RESEARCH PAPER

Phenotype of Dent Disease in a Cohort of Indian Children

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Objective: To describe the clinical and genotypic features of Dent disease in children diagnosed at our center over a period of 10 years.

Design: Case series.

Setting: Pediatric Nephrology Clinic at a referral center in Northern India.

Methods: The medical records of patients with Dent disease diagnosed and followed up at this hospital from June 2005 to April 2015 were reviewed. The diagnosis of Dent disease was based on presence of all three of the following: *(i)* low molecular weight proteinuria, *(ii)* hypercalciuria and *(iii)* one of the following: nephrolithiasis, hematuria, hypophosphatemia or renal insufficiency, with or without mutation in *CLCN5* or *OCRL1* genes.

Results: The phenotype in 18 patients diagnosed with Dent disease during this period was characterized by early age at onset (median 1.8 y), and polyuria, polydipsia, salt craving, hypophosphatemic rickets and night blindness. Rickets was associated with severe deformities, fractures or loss of ambulation in six patients. Nephrocalcinosis was present in three patients, while none had nephrolithiasis. Generalized aminoaciduria was seen in 13 patients, two had glucosuria alone, and one had features of Fanconi syndrome. Over a median follow up of 2.7 years, one patient developed renal failure. Genetic testing (*n*=15) revealed 5 missense mutations and 3 nonsense mutations in *CLCN5* in 13 patients. Five of these variations (p.Met504Lys, p.Trp58Cys, p.Leu729X, p.Glu527Gln and p.Gly57Arg) have not been reported outside the Indian subcontinent.

Conclusion: Our findings suggest a severe phenotype in a cohort of Indian patients with Dent disease.

Keywords: *CLCN5, Hypophosphatemic rickets, Night blindness, Polyuria.*

Dent disease is an X-linked disorder of proximal tubular function characterized by low molecular weight proteinuria, the most consistent feature, as well as hypercalciuria, nephrocalcinosis, nephrolithiasis and progressive renal failure [1,2]. The condition presents in boys during early childhood with symptoms of renal stones (pain abdomen, hematuria), bone pains or deformities due to rickets or with incidentally detected low molecular weight proteinuria. Progression to end stage renal disease (ESRD) occurs between 3rd to 5th decades in 30-80% of affected males.

The disease is caused in 60% patients by inactivating mutations in the *CLCN5* gene, located on Xp11.22 encoding a 746 amino acid Cl^-/H^+ exchanger (Dent disease 1) [4]; 15% of patients show mutations in the *OCRL1* gene (Dent disease 2) on chromosome Xq25, which encodes phosphatidylinositol 4,5-biphosphate 5-phosphatase [5,6]. There is genetic heterogeneity and no genotype phenotype correlations are established [6-8]. A previous report on three patients with Dent disease from this center emphasized the early onset of symptoms and occurrence of night blindness, presumably secondary to urinary wasting of retinol binding protein (RBP) [9]. In this study, we now report our experience on diagnosis and management of a cohort of 18 patients with the condition, including follow-up of those reported previously.

METHODS

The medical records of patients with Dent disease diagnosed and followed up at this hospital from June 2005 to April 2015 were reviewed. Three patients diagnosed before this period were also included. The diagnosis of Dent disease was based on presence of all three of the following: *(i)* low molecular weight proteinuria, defined as increased excretion of β_2 microglobulin >1500 µg/L (normal <300 µg/L), *(ii)* hypercalciuria (urinary calcium excretion >4 mg/kg/day or calcium creatinine ratio, U_{Ca}/U_{Cr} >0.2 mg/mg), and *(iii)* one of the following: nephrolithiasis, hematuria, hypophosphatemia or renal insufficiency, with or without mutation in *CLCN5* or *OCRL1* genes. Patients with other causes of proximal tubular dysfunction (proximal renal tubular acidosis), hypercalciuria (distal renal tubular acidosis, idiopathic hypercalciuria), refractory rickets

(familial hypophosphatemic rickets, vitamin D dependence) and nephrolithiasis were excluded.

