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Clinical and Biochemical Association between Single-Nucleotide Polymorphism of the Uromodulin Gene and Albuminuria in Patients with Type-2 Diabetes Mellitus

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ABSTRACT

Objective: *This study aimed to investigate the association of single nucleotide polymorphism (SNP) of UMOD gene (three SNP at promoter region; rs13333226, rs12917707, and rs4293393) with albuminuria and other renal function biomarkers in patients with type-2 diabetes mellitus.* ***Methods:*** *A case-control study design was employed to enroll 120 subjects, where 30 healthy subjects (as control group), 30 diabetic patients with normo-albuminuria (as first case group), 30 diabetic patients with microalbuminuria (as second case group) and 30 diabetic patients with macroalbuminuria (as third case group) were involved. Blood and urine samples were collected from the patients during their visits to diabetic clinics. Age, gender and BMI were taken for each participant. Fasting serum glucose (FSG), serum creatinine and blood urea were measured by a spectrophotometer, serum cystatin-c by ELISA technique, HbA1c was measured by the CLOVER A1c system, urinary albumin was measured by turbidimetric end-point method, (UACR) urinary albumin creatinine ratio were estimated, eGFR was also calculated. A whole blood sample was used for DNA extraction, finally, real time PCR technique was used for the determination of SNP genotype.* ***Results:*** *The results showed that the G minor allele of rs13333226 has a protective factor in patients with albuminuria. Also, the common variant AA of rs13333226 genotype was associated with a reduction in GFR. For the rs12917707 the common variant GG was associated with the development of albuminuria in diabetic patients and with the reduction in GFR. Finally, the frequency of (rs4293393) CC genotype was common and associated with the development of albuminuria in diabetic patients when compared with healthy controls.* ***Conclusions:*** *SNP in the regulatory region of the UMOD gene has a role in the protection from albuminuria in diabetes mellitus patients.*

Keywords: Uromodulin, Albuminuria, UMOD gene, Microalbuminuria, Diabetes mellitus, Nephropathy

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is considered as a major health problem throughout the world. Diabetes mellitus (DM) is the most frequent cause of chronic kidney failure in both developed and developing countries [1].

Albuminuria is the first sign of diabetic nephropathy (DN), also renal function as measured by glomerular filtration rate (GFR) may decline before the development of albuminuria, demonstrating that there is an earlier phase of kidney damage that could be detected and targeted with interventions. On the other hand, kidney damage can progress even when albuminuria has regressed, thus annual screening for DN should also include urine albumin in addition to measurement of serum creatinine and estimation of GFR [2].

An early sign of diabetic nephropathy is microalbuminuria, which is defined as levels of albumin ranging from 30-300 mg/24-h urine collection or albumin creatinine ratio (ACR) ranging from (30-300) mg/g. Overt albuminuria, macroalbuminuria is defined as a urinary albumin excretion of more than 300 mg/24-h or (ACR) more than 300 mg/g [3].

Uromodulin (tamm-Horsful glycophosphatidylinositol-anchored glycoprotein) is exclusively expressed by epithelial cells of the thick ascending limb of Henle's loop (TAL). The released protein is excreted in the urine at a rate of 20-100 mg/d and represents the most abundant urinary protein in the healthy individual and is the main constituent of hyaline urinary casts [4]. Functions attributed to uromodulin include protection against urinary tract infections; prevention of renal calculi formation by reducing aggregation of calcium crystals and influencing transport processes by regulating the activity of the sodium-potassium-chloride co-transporter (NKCC2) [5].

UMOD gene is located on chromosome 16p12.3-16p13.11 (which is in the short (p) arm of chromosome 16) and is composed of 11 exons (Molecular Location: base pairs 20,333,051-20,356,301 on chromosome 16) [6].

UMOD is the most abundant transcript expressed in the human kidney [7]. Within the kidney, uromodulin is essentially distributed within the thick ascending limb (TAL) segment. Deep RNA sequencing in micro dissected rat nephron segments showed nearly 10-fold higher levels of uromodulin mRNA in the cortical TAL than in the medullary TAL and low levels in the distal convoluted tubule (DCT) [8].

