

Prevalence of UMOD gene mutation among Saudi patients with kidney failure

Abstract

Background: Mutations in the uromodulin (UMOD) gene lead to a dominant hereditary renal disease, which may ultimately result in kidney failure. Therefore, the aim of this study was to assess the burden of UMOD associated renal among Saudi patients with renal failure (RF).

Methodology: PCR amplification of 10 exons (Forward and reverse) enclosed in the UMOD gene is done on the patient's genomic DNA of 103 Saudi patients with RF.

Results: Of the 103 patients, UMOD gene mutation was identified in 10/103 (9.7%).
Conclusion: UMOD gene mutation is relatively prevalent among Saudi patients with RF. Further evaluation of different mutations in this gene is important for overall assessment of its role in RF among Saudi population.

Keywords: UMOD gene, renal failure, CKD, MCKD2, PHCs, FJHN, erythrocyte lysis, PCR, nitrogenous, metabolites, UMOD, GALNT11, CDH23, ependorf-master cycler, MEOX2, GALNT11, IL1RAP, NPPA, HPCAL1

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Abbreviations: CKD, chronic renal disease; RF, renal failure; PHCs, primary health care centers; THP, tamm-horsfall protein; MCKD2, medullary cystic kidney disease-2; FJHN, familial juvenile hyperuricemia nephropathy; DS, digestion buffer; RT, room temperature; PCR, polymerase chain reaction; GWAS, genome-wide association studies; SNPs, single-nucleotide polymorphisms

Introduction

Chronic renal failure (RF) or chronic renal disease (CKD), is a slow progressive loss of kidney function over a period of prolonged times. Ultimately the patient has permanent kidney failure.¹ Chronic renal disease is a substantial public health problem which significantly raises the likelihood of adverse outcomes and high health-care costs. The 2011 report of the World Health Organization General Assembly on non-communicable diseases identified chronic kidney disease as a worldwide health issue posing a heavy economic burden.² There is now undoubted evidence that CKD can be detected using simple laboratory tests, and that treatment can prevent or delay complications of diminished kidney function, slow the progression of kidney disease, and decrease the risk of many CKD associated diseases.³ Risk factors for progress of CKD, mainly chronic kidney failure, would involve susceptibility factors and initiation factors.⁴ Prevention of adverse consequences of CKD could be enabled by assessing individuals with risk factors, to allow earlier detection, and by risk factor reduction in individuals without CKD, to prevent or slow the progress of the disease. The difficulty of identifying the early stages of CKD makes it challenging to determine whether the risk factors so far identified relate more to susceptibility, initiation, or progression.⁵ A multi-center cross sectional survey included 5000 Saudi selected from 30 primary health care centers (PHCs) in Kingdom of Saudi Arabia (KSA) has

reported a prevalence of 9.4%. CKD prevalence in the general Saudi population is noticeably high, as there is close homology within Saudi population in different regions.⁶ Many factor have been studied and found to contribute to the burden of CKD in KSA, including; hypertension^{7,8} diabetes,⁹ obesity^{10,11} and other factors.¹² However, with diverse causes of CKD and renal failure, genetic factors are frequently missed. The human UMOD gene is located on chromosome 16 and encodes the most common protein in human urine, Tamm-Horsfall protein (THP). Uromodulin is a glycoprotein that is encoded by the UMOD gene.^{13,14} Uromodulin is the most plentiful protein expelled in familiar urine.¹⁵ This protein acts as a constitutive inhibitor of calcium crystallization in renal fluids. The excretion of uromodulin in urine gives defense against urinary tract infections. Defects in UMOD gene are linked to the autosomal dominant renal disorders medullary cystic kidney disease-2 (MCKD2) and familial juvenile hyperuricemia nephropathy (FJHN).¹⁶ Rare mutations in UMOD cause mendelian forms of kidney disease.¹⁴ Therefore, our objective of this study was to detect the possible mutation in this UMOD gene among Saudi patients with renal failure.

Materials and methods

In this study 103 patients with RF (full coverage) were selected during a multi-center cross sectional survey (for chronic Kidney disease) included 5000 Saudi selected from 30 primary health care centers (PHCs) in Hail Region, Kingdom of Saudi Arabia (KSA). All study subjects were receiving dialysis. Whole blood was used to obtain DNA.

