University of Zurich[UZH]

ZIHP

Uromodulin-associated Nephropathies

Prof. Dr. Med. O. Devuyst

KDIGO Conference on ADTKD
Boston, Sept. 10, 2014

UniversitätsSpital Zürich

KIDNEY CONTROL OF HOMEOSTASIS
SWISS NATIONAL CENTRE OF COMPETENCE IN RESEARCH

Uromodulin-associated Nephropathies

- From Tamm-Horsfall protein to Uromodulin
- FJHN – MCKD2 and *UMOD* mutations
- Clinical characteristics
- Diagnosis
- Mechanism of disease
- Introduction of the key questions

A MUCOPROTEIN DERIVED FROM HUMAN URINE WHICH REACTS WITH INFLUENZA, MUMPS, AND NEWCASTLE DISEASE VIRUSES

By IGOR TAMM, M.D., and FRANK L. HORSFALL, Jr., M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

J Exp Med, January 1, 1952

Science, 3 April 1987

Identification of Human Uromodulin as the Tamm-Horsfall Urinary Glycoprotein

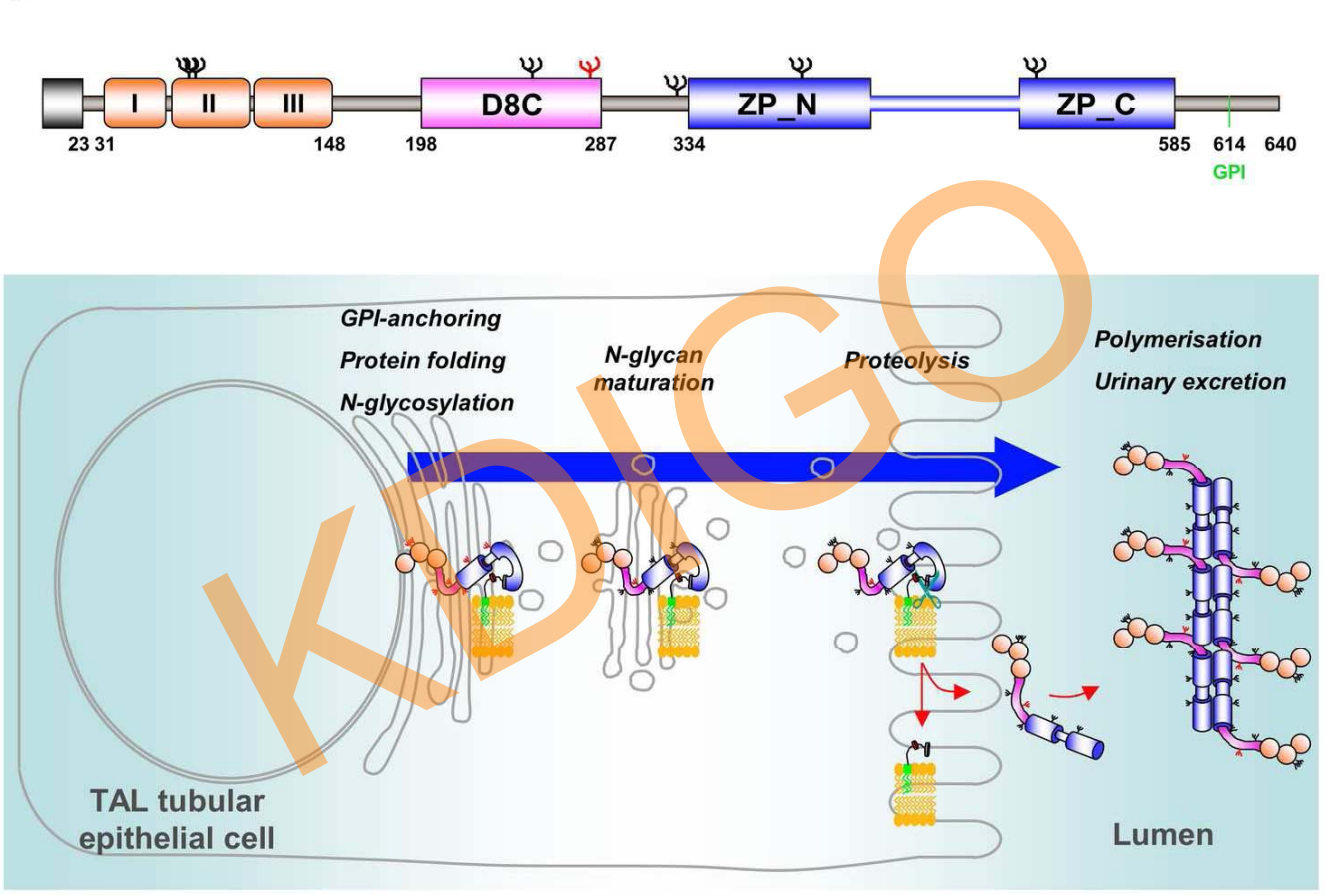
Diane Pennica, William J. Kohr, Wun-Jing Kuang, Debbie Glaister, Bharat B. Aggarwal, Ellson Y. Chen, David V. Goeddel

Uromodulin (Tamm-Horsfall Protein) is the most abundant protein in nomal human urine: 50-100 mg/day

RNAs isolated from 150 different tissues and cell lines:

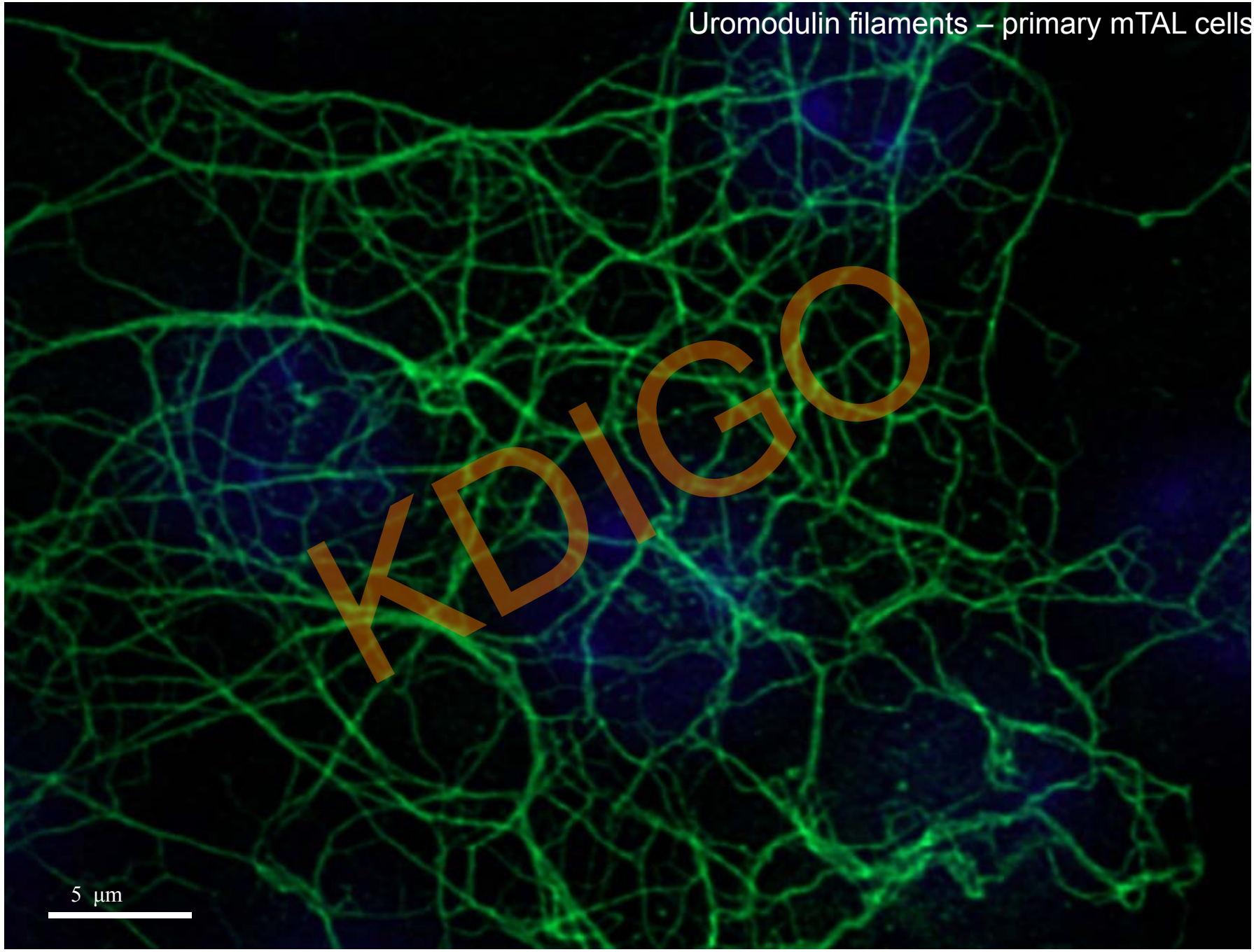
uromodulin mRNA detected only from human adult kidney.

Structure and Traffic of Uromodulin



- 640 AA, 48 cysteines, 7 N-glycosylation (25-30% carbohydrate content)
- 3 EGF + central domain + zona pellucida domain; C-terminus : GPI anchor in ER
- Proteolytic cleavage (524-525) → urine excretion & polymerisation → filaments

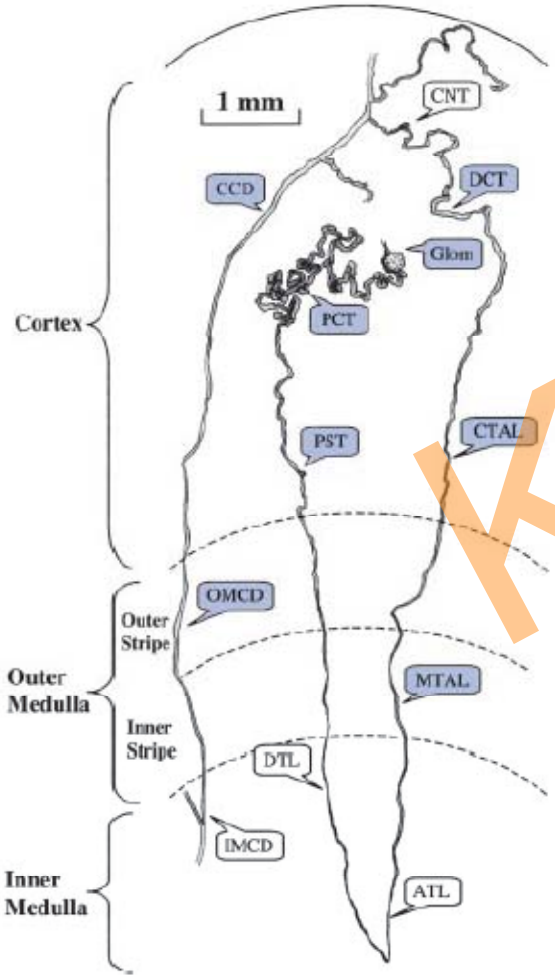
Rampoldi et al. Kidney Int 2011, 80

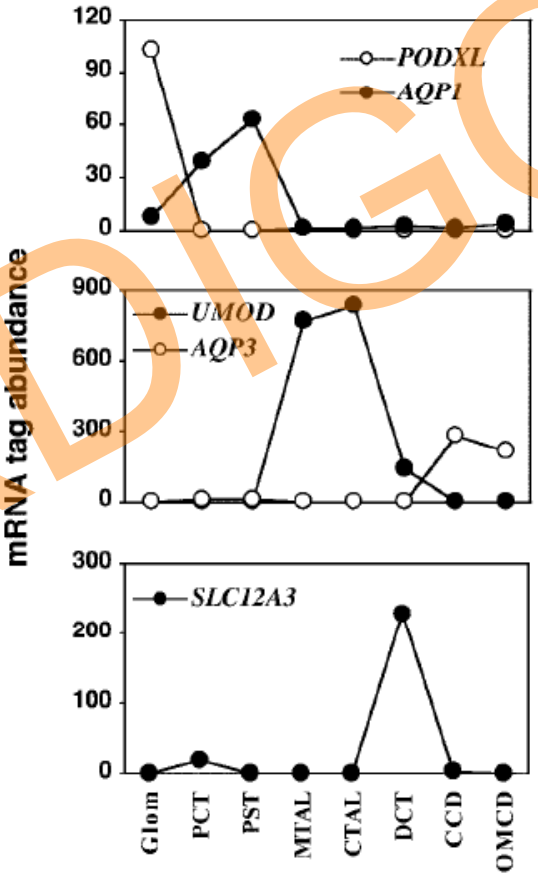

Uromodulin filaments – primary mTAL cells
KDIGO
5 µm

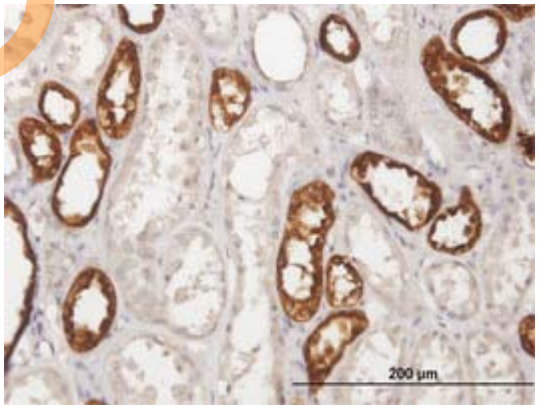
A panoramic view of gene expression in the human kidney

