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CASE REPORT

Accidentally detected nephrocalcinosis in a boy with a homozygous R396W mutation in the *CYP24A1* gene – 7-year follow-up

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ABSTRACT

Nephrocalcinosis can manifest as frequent urination, haematuria and recurrent urinary tract infections, as well as a decrease in bone mineral density, increasing the risk of osteoporosis. Excessive urinary calcium excretion may have a genetic basis, including mutations within the genes encoding vitamin D_3 metabolising enzymes. This paper presents a report of a 7-year follow-up of a boy in whom abdominal ultrasound incidentally detected nephrocalcinosis. The patient was confirmed to have a homozygous R396W mutation in the *CYP24A1* gene, which encodes an enzyme responsible for inactivating calcitriol and protecting the cell from vitamin D_3 intoxication. Radiological examinations showed a decrease in bone mineral density in the spine. Genetic factors, especially those related to abnormal vitamin D_3 metabolism, should be considered in the differential diagnosis of incidentally detected nephrocalcinosis. Children with excessive urinary calcium excretion are at particular risk of developing skeletal complications, including osteopenia and osteoporosis.

KEY WORDS:

paediatrics, bone density, calcium, nephrocalcinosis, vitamin D_3 24-hydroxylase.

INTRODUCTION

Nephrocalcinosis (NC), defined as the deposition of precipitated oxalate or calcium phosphate salts within the tubules, tubular epithelium and/or interstitial tissue of the kidney, may initially be asymptomatic. However, in the long term it significantly increases the risk of developing chronic kidney disease [1]. The diagnosis is made based on the characteristic radiological picture obtained either by ultrasound or computed tomography. The most common image of NC is calcification around (rims) the entire pyramids of the renal medulla with or without accompanying calcific deposits; the lesions always occur bilaterally.

Three basic types of hypercalciuria (HC) are distinguished: absorptive, renal and resorptive. Absorptive HC may be related to direct increased calcium absorption (type I), excessive action of the hormonally active form of vitamin D_3 – $1.25(OH)_2D_3$ (type II) – or decreased phosphorus absorption (type III). Renal HC results from decreased renal calcium absorption, whereas resorptive HC is caused by increased bone turnover and osteolysis [2, 3]. Apart from symptoms such as abdominal pain and haematuria/cirrhosis, HC can also cause kidney stones, recurrent urinary tract infections and decreased bone mineral density, thus increasing the risk of developing osteoporosis [3, 4].

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In the paediatric population, calcium hypersecretion and NC are more likely to have a genetic background than in adults – to date, a number of defects have been identified that may be responsible for this process [2]. One of these is a mutation of the *CYP24A1* gene encoding vitamin D_3 24-hydroxylase, a mitochondrial cytochrome P-450 oxidase responsible for catalysing the hydroxylation reaction of both $25(OH)D_3$ and $1.25(OH)_2D_3$. As a result of the reaction, calcitriol is inactivated and converted into a water-soluble metabolite, calcitroic acid, which is then removed from the body to protect the cell from vitamin D_3 intoxication. A loss-of-function mutation of the enzyme results in an up to ten-fold prolongation of the serum half-life of $1.25(OH)_2D_3$ and disruption of calcium-phosphate homeostasis [5, 6].

To date, there are few data on the clinical course, bone complications or long-term follow-up of children with a defect in the *CYP24A1* gene. Furthermore, treatment of this rare disorder remains a challenge. Here we present a case of a patient with a mutation in the *CYP24A1* gene in order to show the unusual presentation and discuss diagnostic and therapeutic considerations.

CASE REPORT

In 2015, a six-year-old Caucasian boy (P.M.) presented with his mother to the Emergency Department of University Clinical Hospital No. 4, presently the University Paediatrics Centre of the Medical University of Lodz, due to an upper respiratory tract infection and severe abdominal pain. In the ED, the boy underwent abdominal ultrasound which showed increased echogenicity of the kidney pyramids and a simple cyst, 7 mm in diameter, in the upper pole of the left kidney. The patient's family history of urinary tract diseases was unremarkable. The boy was referred for further evaluation and treatment to the Department of Paediatric Endocrinology, Diabetology and Nephrology.