Rare mutations in the UMOD gene have been found to cause uromodulin associated kidney disease. These mutations alter the structure of the protein, preventing its release from kidney cells. Abnormal buildup of uromodulin may trigger the self-destruction (apoptosis) of cells in the kidneys, causing kidney disease [9]. Recent genome wide association studies have shown that modifications in the UMOD gene are associated with an increased risk of chronic kidney disease (CKD), nephrolithiasis, and hypertension, and uromodulin associated single nucleotide polymorphisms may either predispose an individual to CKD or accelerate its progression [10].

In chronic kidney diseases (CKDs) of various etiologies, urinary excretion of uromodulin is usually decreased in parallel with the glomerular filtration rate (GFR) [11]. In addition, uromodulin excretion seems to be determined by common polymorphisms of the UMOD gene region. Genome wide association studies have consistently shown an association between single nucleotide polymorphisms in the UMOD gene region and estimated glomerular filtration rate (eGFR) [12].

PATIENTS AND METHOD

This study was conducted at the diabetic outpatient clinic in Al-Imamain Alkadhimain Medical city in Baghdad from the 1st of May 2016 to 25th of December 2017. The practical part was conducted at the laboratories of the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Study Groups

The study included 90 subjects (male and female) with type 2 diabetes mellitus patients diagnosed according to the American Diabetes Association (ADA) guidelines (American Diabetes Association 2015) aged 35-65 years. The patients were divided into 3 groups: Normoalbuminuria (n=30) with urinary albumin to creatinine ratio (ACR) <30 mg/g creatinine. Microalbuminuria (n=30) with (ACR) = 30-299 mg/g creatinine. Macroalbuminuria (n=30) with (ACR) >300 mg/g creatinine. As well as a control group (n=30) healthy volunteers, with age and sex matched with patient groups.

Anthropometric measurements, such as weight, height, BMI, systolic and diastolic blood pressure was assessed. A 10 ml of blood was aspirated from each patient and control subjects (2 ml in EDTA tube for HbA1c and 6 ml in the plain tube for measurement of biochemical parameters in serum and 2 ml in EDTA tube for molecular analysis). A 24 hour urine was collected for measurement of biochemical parameters in urine. QuicK-DNA™ Miniprep Kit was used for purification of high quality DNA from whole blood. Agarose gel electrophoresis was adopted to determine the presence and integrity of the DNA pieces. Nanodrop NAS-99 spectrophotometer was used for evaluating the concentration and purity of DNA in samples. The TaqMan® SNP Genotyping Assays used TaqMan® 5´-nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples.

Statistical Analysis

Data were entered into SPSS statistical software (version. 17.0). Descriptive data analysis was first done to describe the participants demographic and biochemical criteria. The mean and standard error (SE) was used to describe the continuous variables. The p-value less than 0.05 was considered significant, and less than 0.01 was considered highly significant. Allele and genotype frequencies were given as percentage frequencies, the genotype frequencies were

first tested for their agreement with Hardy-Weinberg equilibrium (HWE), and a significant difference between the observed and expected genotype frequencies was assessed by Pearson's Chi-square test. The association between UMOD gene SNP and diabetic nephropathy was presented in terms of odds ratio (OR), and a significant difference was assessed by two-tailed Fisher exact probability.

RESULTS

Total 120 (30 normalbuminuria, 30 microalbuminuria, 30 macroalbuminuria, and 30 healthy control) subjects were enrolled in this study. The anthropometric measurements and clinical characteristics of different study groups as, age, gender, BMI, diastolic blood pressure, systolic blood pressure and the duration of diabetes mellitus was analyzed by using one way ANOVA test, as illustrated in Table 1.

Table 1 mean value ± SE of age, gender, BMI, systolic BP, diastolic c BP and duration of diabetes mellitus of different studied groups

