




# Role of Transcription Factor 7 like 2 and Silent Information Regulator 1 Genes in the Development of Cardiovascular Complications in a Group of Egyptian Patients with Chronic Kidney Disease

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## Abstract

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**Keywords:** Chronic kidney disease; Cardiovascular diseases; Polymerase chain reaction; Single nucleotide polymorphisms; Silent information regulator 1; Transcription factor 7-like 2

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**BACKGROUND:** Sirtuins silent information regulator 1 (SIRT) is histone deacetylases that act as antioxidants and involved in the pathogenesis of cardiovascular diseases (CVD) which are the major complications of chronic kidney disease (CKD). Transcription factor 7-like 2 (TCF7L2) genetic polymorphisms could contribute to the risk of CVD as TCF7L2 proteins regulate vascular remodeling.

**AIM:** We tried to demonstrate the role of genetic polymorphisms: rs7069102 and rs10823108 in SIRT1 gene and rs7903146 in TCF7L2 gene in the development of CVD in CKD Egyptian patients.

**METHODS:** This study included 120 CKD patients (60 with CVD and 60 without CVD) and 60 age and sex-matched healthy subjects as a control group. Routine laboratory investigations were performed and genotyping for candidate single nucleotide polymorphisms was done by Taqman-real-time polymerase chain reaction.

**RESULTS:** The frequency of the C allele of rs7069102 was significantly higher in CKD patients with CVD as compared to the normal control group ( $p < 0.001$ ) and as compared to CKD patients without CVD ( $p < 0.001$ ). Percentages of AG and GG genotypes of rs10823108 were significantly higher in CKD patients with CVD as compared to the normal control group ( $p = 0.002, 0.035$ , respectively). The frequency of the T allele of rs7903146 was significantly higher in CKD patients with CVD as compared to the normal control group ( $p < 0.001$ ).

**CONCLUSION:** We found that C allele of rs7069102, GG and AG genotypes of rs10823108 in the SIRT1 gene and T allele of rs7903146 in TCF7L2 gene have a potential role in the pathogenesis and the risk of CVD development in CKD Egyptian patients.

## Introduction

Chronic kidney disease (CKD) is a common chronic disease affecting many people worldwide. CKD is diagnosed by reduction of estimated glomerular filtration rate (eGFR)  $<60$  mL/min/1.73 m<sup>2</sup> for at least 3 months [1]. The global incidence and prevalence of CKD had markedly increased over the past 20 years. Mortality due to CKD has also increased dramatically [2]. End-stage renal disease (ESRD) is usually associated with other chronic diseases such as diabetes and hypertension. The most important cause for the increased morbidity and mortality among ESRD patients is cardiovascular diseases (CVD); the prevalence of CVD is significantly elevated in patients with CKD that can't be attributed to traditional risk factors such as diabetes and hypertension alone [3]. Several investigators had studied the risk of CVD to know the exact pathogenic mechanisms that could explain the higher risk of CVD in CKD patients. The identification of

genetic factors that are associated with the pathogenesis of CVD in CKD patients could help in the improvement of diagnostic tools and risk assessment of CVD in CKD patients as well as modification of therapy [4].

The transcription factor 7-like 2 (TCF7L2) gene is located on chromosome 10q25.3, which contains 215,863 bases, including 17 exons, encoding 596 amino acids. The TCF7L2 is a member of the TCF/lymphoid enhancer factor family. It is a downstream regulator in the Wnt signaling pathway, which regulates the proliferation of pancreatic beta-cell; thus, it stabilizes glucose levels in the body through the regulation of insulin secretion. Interestingly, TCF7L2 protein encoded by the TCF7L2 gene and could increase the risk of CVD as it is a major regulator in the endothelial cell growth proliferation, and thus, it affects vascular remodeling [5]. TCF7L2 gene is one of the candidate genes predisposing to diabetes. Many single nucleotide polymorphisms (SNPs) in the TCF7L2 gene were associated with increased risk of type 2 diabetes, especially the SNP: rs7903146, which

is located in the intron 3. This SNP is also linked to coronary atherosclerosis in diabetic patients [6].

Sirtuins silent information regulator (SIRT1) is a family of histone deacetylases regulating gene expression [7]. Sirtuin proteins are categorized according to their subcellular localization. SIRT1 is a nuclear protein and is also present in the cytoplasm [8]. Due to their ability to target post-translational histone modifications, sirtuins are vital for many processes such as DNA repair and antioxidant activity through promoting gene silencing and transcriptional repression. SIRT1 is mainly a nuclear protein; however, it can exit the nucleus into the cytoplasm under ischemic conditions [7], [9]. SIRT1 was reported to be associated with oxidative stress in an ischemic environment through the repression of its downstream targets [5]. Some stress-related genes are targets of the SIRT1 gene, such as mTOR, PI-3K, PPAR- $\gamma$ , and p53. SIRT1 has been recognized as a regulator of the cardiovascular system, and it has anti-atherosclerotic capabilities through the prevention of stress-related complications. Activation of SIRT1 function by drugs was reported to reduce oxidative injury-induced endothelial cell death [10]. Accordingly, we tried to investigate the role of SNPs of rs7069102 and rs10823108 in the SIRT1 gene as well rs7903146 in the TCF7L2 gene in the development of CVD in CKD Egyptian patients.

