Genetic evaluation of paediatric nephrocalcinosis: phenotype-driven genetic panels reveal a rare diagnosis

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ABSTRACT

Monogenic causes of paediatric nephrocalcinosis are associated with extensive phenotypic variability. We report a 14-year-old male who presented at 8 years of age with incidentally identified nephrocalcinosis alongside growth impairment and dental anomalies. Extensive genetic investigation confirmed a molecular diagnosis of Bartter syndrome type II. This is exceptional in both late presentation and the presence of amelogenesis imperfecta, a very rare association of inherited tubulopathies. Details of the nephrocalcinosis gene panel analysed and associated phenotypes are presented to highlight the utility of a phenotype-driven genetic panel in resolving an atypical presentation of nephrocalcinosis, allowing precise diagnosis, tailored therapy and prognostication.

Keywords: amelogenesis imperfecta, Bartter syndrome, KCNJ1, tubulopathy

BACKGROUND

The overall incidence of paediatric nephrocalcinosis/nephrolithiasis is uncertain, and likely underestimated in reports due to asymptomatic cases [1]. Preterm infants are at greater risk due to renal tubular immaturity, and use of medications and nutritional supplements that promote calcium salt deposition.

Monogenic causes, including tubulopathies, must be considered in the investigation of nephrocalcinosis. Correct and prompt diagnosis gives the opportunity for earlier intervention to delay progression of renal dysfunction or development of nephrolithiasis [2].

CASE REPORT

An 8-year-old male was referred to the nephrology clinic with bilateral nephrocalcinosis, identified during investigation of recurrent urinary tract infections. He had significant thirst and marked nocturnal enuresis despite previously achieving daytime continence. Height and weight were <0.4th centile. He had carious, irregular, hypomineralized dentition; all other ectodermal structures were normal with no family history of renal, skeletal or dental conditions.

Investigations revealed mild hypokalaemia (3.4 mmol/L) but normal serum bicarbonate (25 mmol/L), creatinine, calcium, phosphate, magnesium, alkaline phosphatase and parathyroid...
Table 1. Gene panel—20 genes associated with nephrolithiasis and nephrocalcinosis

