Comprehensive genetic analysis reveals complexity of monogenic urinary stone disease

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SUPPLEMENTAL METHODS

NGS library generation and sequencing: We employed the SureSelect (Agilent) method (capture probes designed using SureDesign) to capture coding exons, plus 50bp flanking IVS regions and the UTRs of the selected genes. A tiling density of 5 probes overlapping with every base was used to improve the capture efficiencies and the least stringent masking of repeat regions to include repeats shorter than the length of the sequencing libraries (~250bp fragments). In addition, the appropriate boosting parameters were set to different levels of probe replications in different regions to minimize the local coverage differences (e.g. between regions of different GC contents).

Paired-end indexed libraries were prepared using the NEBNext Ultra library preparation kit with Illumina barcode adaptors. Targeted sequence was captured using the SureSelect Custom capture protocol on an Agilent Bravo liquid handler (RKSC Mayo Laboratory) following the manufacturer's protocol, with the captured DNA fragmented using the Covaris E-220 sonicator. Adapter ligated DNA fragments were size selected to enrich for ~350bp fragments using a double SPRI bead purification. The concentration and size distribution of the libraries were determined on an Agilent Bioanalyzer DNA 1000 chip. Individual patient samples (n=24) were pooled before capture and two pools (48 samples) sequenced on a HiSeq4000 system lane (Illumina). The degree of multiplexing resulted in an average sequencing depth per base of ~800 reads.

Variant identification: Read QC and alignment, variant calling, and variant/gene annotation were performed using the Mayo in-house analytic pipeline GenomeGPS (GGPS). The Accession numbers of the transcripts and proteins are shown in Table S3. Specifically**,** read qualities were examined by FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc) and reads aligned to the reference genome (hg19) using alignment tools BWA and Samtools, duplicate reads eliminated using Picard, and realignment and recalibration performed through GATK before variant calling. The obtained VCF files were then analyzed by the RKSC SVS pipeline (Golden Helix: Version 8.9.0). A series of filters were applied: quality filter of read depth ≥10x and genotype quality ≥20, removal of Genome Aggregation Database (gnomAD)[1] variants with a minor allele frequency >1%, characterization of coding and non-coding SNVs within +6 and -20bp of the splice site (in secondary analysis some deeper intronic changes were assessed), and subsequent removal of SNPs predicted to be neutral by 4/6 dbNSFP tools (SIFT, PolyPhen-2 HVAR, MutationTaster, Mutation Assessor, FATHMM, and FATHMM MKL). CNV screening by LOG2 ratio of reads of the NGS was applied to all genes in the panel[2]. Microarray analysis (ThermoFisher Cytoscan HD) was employed to further characterize large CNV abnormalities.

Variant evaluation: Possible pathogenic variants were verified by Sanger sequencing, or multiplex ligation-dependent probe amplification (MLPA) for CNV, and traced in families where possible. Variants of unknown significance (VUS) were further assessed for previous description (HGMD[3], ClinVar[4] and the literature), frequency in gnomAD[1], ortholog and domain conservation evaluated by multisequence alignments, and evaluation for splicing using the Berkeley Drosophila Genome Project (BDGP) Splice Site Prediction by Neural Network and Genomnis Human Splice Finder (HSF) sites[5, 6].

Reporting of results was according to the ACMG guidelines (https://www.medschool.umaryland.edu/genetic_variant_interpretation_tool1.html/)[7].

References

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SUPPLEMENTAL FIGURE

Figure S1

FIGURE S1: DDN26 has an inframe duplication of *SLC34A1* (**A**) DDN26 has an inframe duplication of *SLC34A1,* c.460_480dup (p.Ile154_Val160dup). In addition, we detected a possible novel atypical splicing change of *SLC34A3*, c.561-8G>A that may result in the insertion of two amino acids, p.Glu186_Arg187insSerHis, and has been classed as a VUS.