Groups		Age (years)	Gender		Body Mass Index kg/m²	Diastolic BP mmHg	Systolic BP mmHg	Duration of DM years
			Male No (%)	Female No (%)				
Normo-albuminuria	Mean ± SE	48.46 ± 1.70	13 (43%)	17 (57%)	30.59 ± 0.84	87.66 ± 2.20	134.00 ± 2.85	4.93 ± 0.66
Micro-albuminuria	Mean ± SE	50.26 ± 1.50	12 (40%)	18 (60%)	29.41 ± 0.93	90.00 ± 2.06	135.33 ± 2.91	8.26 ± 0.73
Macro-albuminuria	Mean ± SE	52.46 ± 1.78	17 (57%)	13 (43%)	29.27 ± 0.75	93.93 ± 2.58	143.66 ± 3.37	11.13 ± 0.81
control	Mean ± SE	48.20 ± 1.69	16 (53%)	14 (47%)	27.61 ± 0.47	84.66 ± 1.37	126.83 ± 2.01	-------
ANOVA p-value		0.163	0.094	0.071	0.063	0.001**	0.019*	<0.001**
*The differences are significant (p<0.05); **the differences is highly significant (p<0.01)								

Table 2 summarizes the allele frequencies (UMOD gene rs13333226) genotyping of different study subjects. The homozygous wild genotype (AA) was most abundant than (AG) and (GG) genotypes in microalbuminuria group with a frequency of (60%), in macroalbuminuria group (56.67%), in normoalbuminuria group (46.67%) and (26.67%) in control group. Whereas, the highest percentage of the heterozygous genotype (AG) in the control group was 70% with an A allele frequency of 61.67% and G allele frequency of 38.33% only.

Table 2 Genotyping of (rs13333226) UMOD gene polymorphism with allele frequency in the study groups

Group			AA	AG	GG	A	G	Total	p-value
Normoalbuminuria	O	No.	14	15	1	43	17	30	0.01
		%	46.67%	50.00%	3.33%	71.67%	28.33%		
	E	No.	15.41	12.18	2.41	-	-		
		%	51.36%	40.61%	8.03%	-	-		
Microalbuminuria	O	No.	18	12	0	48	12	30	
		%	60.00%	40.00%	0.00%	80.00%	20.00%		
	E	No.	19.2	9.6	1.2	-	-		
		%	64.00%	32.00%	4.00%	-	-		
Macroalbuminuria	O	No.	17	13	0	47	13	30	
		%	56.67%	43.33%	0.00%	78.33%	21.67%		
	E	No.	18.41	10.18	1.41	-	-		
		%	61.36%	33.94%	4.7%	-	-		
Control	O	No.	8	21	1	37	23	30	
		%	26.67%	70.00%	3.33%	61.67%	38.33%		
	E	No.	11.41	14.18	4.41	-	-		
		%	38.03%	47.28%	14.69%	-	-		
Total			57	61	2	175	65	120	

To evaluate the significance of UMOD rs13333226 A/G genotyping results, Chi-square test was used to investigate the odds ratio (OR), significance of genotyping and risk factor for the development of albuminuria in patients group when compared with healthy control group, it showed that the patients with homozygous AA genotype were at significantly

higher risk than heterozygous AG genotype to have albuminuria when compared with control subjects (OR=4.12, p=0.01, CI: 1.38-12.27 for microalbuminuira group and OR=3.59, p=0.02, CI: 1.21-10.63 for macroalbuminuria). Also, it showed that the G minor allele has a protective factor in both microalbuminuria and macroalbuminuria groups (OR=0.40, p=0.029, CI: 0.17-0.91 and OR=0.44, p=0.049, CI: 0.19-0.99) rspectively. However, normoalbuminuric patients didn't have this significant risk, as shown in Table 3.

Table 3 Odds ratio (OR), p-value and Confidence interval (CI) of the UMOD rs13333226 A/G genotypes in different diabetic patients groups versus the control group

CI	Fisher' P	O.R		
0.81-7.09	0.111	2.40	AA	Normoalbuminuria vs. control
0.05-16.7	1.000	1.00	GG	
0.14-1.12	0.116	0.42	AG	
0.73-3.38	0.246	1.57	A	
0.29-1.36	0.252	0.63	G	
1.38-12.27	0.010	4.12	AA	Microalbuminuria vs. control
-	-	-	GG	
0.09-0.83	0.021	0.28	AG	
1.09-5.64	0.029	2.48	A	
0.17-0.91	0.029	0.40	G	
1.21-10.63	0.020	3.59	AA	Macroalbuminuria vs. control
-	-	-	GG	
0.11-0.94	0.039	0.32	AG	
1.00-5.02	0.048	2.24	A	
0.19-0.99	0.0.490	0.44	G	

ANOVA test was used to stratify the glomerular filtration rate (eGFR-based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance) according to different genotypes of UMOD gene rs13333226 polymorphism in different study groups as shown in Table 4. The mean ± SE of the GFR in patients with macroalbuminuria was significantly increased (p=0.03) in the AG genotype carrier as compared to the GFR in the AA genotype. There were no statistically significant differences in the mean ± SE of the GFR in patients with normalbuminuria and microalbuminuria groups or in control group carrying the AA, AG or GG genotypes of the UMOD gene (rs13333226) polymorphism.