| Disorder incidence | Phenotype | Associated gene | Inheritance pattern | Mutational spectrum | Benefit of genetic diagnosis and clinical significance |
|--------------------|-----------|----------------|---------------------|--------------------|------------------------------------------------------|
| 1,25(OH)D-24 hydroxylase deficiency < 1 100 000 | Early onset hypercalcaemia, hypophosphataemia, hypercalciuria, decreased intact PTH, medullary nephrocalcinosis | CYP24A1 | AR | Predominantly missense Deletions reported | Variable presentation; rarely in adulthood. Typically faltering growth, hypotonia, vomiting, constipation and/or polyuria. Association with corneal calcification and osteoporosis. Management: restrict vitamin D. Presentation: infancy to adulthood, may present with ESRD. May require biopsy to diagnose. Can recur after transplantation if untreated. Management: xanthine oxidase inhibitor/low purine diet. |
| APRT deficiency 1/15 000–50 000 (ethnicity dependent) HTZ 1/90 | Accumulation of 2,8-DHA in kidney leading to urinary stones/nephrocalcinosis | APRT | AR | Missense (70%) Nonsense | Wide range of onset depending on underlying genetic diagnosis and phenotype. Biochemistry can be non-specific. Difficult to diagnose clinically. Management: correction of electrolyte abnormalities crucial. Lower specificity of biochemical methods to distinguish homozygous and heterozygous individuals. Age-dependent variability in urinary cysteine levels. Stones can be composed of cysteine or calcium. Kidney stone formation can occur in HTZ individuals. Recurrent stones < 3 years of age: 14–18%. Management: therapy to alkalinise urine/chelation therapy. |
| Barter syndrome | Classical presentation: hyperreninemic, hyperaldosteronism, hypokalaemia, nephrocalcinosis Potential to develop ESRD | SLC12A1, KCNJ1, CLCNKB, CASR | AR, AR, AR, AD | <50 bp deletions Missense/nonsense Missense/nonsense Small duplication | Many cases go undetected until CKD/ESRD develops due to clinical heterogeneity and non-specific imaging findings. Renal biopsy findings: FSGS/nephrocalcinosis/fibrosis. Genetic testing can be confirmatory. Management: monitoring of renal function, treatment of extra renal features (Lowe). |
| Cystinuria 1/2500–7000 (ethnicity dependent) Account for up to 1% all stones worldwide (25% of paediatric nephrocalcinosis) | Defect in proximal tubular reabsorption of filtered cysteine leading to recurrent stone formation 50% of affected individuals present with stones in the first decade of life Can lead to CKD | SLC3A1, SLC7A9 | AR, AD/AR | Point mutations Multi-exon deletions Small genomic rearrangements | Many cases go undetected until CKD/ESRD develops due to clinical heterogeneity and non-specific imaging findings. Renal biopsy findings: FSGS/nephrocalcinosis/fibrosis. Genetic testing can be confirmatory. Management: monitoring of renal function, treatment of extra renal features (Lowe). |
| Dent disease Lowe syndrome Dent: <1/1 000 000 Lowe: 1/500 000 | Renal tubular disorder characterized by proteinuria, hypercalciuria, nephrocalcinosis/nephrolithiasis CKD/ESRD can occur third to fifth decade in 30–80% affected males Lowe: similar renal phenotype but with multisystem extra renal features of congenital cataracts, glaucoma, intellectual disability, postnatal growth retardation | CLCN5, OCRL1 | XR | 100 different nonsense/missense variants reported 70% non-sense with absent protein production | Many cases go undetected until CKD/ESRD develops due to clinical heterogeneity and non-specific imaging findings. Renal biopsy findings: FSGS/nephrocalcinosis/fibrosis. Genetic testing can be confirmatory. Management: monitoring of renal function, treatment of extra renal features (Lowe). |
| Distal renal Tubular acidosis | Acidaemia, hypokalaemia, growth impairment, nephrocalcinosis, nephrolithiasis, haemolytic anaemia, spheroctysis/elliptocytosis | SLC4A1 | AD/AR | Missense | Management: bicarbonate and potassium replacement. Monitor CKD. |
| Disorder incidence | Phenotype | Associated gene | Inheritance pattern | Mutational spectrum | Benefit of genetic diagnosis and clinical significance |
|---------------------|-----------|-----------------|---------------------|--------------------|-------------------------------------------------------|
| Familial hypomagnesemia with hypercalciuria | Acidosis, sensorineural hearing loss, rickets, osteomalacia | ATP6VIB1, ATP6V0A4 | AR | Missense | Management: audiometry |
| <1/1 000 000 | Renal magnesium wasting, hypercalciuria and nephrocalcinosis | CLDN16, CLDN19 | AR | Splice site | Predominantly missense mutations |
| | ESRD by adolescence/early adult life | | | | Biochemical triad of hypomagnesemia, hypercalciuria and nephrocalcinosis, alongside distal renal tubular acidosis |
| | Severe ocular involvement associated with | | | | Renal histology not diagnostic/specific |
| | CLDN19 | | | | CKD distinguishes from other magnesium wasting disorders |
| | | | | | Management: diagnosis guides pharmacotherapy, monitoring of CKD, evaluation for KRT |
| Hypophosphataemia with rickets with hypercalciuria | Renal phosphate wasting with calcium stones | SLC34A3 | AR | Missense/frame shift | Biochemical parameters can be normal |
| Infantile hypercalcemia | Variable associated features of slow growth, short stature, muscle weakness, arthralgia | SLC34A1 | AD/AR | | Molecular diagnosis can permit bone protection |
| Primary Fanconi renitubular syndrome | Inherited disorders where hepatic enzyme deficiencies result in overproduction of oxalate, leading to calcium oxalate stones | AGXT | AR | 4 recurrent missense variants | Early presentation non-specific: faltering growth, nausea |
| <1/100000 | ESRD may occur as young as 4 months of age | GRHPR | | Exon 1 and 2 hotspots | 24-h urinary oxalate may aid diagnosis, may require liver biopsy to confirm |
| | Systemic oxalosis can cause multiorgan manifestations | HOGA1 | | | Delay in diagnosis/misdiagnosis can delay therapy and risk renal transplant with systemic oxalosis |
| Primary hyperoxaluria | | | | | Management: specific mutation p.Gly170ARG managed with pyridoxine. Combined kidney/liver transplant |
| 1/100 000 | | | | | 2,8-DHA: 2,8-dihydroxyadenine, AD: autosomal dominant, AGXT: alanine-glyoxylate and serine-pyruvate aminotransferase, APRT: adenine phosphoribosyltransferase, AR: autosomal recessive, ATP6VIB1, ATP6V0A4: Vacuolar ATP-ase, CASR: calcium-sensing receptor, CKD: chronic kidney disease, CLCN5: chloride voltage-gated channel 5, CLCNKB: chloride voltage-gated channel Kb, CLDN16: Claudin-16, CLDN19: Claudin-19, CYP24A1: cytochrome P450 family 24 subfamily A member 1, ESRD: end-stage renal disease, FSGS: focal segmental glomerulosclerosis, GRHPR: glyoxylate reductase/hydroxypyruvate reductase, HOGA1: 4-hydroxy-2-oxoglutarate aldolase 1, HTZ: heterozygote, KCNJ1: potassium inwardly rectifying channel subfamily J member 1, KRT: kidney replacement therapy, OCRL1: inositol polyphosphate 5-phosphatase OCRL-1, SLC: solute carrier family (family number, followed by member number), i.e. SLC3A1: solute carrier family 3 member 1, XR: X-linked recessive. |
hormone (PTH). Serum 1,25-dihydroxycholecalciferol was elevated (229 pmol/L, normal 20–120 pmol/L). Urinary biochemistry revealed hypercalcuria (urinary calcium creatinine ratio 1.64 mmol/mmol, normal 0.04–0.08 mmol/mmol) and mild proteinuria (urine protein creatinine ratio 86 mg/mmol creatinine, normal <20). Radiological bone age was 4.85 years at a chronological age of 8.37 years. Hormonal axes, including growth hormone, and array CGH were normal [arr (1-22)x2, (XY)x1].

