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 D3-24-hydroxylase were discovered. The defect in vitamin D degradation leads to an accumulation of active 1,25(OH)2D3 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)2D3 were reported: while some authors observed elevated levels of 1,25(OH)2D3 (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)2D3 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 D2. 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)2D3 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...
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 neturalization 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. 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)2D3 of 109 pg/mL. Calcium excretion in a spot urine sample was elevated (0.32 g/g, reference range < 0.25). Both kidney stones were removed (Table 1). During a follow-up of three years, there is no recurrence 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-D3 to 24,25-(OH)2-D3 ratio enabled 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

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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             |              |              |
| S-Ca (mmol/L) (2.1–2.6)         | 2.7          | 3.0          |
| iPTH (pg/mL) (14–72)            | 2.8          | 9.7          |
| 25-OH-D3 (pg/mL) (20–65)        | 42           | 73           |
| 1,25(OH)2-D3 (pg/mL) (17–74)    | nd           | 109          |
| Ca/creatinine ratio (mg/mg)     | 0.68 (< 0.60) | 0.32 (< 0.22) |
| 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-D3 (pg/mL) (20–65)        | 43           | 82           |
| 1,25(OH)2-D3 (pg/mL) (17–74)    | 69           | 81           |
| Ca/creatinine ratio (mg/mg)     | 0.32         | 0.30         |

*a Reference values for Ca/creatinine ratios according to Sargent et al. J Pediatr 1993.*
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 symptoms when the mother is exogenous vitamin D deficiency, a recent study concluded that 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)2D3 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). 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)2D3 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)2D3 production despite being able to limit vitamin D activation under normal circumstances.
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Present at all ages with diverse clinical symptoms ranging from early severe symptomatic hypercalcinemia 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 Hypercalcinemia (IIH) appears superseded and insufficient to describe our current knowledge of the spectrum of disease caused by CYP24A1 deficiency. Many question 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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