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Study the Association of Uromodulin Gene rs13332878 with Chronic Kidney Disease

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Abstract

Uromodulin is the most abundant protein ordinary excreted in urine which could be used as a biomarker to diagnose kidney diseases. However, evidence suggests that it regulates salt transport, protects against urinary tract infection and kidney stones, and has a role in kidney damage and innate immunity. This study aimed to understand the association of uromodulin gene rs13332878 with chronic kidney disease. More than 100 people were selected for the study and the samples collected from the under study subjects were divided into two groups. 70 chosen subjects were under the dialysis with kidney failure, and aged between 18-88 years. The second group included 30 samples from healthy individuals, used as control. One of the ways used to identify the genotype was the tetra-primers amplification refractory mutation system–polymerase chain reaction (ARMS–PCR). Regarding the results of SNP (rs13332878), there were three genotypes: GG, CG and CC. Genotype GG was shown in the size 288bp, genotype CG was shown in the size 288+577bp, whereas genotype CC was shown in the size 577bp. The results of frequency genotypes that appeared for GG, GC and CC were 74.29%, 17.14% and 8.57% respectively in patients. Whereas in the control, the frequency of genotypes was GG 3.33%, GC 16.67% and CC 80%. It was observed that less than 1 odds ratio meant that the genotypes were to be considered as a preventive factor. While more than 1 OR meant that the risk had increased (P-value 0.01). No significant differences for the genotypes GC were found when comparing patients and the control groups (P> 0.01). So, we can conclude that there was an association between SNP (rs13332878) of uromodulin with kidney disease and genotype GG should be considered a risk factor while CC genotype represents a preventive factor.

Keywords: rs13332878, Kidney disease, Uromodulin

دراسة ارتباط جين Uromodulin rs13332878 مع مرض الكلى المزمن

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الخلاصة

اليوروموديلين هو البروتين الأكثر وفرة الذي يفرز في المعتاد عند الادرار هو علامة بيولوجية يمكن استخدامها لتشخيص اضطرابات الكلى في وقت مبكر. ومع ذلك ، تشير الدلائل إلى أنه ينظم نقل الملح ، ويحمي من عدوى المسالك البولية وحصى الكلى والمناعة الذاتية للكلى ويعتبر مؤشر لتلف الكلى. هدفت هذه

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الدراسة إلى التعرف على دور ارتباط جين rs13332878 والخاص باليوروموليين بأمراض الكلى المزمنة . تضمنت الدراسة 100 عينة وتم تقسيم عينات الدراسة إلى مجموعتين ، حيث تم اختيار 70 عينة تحت غسيل الكلى والمصابين بالفشل الكلوي و تتراوح أعمارهم بين 18–88 سنة ، اما المجموعة الثانية 30 عينة تمثل السيطرة. الطريقة المستخدمة لتحديد النمط الجيني هي نظام Tetra–primers (ARMS–PCR) . فيما يتعلق بالنتائج (rs13332878) SNP ، أظهر التركيب الجيني GG في حجم 288 bp و أظهر النمط الجيني CG في بحجم 288 + 577 ، بينما أظهر التركيب الوراثي CC بحجم 577. كانت نتائج التكرار الجيني لـ74.29) GG ٪ (و 17.14) GC٪ (و8.57) CC ٪ (في المرضى. في حين كانت الطرز الوراثية في السيطرة3.33) GG ٪ (و 16.67) GC٪ (و 80) CC٪ (. لوحظ عندما تكون نسبة الأرجحية أقل من 1 يعني أن الطرز الجينية تعتبر عاملاً وقائيًا ، بينما تعني نسبة الأرجحية أكثر من 1 عاملاً ممرض عند قيمة $P \leq 0.01$ لا توجد فروق ذات دلالة إحصائية للطرز الجينية GC عند المقارنة بين المرضى والسيطرة .($P > 0.01$) ووجود ارتباط بين SNP (rs13332878) و Uromodulin في أمراض الكلى حيث يمثل النمط الجيني GG كعامل خطر ويمثل CC كعامل وقائي.

Introduction

Uromodulin, also known as Tamm-Horsfall protein, is a 95-kDa glycoprotein encoded by the UMOD gene on chromosome 16p12.3.14,15 [1]. The thick ascending limb (TAL) and early distal convoluted tubule in the kidney are the only places in the body where it is produced. Uromodulin is released in greater amounts in the urinary system, where it is the most abundant protein under physiological conditions and has anti-inflammatory, anti-infective and electrolyte-handling properties [2].

