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Clinical Report

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Medullary nephrocalcinosis in an adult patient with idiopathic infantile hypercalcaemia and a novel CYP24A1 mutation

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Abstract

Idiopathic infantile hypercalcaemia (IIH) is an autosomal recessively inherited disease, presented in the first year of life with hypercalcaemia, precipitated by normal amounts of vitamin D supplementation. Recently loss-of-function mutations in the CYP24A1 gene, which encodes the vitamin D-metabolizing enzyme 24-hydroxylase, have been found in these patients. We describe a young man homozygous for a novel missense mutation (c.628T>C) of the CYP24A1 gene. He had suffered from severe hypercalcaemia in early childhood. At age 29 he presented with medullary nephrocalcinosis, chronic kidney disease (CKD) stage 2, microalbuminuria, mild hypertension and nephrogenic diabetes insipidus. He had mild hypercalcaemia and moderate hypercalciuria. As a novel finding, fibroblast growth factor 23 (FGF23) was elevated.

Keywords: nephrocalcinosis; hypercalcaemia; fibroblast growth factor 23

Introduction

Vitamin D hormones play a central role in calcium homeostasis. Tight control of the vitamin D system requires inactivation of its active compound 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) through 24-hydroxylation by means of the enzyme 24-hydroxylase (CYP24A1) and degradation to calcitroic acid. Similarly, 25-hydroxyvitamin D_3 ($25(OH)D_3$), the precursor of $1,25(OH)_2D_3$, is converted to $24,25(OH)_2D_3$, precluding its further activation [1]. CYP24A1 is a mitochondrial enzyme found mainly in the kidney, bone and intestine and probably in all cells that express the vitamin D receptor. In genome-wide association studies, variants at the CYP24A1 locus on chromosome 20q13 were associated with $25(OH)D_3$ levels [2, 3]. CYP24A1 knockout mice have severe hypercalcaemia, which is fatal in half of the animals. The others will develop nephrocalcinosis when given vitamin D [4, 5]. Recently, loss-of-function mutations in the CYP24A1 gene have been found in some patients suffering from the rare human disease idiopathic infantile hypercalcaemia (IIH) [6]. We here describe a young man, who had suffered from IIH and is homozygous for a novel missense mutation in the CYP24A1 gene.

Case report

A 29-year-old man was admitted for evaluation of an elevated serum creatinine of 1.40 mg/dL (estimated glomerular filtration rate, eGFR, 70 mL/min/1.73 m^2). He suffered from mild hypertension treated with 8 mg of candesartan. Urine analysis revealed microalbuminuria (albumin/creatinine 36 mg/g). A renal sonogram demonstrated medullary nephrocalcinosis (Figure 1). The patient also suffered from mild nephrogenic diabetes insipidus with isosthenuria and 3 to 4 L of urine per day.

Medical history revealed that the patient had suffered from IIH, manifesting in his third month of life, when he was admitted for failure to thrive. He was noted to have total serum calcium of 4.2 mmol/L, hypercalciuria and nephrocalcinosis. He had received 800 IU of vitamin D_3 supplementation per day at that time. His serum $25(OH)D_3$ level was seen to be elevated to 123 ng/mL, and vitamin D was stopped. The child was treated with prednisolone and put on a calcium-free formula. Serum calcium normalized quickly. Further development under low calcium alimentation was normal. Total serum calcium levels remained in the upper range of normal or slightly elevated (2.4–2.6 mmol/L), but hypercalciuria (0.35–0.45 g calcium/g creatinine) persisted. Intact PTH levels measured on multiple occasions were always suppressed. The $25(OH)D_3$ levels were elevated during the first 4 years of life (range 110–609 ng/mL) and then returned to normal. The $1,25(OH)D_3$ was first determined at 13 years of age and was in the upper range of normal (65 pg/mL). The current results of calcium and vitamin D metabolism are shown in Table 1. Bone density determined by DEXA was normal (lumbar spine 1.247 g/cm^2, *T* score 0.2; femoral neck 1.175 g/cm^2, *T* score 0.7).

