

SUPPLEMENTARY MATERIAL

Genetic and Clinical Predictors of Age of End-Stage Kidney Disease in Individuals with Autosomal Dominant Tubulo-Interstitial Kidney Disease due to *UMOD* mutations

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1. Supplementary Table S1. Characteristics for each *UMOD* mutation in the study. Range of ESKD is earliest age of ESKD and latest age of ESKD.

<i>UMOD</i>			ADTKD- <i>UMOD</i> Individuals (n)			Age of ESKD (years)		Families linked to rs4293393 variant (n)				References
Mutation	Domain	Exon	Families	Genotyped	Historic	Median	Range	major (A)	minor (G)	unknown	<i>in vitro</i> score	Citations given in supplemental references. If novel, investigator initials given. ClinVar accession number given if known
p.C32T	EGF1	3	1	1	0	--		1	0	0	--	novel, S.K.
p.C32W	EGF1	3	1	3	1	46	41-52	1	0	0	2	1-4
p.C32Y	EGF1	3	1	4	0	--	--	1	0	0	2	4-6
p.E34K	EGF1	3	1	3	0	--	--	1	0	0	--	4, 5
p.A39T	EGF1	3	4	10	0	66	54-75	3	0	1	1	4
p.C41Y	EGF1	3	1	1	1	44	44	1	0	0	--	novel, A.J.B.
p.C50S	EGF1	3	1	2	0	24	21-27	1	0	0	1	4, 7, 8
p.C52S	EGF1	3	1	3	4	41	30-47	0	1	0	--	4
p.C52Y	EGF1	3	1	4	2	45	43-47	0	1	0	--	4, 9, 10
p.G55S	EGF1	3	1	6	1	56	44-60	1	0	0	--	11
p.D59A	EGF1	3	1	2	0	60.5	59-62	1	0	0	2	4, 5, 7, 12, 25
p.D59Y	EGF1	3	2	7	0	45	41-49	2	0	0	--	novel, G.C., G.G.
p.T62P	EGF1	3	1	2	0	--	--	0	0	1	--	10, 13, ClinVar: VCV000318297.1
p.C63S	EGF1	3	1	2	1	36.5	32-46	1	0	0	--	4, 14
p.D67G	EGF2	3	1	1	0	41	41	1	0	0	--	novel, D.G., A.A.
p.E68K	EGF2	3	2	6	6	48	32-76	2	0	0	--	4, 13
p.C69R	EGF2	3	1	1	0	--	--	1	0	0	--	4, 5
p.N76K	EGF2	3	1	1	2	28	19-33	1	0	0	--	4
p.N76S	EGF2	3	1	1	0	26	26	0	0	1	--	novel, D.G., A.A.
p.C77R	EGF2	3	1	3	0	39	39	1	0	0	--	4
p.C77S	EGF2	3	1	2	0	32	32	0	1	0	--	4, 15, ClinVar: VCV000448849.1
p.C77Y	EGF2	3	1	4	0	48	48	1	0	0	2	4, 7, 12, 16, 17, ClinVar: VCV000012258.1
p.C83R	EGF2	3	1	5	1	46	46	0	1	0	--	novel, A.J.B.
p.C83S	EGF2	3	1	2	0	20.5	18-23	1	0	0	3	novel, G.C., G.G.
p.C83W	EGF2	3	1	2	0	27	27	1	0	0	--	2

2 - Predictors of Progression in Individuals with ADTKD-*UMOD*

UMOD			ADTKD-UMOD Individuals (n)			Age of ESKD (years)		Families linked to rs4293393 variant (n)				References
Mutation	Domain	Exon	Families	Genotyped	Historic	Median	Range	major (A)	minor (G)	unknown	<i>in vitro</i> score	Citations given in supplemental references. If novel, investigator initials given. ClinVar accession number given if known
p.N85K	EGF2	3	1	1	1	55	44-66	1	0	0	--	19, ClinVar: VCV000562434.1
p.N85S	EGF2	3	1	1	1	41	41	1	0	0	--	novel, A.J.B., ClinVar: VCV000384591.1
p.G88D	EGF2	3	8	23	0	65.5	55-76	7	0	1	1	4
p.G88V	EGF2	3	2	2	0	51.5	44-59	1	1	0	--	13
p.S91del	EGF2	3	5	13	4	50	37-66	5	0	0	2	13
p.C92G	EGF2	3	1	3	0	31.5	23-40	0	0	1	--	novel, O.D.
p.C92Y	EGF2	3	2	6	3	42.5	40-52	2	0	0	--	4
p.V93-G97delinsAASC	EGF2	3	11	44	30	48	27-75	10	0	1	2	2, 12, 18, 20, 21
p.C94W	EGF2	3	1	2	5	41	28-53	1	0	0	--	4, 23
p.C94Y	EGF2	3	1	2	0	42	42	1	0	0	--	novel, D.G., A.A.
p.G103C	EGF3	3	1	6	5	36	24-49	1	0	0	--	2, 4, 12, 24, ClinVar: VCV000012257.1
p.G105C	EGF3	3	1	1	0	47	47	1	0	0	--	novel, D.G., A.A.
p.C106F	EGF3	3	10	26	21	55	35-72	10	0	0	1	4, 19, 23, ClinVar: VCV000094129.3
p.C106G	EGF3	3	2	7	4	51.5	35-68	1	1	0	--	4, 23
p.C106Y	EGF3	3	1	4	2	70	60-84	1	0	0	2	4, 7, 21, 23
p.C112R	EGF3	3	2	5	0	63	59-64	2	0	0	4	4, 5, 7, 12
p.C112Y	EGF3	3	1	4	0	22	22	1	0	0	--	4, 17, 25, 26
p.C120R	EGF3	3	1	2	0	36	36	1	0	0	--	27
p.C126R	EGF3	3	1	3	0	--	--	1	0	0	4	4, 5, 7, 12, 16, 25 ClinVar: VCV000012259.1
p.N128S	EGF3	3	3	10	2	45	41-78	2	1	0	4	2, 4, 12, 28, ClinVar: VCV000012260.1
p.C135F	EGF3	3	2	4	2	45	44-46	2	0	0	--	4
p.C135S	EGF3	3	1	1	1	41.5	35-48	1	0	0	--	2, 4, 5, 7, 12
p.C135W	EGF3	3	1	5	1	36	36	1	0	0	--	novel, A.J.B.
p.C135Y	EGF3	3	2	14	3	41	37-47	2	0	0	4	4, 5
p.C137R	EGF3	3	1	2	0	48	48	0	0	1	--	novel, G.C., G.G.