Danielle Chabardès-Garonne*†, Arnaud Méjean‡, Jean-Christophe Aude*, Lydie Cheval†, Antonio Di Stefano†, Marie-Claude Gaillard*, Martine Imbert-Teboul†, Monika Wittner†, Chanth Balian‡, Véronique Anthouard§, Catherine Robert§, Béatrice Ségurens§, Patrick Wincker§, Jean Weissenbach§, Alain Doucet†, and Jean-Marc Elalouf*†¶

13710–13715 | PNAS | November 11, 2003 | vol. 100 | no. 23

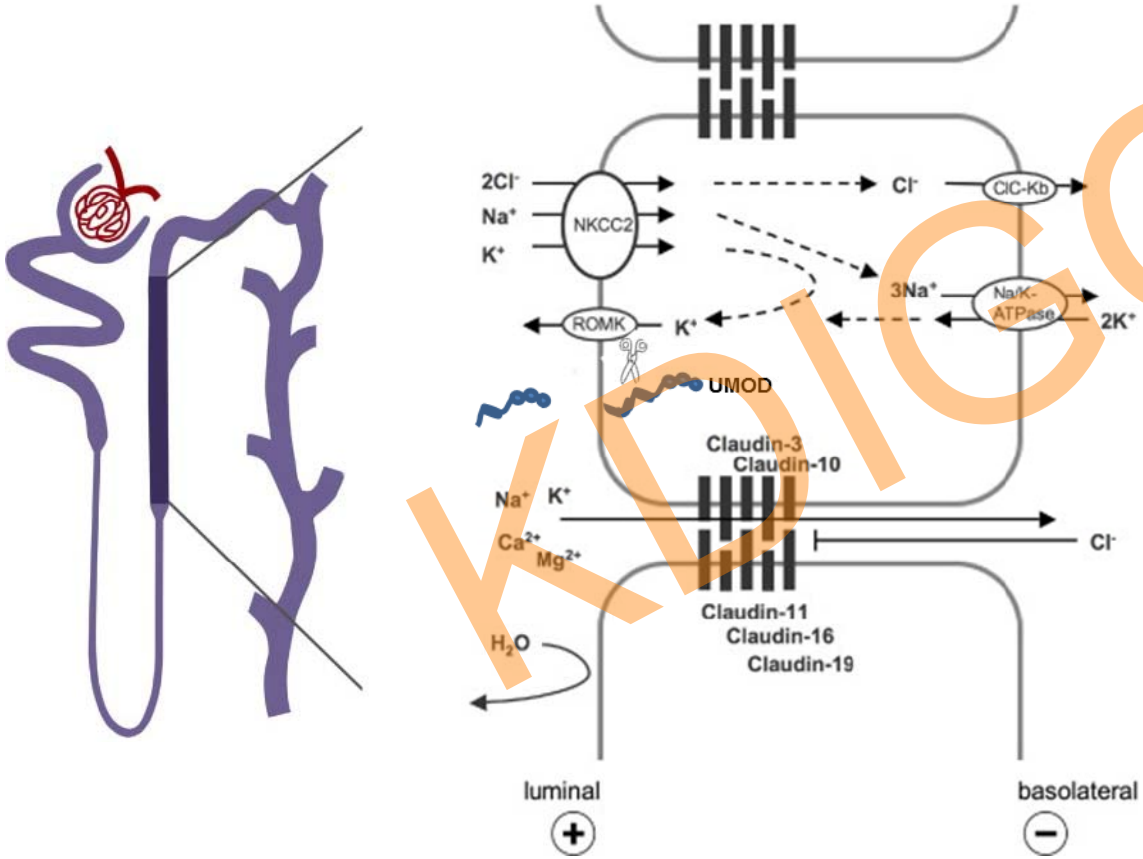






Exclusive expression in TAL segment

TAL Segment: Central Role in Homeostasis



- Handling of NaCl:
  - Blood pressure
  - Urinary concentration
- Diluting segment
- Handling of Ca^{2+} & Mg^{2+}:
  - Biomineralization
- Secretion of uromodulin

Uromodulin: Properties and Pathophysiology

- Filaments, with tendency to gelation/aggregation
- Interaction with IgG, light chains, C1, ILs
- Binding and activation of leukocytes
- Binding to uropathogenic strains of E. Coli

- *Pathophysiology (KO mouse model):*
  - Cast formation : gelification (Bence-Jones, contrast, ischemia)
  - Interstitial nephropathy : autoimmune deposits; binding to T cells
  - Defense against urinary tract infection
  - Protection against stones : inhibitor of Ca^{2+} oxalates aggregation

Renal Phenotype of Uromodulin-null Mice

- No glomerular defects
- *Changes in TAL*:
  - ↑ intracellular NKCC2 (vesicles) - ↓ p-NKCC2 (membrane)
  - ↑ intracellular ROMK
  - ↓ response to furosemide
- *Discrete NaCl loss → compensatory changes in distal nephron*:
  - ↑ abundance of NCC - ↑ volume of DCT

- *Aid to surface expression of **ROMK***
- *Facilitates baseline phosphorylation of **NKCC2***

Bachmann et al. AJP 288, 2005; Renigunta et al. JBC 286, 2011; Mutig et al. JBC286, 2011

Two Rare Disorders: FJHN and MCKD2

Familial juvenile hyperuricemic nephropathy (FJHN, MIM 162000) is a rare autosomal dominant condition characterized by abnormal tubular handling of urate associated with progressive renal failure.

- Presentation: gout or hyperuricemia occurring in a young normotensive subject of either gender, absence of a purine synthesis disorder, with low FEurate.
- CKD appears between 15 and 40 yr of age; ESRD within 10 to 20 yr.
- Biopsy: chronic interstitial nephritis, with thickening and splitting of TBM.

- **Marked thickening of TBM**: also observed in nephronophthisis and medullary cystic kidney disease (MCKD) group of diseases.
- History of **gout and/or hyperuricemia** also reported in MCKD patients
- ***Mapping FJHN to 16p11, close to MCKD2 locus on 16p12.***

J Am Soc Nephrol 12: 2348–2357, 2001

Familial Juvenile Hyperuricemic Nephropathy and Autosomal Dominant Medullary Cystic Kidney Disease Type 2: Two Facets of the Same Disease?

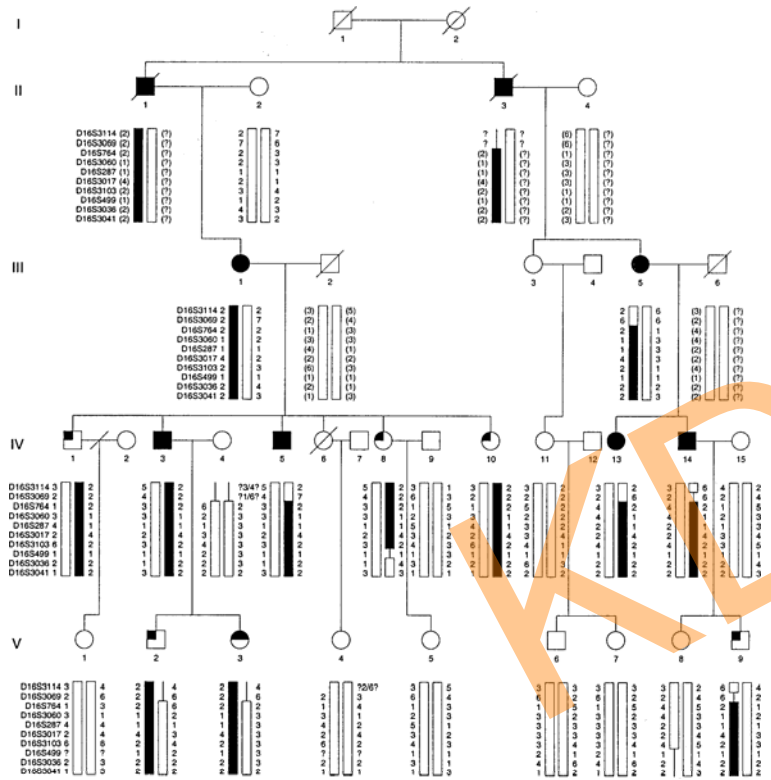
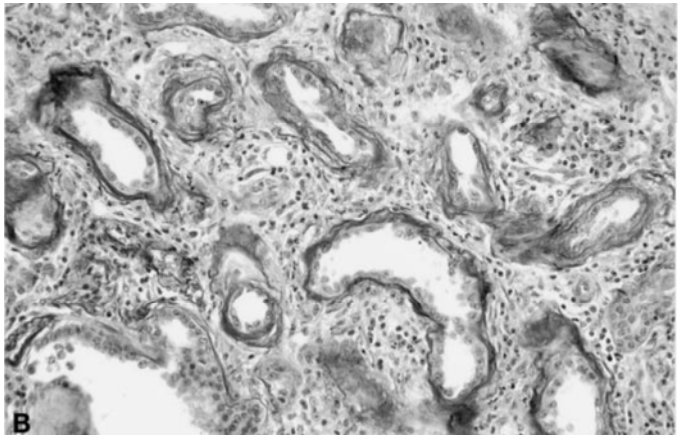


Table 1. Clinical, biochemical, and histologic characteristics of investigated subjects[a]

Patient No.	Gender	Phenotype	At Screening					Gout/Age (yr) at First Attack	ESRF/ Age (yr)	Renal Histologic Features
			Age (yr)	FE_{ur} (%)	GFR (ml/min)	Serum Creatinine Concentration (mg/dl)	Serum Uric Acid Concentration (mg/dl)			
III-1	F	FJHN		ND				+/?	+/63	ND
III-5	F	FJHN		ND				+/19	+/54	ND
IV-1	M	FJHN	34	4.6	108	1.4	9.5	–	–	ND
IV-3	M	FJHN	32	ND		4.4	13.4	+/32	+/36	TIN
IV-5	M	FJHN	19	ND		1.3	High	+/8	+/28	ND
IV-8	F	FJHN	30	5.5	88	0.9	5.7	–	–	ND
IV-10	F	FJHN	22	6.6	124	1.1	6.1	–	–	ND
IV-11	F	NL	36	ND		0.85	3.7	–	–	ND
IV-13	F	FJHN	31	ND		1.4	10.7	+/18	+/40	TIN
IV-14	M	FJHN	38	4.3		3.0	3.3	+/26	+/42	TIN
V-1	F	UD		ND		ND	ND	–	–	ND
V-2	M	FJHN	10	5.1	79	0.9	7.2	–	–	ND
V-3	F	FJHN	7	6.4	43	0.7	5.4	–	–	ND
V-4	F	NL	6	14.3		0.6	3.7	–	–	ND
V-5	F	UD		ND		ND	ND	–	–	ND
V-6	M	NL	14	ND		0.8	5.1	–	–	ND
V-7	F	NL	2	ND		0.4	2.8	–	–	ND
V-8	F	NL	13	9.8	84	0.8	4.6	–	–	ND
V-9	M	FJHN	16	5.7	103	1	7.4	–	–	ND



