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CLINICAL SERIES

Genetic study in patients with nephrocalcinosis

Estudio genético en pacientes con nefrocalcinosis

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What do we know about the subject matter of this study?

The incidence of nephrocalcinosis is increasing in children, and metabolic or genetic causes are the most frequent in this age group. This entity can progress to chronic kidney disease therefore early diagnosis and treatment is essential.

What does this study contribute to what is already known?

We present ten cases of nephrocalcinosis of genetic origin in pediatric age. The importance of genetic analysis in patients and family members is emphasized, as well as the need to review it from time to time in patients with mutations of uncertain clinical significance, since new mutations not described at the time of the initial study can be reported or reclassified according to the availability of new evidence in databases, functional assays, or structural analysis.

Abstract

Nephrocalcinosis can be inherited or acquired and may progress to chronic kidney disease. **Objective**: to analyze the clinical, biochemical, and genetic characteristics of patients with genetic nephrocalcinosis and to establish a correlation between genotype and phenotype. **Patients and Method**: patients under 18 years of age with nephrocalcinosis followed up in a tertiary hospital in Madrid between 2013 and 2022 were studied. Inclusion criteria: nephrocalcinosis of monogenic etiology. Exclusion criteria: incomplete data. Demographic, biochemical, and imaging data were collected; a genetic study was performed with a gene panel in five patients, exome and splicing regions in one patient, and PCR amplification and Sanger sequencing in four patients. The study was approved by the ethics committee. **Results**: ten patients (70% male) with a median age at diagnosis of 21.5 months were included. A mutation in the *CYP24A1* gene was detected in four patients, three of whom also had a mutation in the *SLC34A1* gene, causative of infantile idiopathic hypercalcemia; three patients had mutations in the *SLC34A3* gene, causing hereditary hypophosphatemic rickets with hypercalciuria and nephrocalcinosis; one patient diagnosed with distal renal tubular acidosis type 1 presented two pathogenic variants in heterozygosis in the *ATP6V0A4* gene; one patient diagnosed with Bartter syndrome presented two variants in the *CLCNKB* gene; finally, a homozygous mutation in the *CLDN19* gene

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was detected in a patient with familial hypomagnesemia with hypercalciuria and nephrocalcinosis. Sixteen family members were studied and mutations were detected in 11 of them, all with no symptoms. **Conclusions**: the genetic study of patients with nephrocalcinosis allows etiological diagnosis, detection of asymptomatic affected relatives, and establishing treatment, which improves long-term renal prognosis. It also allows genetic counseling and advice to be given.

Introduction

The term nephrocalcinosis is used to describe the deposition of calcium oxalate and/or calcium phosphate in the interstitial tissue and/or renal tubules[1,2]. It may manifest with signs or symptoms of chronic kidney disease or be diagnosed incidentally[3].

Although it is less frequent in children than in adults, an increase in its incidence has been reported in this age group[4-7]. Nephrocalcinosis can affect children of any age, but it is more frequent in the first years of life, especially in premature infants, in whom a prevalence of up to 40%[8-11] has been described. They are a population at risk due to factors such as the innate immaturity of their kidneys, hypocitraturia, parenteral nutrition, and the medications they sometimes receive, such as furosemide and corticosteroids[10,12].

Nephrocalcinosis occurs in the context of various genetic, metabolic, or acquired diseases[2], the former being more frequent in pediatrics. It is associated with conditions that produce hypercalcemia and/or hyperphosphatemia and/or increased urinary excretion of calcium, phosphate, or oxalate, as well as hypocitraturia[3]. Predisposing diseases are idiopathic hypercalciuria or primary/secondary hyperoxaluria. A variety of renal tubular and metabolic diseases due to mutations in genes involved in calcium metabolism such as Bartter syndrome, type 1 distal renal tubular acidosis, or familial hypomagnesemia with hypercalciuria and nephrocalcinosis[3,7,8,13-15] are also predisposing diseases. Some anatomical alterations may favor its development, such as the medullary sponge kidney (Table 1).

According to the degree of renal involvement, nephrocalcinosis has been subdivided as follows: molecular or chemical, where the increase in calcium is intracellular and not visible microscopically or through imaging. This type is usually observed in patients with hypercalciuria and can be reversed when the hypercalciuria is corrected[2,3]. Microscopic nephrocalcinosis is detected through biopsy—mineral deposits seen under optical microscopy—but not through imaging; and macroscopic nephrocalcinosis, where calcium deposits are visible using imaging techniques[1,2].