Table 4 Comparison of the mean ± SE of the eGFR- based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance in different genotypes of (rs13333226) SNP of the UMOD gene in the study groups

Groups	UMOD rs13333226 Genotyping	eGFR-Cr ml/ min./1.73m^2	eGFR-Cy ml/ min./1.73m^2	Cr.Cl ml/min
Normoalbuminuria	AA	94 ± 6.7	96 ± 5.8	93 ± 6.7
	GG	121 ± 4.9	126 ± 3.8	135 ± 4.3
	AG	96 ± 7.7	102 ± 5.2	105 ± 6.0
	p-value	0.66	0.35	0.16
Microalbuminuria	AA	90 ± 6.9	89 ± 5.7	85 ± 6.1
	GG	-	-	-
	AG	92 ± 7.0	90 ± 8.1	87 ± 7.2
	p-value	0.81	0.95	0.76
Macroalbuminuria	AA	73 ± 6.9	82 ± 5.5	74 ± 6.5
	GG	-	-	-
	AG	92 ± 8.2	98 ± 7.2	95 ± 8.1
	p-value	0.03	0.05	0.02
Control	AA	110 ± 11.2	107 ± 8.3	130 ± 8.8
	GG	119 ± 4.0	112 ± 3.7	127 ± 2.2
	AG	121 ± 3.6	110 ± 4.0	120 ± 2.8
	p-value	0.20	0.24	0.37

Total	AA	95 ± 3.7	96 ± 3.0	90 ± 3.6
	GG	97 ± 2.6	98 ± 2.2	123 ± 1.5
	AG	98 ± 3.8	99 ± 3.1	99 ± 3.6
	p-value	0.17	0.23	0.06

Table 5 summarizes the allele frequencies (UMOD gene rs12917707) genotyping of different study subjects. The homozygous wild genotype (GG) is the most abundant than (GT) and (TT) genotypes in macroalbuminuria group with a frequency of (53.33%), in microalbuminuria group (50%), in normoalbuminuria group (43.33%) and (26.67%) in control group. Whereas, the highest percentage of the heterozygous genotype (GT) in the control group was (66.67%) with a G allele frequency of 60% and T allele frequency of 40% only, also the highest percentage of heterozygous genotype (GT) in normoalbuminuria group was (50%) with a G allele frequency of 68.33% and T allele frequency of 31.67% only.

Table 5 Genotyping of (rs12917707) UMOD gene polymorphism with allele frequency in the study groups

Group			GG	GT	TT	G	T	Total	p-value
Normoalbuminuria	O	No.	13.00	15.00	2.00	41.00	19.00	30	0.01
		%	43.33%	50.00%	6.67%	68.33%	31.67%		
	E	No.	14.01	12.98	3.01	-	-	-	
		%	46.69%	43.28%	10.03%	-	-		
Microalbuminuria	O	No.	15.00	14.00	1.00	44.00	16.00	30	
		%	50.00%	46.67%	3.33%	73.33%	26.67%		
	E	No.	16.13	11.73	2.14	-	-	-	
		%	53.77%	39.10%	7.13%	-	-		
Macroalbuminuria	O	No.	16.00	14.00	0.00	46.00	14.00	30	
		%	53.33%	46.67%	0.00%	76.67%	23.33%		
	E	No.	17.70	10.70	1.60	-	-	-	
		%	59.00%	35.67%	5.33%	-	-		
Control	O	No.	8.00	20.00	2.00	36.00	24.00	30	
		%	26.67%	66.67%	6.66%	60.00%	40.00%		
	E	No.	10.80	14.40	4.80	-	-	-	
		%	36.00%	48.00%	16.00%	-	-		
Total			52	63	5	167	73	120	

To evaluate the significance of UMOD rs12917707 G/T genotyping results, Chi-square test was used to investigate the odds ratio (OR), significance of genotyping and risk factor for the development of albuminuria in patients group when compared with healthy control group, it showed that the patients with homozygous GG genotype were at significantly higher risk than heterozygous GT genotype to have albuminuria only in macroalbuminuria when compared with control subjects OR=3.14, p=0.037, CI: 1.06-9.26. However, both normoalbuminuria and microalbuminuria patients didn't have this significant risk, as shown in Table 6.