- Autosomal dominant
- Hyperuricemia (low FEurate) during childhood
- Chronic intersitital nephritis (thickening TBM)
- Progressive renal failure - adulthood

ORIGINAL ARTICLE

Mutations of the *UMOD* gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy

T C Hart, M C Gorry, P S Hart, A S Woodard, Z Shihabi, J Sandhu, B Shirts, L Xu, H Zhu, M M Barmada, A J Bleyer

J Med Genet 2002;**39**:882–892

0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(3):1398–1401

doi: 10.1210/jc.2002-021973

UROMODULIN Mutations Cause Familial Juvenile Hyperuricemic Nephropathy

J. J. O. TURNER*, J. M. STACEY*, B. HARDING, P. KOTANKO, K. LHOTTA, J. G. PUIG, I. ROBERTS, R. J. TORRES, R. V. THAKKER

J Am Soc Nephrol 14: 2883–2893, 2003

A Cluster of Mutations in the UMOD Gene Causes Familial Juvenile Hyperuricemic Nephropathy with Abnormal Expression of Uromodulin

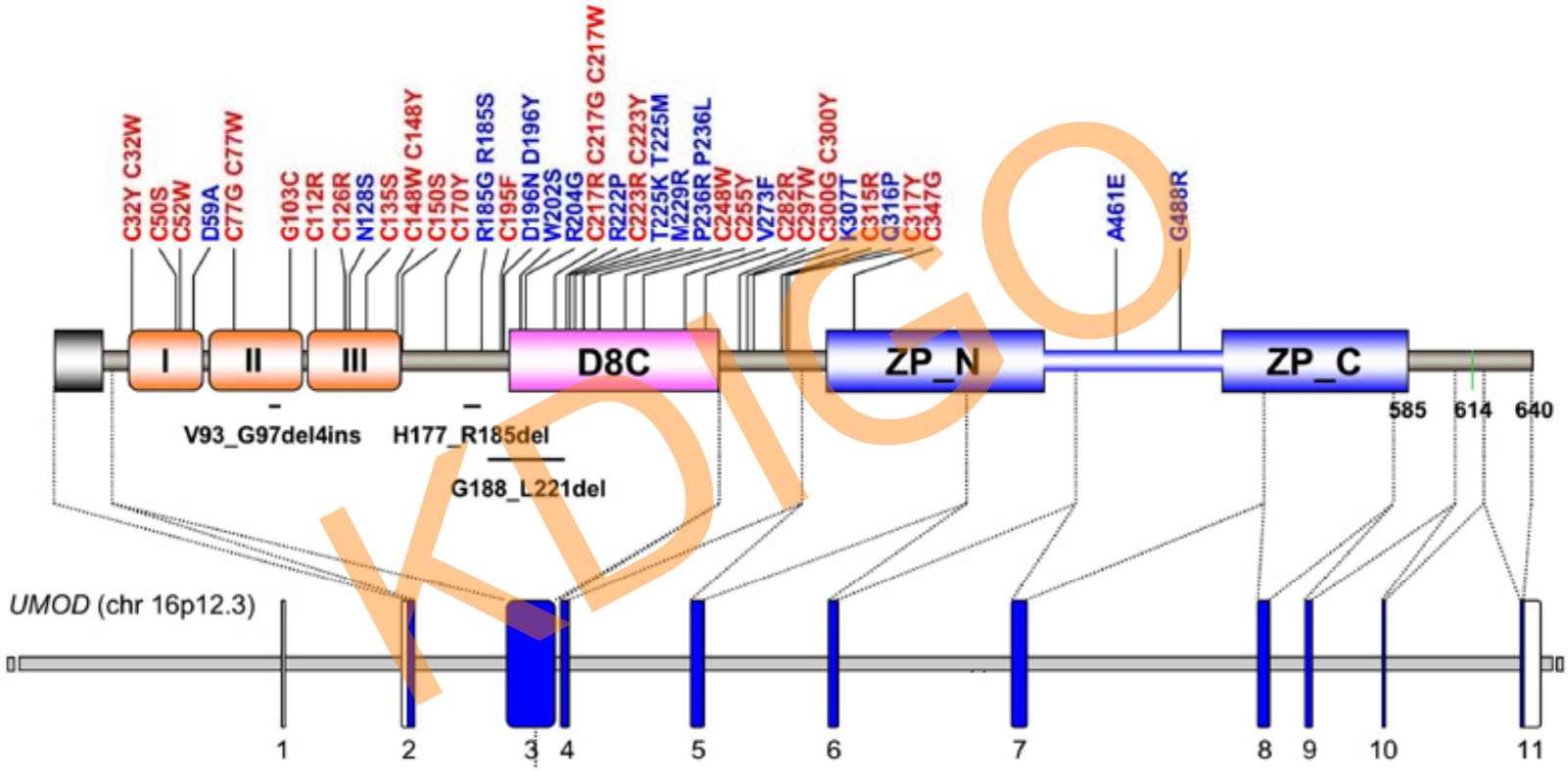
KARIN DAHAN,* OLIVIER DEVUYST,† MICHÈLE SMAERS,*
DIDIER VERTOMMEN,† GUY LOUTE,§ JEAN-MICHEL POUX,‖ BÉATRICE VIRON,¶
CHRISTIAN JACQUOT,# MARIE-FRANCE GAGNADOUX,**
DOMINIQUE CHAUVEAU,†† MATHIAS BÜCHLER,‡‡ PIERRE COCHAT,§§
JEAN-PIERRE COSYNS,‖‖ BÉATRICE MOUGENOT,¶¶ MARK H. RIDER,‡
CORINNE ANTIGNAC,## CHRISTINE VERELLEN-DUMOULIN*, and YVES PIRSON†

Human Molecular Genetics, 2003, Vol. 12, No. 24 **3369–3384**
DOI: 10.1093/hmg/ddg353

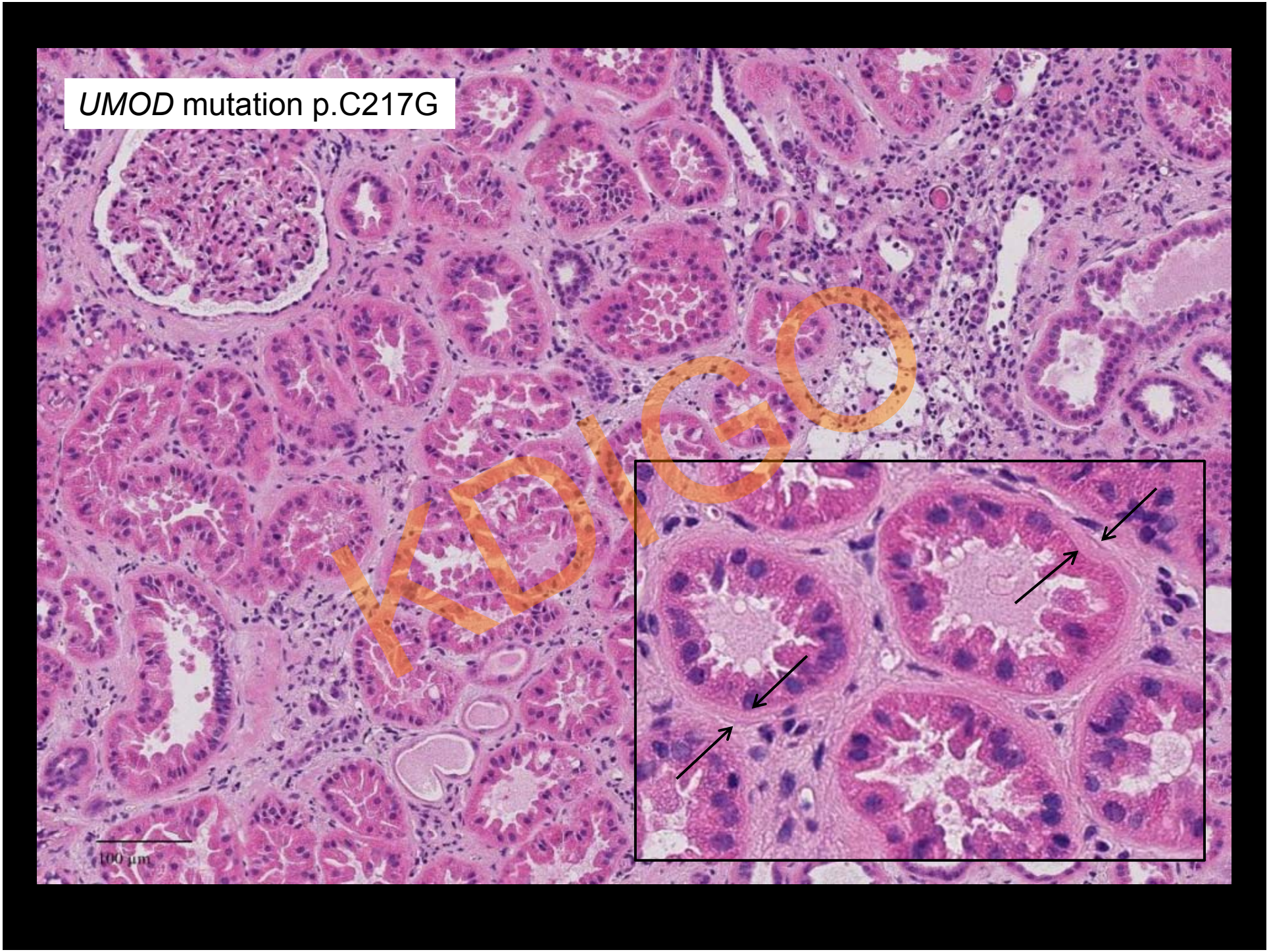
Allelism of MCKD, FJHN and GCKD caused by impairment of uromodulin export dynamics

Luca Rampoldi[1], Gianluca Caridi[2], Daniela Santon[3], Francesca Boaretto[3], Ilenia Bernascone[1], Giuseppe Lamorte[1], Regina Tardanico[4], Monica Dagnino[2], Giacomo Colussi[5], Francesco Scolari[4], Gian Marco Ghiggeri[2], Antonio Amoroso[3] and Giorgio Casari[1,*]

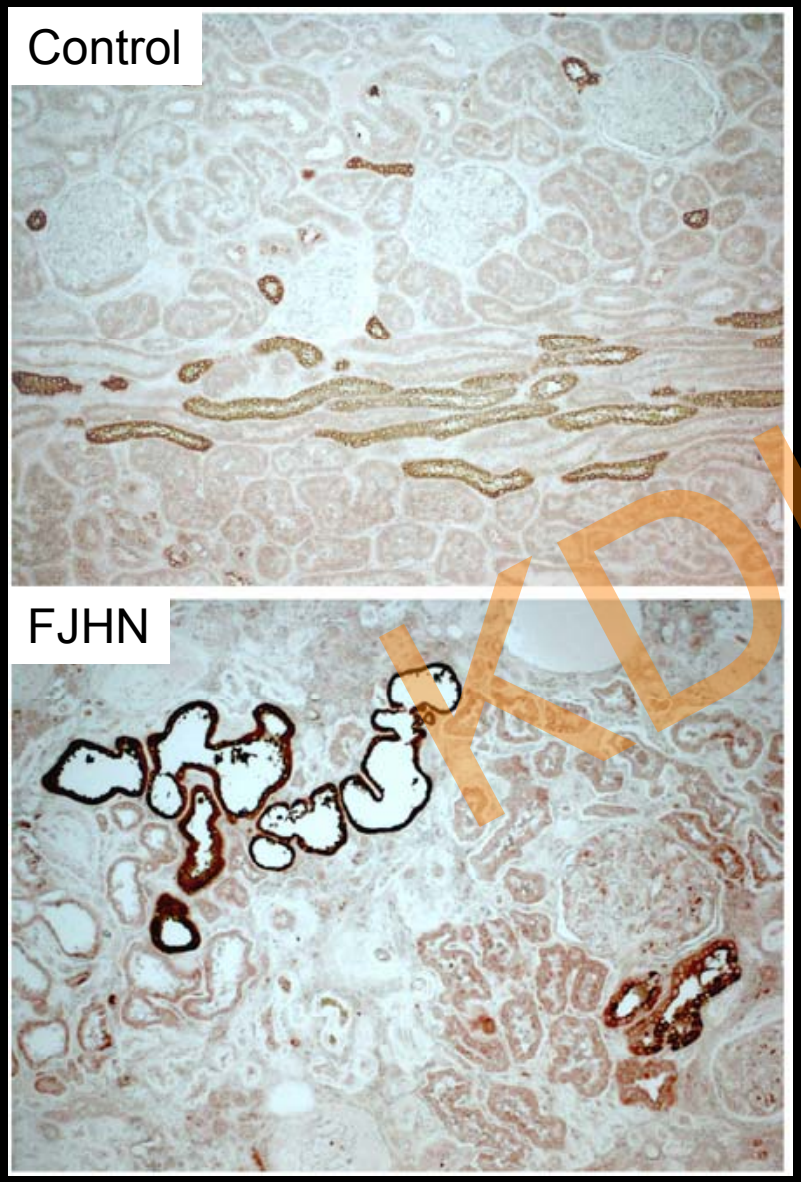
Uromodulin Mutations Associated with FJHN