In clinical practice, the term nephrocalcinosis refers to macroscopic deposits. It may be uni- or bilateral and localized in one or more segments of the kidney[2]. It most often affects the renal medulla and is diagnosed mainly by renal ultrasound. Radiologically, medullary nephrocalcinosis can be classified into grades I to III, according to the extent of medullary hyperechogenicity[8,16,17].

Cases of cortical nephrocalcinosis are much less frequent and are associated with cortical necrosis, chronic glomerulonephritis, pyelonephritis, primary/secondary oxalosis, autosomal recessive polycystic kidney disease, or benign nodular cortical nephrocalcinosis[2,8].

It is a chronic pathology, with slow, progressive development, often diagnosed incidentally in asymptomatic patients[3]. It may present with symptoms caused by nephrocalcinosis itself, such as colicky lumbar pain, nocturia, polyuria, or polydipsia, or with symptoms of the underlying pathology, such as pain or bone malformations in rickets, growth delay, or cramps in tubular acidosis, or hypomagnesemia[3,7,11].

Renal prognosis depends on the underlying cause and most patients have good renal prognosis[3]. However, it is important to detect the causes that can progress to end-stage renal disease, in order to delay its development. In recent years, several genetic alterations associated with metabolic disorders that predispose to its development have been described. The most important are alterations in tubular calcium transport causing hypercalciuria, but also mutations that produce hyperphosphaturia, hyperoxaluria, and hypocitraturia[2,18] (Table 2).

Up to 30 genes associated with nephrolithiasis/nephrocalcinosis, with autosomal dominant, autosomal recessive, or X-linked inheritance, have been described[19]. Among them are *CLCN5*, *CASR*, *CLDN16*, *CLDN19*, *ADCY10*, *CYP24A1*, or *SLC34A1*.

Daga et al.[4] and Braun et al.[6] described an incidence of monogenic etiology in 20% and 16.8% of patients with nephrocalcinosis/nephrolithiasis under 18 years of age, respectively.

The objective was to analyze the clinical, biochemical, and genetic characteristics of patients with genetic nephrocalcinosis in order to establish a correlation between genotype and phenotype.

Table 1. Causes of nephrocalcinosis

Hypercalciuria	Hyperphosphaturia	Hyperoxaluria
With hypercalcemia	With hyperphosphatemia	
Primary hyperparathyroidism Sarcoidosis Childhood hypercalcemia type 1 and 2 Vitamin D poisoning Milk and alkaline syndrome Williams- Beuren syndrome Congenital hypothyroidism	Tumor lysis syndrome Sodium phosphate laxatives	Primary hyperoxaluria Secondary hyperoxaluria
Without hypercalcemia	Without hyperphosphatemia	Others
Idiopathic hypercalciuria Medullary sponge kidney Neonatal nephrocalcinosis Prolonged immobilization Loop diuretics Corticosteroids Hereditary tubulopathies - Bartter syndrome - Distal renal tubular acidosis type 1 - Familial hypomagnesemia with hypercalciuria and nephrocalcinosis - Autosomal dominant hypocalcemia - Dent 's disease - Lowe syndrome Chronic hypokalemia Tyrosinemia type I Cystic fibrosis	Hereditary tubulopathies: - Dent 's disease I and II - Lowe syndrome - X-linked hypophosphatemic rickets , autosomal dominant and recessive - Hypophosphatemic rickets with hypercalciuria	Hypocitraturia Prematurity

Adapted from: Sayer JA. 2024[3]

Patients and Method

Retrospective observational study in patients under 18 years of age with follow-up in the Nephrology Department of a tertiary hospital in Madrid, diagnosed between 2013 and 2022. Inclusion criteria were diagnosis of nephrocalcinosis of monogenic etiology under follow-up in the Pediatric Nephrology consultation on the dates described. Patients with incomplete data in their clinical history (mainly loss to follow-up) were excluded.

The following data were collected at diagnosis: sex, age, etiology, degree of medullary nephrocalcinosis, analytical data [serum levels of 25(OH)D3, 1,25(OH) D3, calcium, ionic calcium, parathyroid hormone, phosphate, creatinine, glomerular filtration rate estimated according to the modified Schwartz formula, urinary indices (calcium/creatinine, citrate/creatinine, calcium/citrate), urinary pH], and treatment received. The ultrasounds were performed by different radiologists from the same department with uniform criteria defined according to a radiological nephrocalcinosis scale that grades renal echogenicity from 0 to III, with 0 being no nephrocalcinosis, I mild, II moderate, and III severe[8,16,17,20].

