ORIGINAL ARTICLE

ASSOCIATION OF GENETIC POLYMORPHISM AND EXPRESSION OF UMOD GENE AND CHRONIC KIDNEY DISEASE

DOI: 10.36740/WLek202109209

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ABSTRACT

The aim: This study is designed to investigate the possible association of genetic polymorphism expression of UMOD gene and chronic kidney disease in population.

Materials and methods: In the current study, the single-nucleotide polymorphisms (SNPs) were genotyped in the promoter region of the UMOD gene in CKD patients to assess its association with the kidney outcome of CKD. So 50 patients' blood samples suffered from CKD were collected. Among these patients, 21 were men and 29 women, aged 35 – 85 years old. Another group included 50 healthy subjects. DNA was extracted from all blood samples with EDTA using Quick DNA miniprep Kit ZYMO, (Cat№ D3025) according to manufacturer's instructions. Genotyping of 1 common polymorphisms (rs4293393) of the UMOD gene was done and the RNA was extracted and converted to cDNA and a set of primers was used to amplify specific region within the UMOD gene; another set was used to amplify the GAPDH gene to use it in calculation as a reference gene.

Results and conclusions: After statistical analysis, the results showed that there could be association between having CC mutant polymorphism in UMOD gene and having CKD.

KEY WORDS: Chronic Kidney disease, Uromodulin protein, genetic polymorphism

Wiad Lek. 2021;74(9 p.II):2297-2300

INTRODUCTION

Chronic kidney disease is a general term for heterogeneous disorders affecting kidney structure and function. [1] It is a worldwide public health problem with an increasing incidence and prevalence, poor outcomes, and high cost. Outcomes of chronic kidney disease include not only kidney failure but also complications of decreased kidney function and cardiovascular disease. [2] So, it is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or both. Prevalence is estimated to be 8–16% worldwide. Complications include increased all-cause and cardiovascular mortality, kidney-disease progression, acute kidney injury, cognitive decline, anemia, mineral and bone disorders, and fractures. Worldwide, diabetes mellitus is the most common cause of chronic kidney disease, but in some regions other causes, such as herbal and environmental toxins are more spreaded [3].

The UMOD gene contributes to producing a protein called uromodulin. This protein is produced by the kidneys and then excreted from the body with the urine. The function of uromodulin remains unclear, although, it is known to be the most abundant protein in the urine of healthy individuals. Despite compelling genetic evidence for the association between UMOD risk variants and disease susceptibility in the general population, the underlying biological mechanism is not understood. Uromodulin overexpression in transgenic mice led to salt-sensitive hypertension and to the presence of age-dependent renal lesions similar to those observed in elderly individuals homozygous for UMOD promoter risk variants [4]. Rare mutations in UMOD have previously been described as a cause of monogenetic forms of kidney disease [5]. The UMOD gene is exclusively transcribed in the kidneys [6]. Previous studies have indicated that UMOD mutations contribute to familial juvenile hyperuricemia nephropathy, medullary cystic kidney disease 2 [7] and that promoter variants of the UMOD gene are associated with the estimated glomerular filtration rate (eGFR), blood pressure, plasma uric acid level and incidence of CKD [8-10]. In the current study, the single-nucleotide polymorphisms (SNPs) were genotyped in the promoter region of the UMOD gene in CKD patients to assess its association with the kidney outcomes of CKD.

THE AIM

This study is designed to investigate the possible association of genetic polymorphism expression of UMOD gene and chronic kidney disease in population.

