 ml) was collected in ethylenediaminetetraacetic acid tube for genetic analysis. Genomic DNA was extracted based on the spin column kit method. The DNA samples were stored at −20°C until analysis. Genotyping for TCF7L2 gene rs7903146 (C/T) and ATP2B1 gene rs11105354 (A/G) was carried out through polymerase chain reaction (PCR) in a final volume of 25 µl reaction and analyzed by agarose gel electrophoresis. The primers of both genes and their products were as follows: TCF7L2 Gene-3'-GCCTCAAAACCTAGCACAGC-5' and 5'-GTGAAGTGCCCAAGCTTCTC-3' and ATP2B1 Gene-3'-CTTTGCCAGTGAAAGTGCAG-5' and 5'-TGGGTGGGAAAGTTTTTCTG-3'; product size-TCFL2 gene– 221 and ATP2B1 gene-159. The PCR steps were as follows: initial DNA denaturation at 95°C for 5 min ➔ Denaturation at 95°C for 30 s ➔ Annealing at 63°C for 30 s ➔ Extension at 72°C for 30 s followed by amplification of Steps 2–4 by 35 cycles and final extension at 72°C for 5 min.

The PCR final volume of 25-µl reaction sample contained Red Dye PCR Master Mix of 10 µl, primer mix of 10 µl, and purified DNA sample of 5 µl. The PCR products were analyzed by agarose gel electrophoresis [Figure 1]. The PCR master mix, probes, and reagents were procured from the company Helini Biomolecules, Chennai.

Three milliliters of blood was collected for estimation of biochemical parameters such as glucose, urea, creatinine, and lipid profile and was estimated by the International Federation of Clinical Chemistry and Laboratory Medicine-recommended method using fully automated autoanalyzer and insulin by chemiluminescence technology using Roche diagnostic kit in automated hormone analyzer.

Statistical analysis

Descriptive and inferential statistical analysis was carried out in the present study. The C and T allele distributions of TCF7L2 gene rs7903146 and ATP2B1 gene rs11105354 (A/G) were studied in CKD patients. The SPSS 17 (SPSS Inc, Chicago), MedCalc version 9.0.1 (MedCalc Software, Ostend, Belgium), were used for data analysis. The present study was verified for the Hardy–Weinberg equilibrium, and Chi-square value was <3.841, so the two populations were same and said to be in the Hardy–Weinberg equilibrium.

Results

The mean of age (years), height (cm), and weight (kg) in cases and controls was 54.88 ± 14.88 and 54.54 ± 14.12, 164.3 ± 6.0 and 163.45 ± 5.8, and 65.8 ± 9.17 and 64.8 ± 8.97, respectively, and were comparable [Table 1]. In the present study, the biochemical parameters such as glucose, insulin, urea, creatinine, glycated albumin, triglyceride, and very-low-density lipoprotein (VLDL) cholesterol were significantly elevated in CKD cases when compared to controls.

In the present study, T allele frequency was observed in 12 controls and 23 cases (odds ratio [OR] = 2.2, 95% confidence interval [CI]: 1.0–4.7). CC genotype was observed in 38 controls and 27 cases, and CT genotype was seen in 12 controls and 22 cases and was significantly associated with CKD (OR = 2.5, $P < 0.05$). C allele frequency was found in 76 cases and 88 controls. TT genotype was found to be strongly associated with CKD patients (OR = 4.2), and T allele was found to be significantly associated with CKD patients (OR = 2.2, $P < 0.05$) [Table 2].

In the present study, GG genotype was observed in 33 controls and 21 cases, and AG genotype was observed

in 20 cases and 11 controls (OR = 2.7, 95% CI: 1.1–6.6) and was strongly associated with CKD [Table 3]. The AA genotype was observed in nine cases and six controls. G allele was found in 62 cases and 77 controls. A allele was observed in 38 cases and 23 controls and is significantly associated with CKD (OR = 2, 95% CI: 1.1–3.8).