The genetic study of the patients was performed in different centers according to the suspected pathology. The technique used for genetic analysis varied according to the laboratory and clinical suspicion, using gene panels in five patients, exome and splicing region analysis in one patient, and PCR amplification and Sanger sequencing in four patients. All patients and their parents were referred to the Clinical Genetics consultation where the presence of the specific mutation was investigated and genetic counseling for future pregnancies was provided.

The study was approved by the Clinical Ethics Committee of the hospital. Clinical data were recorded anonymized in a Microsoft Excel database.

Results

Ten patients from nine unrelated families were included; 70% were male (Table 3). The median age at diagnosis was 21.5 months (IQR 28 months). Three of

the ten patients (two of them related) had mutations in two different genes (*CYP24A1* and *SLC34A1*).

A mutation in the *CYP24A1* gene was detected in four patients[1-4]. Patient 1 was diagnosed incidentally during the study due to enuresis. He presented normal serum phosphate values, hypercalcemia with hypercalciuria and hypocitraturia, suppressed parathyroid hormone, and calcidiol and calcitriol within normal values. Radiologically, he presented bilateral grade II nephrocalcinosis. This patient had two heterozygous mutations, one of the variants inherited from his father (asymptomatic) and the other variant of unknown origin, since it was an *in vitro* fertilization with ovodonation (*de novo* variant vs. donor carrier). He received hydrochlorothiazide and potassium citrate.

The other three patients (2, 3, and 4) with mutations in the *CYP24A1* gene had a heterozygous variant in *CYP24A1* and also had a heterozygous mutation in the *SLC34A1* gene. The clinical presentation at diagnosis was acute pyelonephritis in patients 2 and 3 (the latter also associated with growth failure), and isolated growth failure in patient 4. All three had bilateral grade II-III nephrocalcinosis, hypercalcemia (corrected after early childhood), hypophosphatemia with hyperphosphaturia, hypercalciuria, hypocitraturia, suppressed parathyroid hormone, and elevated calcitriol. Patient 2 inherited both heterozygous variants (*CYP24A1* and *SLC34A1*) from his mother (asymptomatic). The genetic study of his father and brother was negative. Patients 3 and 4 were siblings and both inherited a heterozygous variant in the *CYP24A1* gene and two mutations in the *SLC34A1* gene in trans configuration, one inherited from each parent. The parents were asymptomatic. As a treatment, patient 2 received potassium citrate only, while patients 3 and 4 were additionally treated with hydrochlorothiazide and oral phosphate supplementation.

Mutations in the *SLC34A3* gene were detected in three patients (numbers 5, 6, and 7). Patient 5 was diagnosed because of vomiting and abdominal pain, and patients 6 and 7 were studied after acute pyelonephritis. Patient 6 had bilateral grade I nephrocalcinosis, while patients 5 and 7 had bilateral grade II nephrocalcinosis. All three had normal calcemia and phosphatemia, normal calcidiol, elevated calcitriol, normal/lower limit of normal parathyroid hormone, hypercalciuria, and marked hypocitraturia. All had isolated mutations in the *SLC34A3* gene and were inherited from only one parent, who was asymptomatic in all cases. All three received potassium citrate, and patient 6 also received hydrochlorothiazide.

Patient 8, diagnosed with type 1 distal renal tubular acidosis, presented at the onset dehydration with metabolic acidosis, hypocitraturia, and inappropriately alkaline urine pH without alteration of calcium phosphate metabolism. He presented bilateral grade II nephrocalcinosis and the genetic study showed two pathogenic heterozygous variants in the *ATP6V0A4* gene. The parents declined the genetic study. This patient was treated with potassium citrate, bicarbonate, and oral potassium supplements.

Patient 9, diagnosed with type III Bartter syndrome, was 19 months at the onset, and presented with polyuria, polydipsia, vomiting, growth failure, and bilateral grade II nephrocalcinosis. He presented with lower-limit phosphatemia, hypokalemic metabolic alkalosis, normal parathyroid hormone and calcidiol, elevated calcitriol, hypercalciuria, and hypocitraturia. Two heterozygous variants were found in the *CLCNKB* gene and in trans position, each inherited from one parent. He was treated with indomethacin, spironolactone, and oral potassium and magnesium.

Patient 10, affected by familial hypomagnesemia with hypercalciuria and nephrocalcinosis, was diagnosed after acute pyelonephritis and presented with bilateral grade II nephrocalcinosis and maculopathy in the left eye. The following stand out: hypomagnesemia, hyperuricemia, elevated parathyroid hormone and calcitriol levels (all in the high range), normal calcidiol, hypercalciuria, and hypocitraturia. A homozygous mutation in the *CLDN19* gene inherited from both parents was detected. She received hydrochlorothiazide, calcidiol, potassium citrate, and magnesium. All patients had a normal glomerular filtration rate at diagnosis.