Chronic kidney disease (CKD) is a common disorder that is increasing around the world. It's related to the progression of end-stage renal disease (ESRD) [3]. The rising number of patients with ESRD treated by renal replacement therapy, dialysis or transplantation reflects the worldwide increase in the number of patients with CKD [4]. Despite the fact that the majority of initial studies focused on candidate genes, the majority of findings were not validated, hence reflecting a limited ability to recognize pathophysiologic genes related to CKD [5]. A genome-wide association study (GWAS) is a strong hypothesis-free tool for deciphering this component through association studies of CKD with millions of genetic variations spread across the genome [6]. Since the first GWAS was published in 2005, this strategy has resulted in the finding of new loci for a variety of human illnesses and traits [7]. We examined the recent successes of GWAS meta-analyses on renal phenotypes [8]. As previously reported, even for people with the some form of kidney disease, the rate of long-term renal function failure differs greatly [9, 10]. CKD is normally observed by routine screening with a blood chemistry profile and urine samples, or as a result of an unusual discovery [11]. Patients with advanced CKD can experience fatigue, nausea, vomiting, metallic taste, unintended weight loss, pruritus, mental state changes, dyspnea or peripheral edema [12-14]. Recently it has been shown that UMOD regulates salt reabsorption along the thick ascending limb by acting on renal outer medullary potassium channels. Furthermore, one of the prevalent UMOD variants, rs13333226, has been linked to HTN and cardiovascular risk in European populations in a genome-wide association analysis [15, 16].

Aim of study

Our research aimed to understand the role of uromodulin gene, rs13332878, association with chronic kidney disease

Material and Method

Sample Collection and Ethical Consent

Samples were collected from Al-Karama Hospital (Al-Hayat Dialysis Center), The understudy samples were divided into two groups. There were 70 patients with kidney failure before dialysis, aged between 18-88 years. The second group included 30 samples from apparently healthy individuals used as control, With the average age of 35 years.. This study was approved by Ethical Review Board, College of Science, Biology Department in the University of Baghdad in October 15, 2020 (Ref.: CSEC/1020/0056).

In addition to the study of the relationship of *UMOD* gene with kidney failure, the relationship of rs13332878 with age and gender was also studied. To verify that the patients had kidney failure, urea level and creatinine in blood serum were measured and studied.

Genetic Analysis

The DNA was extracted from samples using the Korean company's [17] DNA extraction kit. Genotyping was performed by Tetra ARMS–PCR. Electrophoresis was used to identify the results of PCR in the presence of standard DNA in order to differentiate between the bunds size of the PCR results on an agarose gel. PCR reaction was at a final volume of 25µl, consisting of Taq PCR PreMix 5µl (Bioneer/Korea), outer primer 0.5µl, reverse primer 0.5µl, inner primer 0.5µl, reverse primer 0.5µl, DNA 1.5 µl and distilled water 16.5 µl. The thermo-cycling PCR conditions included an initial enzyme activation step at 95°C∘ for 10 min and 50 cycles of denaturation at 95°C for 15s, and annealing and extension at 60°C for 60s. Primers used in this study with their sequences [18] are shown in Table 1.

Table 1: Primer product (rs13332878)

Primer	Sequence	Product size
Forward OUT Primer	5'-GAACCATCCCCTGTCCTGAG - 3'	577
Reverse	5'-GGGACTCCCTGAGTAGAACC - 3'	
Forward INN Primer	5'-CAGGCCTGCAGGCAGTGGCTC - 3'	288
Reverse	5'-CTCTCTCCTCCCCAGCTTCTA - 3’	

Statistical Analysis

The results were analyzed by SAS program (Statistical Analysis System 2012) to analyze the differences between the parameters in the current study. ANOVA (Analysis of Variation-ANOVA) and Chi-square tests were used for comparison among the groups. Michael H. Court calculator online (2005 - 2008) was applied for the studied SNPs to investigate Hardy-Weinberg equilibrium, where if the p-value > 0.05, the population coordinated with HWE. Odds ratio (OR) was calculated by the statistical software epidemiological (WINPEPI version 11.65) to detect the risk of genotypes. It was applied by using Chi-square, and Fisher's exact probability. The results were considered significant when the statistical probability was less than 0.05 and 0.01.