Table 6 Odds ratio (OR), p-value and Confidence interval (CI) of the UMOD rs12917707 G/T genotypes in different diabetic patients groups versus the control group

CI	Fisher' P	O.R		
0.71-6.22	0.179	2.10	GG	Normoalbuminuria vs. control
0.13-7.60	1.000	1.00	TT	
0.17-1.41	0.192	0.50	GT	
0.67-3.04	0.342	1.43	G	
0.32-1.47	0.342	0.69	T	
0.93-8.10	0.066	2.75	GG	Microalbuminuria vs. control
0.04-5.62	0.561	0.48	TT	
0.15-1.24	0.120	0.43	GT	
0.84-3.96	0.123	1.83	G	
0.25-1.17	0.123	0.54	T	

1.06-9.26	0.037	3.14	GG	Macroalbuminuria vs. control
-	-	-	TT	
0.12-0.96	0.045	0.35	GT	
0.99-4.82	0.048	2.69	G	
0.20-1.00	0.050	0.45	T	

ANOVA test was used to stratify the glomerular filtration rate (eGFR-based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance) according to different genotypes of UMOD gene rs12917707 polymorphism in different study groups as shown in Table 7. The mean ± SE of the GFR in patients with macroalbuminuria was significantly increased (p=0.01) in the GT genotype carrier as compared to the GFR in the GG genotype. On the other hand, there were no statistically significant differences in the mean ± SE of the GFR in patients with normoalbuminuria and microalbuminuria and/or in control group carrying the GG, GT or TT genotypes of the UMOD gene (rs12917707) polymorphism.

Table 7 Comparison of the mean ± SE of the eGFR-based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance in different genotypes of (rs12917707) SNP of the UMOD gene in the study groups

Groups	UMOD rs12917707 Genotyping	eGFR-Cr ml/ min./1.73m^2	eGFR-Cy ml/ min./1.73m^2	Cr.Cl ml/min.
Normoalbuminuria	GG	91 ± 7.6	95 ± 7.2	96 ± 8.2
	TT	102 ± 4.9	111 ± 3.8	112 ± 12.7
	GT	99 ± 6.9	102 ± 3.9	103 ± 4.9
	p-value	0.74	0.52	0.6
Microalbuminuria	GG	95 ± 7.4	98 ± 7.3	89 ± 6.7
	TT	91 ± 4.9	90 ± 4.6	84 ± 4.6
	GT	89 ± 6.3	83 ± 4.5	81 ± 5.8
	p-value	0.18	0.23	0.13
Macroalbuminuria	GG	76 ± 8.0	84 ± 7.3	71 ± 8.2
	TT	-	-	-
	GT	92 ± 7.0	98 ± 5.2	88 ± 5.7
	p-value	0.01	0.03	0.01
Control	GG	123 ± 8.2	113 ± 7.4	122 ± 3.0
	TT	116 ± 6.5	105 ± 4.5	115 ± 3.6
	GT	118 ± 5.2	112 ± 4.8	117 ± 3.9
	p-value	0.84	0.89	0.71
Total	GG	98 ± 4.1	99 ± 3.7	90 ± 3.6
	TT	96 ± 2.7	96 ± 2.2	133 ± 1.5
	GT	97 ± 3.6	97 ± 2.7	99 ± 3.6
	p-value	0.98	0.85	0.93

Table 8 summarizes the allele frequencies (UMOD gene rs4293393) genotyping of different study subjects. In all patients groups, the homozygous wild genotype (CC) was most abundant than (CT) and (TT) genotypes, in macroalbuminuria group with a frequency of (66.67%), in microalbuminuria group (73.33%), and in normoalbuminuria group (63.33%). Whereas, the highest percentage of the heterozygous genotype (CT) in the control group was (63.33%) with a C allele frequency of 78.33% and T allele frequency of 21.67% only.