SUPPLEMENTAL TABLES

Table S1: Genes on the 90 gene and 102 gene panels

	Gene Name	NM	Gene ID	Chr
1	ABCG2	NM_004827.2	9429	4q22
2	ACLY	NM_001303274.1	47	17q12-q21
3	ACO2	NM_001098.2	50	22q13.2
4	ADCY10	NM_018417.5	55811	1q23.3-q24
5	AGTR1	NM_031850.3	185	3q21-q25
6	AGXT	NM_000030.2	189	2q36-q37
7	AHSG	NM_001622.2	197	3q27
8	ALPL	NM_000478.4	249	1p36.12
9	AMBP	NM_001633.3	259	9q32-q33
10	APLN	NM_017413.4	8862	Xq25-q26.3
11	APLNR	NM_005161.4	187	11q12
12	APRT	NM_000485.2	353	16q24
13	AQP2	NM_000486.5	359	12q12-q13
14	ATP2B1	NM_001682.2	490	12q21.3
15	ATP6V0A4	NM_020632.2	50617	7q33-34
16	ATP6V1B1	NM_001692.3	525	2p13.1
17	ATP4B	NM_000705.3	496	13q34
18	ATP1A1	NM_001160233.1	476	1p21
19	ATP1A2	NM_000702.3	477	1q23.2
20	AVP	NM_000490.4	551	20p13
21	AVPR2	NM_000054.4	554	Xq28
22	BSND	NM_057176.2	7809	1p32.1
23	CA2	NM_000067.2	760	8q22
24	CALB1	NM_004929.3	793	8q21.3-q22.1
25	CASR	NM_001178065.1	846	3q21.1
26	CAV1	NM_001753.4	857	7q31.1
27	CLCN5	NM_001127899.3	1184	Xp11.22
28	CLCNKB	NM_000085.4	1188	1p36
29	CLDN14	NM_144492.2	23562	21q22.3
30	CLDN16	NM_006580.3	10686	3q28
31	CLDN19	NM_148960.2	149461	1p34.2
32	CYP24A1	NM_000782.4	1591	20q13
33	CYP27B1	NM_000785.3	1594	12q14.1
34	CYP2R1	NM_024514.4	120227	11p15.2
35	DGKH	NM_178009.4	160851	13q14.1
36	DOLPP1	NM_020438.4	57171	9q34.1
37	DPM2	NM_003863.3	8818	9q34.13
38	ECM2	NM_001393.3	1842	9q22.3
39	F2	NM_000506.3	2147	11p11
40	FAM188B	NM_032222.2	84182	7p14.3
41	FAM20A	NM_017565.3	54757	17q24.2
42	FGF23	NM_020638.2	8074	12p13.3
43	GLS	NM_014905.4	2744	2q32-q34
44	GLUD1	NM_005271.3	2746	10q23.3
45	GRHPR	NM_012203.1	9380	9q12
46	HNF4A	NM_000457.4	3172	20q13.12
47	HOGA1	NM_138413.3	112817	10q24.2
48	HPRT1	NM_000194.2	3251	Xq26.1
49	HSPG2	NM_001291860.1	3339	1p36.1-p34
50	IGFBP7	NM_001253835.1	3490	4q12
51	KCNJ1	NM_000220.4	3758	11q24
52	KL	NM_004795.3	9365	13q12
53	OCRL	NM_000276.3	4952	Xq25-Xq26.1
54	OGDH	NM_002541.3	4967	7p14-p13
55	OXSR1	NM_005109.2	9943	3p22.2
56	PCK1	NM_002591.3	5105	20q13.31
57	PRPS1	NM_002764.3	5631	Xq22.3
58	RHBG	NM_020407.4	57127	1q21.3
59	RHCG	NM_016321.2	51458	15q25
60	SLC12A1	NM_000338.2	6557	15q15-q21.1
61	SLC12A3	NM_000339.2	6559	16q13
62	SLC13A2	NM_001145975.1	9058	17p13.2
63	SLC17A3	NM_001098486.1	10786	6p21.3
64	SLC22A12	NM_144585.3	116085	11q13.1
65	SLC22A6	NM_004790.4	9356	11q12.3
66	SLC22A8	NM_004254.3	9376	11q11
67	SLC26A1	NM_213613.3	10861	4p16.3
68	SLC26A6	NM_022911.2	65010	3q21.3
69	SLC2A9	NM_020041.2	56606	4p16-p15.3
70	SLC34A1	NM_003052.4	6569	5q35
71	SLC34A3	NM_001177316.1	142680	9q34
72	SLC38A3	NM_006841.5	10991	3p21.3
73	SLC3A1	NM_000341.3	6519	2p16.3
74	SLC4A1	NM_000342.3	6521	17q21-q22
75	SLC4A4	NM_001098484.2	8671	4q21
76	SLC4A7	NM_003615.4	9497	3p22
77	SLC5A12	NM_178498.3	159963	11p14.2
78	SLC5A8	NM_145913.3	160728	12q23.1-q23.2
79	SLC7A9	NM_014270.4	11136	19q13.1
80	SLC7A13	NM_138817.2	157724	8q21.3
81	SLC8A1	NM_021097.2	6546	2p23-p22
82	SLC9A3	NM_004174.2	6550	5p15.3
83	SLC9A3R1	NM_004252.4	9368	17q25.1
84	SPP1	NM_001251830.1	6696	4q21-q25
85	STK39	NM_013233.2	27347	2q24.3
86	TRPV5	NM_019841.6	56302	7q35
87	UMOD	NM_001278614.1	7369	16p12.3
88	VDR	NM_001017536.1	7421	12q12-q14
89	WNK4	NM_032387.4	65266	17q21-q22
90	XDH	NM_000379.3	7498	2p23.1
91	ANO4	NM_001286615	121601	12q23.1
92	BAZ1B/WSTF	NM_032408.3	9031	7q11.23
93	CLDN3	NM_001306	1365	7q11.23
94	CLDN4	NM_001305.4	1364	7q11.23
95	HIP1	NM_005338.6	3092	7q11.23
96	LRP2	NM_004525.2	4036	2q31.1
97	OXGR1	NM_001346194	27199	13q32.1
98	PBX1	NM_002585.3	5087	1q23.3
99	SLC26A11	NM_001166347.1	284129	17q25.3
100	SLC39A10	NM_001127257.1	57181	2q32.3
101	TBL2	NM_012453	26608	7q11.23
102	TRPM6	NM_017662	140803	9q21.13