Sequence analysis of the CYP24A1 gene was performed in the patient. For this purpose, all exons including intron–exon boundaries of the CYP24A1 gene were amplified by the polymerase chain reaction (PCR) according to a previously published protocol (PMID: 22337913). PCR products were directly sequenced using an ABI 3130 DNA Analyser (Applied Biosystems).

Sequencing analysis revealed that the patient was homozygous for three common DNA polymorphisms (rs2296241, rs2762934 and rs6022987) and additionally for a yet undescribed nonsynonymous mutation in exon 4 (c.628T>C), causing tryptophan to be replaced with arginine in codon 210 (W210R) (Figure 2). *In silico* analysis of the W210R mutation using the SIFT tool (http://sift.jcvi.org; PMID: 19561590) and the PolyPhen-2 tool (http://genetics.bwh.harvard.edu/pph2/index.shtml; PMID: 20354512) consistently showed a probably damaging effect on the protein, reaching highest possible scores of the two algorithms (SIFT score: 0.00; PolyPhen-2 score: 1.000).

Both parents and the two siblings of the patient were genotyped for the presence of the W210R variant by sequence analysis of exon 4. All family members carried the heterozygous genotype of mutation c.628T>C (Figure 3).

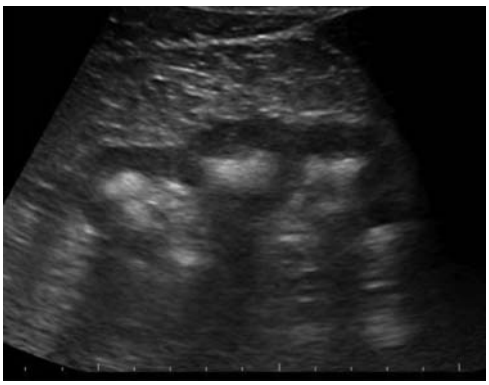

Fig. 1. Ultrasound of the right kidney shows marked medullary nephrocalcinosis. The kidney is reduced in size with rarefication of the parenchyma.

To assess the frequency of the W210R variant in the local population, 514 DNA samples of patients previously recruited for study purposes at Feldkirch Academic Teaching Hospital (PMID: 19135198) were genotyped for the mutation. Genotyping was carried out with the 5′ nuclease assay on a LightCycler® 480 Real-Time PCR System (F. Hoffmann-La Roche Ltd., Basel, Switzerland) using TaqMan® MGB probes and PCR primers obtained from the Assay-by-design™ service (Applied Biosystems, Forster City, CA, USA). Genotyping was successful in all patients. None of the patients were found to carry the W210R mutation.

Table 1 shows laboratory parameters of the patient's parents and his younger brother and sister. No major abnormalities in calcium metabolism were evident. However, the younger brother had a borderline total serum calcium level and elevated $1,25(OH)D_3$. A renal sonogram of the family members showed no nephrocalcinosis or renal calculi.

Serum levels of fibroblast growth factor 23 (FGF23) were measured by enzyme-linked immunosorbent assay in all family members (C-terminal FGF23 assay, Immunotopics, San Clemente, CA). Whereas serum levels in the heterozygous family members were normal, the patient's FGF23 was considerably elevated (3- to 4-fold compared with patients with a similar eGFR as measured using the same assay) (Table 1) [7]. Consequently, the patient also had moderate renal phosphate wasting with a reduced tubular reabsorption of phosphate and tubular maximum reabsorption of phosphate per litre of GFR (Table 1).