Clinical and biochemical details were recorded at diagnosis and at follow up. Standard deviation score (SDS) for weight and height were calculated using World Health Organization charts and AnthroPlus v.1.0.4 calculator (www.who.int/growthref/tools/en). Blood levels of calcium, phosphate, alkaline phosphatase, electrolytes, creatinine, pH, bicarbonate, 25-hydroxyvitamin D and parathormone were measured and compared to age-appropriate cut-offs. Timed urinary excretion of phosphate and creatinine was used to estimate tubular maximum for phosphate reabsorption/glomerular filtration rate (TmP/GFR); estimated glomercular filteration rate (eGFR) by the modified Schwartz formula was used to classify stages of chronic kidney disease (CKD) [10]. Height SDS, blood creatinine, eGFR and calcium excretion were compared at presentation and follow-up by Wilcoxon signed rank test (SPSS version 15.0, SPSS Inc., Chicago).

Genomic DNA, isolated from peripheral blood leukocytes, was amplified by polymerase chain reaction (PCR) using primers for all exons of the *CLCN5* gene [11], followed by Sanger sequencing. In case the *CLCN5* sequence was negative for variations, the *OCRL1* gene was screened. Pathogenecity prediction software, sorting intolerant from tolerant (SIFT, *http://sift.jcvi.org/*) and PolyPhen2 (*http://genetics.bwh.harvard.edu/pph2/*) were used to predict the effect of novel exonic variations.

RESULTS

Clinical and biochemical data on 18 patients with Dent disease are summarized in ***Table* I**. Detailed data are shown in ***Web Table* I**. All patients except two pairs (maternal uncle and nephew Pt. 6, 7; brothers Pt.11, 12) were unrelated. One patient had a history of similar illness in maternal uncle who had end stage renal failure and renal transplantation at 38-years of age. The median age at onset of symptoms was 1.8 years and that at diagnosis was 8 years. All patients had short stature with median height SDS at presentation of -3.6. The presenting features included bony deformities due to rickets and complaints of polyuria, polydipsia and craving for salty foods. One or more episodes of night blindness, manifested on multiple occasions at variable periods after onset of symptoms in 12 patients (66.7%), were responsive to therapeutic doses of vitamin A. One patient presented with persistent nephrotic range proteinuria without edema; Dent disease was suspected based on the presence of rickets, low molecular weight proteinuria and hypercalciuria. At presentation, the median eGFR was 72 mL/min/1.73 m^2; 14 (77.8%) and 5 (27.8%) patients had eGFR <90 mL/min/1.73 m^2 and <60 mL/min/1.73 m^2, respectively. Medullary nephrocalcinosis was present in two patients at diagnosis and in one on follow up. None of the patients had nephrolithiasis.

All patients had radiological evidence of rickets with normal blood levels of calcium and alkaline phosphatase. Hypophosphatemia was seen in 17 (94.4%) patients with median blood level of phosphate 2.6 mg/dL; and TmP/GFR below 4 mg/dL in all. Serum 25-hydroxyvitamin D level was low (<30 ng/mL) in 5 of 12 patients tested. Ten patients showed normal levels of parathormone and two each with renal dysfunction and vitamin D deficiency had elevated levels. Many of the patients had received therapeutic dose of vitamin D before presenting to our center. We administered vitamin D only if the patient showed low levels of 25-OD vitamin D. Rickets was refractory to therapeutic doses of vitamin D in all patients. Additional abnormalities of proximal tubular function were generalized aminoaciduria in 13 (72.2%), glucosuria in 2 and normal anion gap metabolic acidosis in 2 patients including one with features of Fanconi syndrome. All patients received therapy with citrate and

