Case Report

Hereditary Hypercalcemia Caused by a Homozygous Pathogenic Variant in the CYP24A1 Gene: A Case Report and Review of the Literature

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1. Introduction

Loss of function mutations of CYP24A1 gene, which is involved in vitamin D catabolism, cause vitamin D-mediated PTH-independent hypercalcemia. The phenotype varies from life-threatening forms in the infancy to milder forms in the adulthood. Case Presentation. We report a case of a 17-year-old woman with a history of nephrolithiasis, mild PTH-independent hypercalcemia (10.5 mg/dL), and high serum 1,25(OH)2D concentrations (107 pg/mL). Other causes of hypercalcemia associated with the above biochemical signature were excluded. Family history revealed nephrolithiasis in the sister. Blood testing in first-degree relatives showed serum PTH in the low-normal range and 1,25(OH)2D at the upper normal limit or slightly elevated. The CYP24A1 gene analysis revealed a known homozygous loss-of-function pathogenic variant (c.428_430delAAG, rs777676129, p.Glu143del). The panel of vitamin D metabolites evaluated by liquid chromatography showed the typical profile of CYP24A1 mutations, namely, low 24,25(OH)2D3, elevated 25(OH)D3:24,25(OH)2D3 ratio, and undetectable 1,24,25(OH)3D3. The parents and both the siblings harbored the same variant in heterozygosis. We decided for a watchful waiting approach and the patient remained clinically and biochemically stable over a 24-month followup. Conclusion. CYP24A1 gene mutations should be considered in cases of PTH-independent hypercalcemia, once that more common causes (hypercalcemia of malignancy, granulomatous diseases, and vitamin D intoxication) have been ruled out.

Pathogenic variants (PVs) in the human cytochrome P450 24 subfamily A member 1 (CYP24A1) gene are associated with Idiopathic Infantile Hypercalcemia (IIH, OMIM 143880), a rare disease recently related to vitamin D catabolism impairment [1]. The CYP24A1 gene encodes a 24-hydroxylase enzyme, which catalyzes the degradation of the active form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)2D] by multiple pathways [2]. CYP24A1 loss of function leads to an increase in serum 1,25(OH)2D concentration, which may be associated with various degrees of hypercalcemia and hypercalciuria and low-or-undetectable plasma parathyroid hormone (PTH) levels. The phenotype of IIH embraces a wide range of clinical scenarios [3], from severe forms diagnosed early in the infancy (severe hypercalcemia associated with dehydration, vomiting, nephrocalcinosis, and sometimes death) [4] to milder forms, often diagnosed in the adulthood during workout for recurrent nephrolithiasis [5]. Since the recognition in 2011[1] that PVs in CYP24A1 are responsible for IIH, a large number of cases have been reported, leading to an increased insight into the diagnostic and therapeutic management of this disease [6, 7].
Herein we describe a case of recurrent nephrolithiasis and moderate PTH-independent hypercalcemia of undetermined origin referred to our outpatient clinic for further investigation. The familial nature of hypercalcemia prompted us to search for genetic causes and we identified a loss of function variant in the \textit{CYP24A1} gene.

### 2. Case Presentation

A 17-year-old woman was referred to the Endocrine Unit of the University Hospital of Pisa for further evaluation of hypercalcemia associated with undetectable/low PTH levels.

Her clinical history was unremarkable except for a previous admission to the local Emergency Unit for renal colic 3 years before; an abdominal ultrasound revealed unilateral kidney stones. On that occasion, the patient was treated with analgesics and hydration and no further investigations were performed. One year later she underwent extracorporeal shockwave lithotripsy for the recurrence of renal colics. At that time, routine blood tests revealed hypercalcemia [12.4 mg/dL; (reference range 8.4-10.2)], hypercalciuria [390 mg/24h, (100-300)], and undetectable PTH (< 4 pg/mL; NV 8-40) and a 25-hydroxyvitamin D [25(OH)D] level of 37.4 ng/mL. The family history was unremarkable with the exception of nephrolithiasis in the sister.

