

## Juvenile onset IIH and *CYP24A1* mutations

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### ABSTRACT

The term Idiopathic infantile hypercalcemia (IIH) was first introduced almost 70 years ago when symptomatic hypercalcemia developed in children after receiving high doses of vitamin D for the prevention of rickets. The underlying pathophysiology remained unknown until recessive mutations in *CYP24A1* encoding Vitamin D<sub>3</sub>-24-hydroxylase were discovered. The defect in vitamin D degradation leads to an accumulation of active 1,25(OH)<sub>2</sub>D<sub>3</sub> with subsequent hypercalcemia. Enhanced renal calcium excretions lead to hypercalciuria and nephrocalcinosis. Meanwhile, the phenotypic spectrum associated with *CYP24A1* mutations has significantly broadened. Patients may present at all age groups with symptoms originating from increased serum calcium levels as well as from increased urinary calcium excretions, i.e. kidney stones. Possible long term sequelae comprise chronic renal failure as well as cardiovascular disease. Here, we present a family with two affected siblings with differing clinical presentation as an example for the phenotypic variability of *CYP24A1* defects.

### 1. Introduction

Idiopathic infantile hypercalcemia (IIH) was first described as a novel clinical entity when symptomatic hypercalcemia developed in infants after receiving high doses of vitamin D (approximately 4000 IE/day) for the prevention of rickets in Great Britain in the 1950s (Lightwood and Stapleton, 1953; Fanconi, 1951). Affected children present with failure-to-thrive, weight loss, dehydration, polyuria, muscular hypotonia, and lethargy, typical symptoms of severe hypercalcemia. Concomitant hypercalciuria typically leads to the development of early nephrocalcinosis. Infants who receive daily oral vitamin D for the prevention of rickets usually present in the first year of life. Metabolic studies indicated that the hypercalcemia was due to an increased intestinal calcium uptake and associated with an increased renal calcium excretion (Morgan et al., 1956). A potential connection to vitamin D supplementation was early recognized and IIH was suspected to represent a state of vitamin D hypersensitivity in children being especially susceptible to vitamin D (Morgan et al., 1956). Despite these early advancements, the exact link between vitamin D supplementation and the development of hypercalcemia in affected infants remained obscure. Especially, contradictory data regarding serum levels of active 1,25(OH)<sub>2</sub>D<sub>3</sub> were reported: while some authors observed elevated levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> (McTaggart et al., 1999), other affected children were found to have levels within the normal range (Nguyen et al., 2010). Though it remained unknown if IIH at all represented a hereditary disease, single familial cases pointed to a genetic background,

pedigree analyses indicated an autosomal-recessive inheritance (McTaggart et al., 1999).

Finally, in 2011, recessive mutations in *CYP24A1* were discovered as the first underlying genetic defect in IIH (Schlingmann et al., 2011). The *CYP24A1* gene codes for the cytochrome P450 enzyme 24-hydroxylase, a multi-catalytic enzyme responsible for a 5-step, vitamin D-inducible, C-24-oxidation pathway that converts 1,25(OH)<sub>2</sub>D<sub>3</sub> into water soluble calcitroic acid thereby inactivating it (Makin et al., 1989). The initial patient cohort consisted of six infants who received daily vitamin D supplementation in the range of 500 IU, and four children who, in the 1980s in the German Democratic Republic, had been given single doses of 600,000 IU vitamin D<sub>2</sub>. Whereas the first cohort developed symptoms after several months indicating a critical role of a certain cumulative dose of exogenous vitamin D, bolus doses in the second cohort resulted in the development of symptoms resembling acute vitamin D toxicity within days to weeks after administration. After acute treatment, serum calcium levels largely normalized, but tended to be continuously at the upper normal limit during follow-up. Levels of iPTH remained suppressed in the majority of patients and levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> mostly persisted within the upper normal range, reflecting a continuous activation of vitamin D metabolism (Schlingmann et al., 2011). Later, mutations in *SLC34A1* encoding renal proximal-tubular sodium-phosphate co-transporter NaPi-IIa were discovered as a second genetic defect underlying IIH (Schlingmann et al., 2016). Patients with NaPi-IIa defects share critical phenotypic and biochemical features of patients with *CYP24A1* defects but in addition

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**Table 1**  
Clinical and laboratory data of patients F2.1 and F2.2.