3 - Predictors of Progression in Individuals with ADTKD-UMOD

UMOD			ADTKD-UMOD Individuals (n)			Age of ESKD (years)		Families linked to rs4293393 variant (n)				References
Mutation	Domain	Exon	Families	Genotyped	Historic	Median	Range	major (A)	minor (G)	unknown	<i>in vitro</i> score	Citations given in supplemental references. If novel, investigator initials given. ClinVar accession number given if known
p.C148R	EGF3	3	1	6	0	33	28-41	1	0	0	4	4, 5
p.C148S	EGF3	3	2	7	4	51	32-60	2	0	0	--	4
p.C148Y	EGF3	3	4	14	11	44	25-66	4	0	0	4	2, 4, 13, 24, ClinVar: VCV000012255.1
p.C150S	Cys-Rich 1	3	1	6	0	49	42-59	1	0	0	4	4, 17, 21
p.C150Y	Cys-Rich 1	3	1	1	0	--	--	1	0	0	--	novel, D.G., A.A.
p.G153C	Cys-Rich 1	3	1	5	0	57	57	1	0	0	--	29
p.C170Y	Cys-Rich 1	3	2	7	0	58	58	2	0	0	--	4, 5, 7, 10, 12, 25
p.D172H	Cys-Rich 1	3	2	10	2	--	--	2	0	0	--	4, 5
p.P173L	Cys-Rich 1	3	1	2	1	--	--	1	0	0	--	4
p.P173T	Cys-Rich 1	3	1	2	0	51	51	1	0	0	--	novel, G.C., G.G.
p.C174R	Cys-Rich 1	3	1	5	2	63.5	63-64	1	0	0	--	4, 7
p.H177-R185del	Cys-Rich 1	3	25	108	65	46	20-87	25	0	0	3	12, 24, ClinVar: VCV000012254.1
p.R178P	Cys-Rich 1	3	9	21	27	53	39-79	8	0	1	4	4, 15, ClinVar: VCV000586921.1
p.L180P	Cys-Rich 1	3	2	8	4	62	38-75	2	0	0	--	novel, A.J.B., O.D., S.K., ClinVar: VCV000196361.1
p.R185C	Cys-Rich 1	3	3	4	1	38	38	2	1	0	--	4, 5, 15, 25, ClinVar: VCV000586922.1
p.R185G	Cys-Rich 1	3	1	4	2	42	42	1	0	0	--	1-4
p.R185H	Cys-Rich 1	3	3	9	0	58	56-62	3	0	0	--	4, 5
p.R185S	Cys-Rich 1	3	1	10	0	33	27-40	1	0	0	--	5, 7, 12
p.D196A	Cys-Rich 1	3	1	2	0	40	40	1	0	0	--	4, 5, 7, 16, 30
p.D196Y	Cys-Rich 1	3	3	7	0	38	28-48	2	1	0	--	1, 5, 7, 21
p.G201D	Dom 8 Cys	3	1	1	0	--	--	1	0	0	--	novel, O.D.
p.W202C	Dom 8 Cys	3	1	1	0	55	55	1	0	0	--	4, 9
p.W202R	Dom 8 Cys	3	1	5	0	38	38	1	0	0	--	novel, G.C., G.G.
p.W202S	Dom 8 Cys	3	2	3	1	38	34-50	1	0	0	--	2, 4, 12
p.Y203D	Dom 8 Cys	3	1	3	2	62	59-71	1	0	0	--	novel, A.J.B.