Shaded genes are only present on the 102 gene panel

Table S2: Details of novel Sanger detected PH and DD gene pathogenic variants

Family ID	Variant description	Variant type	GnomAD freq	Clin Var	Splicing evaluation		Missense evaluation			ACMG evaluation		Other allele	Phenotype
					HSF	BDGP	Pre	Ortho	Dom	Class	Evidence		
***AGXT*: PH1**													
PH1-01	c.165_172dup (p.Ala294fs21*)	F/S Dup	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	c.725dupT (p.Lys242fs)	M, On 3y, BNL, NC
PH1-02	c.166-11_-8del_insTGCATGCAGAT (p.Ile56fs)	Splice	None	None	78.3 to 78.1, new -9, 80	0.96 to 0.51, new -9, 0.47	NA	NA	NA	LP V	PM2, PM3, PP3, PP4	c.166-11_-8del_insTGCATGCAGAT (p.Ile56fs)	M, C, On 1.5y, BNL, COD/COM
PH1-03	c.191T>A (p.Ile64Asn)	Mis	None	None	NA	NA	3/3	7/7	2/9	LP V	PM2, PM5, PP3, PP4	c.577delC (p.L193fs18*)	F, On 6y, ESKD, CHF, >U/Ox, PN
PH1-04	c.224delC (p.Thr75fs44*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.358G>A (p.Gly120Arg)	F, On 2y, NC, >U/Ox
PH1-05	c.352C>T (p.Arg118Cys)	Mis	18/ 260376	1x VUS; 1x PATH	NA	NA	3/3	7/7	6/9	VUS	PM5, PP3, PP4, PP5	c.33dupC (p.Pro11fs33X)	F, Onset 17y, NL, >U/Ox, CKD IV
PH1-04	c.358G>A (p.Gly120Arg)#	Splice	1/ 222688	1x VUS	95.9 to 85.8	0.99 to 0.74	3/3	7/7	NA	LP V	PM2, PM3, PP3, PP4	c.224delC (p.Thr75fs44*)	F, On 2y, NC, >U/Ox
PH1-06												c.358G>A (p.Gly120Arg)	F, C, On 3m: EK, CKD, MBD
PH1-07	c.638C>A (p.Ala213Asp)	Mis	1/ 31388	None	NA	NA	2/3	6/7	NA	VUS	PM2, PP3, PP4	c.774G>T (p.Arg258Ser)	M: An, HTN, ESKD
PH1-08	c.766delC (p.Gln256fs16*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.766delC (p.Gln256fs16*)	M, On 4y
PH1-07	c.774G>T (p.Arg258Ser)	Mis	6/ 282566	None	NA	NA	3/3	7/7	NA	VUS	PP3, PP4	c.638C>A (p.Ala213Asp)	M: An, HTN, ESKD
PH1-09	c.824G>T (p.Ser275Ile)	Mis	1/ 251318	None	NA	NA	3/3	6/7	1/9	LP V	PM2, PM5, PP3, PP4	c.508G>A (p.Gly170Arg)	M, On 14y, >U/Ox
PH1-14	(c.846+?_c.1179+?) del (p.Gly283fs)	L del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	(c.846+?_c.1179+?)del (p.Gly283fs)	M, On 5y, BNL
PH1-10	c.847-12_-6del (p.Gly283fs)	Splice	None	None	91.5 to 41.0	0.59 to 0.14	NA	NA	NA	VUS	PM2, PP3, PP4	c.976delG (p.Ala325fs)	F, On 1y, Hm, Au, Ur, MC
PH1-11	c.869_882dup (p.Ala294fs21*)	F/S Dup	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.869_882dup (p.Ala294fs21*)	M, On 3y: NC, NL, >U/Ox
PH1-12	c.959_960delCA (p.Thr320fs9*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.846+1G>T (p.Gln282fs)	F, Onset 1y, >U/Ox, NC, HTN, L Tx,
PH1-13	c.995G>A (p.Trp332*)	Nons	1/ 31384	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	c.508G>A (p.Gly170Arg)	M, On 25y: NL, SC, Neph
***GRHPR*: PH2**													
PH2-01	c.109_118delinsCAC (p.Asp37fs6*)	F/S InDel	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	c.493+2T>A (p.Gly165fs)	M, On1y: NL, NC >U/Ox
PH2-02	c.501delC (p.Ala167fs5*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.501delC (p.Ala167fs5*)	M; CKD3, >U/Ox
PH2-03	c.783delT (p.Gly261fs8*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.783delT (p.Gly261fs8*)	F: NL, >U/Ox, ESKD, HTN