On admission to the clinic, the boy's condition was described as fairly good. On physical examination, signs of an upper respiratory tract infection were observed and laboratory tests showed elevated inflammatory markers. During the hospitalisation, the antibiotic therapy initiated on an outpatient basis was continued and antipyretics were administered, which resulted in a reduction in inflammatory markers and improvement in the patient's general condition. No peripheral oedema was observed during the stay at the clinic, blood pressure values were within the reference range and diuresis was normal. Additional tests performed before discharge showed increased urinary calcium excretion (Ca/Cr: 0.6 mg/mg, N: < 0.28) with normal serum calcium levels (Table 1). Due to the abnormal kidney ultrasound and HC, further hospitalisation was planned for more detailed work-up. After one month, daily calcium excretion was determined on an outpatient basis and was 6.8 mg/kg b.w./day (N: < 4).

The boy's subsequent hospitalisation showed a reduced parathormone (PTH) level (6.18 pg/ml, N: 15–68.3) and $25(OH)D_3$ (21.3 ng/ml, N: 30–100) with normal serum levels of calcium, phosphorus, magnesium and $1.25(OH)_2D_3$ (81.6 pg/ml, N: 25–86.5). Thyroid hormone concentrations (FT3, FT4, TSH) remained within reference limits. The Pak test, modified by Stapleton, yielded a Ca/Cr ratio of 0.15 mg/mg before calcium loading and 0.37 mg/mg after loading, confirming the suspicion of absorptive hypercalciuria [7]. Decreased magnesium and phosphate excretion and elevated urinary uric acid concentrations were also observed. Urinary excretion of citrate (308.24 mg/g creatinine, N: > 18), oxalate (0.18 mmol/1.73 m^2/24 h, N: < 0.5) and calcium remained within reference limits. The boy was advised to follow a diet with an even daily calcium supply of 600–800 mg/day, vitamin D_3 supplementation of 500 IU/day and further assessment at the Nephrology Outpatient Clinic.

At the first follow-up visit two months after hospitalisation, the boy reported no complaints. The Ca/Cr ratio was 0.32 mg/mg. A densitometric study was performed, with a *Z*-score of 1.2 in the total body protocol and –1.7 in the spine protocol. Further observation for osteoporosis was recommended. Kidney ultrasound performed during a one-day hospitalisation in February 2017 showed bilateral NC, and cysts in the left kidney (5 mm and 3.7 mm).

In February 2018, due to persistent vitamin D_3 deficiency (25(OH)D3: 25.3 ng/ml), the supplementation dose was increased to 1000 IU/day. Genetic diagnosis based on Sanger sequencing was also performed, demonstrating a homozygous R396W mutation in *CYP24A1*. The test was conducted in a laboratory in the Department of General Paediatrics, Muenster, Germany, as part of the activities of the Polish Registry of Inherited Tubulopathies (POLtube; https://ptnfd.org./poltube).

In 2019, Pak's oral calcium loading test with Stapleton's modification was repeated, showing renal hypercalciuria (Ca/Cr ratio before loading: 0.33 mg/mg; after loading: 0.6 mg/mg) [7]. Vitamin D_3 supplementation was discontinued, sunlight avoidance advised, and hydrochlorothiazide (HTZ) recommended at a dose of 12.5 mg per day in two divided doses (0.4 mg/kg b.w./day). However, the child's parents did not comply with medical recommendations and did not give him the medication regularly.

One year later, in August, a follow-up hospitalisation showed significant HC (calcium excretion: 9.83 mg/kg b.w./24 h) and persistently reduced PTH levels (6.7 pg/ml). The $25(OH)D_3$ concentration, despite the lack of supplementation, was normal at 63 ng/ml. Urinary citrate concentration remained within reference limits. The boy's parents were interviewed, the need for chronic treatment with HTZ was explained, and the total daily dose was planned to be increased to 25 mg in two divided doses (0.7 mg/kg b.w./day).

