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Case Report

Novel *CYP24A1* Mutation in a Young Male Patient with Nephrolithiasis: Case Report

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Keywords
CYP24A1 · Nephrolithiasis · Vitamin D 24-hydroxylase · 1,25-dihydroxy vitamin D3 · Hypercalcemia

Abstract
Background/Aims: The *CYP24A1* gene encodes the vitamin D 24-hydroxylase enzyme, which hydroxylates active forms of vitamin D into inactive forms. Biallelic mutations in the *CYP24A1* gene can lead to elevated levels of active vitamin D metabolites and, consequently, to hypercalcemia, hypercalciuria, nephrocalcinosis, and nephrolithiasis; however, monoallelic mutations have been associated only with milder phenotypes. In the present manuscript, we report the case of a young male patient who presented hypercalcemia and nephrolithiasis, suppressed parathormone, and elevated 1,25 dihydroxy vitamin D levels. ***Methods:*** Biochemical analyses were performed on Cobas 8000, F. Hoffmann-La Roche AG, Basel, Switzerland. The proband was initially evaluated for occult malignancies by body imaging, serum electrophoresis, and tumor markers, which did not reveal any pathology. DNA samples of the proband and his sibling were then examined using Sanger sequencing. ***Results:*** Genetic analysis revealed 2 compound heterozygous *CYP24A1* mutations (p.L148P and p.R223*). The novel nonsense *CYP24A1* mutation, p.R223*, was also found heterozygously in other family members with a medical history of nephrolithiasis. ***Conclusions:*** The identification of this gene mutation causing hypercalcemia, hypercalciuria, and renal stones allows the specific management of endogenous vitamin D production.

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Introduction

The *CYP24A1* gene encodes a member of the cytochrome P450 superfamily known as vitamin D 24-hydroxylase. This mitochondrial protein is largely present within the intestine and kidneys, catalyzing the hydroxylation of the major active forms of vitamin D, 1,25-dihydroxy vitamin D3 (1α,25$[OH]_2D_3$) and 25-hydroxyvitamin D3 (25$[OH]D_3$), into inactive forms [1]. The latter is a pre-hormone produced in the liver through the enzymatic hydroxylation of cholecalciferol/ergocalciferol (cholecalciferol 25-hydroxylase, CYP2R1) [2], which is then converted into 1α,25$[OH]_2D_3$ (by 25$[OH]D_3$ 1-alpha-hydroxylase, CYP27B1), a process that mainly occurs in the kidney's proximal tubule [3]. 25$[OH]D_3$ is the precursor of the major active form of vitamin D (1α,25$[OH]_2D_3$); however, it is also capable of activating the vitamin D receptor despite its lower affinity [4, 5].

1α,25$[OH]_2D_3$, along with parathormone (PTH) and calcitonin, is an essential regulator of calcium-phosphate metabolism, stimulating calcium and phosphate absorption through the intestines and kidneys in addition to enhancing bone remodeling [6–8]. Further, CYP24A1 is upregulated by 1α,25$[OH]_2D_3$, thus ensuring a strict feedback regulation. Loss-of-function mutations in *CYP24A1* can lead to elevated levels of 1α,25$[OH]_2D_3$ and 25$[OH]D_3$ that can cause absorptive hypercalcemia and hypercalciuria, which leads to complications such as nephrocalcinosis and nephrolithiasis. Calcium deposition in mitochondrial structures and the consequent altered metabolism damage the renal epithelium and provoke tubular necrosis, potentially resulting in chronic kidney disease [9].

The present case report describes a compound heterozygous *CYP24A1* mutation in a young male patient and a heterozygous nonsense *CYP24A1* in family members affected by renal stone disease.

Case Report

We report the case of a male Czech patient of Caucasian descent whom initially presented a renal colic at 19 years of age. Renal ultrasonography showed bilateral nephrolithiasis and nephrocalcinosis. Prior to this renal colic episode, the patient had experienced backache and visible hematuria several times after performing sport activities. He did not notice any other health issues. Therapeutic intervention consisted of left-sided extracorporeal shock wave lithotripsy.