Variable	Patient F2.1	Patient F2.2
Age at presentation	10 months	13 years
Vitamin D prophylaxis	400 IU/day	400 IU/day
Clinical symptoms	Failure to thrive	Nephrolithiasis
Laboratory findings		
At initial presentation		
S-Ca (mmol/L)(2.1–2.6)	2.7	3.0
iPTH (pg/mL)(14–72)	2.8	9.7
25-OH-vit D <sub>3</sub> (ng/mL)(20–65)	42	73
1,25-(OH) <sub>2</sub> -vit D <sub>3</sub> (pg/mL)(17–74)	nd	109
Ca/creatinine ratio (mg/mg)	0.68 (< 0.60) <sup>a</sup>	0.32 (< 0.22) <sup>a</sup>
At last follow-up		
S-Ca (mmol/L)(2.1–2.6)	2.28	2.8
iPTH (pg/mL) (14–72)	14.4	11.9
25-OH-vit D <sub>3</sub> (ng/mL) (20–65)	43	82
1,25-(OH) <sub>2</sub> -vit D <sub>3</sub> (pg/mL) (17–74)	69	81
Ca/creatinine ratio (mg/mg)(< 0.22) <sup>a</sup>	0.32	0.30

<sup>a</sup> Reference values for Ca/creatinine ratios according to Sargent et al. *J Pediatr* 1993.

exhibit phosphate depletion due to primary renal phosphate wasting.

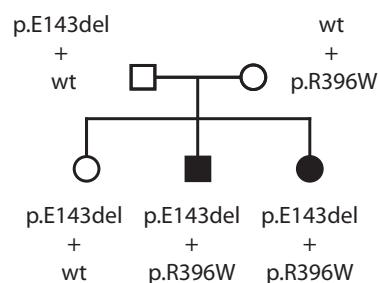
We here present a family with two affected siblings displaying different phenotypic presentations of *CYP24A1* associated disease in infancy and adolescence, respectively.

## 2. Case report

The female index patient (F1.1) was born at term. The neonatal period was uneventful. The infant was breastfed and received regular vitamin D prophylaxis for the prevention of rickets with a dose of 400 IU per day. She presented at the age of 10 months with failure to thrive. The laboratory work-up revealed borderline hypercalcemia and a suppressed iPTH level (see Table 1). Renal function tests and blood gases were normal. Urine analysis revealed hypercalciuria (12 mg/kg/day) and renal ultrasound demonstrated severe nephrocalcinosis. Genetic analyses excluded Williams syndrome (chr7q11.23 deletion (Perez Jurado et al., 1996)). Under the suspected diagnosis of IIH, a low-calcium diet was initiated and vitamin D prophylaxis was stopped. Therapeutic regimens led to a normalization of serum calcium levels while iPTH levels remained suppressed. While urine analyses demonstrated a normalization of calcium excretions, follow-up ultrasound examinations showed persistent nephrocalcinosis. At the age of three years, the low-calcium diet was stopped which led to a re-occurrence of hypercalciuria and suspected aggravation of nephrocalcinosis. The patient was subsequently treated with citrate as well as hydrochlorothiazide until the age of nine years. Thereafter, serum calcium levels and calcium excretions have remained in the upper normal range, nephrocalcinosis has persisted without further aggravation, and renal function is normal.

