The Association of Uromodulin Genotype with Renal Cancer Aggressiveness

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INTRODUCTION

Uromodulin (UMOD) or Tamm-Horsfall protein, is a glycoprotein encoded by the *UMOD* gene and represents the most common protein in humans' urine. To date, the physiological roles of uromodulin are not yet completely elucidated. Several studies demonstrated a compelling evidence of an association between *UMOD* gene variants and chronic kidney disease and cardiovascular side effects development or with increased levels of gene expression and urinary UMOD production. Notably, in the context of Renal Cell Carcinoma (RCC) patients, previous investigations reported a significant *UMOD* downregulation. Nonetheless, no information regarding the relationship between *UMOD* genotype variants and RCC clinical phenotype is currently available

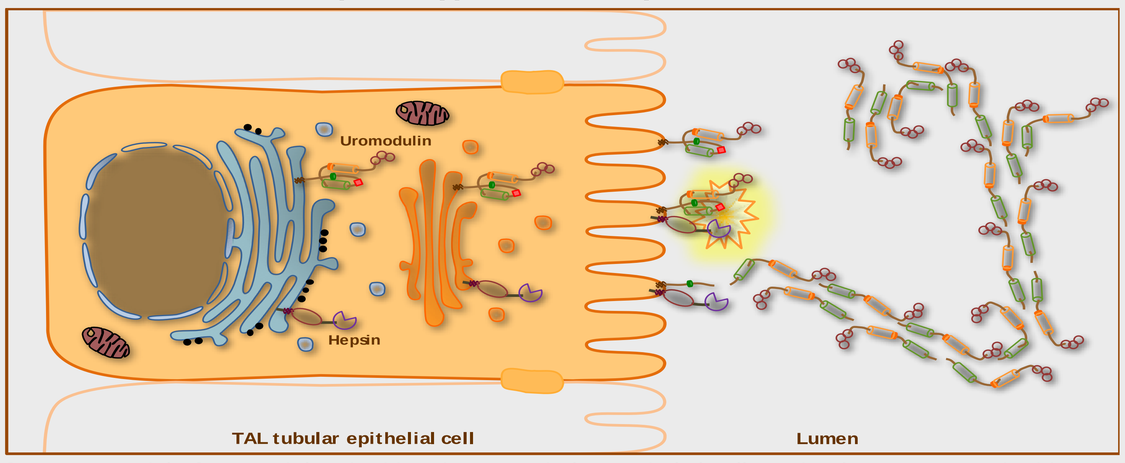

Figure 1: Model of uromodulin shedding by TAL tubular epithelial cells and polymerisation. Shedding by hepsin at the uromodulin consensus cleavage site (red diamond), likely occurring at the plasma membrane, generates polymerisation-competent species that are assembled into polymeric filaments within the tubular lumen (Brunati et al, Elife 2015)

MATERIAL and METHODS

After ethics committee's approval (protocol #2007/290820007/V3), clinical data were prospectively collected for 211 consenting patients diagnosed with a renal mass at computed tomography or magnetic resonance imaging and subsequently treated with radical or partial nephrectomy (2011-2015). Genomic DNA was extracted from white blood cells or normal kidney tissue using the QIAamp DNA Mini Kit (Qiagen). Purity and concentration of DNA recovered were determined with NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies). Genotyping of the single nucleotide polymorphism (SNP) rs4293393 was performed using 5' nuclease allelic discrimination assays with allele-specific MGB probes (TaqMan SNP Genotyping Assay, ID C__27865986_10, Applied Biosystems). Genotype clustering and calling were carried out by using StepOne™ software v2.2.2 (call rate 99.6%). The outcomes of the study were patient and cancer characteristics (Supplementary material 1). Due to the higher frequency of *UMOD* rs4293393 risk allele T relative to the lower frequency of the protective allele C in the general population[8], homozygous CC patients (n=10) and heterozygous patients (n=60) were grouped together and then compared to homozygous TT patients (n=141). Medians and interquartile ranges or frequencies and proportions were reported for continuous or categorical variables, respectively. Mann-Whitney (continuous variables) and Chi-square tests (categorical variables) were used to investigate the statistical significance of differences in patient and cancer characteristic after stratification for *UMOD* genotype. All statistical tests were performed using the RStudio graphical interface v.0.98 for R software environment v.3.0.2. All tests were two-sided with a significance level set at p<0.05.

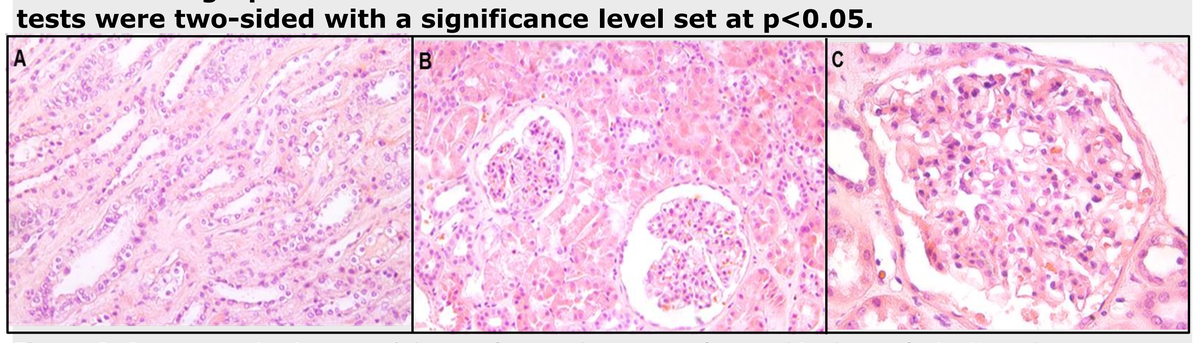

Figure 2: Representative images of the renal parenchyma samples used in the analysis. Note that glomeruli's density is within the normal range. A normal renal glomerulus is shown in C. (H&E stain; 10x and 20x)