TABLE I SUMMARY OF CLINICAL AND BIOCHEMICAL FEATURES OF PATIENTS WITH DENT DISEASE

Parameter	*Value*
Age at onset (y)	1.8 (0.3,8)
Age at diagnosis (y)	8.0 (1.5,14)
Height SDS at presentation	–3.6 (-8.4,-1.9)
Time from onset to diagnosis (y)	4.0 (1,13)
Polyuria, polydipsia, *n* (%)	16 (88.9)
Salt preference, *n* (%)	9 (50.0)
Rickets, *n* (%)	18 (100)
Night blindness, *n* (%)	12 (66.7)
Serum creatinine (mg/dL)	0.6 (0.3,1.3)
eGFR at presentation (mL)	72 (28,126) mL/min/1.73 m^2
Hypokalemia, *n* (%)	13 (72.2)
Serum phosphate (mg/dL)	2.6 (2.4,3) dL
TmP/GFR (mg/dL)	1.7 (1.1,3.6)
24-hr urine protein (mg)	1150 (520,3400)
24-hr urine calcium (mg/kg)	8.1 (3, 20)
Aminoaciduria, *n* (%)	13 (72.2)
Follow up duration (y)	2.7 (0.3,20.6) y
Height SDS	–4.3 (-8.4, -1.5)
eGFR (mL/min/1.73 m^2)	66 (28, 120)
24-hr urine calcium (mg/kg)	7 (2, 17.6)

Values as median (range) unless specified otherwise.

phosphate supplements; 3 also received hydrochlorothiazide for treatment of hypercalciuria for a brief duration. Hypokalemia prompted discontinuation of the same.

Follow-up data were available for all, except one (***Web Table* I**). At a median (range) follow-up of 2.7 (0.3-20.6) years, the median (range) height SDS was -4.3 (-8.4 to -1.5), similar to that at diagnosis (*P*=0.81). Radiological healing of rickets occurred in 9 of 12 patients with more than 6 months follow-up. Bony deformities persisted and three patients underwent corrective osteotomy. The median (range) eGFR at follow up, 66 (28-120) mL/min/1.73 m^2, was also similar to that at diagnosis (*P*=0.80). Seven patients, including one with nephrocalcinosis showed decline in renal function; 4 showed >25% decline in eGFR. An additional patient had CKD stage IV at 9.5 years of age. Kidney biopsy in his sibling showed global sclerosis in most glomeruli and significant interstitial fibrosis and tubular atrophy. Hypercalciuria persisted at follow-up (median 7.0; range 2-17.6 mg/kg/day), without change from baseline (*P*=0.78).

Genetic analysis: Sequence analysis of the *CLCN5* gene in 15 patients revealed eight mutations in exons 3, 7, 9, 10, 11 and 12 in 13 patients (***Web Table* II, *Fig*. 1**) [12-15]. Five were missense mutations (p.Ser244Leu, p.Met504Lys, p.Trp58Cys, p.Glu527Gln and p.Gly57Arg), and three were nonsense mutations (p.Leu729X, p.Arg648X and p.Arg637X). Two patients did not show mutations in the *CLCN5* or *OCRL1* genes. Both the novel missense mutations (p.Glu527Gln and p.Gly57Arg) were predicted to affect protein function by either SIFT or PolyPhen2 computer programs. All three nonsense mutations including one novel mutation (p.Leu729X) were expected to be pathogenic.

DISCUSSION

The present report describes a severe phenotype of Dent disease. Most patients showed pathogenic variants involving the *CLCN5* gene. The median age at diagnosis was similar to a cohort of 117 European patients and pooled data from 377 patients [16]. However, patients in this report showed relatively early onset of symptoms compared to other series (***Table* II**) [5,16-18]. While rickets is reported in infants with Dent disease, early onset with polyuria, polydipsia and night blindness is not described [1,12-19]. We also report the occurrence of renal failure in the first decade of life in patients with a common mutation in *CLCN5*, p.Ser244Leu.

Most series report rickets in up to one-third patients with Dent disease 1 [5,16-18]. Wrong, *et al*. [2] reported rickets in 40% patients and hypophosphatemia in one-third patients, with satisfactory response to vitamin D therapy. All our patients showed refractory rickets with