Blood DNA extraction

DNA was extracted from the whole blood employing the following

steps: 1.5ml of blood was added to a centrifuge tube followed by addition of 2 volume of erythrocyte lysis buffer (RS), then the vortex mixed upside down or back and forth, centrifuged at 5000rpm for 3minutes. The obtained supernatant was discarded and white or pale red precipitate was collected. Thereafter, 200ul digestion buffer (DS) was added, vortex to form completely uniform suspension, then for the removal of RNA, 4ul RNase was added, vortex 5 second and incubated at RT 5minutes. Thereafter, 20ul proteinase K and 220ul lysate (MS0) was added. vortex and incubated in water bath at 65°C for 15minutes, then 220ul ethanol was added, upside -down mixed until flocculent precipitate occurred. A brief centrifugation to remove the inner wall of the tube cap drops, and solution was transferred to purification column. Centrifuged at 12.000rpm for 1minute, and discarded the filtrate. Then 500ul of protein solution PS was added, centrifuged at 12.000rpm for 1minute, discarded the filtrate. Then 500ul Rinse (PE) was added, centrifuged at 12.000rpm for 3minutes, to completely remove the residual liquid purification column. Purification columns placed on a new 1.5ml centrifuge tube, to the center, dropping 30-100ul eluent TE, incubated at Room Temperature (RT) for 2minutes. Then centrifuged at 12.000rpm for 2minutes, bottom of the tube was containing high- purity genomic DNA. DNA was stored at -20C for subsequent amplification. Polymerase Chain Reaction (PCR) amplification of 10 exons contained in the UMOD gene is implemented on the patient's genomic DNA extracted from whole blood. Direct sequencing of amplification products is done in both forward (GAGCGGCTCAGAGAACTTCAGTGG) and reverse (CCCGTGTCTGTGTTACATTCATC) directions (Primer Sequence (5'-3'), amplification (529 bp)), using PCR method. PCR amplification of the UMOD gene was performed as designated in Table 1 & 2. The program used for amplification at the thermal cycler (Eppendorf - Master cycler) from (Beijing Aidlab Biotechnology Co., Ltd). The genotyping of the single nucleotide polymorphism (SNP) at the UMOD locus was performed. Variant rs12917707 of UMOD gene was genotyped by using the ABI Real time TaqMan allelic discrimination assay.

Table 1 Reaction mixture

DNA template	10µl
Primer 1	1µl
Primer 2	1µl
2xTaq PCR Master Mix	12.5µl
ddH ₂ O	Up to 25µl

DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; ddH₂O, double-distilled water

Table 2 PCR steps

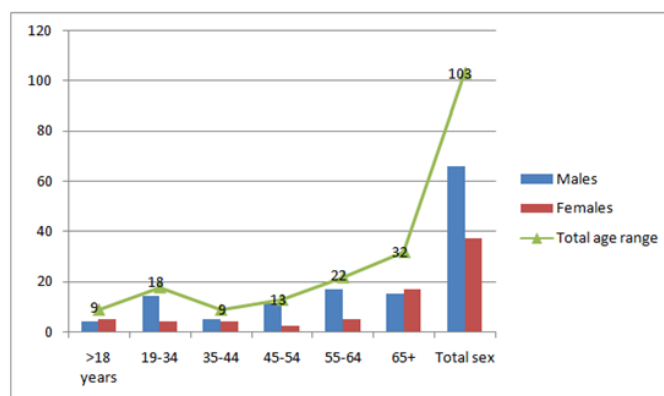
Steps	Temperature	Time	Cycles
0	94°C	Pause	
1	94°C	5min	1
	94°C	30sec	
2	55°C	30sec	30
	72°C	1min	
3	72°C	5min	1
4	4°C	Storage	

Ethical consent

The study was approved by Ethical Review Board, College of Medicine, and University of Hail. This in addition to the fact that, the authors followed the tenants of the Declaration of Helsinki. All participants were consented before inclusion and collection of their demographical data.