TABLE 1. Values of serum biochemical indices

Age	Date	Creatinine [mg/dl] (N: 0.3–0.7)	Schwartz eGFR [ml/min] (N: > 90)	Urea [mg/dl] (N: 16.6–48.5)	Sodium [mmol/l] (N: 136–145)	Potassium [mmol/l] (N: 3.5–5.1)	Calcium [mg/dl] (N: 8.4–10.2)	Magnesium (N: 1.8 – 2.5)	Phosphorus [mg/dl] (N: 3.72–5.58)	Uric acid [mg/dl] (N: 3.4–7.0)	Parathormone [pg/ml] (N: 15–68.3)	Calcium/creatinine in urine [mg/mg] (N: 5–10 y.o.: < 0.21, N: >10 y.o.: < 0.16)	Calcium in 24 h urine collection [mg/kg b.w./24 h] (N: 1–4)
6	19.02.2015	0.45		19	*133*	4.1	9.4	1.8	5.1	*2.6*			
	24.02.2015						9.7	2.2					
	24.02.2015	0.31		11	141	4.3	8.3		4.8	*2.1*			7
	30.04.2015	0.35	143	26	138	4.2	9.8	2.2		*3.3*	*9.9*	**0.60**	3
	05.05.2015						9.7	2.0	4.5		*6.2*	**0.22/0.37***/0.15	
	18.12.2015	0.39	175	23	138	4.1	9.7	2.1	5.0	4.0	*10.0*	**0.29**	**4/6**
7	30.08.2016	0.55	98	25			9.4	1.8	4.3	*3.1*	*5.5*	**0.50**	**11**
	01.09.2016						9.4		4.9				**8**
	27.10.2016	0.53		29						3.5			
	31.10.2016	0.54	133			4.1	9.6					**0.28**	
8	21.02.2017	0.59	123	34	138	4.4	9.9	1.9	4.5	*2.5*	*9.1*	0.14	
9	15.02.2018	0.52	109	17			9.4	1.9	4.2	*2.8*	15.4	**0.31**	3
	18.10.2018	0.6	126	22			9.9	2.0	4.4	*3.2*	*7.9*		**5**
10	09.05.2019	0.57	138	30	137	4.1	9.7	1.9	4.4	3.4	*7.4*	**0.43/0.60*/0.33**	**8**
11	20.08.2020	0.64	97	25	140	4.2	9.9	2.0	4.4	38	*6.7*	**0.24**	**10**
12	03.11.2021	0.72	121	31	135	4.4	10.3		4.6		*4.4*	**0.15**	
	10.01.2022	0.7		37	137	3.9	9.2		4.7		21.2	0.08	

*Bold – value above the upper limit of normal range, italic – value below the lower limit of normal range. *Measurement after oral calcium loading in the Pak test with Stapleton's modification [7].*

At follow-up, normalisation of the Ca/Cr ratio was achieved and the dose of HTZ was reduced to 12.5 mg per day in two divided doses (0.3 mg/kg/day) in November 2021. Densitometry in the total body projection yielded a *Z*-score of 0.1, and in the spine projection a *Z*-score of –2.2. A spinal X-ray showed vertebral bodies with normal height and fine internal structure. The border plates were properly calcified and slightly concave in the lumbar vertebrae. Since there was no history of fractures, the diagnostic criteria for osteoporosis were not met.

At the appointments, the boy did not complain of any symptoms, and no peripheral oedema or other significant abnormalities were found on physical examination. Blood pressure readings, measured both in casual measurements and by ABPM, did not deviate from the reference values. Similarly, general urine tests performed regularly did not show deviations from the norm. The ultrasound image of the urinary tract had remained stable since 2017. Follow-up laboratory tests performed in January 2022 showed normalisation of serum PTH levels (21.2 pg/ml) and a further reduction in the child's urinary Ca/Cr ratio (0.08 mg/mg). Currently, the boy is physically active, he plays in a school football team and is developing properly.

DISCUSSION

Vitamin D_3, as a prohormone, is produced in skin cells during exposure to ultraviolet radiation; it is also obtained from food. It then goes through a multi-step activation process involving 25-hydroxylase contained mainly in liver cells and a 1α-hydroxylase present in kidney cells. The formed $1.25(OH)_2D_3$, after binding to the vitamin D receptor (VDR), has a number of functions, including the regulation of calcium-phosphate metabolism [8]. It actively promotes the absorption of calcium and phosphate in the small intestine, as well as the reabsorption of these compounds in the kidneys, thereby increasing their serum concentrations. Parathormone, which, along with vitamin D_3 and calcitonin, is considered the main regulator of calcium-phosphate metabolism, is reversibly inhibited by $1.25(OH)_2D_3$ [9, 10].