At admission, physical examination was normal, with no evidence of major bone abnormalities. Lab tests confirmed hypercalcemia, hypercalciuria, and low/undetectable PTH levels; bone turnover markers were slightly above the upper limit of adult reference range (Table 1). Routine biochemistry was normal. Chest X-ray and abdominal and neck ultrasound were unremarkable. The long lasting hypercalcemia, the negative medical history beyond nephrolithiasis, and the normal imaging studies made unlikely the hypothesis of paraneoplastic hypercalcemia. Further evaluation revealed elevated serum levels of 1,25(OH)\textsubscript{2}D suggesting vitamin D-dependent hypercalcemia. A granulomatous disease could be ruled out on the basis of normal serum concentration of angiotensin converting enzyme and the absence of specific signs at chest X-rays.

Because of the young age of the patient and the family history of nephrolithiasis, biochemical tests were performed in first-degree relatives. Total and ionized serum calcium, phosphate, PTH, and 1,25(OH)\textsubscript{2}D levels were in the normal range in both parents, who had a low vitamin D status. Interestingly, in the siblings PTH concentration was in the low-normal range and 1,25(OH)\textsubscript{2}D at the upper normal limit or slightly elevated (Table 2). The latter findings, together with the biochemical profile of the patient, suggested that hypercalcemia might be due to an impairment of the CYP24A1 catabolic pathway. The genetic analysis in the proband was made using High Resolution Melting Analysis (HRMA) [8] and further confirmed using gene amplification and sequencing [9], revealing a known homozygous PV (c.428_430delAAG, rs777676129, p.Glu143del) in the \textit{CYP24A1} gene (Figure 1(a)). The same heterozygous variant was detected in the parents.

### Table 1: Clinical and biochemical data at admission to our clinic.

| Analyte                            | Result   | Normal adult reference range |
|------------------------------------|----------|-----------------------------|
| Total calcium (mg/dL)              | 10.5     | 8.6-10.2                    |
| Ionized calcium (mmol/L)           | 1.35-1.36-1.45* | 1.13-1.32                   |
| Phosphate (mg/dL)                  | 3.1      | 2.7-4.5                     |
| Magnesium (mg/dL)                  | 2.02     | 1.7-2.2                     |
| Albumin (g/dL)                     | 4.6      | 3.6-5.2                     |
| PTH (pg/mL)                        | < 4-7*   | 8-40                        |
| Calcitonin (pg/mL)                 | < 2      | < 11.5                      |
| 25-hydroxy vitamin D (ng/mL) \(\textsuperscript{\text{§}}\) | 30.3     |                             |
| 1,25-dihydroxyvitamin D (pg/mL) \(\textsuperscript{\text{∫}}\) | 107      | 20-67                       |
| Osteocalcin (ng/mL)                | 61.6     | 6.8-34                      |
| Bone-specific alkaline phosphatase (mcg/L) | 23       | 2-20                        |
| Carboxy-terminal collagen crosslinks (ng/mL) | 1.042    | 0.112-0.738                 |
| Urine calcium (mg/24h)             | 150-410-455* | 100-321                     |
| Urine phosphates (mg/24h)          | 697-875* | 400-1300                    |
| Urine magnesium (mg/24h)           | 140-164* | 60-120                      |
| Creatinine (mg/dL)                 | 0.69-0.72+ | 0.5-0.9                     |
| Urine creatinine (mg/24h)          | 1050-1085-1148* | 740-1570                  |
| Angiotensin converting enzyme (U/L) | 80       | 65.8-114.4                 |

\(\textsuperscript{\text{§}}\): 25(OH)D was assayed at University of Pisa laboratory as total 25(OH)D (i.e. the sum of 25(OH)D\textsubscript{2} + 25(OH)D\textsubscript{3}) using a chemiluminescence immunoassay (IDS-iSYS, Immunodiagnostics systems, Boldon, Tyne and Wear, UK)