4 - Predictors of Progression in Individuals with ADTKD-UMOD

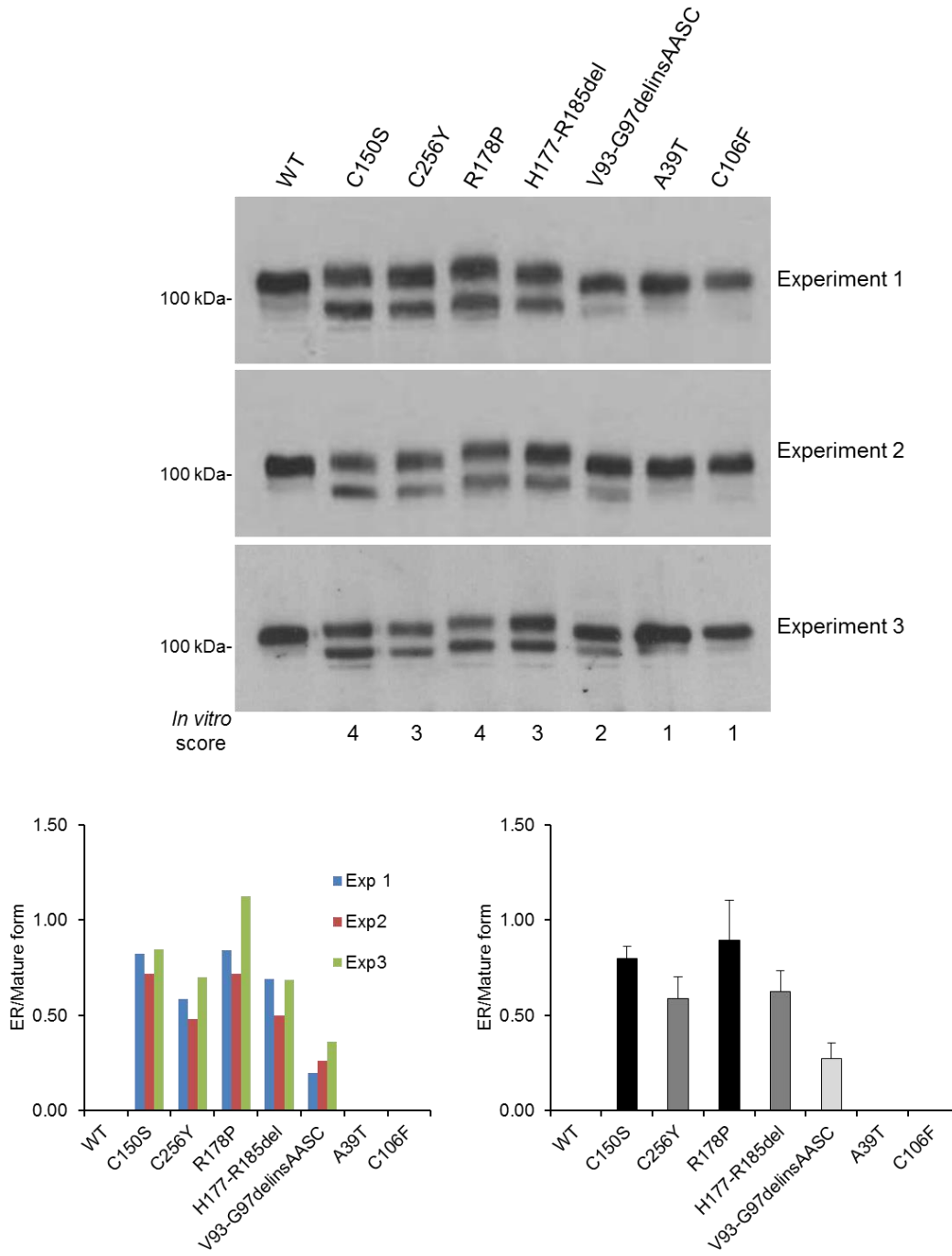
UMOD			ADTKD-UMOD Individuals (n)			Age of ESKD (years)		Families linked to rs4293393 variant (n)				References
Mutation	Domain	Exon	Families	Genotyped	Historic	Median	Range	major (A)	minor (G)	unknown	<i>in vitro</i> score	Citations given in supplemental references. If novel, investigator initials given. ClinVar accession number given if known
p.G209D	Dom 8 Cys	3	1	3	0	46	46	1	0	0	--	novel, G.C., G.G.
p.G210D	Dom 8 Cys	3	1	2	0	--	--	1	0	0	--	4, 13
p.G210R	Dom 8 Cys	3	1	3	0	--	--	1	0	0	2	31
p.G210S	Dom 8 Cys	3	1	2	0	--	--	1	0	1	--	4, 5, 19, ClinVar: VCV000562320.1
p.R212C	Dom 8 Cys	3	3	9	0	65	43-65	3	0	0	3	4
p.C217F	Dom 8 Cys	3	1	1	0	32	32-32	1	0	0	--	novel, G.C., G.G.
p.C217G	Dom 8 Cys	3	1	2	0	37	37	1	0	0	3	4, 5, 7, 12, 25, ClinVar: VCV000039418.1
p.C217R	Dom 8 Cys	3	1	5	0	50	50	1	0	0	3	2, 4, 12, 24, ClinVar: VCV000012256.1
p.P219L	Dom 8 Cys	3	1	3	0	--	--	1	0	0	--	novel, C.I., F.S.
p.R222P	Dom 8 Cys	3	1	10	0	51.5	29-67	1	0	0	--	4, 5, 7, 12, 25
p.C223G	Dom 8 Cys	3	1	2	0	56	56	1	0	0	--	32
p.C223R	Dom 8 Cys	3	1	1	0	--	--	1	0	0	4	1-4, 7, 16
p.C223Y	Dom 8 Cys	3	2	4	1	48	38-50	2	0	0	--	2, 4, 7, 12, 33
p.T225M	Dom 8 Cys	3	2	12	0	38	38	2	0	0	--	4, 5, 7, 12, 25
p.M229R	Dom 8 Cys	3	2	8	0	50	34-62	2	0	0	3	2, 4, 6, 7
p.W230R	Dom 8 Cys	3	1	4	0	60	57-63	1	1	0	--	4, 7, 13, 21, 34
p.H235Q	Dom 8 Cys	3	2	5	0	51.5	45-58	0	2	0	--	4
p.P236L	Dom 8 Cys	3	4	13	7	45	43-67	4	0	0	3	5, 6, 12, ClinVar: VCV000521547.1
p.P236R	Dom 8 Cys	3	4	8	1	40	24-50	3	0	1	4	4, 35
p.P236S	Dom 8 Cys	3	1	3	0	--	--	1	0	0	--	4
p.R245P	Dom 8 Cys	3	1	3	0	52	52	1	0	0	--	novel, D.G., A.A.
p.K246-S252del	Dom 8 Cys	3	1	2	0	43.5	39-48	1	0	0	--	36
p.C248W	Dom 8 Cys	3	3	4	2	71	71	2	1	0	--	2, 4, 7, 12, 20, 37, ClinVar: VCV000287876.1
p.H250Q	Dom 8 Cys	3	1	1	0	--	--	0	0	1	--	4
p.C255Y	Dom 8 Cys	3	1	5	0	56	40-78	1	0	0	--	2, 9, 12, 28, 38, ClinVar: VCV000012261.1
p.C256Y	Dom 8 Cys	3	1	5	0	46	39-60	1	0	0	3	4