Table 8 Genotyping of (rs4293393) UMOD gene polymorphism with allele frequency in the study groups

Group			CC	CT	TT	C	T	Total	p-value
Normoalbuminuria	O	No.	19.00	11.00	0.00	49.00	11.00	30	0
		%	63.33%	36.67%	0.00%	81.67%	18.33%		
	E	No.	20.01	8.98	1.01	-	-		
		%	66.70%	29.93%	3.37%	-	-		
Microalbuminuria	O	No.	22.00	8.00	0.00	52.00	8.00	30	
		%	73.33%	26.67%	0.00%	86.67%	13.33%		
	E	No.	22.54	6.93	0.53	-	-		
		%	75.13%	23.11%	1.76%	-	-		
Macroalbuminuria	O	No.	20.00	10.00	0.00	50.00	10.00	30	
		%	66.67%	33.33%	0.00%	83.33%	16.67%		
	E	No.	20.84	8.33	0.83	-	-		
		%	69.46%	27.78%	2.76%	-	-		
Control	O	No.	11.00	19.00	0.00	41.00	19.00	30	
		%	36.67%	63.33%	0.00%	78.33%	21.67%		
	E	No.	14.01	12.98	3.01	-	-		
		%	46.69%	43.28%	10.03%	-	-		
Total			72	48	0	192	48	120	

To evaluate the significance of UMOD rs4293393 C/T genotyping results, Chi-square test was used to investigate the odds ratio (OR), significance of genotyping and risk factor for the development of albuminuria in patients group when compared with healthy control group, it showed that the patients with homozygous CC genotype were at significantly higher risk than heterozygous CT genotype to have albuminuria when compared with control subjects (OR=3.45, p=0.022, CI: 1.19-9.99 for macroalbuminuria and OR=4.75, p=0.005, CI: 1.58-14.24 for microalbuminuira group). Also, it showed that the T minor allele has a protective factor in both microalbuminuria and macroalbuminuria groups (OR=0.33, p=0.019, CI: 0.13-0.83 and OR=0.43, p=0.05, CI: 0.18-1.03) rspectively. However, normoalbuminuric patients didn't have this significant risk, as shown in Table 9.

Table-9 Odds ratio (OR), p-value and Confidence interval (CI) of the UMOD rs4293393 A/G genotypes in different diabetic patients groups versus the control group

Variables		O.R	Fisher' P	CI
Normoalbuminuria vs. control	CC	2.18	0.051	1.04-8.52
	TT	-	-	-
	CT	0.63	0.064	0.11-0.95
	C	2.06	0.038	0.88-4.83
	T	0.48	0.094	0.20-1.13
Microalbuminuria vs. control	CC	4.75	0.005	1.58-14.24
	TT	-	-	-
	CT	0.21	0.005	0.07-0.63
	C	3.01	0.019	1.19-7.57
	T	0.33	0.019	0.13-0.83
Macroalbuminuria vs. control	CC	3.45	0.022	1.19-9.99
	TT	-	-	-
	CT	0.28	0.022	0.10-0.83
	C	2.31	0.048	0.97-5.53
	T	0.43	0.05	0.18-1.03

ANOVA test was used to stratify the glomerular filtration rate (eGFR-based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance) according to different genotypes of UMOD gene rs4293393 polymorphism in different study groups as shown in Table 10. It was found that there were no statistically significant differences in the mean ± SE of the GFR in patients and/or in control group carrying the CC, CT or TT genotypes of the UMOD gene (rs4293393) polymorphism.