A detailed biochemical analysis (Cobas 8000, F. Hoffmann-La Roche AG, Basel, Switzerland) 6 months after the first renal colic episode revealed hypercalcemia (serum calcium levels of 2.87 mmol/L, adjusted for albumin) and hypercalciuria (urinary calcium 11.62 mmol/24 h, urinary ratio calcium/creatinine 0.68 mmol/mmol), preserved renal function estimated glomerular filtration rate ~124 mL/min/1.73 m2 using chronic kidney disease epidemiology collaboration formula, and suppressed PTH levels (<0.4 pmol/L). Increased level of 1α,25$[OH]_2D_3$ (226 pmol/L) and 25$[OH]D_3$ (98.5 nmol/L) within the reference range was found (Table 1). The patient was not under regular medication, had no history of vitamin D or calcium supplementation, no diet or fluid intake abnormalities, and no other dietary supplements or recent history of tanning bed use.

Nephrolithiasis was recurrent and treated by percutaneous nephrolithotomy 3 years later. The chemical analysis of extracted urinary stones revealed calcium phosphate in the form of apatite (50%) and brushite (45%).

Given the persistence of hypercalcemia, the proband was initially evaluated for occult malignancies. Body imaging (skeletal scintigraphy, abdomen ultrasonography, and computed chest tomography scan) did not reveal any pathology. Serum electrophoresis and tumor

Table 1. Characteristics of the proband and his sibling

	Proband	Sibling
Serum calcium, mmol/L (LRR 2.15–2.5 mmol/L)	2.87	2.74
Ionized calcium, mmol/L (LRR 1.13–1.32 mmol/L)	1.54	1.25
Serum phosphorus, mmol/L (LRR 0.81–1.45 mmol/L)	0.93	1.07
Serum magnesium, mmol/L (LRR 0.66–1.07 mmol/L)	0.82	0.88
Serum creatinine, μmol/L (LRR 59–104 μmol/L)	121	90
Urinary calcium, mmol/24 h	11.62	5.18
PTH, pmol/L (LRR 0.5–6.2 pmol/L)	<0.4	3.6
25(OH)D_3, nmol/L (LRR 75–250 nmol/L	98.5	33
1α,25$(OH)_2D_3$, pmol/L (LRR 60–207 pmol/L)	226	126
ALPbf, μg/L (LRR 5.5–22.9 μg/L)	8.6	10
P1NP, μg/L (LRR for men under 30 years not available)	89.36	Not tested
Beta CTx, μg/L (LRR for men under 30 years not available)	0.917	Not tested
Bone densitometry	Total *Z* score: Left hip 0.5 Lumbar spine 0.5	Not performed
Nephrolithiasis	Renal colic at 19 years of age Present bilaterally (USS)	Renal colic at 22 years of age Not present (USS)
Nephrocalcinosis	Present bilaterally (USS)	Not present (USS)
CYP24A1 mutation	p.L148P + p.R223*	p.R223*

Basic initial clinical characteristic of the male patient and his sister. The examination was performed in June.

PTH, parathyroid hormone; ALP_{bf}, alkaline phosphatase – bone fraction; P1NP, procollagen type 1 N terminal, propeptide; CTx, crosslaps; LRR, lab reference range; USS, ultrasound scan; 1α,25$(OH)_2D_3$, 1,25-dihydroxy vitamin D3; *, mutation resulting in a premature stop codon.

makers alpha-fetoprotein, carcinoembryonic antigen, C15, C19, and chromogranin – A were within reference range. Bone densitometry (DEXA – dual-energy x-ray absorptiometry) and bone turnover markers were also within the reference range for age, gender, and body mass index (Table 1).

The patient's family history was informative. Both the patient's mother and maternal uncle suffered from nephrolithiasis since their teenage years. The patient's sister (23 years old) underwent an episode of renal colic (with spontaneous passage of a stone, at 22 years of age).