5 - Predictors of Progression in Individuals with ADTKD-UMOD

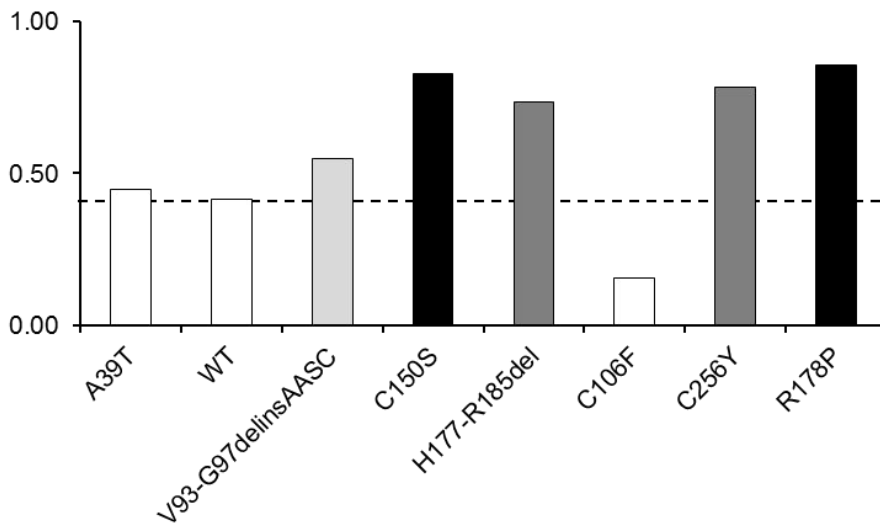
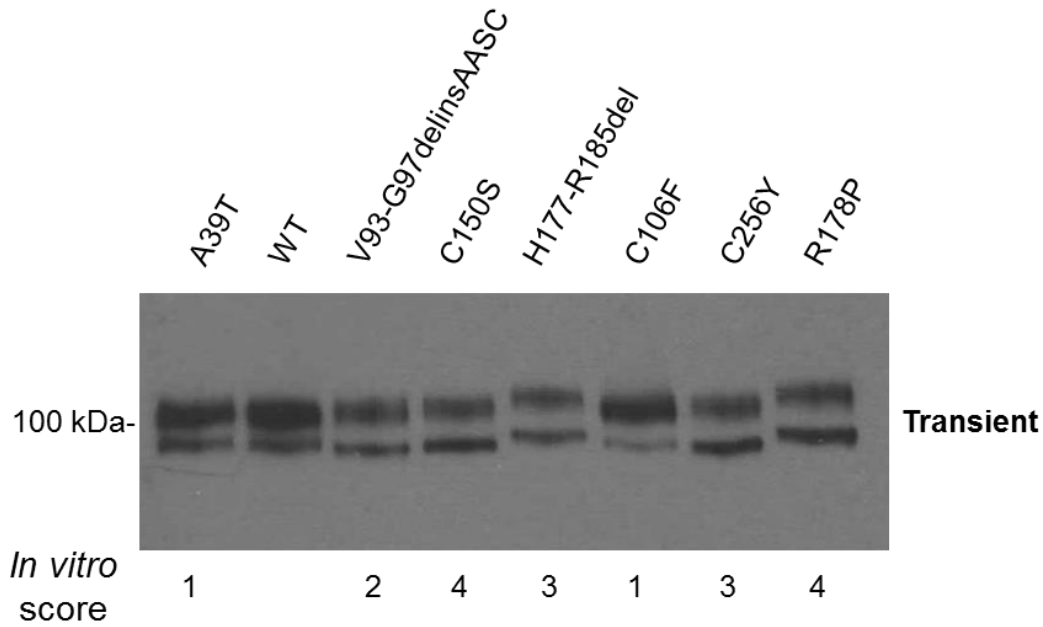
UMOD			ADTKD-UMOD Individuals (n)			Age of ESKD (years)		Families linked to rs4293393 variant (n)				References
Mutation	Domain	Exon	Families	Genotyped	Historic	Median	Range	major (A)	minor (G)	unknown	<i>in vitro</i> score	Citations given in supplemental references. If novel, investigator initials given. ClinVar accession number given if known
p.L257P	Dom 8 Cys	3	2	7	2	46	38-59	2	0	0	--	4
p.W258C	Dom 8 Cys	3	1	1	1	35	35	0	1	0	--	19, ClinVar: VCV000562394.1
p.C267F	Dom 8 Cys	3	1	2	1	63	52-74	1	0	0	--	4
p.G269C	Dom 8 Cys	3	1	1	2	59	49-69	1	0	0	--	4
p.G270C	Dom 8 Cys	3	1	3	0	28	28	1	0	0	--	4
p.Y272del	Dom 8 Cys	3	1	2	0	62	62	1	0	0	--	novel, O.D.
p.V273F	Dom 8 Cys	3	1	3	0	--	--	1	0	0	4	2, 4, 7, ClinVar: VCV000012264.1
p.V273L	Dom 8 Cys	3	1	3	0	--	--	1	0	0	--	5
p.Y274H	Dom 8 Cys	3	1	2	1	48	46-50	1	0	0	--	4
p.C282R	Dom 8 Cys	3	1	2	0	--	--	0	0	1	--	4, 25
p.C282S	Dom 8 Cys	3	1	2	1	32	32	1	0	0	2	4, 5, 12
p.A285E	Dom 8 Cys	3	1	2	0	42	42	1	0	0	--	4, 5, 10
p.Y286C	Dom 8 Cys	3	1	2	2	42.5	34-51	1	0	0	--	novel, A.J.B.
p.C287R	Dom 8 Cys	3	1	1	0	37	37	0	0	1	--	novel, J.C.
p.C287Y	Dom 8 Cys	3	1	1	0	--	--	1	0	0	--	13
p.C297W	Cys-Rich 2	4	1	4	0	39	39	1	0	0	--	4, 5, 39
p.C300G	Cys-Rich 2	4	2	5	1	60	49-71	1	1	0	--	2, 7, 12, 21, 28, 40, ClinVar: VCV000012262.1
p.C306Y	Cys-Rich 2	4	2	5	0	49.5	42-57	2	0	0	--	4, 13
p.C315R	Cys-Rich 2	4	3	6	0	--	--	2	1	0	2	2, 4, 12, 40, 41, ClinVar: VCV000012263.1
p.C315Y	Cys-Rich 2	4	1	5	0	45	45	1	0	0	--	4, 5, ClinVar: VCV000094133.1
p.C317Y	Cys-Rich 2	4	2	6	0	54	54	2	0	0	2	4, 4, 7, 12, 41
p.L333P	Cys-Rich 2	4	1	2	0	--	--	1	0	0	--	novel, C.I., F.S.
p.G488D	ZP	7	2	4	0	49	18-56	2	0	0	--	novel, J.C., ClinVar: VCV000437927.1