MATERIALS AND METHODS

Fifty patients' blood samples suffered from CKD were collected at Al-Yarmook teaching hospital, Baghdad. Among them 21 were men and 29 – women, aged 35 – 85 years old. Another group included 50 healthy subjects. DNA was extracted from all blood samples with EDTA using Quick DNA miniprep Kit ZYMO, (Cat№ D3025) according to manufacturer's instruc-

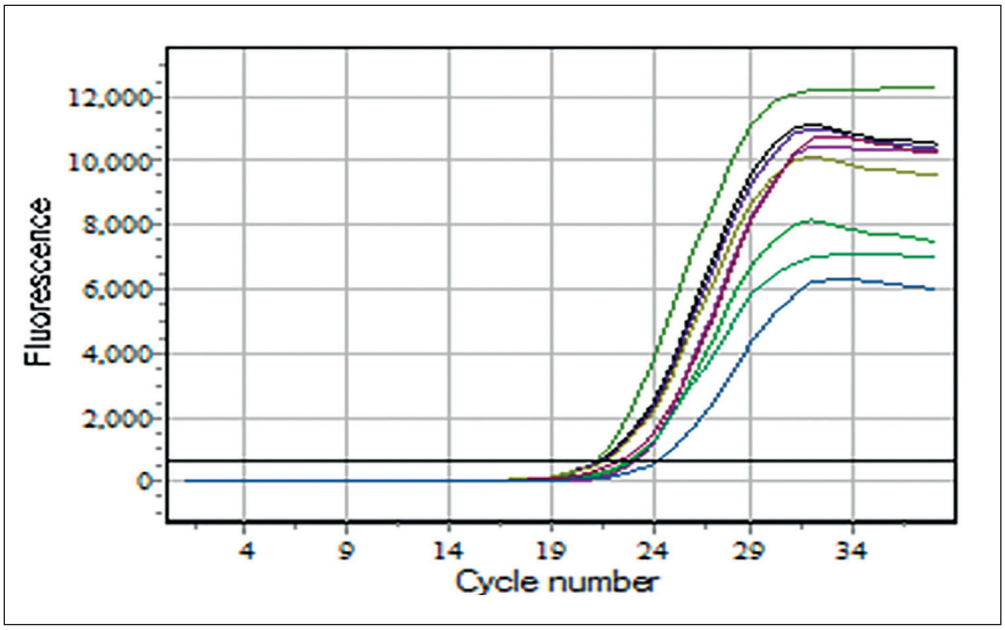

Fig. 1. The result of Real Time qPCR amplification of UMOD gene.

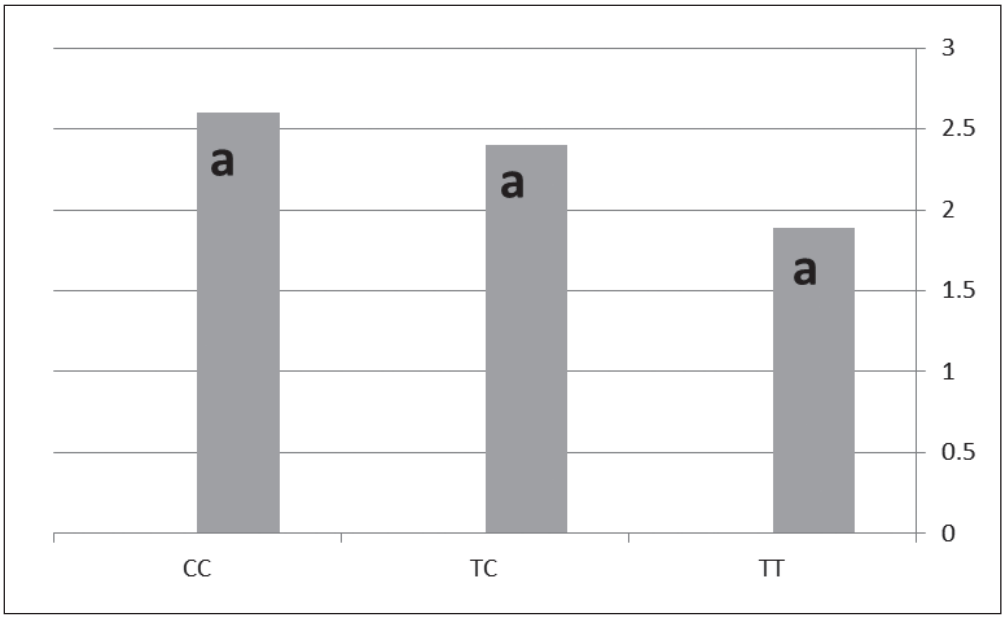

Fig. 2. Genotype distribution of UMOD gene in patients.

