Detection Single Nucleotide Polymorphisms in UMOD *Gene in Patients with Febrile UTI in Hilla Province*

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Abstract

Uromodulin, a glycosylphosphatidylinositol anchored glycoprotein, is exclusively expressed in the thick ascending loop of Henle1 and distal convoluted tubule2 of the mammalian kidney. It is exclusively produced in the kidney and secreted into the urine Mutations lead to uromodulin misfolding and retention in the kidney, where it might stimulate cells of immune system to cause inflammation and progression of kidney disease. Sixty one blood samples were collected from patient with febrile UTI infection whom visit Hilla Teaching hospital /Babylon /Iraq and twenty two samples as a control group**.** *DNA derived by PCR amplification of DNA from blood samples.* Genotyping of *uromodulin* gene was performed using a polymerase chain reaction technique, followed by DNA sequencing. Accordingly, these DNA polymorphisms were confirmed using DNA sequencing. The sequencing results showed the presence of many SNPs determined in the UMOD gene **.**

Key wards:- febrile UTI , Uromodulin , SNp

Introduction

UMOD, also known as Tamm–Horsfall protein, is a glycoprotein expressed exclusively by renal tubular cells lining the thick ascending limb of the loop of Henle (El‑Achkar & Wu,2012). and is the most abundant

protein excreted in the urine under physiologic conditions(Bongiovanni etal,2015). Although its physiologic functions have hitherto remained unclear until recently, substantial progress has been made in highlighting the importance of UMOD in the pathophysiology of medullary cystic kidney disease, UTI, and nephrolithiasis (Iorember & Vehaskari ,2014).For instance, UMOD is believed to be renoprotective against UTI and stone formation and has also been linked to fluid and electrolyte homeostasis, as well as to kidney innate immunity(Rampoldi etal,2011). Furthermore, there has been a renewed interest in the role of UMOD in kidney injury(El‐Achkar & Wu,2012). and in the link of its mutations with the etiology of uromodulin‐associated kidney diseases (UAKD): medullary cystic disease type 2, familial juvenile hyperuricemic nephropathy, and glomerulocystic kidney disease(Iorember & Vehaskari ,2014).

The common features of these diseases are the autosomal dominant inheritance, insignificant urine sediment, and slow progression to end‐stage renal disease, while they are also frequently associated with hyperuricemia and gout (Iorember & Vehaskari ,2014). Other characteristic features include progressive tubulointerstitial damage, impaired urinary concentrating ability, and renal cysts, with evidence pointing at intracellular accumulation of mutant UMOD (due to delayed intracellular trafficking) as a fundamental primary event in the pathogenesis of UAKD(Fugiel etal,2013).

Interestingly, the paradigm of UMOD as a trigger in kidney injury has been challenged by new evidence from a murine model showing a protective role for this protein in AKI, possibly through downregulation of interstitial inflammation(El‐Achkar & Wu,2012). UMOD is expressed by the cells of the thick ascending limb of the loop of Henle, lower UMOD

serum levels may thus reflect a reduction in number or function of these cells in CKD(Risch etal,2014).

Recently highlighted heredity investigations that proved the importance of this protein, since the uromodulin mutations were implicated in dominant tubulointerstitial viscus ill health (Dahan etal,2003; Trudu etal,2013). Several genome-wide association studies (GWASs) investigated a lot of *UMOD* genetic risk loci (such as rs12917707, rs6497476, and rs4293393) were concerned with estimated glomerular filtration rate (eGFR) related to chronic kidney disease (CKD) or upset (Regele etal,2015) . In 2009, the single-nucleotide polymorphisms (SNPs) in the *UMOD* gene were investigated among a big cohort of patients suffering from CKD and found that these SNPs may be implicated to the genetic standing in end-stage renal disease (ESRD) , SNPs are single-nucleotide variations that have arisen randomly over time in the human genome, and it is mostly occurring in noncoding regions (Reznichenko etal,2008). The aim of the study was to analyzes single-nucleotide polymorphism (SNP) in the promoter region of the *UMOD* gene in patients suffering from febrile UTI using polymerase chain reaction –sequencing technique.

Materials and methods

Sampling

Sixty one blood samples were collected from patient with febrile UTI infection whom visit Hilla Teaching hospital /Babylon /Iraq and twenty two samples as a control group**.**

DNA Extraction

The genomic DNA collected from blood and all samples were tested with the nanodrop spectrofotometer to verify and quantify the purity of DNA by measuring absorption in (260/280 nm) according to blood DNA Favorgen- Taiwan extraction kits.