6 - Predictors of Progression in Individuals with ADTKD-UMOD

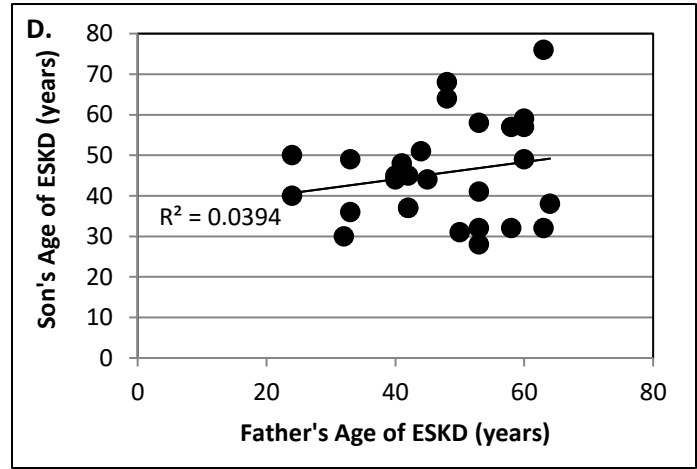
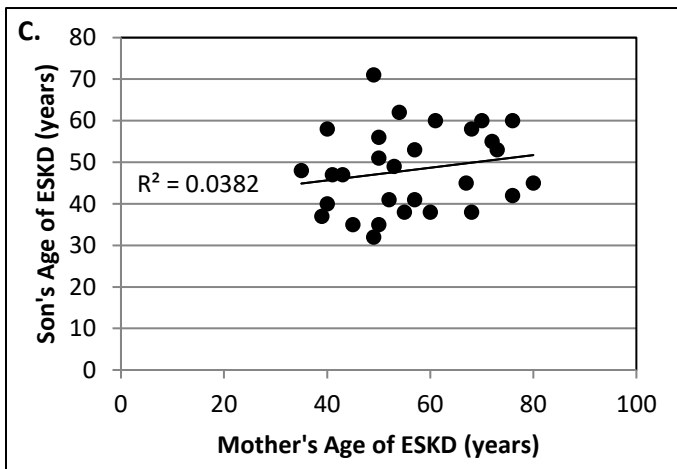
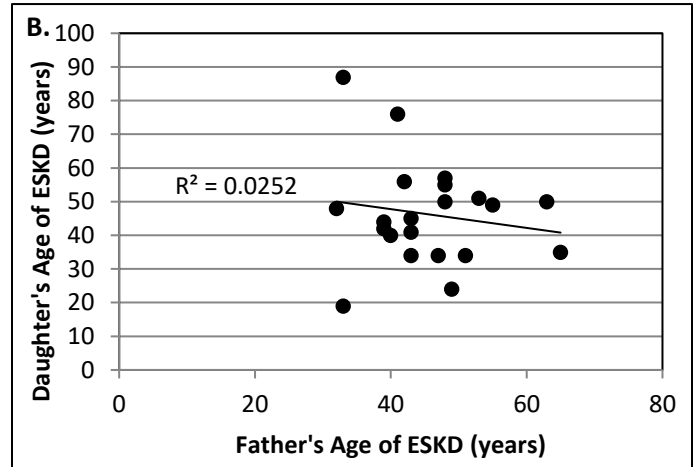
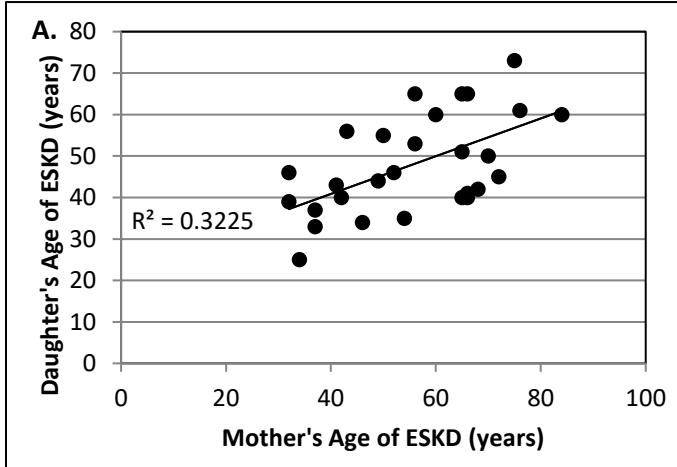
2. Supplementary Figure S1. Western blot analysis of the indicated uromodulin mutant isoforms stably expressed in MDCK cells. Three independent experiments are shown. Graphs represent the quantification of each experiment and the average of the three experiments.



