Supplemental table 1: Overview of underlying mutations and their primary literature report, renal phenotype, Karyotype (KT) and gender

Legend: Age at onset (dx) of proteinuria and Wilms Tumour (WT) unilateral (ul) or bilateral (bl). Immunosuppressive (IS) treatment yes (Y) or no (N), all were irresponsive to steroids, *partial remission with cyclosporin A (CsA), ** no response to CsA.
One patient ($^{+}$) was excluded from correlation analyses as he initially presented with HUS. One patient died aged 5 months (^{t}). In one patient $^{@}$ incidental finding of nephroblastomatosis WT stage 1 when prophylactic bilateral nephrectomy was performed, no chemotherapy. One patient § with concomitant heterozygous *NPHS1* mutation was included in the analysis. 6 proteinuric patients with a median follow up of 7.4 years [5.4-15.4] did have unimpaired renal function. 3 patients without proteinuria did not have impaired renal function at the age of 4.2, 5.8 and 13.1 years.

Abbreviations: FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; DMS, diffuse mesangial sclerosis

Patient number	Exon/ Intron	Mutation	Protein	Age at follow-up (years)	Age at dx proteinuria (months)	Renal Histology	IS treatment	Age at start RRT (months)	eGFR	WT	Age at dx WT (months)	KT/ Gender	Primary literature report
Missense mutations in DNA-binding region													
1[t]	Exon 8	c.1300C>T	p.Arg434Cys	0.4	1	FSGS	N	1		-		**XY/f**	1
2	Exon 8	c.1322A>G	p.His441Arg	12.7	4	DMS	N	13		-		XX/f	this study
3	Exon 8	c.1339G>T	p.Gly447Cys	17.8	3	DMS	Y	3		-		XX/f	2
4	Exon 9	c.1384C>T	p.Arg462Trp	1.1	0.1	-	N	-	60	-		XY/m	3
5	Exon 9	c.1384C>T	p.Arg462Trp	7.6	14	DMS	N	14		ul	14	XX/f	3
6	Exon 9	c.1384C>T	p.Arg462Trp	8.1	10	DMS	N	13		ul[@]	15	XY/m	3
7	Exon 9	c.1384C>T	p.Arg462Trp	18.8	59	DMS	N	59		-		XY/m	3
8	Exon 9	c.1384C>T	p.Arg462Trp	21.9	7	Other	N	7		-		**XY/f**	3
9[+]	Exon 9	c.1384C>T	p.Arg462Trp	6.7	7	DMS	N	7		-		XY/m	3
10	Exon 9	c.1384C>T	p.Arg462Trp	11.7	49	FSGS	Y	52		-		XX/f	3
11	Exon 9	c.1384C>T	p.Arg462Trp	0.3	1	-	N	-	15	-		XX/f	3
12	Exon 9	c.1384C>T	p.Arg462Trp	6.2	17	FSGS	Y	-	≥90	ul	61	XY/m	3
13	Exon 9	c.1384C>T	p.Arg462Trp	3.2	8	DMS	N	8		-		XX/f	3
14	Exon 9	c.1384C>T	p.Arg462Trp	13.9	22	DMS	N	62		ul	22	XY/m	3
15	Exon 9	c.1384C>T	p.Arg462Trp	15.8	7	DMS	N	7		-		- /f	3
16	Exon 9	c.1384C>T	p.Arg462Trp	12.8	16	FSGS	Y**	94		ul	16	XX/f	3
17	Exon 9	c.1384C>T	p.Arg462Trp	19.3	5	DMS	N	21		ul	4	**XY/f**	3
18	Exon 9	c.1385G>A	p.Arg462Gln	16.8	0.1	FSGS	N	4		-		XY/m	1
19	Exon 9	c.1385G>A	p.Arg462Gln	15.3	2	DMS	N	5		-		XY/m	1
20	Exon 9	c.1385G>A	p.Arg462Gln	4.0	0.5	Other	N	0,1		-		XY/m	1
21	Exon 9	c.1385G>C	p.Arg462Pro	6.6	7	DMS	N	7		-		XX/f	4
22	Exon 9	c.1385G>C	p.Arg462Pro	4.3	1	-	N	1		-		XY/m	4
23	Exon 9	c.1390G>A	p.Asp464Asn	0.9	3	FSGS	N	17		-		XY/m	3
24	Exon 9	c.1390G>A	p.Asp464Asn	9.2	19	DMS	N	47		bl	16	XX/f	3
25	Exon 9	c.1390G>A	p.Asp464Asn	1.0	4	DMS	N	4		-		XY/m	3
26	Exon 9	c.1390G>C	p.Asp464His	0.9	3	DMS	N	4		bl	3	XY/m	5
27	Exon 9	c.1391A>G	p.Asp464Gly	2.4	4	-	N	12	-	ul	12	XX/f	3