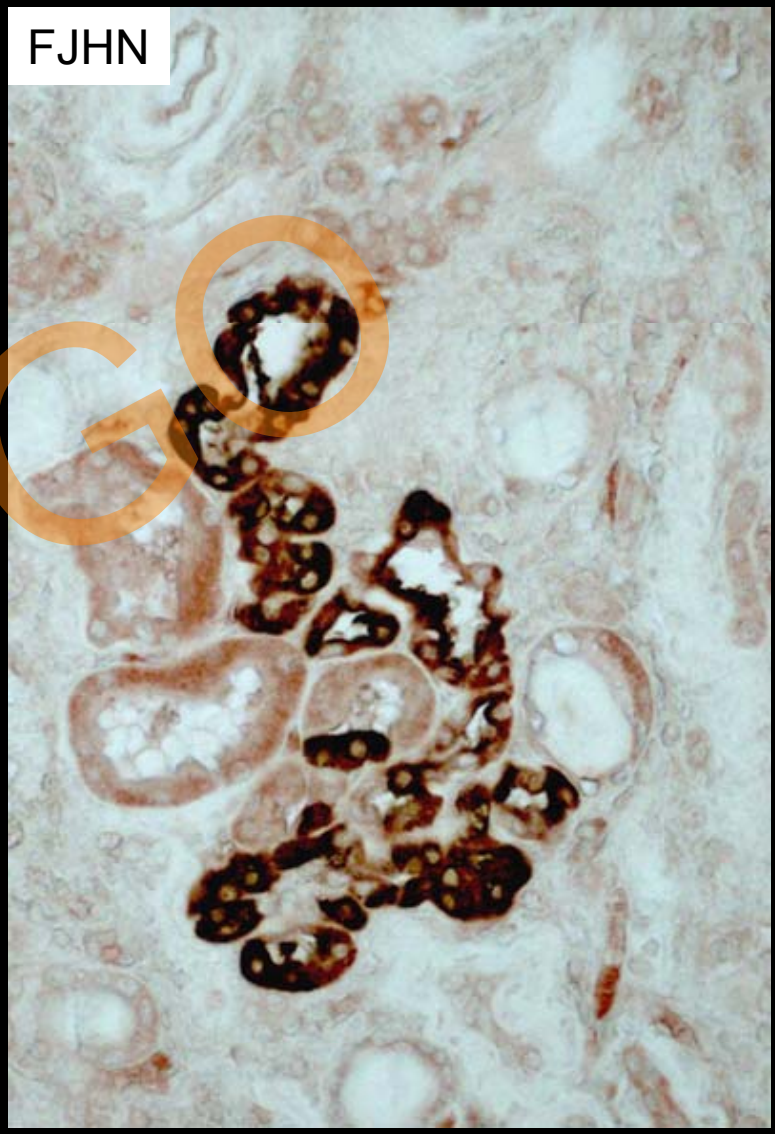


→ 51 mutations, cluster in exons 3 and 4
→ 48/51 missense mutations, 3 in-frame deletions
→ Conserved sequence, **cysteine residues (29/51)**


UMOD mutation p.C217G
100 µm

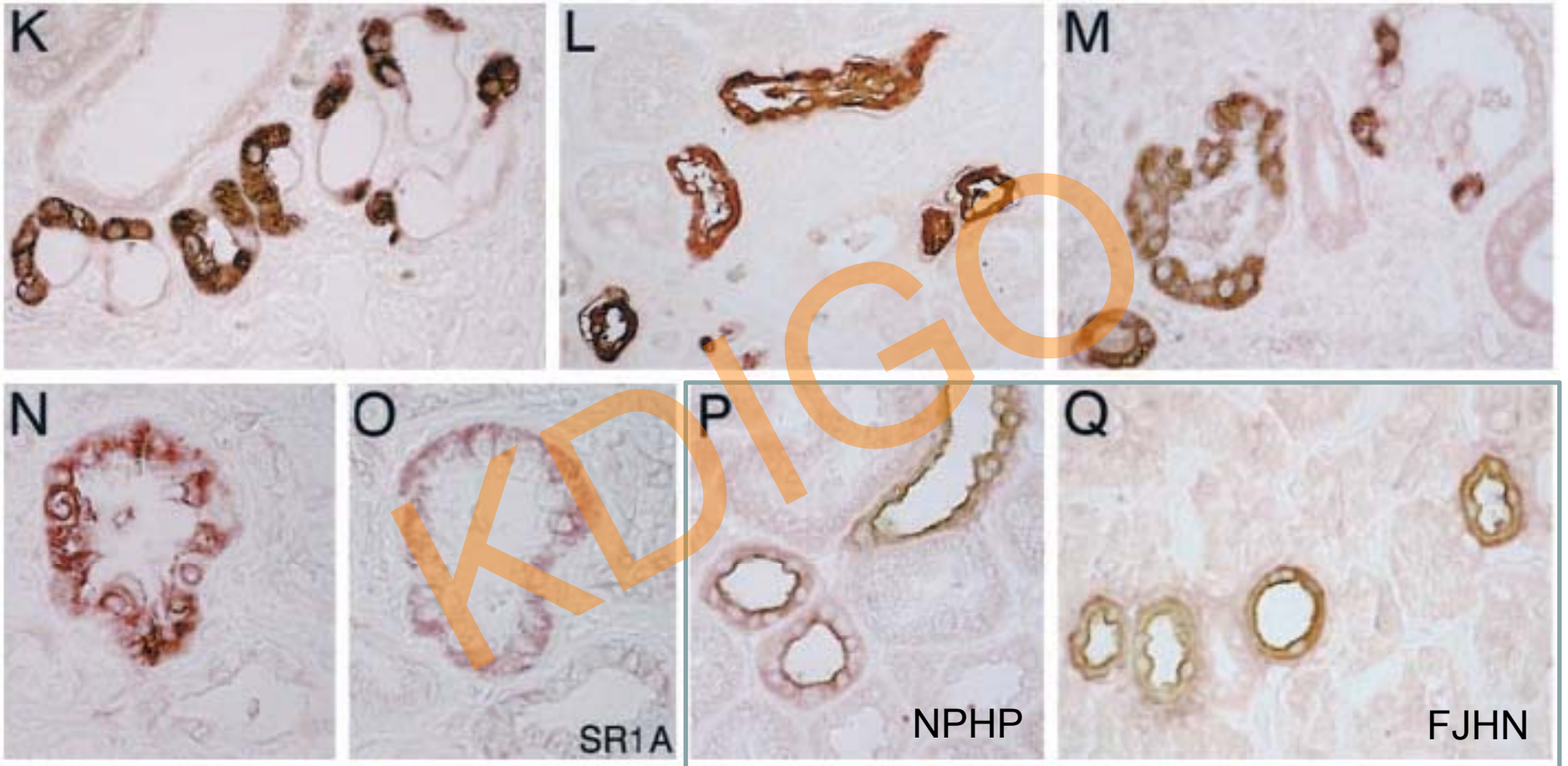
Accumulation of THP in FJHN patients with *UMOD* mutations





Dahan K et al. JASN 14: 2883-93, 2003

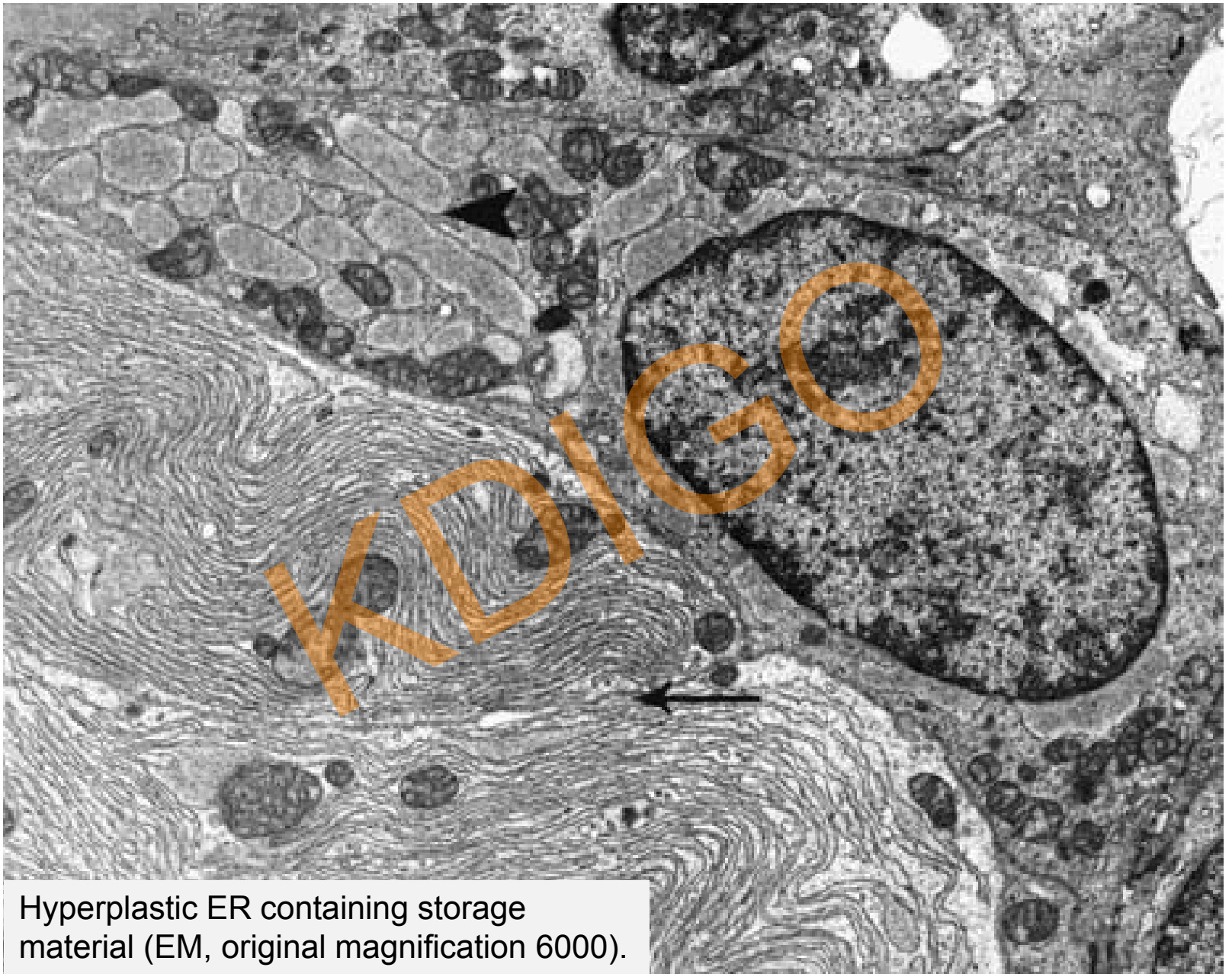
Intense, diffuse, heterogeneous deposits



Intracellular deposits

UMOD-specific deposits

Dahan K et al. JASN 14: 2883-93, 2003



Hyperplastic ER containing storage material (EM, original magnification 6000).