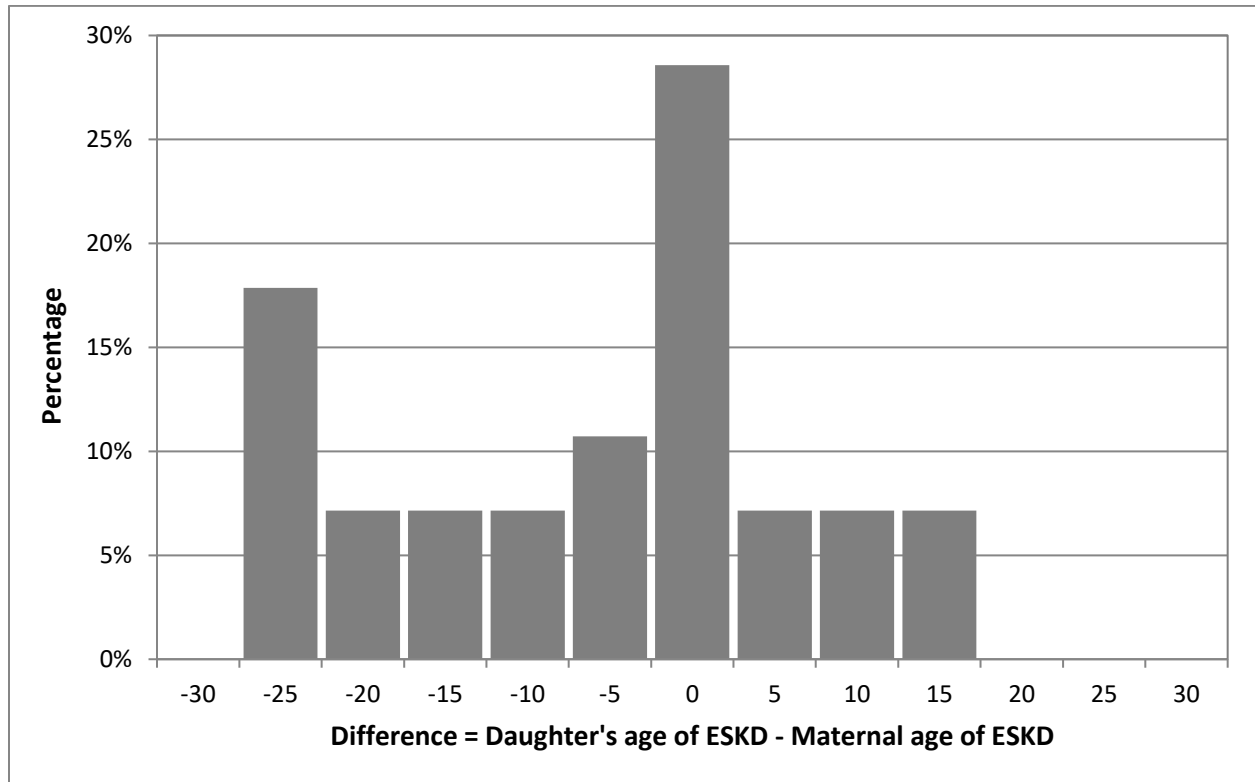
3. Supplementary Figure S2. Western blot analysis of the indicated uromodulin mutant isoforms transiently expressed in MDCK cells. Quantification is shown below. The classification of mutant isoforms is similar to the one obtained in stably transfected MDCK cells, although the difference between the classes is less pronounced due to generally higher levels of ER retained protein (see for instance wild type uromodulin in this blot compared to Supplementary Figure S1).