- CKD patients were subjected to full history taking and clinical examination
- They were stratified according to disease severity by calculation of eGFR using Modification of Diet in Renal Disease equation
- Body mass index (BMI) was calculated by dividing weight by height square ( $\text{kg}/\text{m}^2$ ) and categorized according to the World Health Organization recommendations
- All ESRD patients were recruited from the dialysis unit of Theodor Bilharz Research Institute and they were on regular hemodialysis
- CVD were diagnosed by the electrocardiogram and/or echocardiography
- Patients who had histories of congenital heart disease, rheumatic heart disease, recent myocardial infarction, unstable angina pectoris, acute heart failure, malignancies, major trauma or surgery in the previous 6 months, acute or chronic infectious disease, any kind of immune-mediated disease, and those who were below 18 years old were excluded
- Group III: 60 ethnically-matched donors with no prior medical or family history of CKD were recruited for comparison to a healthy population, randomly selected from individuals attending the outpatient clinic for other reasons. They had normal liver and kidney function tests with no history of hypertension, alcohol abuse, or diabetes.

## Methodology

### **Ethical approval**

Following research ethics, approval from the Institutional Research Board (IRB) of Theodor Bilharz Research Institute (TBRI), (FWA00010609) was obtained before performing the study; the serial number of the protocol is PT (637). Informed consent for the study was obtained from all research participants under the supervision of the IRB. The study was conducted following the ethical principles described by the 1964 Declaration of Helsinki and its later amendments.

### **Study design and participants**

This case-control study was conducted from June 2021 till November 2021. Corresponding to power analysis (80% power with 95% confidence interval), the study groups consisted of 180 age and sex-matched subjects, divided into three groups as follows:

- Group I: included 60 CKD patients with CVD in the form of coronary artery disease, left ventricular hypertrophy, or congestive heart failure
- Group II: included 60 CKD patients without CVD as a pathological control group

### **Laboratory investigations**

4 mm of whole blood samples were obtained after 12 h of fasting into plain tubes, centrifuged for 5 min at 4500 rotations per minute and then serum was stored at  $-20^{\circ}\text{C}$  till the time of analysis. The following biochemical parameters were determined in CKD patients and normal control group by standard biochemical laboratory methods: kidney and liver function tests including serum urea, creatinine, uric acid, calcium, phosphorus, sodium, potassium, alanine aminotransferase, aspartate aminotransferase, total protein, serum albumin, total cholesterol, and triglycerides.

Routine laboratory tests were assayed by the AU 480 chemistry analyzer - Beckman Coulter\*<sup>1</sup> in the clinical chemistry department at TBRI Hospital.

### **Analysis of SNPs**

#### **DNA extraction and quantification of DNA in samples**

2 mm of whole blood samples were collected into a sterile EDTA vacutainer tube and stored at  $-20^{\circ}\text{C}$ . Extraction of genomic DNA from peripheral blood

\* Beckman Coulter Ireland, Inc.: 250 S. Kraemer Blvd., Brea, CA. 92821 USA.

leucocytes of EDTA anticoagulated blood was done using ThermoFisher Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit\*\* (Catalog number: K0781, supplied by Clinilab, Egypt). Quantity of DNA and purity of samples were assessed using Qubit Fluorometric quantification assays (ThermoFisher Scientific, USA\*\*).

### Genotyping assays

Real-time polymerase chain reaction (PCR) with sequence-specific primers was used to define the genetic polymorphism (rs7903146) of the TCF7L2 gene and two SNPs (rs10823108 and rs7069102) of the SIRT1 gene.

Amplification of the extracted DNA and real-time PCR allelic discrimination assay was done using TaqMan® SNP Genotyping Assays (Catalog number: 4351379, supplied by Clinilab, Egypt) and were analyzed on StepOne PCR systems (Applied Biosystems<sup>§2</sup>) according to the protocol proposed by Kristiansen *et al.*, 2001 [11].

The thermal cycling conditions were programmed as the following:

95°C for 10 min, 50 cycles of 92°C for 10 s, and 60°C for 1 min. Allelic discrimination was performed by the Applied Biosystems Step One Real-Time PCR system software. Molecular studies were performed in the molecular research unit in the chemical pathology department, Kasr El-Ainy hospital- Cairo university.

### Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t-test when comparing 2 groups and analysis of variance with multiple comparisons *post-hoc* test when comparing more than 2 groups in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables [12]. For comparing categorical data, Chi-square ( $\chi^2$ ) test was performed. The exact test was used instead when the expected frequency is <5 [13]. Genotype and allele frequencies were compared between groups. Odds ratio (OR) with 95% confidence intervals was calculated using binary logistic regression.  $p < 0.05$  were considered statistically significant.

\*\* ThermoFisher Scientific, Inc.: 81 Wyman St. Waltham CA. 02451 USA.

§ Applied Biosystems: Campus (Foster City, California). 850 Lincoln Centre Dr. Foster City, CA. 94404 USA

## Results

### Demographic, clinical, and laboratory data of the study groups

The demographic, clinical, and laboratory data of the three studied groups are summarized in Table 1. We found that CKD patients with CVD had significantly higher BMI and presence of chronic diseases, namely diabetes mellitus and hypertension as compared to CKD patients without CVD ( $p < 0.001$ ). Duration of hemodialysis was significantly longer in CKD patients with CVD as compared to CKD patients without CVD ( $p < 0.001$ ). Serum urea and creatinine were significantly increased in CKD patients with CVD as compared to the normal control group ( $p < 0.001$ ). Serum calcium and albumin were significantly decreased in CKD patients with CVD as compared to the normal control group ( $p < 0.001$ ). Fasting blood glucose levels were significantly increased in CKD patients with CVD as compared to CKD patients without CVD ( $p = 0.024$ ). Total cholesterol and triglycerides levels, as well as serum phosphorus levels, were significantly increased in CKD patients with CVD as compared to both CKD patients without CVD and the normal control groups ( $p < 0.001$ ).