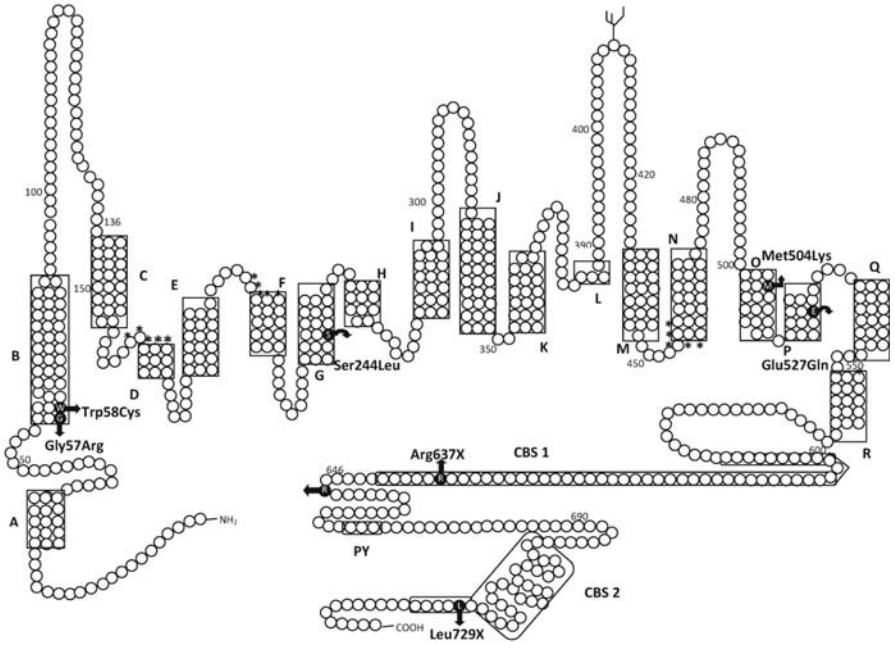

FIG. I *Schematic representation of ClC-5 mutations in 13 patients with Dent disease. The predicted topology of the protein is drawn from Wu, et al. [14] and Dutzler, et al. [15]. ClC-5 consists of 18 á helices, A to R, which are indicated by the boxed areas. Mutations in ClC-5 are shown as solid black circles with the resulting amino acid change indicated besides.*

TABLE II PHENOTYPIC FINDINGS IN PATIENTS WITH DENT DISEASE 1 (*CLCN5* MUTATIONS) IN THE PRESENT AND PREVIOUS REPORTS

Features	*Hoopes [5]*, n=19*	*Sekine [17], n=61*	*Mansour-Hendili [16], n=117*	*Anglani [18], n=47*	*Present study, n=13*
Median age at diagnosis (y)	10	-	7	-	8
Low molecular weight proteinuria, %	100[#]	100[#]	99.0 (*n*=111)	97.9	100[#]
Hypercalciuria, %	100[#]	46.0 (*n*=54)	88.0 (*n*=99)	91.5[#]	100[#]
Nephrocalcinosis, %	89.5 (*n*=19)	38.0 (*n*=53)	61.0 (*n*=102)	83.0	23.1
Nephrolithiasis, %	29.4 (*n*=17)	-	33.0 (*n*=91)	29.8	0
Renal insufficiency[^], %	26.3 (*n*=19)	8.0 (*n*=53)	47.0 (*n*=110)	10.6	38.4
Rickets, %	38.4 (*n*=13)	0 (*n*=61)	15.0 (*n*=93)	36.2	100
Hypophosphatemia, %	50.0 (*n*=18)	-	54.0 (*n*=76)	36.2	92.3
Aminoaciduria, %	75.0 (*n*=8)	-	61.0 (*n*=39)	-	76.9
Hypokalemia, %	35.3 (*n*=17)	-	39.0 (*n*=86)	-	76.9
Metabolic acidosis, %	-	-	13.0 (*n*=68)	-	7.7
Glucosuria, %	38.9 (*n*=18)	-	40.0 (*n*=70)	-	15.4
Concentrating defect, %	-	-	72.1 (*n*=43)	-	84.6

**Data pooled for patients with or without mutations in CLCN5 gene; [#]Considered an essential criterion for diagnosis; [^]Definition varies across reports; renal insufficiency was eGFR<60 mL/min/1.73 m^2 (CKD stage III) in the present study; 1 patient had eGFR <30 mL/min/1.73 m^2 (CKD stage IV).*

hypophosphatemia in 94.4 % cases. Similar to four of our patients, all nine affected boys from two European families with the *CLCN5* mutation p.Ser244Leu, in whom the mutation was first described, showed rickets [20]. However, none of the patients with this mutation from a large pedigree in southern United States had features of rickets [21]. It is unclear whether these phenotypic variations reflect differences in severity of coexistent vitamin D deficiency, dietary and environmental factors, delayed diagnosis, or effect of modifier genes.