Results

This study investigated 103 patients with renal failure, their age ranging from 6 to 99 with a mean age of 51years. Of the 103 patients, 66/103 (64%) were males and 37/103 (36%) were females, giving males' females' ratio of 1.78:1.00, as indicated in Figure 1. As indicated in Figure 2. The distribution of UMOD gene mutations was relatively similar among different age groups within the range from 34 to 66+years with increased percentage of among elder patients (age group 65+ constituting 30% of the total mutations). However, when comparing the percentages within each age range, age group 45-54, representing the highest (18%). Of the 10 patients with UMOD gene mutation, 6/10 (60%) were identified among males and the remaining 4/10(40%) were identified among females. The relative risk (RR) associated with female sex and the 95% confidence interval (CI) was 1.2252 (0.3690 to 4.0685). The association of rs12917707 with renal failure was insignificant and showed some heterogeneity across studied subjects (p-heterogeneity=0.02). In regard to the relationship between UMOD gene mutation and ethnic groups, the majority of cases with UMOD gene mutation were identified among Shammari families representing 70%, as indicated in Figure 3. Regarding the association of Nitrogenous Metabolites with UMOD gene mutations, all patients with UMOD gene mutation were found with high creatinine levels. High urea levels were identified among 70% of patients with UMOD gene mutation. On the other hand 60% of patients with UMOD gene mutation were found with low uric acid levels. Moreover, high blood glucose levels were identified among 70% of patients with UMOD gene mutation, as seen in Table 3.

**Figure 1** Description of the study population by age and sex.

For the association between UMOD gene mutation and minerals, 50% of mutations were found among patients with low Na level and the remaining 50% were identified among those with normal Na levels. For K, 70% of patients with UMOD gene mutation were found with low K levels and the remaining 30% were found with low K levels. For Chloride, 50% of patients with UMOD gene mutation were found with normal chloride levels and 40% with low levels. For Calcium, all of patients with UMOD gene mutation were found with low calcium levels, as indicated in Table 4.

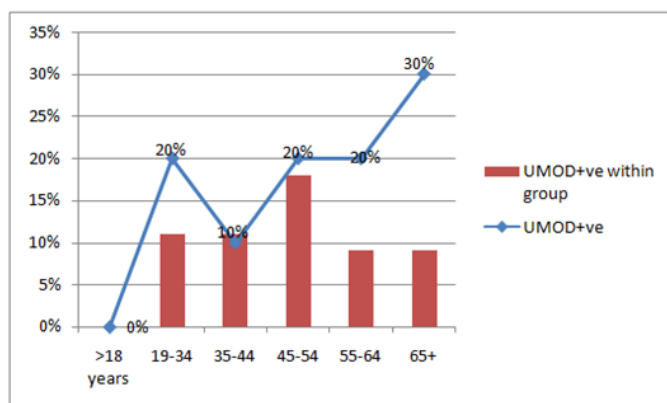


Figure 2 Description of UMOD (Uromodulin) gene mutations by age.

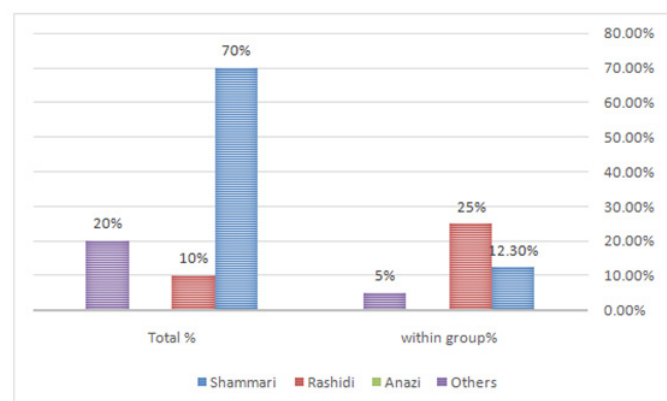


Figure 3 Of the study population by UMOD gene mutation and ethnic groups (Families).