Dental examination was consistent with amelogenesis imperfecta (AI). The patient subsequently had several dental extractions. AI gene panel testing of 22 genes (Supplementary Table S1) did not reveal any pathogenic variants.

A low-salt diet was recommended to reduce urinary calcium excretion. Unfortunately, he developed worsening enuresis, including new daytime wetting. This prompted uroflow assessment, which suggested poor bladder emptying. Cystoscopy demonstrated a lobulated, irregular bladder and urethral mini-valves (not felt to be contributing to his symptoms). Spinal imaging was normal. Management included optimization of stooling, double voiding and excision of the mini-valves.

Persistent hypercalcuria (urinary calcium creatinine ratio varied between 1.5 and 2.3 mmol/mmol) prompted use of chlorothiazide, which was poorly tolerated and quickly discontinued.

Renal function declined, with persistent hypokalaemia, alongside the development of hypochloraemia and alkalosis. He was normotensive with static proteinuria.

Development of a locally available ‘nephrocalcinosis’ genetic panel (Table 1) prompted additional genetic analyses. This confirmed heterozygous variants in KCNJ1, a previously reported frameshift variant c.965del p.(Gly322Alafs*7), and a novel missense variant c.233G>C p.(Arg78Thr). Detection of KCNJ1 variants prompted phenotypic review, and a diagnosis of Bartter syndrome type II (BSII) was made. This led to initiation of ibuprofen (in preference to indomethacin given his existing bladder issues). His estimated glomerular filtration rate (eGFR) was unaffected (59 mL/min/1.73 m² before starting, 60 mL/min/1.73 m² 2 months later) with an associated symptomatic improvement in polyuria and nocturia. Updated imaging identified a poorly functioning left kidney (~10% overall function). He remains under renal, urological and endocrinology surveillance aged 14 years, with consideration of testosterone therapy due to pubertal delay, and chronic kidney disease stage 3A (eGFR of 54 mL/min/1.73 m²).

**DISCUSSION**

Bartter syndrome is characterized by hypokalaemic, hypochloremic metabolic alkalosis and secondary hyperaldosteronism. Five main subtypes are recognized according to clinical manifestations, age of onset and genotype. Biallelic loss-of-function variants lead to impaired functioning of transporters necessary for sodium chloride reabsorption in the thick ascending limb of the loop of Henle [3].

BSII due to KCNJ1 variants classically presents with antenatal polyhydramnios, preterm delivery and severe neonatal salt wasting. Although frequently diagnosed in infancy due to polyuria, dehydration and faltering growth, a late-onset adult phenotype presenting with incidental nephrocalcinosis and mild renal impairment is reported in two patients [4]. Phenotypic variability is recognized in all subtypes of Bartter syndrome; mild/late presentations of BSII may be due to differential effects of specific pathogenic variants on KCNJ1 function.

This case is notable due to the absence of severe salt wasting in infancy, isolated mild hypokalaemia at initial review aged 8 years and the clinical finding of AI. AI is an inherited condition characterized by abnormal enamel development; dental enamel is the most mineralized tissue within the body. The association of AI with nephrocalcinosis is well described in enamel renal syndrome [2], for which mutational analysis was negative for our patient, but is rarely seen in association with BSII [5]. Other groups have hypothesized that biomineralization abnormalities in patients with tubulopathies may affect calcium deposition in enamel.

Our case of late-presenting BSII in association with AI highlights the phenotypic variability of the condition and the need to consider tubular aetiologies in patient with AI, and demonstrates the utility of a nephrocalcinosis phenotype-targeted genetic panel.

**SUPPLEMENTARY DATA**

Supplementary data are available at ckj online.

**CONFLICT OF INTEREST STATEMENT**

The results presented in this paper have not been published previously in whole or part, except in abstract format.

**PATIENT CONSENT**

We would like to thank the patient and his family for allowing us to share their journey.

**DATA AVAILABILITY STATEMENT**

All data pertaining to this manuscript is contained in the manuscript and tables.

**ETHICS STATEMENT**

Specific ethical approval was not required for this research methodology.

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