Discussion

CKD affects 10%–15% of adults globally and increases the risk of various clinical adverse outcomes. CVD is one of the most important complications of CKD.[15] Indeed, up to 50% of people with CKD die due to CVD even before most of them reach end-stage renal disease (ESRD). Clinical guidelines are not consistent regarding whether or how to utilize information on measures of CKD for predicting the risk of CVD.[16] Although both traditional and nontraditional CHD risk factors are common in patients with CKD, the mechanistic basis for the observed accelerated atherosclerosis in CKD remains poorly defined, and the genetic variants may increase susceptibility to both diabetes and kidney disease.[17]

Table 1: Biochemical markers in chronic kidney disease patients and controls

Biochemical markers	Mean±SD		*P*
	Cases	Controls	
Cholesterol (mg/dl)	167.67±45.8	152.4±44.39	0.22
HDL-C (mg/dl)	39.87±2.26	41.1±5.7	0.15
LDL-C (mg/dl)	97.1±39.2	86.7±40.5	0.19
TGL (mg/dl)	157±44.1 mg/dl	91.86±42.21	0.001*
VLDL-C (mg/dl)	32.7±12.1 mg/dl	17.3±11.7	0.001*
TGL:HDL	3.8	3.4	0.04*
Glucose (mg/dl)	122.08±26.3	96.6±12.7	0.001*
Insulin (mIU/ml)	13.0±4.2	9.21±3.81	0.05
Urea (mg/dl)	110.7±43.2	20.4±7.2	0.001**
Creatinine (mg/dl)	5.03±2.3	0.9±0.5	0.001**

**Strongly significant ($P \leq 0.01$); *Moderately significant (P: $0.01 < P \leq 0.05$). HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; TGL: Triglyceride; VLDL-C: Very low-density lipoprotein-cholesterol; SD: Standard deviation

Table 2: Association of transcription factor 7-like 2 gene polymorphism with chronic kidney disease

Gene (*TCF7L2*)	Case	Control	OR	95% CI	*P*
CC	27	38	Reference	-	-
CT	22	12	2.5	1.0-6.0	0.03*
TT	1	0	4.2	0.1-1.0	0.3
CC versus CT + TT	23	12	2.6		0.02*
C	76	88	Reference		
T	23	12	2.2	1.0-4.7	0.04*

*$P<0.05$ Statistically significant; *Moderately significant (P: $0.01 < P \leq 0.05$). OR: Odds ratio; CI: Confidence interval; *TCF7L2*: Transcription factor 7-like 2

Table 3: Association between *ATP2B1* gene polymorphism and chronic kidney disease patients

ATP2B1	Cases	Controls	OR	95% CI	*P*
GG	21	33	Reference	-	-
AG	20	11	2.7	1.1-6.6	0.02*
AA	9	6	2.4	0.7-7.9	0.12
GG versus AG + AA	29	17	2.8	1.2-6.3	0.01*
G	62	77	Reference	-	-
A	38	23	2.0	1.1-3.8	0.02*

*$P < 0.05$ Statistically significant; *Moderately significant (P: $0.01 < P \leq 0.05$). OR: Odds ratio; CI: Confidence interval

In a study done by Sousa AG *et al.*, T allele of rs7903146 was associated with diabetes and in nondiabetic individuals with a higher prevalence and severity of coronary artery disease and cardiovascular events.[18] Baraczynska *et al*.,[5] in a group of 924 healthy participants, documented the following frequencies of rs7903146 genotypes: CC – 53%, CT – 39%, and TT – 8%, and OR for TT genotype was 2.81 and for T allele 1.57. T allele of rs7903146 of TCF7L2 gene confers the risk of developing diabetic nephropathy, and they found for the first time strong relationship between TCF7L2 gene variant rs7903146 and CVD in ESRD.[19] Cauchi *et al*. in a population of 2499 healthy French people observed the following distribution of genotypes: CC – 48.3%, CT – 42.4%, and TT – 9.3%. Munoz *et al*. found the frequencies: CC – 45%, CT – 42%, and TT – 11%. The results of three large population-based trials such as ARIC, FHS, and HAPI suggested that some variants in the TCF7L2 gene are associated with reduced kidney function and CKD progression overall and among participants without diabetes. In the present study, T allele of TCF7L2 gene was observed in 12 controls and 23 cases (OR = 2.2, 95% CI: 1.0–4.7). CC genotype was observed in 38 controls and 27 cases and CT genotype in 12 controls and 22 cases. C allele frequency was found in 76 cases and 88 controls.