\(\textsuperscript{\text{∫}}\): 1,25(OH)\textsubscript{2}D was assayed at University of Pisa laboratory as total 1,25(OH)\textsubscript{2}D (i.e. the sum of 1,25(OH)\textsubscript{2}D\textsubscript{2} + 1,25(OH)\textsubscript{2}D\textsubscript{3}) using a radioimmunoassay (IDS, Immunodiagnostics systems, Boldon, Tyne and Wear, UK)
Table 2: Clinical and biochemical findings in the patient's first degree family members.

|                | I.1 | I.2 | II.1 | II.2 |
|----------------|-----|-----|------|------|
| Age (years)    | 52  | 53  | 26   | 20   |
| History of nephrolithiasis | No  | No  | Yes  | No   |
| Total calcium (mg/dL) | 10  | 9.8 | 9.9  | 9.8  |
| Ionized calcium (mmol/L) | 1.28| 1.23| 1.29 | 1.26 |
| Phosphate (mg/dL)    | 3.6 | 2.9 | 3.9  | 3.1  |
| PTH (pg/mL)          | 17  | 18  | 11   | 8    |
| 25 hydroxyvitamin D (ng/mL) | 7.8 | 17.3| 28.4 | 22.5 |
| 1,25-dihydroxyvitamin D (pg/mL) | 39  | 37  | 72   | 66   |

The reported values are the mean of two independent samples collected in two consecutive days. For family member identification see Figure 1(b). See Table 1 for the normal adult reference range at our laboratory and details about 25(OH)D and 1,25(OH)2D assays.

Reference Sequence: NM_000782.5

AGATCAAACGGTGAAAGGCCCTATCGCGACTACCGCAAGGAAGCTACGGGCTGTGATCTCTGG WILD TYPE

Mother

Father

Proband

Figure 1: (a) Sequences of the CYP24A1 exon 2 obtained by proband and her parents. CYP24A1 gene amplification and sequencing were performed as reported. Sequences of the exon 2 obtained by proband and her parents were shown. Arrows indicate the position of c.428_430delAAG heterozygous and homozygous variant in the parents and the proband, respectively. (b) Family tree.

and the siblings (Figure 1(b)). The parents excluded consanguinity, even though they came from the same small village.

To complete the biochemical profile of vitamin D metabolites, liquid chromatography tandem mass spectrometry (LC-MS/MS) was run on stored serum samples of all the family members. Serum samples were prepared by immunoextraction and derivatized with 4-[(2-(6,7-dimethoxy-4-methyl-3,4-dihydroquinazolinyl)ethyl]-1,2,4-triazoline-3,5-dione (DMEQ-TAD), as reported [10]. We observed that the proband exhibited low 24,25(OH)2D3 (0.42 ng/mL) and elevated 25(OH)D3:24,25(OH)2D3 ratio (118; cutoff >80) which confirmed the diagnosis of impaired CYP24A1 function. A more rigorous chromatographic method[11] was also used to assay the same sample (25(OH)D3:24,25(OH)2D3 ratio = 3117; cutoff >140), which also indicated inappropriately low levels of 24,25(OH)2D3 in the proband. The other family members, who present as heterozygous variants, exhibited essentially normal serum 24,25(OH)2D3 concentrations and 25(OH)D3:24,25(OH)2D3 ratios (Table 3 and Figure 2).
Table 3: Liquid chromatography tandem mass spectrometry analysis results.

| Metabolite                | I.1  | I.2   | II.1 | I.2   | II.3 |
|---------------------------|------|-------|------|-------|------|
| 25(OH)D$_3$ (ng/mL)       | 13.16| 28.88 | 38.03| 28.25 | 49.87|
| 24,25(OH)$_2$D$_3$ (ng/mL)| 0.44 | 1.14  | 1.94 | 1.66  | 0.02 |
| 25(OH)D$_3$/24,25(OH)$_2$D$_3$ ratio | 30.1 | 25.4  | 19.6 | 17.1  | 3117 |
| 1,25(OH)$_2$D$_3$ (pg/mL) | 41.1 | 37.4  | 66.9 | 66.6  | 118.4|
| 1,24,25(OH)$_3$D$_3$ (pg/mL) | <281 | <281  | <281 | <281  | <281 |

Because of the mild hypercalcemia, we did not advise pharmacologic treatments aimed at modulating 1,25(OH)$_2$D$_3$ metabolism and we recommended maintenance of adequate hydration and avoidance of unprotected excessive sunlight exposure. Followup evaluation up to 24 months showed that the patient was in an overall stable condition, with serum calcium concentration slightly above the upper normal limit and renal ultrasound showing no recurrent nephrolithiasis.