Discussion

Our case adds to recent reports indicating that IIH is caused by CYP24A1 deficiency, at least in some patients. Schlingmann *et al.* [6], in the first report, describe CYP24A1 mutations in a cohort of 10 patients. Since then one of the mutations, E143del, has been found in two further families

Table 1. Laboratory parameters of family members with the W210R mutation[a]

Family member	IA	IB	IIA	IIB	IIC
Age (years), gender	57, m	54, f	29, m	27, m	22, f
W210R	Heteroz	Heteroz	Homoz	Heteroz	Heteroz
Creatinine (0.7–1.2 mg/dL)	1.0	0.9	1.4	0.9	0.7
eGFR (>80 mL/min)	79	76	70	111	115
Total serum calcium (2.15–2.55 mmol/L)	2.25	2.33	2.61	2.51	2.36
Ionized calcium (1.12–1.32 mmol/L)	1.09	1.08	1.34	1.17	1.07
Phosphate (0.81–1.45 mmol/L)	1.05	0.95	0.84	0.80	0.75
Intact PTH (15–65 pg/mL)	39	30	13	24	16
25(OH) Vitamin D_3 (30–100 ng/mL)	24.4	20.8	28	18.7	38
$1,25(OH)_2$ Vitamin D_3 (20–63 ng/L)	39	32	41	74	55
$24,25(OH)_2$ Vitamin D_3[b](1,2–2,6 ng/mL)	2.5	2.0	0.6	2.2	5.4
$24,25(OH)_2/25(OH)$	0.10	0.10	0.02	0.12	0.14
FGF23[c] (0–125 RU/mL)	79	76	302	64	48
Urinary Ca/Cr (<0.2 g/g)	0.087	0.052	0.219	0.106	0.047
TRP (82–90%)	85%	84%	70%	79%	81%
TmP/GFR (0.8–1.4 mmol/L)	0.9	0.9	0.6	0.7	0.7

[a]m, male; f, female; Heteroz, Heterozygous; Homoz, Homozygous; eGFR, estimated glomerular filtration rate determined by the CKD-EPI formula; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; Ca/Cr, calcium/creatinine ratio; TRP, tubular reabsorption of phosphate; TmP/GFR, tubular maximum reabsorption of phosphate per litre of GFR.
[b]$24,25(OH)_2$ Vitamin D_3 was determined by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (Labor Limbach, Heidelberg, Germany).
[c]FGF23 was measured using the C-terminal assay.

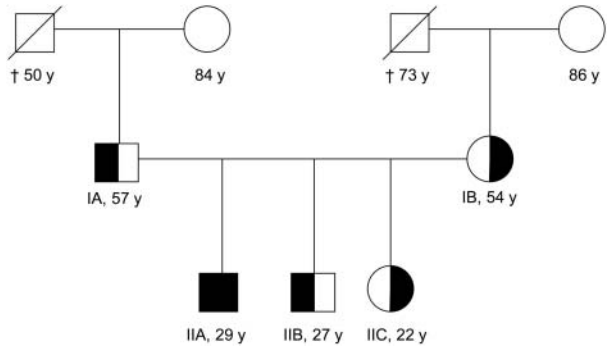

Fig. 2. Family tree.

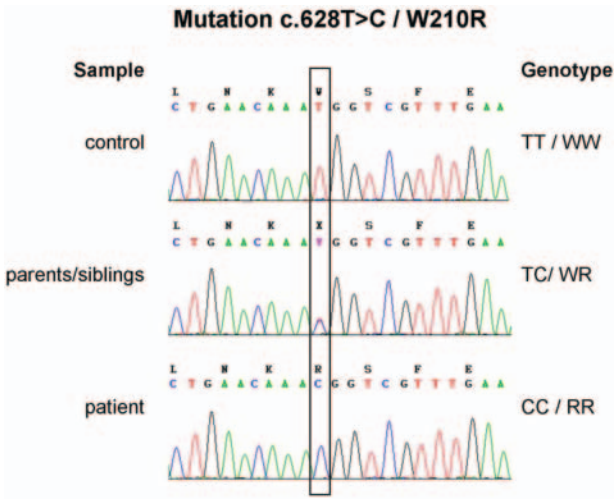

Fig. 3. Sequence analysis of the W210R mutation. Genotyping for the c.628T>C/W210R mutation by DNA sequence analysis. DNA sequencing chromatographs are shown for each genotype and illustrate the transversion of T to C, which causes an amino acid change of tryptophan (W) to arginine (R) in codon 210.