Table 2. Genetic causes of renal hypercalciuria

Proximal tubule
- Dent 's disease
- Hypophosphatemic rickets with hypercalciuria
- Hereditary rickets linked to X, autosomal recessive, autosomal dominant
- Glycogenosis type 1a
- Lowe syndrome
- Tyrosinemia type 1
- Wilson's disease
Loop of Henle
- Bartter syndrome IV
- Familial hypomagnesemia with hypercalciuria and nephrocalcinosis
- Autosomal dominant hypocalcemia
Distal tubule
- Pseudohypoaldosteronism type II
- Distal renal tubular acidosis
- Liddle 's syndrome

Adapted from: Smith J y Stapleton FB. 2024[18]

Table 3. Cases of nephrocalcinosis of genetic origin

Case/Sex/ Age/ Clinical presentation	Genetics/clinical parents	Clinical phenotype/ultrasound data	Analytical parameters	Treatment
Patient 1 Male 6 years Enuresis	**CYP24A1** Two heterozygous mutations c.425_427AAG (p.Gly143del) and c.1226T>C (p.Leu409Ser) Both probably pathogenic Egg donation Father carrier of c.1226T>C (p.Leu409Ser) mutation in heterozygosis, pathogenic Asymptomatic	Infantile hypercalcemia type 1 Bilateral nephrocalcinosis grade II	Maximum calcium level 10.89 mg/dL, normal Cai Normophosphatemia Normal estimated glomerular filtration rate Parathyroid hormone ↓ (7.2 pg/mL) 25 OH vitamin D 45.5 ng/mL Normal 1,25 OH vitamin D Hypercalciuria (6.8 mg/kg/day, Ca/Cr 0.64 mg/mg) Hypocitraturia (Citrate/Cr 0.27 mg/mg) Urine pH 7-8	Hydrochlorothiazide Potassium citrate
Patient 2 Male 6 months Acute pyelonephritis	**CYP24A1** Heterozygous variant c.575A>G (p.Glu192Gly) probably pathogenic **SLC34A1** Heterozygous pathogenic variant c.778G>A (p.Gly260Ser) Mother carries both variants, asymptomatic Father and sibling tested negative	Infantile hypercalcemia type 1 and 2 Bilateral nephrocalcinosis grade III	Hypercalcemia (total calcium 10.92 mg/d, Cai 1.4 mmol/L) corrected Hypophosphatemia at diagnosis (4.1 mg/dL, later normalized) Normal estimated glomerular filtration rate Parathyroid hormone ↓ (7.98 pg/mL) Normal 25 OH vitamin D (38.4 ng/mL) 1.25 OH vitamin D ↑ (139 pg/mL) Hypercalciuria (Ca/Cr 1.3 mg/mg) Hypocitraturia (Citrate/Cr 0.25 mg/mg)	Potassium citrate
Patient 3 Female (twin sister of patient 4) 11 months Acute pyelonephritis and poor weight gain	**CYP24A1** Heterozygous c.427_429del (p.E143del), uncertain clinical significance **SLC34A1** Two pathogenic mutations: c.271_291del21 (p.Val91_97del7aa) and c.1416+3G>A (loss of splice site IVS12), inherited in trans situation (each inherited from a parent) Asymptomatic parents	Infantile hypercalcemia type 1 and 2 (autosomal recessive) Bilateral nephrocalcinosis grade III	Maximum hypercalcemia 14.2 mg/dl, Cai 1.68 mmol/L (corrected at 2 years) Minimum phosphorus 3.14 mg/ dL Normal estimated glomerular filtration rate Parathyroid hormone ↓ (5.7 pg / mL) 25 OH normal vitamin D 1,25 OH vitamin D ↑ 87 pg /ml Hypercalciuria (6.86 mg/kg/day, Ca/Cr 0.59 mg/mg) Hypocitraturia	Hydrochlorothiazide Potassium citrate Phosphorus
Patient 4 Male (twin brother of patient 3) 8 months Poor weight gain	**CYP24A1** Heterozygous c.427_429del (p.E143del) Uncertain clinical significance **SLC34A1** Two pathogenic mutations c.271_291del21 (p.Val91_97del7aa) and c.1416+3G>A (loss of splice site IVS12) Inherited in trans, each inherited from a parent Asymptomatic parents	Infantile hypercalcemia type 1 and 2 (autosomal recessive) Bilateral nephrocalcinosis grade II	Maximum hypercalcemia 11.4 mg/dl, Cai 1.35 mmol/L (corrected at 2 years) Minimum phosphorus 4.59 mg/ dL Normal estimated glomerular filtration rate Parathyroid hormone ↓ (5.5 pg/mL) Normal 25 OH vitamin D 1,25 OH vitamin D ↑ (82 pg/mL) Hypercalciuria (8.29 mg/kg/day, Ca/Cr 0.71 mg/dL) Hypocitraturia	Hydrochlorothiazide Potassium citrate Phosphorus