The patient and the family gave written informed consent for the genetic analysis and the use of their clinical data for scientific purposes, including publication.

3. Discussion

Hypercalcemia is a common disorder, with a prevalence of 1/500 patient in the outpatient setting[12]. Primary hyperparathyroidism is the most common cause of hypercalcemia [13]. Vitamin D-induced hypercalcemia is a heterogeneous group of diseases that includes vitamin D intoxication, granulomatous diseases, and abnormalities of vitamin D metabolism. Hypercalcemia due to loss of function variants in the CYP24A1 gene is a genetic disorder recently described in patients with IIH [1]. Nowadays the name “idiopathic infantile hypercalcemia” is considered a misnomer [6], because in most patients a genetic cause can be identified (namely, a loss-of-function mutation in the CYP24A1 or in the SCL34A1 or large deletions on chromosome 7 causing the Williams-Beuren syndrome), and the clinical phenotype is no longer confined to infancy.

Vitamin D is mainly produced in the skin or supplied by dietary sources. It undergoes an initial activation by 25-hydroxylation in the liver, catalyzed by CYP2R1, thus generating 25(OH)D. A second hydroxylation by 1α-hydroxylase (CYP27B1) takes place mainly in the kidney, but also in several extrarenal tissues, and converts 25(OH)D to 1,25(OH)$_2$D$_3$, the active form of vitamin D [14]. The CYP24A1 gene, located at 20q13.2, encodes the cytochrome P450 component of the mitochondrial 24-hydroxylase enzyme, which catalyzes the degradation of 25(OH)D and 1,25(OH)$_2$D into the multistep 24-oxidation pathway to calcitriol [15, 16]. The CYP24A1 gene has been cloned in animals [17, 18] and humans [19]. Its expression is induced by vitamin D receptor agonists [20] by interacting with a vitamin D response element in the promoter of the gene [18]. Furthermore, many hormones...
involved in bone mineral metabolism regulate the CYP24A1 enzyme. PTH attenuates the 1,25(OH)₂D-mediated induction of the CYP24A1 gene, through a direct effect on the transcription of the gene [21]. Fibroblast growth factor 23 (FGF23) decreases 1,25(OH)₂D levels by inhibiting CYP27B1 expression and inducing CYP24A1 in the kidney [22].

CYP24A1 loss-of-function variants are recognized as a cause of vitamin D-mediated hypercalcemia [23]. As a matter of fact, defective 24-hydroxylase activity results in high 1,25(OH)₂D concentrations and, as a consequence, PTH-independent hypercalcemia with hypercalciuria, in the absence of hypophosphatemia. Twenty-one PVs of CYP24A1 have so far been described in literature [6]. The disease is inherited as a recessive trait and a genotype-phenotype correlation has been postulated [3].

Biiallelic variants, independently of whether in homozygosis or compound heterozygosis, result in a significant phenotype [1, 24], which may range from severe to mild and misrecognized forms [1, 3]. The majority of cases diagnosed in early infancy presents the classic manifestations of IIH, namely, severe hypercalcemia, dehydration, polyuria, vomiting, failure to thrive, nephrocalcinosis, muscular hypotonia, and lethargy, occasionally leading to death [1, 4, 23]. Conversely, cases with biallelic mutations diagnosed in the adulthood commonly present mild to moderate hypercalcemia [23] and recurrent nephrolithiasis [5, 25].