### Genotypic frequency and distribution of the studied SNPs among the study population

The three studied genetic polymorphisms: rs7069102 and rs10823108 in the SIRT1 gene as well as rs7903146 in the TCF7L2 gene were studied and found to differ significantly among the three studied groups ( $p < 0.001$ , 0.001, and 0.009, respectively). The frequency of the studied SNPs is represented in Figures 1-3.

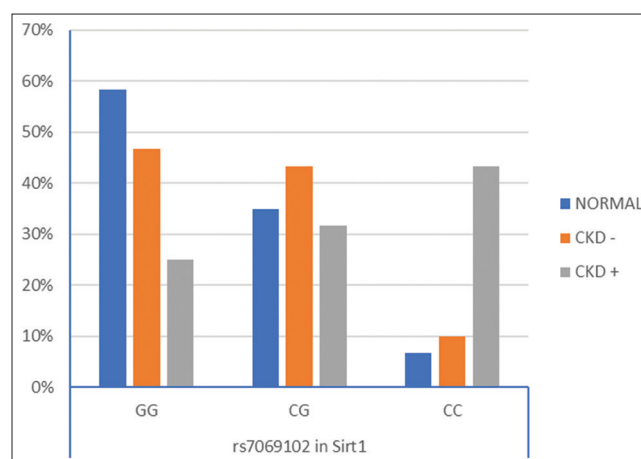


Figure 1: rs7069102 frequency in the study groups: Normal, chronic kidney disease (CKD) without cardiovascular diseases (CVD) (CKD-) and CKD with CVD (CKD+)

The percentage of CC genotype and C allele of rs7069102 in the SIRT1 gene were found to be significantly higher in CKD patients with CVD as

**Table 1: Demographic, clinical, and laboratory characteristics of the study population**

Items	CKD without CVD (n = 60)	CKD with CVD (n = 60)	Normal (n = 60)	p-value
Age (years)*	57.70 ± 6.62	56.88 ± 6.70	54.87 ± 6.85	0.062
Sex** (%)				
Male	43 (71.7)	43 (71.7)	50 (83.3)	0.229
Female	17 (28.3)	17 (28.3)	10 (16.7)	
BMI (kg/m <sup>2</sup> )*	25.03 ± 4.47	28.52 ± 4.38	--	<0.001
Weight (kg)*	71.42 ± 13.32	83.42 ± 12.46	--	<0.001
Duration of dialysis (years)*	6.62 ± 3.24	9.40 ± 3.22	--	<0.001
Systolic blood pressure (mm/Hg)*	132.35 ± 12.55	134.78 ± 14.07	--	0.320
Diastolic blood pressure (mm/Hg)*	84.52 ± 11.15	84.83 ± 8.54	--	0.862
Pulse/minute (number of beats) *	78.27 ± 11.52	76.60 ± 10.84	--	0.416
History of chronic disease** (%)				
Diabetes mellitus	11 (18.3)	10 (16.7)	--	<0.001
Hypertension	28 (46.7)	15 (25.0)	--	<0.001
Systemic lupus erythematosus	0 (0.0)	1 (1.7)	--	
Behcet's disease	0 (0.0)	2 (3.3)	--	
No chronic disease	16 (26.7)	6 (10.0)	--	
Smoking** (%)				
Yes	11 (18.3)	21 (35.0)	--	<0.001
No	44 (73.3)	34 (56.7)	--	
Ex-smoker	5 (8.3)	5 (8.3)	--	
CKD etiology** (%)				
Diabetic Nephropathy	16 (26.7)	35 (58.3)	--	0.001
Hypertensive Nephropathy	29 (48.3)	13 (21.7)	--	0.001
Analgesic Nephropathy	2 (3.3)	1 (1.7)	--	0.001
Obstructive Uropathy	12 (20.0)	8 (13.3)	--	0.001
Vasculitis	0 (0.0)	3 (5.0)	--	0.001
Polycystic kidney disease	1 (1.7)	0 (0.0)	--	0.001
Biochemical data* (%)				
Urea (mg/dl)	88.52 ± 20.64	95.67 ± 21.14‡	25.08 ± 7.68	<0.001
Creatinine (mg/dl)	8.08 ± 1.21	7.81 ± 1.39‡	0.72 ± 0.14	<0.001
Calcium (mg/dl)	8.73 ± 0.78	8.35 ± 0.85‡	10.34 ± 1.01	<0.001
Phosphorus (mg/dl)	4.62 ± 1.28	5.63 ± 1.30‡‡	3.70 ± 0.55	<0.001
Uric acid (mg/dl)	6.02 ± 1.64	5.69 ± 1.66	--	0.271
Glucose (mg/dl)	120.03 ± 50.75	141.83 ± 53.55‡	--	0.024
Cholesterol (mg/dl)	185.37 ± 47.61	239.47 ± 64.47‡‡	151.77 ± 23.76	<0.001
Triglycerides (mg/dl)	114.77 ± 27.04	148.02 ± 38.12‡‡	100 ± 24.78	<0.001
Total protein (g/dl)	7.44 ± 0.62	7.40 ± 0.90	7.38 ± 0.55	0.894
Serum albumin (g/dl)	3.74 ± 0.41	3.75 ± 0.49‡	4.17 ± 0.34	<0.001
ALT (IU/l)	26.13 ± 11.42	28.35 ± 13.40‡	19.55 ± 9.19	<0.001
AST (IU/l)	25.10 ± 12.26	29.43 ± 17.52‡	22.02 ± 6.52	0.008
Na <sup>+</sup> (mmol/l)	137.60 ± 3.56	138.07 ± 3.50	--	0.471
K <sup>+</sup> (mmol/l)	4.47 ± 0.52	4.33 ± 0.62	--	0.176