Polyuria, an important symptom in the our patients, is reported in Dent disease [2]. While formal water deprivation testing was not performed, a defect in urinary concentration is likely, similar to other inherited renal tubular disorders with secondary nephrogenic diabetes insipidus [22]. This may be mediated by downregulation of expression of aquaporin 2 *via* the calcium sensing receptor in apical membrane of medullary collecting duct [22,23]. The high incidence of polyuria in these patients might explain the low prevalence of nephrolithiasis, the former serving as a physiological mechanism preventing stone formation. Moreover, it is also reported that the degree of hypercalciuria may not relate to development of nephrocalcinosis or renal failure [24]. Despite high rates of hypercalciuria and nephrocalcinosis (97.6% and 87.8%, respectively), only 12% of 41 patients of Dent disease showed renal failure [18]. Additional genetic or environmental factors may contribute to the occurrence of nephrocalcinosis/nephrolithiasis and consequent renal dysfunction.

Vitamin A-responsive night blindness, first reported in Dent disease in patients from our center [9], was observed in two-thirds of the present patients, compared to 37.5% in another report [25]. The condition is attributed to high urinary losses of RBP [3,25] and reduced blood levels of retinol and RBP [25], but information on dietary intakes and blood retinol levels is lacking. While insufficient vitamin A intake might predispose patients with Dent disease to clinically overt vitamin A deficiency, none of the unaffected family members developed night blindness.

Previous studies have not attempted grading of CKD, precluding comparisons in different cohorts. Of the 18 patients, 14 (77.8%) showed eGFR<90 mL/min/1.73 m^2 at presentation and 7 showed decline in eGFR on follow up. One patient (Pt. 11) had CKD stage IV at the age of 9.5 years; his younger sibling (Pt. 12) showed glomerular and tubulointerstitial scarring on renal biopsy, emphasizing the risk of progression into renal failure even in patients without nephrocalcinosis. This family with 3 affected boys with renal failure within first decade of life represents a severe phenotype. While genetic testing showed a commonly described mutation, p.Ser244Leu, the occurrence of renal failure in first decade with this mutation is not reported.

We identified 5 missense and 3 nonsense mutations in 13 patients. Five of these 8 mutations (p.Met504Lys, p.Trp58Cys, p.Leu729X, p.Glu527Gln and p.Gly57Arg) have not been reported outside the Indian subcontinent, the

WHAT IS ALREADY KNOWN?

- The phenotype of Dent disease differs across different regions of the world and there are no genotype phenotype correlations.
- Progression to renal failure may occur in third to fifth decades of life.

WHAT THIS STUDY ADDS?

- Dent disease has an early onset with severe symptoms in this cohort of Indian children.
- Renal failure may occur in the first decade of life with the most commonly described *CLCN5* mutation (Ser244Leu).

small numbers preclude any genotype-phenotype correlation. Of the 192 mutations of *CLCN5* that are reported, approximately 17%, 36.5% and 28% are nonsense, missense and frameshift mutations, respectively [16]. The missense mutations p.Glu527Gln and p.Ser244Leu alter ClC-5 α-helices P and G respectively, which interfere with dimer interface formation [14,15]. The mutation p.Met504Lys is expected to disrupt the function of helix O, and p.Trp58Cys and p.Gly57Arg that of helix B, thereby reducing chloride channel function; functional characterization has not been carried out for these mutations.

The study is limited by small sample size, retrospective design, and lack of genetic testing in asymptomatic family members. However, there are important phenotypic differences from previously reported cohorts, including low prevalence of nephrocalcinosis and occurrence of CKD within first decade of life. Apart from the usual features, Dent disease in this cohort of Indian boys has a relatively severe phenotype with early onset of symptoms, hypophosphatemic rickets and night blindness.

Contributions: SB, RT, AS, PH, AB: diagnosis and management of patients; SB, RT: data collection; AS, PH, AB: analytical inputs and review of literature; HC: performed the sequencing of the genes. All authors participated in preparation of the manuscript and approved the final version submitted for publication.