Primer

The targeted sites of the *UMOD* promoter regions were amplified using design-specific primers (Bioneer, IDTDNA - USA). Forward: 5'-GGGGATCTTCTCCCTTGGC-3'; Reverse: 5'-CAACCCACGTCACAGGGAAG-3' (Isam etal,2018) .

PCR was carried out in the reaction mixture containing 1 µl from forward and reverse primers, 12.5 µl of Green Master Mix, and 3 µl of genomic DNA, and the volume of reaction was completed up to 20 µl by adding 2.5 µl of the nuclease-free water. Amplification was carried out in a TRIO Thermal Cycler (Biometra, Germany) programed for 2 min pre-denaturation at 94°C; 30 cycles, 5 min at 94°C (DNA denaturation), 1 min at 61°C (annealing), and 1min at 72°C (extended); and a final extension of 5 min. PCR products were electrophorezed using gel electrophoresis (Cleaver Scientific - UK) in 1% agarose at 75 V for 1 hand visualized by ethidium bromide. Photos were taken using gel documentation system (Cleaver Scientific - UK).

Sequencing of amplified DNA fragment

The amplified fragments were purified using the protocol suggested by Macrogen sequencing company (Macrogen - Korea), and then, they were sequenced using each primer pair. The sequences of the gene fragments analyzed were aligned by multiple sequence alignment program, according to Bio-edit, with the sequences published in the Gen Bank database taken as a reference to identify the polymorphisms.

Results

Genotyping promoter region of the *UMOD* gene

The genomic DNA has been amplified with unique primer for genotyping the ***UMOD*** gene and is done in optimum conditions by thermo-cyclers.

Results showed that the ***UMOD*** gene target sequence in agarose gel is present in a single band (317 bp) (Fig. 1)

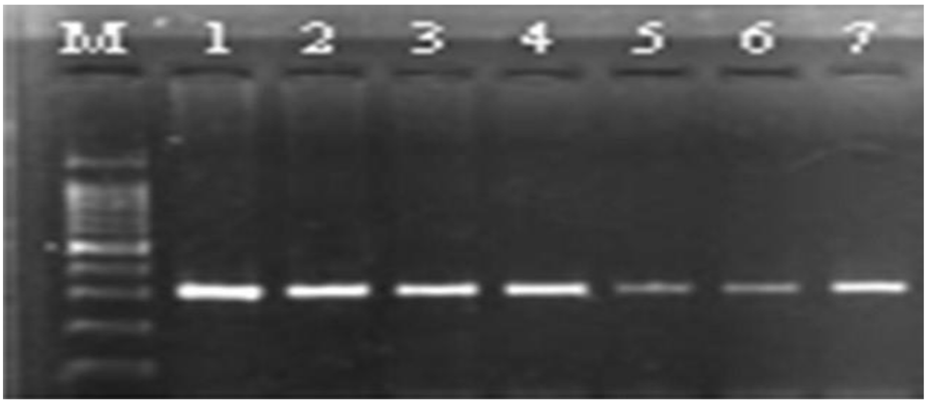

Fig. 1 Agarose gel electrophoresis of amplified products of *UOMD* promoter region of patients with chronic kidney disease and end-stage renal disease and healthy control groups. M: DNA size marker; lane 1-11 refers to the patterns of amplified products of *UMOD* promoter region (317 bp). Electrophoresis conditions: 1% agarose, 75 V, 20 mA for 120 min. The band staining with ethidium bromide