Family ID	Variant description	Variant type	GnomAD freq	ClinVar	Splicing evaluation		Missense evaluation			ACMG evaluation		Other allele	Phenotype
					HSF	BDGP	Pre	Ortho	Dom	Class	Evidence		
***HOGA1*: PH3**													
PH3-01	c.123delT (p.Pro41fs1*)	F/S Del	None	3x PATH	NA	NA	NA	NA	NA	Path Ia	PVS1, PM2, PM3, PP1, PP4	c.700+5G>T (p.Leu233ins17)	F, On 1y, BNL, >U/Ox
PH3-02	c.212G>T (p.Gly71Val)##	Mis	None	None	UC	0.95 to 0.79	3/3	7/7	8/10	LP V	PM1, PM2, PP3, PP4	c.331G>A (p.Gly111Arg)	M, On 16y, BNL, COM, CKD2, >U/Ox
PH3-03	c.234T>A (p.Asn78Lys)	Mis	7/ 251490	None	NA	NA	1/3	4/7	2/10	LP V	PM2, PM3, PP3, PP4	c.208C>T (p.Arg70*)	F, On 6y: NL, dCS, HC, >U/Ox
PH3-02	c.331G>A (p.Gly111Arg)	Mis	9/ 282700	1x LP	NA	NA	3/3	7/7	8/10	LP V	PM1, PM2, PP3, PP4	c.212G>T (p.Gly71Val)	M, On 16y, BNL, COM, CKD2, >U/Ox
PH3-04	c.385G>A (p.Gly129Arg)	Mis	4/ 250696	None	NA	NA	3/3	7/7	10/10	LP V	PM2, PM3, PP3, PP4	c.289C>T (Arg97Cys)	M, On 25y: NL, COM, SC, Neph, >U/Ox
PH3-05												c.385G>A (Gly129Arg)	F, On 2y, BNL, RD
PH3-06	c.533T>A (p.Leu178Gln)	Mis	1/ 251486	None	NA	NA	3/3	7/7	4/10	LP V	PM2, PM3, PP3, PP4	c.700+5G>T (p.Leu233ins17)	F, On 8y, NL, >U/Ox, SS, KC, CKD II
PH3-07	c.533T>C (p.Leu178Pro)#	Mis	None	1x PATH	NA	NA	3/3	7/7	4/10	LP V	PM2, PM3, PP3, PP4	c.535C>A (p.Pro179Thr)	M, On 2y, BNL, CaOx, CKD
	c.535C>A (p.Pro179Thr)#	Mis	None	1x VUS; 1x PATH	NA	NA	2/3	7/7	2/10	LP V	PM2, PM3, PP3, PP4	c.533T>C (p.Leu178Pro)	
PH3-08	c.713delG (p.Gly238fs41*)	F/S Del	1/ 250710	1x VUS	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PM3, PP4	c.700+5G>T (p.Leu233ins17)	M, On 1y, NL, >U/Ox
PH3-09	c.733G>T (p.Val245Phe)	Mis	5/ 282018	None	NA	NA	3/3	7/7	5/10	VUS	PM2, PP3, PP4, PP5	c.818T>C (p.Ile273Thr)	M, On 25y, NL, CaOx, HC, >U/Ox,
PH3-10												c.700+5G>T (p.Leu233ins17)	M, No information
PH3-09	c.818T>C (p.Ile273Thr)	Mis	6/ 246346	None	NA	NA	3/3	7/7	1/10	VUS	PM2, PP3, PP4	c.733G>T (p.Val245Phe)	M, On 25y, NL, CaOx, HC, >U/Ox,
PH3-11	c.943_954dup (p.Glu315_ Arg318dup)	I/F Dup	8/ 251186	1x VUS	NA	NA	NA	NA	NA	LP IV	PM2, PM3, PM4, PP3, PP4	c.943_954dup (p.Glu315_ Arg318dup)	F, On 50y, BNL, >U/Ox HC, CKD3
***CLCN5* :DD1**													
D1-01	c.105+4delA (p.Glu35*)	Splice	None	None	95.9 to 75.5	1.00 to 0.42	NA	NA	NA	LP V	PM2, PP1-M, PP3, PP4	N	M, On 4y, HC, Pr, NC
D1-02	c.122_123delAA (p.Lys41fs11*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	N	M, On 3y, HC Pr, NC
D1-03	c.314_315delCT (p.Ser105fs1*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	N	M, On 7y, Pr, NL, HC
D1-04	c.356G>A (p.Trp119*)	Nons	None	None	NA	NA	NA	NA	NA	Path Ia	PVS1, PP1-S, PM2, PP4	N	M, KTx 35y, Pr, NC, NL
D1-05	c.678C>G (p.Cys226Trp)	Mis	None	None	NA	NA	3/3	6/6	1/7	LP V	PM2, PP1-M, PP3, PP4	N	M, On 17y, Pr, FSGS, HC, VitD def, NL
D1-06	c.1078delG (p.Val360fs3*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ia	PVS1, PP1-S, PM2, PP4	N	M, On 14y, Pr
D1-07	c.1182dupT (p.Leu394fs7*)	F/S Dup	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PP1-M, PP4	N	M, On 3yr, Pr, NC