Table 3 Distribution of nitrogenous metabolites and blood glucose with UMOD gene mutations

Category	UMOD gene mutation			Total
	Variable	Negative	Positive	
Creatinine	Low	1	0	0
	Normal	6	0	6
	High	86	10	103
Urea	Low	6	1	7
	Normal	32	2	34
	High	55	7	62
Uric Acid	Low	51	6	57
	Normal	25	2	27
	High	15	4	19
Blood Glucose	Low	2	0	0
	Normal	30	3	33
	High	61	7	68

*UMOD, Uromodulin

Table 4 Distribution of minerals with UMOD gene mutations

Category	UMOD gene mutation			Total
	Variable	Negative	Positive	
Sodium (Na)	Low	55	5	60
	Normal	37	5	42
	High	1	0	103
Potassium (K)	Low	19	3	22
	Normal	55	7	62
	High	15	1	16
Chloride	Low	22	4	26
	Normal	62	5	67
	High	7	1	8
Calcium	Low	93	10	103
	Normal	0	0	0
	High	0	0	0

Discussion

CKD is a life-long disorder associated with considerable morbidity and mortality due to complications from a progressive loss of kidney function and eventual renal failure. Early diagnosis and detection of the etiologic factors are important to improve the health outcomes of patients with CKD. Uromodulin-associated kidney disease is caused by UMOD gene mutations and leads to end-stage renal disease. However, the recent studies from KSA have reported high prevalence rates of different CKD stages with considerable increase in the CKD associated risk factors.⁶⁻¹¹ Although, these studies have investigated many risk factors that has direct or indirect impact in the etiology of CKD that in most instances might develop to renal failure, they didn't investigate the influence of genetic mutation particularly UMOD gene mutations. To the best of our knowledge this is the first report from KSA in this context. Since, UMOD gene mutations are responsible for the autosomal dominant inheritance of chronic interstitial disease, prevalence rates variations are expected among different populations. Polymorphisms in the UMOD gene have been found responsible for increased urinary uromodulin production and an increased risk of CKD.¹⁷ Families with uromodulin-associated kidney diseases have been reported from the United States, France, the United Kingdom, Morocco, Turkey, and South Korea.¹⁸⁻²³ Furthermore, multiple novel rare variants in the UMOD region were identified, but none were consistently associated with eGFR. Only V458L had modest association with THP levels in the general population and thus could not account for the observed genome-wide association studies (GWAS) signal.²⁴ GWASs have identified multiple loci associated with cross-sectional eGFR, but a systematic genetic analysis of kidney function decline over time is missing. In a study performed a GWAS meta-analysis among 63,558 participants of European descent, initially from 16 cohorts with serial kidney function measurements within the CKDGen Consortium, followed by independent replication among additional participants from 13 cohorts. In stage 1 GWAS meta-analysis, single-nucleotide polymorphisms (SNPs) at MEOX2, GALNT11, IL1RAP, NPPA, HPCAL1, and CDH23 showed the strongest associations for at least one trait, in addition to the known UMOD locus, which showed genome-wide significance with an

annual change in eGFR. In stage 2 meta-analysis, the significant association at UMOD was replicated. Associations at GALNT11 with Rapid Decline (annual eGFR decline of 3ml/min per 1.73m² or more), and CDH23 with eGFR change among those with CKD showed significant suggestive evidence of replication. Combined stage 1 and 2 meta-analyses showed significance for UMOD, GALNT11, and CDH23. Morpholino knockdowns of galnt11 and cdh23 in zebrafish embryos each had signs of severe edema 72h after gentamicin treatment compared with controls, but no gross morphological renal abnormalities before gentamicin administration. Thus, our results suggest a role in the deterioration of kidney function for the loci GALNT11 and CDH23, and show that the UMOD locus is significantly associated with kidney function decline.²⁵ In the present study 70% of patients with UMOD gene mutation were found among Shammari families. Notably, this ethnic group represents around 55% of our studied subjects, see Figure 3.