Patient 5 Male 9 years Vomiting and abdominal pain	**SLC34A3** Heterozygous pathogenic variant (c.448+1G>A) Father carrier of the same variant, asymptomatic Mother tested negative	Hereditary hypophosphatemic rickets with hypercalciuria and nephrocalcinosis Bilateral nephrocalcinosis grade II	Normocalcemia Normophosphatemia Normal estimated glomerular filtration rate Parathyroid hormone 15 pg/mL 25 OH normal vitamin D 1.25 OH vitamin D ↑ (127 pg / mL) Hypercalciuria (9.35 mg/kg/day, Ca/Cr 0.28 mg/mg) Hypocitraturia (Citrate/Cr 0.12 mg/mg)	Hydrochlorothiazide Potassium citrate
Patient 6 Female 2 years and 10 months Acute pyelonephritis	**SLC34A3** Heterozygous mutation c.169T>C (p.Trp57Arg) Variant of uncertain clinical significance Mother carrier of the same variant, asymptomatic Father genetic study negative	Hypophosphatemic rickets with hypercalciuria and nephrocalcinosis Nephrocalcinosis grade I	Normocalcemia Normophosphaemia Normal estimated glomerular filtration rate Normal parathyroid hormone Normal 25 OH vitamin D 1.25 OH vitamin D ↑ (74 pg/mL) Hypercalciuria (9 mg/kg/day, Ca/Cr 0.53 mg/mg) Hypocitraturia (Citrate/Cr 0.38 mg/mg)	Potassium citrate
Patient 7 Male 7 months Acute pyelonephritis	**SLC34A3** Variant c.1453C>T (p.Arg 485Cys) Uncertain clinical significance Father carrier of the same variant, asymptomatic Mother tested negative	Hereditary hypophosphatemic rickets with hypercalciuria and nephrocalcinosis Bilateral nephrocalcinosis grade II	Normocalcemia Normophosphatemia Normal estimated glomerular filtration rate Normal parathyroid hormone Normal 25 OH vitamin D 1.25 OH vitamin D ↑ (156 pg/mL) Hypercalciuria (Ca/Cr 0.53 mg/mg) Hypocitraturia (Citrate/Cr 0.06 mg/mg)	Potassium citrate
Patient 8 Male 3 months Distal renal tubular acidosis	**ATP6V0A4** Two heterozygous pathogenic variants c.1868C>G (p.A529P) and c.2075T>C (p.W598R) Parents declined genetic testing	Distal renal tubular acidosis Bilatearl nephrocalcinosis grade II	Normocalcemia Normophosphatemia Normal estimated glomerular filtration rate Normal parathyroid hormone Normal 25 OH vitamin D Normal 1,25 OH vitamin D Normocalciuria Hypocitraturia (Citrate/Cr 0.1 mg/mg)	Potassium citrate Potassium Bicarbonate
Patient 9 Male 19 months Polyuria and polydipsia	**CLCNKB** Two heterozygous variants c.610G>A (p.Ala204Thr) and c.508G>A (p. Val170Met) in trans position Both probably pathogenic Mother is a heterozygous carrier of the c.508G>A variant Father is a heterozygous carrier of the c.610G>A variant Asymptomatic parents	Bartter syndrome type III Bilateral nephrocalcinosis grade II	Normocalcemia Borderline-low phosphorus (3.7 mg/dL at 17 months) normalized Hypokalemic metabolic alkalosis (7.58, HCO3 27 mmol/L, K minimum 2.4 mEq/mL, Na normal, Cl 94 mEq/L) Normal estimated glomerular filtration rate Normal parathyroid hormone Normal 25 OH vitamin D 1.25 OH vitamin D ↑ (95 pg/mL) Normal renin and aldosterone Hypercalciuria (5.28 mg/kg/day, Ca/Cr 1.62 mg/mg) Hypocitraturia (Citrate/Cr 0.24 mg/mg)	Indomethacin Spironolactone Potassium Magnesium