Family ID	Variant description	Variant type	GnomAD freq	ClinVar	Splicing evaluation		Missense evaluation			ACMG evaluation		Other allele	Phenotype
					HSF	BDGP	Pre	Ortho	Dom	Class	Evidence		
D1-08	c.1252_1255del (p.Pro419Leufs14*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ia	PVS1, PP1-S, PM2, PP4	N	M, On 20y, Pr, HC, NL, KC, NC
D1-09	c.1391_1392dup (p.Ile464fs5*)	F/S Ins	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	N	M, On 16y, Pr, NL, NC
D1-10	c.1574T>G (p.Met525Arg)	Mis	None	None	NA	NA	3/3	6/6	5/10	LP V	PM1, PM2, PP3, PP4	N	M, On 2y, Pr, NC
D1-11	c.1592G>A (p.Gly531Asp)	Mis	None	None	NA	NA	3/3	6/6	5/10	VUS	PM2, PP2, PP3, PP4	N	M, On 9y, Pr, FSGS, NC
D1-12	c.2240A>G (p.Ter747Wext*46)	Non Stop	1/ 182596	None	NA	NA	NA	NA	NA	LP IV	PM2, PM4, PP1-M, PP4	N	M, On 7y, Pr
D1-13	c.(1-?)_(205+1_ 206-1)del (p.Met1fs)	L Del	None	None	NA	NA	NA	NA	NA	Path Ic	PVS1, PM2, PP4	N	M, On 8y, Pr, HC, CKD3
***OCRL*: DD2**													
D2-01	c.1156A>G (p.Arg386Gly)	Mis	None	None	NA	NA	3/3	7/7	10/10	Path IIIb	PP1-S, PM1, PM2, PP3, PP4	N	M, On 4y, Pr, FSGS, GS
D2-02	c.1573A>C (p.Lys525Gln)	Mis	None	1x PATH	NA	NA	6/6	7/7	10/10	Path IIIb	PP1-S, PM1, PM2, PP3, PP4	N	M, On 15y, Pr, RC, CDE, GHD
D2-03	c.1865delG (p.Gly622fs21*)	F/S Del	None	None	NA	NA	NA	NA	NA	Path Ib	PVS1, PM2, PP1-M, PP4	N	M, On 1y, NL, CC, Hyp
D2-04	c.2078C>T (p.Pro693Leu)	Mis	None	1x PATH	NA	NA	3/3	7/7	NA	LP V	PP1-M, PM2, PP3, PP4	N	M, On 8y, Pr