It is still not clear whether different PVs may be associated with different phenotypes. As a matter of fact, the specific PV may influence the extent of the variation in the enzyme activity, thus contributing to the severity of the clinical picture [26]. Specifically, most patients harboring p.Glu143del biallelic mutation present a late-onset clinical picture, which mainly consists in urological manifestations, such as nephrolithiasis and/or nephrocalcinosis [26–29]. Until now, the small number of patients so far reported does not allow drawing significant genotype-phenotype correlations both for the p.Glu143del and for other rarer PVs.

Data about heterozygote carriers are mainly derived from studies involving relatives of index cases carrying biallelic variants. Whether the presence of monoallelic mutation can lead to an overt clinical phenotype is still a matter of debate [24]. Heterozygote carriers usually have a milder biochemical phenotype compared to patients affected by biallelic variants [7, 25, 30, 31], with mild hypercalcemia and less frequently nephrolithiasis [25]. Moreover, others suggest that these patients are mainly asymptomatic and that incidental nephrolithiasis may be due to other causes [7]. This is in keeping with the finding in our kindred, where the clinical and biochemical picture in heterozygous mutation carriers was heterogeneous, thus suggesting that other factors might contribute to the phenotype (see below). Conversely, a study reported two children with monoallelic CYP24A1 intron-exon splice junction mutations (IVS5+1G>A and IVS6-2A>G) with severe hypercalcemia and the classical phenotype of IIH, commonly due to biallelic mutation [31]. The authors postulated that the symptomatic picture could be due to an autosomal dominant inheritance pattern. Additional environmental factors or predisposing conditions, including vitamin D administration [1, 32], sunlight exposure [33], and pregnancy [34], may contribute to the development of a clinically relevant phenotype in patients with either biallelic or monoallelic mutations.

In clinical practice loss-of-function mutations in CYP24A1 should be searched in patients with hypercalcemia and hypercalciuria, associated with low serum PTH concentrations and 1,25(OH)₂D levels in the upper normal range or slightly above. Serum 25(OH)D concentrations can be low, normal, or mildly elevated. The finding of markedly elevated 25(OH)D levels raises the suspicion of vitamin D intoxication. An additional diagnostic clue is the measurement of 24,25(OH)₂D₃, the main product of CYP24A1 [10], that is expected to be decreased. Unfortunately, the assay of this metabolite is not routinely available. Moreover, measurement of absolute 24,25(OH)₂D₃ concentration alone has limited diagnostic value, because low 24,25(OH)₂D₃ can also occur due to low 25(OH)D₃ in addition to CYP24A1 mutation. We observed in patient I.1 (heterozygote carrier) a 24,25-(OH)₂D₃ concentration of 0.56 ng/mL, similar to patient II.3 (proband). Calculation of a 25(OH)D₃:24,25(OH)₂D₃ ratio indicates when 24,25-(OH)₂D₃ concentration is inappropriately low for a given 25(OH)D₃ level. In unaffected individuals serum levels of 24,25(OH)₂D₃ are proportional to those of 25(OH)D and the ratio ranges between 5 and 25. In the patients affected by CYP24A1 loss-of-function mutations, the ratio is markedly increased, up to more than 80 [10, 25, 31] by short method and over 140 by the long method, reported here. Currently, the measurement of the 25(OH)D:24,25(OH)₂D₃ is considered the most accurate screening tool for the identification of patients to be submitted to genetic testing.

Treatment of patient with CYP24A1 PVs is directed towards the control of hypercalcemia. In severe cases, treatment starts with vigorous fluids administration eventually followed by a loop diuretic as furosemide when the patient is adequately hydrated. Other options include calcitonin and bisphosphonates. The use of corticosteroid to reduce intestinal calcium absorption is not advised in the setting of hypercalcemia related to CYP24A1 PVs [35] because its therapeutic benefit requires a functioning CYP24A1 enzyme [36]. Another therapeutic approach aims to modulate the metabolism of 1,25(OH)₂D. Ketoconazole reduces the synthesis of 1,25(OH)₂D by inhibiting the CYP27B1 enzyme and has been effective in patients affected by CYP24A1 loss-of-function mutations in the acute and in the chronic setting [31, 37]. Fluconazole has been proposed as a valid alternative to ketoconazole, especially for the less pronounced long-term toxicity [38].