Nasr et al. Kidney Int 2008

Phenotype and Outcome in Hereditary Tubulointerstitial Nephritis Secondary to *UMOD* Mutations

Guillaume Bollée,[†] Karin Dahan,[‡] Martin Flamant,[§‖] Vincent Morinière,[¶] Audrey Pawtowski,[¶] Laurence Heidet,[**] Didier Lacombe,[††] Olivier Devuyst,[‡‡] Yves Pirson,[‡‡] Corinne Antignac,[†¶§§] and Bertrand Knebelmann[*†]*

- 109 patients, 45 families
- 37 *UMOD* mutations

- Age at diagnosis: 31 years; eGFR 42 ml/min/1.73m2
- Blood pressure: 144/90 mmHg
- Family history of gout and/or renal disease: 89%
- History of gout: 75% men – 50% women
- Age of first gout episode: 21 years
- Renal cysts: 34% (bilat 17%), cortical-medullary

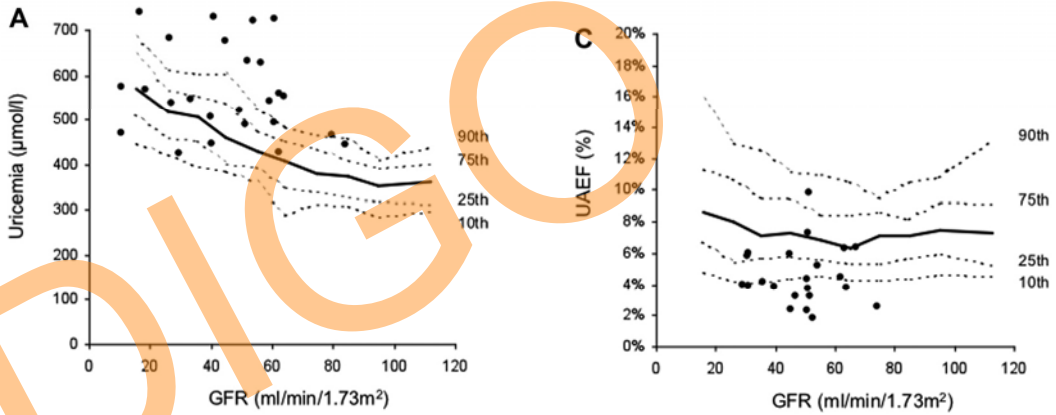
Clin J Am Soc Nephrol 6: 2429–2438, 2011

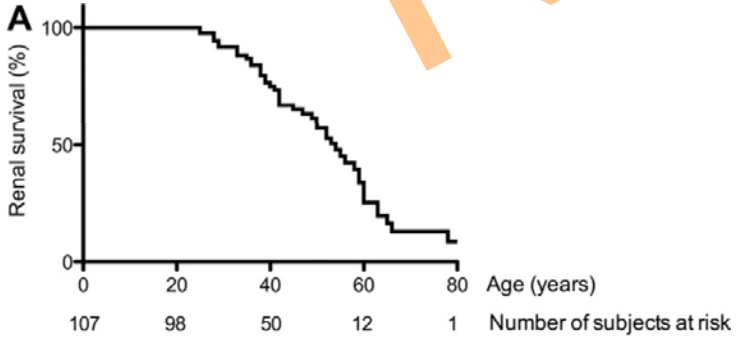
Phenotype and Outcome in Hereditary Tubulointerstitial Nephritis Secondary to *UMOD* Mutations

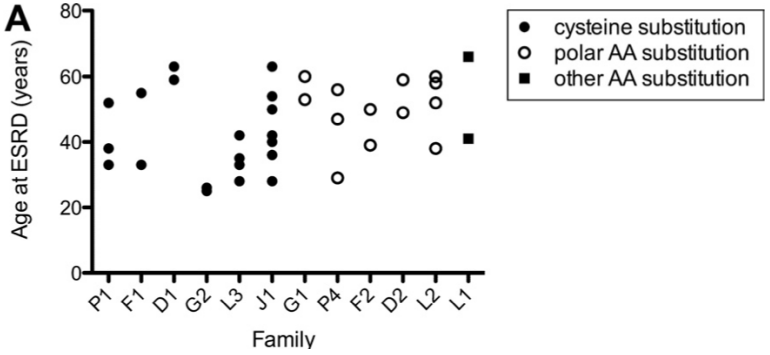
Guillaume Bollée,† Karin Dahan,‡ Martin Flamant,§‖ Vincent Morinière,¶ Audrey Pawtowski,¶ Laurence Heidet,** Didier Lacombe,†† Olivier Devuyst,‡‡ Yves Pirson,‡‡ Corinne Antignac,†¶§§ and Bertrand Knebelmann*†*

- 109 patients, 45 families
- 37 *UMOD* mutations

- Hyperuricemia
- Low FEurate
- Progression
- Intrafamilial variability







Clin J Am Soc Nephrol 6: 2429–2438, 2011

Clinical characteristics – Patients with *UMOD* mutations

- Autosomal dominant inheritance
- Early gout and/or hyperuricemia, due to inappropriate low Feurate (<5%)
- CKD leading to ESRD in adulthood
- Urinary concentrating defect
- Absence or minimal proteinuria, inactive urine sediment

Pathology features – Patients with *UMOD* mutations

- Tubular atrophy & interstitial fibrosis
- Tubular basement membrane thickening and lamellation
- Tubular and glomerular cysts
- Intracellular aggregates in the TAL cells (EM: ER foldings)
- Uromodulin deposits in the TAL cells (immunostaining)

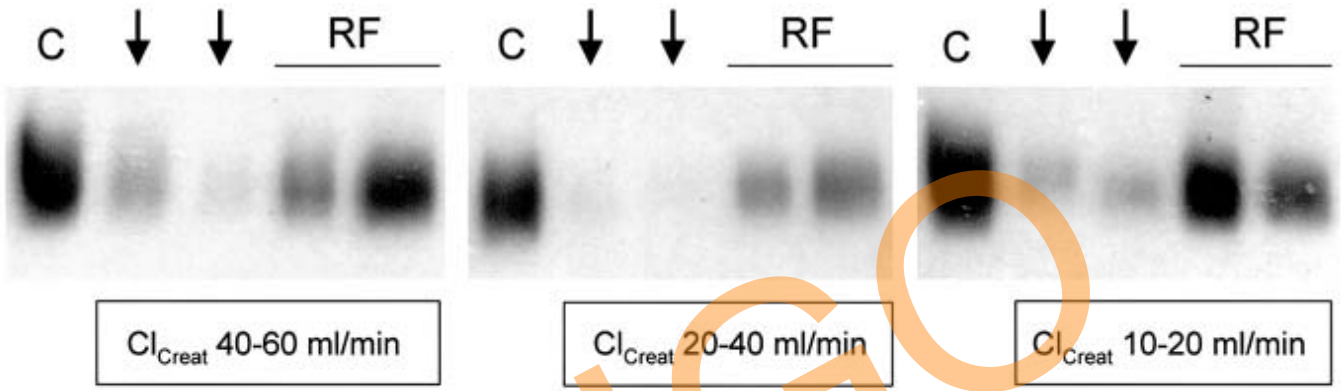
From Dahan et al. 2003; Nasr et al. 2008

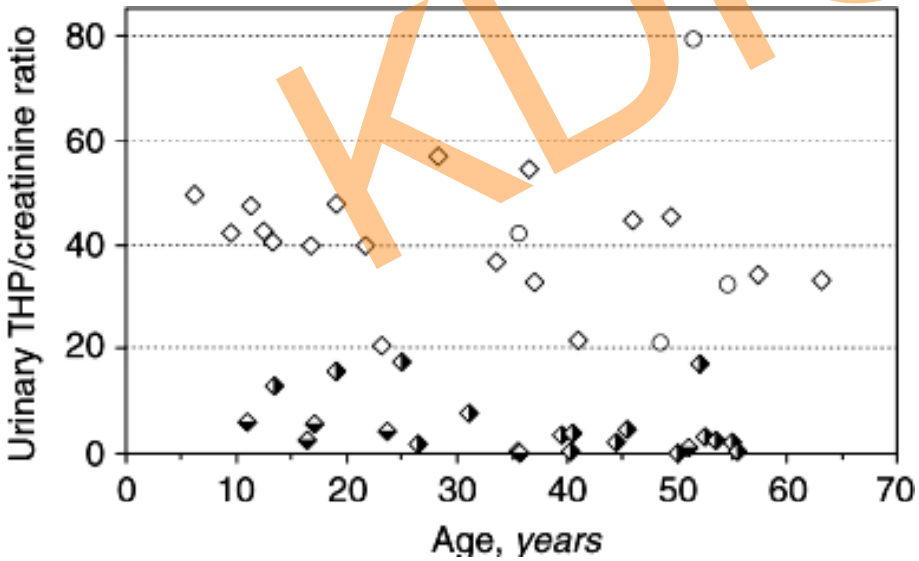
Mutations *UMOD* associated with:

- Glomerulocystic kidney disease (GCKD)
- Unilateral hypoplasia; vesicoureteral reflux (very rare)
- CAKUT : not a frequent cause

Rampoldi L et al. HMG 2003; Lens X et al. AJKD 2005
Wolf MTE et al. Pediatr Nephrol 2009

FJHN : Mutations in *UMOD* decrease THP excretion





ELISA for THP
Families +/- UMOD mutations

- Reduced excretion
- Irrespective of gender, age, GFR, mutation type

Dahan K et al. JASN 14: 2883-93, 2003

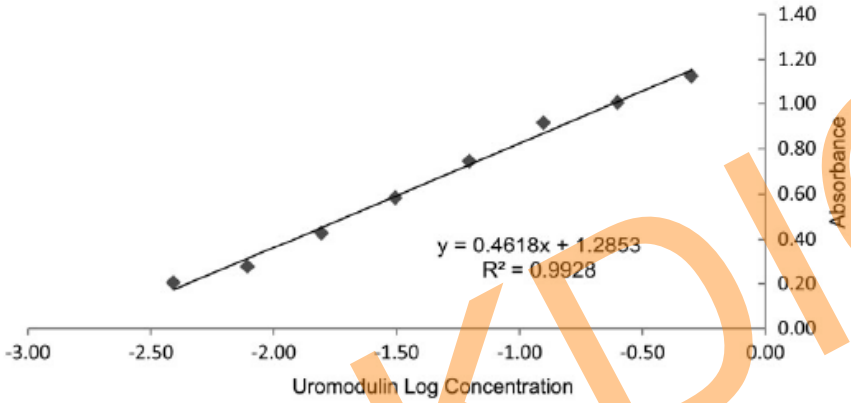
Bleyer et al. Kidney Int 66: 974-7, 2004

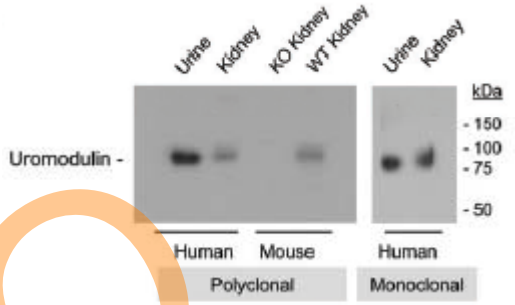
Nephrol Dial Transplant (2013) 0: 1–10
doi: 10.1093/ndt/gft345