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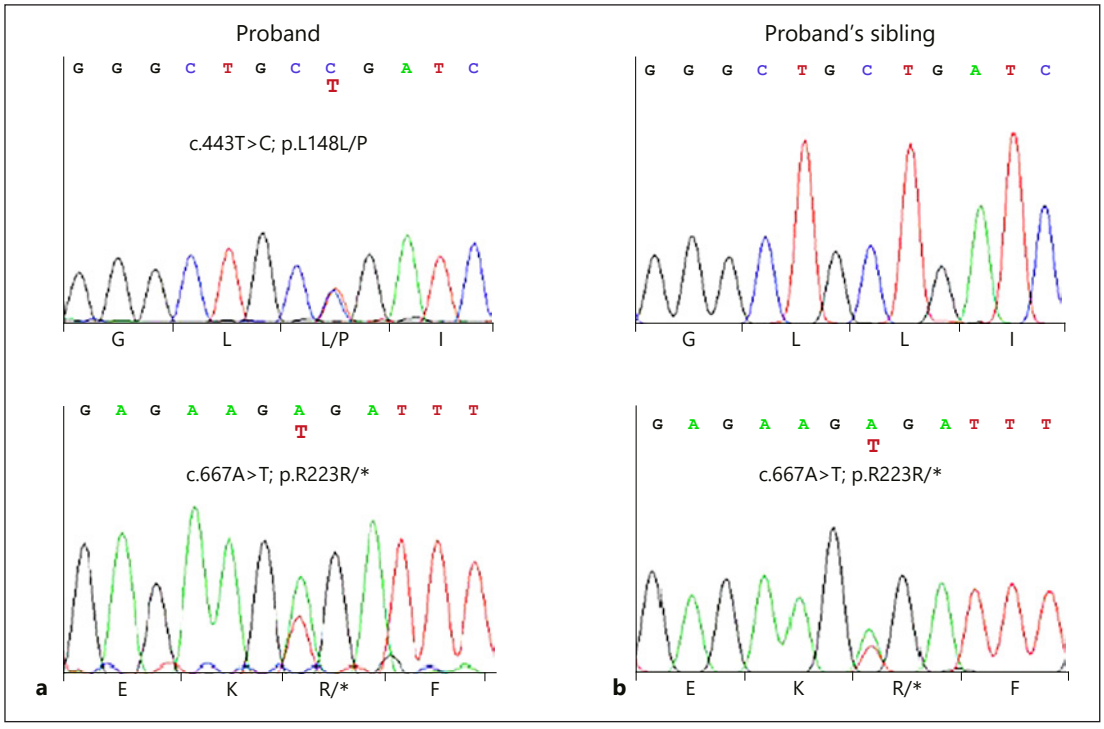

Fig. 1. Sequence chromatograms of the proband and his sister. **a** Combination of a missense mutation (c.443T>C; p.L148P) and nonsense codon mutation (c.667A>T; p.R223*) in the proband; and (**b**) novel nonsense mutation (c.667A>T; p.R223*) in its heterozygous state in the proband's sister (reference sequence NM_000782).

Table 2. In silico analysis of *CYP24A1* variants

	MutationTaster	SIFT	PolyPhen-2	ExAC	gnomAD
c.667A>T p.R223*	Disease causing	NA	NA	3 het alleles in 121,400 alleles	4 het alleles in 246,230 alleles
c.443T>C p.L148P	Disease causing	Deleterious	Probably damaging	19 het alleles in 121,306 alleles	40 het alleles in 276,994 alleles

In silico analysis of *CYP24A1* variants.

het, heterozygous; SIFT, sorting intolerant from tolerant; ExAC, the exome aggregation consortium; gnomAD, the genome aggregation database; NA, not applicable; *, mutation resulting in a premature stop codon.

After this episode, occasional hypercalcemia was detected in this sibling (Table 1). The total calcium levels were tested, obtaining the values of 2.74, 2.31, 2.33, and 2.30 mmol/L in the 6 months period of the study. On the other hand, free calcium levels were estimated in 1.25–1.30 mmol/L. The patient's father had no health problems related to nephrolithiasis or hypercalcemia.

Following informed written consent, DNA samples of the proband and his sibling were examined using Sanger sequencing. Two compound heterozygous pathogenic mutations in the *CYP24A1* gene were identified in the proband; these included a previously reported missense mutation (c.443T>C, p.L148P) and a novel nonsense mutation (c.667A>T, p.R223*). The patient's sister was heterozygous for the nonsense mutation p.R223* and wild type for

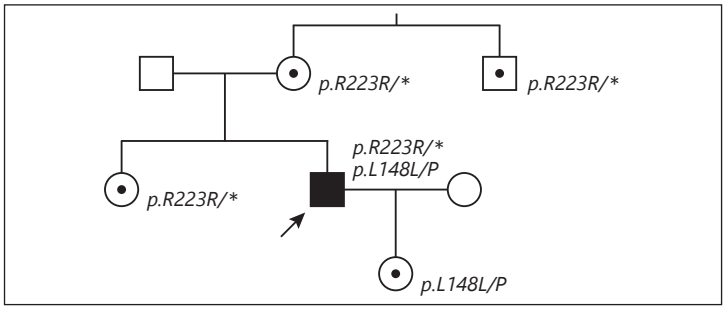

Fig. 2. Family tree of *CYP24A1* mutations in the proband's relatives. The proband is marked with an arrow, using black filling to indicate biallelic mutations. The blank symbols represent unaffected persons, whereas a dot symbolizes a heterozygous load.