Table 10 Comparison of the mean ± SE of the eGFR-based on serum creatinine, eGFR-based on serum Cystatin-c and creatinine clearance in different genotypes of (rs4293393) SNP of the UMOD gene in the study groups

Variables	UMOD rs4293393 Genotyping	eGFR-Cr ml/ min./1.73m²	eGFR-Cy ml/ min./1.73m²	Cr.Cl ml/min.
Normoalbuminuria	CC	95 ± 6.1	99 ± 5.5	99 ± 6.3
	TT	-	-	-
	CT	98 ± 8.7	101 ± 4.7	103 ± 6.4
	p-value	0.74	0.78	0.66
Microalbuminuria	CC	91 ± 5.5	91 ± 5.7	84 ± 5.3
	TT	-	-	-
	CT	88 ± 11.3	86 ± 7.5	83 ± 9.7
	p-value	0.77	0.63	0.89
Macroalbuminuria	CC	89 ± 6.3	95 ± 5.7	83 ± 6.4
	TT	-	-	-
	CT	73 ± 9.7	83 ± 6.7	71 ± 7.9
	p-value	0.15	0.194	0.28
Control	CC	110 ± 9.2	108 ± 6.9	111 ± 6.2
	TT	-	-	-
	CT	124 ± 3.2	114 ± 4.3	122 ± 2.3
	p-value	0.08	0.4	0.06
Total	GG	94 ± 3.2	97 ± 3.0	92 ± 3.2
	TT	-	-	-
	GT	101 ± 4.5	100 ± 3.3	101 ± 4.0
	p-value	0.2	0.49	0.09

DISCUSSION

Although the effect of age and gender in the development of albuminuria in patients with type-2 diabetes mellitus was demonstrated with previous studies [1]. In this study, there were no gender nor age related differences between study groups as shown in Table 1. In part, this was a result of the selection criteria to obtain inter-group matching regarding age and gender.

The key finding of the present study was that a common variant (AA) of rs13333226 in the regulatory region of the UMOD gene was associated with the development of albuminuria in diabetes mellitus patients, in addition, the G minor allele has a protective factor in patients with microalbuminuria and macroalbuminuria. Also, there was a significant reduction in the glomerular filtration rate (GFR) in diabetic patients with macroalbuminuria that carries a common variant AA genotype when compared with those having AG genotype.

These results support the findings of previous studies, where one study was found that a common variant rs13333226 of the UMOD gene was associated with diabetic nephropathy, the G minor allele of rs13333226 was associated with a decreased risk of nephropathy. In addition, it also showd the evidence of association of rs13333226 with eGFR and blood pressure. Notably, the rs13333226 association with diabetic nephropathy was independent of blood pressure and eGFR [13].

In the present study, the common variant GG of (rs12917707) was associated with the development of albuminuria in diabetic patients, and T minor allele had a protective factor in diabetes mellitus patient with macroalbuminuria. In addition, there was a significant reduction in the glomerular filtration rate (GFR) in diabetic patients with macroalbuminuria that carries a common variant GG genotype when compared with those having GT genotype.

In one previous study, which was conducted in ~20000 participants of European ancestry from unselected, population-based cohorts from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, identified a top SNP (rs12917707) located 3.4 kb upstream of UMOD associated with the risk of CKD. The minor T allele of rs12917707 was associated with a 20% reduction in the risk of CKD, and the association was independent of major kidney disease risk factors including older age, male gender, and presence of hypertension or diabetes [14].

The frequency of (rs4293393) CC genotype was common and associated with the development of albuminuria in

diabetic patients with albuminuria when compared with healthy controls. The CT genotype was more frequent in healthy control and diabetic patients with normoalbuminuria than in diabetic patients with albuminuria. And the T minor allele has a protective factor in diabetic patients with albuminuria. But, there was a non-significant correlation between GFR and different genotype in any one of the study groups.

In one previous study, which was an attempt to made a link between UMOD gene variant rs42993393 with kidney disease among north Indian individuals with type 2 diabetes. The frequency of C allele and TC+sCC genotype was found to be different in the overall population of the individuals with diabetes compared to HCs. Further, the frequency of C allele was higher in DN compared to HC and DM individuals, whereas there was no difference between HC and DM. These results indicate that C allele and genotype with C allele may confer the risk of kidney disease in individuals with diabetes [15].

CONCLUSION

From all results above, it can be concluded that the minor allele of UMOD gene was associated clinically and biochemically with the protection from albuminuria in patients with type-2 diabetes mellitus, also it may be concluded that the SNP may interfere with renal function by its correlation with GFR.

RECOMMENDATION

A longitudinal study design with further UMOD gene polymorphism and its relationship with different stages of albuminuria in diabetes mellitus patients. And further functional studies will be needed to answer whether this relationship can be translated into therapeutic targets.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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