S. Youhanna et al. NDT 2013

Original Article

Determination of uromodulin in human urine: influence of storage and processing





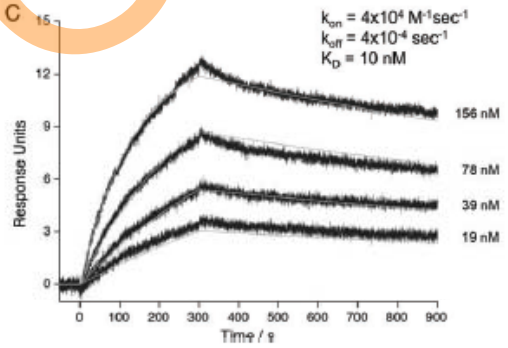


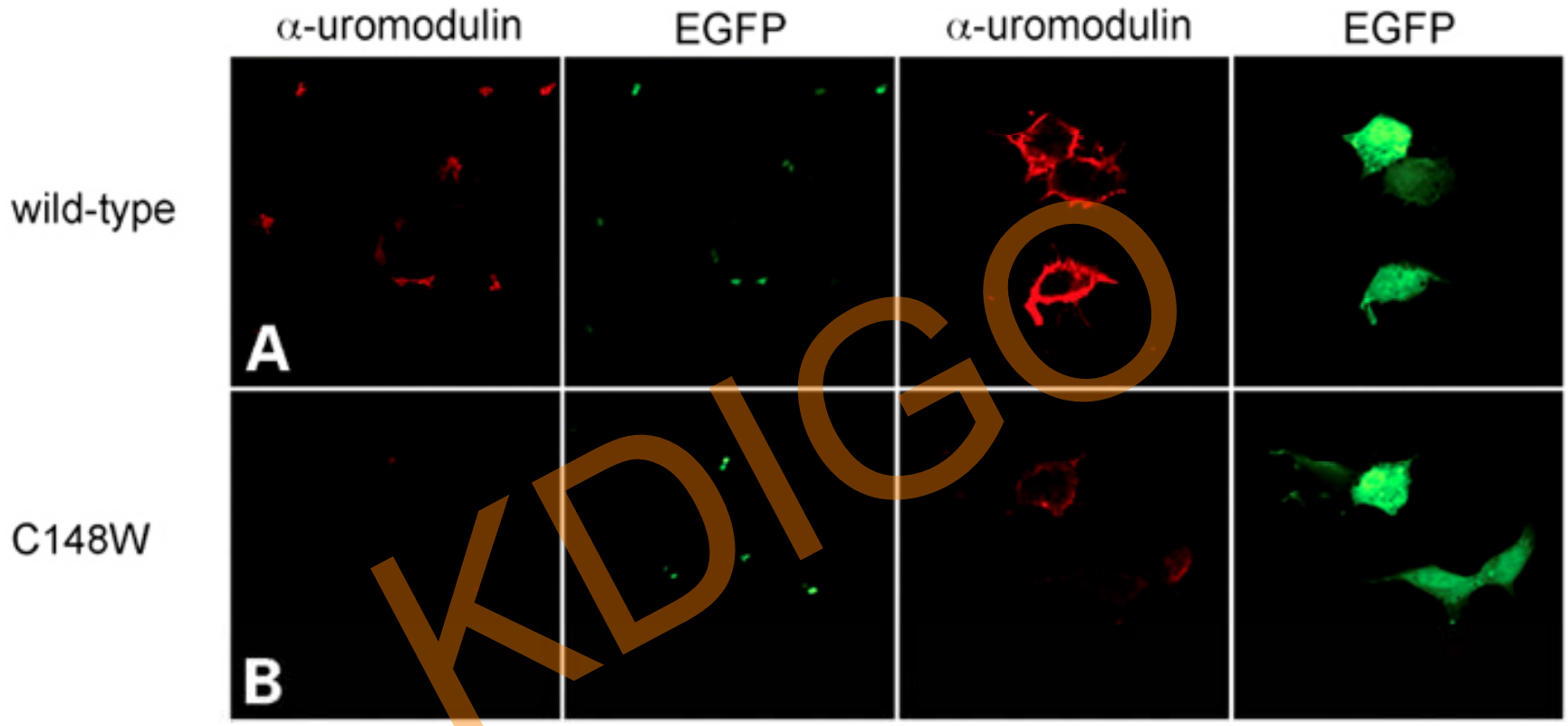
Table 1. Comparison of the characteristics of the in-house ELISA for uromodulin and the commercially available ELISA kits

Kit	Detection range (standard curve) (ng/mL)	Inter-assay variability (%)	Intra-assay variability (%)
In-house	3.9–500	3.28	5.46
MD Bioproduct (Cat. M036020)	2.34–150	11.63	8.36
BioVendor (Cat. RD191163200R)	0.5–32	6.4	2
USCN Life Science, Inc. (Cat. E96918 Hu)	3.13–200	<12	<10

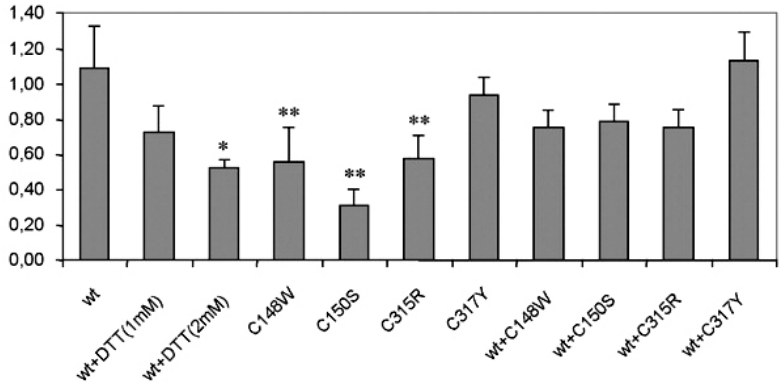
Hypothesis

« Mutations in *UMOD* may critically affect the function and expression of uromodulin, resulting in abnormal accumulation within tubular cells and reduced urinary excretion. »