Rifampin, given its capacity to induce CYP3A4 enzyme, catalyzes a nonspecific hydroxylation of 1,25(OH)₂D to an inactive metabolite, 1,23,25(OH)₃D₃, and has been used with overall good results [39].

Independently of the pharmacologic approaches, it seems reasonable to avoid exogenous vitamin D supplementation, implement a low-calcium diet, and avoid unprotected excessive sunlight exposure, even though the benefit of these approaches remains to be clarified [6].

In the case of females of child-bearing age with biallelic CYP24A1 mutations, it should be noted that the advent of
pregnancy constitutes an added risk for hypercalcemia, as the placenta is a known site of additional 1,25(OH)_{2}D_{3} synthesis. Several cases have been described in which the patient’s hypercalcemia is exacerbated during recurrent pregnancies, a condition which dissipates during nonpregnant periods[28, 34].

4. Conclusion

The patient reported herein represents a typical case of homozygous CYP24A1 loss-of-function mutation discovered in the early adulthood with recurrent nephrolithiasis. About 2 years passed from the initial episode of renal colic to the first measurement of serum calcium and the discovery of hypercalcemia. This is a common finding in many adult patients presenting with nephrolithiasis and hypercalcemia due to PV of the CYP24A1 enzyme [1, 5, 38] and reflects the common attitude to approach the treatment of kidney stones rather than investigating the causes.

The differential diagnosis of hypercalcemia encompasses many different conditions. The possibility of mutation of the CYP24A1 gene as a cause of hypercalcemia should be considered in cases of PTH-independent hypercalcemia, once that more common causes, namely, hypercalcemia of malignancy, granulomatous diseases, activated vitamin D intoxication, have been ruled out.

The clinical expression of the CYP24A1 mutation is heterogeneous both in biallelic (age at diagnosis and severity) and monoallelic members of the same kindred (as in our family). Treatment should be individually tailored, taking into account the risk-benefit ratio. The severity of the clinical manifestations, the patient’s age, the expected side effects of the medication proposed (which should be taken possibly for a lifetime), and the patient’s preference should be taken into account.

Conflicts of Interest

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

[1] K. P. Schlingmann, M. Kaufmann, S. Weber et al., “Mutations in CYP24A1 and idiopathic infantile hypercalcemia,” The New England Journal of Medicine, vol. 365, no. 5, pp. 410–421, 2011.

[2] G. Jones, D. E. Prosser, and M. Kaufmann, “Cytochrome P450-mediated metabolism of vitamin D,” Journal of Lipid Research, vol. 55, no. 1, pp. 13–31, 2014.

[3] D. T. O’Keeffe, P. J. Tebben, R. Kumar, R. J. Singh, Y. Wu, and R. A. Wermers, “Clinical and biochemical phenotypes of adults with monoallelic and biallelic CYP24A1 mutations: evidence of gene dose effect,” Osteoporosis International, vol. 27, no. 10, pp. 3121–3125, 2016.

[4] R. Lightwood and T. Stapleton, “Idiopathic hypercalcemia in infants,” The Lancet, vol. 262, no. 6779, pp. 255–256, 1953.

[5] T. P. Jacobs, M. Kaufman, G. Jones et al., “A lifetime of hypercalcemia and hypercalcuria, finally explained,” The Journal of Clinical Endocrinology & Metabolism, vol. 99, no. 3, pp. 708–712, 2014.

[6] G. Jones, M. L. Kottler, and K. P. Schlingmann, “Genetic diseases of vitamin D metabolizing enzymes,” Endocrinology and Metabolism Clinics of North America, vol. 46, no. 4, pp. 1095–1117, 2017.

[7] A. Molin, R. Baudoin, M. Kaufmann et al., “CYP24A1 mutations in a cohort of hypercalcemic patients: Evidence for a recessive trait,” The Journal of Clinical Endocrinology & Metabolism, vol. 100, no. 10, pp. E1343–E1352, 2015.