Ferguson JF *et al*. in their study found that CKD is associated with coronary artery calcification. Numerous studies have attempted to identify genetic markers for hypertension over the past two decades, but no cross-validated loci in different ethnic groups have thus

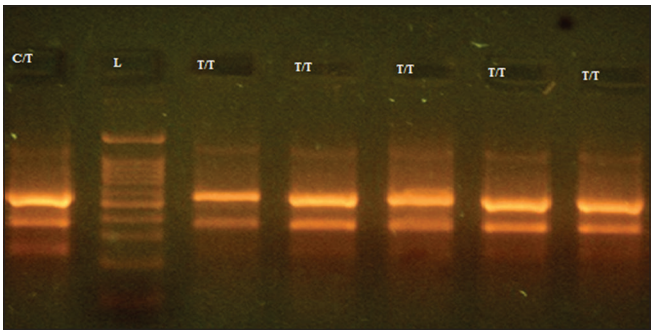

Figure 1: Genotyping result for TCF7L2 gene (rs7903146): Marker (100 bp): Ladder 2, Lane 1 CT (223 and 334), Lane 3, Lane 4, Lane 5, Lane 6, Lane 7 (334 bp): TT Genotype

far been identified except for the Mendelian forms of hypertension.[20] The Millennium Genome Project identified SNPs located upstream or within the ATP2B1 gene as strong susceptible polymorphisms for hypertension in Japanese. Some of these findings have been replicated in individuals of European descent in the Global Blood Pressure Genetics sample and have also been validated in other studies in individuals of European descent, Koreans, and Japanese. In our study, GG genotype was observed in 33 controls and 21 cases and AG genotype was observed in 20 cases and 11 controls (OR = 1.9, 95% CI: 0.8–4.6). The AA genotype was observed in nine cases and six controls. G allele was found in 62 cases and 77 controls. A allele was observed in 38 cases and 23 controls (OR = 2, 95% CI: 1.1–3.8, $P = 0.02$) and is strongly associated with CKD patients.

Dyslipidemia has been established as a well-known traditional risk factor for CVD in the general population, and it is well known that patients with CKD exhibit significant alterations in lipoprotein metabolism.[21] Hypertriglyceridemia is one of the most common quantitative lipid abnormalities seen in patients with CKD. The concentrations of triglyceride-rich lipoproteins (VLDL, chylomicrons, and their remnants) start to increase in early stages of CKD. In our study, serum triacylglycerol levels were higher when compared to controls.

Insulin resistance (IR) is common in patients with mild-to-moderate CKD. IR is also an independent predictor of cardiovascular mortality in ESRD and is increasing recognized as a nontraditional risk factor. IR is linked to protein-energy wasting and malnutrition. Nutritional, metabolic, and cardiovascular complications of renal disease may be a consequence of abnormal insulin action.[22] Therefore, IR may be an important therapeutic target for reduction of cardiovascular mortality in patients with CKD.

Hence, the present study was planned to verify SNPs as CVD risk factors in CKD. Although the magnitude of this risk has been repeatedly discussed in many articles, the trials have conveniently excluded patients with renal impairment. The mechanism underlying the increased risk of cardiovascular events in patients with CKD has not been well-defined. Thus, evidence-based management of CVD in CKD is lacking. This has significant treatment implications, as measures directed at preventing the progression of CKD would prevent cardiovascular morbidity and mortality too.

Limitation

The present pilot study was carried out on a small sample size of fifty CKD patients and fifty healthy controls, and SNPs were assessed by conventional PCR.

Conclusion

The T allele of TCF7L2 and the A allele of ATP2B1 genes are strongly associated with CKD, and in comparison with similar studies, they confer the cardiovascular risk in CKD. Hence, targeting of genetic markers in CKD would prevent cardiovascular morbidity and mortality.

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Conflicts of interest

There are no conflicts of interest.

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