Patient number	Exon/ Intron	Mutation	Protein	Age at follow-up (years)	Age at dx proteinuria (months)	Renal Histology	IS treatment	Age at start RRT (months)	eGFR if no RRT	WT	Age at dx WT (months)	KT/ Gender	Primary literature report
28	Exon 9	c.1394A>C	p.His465Pro	11.9	63	DMS	N	63	-	ul	18	XX/f	6
29	Exon 9	c.1394A>C	p.His465Pro	12.1	15	FSGS	Y	112	-	-		XX/f	6
Missense mutations in other regions													
30	Exon 2	c.745C>T	p.Pro249Ser	13.2	-	-	N	-	≥90	-	-	XY/m	7
31	Exon 7	c.1220A>G	p.His407Arg	17.7	198	FSGS	N	-	60	-	-	- /m	this study
32	Exon 8	c.1008 G>T	p.Gln369His	14.6	0.1	DMS	N	35	-	-	-	XY/m	this study
33	Exon 8	c.1289G>C	p.Arg430Pro	8.5	21	DMS	Y**	21	-	-	-	XY/m	this study
34	Exon 9	c.1357T>C	p.Cys453Arg	4.3	7	DMS	N	15	-	-	-	XY/m	8
35	Exon 9	c.1357T>C	p.Cys453Arg	25.4	35	Other	N	50	-	ul	35	XY/m	8
36	Exon 9	c.1366T>C	p.Cys456Arg	9.3	1	DMS	N	20	-	ul	18	XX/f	6
Truncating mutations													
37	Exon 1	c.369_385del	p.Ala123fs*89	4.3	-	-	N	-	≥90	bl	8	XY/m	this study
38	Exon 1	c.525T>A	p.Cys175*	5.8	-	-	N	-	≥90	bl	12	XY/m	this study
39	Exon 8	c.1288C>T	p.Arg430*	23.1	142	FSGS	N	201	-	bl	11	XY/m	9
40	Exon 8	c.1288C>T	p.Arg430*	19.1	147	FSGS	N	197	-	bl	14	XX/f	9
41	Exon 9	c.1372C>T	p.Arg458*	16.6	94	-	N	199	-	bl	10	XY/m	7
42	Exon 9	c.1372C>T	p.Arg458*	30.4	138	FSGS	N	-	58	-	-	XY/m	7
43	Exon 9	c.1372C>T	p.Arg458*	22.1	38	FSGS	N	193	-	ul	22	- /m	7
44	Exon 9	c.1372C>T	p.Arg458*	12.4	79	Other	N	-	51	ul	16	XY/m	7
Splice site mutations													
45	Intron 9	IVS9+4C>T (c.1432+4C>T)		26.1	116	Other	N	137	-	-	-	**XY/f**	10
46	Intron 9	IVS9+4C>T (c.1432+4C>T)		21.8	47	MCD	Y*	-	≥90	-	-	XX/f	10
47	Intron 9	IVS9+4C>T (c.1432+4C>T)		9.2	8	FSGS	Y*	-	50	-	-	**XY/f**	10
48	Intron 9	IVS9+4C>T (c.1432+4C>T)		8.5	75	FSGS	N	-	≥90	-	-	XY/m	10
49	Intron 9	IVS9+4C>T (c.1432+4C>T)		43.1	83	FSGS	Y	156	-	-	-	XX/f	10
50	Intron 9	IVS9+5G>A (c.1432+5G>A)		21.0	35	FSGS	Y*	218	-	-	-	XX/f	4
51	Intron 9	IVS9+5G>A (c.1432+5G>A)		15.0	48	MCD	Y	-	46	-	-	**XY/f**	4
52	Intron 9	IVS9+5G>A (c.1432+5G>A)		21.0	66	MCD	Y	-	50	-	-	XX/f	4
53	Intron 9	IVS9+5G>A (c.1432+5G>A)		41.0	27	FSGS	Y§	95	-	-	-	XX/f	4