[8, 9]. An additional report described two splice site mutations in another family [10]. Recently another child, which was homozygous for the R396W mutation already reported by Schlingmann *et al*, was described [11]. This patient suffered from severe hypercalcaemia at 4 months of age, which required haemofiltration to lower serum calcium. Whereas Schlingmann *et al*. found homozygous or compound heterozygous mutations in all investigated families, Dauber *et al*. describes mutations in only one out of 28 children with infantile hypercalcaemia [6, 9]. Therefore, IIH seems to be a heterogeneous disease.

IIH (OMIM 143880) is an autosomal recessively inherited disease and was first described in the UK after high-dose vitamin D substitution in milk products was introduced [12]. About 200 cases were observed within 2 years [13–15]. Most of the affected children developed nephrocalcinosis. Usually, after vitamin D substitution is stopped, serum calcium normalizes over the next 3 to 4 years, but patients remain hypercalciuric [16]. Other patients with CYP24A1 mutations may present with nephrolithiasis later in life [8, 10]. The report by Tebben *et al*. suggests that heterozygous individuals may also be clinically affected [10].

The W210R mutation has not been described to date. Genotyping analysis of >500 individuals did not identify any other carrier of the W210R mutation, indicating that the mutation is uncommon in the general population. The frequency of W210R in patients affected by IIH remains to be investigated. Somewhat surprising, our patient is homozygous for the mutation and the CYP24A1 haplotype. A thorough family history revealed that both grandfathers came from the same small Austrian town. Therefore, a remote relationship seems likely.

CYP24A1 is a mitochondrial 514 amino acid protein and has a complex structure of α helices and β strands. It interacts with the mitochondrial membrane, adrenodoxin, haem and vitamin D molecules [17]. Disruption of this structure will impair the function of the enzyme. The new W210R mutation found in our patient is the first to be found in the E helix of CYP24A1. The CYP24A1 mutations described so far are evenly distributed along the molecule (Table 2) [6]. *In vitro* experiments in cells transfected with mutant CYP24A1 have demonstrated a complete loss of function in five and some residual function in one mutant [6]. Although we have no *in vitro* data on the functional consequences of the W210R mutant, *in silico* analysis suggests that the amino acid change is severely damaging. The finding of detectable, albeit low, levels of $24,25(OH)_2D_3$ in our patient would suggest some residual activity of the W210R mutant. As a crude measure of enzyme activity we calculated the $24,25(OH)_2D_3/25(OH)D_3$ ratio (Table 1). This ratio was severely reduced in the homozygous patient by comparison with heterozygous family members, implying that metabolism of $25(OH)D_3$ to $24,25(OH)_2D_3$ is impaired in the homozygous state.

Whereas PTH levels are usually suppressed in IIH, data on $25(OH)_2D_3$ and $1,25(OH)_2D_3$ are inconsistent, with mostly normal but occasionally elevated values being published [8, 10, 18]. One report describes a decrease in $25(OH)_2D_3$ over the years [8]. We also found in part excessively high levels of $25(OH)_2D_3$ in our patient over his first 4 years of life, which normalized thereafter. We, therefore, suggest that continuous improvement of IIH over time is not primarily caused by resistance to $1,25(OH)_2D_3$, but by down-regulation of hepatic conversion of vitamin D3 to $25(OH)D_3$ by means of CYP2R1 (25-hydroxylase). How CYP2R1 activity is regulated, remains largely unknown.

Our patient had a considerably elevated FGF23 level with consequent renal phosphate wasting. Hyperphosphaturia may be an important, hitherto unrecognized, co-factor in the development of nephrocalcinosis in IIH. We suspect that FGF23 production was driven by the (in relation to serum calcium) high $1,25(OH)_2D_3$ level. Whether low $24,25(OH)_2D_3$ might also stimulate FGF23 production is unknown at present, but seems plausible. Both high FGF23 and low PTH probably act in concert to down regulate renal CYP27B1 activity and $1,25(OH)_2D_3$ production [19, 20]. This may be an additional explanation for the continuous improvement in IIH over time. In the normal situation, high FGF23 and low PTH will also stimulate renal expression of CYP24A1 and inactivation of vitamin D metabolites [21, 22]. Obviously, this is impossible for CYP24A1 deficiency. A schematic presentation of the pathogenetic model of IIH is presented in Figure 4.