tions. Genotyping of 1 common polymorphisms (rs4293393) of the *UMOD* gene *was done, using* TaqMan® SNP Genotyping Assays (Cat№ 4331349). For gene expression experiment, the RNA was extracted, using RNA extraction kit (Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA.) and converted to cDNA, using PrimeScriptTM RT reagent Kit (№ RR037A). A set of primers was used to amplify specific region within the UMOD gene; forward primer, CCTTTCTGGCTTCAGGTGCT and the reverse primer, GATGAACCAAGAGGCCACCA. Another set was used to amplify the GAPDH gene to use it with calculation as a reference gene; forward primer, AGGTCATCCCTGAGCTGAA and the reverse primer CTGCTTCACCACCTTCTTGAT. The thermal cycling program was as follows: enzyme activation in 95 C° for 7 min, followed by 40 cycles of two steps (first one was denaturation 95 C° for 20 sec and second step of annealing for 20 sec (55 C°) and extension for 20 sec).

STATISTICAL ANALYSIS

Difference between groups was tested using Student's t-test and Chi square test for continuous and nominal variables, respectively. The allelic and genotype association of SNP were evaluated by Pearson's Chi-square test; and odds ratio (OR) and 95 per cent confidence intervals were determined. For comparison of more than two groups, one-way ANOVA was used. Two-tailed $P<0.05$ was considered significant. All analyses were performed using SPSS 16.0 (SPSS, Chicago IL, USA).

RESULTS AND DISCUSSION

Mutation analysis was achieved by Real Time qPCR amplification of UMOD gene, using specific sets of primers (Figure 1). The genotype and allele frequency of the polymorphism between patients and controls are illustrated in table I.

Table I. Genotype distribution of UMOD gene in healthy control and patients` groups.

Genotype	Controls	Patients	OR.	P-value	CI 95%
TT	31	14	0.24	0.001	0.10 to 0.55
TC	13	17	1.47	0.513	0.63 to 3.44
CC	6	19	4.49	0.005	1.63 to 12.42
Allele frequency					
T	75	45	0.27	0.001	0.15 to 0.50
C	25	55	0.3	0.001	2.02 to 6.66

The homozygous CC mutant genotype was significantly higher in patient than in control group (OR. =4.49, P-0.005 and CI 95% = 1.63-12.42). While the heterozygote TC mutant genotype was higher in patient than in control (OR. =1.47, P-0.513 and CI 95% = 0.63 – 3.44). And the homozygous TT wild type genotype was significantly lower in patient than in control (OR. =0.24, P- 0.001 and CI 95% = 0.10-0.55). The allele frequency of T allele is lower in the patients` group than in control one (OR. =0.27, P-value=0.001 and CI 95%= 0.15-0.50), while C allele is higher in the patients` group than in control one (OR. =0.3, P-value=0.001and CI 95%= 2.02-6.66).

Distribution of genotypes of the UMOD gene in patients are illustrated in Figure 2.

The UMOD is a 80-90 kDa glycoprotein encoded by the UMOD gene. It is the most abundant protein in mammalian urine under physiological conditions. It possesses antimicrobial properties providing defense against uropathogens responsible for urinary tract infections. Its excretion with urine follows proteolytic cleavage of the ectodomain of its glycosyl phosphatidylinositol-anchored counterpart that is situated on the luminal cell surface of the loop of Henle. This protein may act as a constitutive inhibitor of calcium crystallization in renal fluids. The UMOD may be a factor involved in the pathogenic process of kidney disease. Evidence from studies with humans and mice suggest that the role of UMOD is multifaceted [11 – 14]. One study identified that the increased urinary UMOD level was a risk factor for the future incidence of CKD [15].