Results and Discussion

Table 2 shows that there was a significant ($P \leq 0.01$) increase in creatinine concentration in patients (8.13 ±0.30) mg/dl, while in control it was (0.966 ±0.07) mg/dl. Also there was a significant ($P \leq 0.01$) increase in urea concentration in patients (139.61 ±5.37) mg/dl while in control it was (34.27 ±1.81) mg/dl ($P \leq 0.01$).

Table 2: Creatinine and Urea concentration Mean ± SE in patients and control groups.

Group	Mean ± SE	
	Creatinine (mg/dl) Concentration	**Urea (mg/dl) Concentration**
Patients	8.13 ±0.30	139.61 ±5.37
Control	0.966 ±0.07	34.27 ±1.81
T-test	0.938 **	16.521 **
P-value	0.0001	0.0001
**** (P≤0.01).**		

Genetic Detection

The reaction of PCR was carried for the amplification of SNP (rs13332878) site for *UMOD* gene in the optimal conditions, using a specific primer and then the PCR product was detected via electrophoresis in agarose gel (2%) as shown in Figure 1.

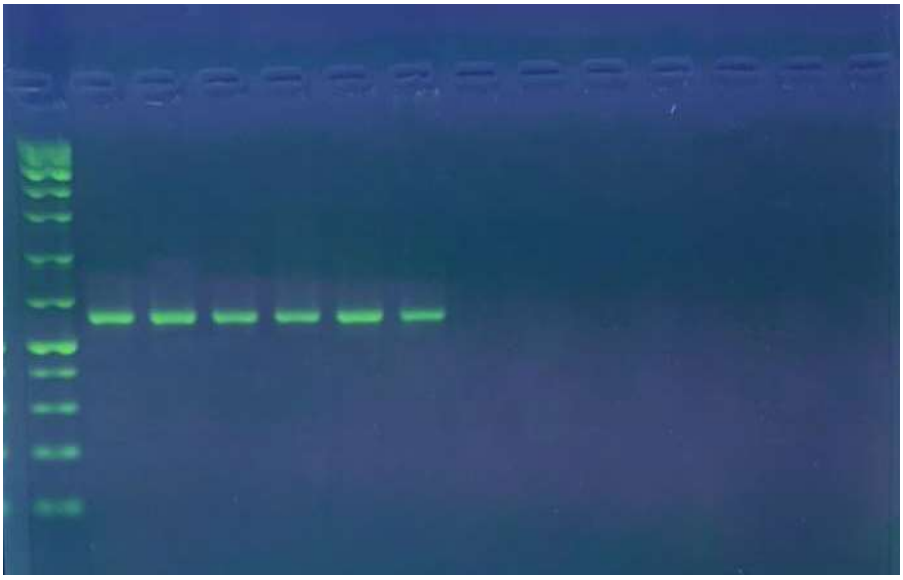

Figure 1: PCR product the band 138 bp of (rs13332878) . The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours (Ladder 1000 plus)

The results of SNP (rs13332878) are shown in Figures 2, 3 and 4 for the patients samples and Figure 5 for the control samples. Where the genotype GG is shown in the size 288bp, CG is shown in the size 288+577bp, whereas CC is shown in the size 577bp.

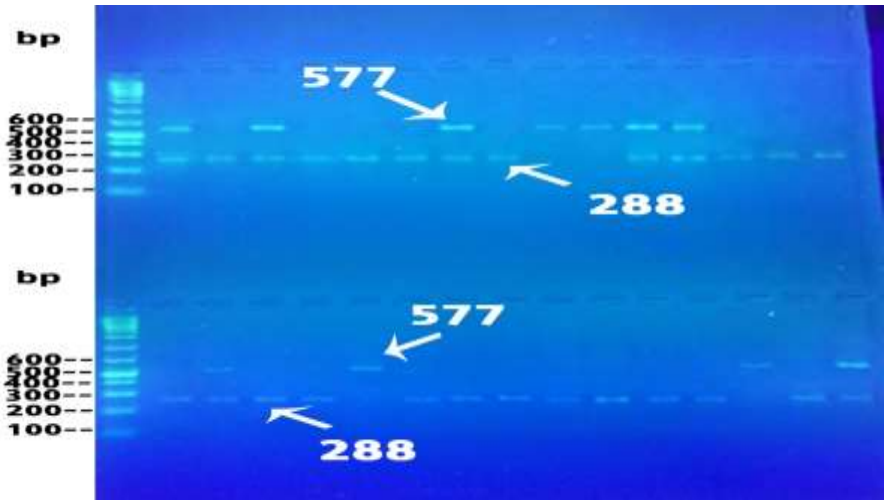

Figure 2: The output of the PCR for UMOD gene (rs13332878) of patients (1-30 samples) represents the reaction results in 288 bp and 577 bp size in agarose gel at a concentration of 2%.