In addition to its role in calcium homeostasis, vitamin D has pleiotropic effects on many organ systems. Whether and how these influences are modified by CYP24A1 deficiency remains unknown.

Table 2. CYP24A1 mutations in IIH

Type of mutation	Mutation	CYP24A1 domain	Reference
Missense	R159Q	C helix	[6]
Missense	W210R	E helix	present study
Missense	E322K	I helix	[6]
Missense	R396W	3a β-sheet	[6, 11]
In-frame deletion	E143del	B helix	[6, 8, 9]
Stop codon	E151X	C helix	[6]
Frame shift	A475fsX490	L helix	[6]
Frame shift	c.445_449 (+1)	between B and C helix	[6]
Splice junction	IVS5+1G>A		[10]
Splice junction	IVS6-2A>G		[10]

In the treatment of the acute infantile phase of the disease, all measures known to reduce serum calcium such as rehydration, prednisolone, calcitonin and bisphosphonates were used with success [23–25]. Discontinuation of any vitamin D supplementation and a low calcium diet are important. Another interesting approach is treatment with ketoconazole. Ketoconazole reduces $1,25(OH)_2D_3$ levels and corrects hypercalcaemia in primary hyperparathyroidism and granulomatous disorders [26]. The drug binds to haem in the catalytic cleft of cytochrome P450 enzymes [1]. Several children and one adult have been treated successfully over several months [10, 18]. Side-effects, such as reduction of other steroid hormones and liver toxicity, will, however, preclude long-term treatment. In our patient, in addition to