4. Supplementary Figure S3. Subgroups comparing parental age of end stage kidney disease (ESKD) with child's age of ESKD. A. Mother's age of ESKD vs. Daughter's age of ESKD. B. Father's age of ESKD vs. Daughter's age of ESKD. C. Mother's age of ESKD vs. Son's age of ESKD. D. Father's age of ESKD vs. Son's age of ESKD.



4. Supplementary Figure S4. Histogram of difference in years between daughter's age of end stage kidney disease (ESKD) vs. mother's age of ESKD.



6. Supplementary Methods: Genetic evaluation and *UMOD* mutational sequencing.

Individuals undergoing *UMOD* mutational analysis for the WF Cohort and some patients in the International Cohort from collaborators P.V., M.Ž., K.H, M.V., A.V., P.C, S.M. K.A.B., G.C, E.G. were analyzed as follows:

Primers	Exon	Cycling Conditions	Reaction Composition
GGTGGAGGCTTGACATC	3	1x 95 °C/2 min, 35x (95 °C/15 sec, 66 °C/20 sec, 70 °C/40 sec), 1x 70 °C/5 min, 1x 15 °C/fe	DMSO 1%, extra MgCl ₂ 2 mM, dNTPs 0.2 mM each, primers 0.3 μM each, gDNA 10 ng
CTCCACGGAGCTGGGGTCTG			
AACTGCTCCGCCAACAGCAG	3B/4	1x 94 °C/5 min, 35x (94 °C/10 sec, 66.4 °C/10 sec, 74 °C/70 sec), 1x 74 °C/10 min, 1x 15 °C/fe	DMSO 4%,extra MgCl ₂ 2 mM, dNTPs 0.2 mM each, primers 0.5 μM each, Deep Vent _R [™] 1:0,0016 U/U, gDNA 10 ng
RP-GGAATAGGGCTCAGATG			
T7-AGCAGCATCCAGGCAC	7	1x 94 °C/5 min, 40x (94 °C/10 sec, 70 °C/15 sec, 74 °C/50 sec), 1x 74 °C/10 min, 1x 15 °C/fe	DMSO 4%, extra MgCl ₂ 2 mM, dNTPs 0.2 mM each, primers 0.1 μM each, gDNA 10 ng
RP-GTCCAAAGACCCCCTCTG			

Where indicated, T7 = AATACGACTCACTATAG, RP = GAAACAGCTATGACCAT are universal primers used for post PCR cycle sequencing.

For other patients in the International cohort, *UMOD* mutational analysis is described by collaborators in the following Supplementary References: E.O., O.D, (5); C.S., L.R, C.I., A.A., D.G., G.C., G.G., F.S, L.R (2); S.C.J., J.C, C.S, R.R. (22); R.J.T. (28); K.L. (16); D.S.(29); D.P.G, C.G. (13); J.R.A., C.F.S. (11).

7. Supplementary Methods: rs4293393 genotyping.

rs4293393 genotyping was performed using genotyping by TaqMan[®] SNP Genotyping Assays with the TaqMan[®] Genotyping Master Mix (Applied Biosystems). 10 μL amplification reactions were set in a MicroAmp[®] Fast Optical 96-Well Reaction Plate as follows: 5 μL of 2x genotyping master mix, 0.5 μL of 20x genotyping assay working solution and 4.5 μL of 3-10 ng/μL gDNA. Plates were processed in StepOnePlus[™] Real-Time PCR System (Applied Biosystems, CA, USA) under Standard Mode thermal cycling conditions: 1x 95°C/10 minutes, 40x (95°C/15 seconds, 60°C/1 minute). Data were analyzed by StepOne[™] Software v2.1 (Applied Biosystems, CA, USA) in autocal default, 2-cluster or single cluster manual calling analytical mode.

For some patients the rs4293393 SNP genotype was analyzed by a pyrosequencing assay on a PyroMark Q96MA apparatus (Qiagen, Hilden, Germany). PCR amplification was carried out with the following primers: 5'biotin-ACCAGATAAAGGAGGCAGACT-3' (forward) and 5'-GAGATCAGCCTTAGAATCCAGA-3' (reverse) with GoTaq Master Mix (Promega Corporation, Madison, WI, USA). Samples were denaturated at 94°C for 5 min, amplified for 35 cycles consisting of 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec

and elongated at 72°C for 7 min. The 5'-biotinylated PCR product of the region including the rs4293393 SNP was immobilized onto streptavidin-coated paramagnetic beads, denaturated by 0.1mol/l NaOH and released according to manufacturer' instructions. The primed single-stranded DNA templates were subjected to real-time sequencing of the region including SNP by using the following reverse primer: 5'TCAGAACCAGAAGGTA -3'.

8. Supplementary Methods: rs4293393 and *UMOD* mutation phase determination

The *UMOD* gene sequence from the promoter to exon 4 was PCR amplified from gDNA by TaKaRa LA Taq DNA Polymerase with GC Buffer I (TaKaRa Bio, France). The 25 µL reaction was composed of 1x GC Buffer I, 0.4 mM each dNTP, 0.2 µM each upper (CAGCAAACACTATCGCAAGGA) and lower (AAGTCATGGCACAGGTAACAC) primer, 1.25 U TaKaRa LA Taq DNA Polymerase, 30 ng template gDNA and ddH₂O and produced amplicons of 6388 bp. DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad, CA, USA) thermal conditions were as follows: 94 °C/5 minutes, 30x (94 °C/15 seconds, 61 °C/30 seconds, 72 °C/6 minutes with 10 seconds increment per cycle), 72 °C/10 minutes and 15 °C temperature. PCR products were separated in 1% agarose gel in TAE buffer (40 mM Tris Base, 20 mM acetic acid, 1 mM sodium EDTA dehydrate) with 0.5x GelGreen dye (Biotium, CA, USA) and purified by PureLink™ Quick Gel Extraction Kit (ThermoFisher Scientific, MA, USA) according to manufacturer's instructions.