Patient 10 Female 3 years Acute pyelonephritis	**CLDN19** Homozygous mutation (p.G20D) in exon 1, pathogenic Both parents carry the same mutation, asymptomatic	Hipomagnesemia familiar con hipercalciuria y nefrocalcinosis Maculopatía ojo izquierdo Nefrocalcinosis bilateral grado II	Normocalcemia Hipomagnesemia (1,08 mg/dL) Hiperuricemia (7,9 mg/dL) Filtrado glomerular estimado normal Paratohormona ↑ (117 pg/mL al diagnóstico, actualmente 155) 25 OH vitamina D normal 1,25 OH vitamina D ↑ (75 al diagnóstico, actualmente 126 pg/ml) Hipercalciuria (13,2 mg/kg/día, Ca/Cr 0,36 mg/mg) Hipocitraturia (Citrato/Cr 0,12 mg/mg)	Hidroclorotiazida Calcidiol Citrato potásico

VALORES NORMALES:

PLASMA

Uric acid:
- 0 days-15 days: 2.8-11.7 mg/dL
- 15 days-1 year: 1.8-6 mg/dL
- 1-12 years: 2.4-7 mg/dL
- 12-19 years: 2.7-7.2 mg/dL

Aldosterone: 1.17-23.6 ng/dL
Bicarbonate: 22-29 mmol/L
Ionic calcium (Cai): 1.15-1-33 mmol/L
Total calcium:
- Children under 1 year: 8.7-11 mg/dL
- Older than 1 year: 8.7-10.7 mg/dL

Chlorine: 97-110 mEq/L
Phosphorus:
- 0 days-8 weeks: 4.8-7.5 mg/dL
- 8 weeks-1 year: 4.7-6.7 mg/dL
- 1-2 years: 4.7-6.7 mg/dL
- 2-6 years: 4.5-6.5 mg/dL
- 6-15 years: 2.7-5.3 mg/dL
- 15-19 years: 2.5-4.5 mg/dL

Magnesium: 1.6-2.4 mg/dL
pH: 7.35-7.45
Potassium:
- 0 days-4 months: 4-6.2-11.7 mEq/L
- 5 months-1 year: 3.7-5.6 mEq/L
- 1-19 years: 3.5-5.3 mEq/L

Sodium: 135-145 mEq/L

25-OH vitamin D: 20-50 ng/mL

1,25-OH vitamin D: 20-54 pg/mL

Parathyroid hormone : 12-88 pg/mL

Renin: 2.9-39.9 pIU/mL

URINE

Calciuria: < 4 mg/kg/day
Calcium/creatinine ratio (Ca/Cr):
- Children under one year of age: 0.03-0.78 mg/mg creatinine
- 1-2 years: 0.05-0.78 mg/mg creatinine
- 2-3 years: 0.02-0.5 mg/mg creatinine
- 3-5 years: 0.02-0.39 mg/mg creatinine
- 5-7 years: 0.01-0.28 mg/mg creatinine
- 7-10 years: 0.01-0.25 mg/mg creatinine
- 10-19 years: 0.01-0.25 mg/mg creatinine

Citrate/creatinine ratio (citrate/Cr): 0.38-0.9 mg/mg creatinine

Phosphorus/creatinine ratio:
- Children under one year of age: 0.33-5.2 mg/mg creatinine
- 1-2 years: 0.33-3.8 mg/mg creatinine
- 2-3 years: 0.33-3.29 mg/mg creatinine
- 3-5 years: 0.33-2.19 mg/mg creatinine
- 5-7 years: 0.33-1.37 mg/mg creatinine
- 7-10 years: 0.33-0.98 mg/mg creatinine
- 10-14 years: 0.22-0.87 mg/mg creatinine
- 14-19 years: 0.22-0.73 mg/mg creatinine

Regarding the clinical evolution of the patients, nephrocalcinosis remained stable in all cases, and all patients also maintained normal renal function.

16 first-degree relatives were studied in the clinical genetics office and mutations were detected in 11 of them, all of whom were asymptomatic.

Discussion

Nephrocalcinosis is an uncommon disease in the pediatric age, and it is usually diagnosed incidentally. Its incidence seems to be increasing in the last decade, perhaps due to its more frequent diagnosis by ultrasound in the study of other pathologies[4,7,11].

In our series, four patients had a mutation in the *CYP24A1* gene, and three of them presented also associated with another mutation in the *SLC34A1* gene. In the process of vitamin D activation, hepatic hydroxylation occurs through 25-hydroxylase, and a second hydroxylation in the kidney by 1α-hydroxylase (*CYP27B1*), generating 1,25-dihydroxyvitamin D3. The *CYP24A1* gene encodes 24-hydroxylase vitamin D, which inactivates 1,25-dihydroxyvitamin D3 and acts on its precursor, 25-hydroxyvitamin D3, producing the inactive metabolite 24,25-dihydroxyvitamin D3. The inhibition of this enzyme leads to an increase in 1,25-dihydroxyvitamin D, causing hypercalcemia with hypercalciuria and nephrocalcinosis[21,22].