The homozygous missense mutation R396W in the *CYP24A1* gene, first described by Schlingmann *et al.* in 2011, was found in a seven-week-old girl who developed hypercalcaemia following oral vitamin D_2 supplementation at a dose of 600 000 IU, which was the standard regimen for rickets prevention at that time. The patient also presented an elevated $25(OH)D_3$ and normal PTH levels. After fluid replacement and systemic corticosteroid therapy, rapid normalisation of serum calcium levels was achieved. No recurrence of hypercalcaemia was observed in infancy or later, and the child's development was normal, suggesting that the high dose of vitamin D_2 was a trigger for the symptoms [11]. P.M. was recommended oral vitamin D_3 supplementation initially at a dose of 500 IU/day and then 1000 IU/day prior to receiving the genetic test result. However, persistently unsatisfactory $25(OH)D_3$ concentrations during the winter months and high normal $1.25(OH)_2D_3$ concentrations suggest poor compliance with the supplementation regimen.

The prevalence of the R396W mutation in the *CYP24A1* gene in the Polish population is estimated at 0.68% [12]. Common laboratory abnormalities reported in patients with known 24-hydroxylase abnormalities include hypercalcaemia, HC, suppression of PTH secretion and elevated or high normal $1.25(OH)_2D_3$ levels in the absence of vitamin D_3 supplementation [13]. In P.M., during a 7-year follow-up, serum calcium concentrations remained at or were only slightly above the upper normal limit. At that time, tests showed persistently decreased PTH levels and increased urinary calcium excretion in daily urine collection, as well as an increased Ca/Cr ratio.

At the age of ten years, the boy was diagnosed with renal HC, although tests performed earlier indicated absorptive type. In their paper, Aladjem *et al.* postulated that these diseases represent a continuum and the division itself may be artificial [14]. This, among other factors, is the cause of the limited usefulness of the Pak test in the diagnosis of HC and the reason why it is no longer routinely applied in clinical practice. Nevertheless, due to the persistently high values of Ca/Cr ratio and calcium excretion, the decision was made to use HTZ in pharmacotherapy as a drug widely administered for the treatment of HC in the paediatric population [3]. However, it is worth emphasizing that HTZ should not be a first-line treatment in *CYP24A1* gene mutation due to the possible side effect of hypercalcaemia, and monitoring of calcium-phosphate balance is recommended during the treatment. The use of this drug should be limited to cases with a *CYP24A1* gene mutation where dietary calcium and salt restriction as well as sunlight avoidance do not have a beneficial effect and in people with kidney stones, as in the case described here. Another drug that may be applied in the treatment of patients with HC, especially those caused by excessive $1.25(OH)_2D_3$ levels, is fluconazole. As a representative of azole antifungal drugs, it has an inactivating effect on 25- and 1α-hydroxylase, causing a reduction in the renal serum concentration of the vitamin D_3 metabolite, consequently decreasing the absorption of calcium from the gastrointestinal tract. During fluconazole therapy, it is worth paying attention to the monitoring of aspartate transaminase (AST) and alanine aminotransferase (ALT), due to the possible occurrence of hepatic reactions [15].

Children diagnosed with HC are at increased risk of osteoporosis and require follow-up bone mineral density (BMD) measurements. P.M. had a persistently reduced BMD value measured by DXA in the spine protocol, but the boy did not meet the diagnostic criteria for osteoporosis [16]. P.M. had no fractures to date and osteocalcin levels remained within reference limits for sex and age. Kidney function, monitored by glomerular filtra-

tion rate, was normal throughout the follow-up period. The boy continues to be physically active, playing sports, which may have a beneficial effect on the increase in bone mass in the future. However, he currently requires ongoing care not only from the Nephrology Clinic but also from the Osteoporosis Treatment Clinic.

CONCLUSIONS

A homozygous R396W mutation in the *CYP24A1* gene can lead to the development of HC and NC in adolescence, without producing general symptoms. Genetic factors, especially those related to abnormal vitamin D_3 metabolism, should be considered in the differential diagnosis of incidentally detected NC. It is essential that assessment of bone metabolism is included in the diagnostic and therapeutic process of patients with excessive urinary calcium excretion and, if indicated, treatment is implemented.

DISCLOSURE

The authors declare no conflict of interest.

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