\*Data are represented as mean ± SD, \*\*Data is represented as a number (percentage), †Significant difference between CKD patients with CVD and healthy control group, ‡Significant difference between CKD patients with CVD and CKD without CVD group of patients, CKD: Chronic kidney disease; CVD: Cardiovascular diseases, BMI: Body mass index, ALT: Alanine aminotransferase, AST: Aspartate transaminase.

compared to the normal control group ( $p < 0.001$  and  $<0.001$ , respectively). Furthermore, the percentage of CC genotype and C allele of rs7069102 in the SIRT1 gene was significantly higher in CKD patients with CVD as compared to CKD patients without CVD ( $p < 0.001$  and  $<0.001$  respectively). Regarding risk estimation for CVD development, for CKD patients with CVD, as compared to the normal control group, we found that rs7069102 CC genotype and C allele carriers are highly susceptible to developing cardiovascular complications than those with (GG/CG) genotypes (for CC genotype OR =

15.167, 95% CI = 4.5-51.06 and  $p < 0.001$ ) (Table 2). Furthermore, we found that CC genotype and C allele carriers of rs7069102 in the SIRT1 gene were 8.08 and 3.1 times more susceptible to develop CVD than non-CC variant carriers (for CC genotype OR = 8.08, 95% CI = 2.7–23.9 and  $p < 0.001$ ) (Table 2).

Regarding rs10823108 in the SIRT1 gene; we found that percentage of both AG and GG genotypes was significantly higher in CKD patients with CVD as compared to the normal control group ( $p = 0.002$  and

**Table 2: Frequency distribution of SIRT1 (rs7069102) genotypes and alleles among the three studied groups**

Genotype	Normal (n = 60) (%)	CKD without CVD (n = 60) (%)	CKD with CVD (n = 60) (%)	p-value	
GG	35 (58.3)	28 (46.7)	15 (25.0)	<0.001	
CG	21 (35.0)	26 (43.3)	19 (31.7)		
CC	4 (6.7)	6 (10.0)	26 (43.3)		
	Normal (n = 60)	CKD without CVD (n = 60)	p-value	OR	95% CI
GG	35 (58.3)	28 (46.7)	Reference		
CG	21 (35.0)	26 (43.3)	0.260	1.548	0.724
CC	4 (6.7)	6 (10.0)	0.365	1.875	0.482
Allele G	91 (75.8)	82 (68.3)	Reference		
Allele C	29 (24.2)	38 (31.7)	0.196	1.454	0.824
	Normal (n = 60)	CKD with CVD (n = 60)	p-value	OR	95% CI
GG	35 (58.3)	15 (25.0)	Reference		
CG	21 (35.0)	19 (31.7)	0.091	2.111	0.887
CC	4 (6.7)	26 (43.3)	<0.001	15.167	4.504
Allele G	91 (75.8)	49 (40.8)	Reference		
Allele C	29 (24.2)	71 (59.2)	<0.001	4.547	2.612
	CKD without CVD (n = 60)	CKD with CVD (n = 60)	p-value	OR	95% CI
GG	28 (46.7)	15 (25.0)	Reference		
CG	26 (43.3)	19 (31.7)	0.480	1.364	0.576
CC	6 (10.0)	26 (43.3)	<0.001	8.089	2.728
Allele G	82 (68.3)	49 (40.8)	Reference		
Allele C	38 (31.7)	71 (59.2)	<0.001	3.127	1.841

CKD: Chronic kidney disease; CVD: Cardiovascular diseases, SIRT1: Silent information regulator 1.

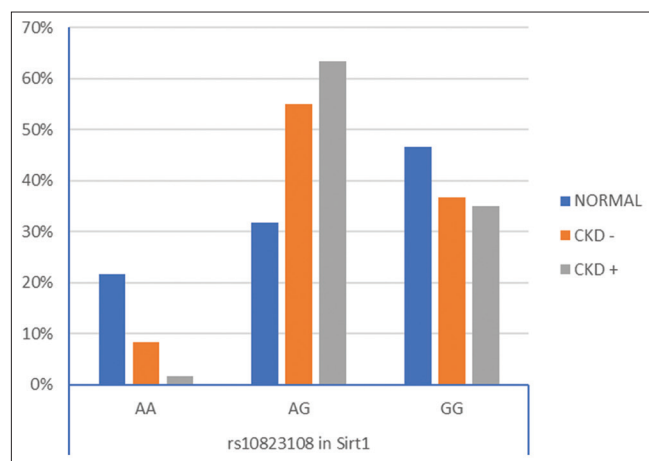


Figure 2: rs10823108 frequency in the study groups Normal, CKD without CVD (CKD-) and CKD with CVD (CKD+)

0.035, respectively). Furthermore, the rs10823108 AG genotype percentage was found to be significantly higher in CKD patients without CVD when compared to the normal control group ( $p = 0.012$ ).