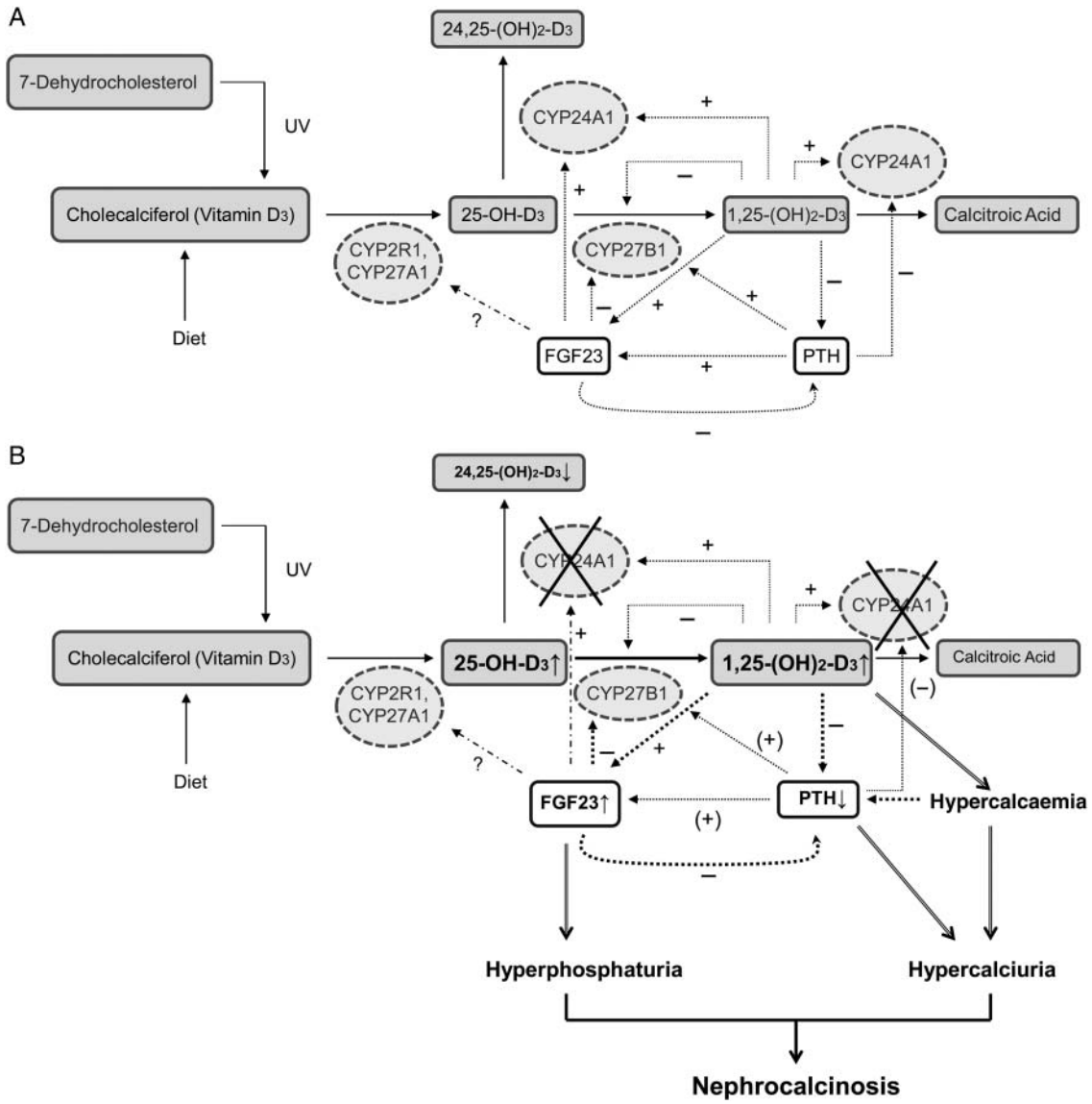

Fig. 4. Mechanisms of vitamin D metabolism and its regulation in health (**A**) and CYP24A1 deficiency (**B**). (A) $25OHD_3$ is activated to $1,25(OH)_2D_3$ by the enzyme CYP27B1. Via the enzyme CYP24A1, $25OHD_3$ is inactivated to $24,25(OH)_2D_3$, $1,25(OH)_2D_3$ to calcitroic acid. In a feed-back loop, $1,25(OH)_2D_3$ inhibits CYP27B1 and enhances the activity of CYP24A1. $1,25(OH)_2D_3$ inhibits PTH secretion and formation, whereas PTH itself activates CYP27B1 and inhibits CYP24A1. $1,25(OH)_2D_3$ stimulates FGF23 secretion which inhibits CYP27B1 and enhances CYP24A1. PTH stimulates FGF23 production, whereas FGF23 decreases PTH secretion. **(B)** Due to the W210R missense mutation, CYP24A1 activity is reduced resulting in increased levels of $25OHD_3$ and reduced levels of $24,25(OH)_2D_3$. Increased $1,25(OH)_2D_3$ (normalizing over years due to the adaptive mechanism as described below, but inadequately high given the hypercalcaemia) suppresses PTH levels and enhances FGF23 secretion. Low PTH and high FGF23 decrease CYP27B1 activity resulting in normal levels of $1,25(OH)_2D_3$ over time. Hypercalcaemia and low PTH cause hypercalciuria which leads to nephrocalcinosis, further aggravated by the hyperphosphaturia caused by high FGF23 levels. PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; $25OHD_3$, 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3. Plus indicates stimulation; minus indicates inhibition; plus sign within parenthesis indicates decreased stimulation; minus sign within parentheses indicates reduced inhibition.

a low calcium diet and avoidance of vitamin D supplements, we recommended sun protection. This measure has already been proposed by others for the acute phase of the disease [18]. As inactivation of vitamin D compounds seems to be the primary problem in patients with IIH and CYP24A1 mutations, suppression of vitamin D synthesis in the skin is a logical approach. We advised our patient to wear appropriate clothing and use sun cream with a high protection factor. In addition, he changed his holiday destination from the Mediterranean to the British Isles. The long-term effect of this approach remains to be seen. Patients should also be aware that in some countries certain foods and food supplements may be fortified with vitamin D and taught to study food labels. Whether an intervention is necessary in family members heterozygous for the mutation is unknown. We would, however, withhold any vitamin D supplementation in these persons.

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Conflict of interest statement. None declared.

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