Missense mutations delay uromodulin export to plasma membrane

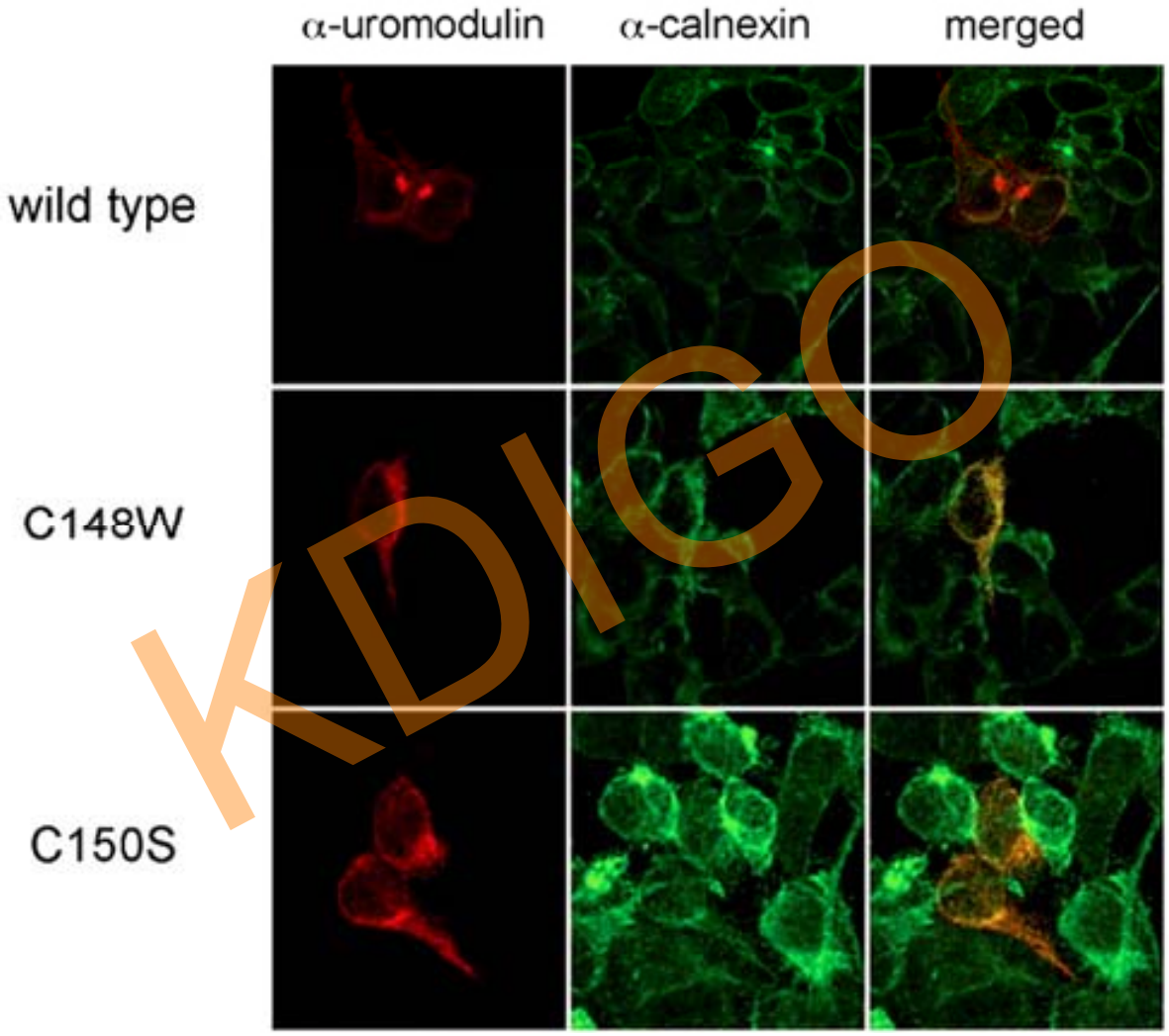


HEK 293- transient co-transfection
EGFP + Uromodulin WT vs. mutated



Rampoldi et al. Hum Mol Genet 12: 3369-84, 2003

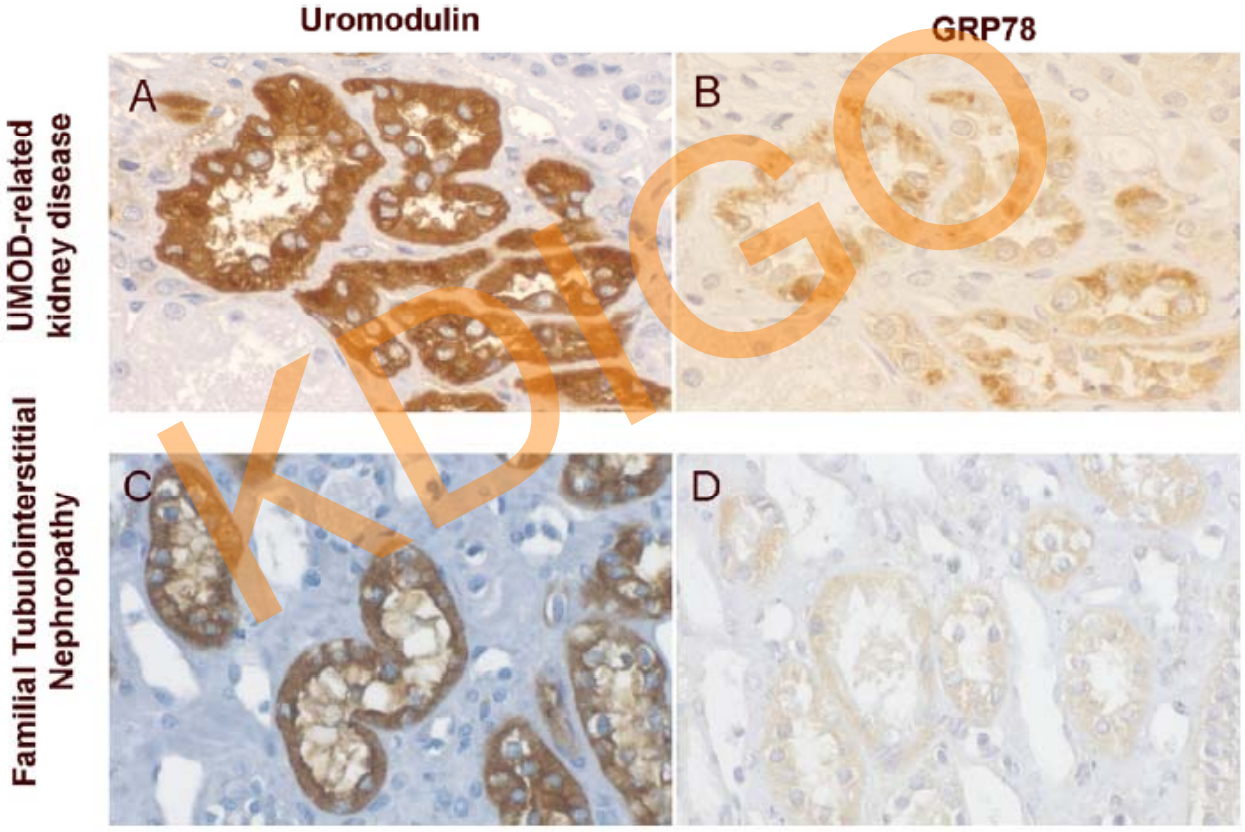
Fate of uromodulin after transfection



→ Mutant protein in ER ⇔ wild-type protein

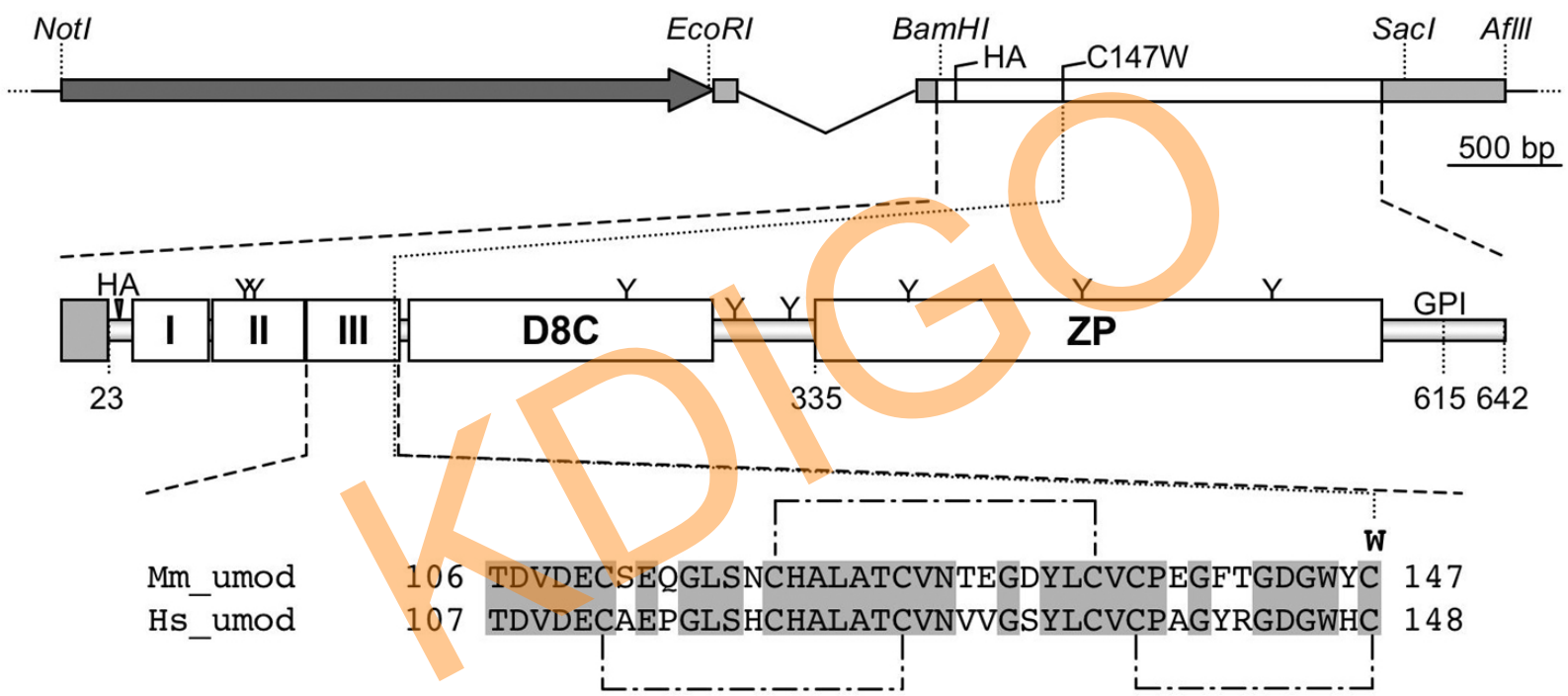
Endoplasmic Reticulum Stress in *UMOD*-Related Kidney Disease: A Human Pathologic Study

Julien Adam, MD,[1] Guillaume Bollée, MD, PhD,[2] Sophie Fougeray, PhD,[3] Laure-Hélène Noël, MD,[3] Corinne Antignac, MD, PhD,[4,5,6] Bertrand Knebelman, MD, PhD,[2] and Nicolas Pallet, MD, PhD[3,7]



Am J Kidney Dis. 2012;59(1):117-121

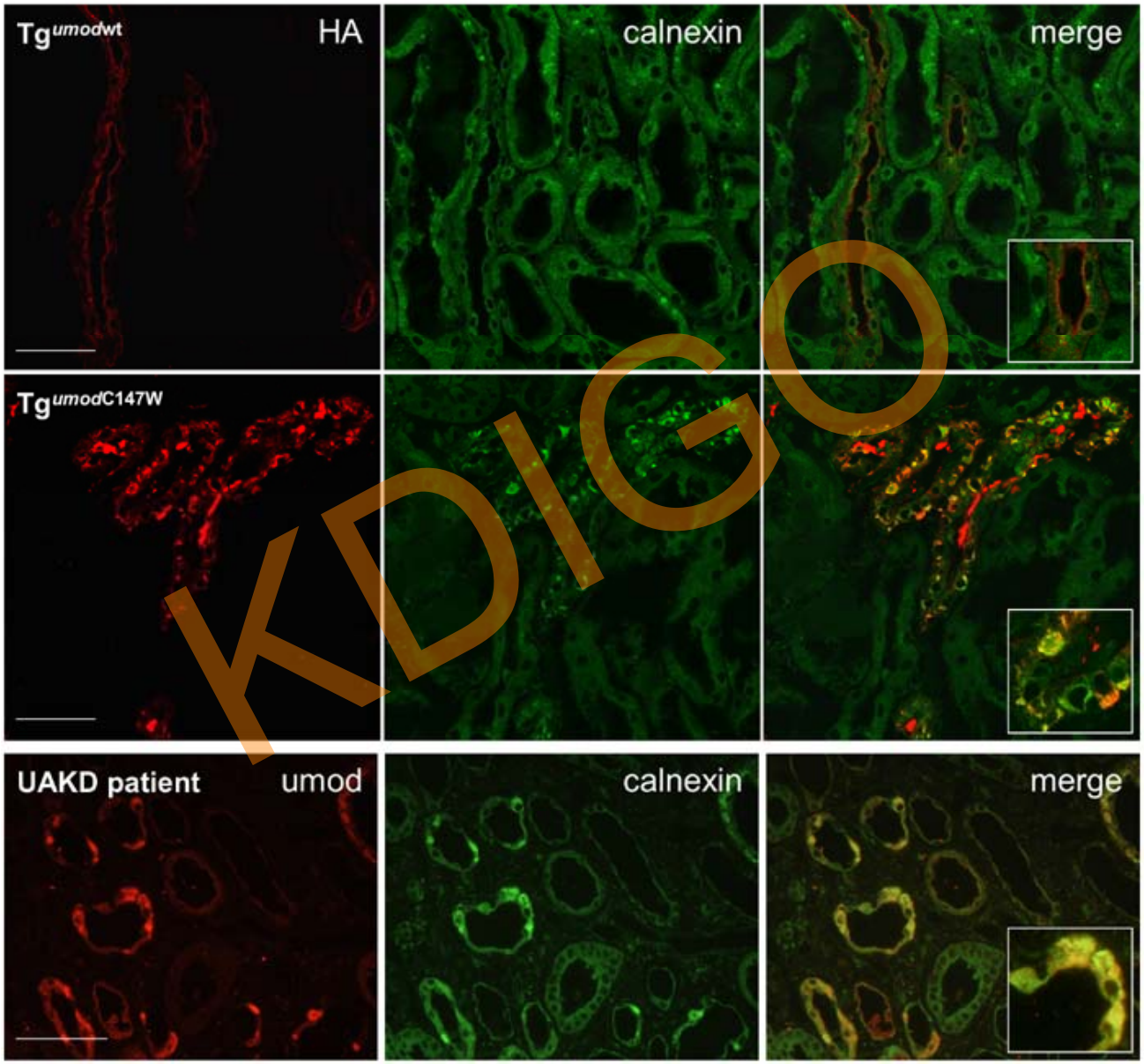
Transgenic uromodulin construct



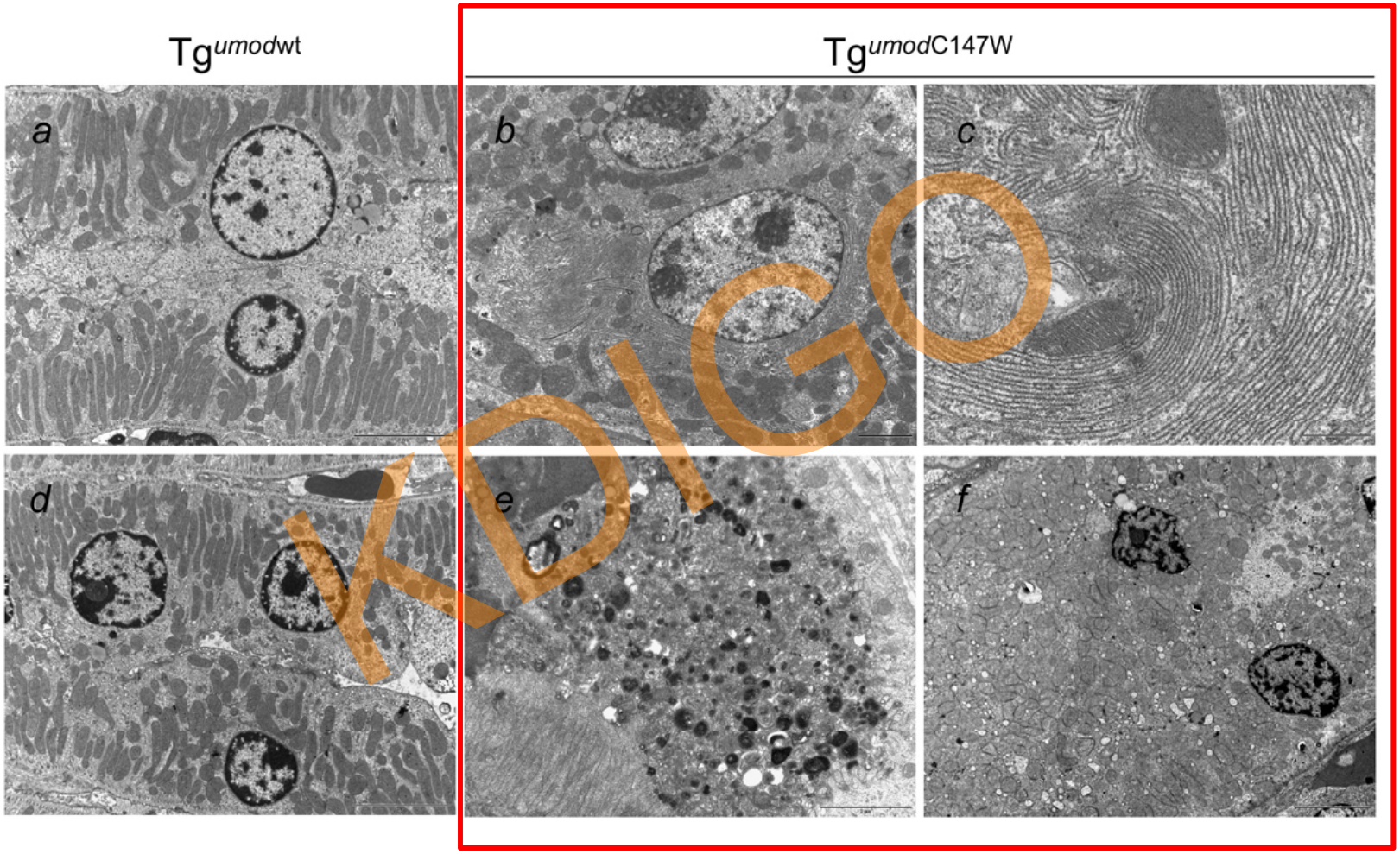
Transgenic wild-type or mutant (C147W) uromodulin