Maternal and paternal amplicons were separated by cloning using the TOPO® XL PCR Cloning Kit (ThermoFisher Scientific, MA, USA). 5 µL TOPO® Cloning reaction incubation time was extended to 25 minutes. Up to 4 µL of cloning reaction was used to transform One Shot® TOP10 chemically competent cells. Transformation reactions were spread on LB plates containing 50 µg/mL kanamycin. At least five resulting colonies from each plate were picked and resuspended in a screening PCR cocktail, in 3 mL of LB medium containing 50 µg/mL kanamycin and streaked on storage LB plates with kanamycin. Additional colonies were streaked on a storage plate for further analysis if necessary. The 25 µL screening PCR cocktail was composed of 1x Red PCR Master Mix (Roalab, Germany), 1 % DMSO, 0.3 µM each upper (GGTGGAGGCTTGACATC) and lower (CTCCACGGAGCTGGGGTCTG) primer and ddH₂O and produced amplicons of 1078 bp (exon 3). DNA Engine Dyad Peltier Thermal Cycler thermal conditions were as follows: 95 °C/2 minutes, 35x (95 °C/15 seconds, 66 °C/20 seconds, 70 °C/40 seconds), 70 °C/5 minutes and 15 °C.

Plasmids from screening PCR positive colonies grown in LB media were isolated with the 5 PRIME FastPlasmid Mini-Prep Kit (5 Prime, Germany), E.Z.N.A.® X-Press Plasmid DNA Mini Kit or the E.Z.N.A.® Plasmid Mini Kit I (Omega Bio-tek, GA, USA). DNA concentrations were determined by NanoDrop 1000 (NanoDrop Technologies, DE, USA).

Plasmids harboring maternal and paternal amplicons were analyzed by the TaqMan® SNP Genotyping Assay for rs4293393 allele and by Sanger sequencing for the *UMOD* mutation. Sequencing was performed by the BigDye® Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, MA, USA) according to manufacturer's instructions in DNA Engine Dyad Peltier Thermal Cycler. Screening PCR primers and primer GGAATAGGGCTCAGATG were used for mutations in exon 3 and 4, respectively. Reactions were purified by the BigDye XTerminator™ Purification Kit and run on a 3500xL Genetic Analyzer equipped with 24

capillaries filled with POP-7™ Polymer (ThermoFisher Scientific, MA, USA). Data were collected using 3500 Series Data Collection Software v3.1 (3500 Series Data Collection Software) and analyzed in SeqMan Pro v10.1.2 (DNASTAR, WI, USA).

9. Supplementary Methods: *in vitro* score determination

Uromodulin expression constructs: cDNA of human wild type uromodulin was cloned in pcDNA 3.1(+) (ThermoFisher, Waltham, MA), and the HA tag was inserted after the leader peptide in between T26 and S27 in the protein sequence (19). The indicated mutant forms were obtained by mutagenesis using the Quickchange Lightning mutagenesis kit (Agilent, Santa Clara, CA) following the manufacturer's instructions. Primers were designed using the software QuikChange® Primer Design Program.

Cell culture conditions: MDCK or HEK cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 200 U/ml penicillin, 200 µg/ml streptomycin and 2 mM glutamine at 37°C, 5% CO₂. Stable populations were generated by transfecting MDCK or HEK cells using lipofectamine 2000 (ThermoFisher) following the manufacturer's protocol. Selection was started 24 h after transfection by adding 0.5 or 0.25 mg/ml G418 (ThermoFisher) for MDCK or HEK cells respectively and was pursued for 1-2 weeks in order to obtain a population of G418-resistant cells.

Western blot: Cells were lysed in octylglucoside lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 60 mM octyl β-D-glucopyranoside, 10 mM NaF, 0.5 mM Sodium orthovanadate, 1 mM glycerophosphate and protease inhibitor cocktail (Sigma)) for 1 h at 4 °C under rotation followed by centrifugation 10 min at 17,000 g. Soluble fractions were quantified by the Bio-Rad Protein Assay (Bio-Rad). 20 µg of each protein lysate were loaded onto 8% reducing SDS-polyacrylamide gel. Transblotted nitrocellulose membranes (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) were incubated with a sheep anti-UMOD antibody (ab9029, Abcam, Cambridge, United Kingdom) followed by incubation with a donkey anti-sheep horseradish peroxidase-conjugated secondary antibody (1:7,500 dilution; Abcam). Protein bands were visualized with the Immobilon Western Chemiluminescent Horseradish Peroxidase Substrate kit (Millipore, Billerica, MA). Using ImageJ software (Schneider et al, Nature Methods, 2012,), we quantified the ratio of ER retained protein (lower molecular-weight, Endo H sensitive) divided by the mature one (higher molecular-weight band, Endo H resistant). Based on this ratio, mutations were divided into 4 subgroups of severity regarding trafficking defect.

10. Supplementary References

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