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WEB TABLE I CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF PATIENTS WITH DENT DISEASE

	Pt. 1	Pt. 2#	Pt. 3	Pt. 4	Pt. 5	Pt. 6*	Pt. 7*	Pt. 8	Pt. 9	Pt. 10	Pt. 11	Pt. 12	Pt. 13	Pt. 14	Pt. 15	Pt. 16	Pt. 17	Pt.18
Age at onset, yr	8	0.3	2	4	1	2	2	6.5	1	4	1	1	0.8	2	0.3	1.5	0.5	6
Age at diagnosis, yr	14	5.3	4	8	5	4	4.5	9.5	14	12	10	8	10.8	12	1.8	4	1.5	8
Height SDS	-2.8	-3.8	-3.8	-3.6	-2.6	-3.5	-6.7	-2.8	-1.9	-5.3	-8.4	-7.6	-8.4	-4.8	-3.0	-2.3	-3.2	-3.24
Onset to diagnosis, yr	6	5	2	4	4	2	2.5	3	13	8	9	7	10	10	1.5	2.5	1	2
Polyuria, polydipsia	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Salt preference	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-
Rickets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Night blindness	+	+	+	+	-	+	+	-	+	+	+	+	-	+	-	-	-	+
Creatinine, mg/dL	0.8	0.6	0.4	0.6	0.74	0.5	0.4	1.06	0.8	0.6	1.3	0.5	0.3	0.9	0.8	0.4	0.4	0.5
eGFR, ml/min/1.73 m^2	76	64	78	62	61	100	87	49	75	66	28	69	126	55	49	98	82	76
Potassium, mEq/L	2.8	3.1	3.9	3.1	3.3	3.2	2.8	2.8	3.7	2.5	3.2	3.1	4.3	3.3	3.5	4.0	3.7	3.3
Calcium, mg/dL	8.7	9.9	9.0	10	10.8	8.6	9.7	9.3	9.4	9.3	8.8	7.8	8.8	8.3	8.9	9.4	8.9	9.7
Phosphate, mg/dL	3.0	2.9	2.0	2.9	2.6	2.8	2.6	4.3	2.4	2.2	2.3	2.7	2.5	2.9	2.8	2.6	2.5	2.5
TmP/GFR, mg/dL	1.1	2.5	1.9	1.9	2.2	1.5	1.7	3.6	2.6	1.3	1.2	1.1	1.5	2.5	NA	2.1	1.5	1.4
Alkaline phosphatase, IU/L	1509	1747	3516	1060	1764	2023	1525	926	3841	2278	3465	5317	1893	2257	1757	1041	537	1897
pH	7.42	7.37	7.43	7.40	7.39	7.38	7.43	7.54	7.42	7.40	7.26	7.36	7.37	7.34	7.31	7.39	7.32	7.33
Bicarbonate, mEq/L	25.6	22.7	20.6	24	21.3	22	21.6	20	22.2	21	15	20	22	21	19.8	19.3	15.4	20.8
25 hydroxyvitamin D, ng/mL	42.8	12	NA	NA	53.5	NA	7	37	35.9	23	42	35.8	NA	NA	24.3	123	19.6	NA
PTH, pg/mL	63.4	24.2	68	72	50.2	NA	29.3	16.6	23.3	15	422	116	NA	NA	373	53.2	170	NA
24-hr urine protein, mg	3400	560	1400	560	1100	2000	1200	1600	1860	1100	640	554	860	840	1230	520	1340	1870
24-hr calcium, mg/kg	3.8	16.6	10.2	20	8	8.2	13	10	9.4	5.3	7	6.8	8.8	5	3.9	13.5	4.6	4
â2-microglobulin, µg/L	57156	NA	53000	32340	88000	68000	68900	99199	72300	39948	78000	89900	64000	129967	19463	28700	38889	40000
Aminoaciduria	+	+	-	-	+	+	+	+	+	-	+	+	+	+	NA	+	+	NA
Follow up duration, yr	2.7	5.8	10	NA	1.8	19	2	1.2	5.4	0.3	0.4	0.4	0.5	1.3	8	4.8	20.6	6.25
Height SDS at follow up	-2.2	-4.2	-3.2	NA	-1.9	-6.2	-5.34	-4.3	-1.5	-5.3	-8.4	-7.6	-8.4	-4.6	-3.9	-1.9	-3.0	-4.64
eGFR follow up, m/min/1.73 m^2	58	96	80	NA	67	52	120	56	78	66	28	57	95	50	36	101	66	107
delta eGFR, ml/min/1.73 m^2/yr	6.7	-5.5	-0.2	NA	-3.3	2.5	-19	-5.8	0.56	-	-	-	-	3.84	1.62	-0.62	0.78	-5.0
24-hr calcium, mg/kg	4.6	17.6	10.6	NA	7.37	9.0	4.7	7.43	10	-	6.8	6.6	9.0	5.5	3.78	7.2	5.0	2