The past medical history of the patient's older brother was unremarkable with an uneventful neonatal period and infancy. He had also received regular vitamin D prophylaxis (400 IU/(day)). At the age of 13 years, he presented with abdominal pain and suspected appendicitis. Abdominal ultrasound revealed a 10 mm concrement in the right renal pelvis as well as a smaller concrement of 6 mm in the left kidney while there was no medullary nephrocalcinosis. Laboratory evaluations demonstrated hypercalcemia of 3 mmol/L, a suppressed iPTH of 9.7 pg/mL, and a level of active 1,25(OH)<sub>2</sub>D<sub>3</sub> of 109 pg/mL (Table 1). The calcium excretion in a spot urine sample was elevated (0.32 g/g, reference range < 0.25). Both kidney stones were removed by ureterorenoscopy. A reduction of oral calcium resulted in a normalization of serum calcium levels as well as urinary calcium excretions. The laboratory testing during follow-up revealed serum calcium levels at the upper limit of the reference range and a persisting suppression of iPTH (Table 1). During a follow-up of three years, there is no

## Family 1 with *CYP24A1* mutations



**Fig. 1.** Pedigree of family 1 with mutated *CYP24A1* alleles indicated. The two affected children are compound-heterozygous for *CYP24A1* mutations p.E143del and p.R396W, whereas both parents and the asymptomatic sister carry one mutated allele each. The two identified mutations represent the two most common mutated *CYP24A1* alleles in the European population, both mutations have been described before, and lead to a complete loss of enzyme function in vitro.

reoccurrence of nephrolithiasis. The older sister as well as both parents are clinically unaffected. Unfortunately, there is no laboratory data on calcium and vitamin D metabolism available. Genetic testing for IIH was initiated that revealed compound-heterozygous mutations p.E143del and p.R396W in the *CYP24A1* gene in both affected siblings while the parents as well as the unaffected sister were found to be heterozygous mutation carriers (Fig. 1).

## 3. Discussion

After the initial description of mutations in *CYP24A1*, the initial findings were substantiated by numerous follow-up-studies. These case series also significantly extended the clinical spectrum caused by *CYP24A1* deficiency demonstrating a wide range of phenotypic presentations that will be summarized and discussed in the following.

*CYP24A1* mutations identified in the initial report were functionally analyzed in an in vitro system demonstrating a complete loss-of-function of the *CYP24A1* enzyme with lack of 24-hydroxylated vitamin D metabolites (Schlingmann et al., 2011). Moreover, an in vivo test method for the determination of 24-hydroxylated vitamin D metabolites in patients' serum was developed (Kaufmann et al., 2014). Calculation of the 25-OH-D<sub>3</sub> to 24,25-(OH)<sub>2</sub>D<sub>3</sub> enables the sensitive detection of patients with bi-allelic *CYP24A1* mutations and lack of 24-hydroxylase enzyme activity irrespective of an individual's vitamin D status (Kaufmann et al., 2014; Kaufmann et al., 2017). Interestingly, a small set of mutations in the *CYP24A1* gene comprising p.E143del, p.R396W, and p.L409S is detected in high frequency in the majority of patients of Caucasian descent irrespective of the severity of biochemical abnormalities, age at manifestation and clinical phenotype. All three variants were already identified in the initial report and shown to lead to a complete loss of enzyme function. They are listed in public exome and genome databases (ExAC, gnomAD) and represent variants that have a significant frequency in the general population (Table 2). Therefore, from the genetic point of view, patients with *CYP24A1* defects display an exceptionally homogenous cohort. This also concerns the number of affected alleles as the vast majority of studies describes patients with bi-allelic *CYP24A1* mutations.

Several studies have described single patients or small case series of typical IIH patients who carried bi-allelic *CYP24A1* mutations and clinically presented with symptomatic hypercalcemia in infancy (Dauber et al., 2012; Fencl et al., 2013; Skalova et al., 2013; Castanet et al., 2013; Dinour et al., 2015). These studies mainly confirmed phenotypic and laboratory characteristics that had been reported in IIH patients since the 1950s and were also reported for the initial cohort of patients with *CYP24A1* mutations. Uniformly describing patients with

**Table 2**  
Most frequent *CYP24A1* loss-of-function mutations.

Gene	Genomic coordinates	Variant (nt)	Variant (aa)	SNP number	ExAC frequency <sup>a</sup>
CYP24A1	20-52789466-CCTT-C	c.427_429delGAA	p.E143del	–	0.000930
CYP24A1	20-52774675-G-A	c.1186C > T	p.R396W	rs114368325	0.001106
CYP24A1	20-52774635-A-G	c.1226T > C	p.L409S	rs6068812	0.001158

CYP24A1 RefSeq-IDs NM\_000782, NP\_000773; UniProt-ID Q07973.