UMOD gene mutations are linked to three autosomal dominant conditions, including familial juvenile hyperuricemic nephropathy; medullary cystic kidney disease type 2, and glomerulocystic kidney disease.²⁶ Most UMOD gene mutations are found to be clustered in exons 4.5 and 8.²⁷ Leading to misfolding of the uromodulin molecule, with the abnormal uromodulin becoming entrapped in the endoplasmic reticulum of the cells of the thick ascending limb of the loop of henle.²⁸ However, one of the limitations in this study was its reliance on the investigation of exon 10. But this in turn may be an advantage exposed the magnitude of mutations in this gene. Although, the number of males patients was higher than females, (66 vs 37), but the entire percentage of females with UMOD gene mutation is relatively higher 10.8% compared to 9% among males. However, there is a lack of literature regarding this issue, which might be a suitable for future search.

Regarding age, most cases with UMOD gene mutation were observed at relatively older patients. This might be expressed by the fact that, uromodulin is known to regulate transport processes in the thick ascending limb, which associated with functional tubular alterations leading to prolonged disease progression.²⁹ Furthermore, 60% of patients with UMOD gene mutation were found with normal creatinine values, hence, 70% of them were found with high urea levels. Moreover, 60% of those with UMOD gene mutation were having low uric acid level. However, changes in the levels of these metabolites can be affected with dialysis, since all of patients in this study were under hemodialysis, though; some studies have reported that mutation of the UMOD gene leads to hyperuricemia.^{17,30} Nevertheless, 70% of patients with UMOD gene mutation were found with high glucose levels. However, no study has indicated these findings, but it might be due to high prevalence rates of diabetes among the study population.^{9,11} On the other hand, there are discrepancies in levels of minerals and UMOD gene mutation, as seen in Table 4. All of patients with UMOD gene mutation were with low calcium levels. The only studies in this context have provided evidence showing that normal uromodulin plays an important role in protecting the urinary system against calcification and propose that reduced expression and/or decreased function of uromodulin could contribute to nephrolithiasis.³¹ In conclusion: The findings of the present study suggest that UMOD gene mutations contribute to the burden of the prevalent CKD in KSA, particularly in Hail region and among Shammari families. Further studies involving more exon clusters is highly recommended.

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Conflict of interest

The author declares no conflict of interest.

References

1. Johnson D. The CARI guidelines: evaluation of renal function. *Nephrology (Carlton)*. 2005;4:S133–S176.
2. Fabrizio Fabrizi, Piergiorgio Messa, Paul Martin. The Unravelling Link between Chronic Kidney Disease and Hepatitis C Infection. *New Journal of Science*. 2014;2014(2014):1–9.
3. Levey AS, Atkins R, Coresh J, et al. Chronic Kidney Disease as a Global Public Health Problem: Approaches and Initiatives– A Position Statement from Kidney Disease Improving Global Outcomes. *Kidney Int*. 2007;72(3):247–259.
4. 27th Bethesda Conference. Matching the Intensity of Risk Factor Management with the Hazard for Coronary Disease Events. September 14–15, 1995. *J Am Coll Cardiol*. 1996;27(5):957–1047.
5. Ginawi IA, Ahmed HG, Ashankyty IM, et al. A survey for potential risk factors for susceptibility to Chronic Kidney Disease in Hail Region, KSA. *Management in Health*. 2013;17(2).
6. Ahmed HG, Ginawi IA, Al-hazimi AM. Prevalence estimates of Chronic Kidney Disease in Hail Region, KSA: in a comprehensive Survey. *International Journal of Science and Research*. 2014;3(7):252–256.
7. Ahmed HG, Ginawi IA, Al-hazimi AM. Prevalence of Hypertension in Hail Region, KSA: in a Comprehensive Survey. *International Journal of Sciences: Basic and Applied Research*. 2014;17(2):288–296.
8. Ahmed HG, Ginawi IA, Haridi HK, et al. Distribution of CKD and Hypertension in 13 Towns in Hail Region, KSA. *Egypt Acad J Biolog Sci*. 2014;6(2):39–45.
9. Ahmed HG, Ginawi IA, Fawaz D, et al. Current Burden of diabetes in Kingdom of Saudi Arabia in an epidemiological survey. *Egypt Acad J Biolog Sci*. 2014;6(2):85–91.
10. Ahmed HG, Ginawi IA, Elsbali AM, et al. Prevalence of Obesity in Hail Region, KSA: in a comprehensive Survey. *Journal of Obesity*. 2014;2014(4):1–5.
11. Ginawi IA, Elsbali AM, Ahmed HG, et al. Prevalence Rates of Diabetes and Obesity in 4 Provinces in Hail Region, KSA. *Egypt Acad J Biolog Sci*. 2014;6(2):47–53.
12. Ginawi IG, Ahmed HG, Al-hazimi AM. Assessment of risk factors for Chronic Kidney Disease in Saudi Arabia. *International Journal of Science Research*. 2014;3(7):446–450.
13. Jeanpierre C, Whitmore SA, Austruy E, et al. Chromosomal assignment of the uromodulin gene (UMOD) to 16p13.11. *Cytogenet Cell Genet*. 1993;62(4):185–187.
14. Kottgen A, Glazer NL, Dehghan A, et al. Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet*. 2009;41(6):712–717.
15. Lau WH, Leong WS, Ismail Z, et al. Qualification and application of an ELISA for the determination of Tamm Horsfall protein (THP) in human urine and its use for screening of kidney stone disease. *Int J Biol Sci*. 2008;4(4):215–222.

