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Exceptional Case

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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: amelogeneis 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

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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
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	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
Bartter 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	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
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 Duplications Small genomic rearrangements	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
Dent disease Lowe syndrome Lowe: 1/500 000 Dent: <1/1 000 000	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% nonsense 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	Acidosis, hypokalaemia, growth impairment, nephrocalcinosis, nephrolithiasis, haemolytic anaemia, spherocytosis/elliptocytosis	*SLC4A1*	AD/AR	Missense	Management: bicarbonate and potassium replacement. Monitor CKD

Table 1. Continued

Disorder incidence	Phenotype	Associated gene	Inheritance pattern	Mutational spectrum	Benefit of genetic diagnosis and clinical significance
	Acidosis, sensorineural hearing loss, rickets, osteomalacia	*ATP6VIB1* *ATP6V0A4*	AR	Missense Splice site	Management: audiometry
Familial hypomagnesemia with hypercalciuria <1/1 000 000	Renal magnesium wasting, hypercalciuria and nephrocalcinosis ESRD by adolescence/early adult life Severe ocular involvement associated with *CLDN19*	*CLDN16* *CLDN19*	AR AR	Predominantly missense mutations	Non-specific presenting features: polyuria, urinary tract infection, renal stones Biochemical triad of hypomagnesemia, hypercalciuria and nephrocalcinosis, alongside distal renal tubular acidosis Renal histology not diagnostic/specific CKD distinguishes from other magnesium wasting disorders Management: diagnosis guides pharmacotherapy, monitoring of CKD, evaluation for KRT
Hypophosphataemia with rickets with hypercalciuria Infantile hypercalcemia Primary Fanconi renotubular syndrome <1/100 000	Renal phosphate wasting with calcium stones Variable associated features of slow growth, short stature, muscle weakness, arthralgia	*SLC34A3* *SLC34A1*	AR AD/AR	Missense/frame shift	Biochemical parameters can be normal Molecular diagnosis can permit bone protection
Primary hypoxaluria 1/100 000	Inherited disorders where hepatic enzyme deficiencies result in overproduction of oxalate, leading to calcium oxalate stones ESRD may occur as young as 4 months of age Systemic oxalosis can cause multiorgan manifestations	*AGXT* *GRHPR* *HOGA1*	AR AR AR	4 recurrent missense variants Exon 1 and 2 hotspots	Early presentation non-specific: faltering growth, nausea 24-h urinary oxalate may aid diagnosis, may require liver biopsy to confirm Delay in diagnosis/misdiagnosis can delay therapy and risk renal transplant with systemic oxalosis Management: specific mutation p.GLy170ARG managed with pyridoxine. Combined kidney/liver transplant

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 hypercalciuria (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 hypercalciuria (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 m^2 before starting, 60 mL/min/1.73 m^2 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 m^2).

DISCUSSION

Bartter syndrome is characterized by hypokalaemic, hypochloraemic 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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