<sup>a</sup> Frequency in the European (non-Finnish) population.

bi-allelic *CYP24A1* mutations, these publications also supported the initial assumption of a primarily recessive trait of “classic” IIH. Furthermore, the initial report as well as one additional study described families in which, after typical manifestation of IIH in a first infant, vitamin D prophylaxis was omitted in a younger sibling who subsequently remained asymptomatic despite sharing the identical bi-allelic *CYP24A1* mutations (Schlingmann et al., 2011; Castanet et al., 2013). On one hand, these findings argue for a certain cumulative dose of exogenous vitamin D needed for the development of symptomatic hypercalcemia, on the other hand, suggest an incomplete penetrance of inherited *CYP24A1* defects. The significant increase in the incidence of IIH that was observed in the 1950s in the UK as well as later in Poland during phases of high vitamin D supplementation (i.e. 4000 IU/day) also suggest a close relationship between dosage of exogenous vitamin D and disease penetrance (Pronicka et al., 2017). The index patient presented here received a currently recommended dose of 400 IU per day vitamin D<sub>3</sub> and manifested in infancy with failure to thrive. Renal ultrasound demonstrated a significant nephrocalcinosis. However biochemical parameters were rather mild contrasting the severe symptomatic hypercalcemia observed in “typical” IIH cases and the diagnosis could have easily been missed. These findings might reflect an early diagnosis in the presence of slowly evolving clinical symptoms patient but could also suggest a generally mild disease phenotype in the index patient.

In addition to cases of “typical” IIH, numerous publications have reported adolescent and adult patients with identical bi-allelic mutations in *CYP24A1* who mainly presented with recurrent kidney stone disease, typically during childhood or young adult age (Nesterova et al., 2013; Dinour et al., 2013; Meusbürger et al., 2013; Colussi et al., 2014; Wolf et al., 2014; Jobst-Schwan et al., 2015). Laboratory examinations mostly demonstrated a milder degree of hypercalcemia, but similarly suppressed iPTH levels compared to infants with “classic” IIH.

Some of these patients were followed for decades and without knowledge of the underlying etiology received diverse medical interventions including parathyroidectomy to treat hypercalcemia and suspected hyperparathyroidism before reliable measurements of iPTH became available (Jacobs et al., 2014). Summarizing published cases with genetically proven *CYP24A1* deficiency, a recent study concluded that more than a third of reported patients with bi-allelic *CYP24A1* mutations presented beyond infancy (Cools et al., 2015). These long-term data obtained in adult patients are crucial for our understanding of possible complications of a sustained activation of vitamin D metabolism caused by *CYP24A1* defects. Particularly, a deterioration of renal function has been reported in a number of cases (Dinour et al., 2013; Colussi et al., 2014; Wolf et al., 2014). However, it remains to be investigated in future studies whether this reported decline in glomerular filtration rate is the consequence of repeated episodes of kidney stone disease, acute kidney injury, and urologic interventions or whether it represents a primary complication of the disease itself.

Moreover, extensive vascular calcifications have been observed in single adult patients suggesting that an augmented 1,25(OH)<sub>2</sub>D<sub>3</sub> activity with subsequent changes in calcium metabolism might also represent a risk factor for coronary artery disease and arterial occlusive disease (Colussi et al., 2014).