References for Supplemental table 1

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Supplemental table 2: Subgroup analysis of 14 patients with missense mutation c.1384C>T leading to p.Arg462Trp

Age at follow up	9.9 (5.5-16.6) years
Nephrotic range proteinuria	14/14 (100%)
Age at diagnosis of proteinuria	9 (6.5-18.3) months
Renal replacement treatment	11/14 (79%)
Age at start of RRT	14 (7-59) months
Renal histology DMS	7/14 (50%)
FSGS	3/14 (22%)
Wilms Tumour (unilateral)	6/14 (43%)
Age at diagnosis of WT	15.5 (12-32) months
Karyotype/gender XY/m	6/14 (43%)
XY/f	2/14 (14%)
XX/f	5/14 (36%)
?/f	1/14 (7%)
XY DSD Cryptorchidism	1/8 (12.5%)
Cryptorchidism, glandular hypospadia	1/8 (12.5%)
Cryptorchidism, penoscrotal hypospadias, +/-micropenis	4/8 (50%)
Sex Reversal (complete gonadal dysgenesis)	2/8 (25%9
XX females with streak ovaries	1/5 (20%)

Supplemental table 3: Allele frequencies and prediction of pathogenicity of *WT1* mutations

Patient number	Genomic Position	CDC	Protein	TGP AF	ESP AF	CG69 AF	NCI-60 AF	GoNL AF	ExAC AF	SIFT Score	SIFT Pred.	PolyP.-2 HumVar Score	PolyP.-2 HumVar Pred.	FATHMM Score	FATHMM Pred.	VEST Score	VEST Pred.	CADD Score	CADD Pred.
Missense mutations in DNA-binding region																			
1	32414251	c.1300C>T	p.Arg434Cys	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.01	Damaging	1	Probably Damaging	1.26	Tolerated	0.969	Damaging	27.4	Pathogenic
2	32414229	c.1322A>G	p.His441Arg	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	0.999	Probably Damaging	-2.18	Damaging	0.963	Damaging	25.9	Pathogenic
3	32414212	c.1339G>T	p.Gly447Cys	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	NA	NA	0.997	Probably Damaging	1.72	Tolerated	0.923	Damaging	33	Pathogenic
4 ; 5 ; 6 ; 7 ; 8 ; 9 ; 10 ; 11 ; 12 ; 13 ; 14 ; 15 ; 16 ; 17	32413566	c.1384C>T	p.Arg462Trp	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.01	Damaging	1	Probably Damaging	1.95	Tolerated	0.953	Damaging	23	Pathogenic
18 ; 19 ; 20	32413565	c.1385G>A	p.Arg462Gln	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.29	Tolerated	0.998	Probably Damaging	1.982	Tolerated	0.859	Tolerated	36	Pathogenic
21 ; 22	32413565	c.1385G>C	p.Arg462Pro	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	1	Probably Damaging	1.95	Tolerated	0.983	Damaging	34	Pathogenic
23 ; 24 ; 25	32413560	c.1390G>A	p.Asp464Asn	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.02	Damaging	0.944	Probably Damaging	1.992	Tolerated	0.102	Tolerated	36	Pathogenic
26	32413560	c.1390G>C	p.Asp464His	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.02	Damaging	0.993	Probably Damaging	2.338	Tolerated	0.961	Damaging	31	Pathogenic
27	32413559	c.1391A>G	p.Asp464Gly	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.17	Tolerated	0.198	Benign	2	Tolerated	0.979	Damaging	26.3	Pathogenic