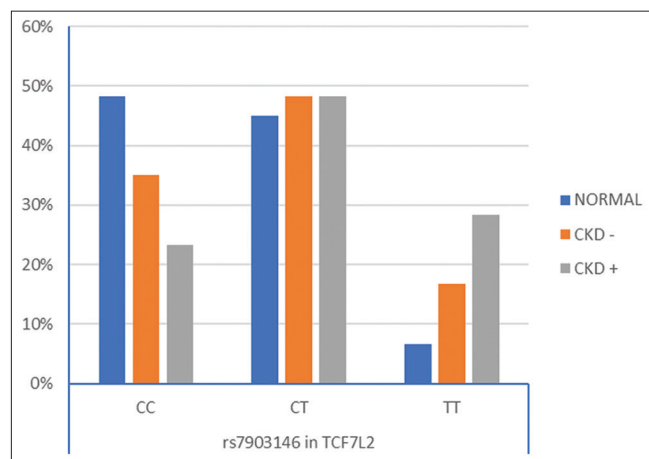


Figure 3: rs7903146 frequency in the study groups Normal, CKD without CVD (CKD-) and CKD with CVD (CKD+)

Carriers of GG genotypes in the SIRT1 gene (rs10823108) were found to be more susceptible to develop CVD than those with non-GG variant (OR = 9.7, 95% CI = 1.18-80.5 and  $p = 0.035$ ) (Table 3).

Moreover, the distribution of the homozygous TT genotype and T allele frequencies in the rs7903146 locus of the TCF7L2 gene among the three groups showed a significant difference compared to homozygous type CC in CKD patients with CVD as compared to the normal control group ( $p = 0.001$  and  $<0.001$ , respectively). The TT genotype of rs7903146 showed a significantly higher risk of cardiovascular complications as compared with carriers of non-TT variant (for TT genotype OR = 8.8, 95% CI = 2.4-31.1 and  $p = 0.001$ ) (Table 4).

### 3.3 Correlation between individual SNPs and other parameters:

We assessed the relation between SIRT1 and TCF7L2 genetic polymorphisms and the laboratory as well as the clinical parameters. We found that there is a significant positive relation between serum glucose level and CC genotype of rs7069102 in the SIRT1 gene ( $p = 0.034$ ) (Table 5). For rs10823108 in the SIRT1 gene, we found that there is a significant positive relation between serum phosphorus level and AG genotype of rs10823108 in the SIRT1 gene ( $p = 0.037$ ) (Table 6). For rs7903146 in the TCF7L2 gene, we found that TT genotype carriers of rs7903146 in TCF7L2 had a significantly higher duration of dialysis ( $p = 0.001$ ). Moreover, we found a significant positive relation between serum triglycerides level and TT genotype of TCF7L2 gene ( $p = 0.030$ ) (Table 7).

## Discussion

Genetic factors are major players in the development of cardiovascular complications especially in CKD patients since CVD represents the main cause of death in CKD patients. SIRT1 is a vital regulator of inflammation and smooth muscle proliferation in the heart. Expression of SIRT1 protein is markedly diminished in inflamed endothelial cells. We investigated

Table 3: Frequency distribution of SIRT1 (rs10823108) genotypes and alleles among the three studied groups

Genotype	Normal (n = 60) (%)	CKD without CVD (n = 60) (%)	CKD with CVD (n = 60) (%)	p-value	
AA	13 (21.7)	5 (8.3)	1 (1.7)	0.001	
AG	19 (31.7)	33 (55.0)	38 (63.3)		
GG	28 (46.7)	22 (36.7)	21 (35.0)		
	Normal (n = 60)	CKD with CVD (n = 60)	p-value	OR	95% CI
AA	13 (21.7)	1 (1.7)	Reference		
AG	19 (31.7)	38 (63.3)	0.002	26.000	3.161
GG	28 (46.7)	21 (35.0)	0.035	9.750	1.181
Allele A	45 (37.5)	40 (33.3)	Reference		
Allele G	75 (62.5)	80 (66.7)	0.500	1.200	0.706
	Normal (n = 60)	CKD without CVD (n = 60)	p-value	OR	95% CI
AA	13 (21.7)	5 (8.3)	Reference		
AG	19 (31.7)	33 (55.0)	0.012	4.516	1.394
GG	28 (46.7)	22 (36.7)	0.233	2.043	0.632
Allele A	45 (37.5)	43 (35.8)	Reference		
Allele G	75 (62.5)	77 (64.2)	0.789	1.074	0.635
	CKD without CVD (n = 60)	CKD with CVD (n = 60)	p-value	OR	95% CI
AA	5 (8.3)	1 (1.7)	Reference		
AG	33 (55.0)	38 (63.3)	0.118	5.758	0.640
GG	22 (36.7)	21 (35.0)	0.169	4.773	0.514
Allele A	43 (35.8)	40 (33.3)	Reference		
Allele G	77 (64.2)	80 (66.7)	0.684	1.117	0.656

CKD: Chronic kidney disease; CVD: Cardiovascular diseases, SIRT1: Silent information regulator 1.