[8] E. De Paolis, A. Minucci, M. De Bonis et al., “A rapid screening of a recurrent CYP24A1 pathogenic variant opens the way to molecular testing for Idiopathic Infantile Hypercalcemia (IIH),” Clinica Chimica Acta, vol. 482, pp. 8–13, 2018.

[9] P. M. Ferraro, A. Minucci, A. Primiano et al., “A novel CYP24A1 genotype associated to a clinical picture of hypercalcemia, nephrolithiasis and low bone mass,” Uroliathisis, vol. 45, no. 3, pp. 291–294, 2017.

[10] M. Kaufmann, J. C. Gallagher, M. Peacock et al., “Clinical utility of simultaneous quantitation of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D by LC-MS/MS involving derivatization with DMEQ-TAD,” The Journal of Clinical Endocrinology & Metabolism, vol. 99, no. 7, pp. 2567–2574, 2014.

[11] M. Kaufmann, N. Morse, B. J. Molloy et al., “Improved screening test for idiopathic infantile hypercalcemia confirms residual levels of serum 24,25-(OH)_{2}D in affected patients,” Journal of Bone and Mineral Research, vol. 32, no. 7, pp. 1589–1596, 2017.

[12] M. W. Yeh, P. H. G. Ituarte, H. C. Zhou et al., “Incidence and prevalence of primary hyperparathyroidism in a racially mixed population,” The Journal of Clinical Endocrinology & Metabolism, vol. 98, no. 3, pp. 1122–1129, 2013.

[13] C. Marcocci and F. Cetani, “Primary hyperparathyroidism,” The New England Journal of Medicine, vol. 365, no. 25, pp. 2389–2397, 2011.

[14] H. F. DeLuca, “Vitamin D: the vitamin and the hormone,” Fed Proc, vol. 33, no. II, pp. 2221–2219, 1974.

[15] G. Jones, D. E. Prosser, and M. Kaufmann, “25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D,” Archives of Biochemistry and Biophysics, vol. 523, no. 1, pp. 9–18, 2012.

[16] G. S. Reddy and K.-Y. Tserng, “Calcitroic acid, end product of renal metabolism of 1,25-dihydroxyvitamin D3 through C-24 oxidation pathway,” Biochemistry, vol. 28, no. 4, pp. 1763–1769, 1989.