NA = not applicable. **Variant Description**: #, c.358G>A (p.G120R) occurs at the last coding base of exon 2 and is predicted to weaken the donor site. ##c.212G>T (p.G71V) occurs at the first coding base of exon 2 and is predicted to weaken the acceptor site. **Variant Type**: L Del, large deletion; Mis, missense, Nons = nonsense; I/F Del, inframe deletion; F/S Del, frameshifting deletion; F/S Dup, frameshifting duplication; F/S InDel, , frameshifting deletion/insertion; Non Stop, stop codon variant. **GnomAD frequency**: frequency in the gnomAD database of "normal individuals". **ClinVar:** times described; PATH, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance. **Splicing evaluation: HSF** = Human Splice Finder, UC, unchanged; **BDGP** = Berkley Drosophila Gene Project, for both normal and variant score shown, and where appropriate N = score of novel site generated; **Missense evaluation**: **Pre** = fraction of predicted damaging pathogenicity scores from: SIFT, PolyPhen-2, Align GVGD; **Ortho** = fraction matching the human sequence in a multisequence alignment (MSA) of orthologs from mammals to fish; **Dom** = fraction matching the human sequence in MSA of conserved domains, NCBI database. **ACMG evaluation**: **Class** = pathogenic classification based on the American College of Medical Genetics (ACMG) guidelines for interpretation of sequence variants: Path = pathogenic, LP = likely pathogenic, VUS = variant of uncertain significance, with subclasses shown; **Evidence** = ACMG evidence supporting the interpretation of sequence variant classification. The evidence is classed as: PVS1 = pathogenic very strong; PS, pathogenic strong, PM, pathogenic moderate; PP, pathogenic supportive (see [18] for details). **Phenotype:** An, anemia; Au, anuria; B, bilateral; C, Consanguineous; CHF, congestive heart failure; COD/COM, calcium oxalate dihydrate/calcium oxalate monohydrate stones; CaOX, calcium oxalate stones; CDE, calcium deposits in eye; CC, congenital cataracts; dCS, duplex collecting system; EK, echogenic kidneys; ESKD, end stage kidney disease; F, female; FSGS, focal segmental glomerular sclerosis; GHD, growth hormone deficiency, Hm, Hematuria; HTN, hypertension; Hyp, hypotonia; KC, kidney cysts; KTx, kidney transplant, LTx, liver transplant; M, male; MBD, mineral bone disease; MC, macular crystals; Neph, nephrectomy; NC, nephrocalcinosis; NL, nephrolithiasis; On, age at disease onset; PN, peripheral neuropathy; Pr, proteinuria; RD, renal dysplasia, SC, staghorn calculus; SS, struvite stones; >U/Ox, high urinary oxalate; Ur, uremia; VitD def, VitD deficiency.

Table S3: Genes with transcript and protein accession numbers

Gene	NM#	NP#
AGXT	NM_000030.3	NP_000021.1
ALPL	NM_000478.6	NP_000469.3
APRT	NM_000485.3	NP_000476.1
ATP6V0A4	NM_020632.3	NP_065683.2
ATP6V1B1	NM_001692.4	NP_001683.2
BSND	NM_057176.3	NP_476517.1
CASR	NM_000388.4	NP_000379.3
CLCNKB	NM_000085.5	NP_000076.2
CLDN16	NM_006580.4	NP_006571.2
CLDN19	NM_148960.3	NP_683763.2
CYP24A1	NM_000782.5	NP_000773.2
CYP27B1	NM_000785.4	NP_000776.1
GRHPR	NM_012203.2	NP_036335.1
HNF4A	NM_175914.4	NP_787110.2
KCNJ1	NM_000220.6	NP_000211.1
SLC12A1	NM_000338.3	NP_000329.2
SLC12A3	NM_000339.3	NP_000330.3
SLC22A12	NM_144585.4	NP_653186.2
SLC26A1	NM_213613.4	NP_998778.1
SLC34A1	NM_003052.5	NP_003043.3
SLC34A3	NM_080877.2	NP_543153.1
SLC3A1	NM_000341.4	NP_000332.2
SLC4A1	NM_000342.4	NP_000333.1
SLC7A9	NM_014270.5	NP_055085.1
SLC9A3R1	NM_004252.5	NP_004243.1
WNK4	NM_032387.5	NP_115763.2