There are two phenotypes derived from this mutation: idiopathic infantile hypercalcemia or infantile hypercalcemia type 1, with its onset in infancy and presents with symptomatic hypercalcemia, dehydration, vomiting, growth failure, and nephrocalcinosis; and the adult/late form, which is milder, presenting with nephrolithiasis, hypercalciuria, and nephrocalcinosis and is usually diagnosed incidentally[23]. Laboratory findings include hypercalcemia with normal-high calcitriol, suppressed parathyroid hormone, and hypercalciuria. Treatment consists of a low-calcium, low-oxalate diet, intravenous fluid therapy, avoiding vitamin D supplements and sun exposure. Some antifungals (fluconazole, ketoconazole) have shown efficacy since by inhibiting cytochrome P450, they decrease 1α hydroxylase activity. Recent studies have shown that rifampicin is a potent *CYP3A4* inducer and reduces 1,24-dihydroxyvitamin D3 levels, improving hypercalcemia[24].

There is a subgroup within idiopathic infantile hypercalcemia due to an inactivating mutation in the *SLC34A1* gene, which encodes the sodium-phosphate cotransporter IIa (NaPi-IIa) and is usually associated with hypophosphatemia. This cotransporter, together with its counterpart NaPi-IIc (*SLC34A3*), re-absorbs up to 80% of the filtered phosphate. This subgroup has been classified as infantile hypercalcemia type 2 and has autosomal recessive inheritance. It has the same course as idiopathic infantile hypercalcemia, but it also associates hypophosphatemia due to loss at the proximal tubule level and inhibition of FGF-23, which increases calcitriol and worsening of hypercalcemia and hypercalciuria. Although it is an autosomal recessive disease, patients with mutations in one of the two alleles may have hypercalciuria, with a milder phenotype. Treatment is similar to idiopathic infantile hypercalcemia[23,25]. It may require phosphate, either associated with or without thiazides, which could correct or minimize hypercalciuria, but should be used with caution due to the risk of calcium phosphate lithiasis.

We detected a mutation in the *CYP24A1* gene in four patients[1-4]. In patient 1, since a pathogenic variant was found in the father, it was assumed that the variants were in trans position, and their clinical phenotype was interpreted as the result of the combination of both pathogenic variants, confirming that the patient had infantile hypercalcemia type 1.

In the case of patient 2, who inherited both maternal variants, the mother's phenotype was likely milder, with the disappearance of nephrocalcinosis and the clinical symptoms in early childhood, as is often the case in infantile hypercalcemia. The presence of other genetic amplifying factors in the patient or inhibitory factors in the mother that justify different phenotypes cannot be ruled out.

In patients 3 and 4 (siblings), the fact that they inherited two pathogenic variants in the *SLC34A1* gene in trans position could account for the more severe clinical phenotype compared to their parents. In addition, they presented a variant in the *CYP24A1* gene that was considered of uncertain clinical significance.

Mutations in the *SLC34A3* gene (NaPi-IIc) are responsible for hereditary hypophosphatemic rickets with hypercalciuria and nephrocalcinosis[26,27]. Hypophosphatemia produces elevated calcitriol, increasing intestinal absorption of calcium and phosphate which suppresses parathyroid hormone leading to hypercalciuria and nephrocalcinosis. These patients may also present a phenotype similar to patients with *SLC34A1* mutations.

Mutations in the *SLC34A3* gene were detected in three patients. All had isolated mutations in the *SLC34A3* gene and were inherited from one parent, who was asymptomatic in all cases. It is possible that the patients had other undetected mutations, or that the parents had undetected biochemical alterations or undiagnosed nephrocalcinosis.

Type 1 distal renal tubular acidosis occurs due to an inability of the α-intercalated cells in the distal tubule to secrete acid[28]. It presents with hyperchloremic metabolic acidosis with normal anion gap, inappro-

priately alkaline urine, hypocitraturia, hypercalciuria, and nephrocalcinosis[29]. Its etiology can be hereditary or acquired[28] and, among the former, there are different forms of inheritance such as autosomal dominant (*SLC4A1* mutations, milder, and of late onset[30]); autosomal recessive with deafness (*ATP6V1B1* mutations, severe metabolic acidosis, growth retardation, rickets, early nephrocalcinosis, and sensorineural hearing loss); autosomal recessive without deafness (*ATP6V0A4* mutations). These mutations account for 80-85% of cases of primary distal renal tubular acidosis. In the remaining 15-20% of cases, no pathogenic mutation is observed. Treatment is based on early administration of potassium citrate to prevent the development of nephrocalcinosis[31]. Patient 8, diagnosed with type 1 distal renal tubular acidosis, had two compound heterozygous variants (different alleles) in the *ATP6V0A4* gene, compatible with the suspected disease.