According to the previous report published in 2012, CKD prevalence increases with age and affects >10% of the Chinese adult population(16). Another study also showed that CKD can progress to end-stage renal disease (ESRD) that requires dialysis or transplantation.

Other study has indicated that UMOD mutations contribute to familial juvenile hyperuricemia nephropathy, medullary cystic kidney disease 2 [7] and that promoter variants of the UMOD gene associated with the estimated glomerular filtration rate (eGFR), blood pressure, plasma uric acid level and incidence of CKD [8].

CONCLUSIONS

After statistical analysis, the results showed that there could be association between having CC mutant polymorphism in UMOD gene and having CKD.

REFERENCES

1. Levey A. S., Coresh J. Chronic kidney disease. The lancet.2012; 379(9811): 165-180.
2. Levey A. S., Coresh J., Balk E. et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Annals of internal medicine. 2003;139(2): 137-147.
3. Jha V., Garcia-Garcia G., Iseki K. et al. Chronic kidney disease: global dimension and perspectives. The Lancet. 2013; 382(9888): 260-272.
4. Trudu M., Janas S., Lanzani C. et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. Nature medicine.2013; 19(12): 1655.
5. Hart T.C., Gorry M.C., Hart P.S. et al. Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. J Med Genet. 2002;39: 882–892.
6. Serafini-Cessi F., Malagolini N., Cavallone D. Tamm-Horsfall glycoprotein: Biology and clinical relevance. Am J Kidney Dis. 2003;42: 658–676.
7. Hart T.C., Gorry M.C., Hart P.S. et al. Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. J Med Genet. 2002;39:882–892. doi: 10.1136/jmg.39.12.882.
8. Padmanabhan S., Melander O., Johnson T. et al. Global BPgen Consortium: Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet. 2010;6:e1001177. doi: 10.1371/journal.pgen.1001177.
9. Köttgen A., Pattaro C., Böger C.A. et al. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010;42:376–384. doi: 10.1038/ng.568.
10. Köttgen A., Glazer N.L., Dehghan A. et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet. 2009;41:712–717. doi: 10.1038/ng.377.
11. El-Achkar T.M., Wu X.R., Rauchman M. et al. Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression. Am J Physiol Renal Physiol. 2008;295:534–544. doi: 10.1152/ajprenal.00083.2008.
12. Sejdiu I., Torffvit O. Decreased urinary concentration of Tamm-Horsfall protein is associated with development of renal failure and cardiovascular death within 20 years in type 1 but not in type 2 diabetic patients. Scand J Urol Nephrol. 2008;42:168–174. doi: 10.1080/00365590701644691.
13. Zaucke F., Boehnlein J.M., Steffens S. et al. Uromodulin is expressed in renal primary cilia and UMOD mutations result in decreased ciliary uromodulin expression. Hum Mol Genet. 2010;19:1985–1997. doi: 10.1093/hmg/ddq077.
14. Prajczer S., Heidenreich U., Pfaller W. et al. Evidence for a role of uromodulin in chronic kidney disease progression. Nephrol Dial Transplant. 2010;25:1896–1903. doi: 10.1093/ndt/gfp748.

15. Köttgen A., Pattaro C., Böger C.A. et al. New loci associated with kidney function and chronic kidney disease. Nat Genet. 2010;42:376–384. doi: 10.1038/ng.568.
16. Zhang L., Wang F., Wang L. et al. Prevalence of chronic kidney disease in China: A cross-sectional survey. Lancet. 2012;379:815–822. doi: 10.1016/S0140-6736(12)60033-6.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received: 29.06.2021
Accepted: 30.08.2021

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article