**Table 4: Frequency distribution of TCF7L2 (rs7903146) genotypes and alleles among the three studied groups**

Genotype	Normal (n = 60)	CKD without CVD (n = 60)	CKD with CVD (n = 60)			p-value
CC	29 (48.3)	21 (35.0)	14 (23.3)			0.009
CT	27 (45.0)	29 (48.3)	29 (48.3)			
TT	4 (6.7)	10 (16.7)	17 (28.3)			
	Normal (n = 60)	CKD without CVD (n = 60)	CKD with CVD (n = 60)	OR	95% CI	
CC	29 (48.3)	21 (35.0)	Reference			
CT	27 (45.0)	29 (48.3)	0.314	1.483	0.688	3.198
TT	4 (6.7)	10 (16.7)	0.059	3.452	0.952	12.521
allele C	85 (70.8)	71 (59.2)	Reference			
allele T	35 (29.2)	49 (40.8)	0.059	1.676	0.980	2.865
	Normal (n = 60)	CKD with CVD (n = 60)	CKD without CVD (n = 60)	OR	95% CI	
CC	29 (48.3)	14 (23.3)	Reference			
CT	27 (45.0)	29 (48.3)	0.058	2.225	0.974	5.080
TT	4 (6.7)	17 (28.3)	0.001	8.804	2.492	31.105
Allele C	85 (70.8)	57 (47.5)	Reference			
Allele T	35 (29.2)	63 (52.5)	<0.001	2.684	1.576	4.571
	CKD without CVD (n = 60)	CKD with CVD (n = 60)	CKD without CVD (n = 60)	OR	95% CI	
CC	21 (35.0)	14 (23.3)	Reference			
CT	29 (48.3)	29 (48.3)	0.350	1.500	0.641	3.509
TT	10 (16.7)	17 (28.3)	0.076	2.550	0.907	7.165
Allele C	71 (59.2)	57 (47.5)	Reference			
Allele T	49 (40.8)	63 (52.5)	0.071	1.602	0.961	2.669

CKD: Chronic kidney disease; CVD: Cardiovascular diseases, TCF7L2: Transcription factor 7-like 2.

**Table 5: Correlation between rs7069102 in SIRT1 genotypes in both CKD groups and the laboratory data**

Parameters	rs7069102 in SIRT1			p-value
	GG	CG	CC	
BMI (kg/m <sup>2</sup> )	26.13 ± 4.71	27.43 ± 5.12	26.74 ± 4.22	0.439
Duration of dialysis (years)	7.86 ± 3.82	7.69 ± 3.38	8.66 ± 3.26	0.382
Systolic blood pressure (mm/Hg)	133.8 ± 15.96	134.16 ± 11.16	132.38 ± 12.59	0.837
Diastolic blood pressure (mm/Hg)	83.95 ± 11.37	84.22 ± 8.12	86.28 ± 10.16	0.562
Calcium (mg/dl)	8.66 ± 0.71	8.45 ± 0.96	8.51 ± 0.82	0.505
Phosphorus (mg/dl)	4.98 ± 1.42	5.24 ± 1.64	5.17 ± 0.89	0.683
Glucose (mg/dl)	121.56 ± 53.88	125.91 ± 40.47	150.59 ± 63.27	0.045
Cholesterol (mg/dl)	196.05 ± 55.82	220.96 ± 54.93	222.41 ± 77.27	0.100
Triglycerides (mg/dl)	126.98 ± 34.88	133.40 ± 35.49	134.50 ± 41.83	0.618
ESRD Etiology (%)				
Diabetic nephropathy	13 (30.2)	20 (44.4)	18 (56.3)	0.001
Hypertensive nephropathy	16 (37.2)	21 (46.7)	5 (15.6)	
Analgesic nephropathy	3 (7.0)	0 (0.0)	0 (0.0)	
Obstructive Uropathy	11 (25.6)	3 (6.7)	6 (18.8)	
Vasculitis	0 (0.0)	0 (0.0)	3 (9.4)	
Polycystic kidney disease	0 (0.0)	1 (2.2)	0 (0.0)	

CKD: Chronic kidney disease, SIRT1: Silent information regulator 1, ESRD: End stage renal disease.

the role of two SNPs in the SIRT1 gene (rs706 9102 and rs10823108) in the development of cardiovascular

**Table 6: Correlation between rs10823108 in SIRT1 in both CKD groups and the laboratory data**

Parameters	rs10823108 in SIRT1			p-value
	AA	AG	GG	
BMI (kg/m <sup>2</sup> )	23.72 ± 5.22	27.29 ± 4.84	26.35 ± 4.40	0.159
Duration of dialysis (years)	8.17 ± 2.23	7.96 ± 3.90	8.07 ± 2.99	0.807
Systolic blood pressure (mm/Hg)	126.67 ± 5.16	133.55 ± 12.42	134.56 ± 15.36	0.401
Diastolic blood pressure (mm/Hg)	85.00 ± 5.48	83.24 ± 9.22	87.00 ± 11.08	0.144
Calcium (mg/dl)	8.85 ± 0.20	8.43 ± 0.89	8.68 ± 0.78	0.188
Phosphorus (mg/dl)	3.83 ± 0.60	5.30 ± 1.45	5.03 ± 1.25	0.036
Glucose (mg/dl)	145.67 ± 80.62	132.04 ± 47.93	127.05 ± 57.74	0.700
Cholesterol (mg/dl)	214.67 ± 92.99	222.62 ± 60.74	195.26 ± 58.80	0.076
Triglycerides (mg/dl)	126.50 ± 41.97	132.20 ± 37.76	130.74 ± 35.61	0.928
ESRD Etiology (%)				
Diabetic nephropathy	3 (50.0)	30 (42.3)	18 (41.9)	0.161
Hypertensive nephropathy	1 (16.7)	30 (42.3)	11 (25.6)	
Analgesic nephropathy	0 (0.0)	0 (0.0)	3 (7.0)	
Obstructive Uropathy	2 (33.3)	9 (12.7)	9 (20.9)	
Vasculitis	0 (0.0)	1 (1.4)	2 (4.7)	
Polycystic kidney disease	0 (0.0)	1 (1.4)	0 (0.0)	