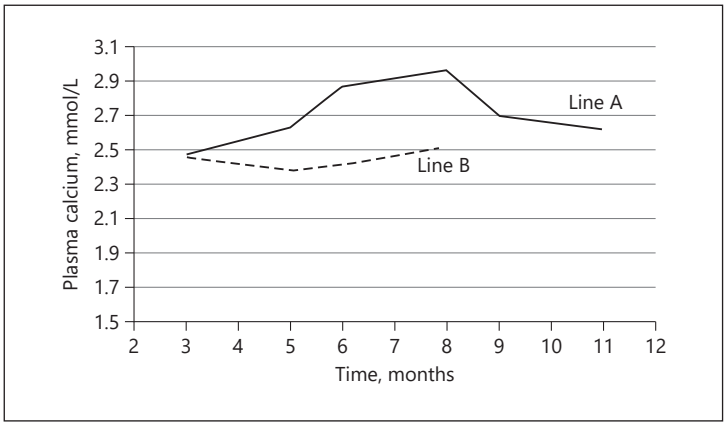

Fig. 3. Representation of annual calcium level fluctuations in the plasma of the proband. Line A – no regime intervention. Line B – with regime intervention.

the remaining allele (Fig. 1; Table 2). Segregation analysis revealed that the mutation p.R223* was also present in heterozygous state in the patient's mother and maternal uncle, both of whom had a history of renal stones. The patient's father was wild type for both alleles. Further, the heterozygous missense mutation (p.L148P) was found in the patient's newborn daughter, which also presented a wild-type status for the second allele (Fig. 2).

Serum biochemistry was performed repeatedly in the proband through different seasons of the year. Total serum calcium levels ranged from 2.38 to 2.96 mmol/L (Fig. 3), with a tendency toward higher levels during summer and becoming lower at the end of winter. We also observed corresponding plasma-level fluctuations of $25[OH]D_3$ (mean values: [1] without the regime intervention summer – 106.25 nmol/L; [2] with the regime intervention summer/winter – 97.1/52.35 nmol/L; summer April – September/winter October/March) and $1\alpha,25[OH]_2D_3$ (mean values: [1] without the regime intervention summer – 204.5 pmol/L; [2] with the regime intervention summer/winter – 120 /98.5 pmol/L; summer April–September/winter October/March), with the highest values observed during August (summer) and the lowest in February (winter).

The patient was treated empirically with a fluid regimen, restriction of dairy products, and L-methionine 500 mg 3 times a day. Consistent use of sunscreens, avoidance of tanning beds, and products containing vitamin D was recommended.

Discussion

The human *CYP24A1* gene encodes a mitochondrial protein of 514 amino acids possessing 23- and 24-hydroxylase activity. This gene is mostly expressed in tissues responsive to $1\alpha,25[OH]_2D_3$, which includes kidneys, intestines, and skin [10–12].

CYP24A1 mutations cause the reduced function of the vitamin D 24-hydroxylase enzyme, leading to persistent active metabolites of vitamin D, 1α,25$[OH]_2D_3$ and 25$[OH]D_3$. Increased levels of these active forms of vitamin D can lead to hyperabsorptive hypercalcemia and hypercalciuria and, consequently, to nephrolithiasis and nephrocalcinosis. Although loss-of-function mutations in *CYP24A1* represent a relatively rare metabolic disorder [10], it should always be considered as the underlying cause of calcium nephrolithiasis, especially in young patients with positive family history of nephrolithiasis.

In the present case, the young male patient showed symptoms of hypercalcemia and hypercalciuria, elevated 1α,25$[OH]_2D_3$ level, and suppressed PTH, which are the typical biochemical characteristics of patients carrying a loss-of-function mutation in *CYP24A1*. Although the patient's biochemical characteristics, in addition to the positive family history and the young age of the patient, were indicative of an underlying genetic disorder, more common causes for the increased levels of 1α,25$[OH]_2D_3$, such as exogenous supply and endogenous overproduction, should be excluded first. Thus, the patient was carefully interrogated about his diet regimen and supplementation. Further, to exclude an occult malignancy as a potential source of 1α,25$[OH]_2D_3$ overproduction, body imaging and serum tumor marker analyses were performed. However, no pathologic result was found and thus there was no indication of exogenous or endogenous cause for the increased levels of 1α,25$[OH]_2D_3$.