Bartter syndrome is a rare tubulopathy, with a prevalence of 1/40,000-50,000[32]. It is caused by various mutations in genes encoding proteins involved in the reabsorption of sodium chloride in the thick ascending limb of the loop of Henle, causing fluid and electrolyte loss. It is characterized by significant polyuria (due to saline loss), secondary hyperaldosteronism resulting in hypochloremic hypokalemic metabolic alkalosis, and normal/low blood pressure[32–36]. In patient 9, two heterozygous variants in the *CLCNKB* gene were found in trans position, confirming the clinical suspicion of Bartter syndrome.

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is caused by mutations in the *CLDN16* and *CLDN19* genes, which encode claudin 16 and 19 (CLDN16 and CLDN19). The claudins reabsorb 25% of the calcium and 60% of the magnesium in the thick ascending limb of the loop of Henle[37,38]. These mutations are transmitted in autosomal recessive inheritance[1,38,39]. 50 different mutations have been identified for the *CLDN16* gene, while only 13 mutations have been described in the *CLDN19* gene, the most frequent form in Spain[40].

Its onset occurs in childhood with recurrent urinary tract infections, polyuria/polydipsia, short stature, vomiting, and early nephrocalcinosis, progressing to chronic kidney disease in adolescence[40]. Renal loss of magnesium and calcium is characteristic, although hypomagnesemia is not always present. Mutations in the *CLDN19* gene are also associated with ocular alterations since this gene is also expressed in the retinal epithelium[37]. Treatment, consisting of magnesium and thiazides, is not always effective[40]. Most of these patients require renal replacement therapy before adulthood, although the definitive treatment is renal transplantation (the disease does not recur in the graft)[37]. Patient 10, affected by familial hypomagnesemia with hypercalciuria and nephrocalcinosis, carried a homozygous mutation in the *CLDN19* gene inherited from both parents, compatible with her diagnosis.

In our series, the diagnosis of nephrocalcinosis in most patients was made incidentally when performing abdominal-renal ultrasound in the context of urinary tract infection, growth failure, metabolic acidosis, suspected structural alterations, or uropathies. Both in the patient diagnosed with Bartter syndrome and in the patient with distal renal tubular acidosis, nephrocalcinosis was detected in their evolution during follow-up when serial ultrasounds were performed looking for it. Therefore, we believe it is essential to perform abdominal-renal ultrasound, a non-invasive test, in the diagnosis and follow-up of pediatric patients with urinary symptoms (recurrent urinary tract infection, polyuria, recurrent abdominal pain, or colic), which may indicate underlying pathology. When nephrocalcinosis is detected, it is necessary to evaluate renal tubular function, looking for metabolic acidosis/alkalosis, as well as electrolyte alterations (calcium phosphate metabolism, vitamin D, parathyroid hormone, and magnesium).

A family history of nephrolithiasis and/or nephrocalcinosis may point to a genetic etiology, and the study of first-degree relatives of patients with nephrocalcinosis is recommended in order to detect undiagnosed affected relatives. Genetic testing, in addition to enabling early diagnosis and slowing the progression of kidney disease in its initial stages, allows for causal diagnosis in cases that present with analytical findings indicative of end-stage chronic kidney disease, as seen in some cases of distal renal tubular acidosis[30]. The finding of the mutated gene allows us to know the pathophysiological mechanism responsible for nephrocalcinosis and to offer treatment. The discovery of new mutations may help in the diagnosis of cases of unknown origin.

The genetic analysis of our patients highlights the importance of performing this study both in patients with suspicion of a specific disease and those patients with nephrocalcinosis without other findings. Sometimes, mutations of uncertain origin are found that may or may not be pathogenic, thus it is important to update the genomic databases and to perform periodic reviews with updates of the genetic study of patients with variants of uncertain clinical significance since scientific advances may provide new relevant information on mutations that are not known.

Conclusions

Genetic diagnosis helps to identify affected patients early and provides the opportunity to offer specific treatment that slows the progression of nephrocalci-

nosis. It has important implications for asymptomatic family members who are carriers of the disease (healthy or undiagnosed patients) since early follow-up and treatment can avoid long-term complications. In the future, the discovery of new mutations may help in the diagnosis of cases where the cause remains unknown.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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