Another clinical presentation that deserves special attention is a

primary manifestation of *CYP24A1* mediated disease in women during pregnancy or shortly after labour (Dinour et al., 2015; Shah et al., 2015; Woods et al., 2016; Kwong and Fehmi, 2016). Of note, all reported women carried bi-allelic *CYP24A1* mutations. These cases demonstrate that women with *CYP24A1* defects are at risk to develop severe clinical symptoms during pregnancy which represents a state of increased 1,25(OH)<sub>2</sub>D<sub>3</sub> production despite being able to limit vitamin D activation under normal circumstances. Interestingly, two of these women had presented with kidney stones prior to their pregnancies (Dinour et al., 2015; Shah et al., 2015). Clinical disturbances of hypercalcemia in pregnancy include nausea, vomiting, fatigue, and polyuria, but more importantly mother and child are put at risk for severe complications such as arterial hypertension, nephrolithiasis, pancreatitis, life threatening hypercalcemic crises, and fetal demise (Woods et al., 2016; Norman et al., 2009). Moreover, neonatal morbidity and mortality are increased due to intrauterine growth restriction, low birth weight, and preterm delivery (Rey et al., 2016).

The clinical findings as well as biochemical changes observed in the affected brother presented here are characteristic for a “late” manifestation of IIH. He had remained clinically asymptomatic before, showed lack of overt hypercalcemia, and renal ultrasound revealed no nephrocalcinosis that represents an almost uniform finding in young patients. Clinical presentation with nephrolithiasis as well as age at initial manifestation can definitely be considered typical for “late” IIH. One could speculate if a determination of iPTH levels at an earlier age would have yielded suppressed values as this parameter is considered a sensitive diagnostic tool also for asymptomatic and normocalcemic patients (Molin et al., 2015; Sayer, 2015).

So far, only a limited number of studies provide clinical and laboratory data for individuals with heterozygous *CYP24A1* mutations mostly examining family members of index patients with bi-allelic *CYP24A1* mutations (Colussi et al., 2014; Cools et al., 2015; Molin et al., 2015). These demonstrate similar, albeit milder biochemical abnormalities comprising increased levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> and low levels of iPTH, and, at the same time, show an increased frequency of kidney stones. The finding of a single heterozygous *CYP24A1* mutation observed in a patient who developed symptomatic hypercalcemia after vitamin D bolus prophylaxis (600.000 IU p.o.) as well as the detection of a mild degree of hypercalcemia in a cohort of clinically asymptomatic neonates carrying a single heterozygous *CYP24A1* mutation also support the assumption of a milder, but detectable phenotype caused by *CYP24A1* haploinsufficiency (Schlingmann et al., 2011; Molin et al., 2015). Heterozygous mutation carriers may be prone to develop clinically evident disease only under circumstances promoting increased vitamin D activation, i.e. prematurity/early infancy, high doses of exogenous vitamin D, or during pregnancy (see above). Especially in account of the observed frequency of heterozygous *CYP24A1* variants in the general population, these findings demand larger studies exploring the spectrum of biochemical changes and clinical phenotypes in carriers of a single heterozygous *CYP24A1* mutation.

In contrast to a spectrum of well-defined therapeutic measures for acute symptomatic hypercalcemia (Rodd and Goodyer, 1999; Davies and Shaw, 2012), specific therapeutic strategies for patients with *CYP24A1* defects who display a mild degree of hypercalcemia but present with recurrent renal symptoms are only insufficiently examined

to date. Usually, regular vitamin D supplementation is stopped in affected infants and older patients and their parents are advised to avoid vitamin D supplements during later life. In this context, the identification of patients with *CYP24A1* defects, i.e. by genetic testing or analysis of 24-hydroxylated metabolites, could enable the selective omission of vitamin D supplementation in individuals at risk before development of clinical symptoms as shown for two younger siblings of hypercalcemic patients with *CYP24A1* defects (Schlingmann et al., 2011; Castanet et al., 2013). As a tendency to higher serum calcium levels and higher urinary calcium excretions has been observed during summer months, it might be recommended to avoid excessive sunlight exposure and prevent seasonal peaks of cutaneous vitamin D synthesis (Figueres et al., 2015). The authors conclude that although IIH typically manifests after vitamin D supplementation, sunlight exposure and thus cutaneous vitamin D synthesis may critically influence the clinical course during follow-up. On the other hand, little is known about the risk of vitamin D deficiency with low levels of 25(OH)<sub>2</sub>D<sub>3</sub> in patients with *CYP24A1* defects. While these might prevent excessive vitamin D activation via renal 1 $\alpha$ -hydroxylase, very low 25(OH)<sub>2</sub>D<sub>3</sub> levels might be insufficient for extrarenal 1,25(OH)<sub>2</sub>D<sub>3</sub> synthesis.