Accordingly, the prior analyses were followed by sequencing *CYP24A1* in search of pathogenic variants. This revealed 2 pathogenic *CYP24A1* alleles; the first was a known missense mutation (p.L148P) that results in 25–50% decreased activity of CYP24A1 [10, 13, 14]. This mutation was found in the patient's newborn daughter as well; however, it was not found in either of the patient's parents. The patient's father presented a wild-type genotype which is consistent with the lack of nephrolithiasis symptoms. Therefore, this *CYP24A1* missense mutation (p.L148P) may have arisen de novo or nonpaternity should be considered.

The second *CYP24A1* mutation found in the proband was a novel nonsense mutation (p.R223*). This mutation was also found heterozygously in the patient's sister, mother, and maternal uncle. All of these relatives had symptoms of nephrolithiasis since their youth. The patient's sister had evidence of occasional hypercalcemia. Her abdomen ultrasonography did not reveal nephrolithiasis or nephrocalcinosis. The familiar medical history on the maternal side suggests that the novel allele (p.R223*), even in its heterozygous state, may have a significant impact on calcium metabolism and leads to nephrolithiasis.

The hypercalcemia with hypercalciuria causing progressive nephrolithiasis and nephrocalcinosis in our young male patient was caused by compound biallelic *CYP24A1* mutations that led to increased activity of the vitamin D metabolite 1α,25$[OH]_2D_3$. The management of our patient included:

Biochemical and Body Imaging Monitoring

Blood samples from the proband were analyzed repeatedly at different seasons of the year; however, regular examination could not be achieved due to poor compliance by the patient. Fluctuations of vitamin D plasma levels during seasonal sunlight exposure were detected, as described previously [15], with the highest values observed during August (summer) and the lowest values during February (winter). Corresponding fluctuations of calcemia were noticed. Further examination by abdomen ultrasonography showed progressive nephrolithiasis.

Bone densitometry was also performed to identify any loss of bone tissue, which is an essential examination tool in patients with calcium/phosphate metabolism disorders. Despite that vitamin D is crucial in sufficient quantity for bone formation and mineralization, high levels can also result in inadequate bone resorption [16]. In addition, basic bone turnover markers were examined, with P1NP and Beta CTx showing a very modest increase (in relation

to the age, gender, and BMD of the patient), DXA showed values entirely within the normal range and the patient had no medical history of bone fractures. Therefore, we suggest that a calcium/phosphate metabolic disorder related to *CYP24A1* mutations (p.L148P, p.R223*) does not seem to have a negative influence on bone quality during the young age of the afflicted patient. However, bone quality could be influenced in a positive manner by the active sport habits of the proband, as we know that physical activity contributes markedly to increased bone density [17].

Regimen Arrangement and Medication

A high fluid intake, avoiding unnecessary sun exposure and tanning beds, and minimization of dietary vitamin D intake was recommended. Supplementation with L-Methionine was recommended for urinary acidification to prevent calcium phosphate stone formation [18].

The successful short-term therapy with azole agents has been described in patients with *CYP24A1* mutations for their ability to inhibit cytochrome P450-dependent enzyme systems and thus inhibiting liver metabolism of vitamin D [10, 19]. Regardless, long-term therapy with azole agents is not suitable because of its potentially serious side effects, including hepatotoxicity. Recently, encouraging results have shown that low doses of fluconazole could be considered in the treatment of patients with *CYP24A1* mutations due to its favorable side-effects profile [20, 21].

Conclusions

Biallelic mutations in the *CYP24A1* gene can lead to elevated levels of active vitamin D metabolites, hypercalcemia, hypercalciuria, and nephrolithiasis. In the present case, the genetic analysis of the *CYP24A1* gene in a young male patient revealed 2 compound heterozygous mutations, p.L148P and p.R223*. The latter represents a novel nonsense mutation that was also found in the maternal members of the patient's family, whom also had evidence of renal stone disease. The identification of this molecular genetic cause of renal stones enables the specific management of these patients to minimize endogenous vitamin D production.

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Statement of Ethics

Ethical approval for this study was provided by Newcastle Upon Tyne Research Ethics Committee. The patients supplied written informed consent for the publication of this case.

Disclosure Statement

The authors declare no conflicts of interest.

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Author Contributions

J.J., R.H., L.P., and V.P.: performed clinical studies, conceived the study, and wrote the draft. S.A. and J.A.S.: performed molecular genetic analysis. J.A.S.: performed in silico analysis and edited the manuscript.

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