[17] Y. Ohyama and K. Okuda, “Isolation and characterization of a cytochrome P-450 from rat kidney mitochondria that catalyzes the 24-hydroxylation of 25-hydroxyvitamin D3,” The Journal of Biological Chemistry, vol. 266, no. 14, pp. 8690–8695, 1991.
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18. Y. Ohyama, M. Noshiro, Y. Kato et al., “Structural Characterization of the Gene Encoding Rat 25-Hydroxyvitamin D3 24-Hydroxylase,” Biochemistry, vol. 32, no. 1, pp. 76–82, 1993.
19. K.-S. Chen, J. M. Prahl, and H. F. Deluca, “Isolation and expression of human 1,25-dihydroxyvitamin D3 24-hydroxylase cDNA,” Proceedings of the National Academy of Sciences of the United States of America, vol. 90, no. 10, pp. 4543–4547, 1993.
20. G. Jones, S. A. Strugnell, and H. F. DeLuca, “Current understanding of the molecular actions of vitamin D,” Physiological Reviews, vol. 78, no. 4, pp. 1193–1231, 1998.
21. M. Kaufmann, S. M. Lee, J. W. Pike, and G. Jones, “A high-calcium and phosphate rescue diet and VDR-expressing transgenes normalize serum Vitamin D metabolite profiles and renal Cyp27b1 and Cyp24a1 expression in VDR null mice,” Endocrinology, vol. 156, no. 12, pp. 4388–4397, 2015.
22. T. Shimada, H. Hasegawa, Y. Yamazaki et al., “FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis,” Journal of Bone and Mineral Research, vol. 19, no. 3, pp. 429–435, 2004.
23. P. J. Tebben, R. J. Singh, and R. Kumar, “Vitamin D-mediated hypercalcemia: Mechanisms, diagnosis, and treatment,” Endocrine Reviews, vol. 37, no. 5, pp. 521–547, 2016.
24. T. O. Carpenter, “CYP24A1 loss of function: Clinical phenotype of monoallelic and biallelic mutations,” The Journal of Steroid Biochemistry and Molecular Biology, vol. 173, pp. 337–340, 2017.
25. M. Cools, S. Goemaere, D. Baetens et al., “Calcium and bone homeostasis in heterozygous carriers of CYP24A1 mutations: A cross-sectional study,” Bone, vol. 81, pp. 89–96, 2015.
26. T. Jobst-Schwane, A. Pannes, K. P. Schlingmann, K.-U. Eckardt, B. B. Beck, and M. S. Wiesener, “Discordant clinical course of vitamin-D-hydroxylase (CYP24A1) associated Hypercalcemia in Two Adult Brothers with Nephrocalcinosis,” Kidney and Blood Pressure Research, vol. 40, no. 5, pp. 443–451, 2015.
27. H.-F. Ji and L. Shen, “CYP24A1 mutations in idiopathic infantile hypercalcemia,” The New England Journal of Medicine, vol. 365, no. 18, pp. 1741–1743, 2011.
28. D. Dinour, M. Davidovits, S. Aviner et al., “Maternal and infantile hypercalcemia caused by vitamin-D-hydroxylase mutations and vitamin D intake,” Pediatric Nephrology, vol. 30, no. 1, pp. 145–152, 2015.
29. D. Dinour, P. Beckerman, L. Ganon, K. Tordjman, Z. Eisenstein, and E. J. Holtzman, “Loss-of-function mutations of CYP24A1, the vitamin D 24-hydroxylase gene, cause long-standing hypercalciuric nephrolithiasis and nephrocalcinosis,” The Journal of Urology, vol. 190, no. 2, pp. 552–557, 2013.
30. A. Dauber, T. T. Nguyen, E. Sochett et al., “Genetic defect in CYP24A1, the vitamin D 24-hydroxylase gene, in a patient with severe infantile hypercalcemia,” The Journal of Clinical Endocrinology & Metabolism, vol. 97, no. 2, pp. E268–E274, 2012.
31. P. J. Tebben, D. S. Milliner, R. L. Horst et al., “Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to CYP24A1 mutations: Effects of ketoconazole therapy,” The Journal of Clinical Endocrinology & Metabolism, vol. 97, no. 3, pp. E423–E427, 2012.
32. M. Castanet, E. Mallet, and M.-L. Kottler, “Lightwood syndrome revisited with a novel mutation in CYP24 and vitamin D supplement recommendations,” Journal of Pediatrics, vol. 163, no. 4, pp. 1208–1210, 2013.
33. M.-L. Figueres, A. Linglart, F. Bienaime et al., “Kidney function and influence of sunlight exposure in patients with impaired 24-hydroxylation of vitamin D due to cyp24a1 mutations,” American Journal of Kidney Diseases, vol. 65, no. 1, pp. 122–126, 2014.
34. A. D. Shah, E. C. Hsiao, B. O’Donnell et al., “Maternal hypercalcemia due to failure of 1,25-dihydroxyvitamin-D3 catabolism in a patient With CYP24A1 mutations,” The Journal of Clinical Endocrinology & Metabolism, vol. 100, no. 8, pp. 2832–2836, 2015.
35. G. Colussi, L. Ganon, S. Penco et al., “Chronic hypercalcaemia from inactivating mutations of vitamin D 24-hydroxylase” (CYP24A1): Implications for mineral metabolism changes in chronic renal failure,” Nephrology Dialysis Transplantation, vol. 29, no. 3, pp. 636–643, 2014.
36. R. St-Arnaud, “CYP24A1-deficient mice as a tool to uncover a biological activity for vitamin D metabolites hydroxylated at position 24,” The Journal of Steroid Biochemistry and Molecular Biology, vol. 121, no. 1-2, pp. 254–256, 2010.