CKD: Chronic kidney disease, SIRT1: Silent information regulator 1, ESRD: End stage renal disease.

complications in a group of CKD Egyptian patients. We demonstrated the presence of an association between the C allele of the SIRT1 rs7069102 polymorphism and CVD development in CKD patients. There was a significant increase in the SIRT1 (rs7069102) CC genotypes and C allele frequency among CKD patients with CVD than the normal control group and also a significant increase in the risk of CVD among CC genotype carriers (OR = 15.167, 95% CI = 4.5–51.06 and p < 0.001) and C allele carriers (OR = 4.547, 95% CI = 2.612–7.914 and p < 0.001). Moreover, there was a significant difference in the SIRT1 (rs7069102) genotypes frequency when compared between CKD patients with CVD and those without CVD (p < 0.001); as the CC genotype was significantly higher in CKD patients with CVD than in those without CVD, also we found that CC genotype carriers in CKD patients with CVD were at higher risk for CVD development when compared to CKD patients without CVD (OR = 8.089 and 95% CI = 2.728–23.983) and the same result was also found for C allele (OR = 3.127 and 95% CI = 1.841–5.310); finding that CKD patients with CVD harboring

**Table 7: Correlation between rs7903146 in TCF7L2 in both CKD groups and the laboratory data**

Parameters	rs7903146 in TCF7L2			p-value
	CC	CT	TT	
BMI (kg/m <sup>2</sup> )	26.55 ± 5.09	27.02 ± 4.93	26.54 ± 3.94	0.861
Duration of dialysis (years)	6.69 ± 4.00	8.07 ± 3.18	9.59 ± 2.89	0.001
Systolic blood pressure (mm/Hg)	133.89 ± 12.03	134.00 ± 13.35	132.22 ± 15.21	0.839
Diastolic blood pressure (mm/Hg)	82.03 ± 11.14	85.34 ± 8.83	86.67 ± 10.00	0.145
Calcium (mg/dl)	8.70 ± 0.77	8.42 ± 0.84	8.60 ± 0.90	0.263
Phosphorus (mg/dl)	5.20 ± 1.32	5.10 ± 1.26	5.10 ± 1.74	0.931
Glucose (mg/dl)	130.14 ± 41.83	126.62 ± 53.64	141.22 ± 64.43	0.499
Cholesterol (mg/dl)	198.20 ± 46.50	215.53 ± 58.47	224.15 ± 84.81	0.237
Triglycerides (mg/dl)	121.17 ± 33.56	131.03 ± 35.76	145.41 ± 40.17	0.036
ESRD Etiology (%)				
Diabetic nephropathy	15 (42.9)	22 (37.9)	14 (51.9)	0.540
Hypertensive nephropathy	11 (31.4)	23 (39.7)	8 (29.6)	
Analgesic nephropathy	1 (2.9)	1 (1.70)	1 (3.7)	
Obstructive Uropathy	8 (22.9)	10 (17.2)	2 (7.4)	
Vasculitis	0 (0.0)	1 (1.7)	2 (7.4)	
Polycystic kidney disease	0 (0.0)	1 (1.7)	0 (0.0)	

CKD: Chronic kidney disease, TCF7L2: Transcription factor 7-like 2, ESRD: End stage renal disease

CC genotype and C allele are more susceptible to develop CVD 15.167 and 4.5 times, respectively, than those with (GG/CG) genotypes.

Following our results, a study investigated the relationship between rs7069102 genetic polymorphism and the risk of myocardial infarction, showing that patients carrying the C allele of the rs7069102 have an increased risk of myocardial infarction. This study found that rs7069102 polymorphism could affect the expression of SIRT1 protein and CC genotype might lead to over-expression of SIRT1 protein in CVD patients; this increase in SIRT1 protein levels could be understood as a compensatory mechanism for enhancing the production of e-NOS to combat high levels of oxidative stress in CKD patients with CVD [14]. Another study revealed that CKD patients carrying the C variant of rs7069102 in the SIRT1 gene had an increased incidence of concentric left ventricular hypertrophy [15], which also comes in accordance with our results.

Regarding rs10823108 SNP in the SIRT1 gene, we found a significant association between the AG and GG genotypes of rs10823108 in the SIRT1 gene and CVD development in CKD patients. The percentage of both AG and GG genotypes was significantly increased in CKD patients with CVD as compared to the normal control group ( $p = 0.002$  and  $0.035$ , respectively). Carriers of GG genotype in the SIRT1 gene (rs10823108) are more susceptible to developing CVD than those with other SIRT1 rs10823108 genotypes (OR = 9.7 and 95% CI = 1.18–80.5), denoting that GG genotype carriers of rs10823108 in the SIRT1 gene are 9.7 times more susceptible to develop CVD. Up to the best of our knowledge, our present study is the first one to investigate rs10823108 and CVD development in CKD patients.