Specifically, a low-calcium diet as implemented in the presented cases is not recommended on a routine basis and requires close monitoring due to its potential to induce calcium deficiency and iatrogenic rickets as well as an increase in nutritional oxalate absorption that may aggravate the risk for developing renal calcifications and oxalate kidney stones. A similar caution should be applied to the use of sodium cellulose phosphate (SCP), a non-absorbable cation exchange resin used for the removal of excess calcium from the body though SCP has been successfully used in patients with infantile hypercalcemia over a period of several years without relevant side effects (Huang et al., 2006; Mizusawa and Burke, 1996).

Often, corticosteroids are used for the treatment of symptomatic hypercalcemia as they are known to inhibit enteral calcium absorption. Next to this intestinal effect, corticosteroids have been shown to induce *CYP24A1* expression (Jones et al., 2012). However, the therapeutic efficacy of corticosteroids in patients with *CYP24A1* defects was studied in detail by Colussi and colleagues who report a failure of corticosteroids to lower serum calcium levels, decrease urinary calcium excretions, and normalize iPTH levels (Colussi et al., 2014). These findings might indicate that the therapeutic effect in other disorders such as granulomatous disease mainly involves the induction of *CYP24A1* rather than the intestinal effect.

Several studies have evaluated the use of ketoconazole in *CYP24A1*-mediated hypercalcemia at least for periods of several months (Nguyen et al., 2010; Nesterova et al., 2013; Tebben et al., 2012). Next to an inhibition of fungal cytochrome P450 enzymes azole derivatives such as ketoconazole and fluconazole also suppress mammalian cytochrome P450 enzymes including 1 $\alpha$ -hydroxylase (*CYP27B1*) (Nguyen et al., 2010; Sayers et al., 2015). As the use of ketoconazole is limited by its side-effects and toxicity, fluconazole was introduced as a therapeutic alternative (Sayers et al., 2015). The authors demonstrated that even low doses produce a significant decline of 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in patients with bi-allelic *CYP24A1* defects and a stabilization of serum calcium levels (Sayers et al., 2015). Very recently, a gain-of-function mutation has been identified in *CYP3A4* leading to vitamin D-dependent rickets type 3 (Roizen et al., 2018). The affected patients exhibited low 25-OH-D<sub>3</sub> as well as low 1,25(OH)<sub>2</sub>D<sub>3</sub> levels. In vitro studies demonstrated a 10-fold higher *CYP3A4* activity in inactivating 1,25(OH)<sub>2</sub>D<sub>3</sub> compared to wildtype *CYP3A4* and even a 2-fold higher activity compared to *CYP24A1*. These new findings further support earlier observations of the same group that a targeted induction of *CYP3A4* by rifampin might be used to normalize elevated vitamin D metabolites in patients with *CYP24A1* defects (Hawkes et al., 2017).

In summary, following the identification of *CYP24A1* mutations as the underlying genetic defect in IIH, numerous studies have identified a large number of patients with identical *CYP24A1* defects who clinically

present at all ages with diverse clinical symptoms ranging from early severe symptomatic hypercalcemia to kidney stone disease in normocalcemic adults. An example for the phenotypic variability in siblings with an identical genetic defect was presented here. In the light of these findings, every single part of the original term Idiopathic Infantile Hypercalcemia (IIH) appears superseded and insufficient to describe our current knowledge of the spectrum of disease caused by *CYP24A1* deficiency. Many questions concerning the long-term outcome and potential treatments to prevent disease complications remain to be answered. This is especially true considering the frequency of proven loss-of-function mutations in *CYP24A1* in the general population as well as the genetic heterogeneity demonstrated by identification of mutations in renal phosphate co-transporters NaPi-IIa and NaPi-IIc producing similar clinical and biochemical phenotypes including an imminent deterioration of renal function (Schlingmann et al., 2016; Dasgupta et al., 2014; Dinour et al., 2016).

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