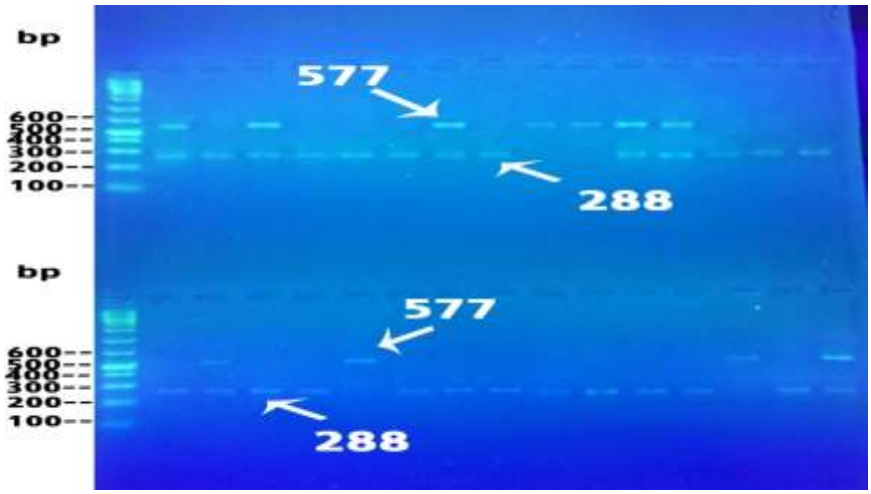

Figure 3: The output of the PCR for UMOD gene (rs13332878) of patients (31-60 samples) represents the reaction results in 288 bp and577 bp size in agarose gel at a concentration of 2%.

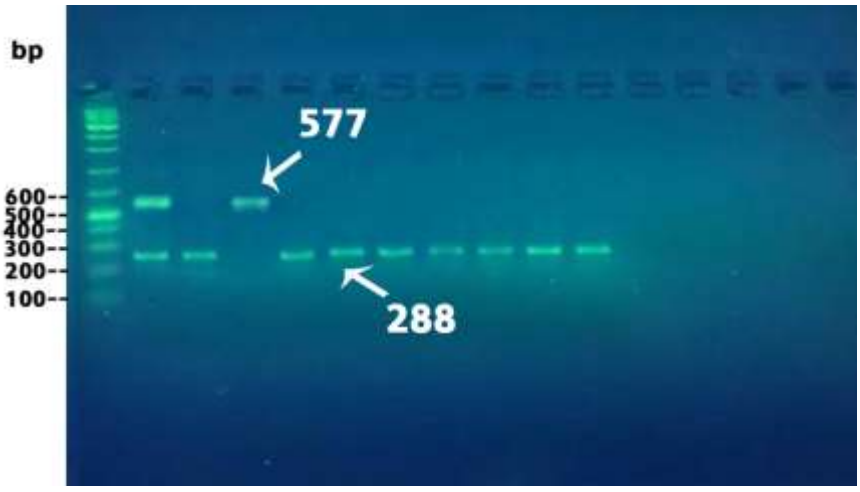

Figure 4: The output of the PCR for UMOD gene (rs13332878) of patients (61-70 samples) represents the reaction results in 288 bp and577 bp size in agarose gel at a concentration of 2%.