The presence of an association of SIRT1 genetic polymorphisms with the pathogenesis and increased risk of diabetic kidney disease (DKD) and its complications was confirmed by recent studies. A recent study showed that the GG and AG genotypes in the rs10823108 locus were associated with increased DKD risk [16]. As well as the C allele, CC and CG genotypes of rs7069102 were more common in the group of diabetic nephropathy patients when compared with the GG genotype carriers (OR = 1.94 and  $p = 0.02$ ) [17], which goes hand in hand with our study as we found a significant positive correlation between serum glucose levels and the CC genotype of rs7069102 in the SIRT1 gene ( $p = 0.034$ ). Besides, SIRT1 induces lipolysis in the adipose tissue, insulin secretion, and insulin sensitivity, acting as a powerful anti-diabetic factor [18]. So that we can conclude that SIRT1 rs7069102 could be used as a genetic marker to assess the cardiovascular risk among diabetic nephropathy patients.

Moreover, we found that there is a significant positive correlation between serum phosphorus levels and the AG genotype of rs10823108 in the SIRT1 gene

( $p = 0.037$ ). High serum phosphate level is a constant feature in CKD patients with CVD and has been significantly correlated with increased cardiovascular risk in CKD patients. Reduction of phosphate is crucial to prevent CVD risk in CKD patients [19]. Thus, it is vital to investigate the role of genetic polymorphisms that have a positive correlation with high levels of phosphorus in CKD patients with CVD as it might affect therapy. Hence, the importance of our study findings, which may indicate that the AG& GG genotypes of rs10823108 could aid in CVD pathogenesis in CKD patients through elevation of serum phosphorus.

TCF7L2 is a member of a family of TCFs that have a role in the pathogenesis of CVD through modulation of smooth muscle cell proliferation. The TCF7L2 rs7903146 genetic polymorphism in the TCF7L2 gene was reported previously to be associated with diabetic coronary atherosclerosis and CKD progression to ESRD. Our study revealed a significant association between the T allele and TT genotypes of rs7903146 in the TCF7L2 gene and CVD development in CKD patients. The TT genotype and T allele frequencies of rs7903146 in the TCF7L2 gene were significantly increased in CKD patients with CVD as compared to the normal control group ( $p < 0.001$  and  $<0.001$ , respectively). Moreover, we found that rs7903146 in the TCF7L2 gene TT genotype and T allele carriers are 8.8 and 2.6 times, respectively, more susceptible to develop CVD (for TT genotype OR = 8.8, 95% CI = 2.4–31.1 and  $p = 0.001$ ). A previous study performed on a group of ESRD patients with CVD compared to a healthy control group found a significant association between the TCF7L2 gene rs7903146 and risk of CVD occurrence in ESRD patients [20], which supported our results. Another study done on a group of CKD patients compared to a group of healthy controls showed that the T allele of the TCF7L2 gene rs7903146 is a genetic risk factor for CVD in CKD patients [21], which also comes in accordance with our results.

TCF7L2 is well-known to be one of the diabetes-susceptibility genes, it could elevate the risk of renal disease by two mechanisms, the first mechanism is through its effect on diabetes susceptibility, and DM furtherly will cause renal damage. The second mechanism is through the TCF4 protein, expressed by the TCF7L2 gene. TCF4 protein is important for kidney development and thus could affect the progression of kidney disease. These findings are supported by a study performed on 2 groups of diabetic patients, one group with DKD and the other group without DKD, which found that patients harboring the TT genotype of rs7903146 were at higher risk to acquire the nephropathy than the CC genotype carriers ( $p = 0.02$ ), elucidating that the T allele confers genetic risk to develop nephropathy and its complications such as CVD [22], which also come in accordance of our results. A recent study performed on CKD patients with CVD compared to healthy controls reported that the T allele of rs7903146 had a significant

association with CVD development in CKD patients (OR = 2.2 and  $p < 0.05$ ) [21]. Another study performed on ESRD with CVD compared to a group of healthy controls found that CKD patients carrying the T allele of rs7903146 in the TCF7L2 gene were 1.57 times more susceptible for developing CVD than C allele carriers (OR = 1.57 and 95 % CI = 1.31–1.90), which comes in harmony with the results of our study [21].

As hypertriglyceridemia is one of the traditional risk factors for CVD development in CKD patients; Kulkarni *et al.*, 2019 found that serum triglycerides levels were significantly increased in CKD patients with CVD as compared to normal controls, which is matched with our results as we elucidated a significant positive correlation between serum triglycerides levels and the TT genotype of TCF7L2 gene ( $p = 0.030$ ), confirming that the T allele of rs7903146 in the TCF7L2 gene confers high risk for CVD development in CKD patients [21].

Our study was performed to assess the genetic polymorphisms as molecular risk factors in the pathogenesis of CVD in CKD patients. The exact mechanism which can fully explain the exaggerated prevalence of cardiovascular morbidity and mortality in patients with CKD is not fully understood yet. Therefore, traditional management of CVD in CKD patients is not enough to hinder the progression of CVD in these patients, so investigating genetic risk factors could have a significant impact on the treatment, and also we can use them for early prediction of CVD in diabetic patients to protect diabetic patients from both CKD and CVD.

Further research is needed to confirm our results and to clarify the impact of the studied SNPs on the pathogenesis and risk of CVD complications in CKD patients, and also to identify more genetic variants as they could be used as susceptibility markers for CVD in CKD patients.

## Conclusion

To conclude, we found a significant role of rs7903146 in the TCF7L2 gene and SIRT1 rs7069102 and rs10823108 genetic polymorphisms in the development of cardiovascular events in CKD patients. This implies that these molecular variants could be used as markers for susceptibility for CVD development in CKD Egyptian patients.

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