CONCLUSION

In our study cohort the observed results do not support the hypothesis of a relationship between *UMOD* genotype and general health status of RCC patients (Table1). Conversely, a relationship between *UMOD* genotype and a more aggressive clinical and pathological RCC phenotype (Table 2) was discovered. Patients with TT genotype were more frequently diagnosed with cM1 RCC at pre-operative staging and were more frequently diagnosed with pT3-pT4 and lymphovascular invasive RCC at post-operative histologic examination.The association with clinical metastasis and lymphovascular invasion were also confirmed after multivariable analyses including clinical predictors of aggressive RCC.

To explain this new exciting findings we hypothesized that uromodulin might play a relevant immunomodulatory role, eventually influencing the immune response against RCC. In this light, TT patients might be characterized by a less efficient immune response to RCC which is operational either at local (higher proportion of aggressive primary) and systemic level (higher proportion of metastatic disease at diagnosis).

AIM of THE STUDY and CLINICAL RELEVANCE

→To investigate the relationship between Renal Cell Carcinoma and UMOD genotype

→ To discover if UMOD genotype could be a potential predictive biomarkers of renal cancer aggressiveness

→ To establish a personalized surgical and medical treatment based on different UMOD genotype for RCC patients

RESULTS

Table 1. Association between *UMOD* genotype and patient characteristics in 211 cases diagnosed with renal mass and treated with surgery, 2011-2015.

Variable	Overall Population (n=211)	Genotype TC-CC (n=70, 33%)	Genotype TT (n=141, 67%)	P value
BMI				1
Median	25.8	25.7	25.9	
IQR	23.3-28.7	23.6-27.8	23.1-29	
CCI				0.6
0	96 (45)	31 (44)	65 (46)	
1	55 (26)	17 (24)	38 (27)	
2	30 (14)	9 (13)	21 (15)	
3	13 (6)	5 (7)	8 (6)	
4	9 (4)	3 (4)	6 (4)	
≥5	8 (4)	5 (7)	3 (2)	
Hypertension				0.6
No	101 (48)	36 (51)	65 (46)	
Yes	110 (52)	34 (49)	76 (54)	
Diabetes				0.5
No	173 (82)	56 (80)	117 (83)	
Yes	38 (18)	14 (20)	24 (17)	
Preoperative Hb, g/dL				0.06
Median	13.6	14	13.6	
IQR	12.5-14.7	12.6-15.2	12.4-14.5	
Pre-operative eGFR				0.3
Median	87	90	86	
IQR	63-97	67-99	63-96	
Post-operative eGFR				0.7
Median	59	61	58	
IQR	47-80	48-81	47-78	

Data presented as frequencies and percentages unless otherwise specified.
IQR: Interquartile range.
BMI: body mass index. CCI: Charlson comorbidity index. Hb: Haemoglobin. eGFR: estimated glomerular filtration rate.

Table 2a. Association between *UMOD* genotype and cancer characteristics in 211 cases diagnosed with renal mass and treated with surgery, 2011-2015.

Variable	Overall Population (n=211)	Genotype TC-CC (n=70, 33%)	Genotype TT (n=141, 67%)	P value
Clinical Metastasis				0.001
cM0	184 (87)	69 (99)	115 (82)	
cM1	27 (13)	1 (1)	26 (18)	
Malignancy				0.3
Kidney cancer	189 (90)	60 (86)	129 (91)	
Benign Mass	22 (10)	10 (14)	12 (9)	
Focality				0.1
Monofocal	201 (95)	64 (91)	137 (97)	
Multifocal	10 (5)	6 (9)	4 (3)	
Pathologic size, mm				0.1
Median	53	50	56	
IQR	32-80	30-65	35-80	

Table 2b. Association between *UMOD* genotype and cancer characteristics in 189 cases diagnosed with pathologically confirmed renal cell carcinoma and treated with surgery, 2011-2015.

Variable	RCC Patients (n=189)	Genotype TC-CC (n=60, 32%)	Genotype TT (n=129, 68%)	P value
Pathologic T stage				0.047
pT1 - pT2	121 (64)	45 (75)	76 (59)	
pT3 - pT4	68 (36)	15 (25)	53 (41)	
Pathologic N stage				0.4
pN0	68 (36)	19 (32)	49 (38)	
pN1	11 (6)	2 (3)	9 (7)	
pNx	110 (58)	39 (65)	71 (55)	
Pathologic Grade				0.1
G1 - G2	109 (58)	40 (67)	69 (53)	
G3 - G4	80 (42)	20 (33)	60 (47)	
Necrosis[1]				0.3
No	83 (44)	30 (51)	53 (41)	
Yes	105 (56)	29 (49)	76 (59)	
Lymphovascular invasion[1]				0.02
No	144 (76)	52 (87)	92 (71)	
Yes	44 (24)	7 (13)	37 (29)	

Data presented as frequencies and percentages unless otherwise specified.
[1]Missing for one RCC case

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