Bernascone et al. Hum Mol Genet 2010

Mutant Uromodulin is Retained in ER



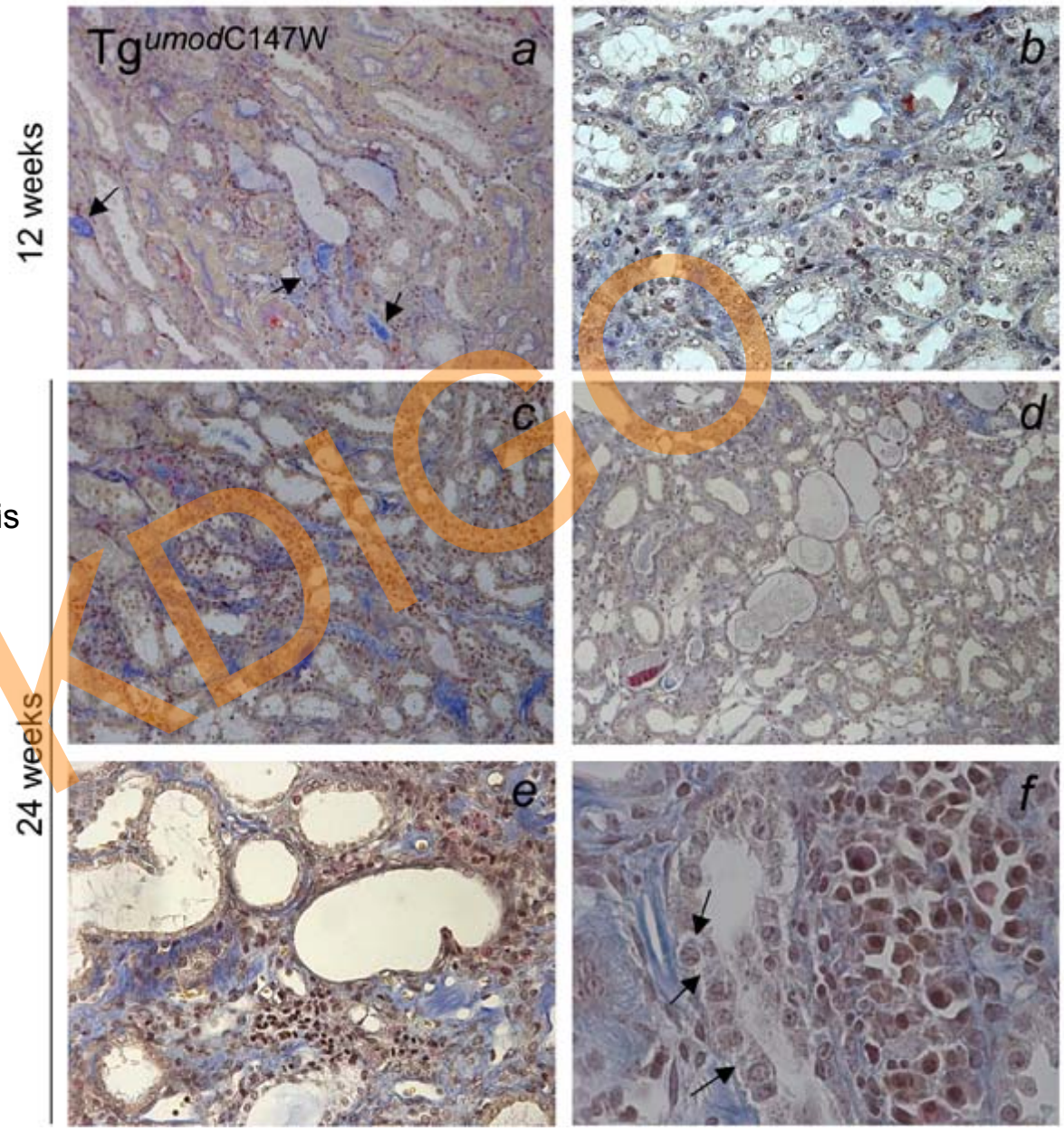
Expanded ER with folded membranes, cytosplasmic accumulation



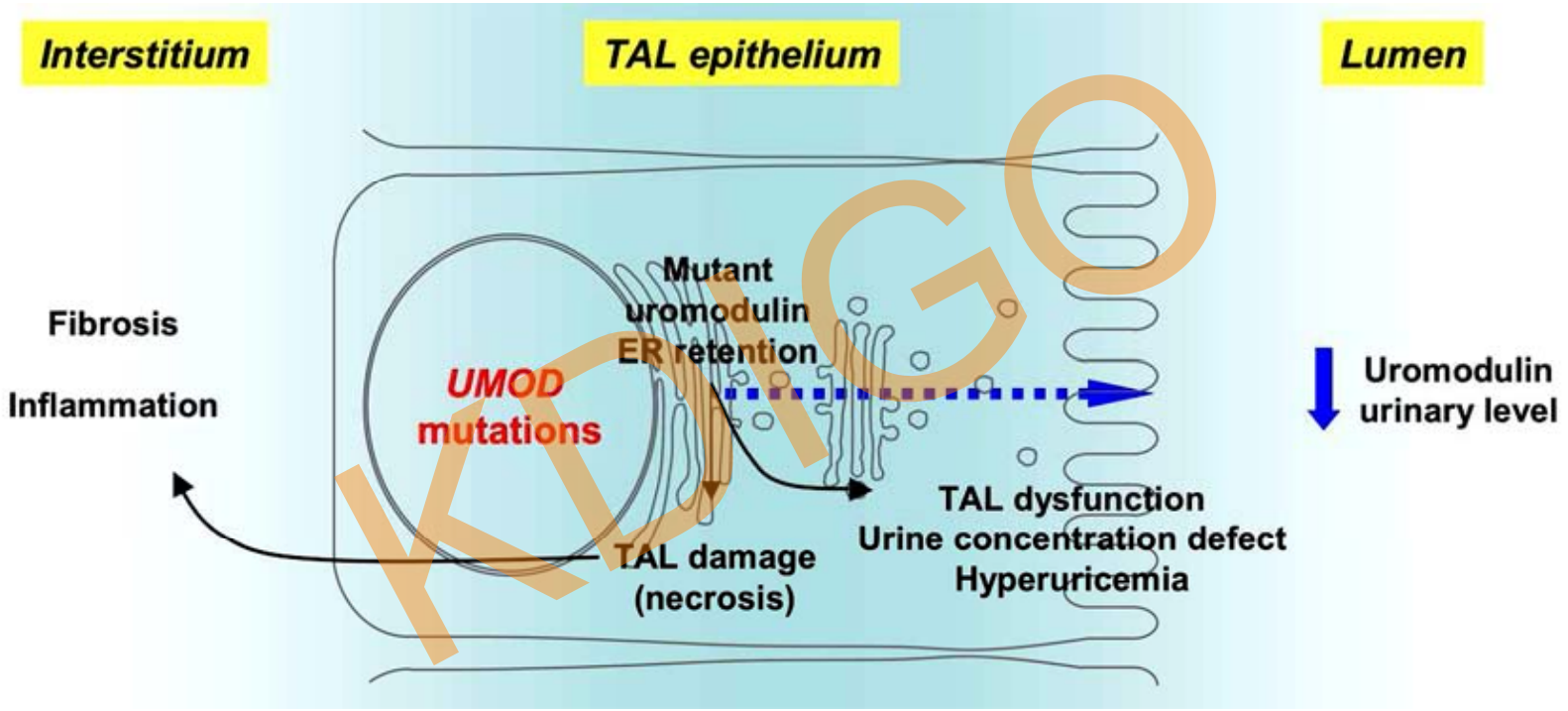
Bernascone et al. Hum Mol Genet 2010

Progressive tubulo-interstitial damage in Tg^{UmodC147W} mice

- Tubular dilations, cysts
- Protein casts
- Interstitial damage & fibrosis
- Cell detachment



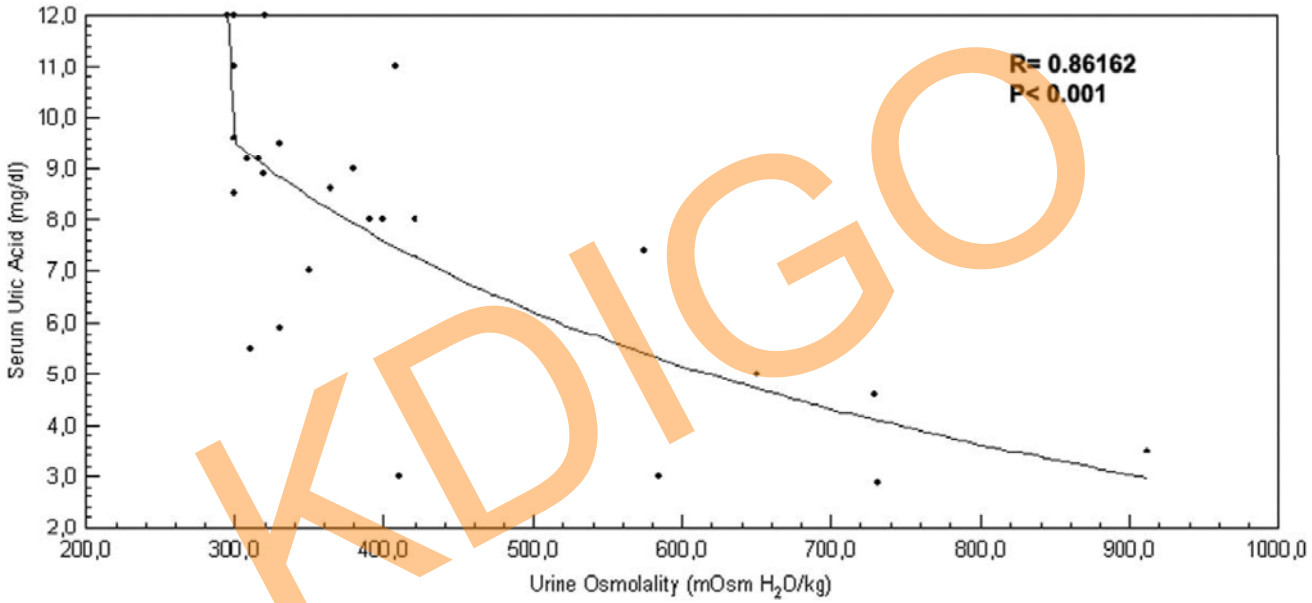
Pathophysiology of Uromodulin-associated Kidney Disease



Central role of TAL dysfunction ?

Kidney Int (2011) **80**, 338–347

Hyperuricemia Correlates with Concentrating Defect



Correlation between serum uric acid level and urine osmolality in 26 patients with *UMOD* mutations:

Hyperuricemia secondary to urinary concentrating defect – TAL dysfunction ?

Scolari et al. Am J Kidney Dis 44, 2004

Uromodulin-associated Nephropathies: Questions

- Diagnostic criteria justifying genetic testing ?
- Sequence of genetic testing ?
  - *UMOD* > *HNF1B* > *REN* : *criteria - algorithm*
  - Hot spot *UMOD* ?
- Causality of *UMOD* allelic variants ?
- Diagnostic value: uromodulin in urine ?
- Renal biopsy and immunostaining ?
- MCKD2 = ? MCKD1 – guide for *MUC1* testing ?
- Management of hyperuricemia and gout ?

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SEVENTH FRAMEWORK PROGRAMME

UCL Université catholique de Louvain

Inserm
Institut national de la santé et de la recherche médicale

UMC St Radboud

TeleThon

Great Ormond Street Hospital

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MARIE CURIE ACTIONS