16. UMOD uromodulin [Homo sapiens (human)]. 2015.
17. Bleyer AJ, Živná Mb, Knoch S. Uromodulin–Associated Kidney Disease. *Nephron Clin Pract.* 2011;118(1):c31–c36.
18. Hart TC, Gorry MC, Hart PS, 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(12):882–892.
19. Dahan K, Devuyt O, Smaers M, et al. A Cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol.* 2003;14(11):2883–2893.
20. Rampoldi L, Caridi G, Santon D, et al. Allelism of MCKD, FJHN and GCKD caused by impairment of uromodulin export dynamics. *Hum Mol Genet.* 2003;12(24):3369–3384.
21. Nasr SH, Lucia JP, Galgano SJ, et al. Uromodulin storage disease. *Kidney Int.* 2008;73:971–976.
22. Wolf MT, Hoskins BE, Beck BB, et al. Mutation analysis of the Uromodulin gene in 96 individuals with urinary tract anomalies (CAKUT). *Pediatr Nephrol.* 2009;24(1):55–60.
23. Lee DH, Kim JK, Oh SE, et al. A case of familial juvenile hyperuricemic nephropathy with novel uromodulin gene mutation, a novel heterozygous missense mutation in Korea. *J Korean Med Sci.* 2010;25(11):1680–1682.
24. Köttgen A, Yang Q, Shimmin LC, et al. Association of Estimated Glomerular Filtration Rate and Urinary Uromodulin Concentrations with Rare Variants Identified by UMOD Gene Region Sequencing. *PLoS ONE.* 2012;7(5):e38311.
25. Mathias Gorsk, Adrienne Tin, Maija Garnaas, et al. Genome –wide association study of kidney function decline in individuals of european descent. *Kidney Int.* 2015;87:1017–1029.
26. Scolari F, Caridi G, Rampoldi L, et al. Uromodulin storage diseases: clinical aspects and mechanisms. *Am J Kidney Dis.* 2004;44(6):987–999.
27. Bollee G, Dahan K, Flamant M, et al. Phenotype and outcome in hereditary tubulointerstitial nephritis secondary to UMOD mutations. *Clin J Am Soc Nephrol.* 2011;6(10):2429–2438.
28. Williams SE, Reed AA, Galvanovskis J, et al. Uromodulin mutations causing familial juvenile hyperuricaemic nephropathy lead to protein maturation defects and retention in the endoplasmic reticulum. *Hum Mol Genet.* 2009;18(16):2963–2974.
29. Labriola L, Olinger E, Belge H, et al. Paradoxical response to furosemide in uromodulin–associated kidney disease. *Nephrol Dial Transplant.* 2015;30(2):330–335.
30. Bleyer AJ, Woodard AS, Shihabi Z, et al. Clinical characterization of a family with a mutation in the uromodulin (Tamm–Horsfall glycoprotein) gene. *Kidney Int.* 2003;64(1):36–42.
31. Liu Y, Mo L, Goldfarb DS, et al. Progressive renal papillary calcification and ureteral stone formation in mice deficient for Tamm–Horsfall protein. *Am J Physiol Renal Physiol.* 2010;299(3):F469–E478.