**Pt. 6 and 7 had nephrocalcinosis at initial diagnosis; #Pt. 2 developed nephrocalcinosis on follow up at 6-yr of age; ^Delta eGFR computed for patients with follow up >1 yr; SDS standard deviation score; eGFR estimated glomerular filtration rate; TmP/GFR tubular maximum for phosphate/GFR; PTH parathormone; NA not available.*

WEB TABLE II RESULTS OF GENETIC TESTING FOR *CLCN5* GENE IN 15 OF 18 PATIENTS. *OCRL1* GENE WAS SEQUENCED IF NO MUTATION WAS FOUND IN *CLCN5*

	*Mutation**	*Location*	*Type of mutation*	*Amino acid change**	*Location of mutation^*	*SIFT[@] score*	*PolyPhen-2[##] (sensitivity; specificity)*
Pt. 1	c.731C>T	Exon 7	Missense	p.Ser244Leu[#]	Helix G	Damaging, 0	Probably damaging; score 0.999 (0.14; 0.99)
Pt. 2	c.1511T>A	Exon 9	Missense	p.Met504Lys[$]	Helix O	Damaging, 0	Probably damaging; score 0.992 (0.70; 0.97)
Pt. 3	c.2186T>G	Exon 12	Nonsense	p.Leu729X[$]	CBS-2 domain	-	-
Pt. 4	c.1511T>A	Exon 9	Missense	p.Met504Lys[$]	Helix O	Damaging, 0	Probably damaging; score 0.992 (0.70; 0.97)
Pt. 5	c.731C>T	Exon 7	Missense	p.Ser244Leu[#]	Helix G	Damaging, 0	Probably damaging; score 0.999 (0.14; 0.99)
Pt. 6	c.174G>C	Exon 3	Missense	p.Trp58Cys[$]	Helix B	Damaging, 0	Probably damaging; score 1.000 (0.00; 1.00)
Pt. 7	c.174G>C	Exon 3	Missense	p.Trp58Cys[$]	Helix B	Damaging, 0	Probably damaging; score 1.000 (0.00; 1.00)
Pt. 8	c.1579G>C	Exon 10	Missense	p.Glu527Gln	Helix P	Damaging, 0	Probably damaging; score 1.000 (0.00; 1.00)
Pt. 9	c.1942C>T	Exon 11	Nonsense	p.Arg648X[#]	Between CBS domains	-	-
Pt. 10	c.169G>C	Exon 3	Missense	p.Gly57Arg	Helix B	Tolerated, 0.08	Probably damaging; score 0.999 (0.14; 0.99)
Pt. 11	c.731C>T	Exon 7	Missense	p.Ser244Leu[#]	Helix G	Damaging, 0	Probably damaging; score 0.999 (0.14; 0.99)
Pt. 12	c.731C>T	Exon 7	Missense	p.Ser244Leu[#]	Helix G	Damaging, 0	Probably damaging; score 0.999 (0.14; 0.99)
Pt. 13	c.1909C>T	Exon 10	Nonsense	p.Arg637X[‡]	CBS-1 domain	-	-
Pt. 14, 15	No mutations identified in *CLCN5* or *OCRL1* genes						

CBS cystathionine beta synthase. ***Numbering according to cDNA sequence (GenBank NM_001282163.1) & protein sequence (GenBank NP_001269092.1) with A of first coding methionine no. 1; [#]Reported by Lloyd [12]; [$]Sethi [9]; [‡]Takemura [13]; ^Predicted topology adopted from Wu [14]; [@]Sorting intolerant from tolerant (SIFT) predicts substitutions with a score <0.05 as damaging and others as tolerated (http://sift.jcvi.org/); [##]PolyPhen-2 score represents the probability that a substitution is damaging; values near 1 are predicted to be deleterious (http://genetics.bwh.harvard.edu/pph2).*