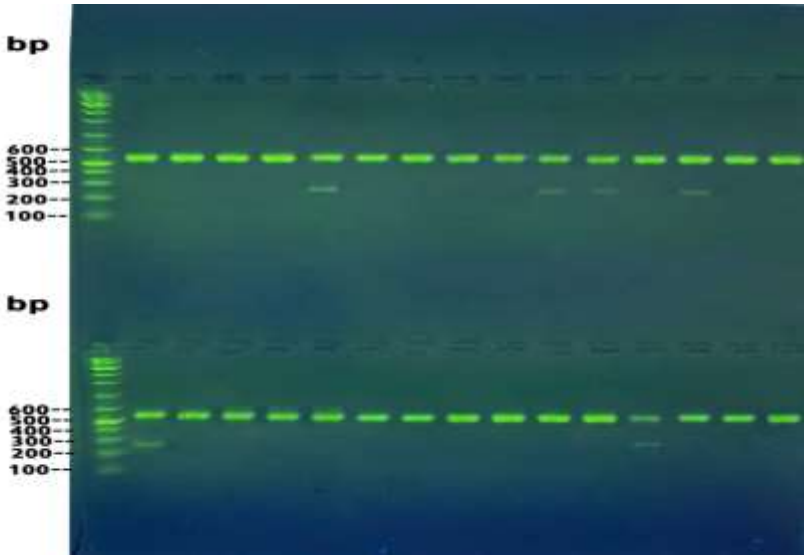

Figure 5: The output of the PCR for UMOD gene (rs13332878) of patients (70-100 samples) represents the reaction results in 288 bp and577 bp size in agarose gel at a concentration of 2%.

Table 3 shows the genotypes distribution for rs13332878 compatibility with Hardy-Weinberg equilibrium in healthy subjects while in patients it was not compatible. The results of Hardy–Weinberg equilibrium should be no less than <0.05 because of the heterogeneous population. The population in Hardy–Weinberg equilibrium has a large sample size, does random mating and has no migration [19]. The results of frequency genotypes in patients were GG 74.29%, GC 17.14% and CC 8.57%. Whereas in control, the frequency of genotypes was GG 3.33%, GC 16.67% and CC 80%. There were significant differences for the genotypes GG (OR=83.8), and CC (OR=0.02) when compared between patients and control (P≤ 0.01). In Table2, it has been observed that less than 1 OR meaning the genotypes are to be considered as a preventive factor. While OR more than 1 means the risk has increased [20]. There were no significant differences for the genotypes GC when comparing patients and control groups (P> 0.01) as shown in Table 3.

Table 3: The genotype frequency and Hardy-Weinberg Equilibrium (HWE) for rs13332878 studied groups.

Groups			Genotype frequency			
			GG	**GC**	**CC**	HWE P ≥0.05
Controls (N=30)	Observed	N	1	5	24	**0.3**
		%	3.3	16.7	80	
	Expected	N	0.4	6.2	23.4	
		%	1.3	20.7	78	
Patients (N= 70)	Observed	N	52	12	6	**0.0009**
		%	74.3	17.1	8.6	
	Expected	N	48.1	19.9	2.1	
		%	68.7	28.4	3	

Table 3: Genotype and Allele frequency of UMOD gene (rs13332878) SNP in patients and control groups.

SNP:rs13332878 Genotype	Patients No. (%)	Control No. (%)	Odd ratio (IC:95%)	P-value	Chi-Square (χ^2)
GG	52 (74.3%)	1 (3.3%)	83.8(10.99 - 638.48)	0.0001	13.44 **
GC	12 (17.1%)	5 (16.7%)	1.03(0.33- 3.20)	0.894	0.339 NS
CC	6 (8.6%)	24 (80 %)	0.02(0.01-0.08)	0.0001 **	14.61 **
Allele Frequency					
G	0.83	0.12		--	--
C	0.17	0.88		--	--
** (P≤0.01).					

Table 4: The relationship of rs13332878 within same sex

Male						
Genotype	patient	(%)	Control	(%)	Chi^2	P-value
GG	24	(68.57)	1	(3.33)		
GT	8	(22.86)	5	(16.67)		
TT	3	(8.57)	24	(80.00)	38.03	0.00**
Sum	35	(100)	30	(100)		
Ch^2	2.86		1.054			
P-value	0.091	NS	0.305	NS		
Female						
Genotype	patient	(%)	Control	(%)	Chi^2	P-value
GG	28	(80.00)	1	(3.33)		
GT	4	(11.43)	5	(16.67)		
TT	3	(8.57)	24	(80.00)	41.44	0.00**
Sum	35	(100)	30	(100)		
Ch^2	9.87		1.054			
P-value	0.002**		0.305	NS		

* Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; NS Non-significant

The results indicated that there were significant differences in males when comparing patients and the control groups (Table 4). Significant differences in females were observed when patients and control ($P \leq 0.01$) were compared. Moreover, there were no significant differences within patients and control groups both for males and females ($P > 0.05$) (Table 4).

Conclusion

It was concluded that there was an association between SNP (rs13332878) and uromodulin in kidney disease, where genotype GG represented etiological factor and CC represented protective factor. Also, there was a significant difference between males and females when comparing patients and the control groups.

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