28 ; 29	32413556	c.1394A>C	p.His465Pro	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	0.999	Probably Damaging	1.61	Tolerated	0.973	Damaging	25.7	Pathogenic
Missense mutations in other regions																			
30	32450067	c.745C>T	p.Pro249Ser	0.04 %	0.04 %	0.00 %	0.00 %	0.10 %	0.04 %	0.32	Tolerated	0.016	Benign	-1.84	Damaging	0.729	Tolerated	20.9	Pathogenic
31	32417832	c.1220A>G	p.His407Arg	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.02	Damaging	0.786	Possibly Damaging	1.32	Tolerated	0.851	Tolerated	32	Pathogenic
32	32421584	c.1008C>A	p.Ser336Arg	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.42	Tolerated	0.065	Benign	-1.94	Damaging	0.304	Tolerated	13.34	Nonpathogenic
33	32414262	c.1289G>C	p.Arg430Pro	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	0.954	Probably Damaging	2.12	Tolerated	0.974	Damaging	32	Pathogenic
34 ; 35	32413593	c.1357T>C	p.Cys453Arg	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	1	Probably Damaging	-1.96	Damaging	0.995	Damaging	24.7	Pathogenic
36	32413584	c.1366T>C	p.Cys456Arg	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0	Damaging	0.999	Probably Damaging	-2.05	Damaging	0.992	Damaging	26.6	Pathogenic
Truncating mutations																			
37	32456507	c.369_385delGCGGAGCCGGTGGCGGC	p.Ala123fs	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
38	32456367	c.525T>A	p.Cys175*	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	1	Tolerated	NA	NA	NA	NA	NA	NA	39	Pathogenic
39 ; 40	32414263	c.1288C>T	p.Arg430*	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	1	Tolerated	NA	NA	NA	NA	NA	NA	40	Pathogenic
41 ; 42 ; 43 ; 44	32413578	c.1372C>T	p.Arg458*	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	1	Tolerated	NA	NA	NA	NA	NA	NA	40	Pathogenic

All cDNA and protein positions are relative to transcript NM_024426.4
TGP AF = 1000 genomes project allele frequency
ESP AF = Exome Sequencing Project allele frequency
CG69 AF = Complete Genomics sequencing project allele frequency
NCI-60 AF = National Cancer Institute sequencing project allele frequency
GoNL AF = Genomes of the Netherlands sequencing project allele frequency
ExAC AF = Exome Aggregation Consortium allele frequency

SIFT = Functional consequence prediction tool, Damaging: [0 ≤ Score ≤ 0.05]; Tolerated [0.06 ≤ Score ≤ 1]
PolyPhen-2 = Functional consequence prediction tool, Probably Damaging: [0.909 ≤ Score ≤ 1]; Possibly Damaging: [0.447 ≤ Score ≤ 0.908]; Benign: [0 ≤ Score ≤ 0.446]
FATHMM = Functional consequence prediction tool. Damaging: [-18.09 ≤ Score < -1.5]; Tolerated: [-1.15 ≤ Score ≤ 11.00]
VEST = Functional consequence prediction tool. [0 ≤ Score ≤ 1].
CADD = Functional consequence prediction tool. Non-pathogenic: [0 ≤ Score ≤ 15]; Pathogenic: [15 < Score ≤ 99]