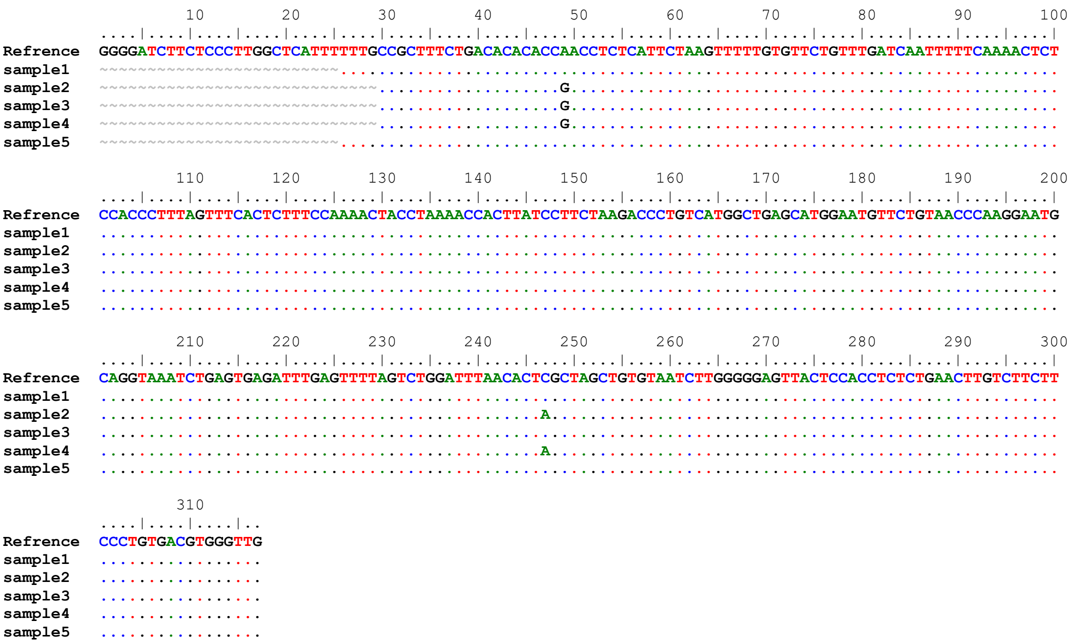

Fig. 2: Sequences alignment ID: AY061638.1 results for Homo sapiens, *UOMD* gene promoter region by Bio Edit program version 7.2.5.

Discussion

The genomic DNA was amplified using special primers for ***UOMD*** gene genotyping. The findings show that one band (317 bp) of the ***UOMD*** gene target sequence has been present.

The biological effectiveness of uromodulin is still rather mysterious, and it may have ability to conserve water/electrolyte balance inside the TAL depending on its gelification and physicochemical characteristics. In addition, it has protective effects against urinary tract infections (UTI) in Umod knockout mice (Bates etal,2004). This function is due to the ability of the uromodulin through its rich-mannose moiety to bind with the fimbria of bacteria such as *Escherichia coli* (Serafini-Cessi etal,2003).

variation in UMOD, some other variations have also been studied in diseases with renal impairment. Gómez *et al* found a missense mutation p.V458L in which leucine variant was more frequent in individuals with reduced GFR as compared to healthy individuals with normal GFR. Associations of *UMOD* rs13333226 G allele with hypertension,. However, Cui *et al* reported association of rs13333226G allele with slower decline in renal function in individuals with CKD. A study of *UMOD* variant rs12917707 in Italian diabetic cohort, no association was found with renal function(Prudente etal,2016).

Depending on the finding of Köttgen *et al*. (2010) who revealed that the SNP rs4293393 positioned at 300 bp upstream of the uromodulin encoding gene, is related to increasing a risk of febrile UTI in the Icelandic population. The rs4293393 variation was additionally connected with uric acid levels increasing and amplified risk of arthritis, a distinction with a lower danger of creation of calcium-containing urinary organ stones within the Icelandic population. The relationship between the rs4293393 or rs12917707 variants with creatinine levels increasing was definitely in a

meta-analysis of five Europeans isolates (EUROSPAN) and at intervals the large European cluster (Chambers et al., 2010).

The human GWASs all systematically show the uromodulin decreasing alleles (of rs12917707, rs4293393, rs13333226) to be related to higher GFR (Kottgen *et al*., 2009). Though lower GFR could indicate nephritic injury and reduced functioning tubular mass leading to lower urinary uromodulin as seen in clinical studies, the single-nucleotide polymorphism–GFR association implies a potential causative association between UMOD variables on GFR, If this were the case, then absence of UMOD in UMOD−/− mice should also show high GFR. However the UMOD−/− mice show significantly decreased urinary GFR compared with wild-type mice, one suggested explanation is related to the finding that adjusted 24-hour uromodulin excretion (reported as μg/ ml of creatinine clearance) is increased in patients with UTI and that 24-hour uromodulin excretion increases in diabetic patients with early kidney disease but without a significantly decreased GFR (Padmanabhan *et al*., 2010).

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