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Authors

Woods, Gina N
Saitman, Alec
Gao, Hanlin
et al.

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CASE REPORT

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A Young Woman With Recurrent Gestational Hypercalcemia and Acute Pancreatitis Caused by CYP24A1 Deficiency

Gina N Woods,[1] Alec Saitman,[2] Hanlin Gao,[3] Nigel J Clarke,[4] Robert L Fitzgerald,[2] and Nai-Wen Chi[1]

[1]Department of Medicine, University of California, San Diego, CA, USA
[2]Department of Pathology, University of California, San Diego, CA, USA
[3]Fulgent Diagnostics, Temple City, CA, USA
[4]Mass Spectrometry R&D Department, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, USA

ABSTRACT

The *CYP24A1* gene encodes a mitochondrial 24-hydroxylase that inactivates $1,25(OH)_2D$. Loss-of-function mutations in *CYP24A1* cause hypercalcemia, nephrolithiasis and nephrocalcinosis. We describe a woman with CYP24A1 deficiency and recurrent gestational hypercalcemia. Her first pregnancy, at age 20, resulted with the intrauterine demise of twin fetuses. Postpartum, she developed severe hypercalcemia (14 mg/dL), altered mental status, and acute pancreatitis. Her PTH was suppressed (6 pg/mL) and her $1,25(OH)_2D$ was elevated (165 and 195 pg/mL on postpartum day 1 and 5, respectively). Between one and three months postpartum, her serum calcium decreased from 11.4 to 10.2 mg/dL while her $1,25(OH)_2D$ level decreased from 83 to 24 pg/mL. Her 24-hour urine calcium was 277 mg. Six months postpartum, she became pregnant again. At 14 weeks, her albumin-corrected calcium level was 10.4 mg/dL and her $1,25(OH)_2D$ level exceeded 200 pg/mL. To establish the diagnosis of CYP24A1 deficiency, we showed her $24,25(OH)_2D$ level to be undetectable (<2 ng/mL). Exon sequencing of the *CYP24A1* gene revealed a homozygous, 8-nucleotide deletion in exon 8, causing an S334V substitution and premature termination due to a frame shift (c.999_1006del, p.Ser334Valfs*9). To prevent hypercalcemia, she was advised to discontinue prenatal vitamins, avoid sun exposure and calcium-rich foods, and start omeprazole and a calcium binder (250 mg K-Phos-neutral with meals). Despite these measures, both hypercalcemia (11.5 mg/dL) and acute pancreatitis recurred. Labor was induced and a healthy, normocalcemic boy was delivered. In the absence of lactation, maternal hypercalcemia resolved within 2 months. This report shows that CYP24A1-deficient subjects may be normocalcemic at baseline. Hypercalcemia may be unmasked by pregnancy through the routine use of calciferol-containing prenatal vitamins, increased 1-alpha hydroxylation of VitD by the placenta and maternal kidney, and production of PTHrP by the uteroplacental unit. CYP24A1 deficiency should be considered in patients with unexplained vitamin D-mediated hypercalcemia. © 2016 American Society for Bone and Mineral Research.

KEY WORDS: CYP24A1; HYPERCALCEMIA; PANCREATITIS; VITAMIN D

Introduction

The *CYP24A1* gene encodes a mitochondrial 24-hydroxylase that metabolizes both 25OHD and $1,25(OH)_2D$. The induction of *CYP24A1* gene expression by both hypercalcemia and $1,25(OH)_2D$ provides a safeguard against vitamin D-induced hypercalcemia.[1] Conversely, inactivating mutations in *CYP24A1* can cause hypercalcemia. Following the introduction of vitamin D fortification of infant formula in Great Britain in the 1950s, Lightwood described an epidemic of idiopathic infantile hypercalcemia and postulated supranormal sensitivity to vitamin D as the culprit.[2,3] Subsequently, Schlingman identified loss-of-function mutations in *CYP24A1* in a kindred of idiopathic infantile hypercalcemia.[4] CYP24A1 deficiency has since been reported to cause hypercalcemia in all age groups.[5]

During pregnancy, 25OHD 1-alpha hydroxylase is expressed by the placenta and upregulated in the maternal kidney, leading to a physiological doubling of maternal $1,25(OH)_2D$ levels.[6] Because most prenatal vitamin supplements provide 400 IU/day of vitamin D, one might expect pregnancy to increase the phenotypic penetrance of CYP24A1 deficiency. However, only two cases of gestational hypercalcemia have been attributed to CYP24A1 deficiency.[7,8] Herein we report the case of a 20-year-old woman with a homozygous inactivating *CYP24A1* mutation who developed recurrent gestational hypercalcemia and pancreatitis.

Patient and Methods

Case report

A 20-year-old primipara was referred to our medical center at 23 weeks of gestation because of severe maternal hypertension and intrauterine growth restriction of twin fetuses. After the

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Address correspondence to: Gina Woods, MD, 3350 La Jolla Village Drive, 111G, San Diego, CA 92161, USA. E-mail: gwoods@ucsd.edu

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demise of both fetuses, labor was induced at 26 weeks. Immediately postpartum, the patient's mental status became altered and hypercalcemia was noted at 14 mg/dL (albumin 3.0 g/dL). She was treated with saline infusion and one dose of subcutaneous calcitonin (4 U/kg). Biochemical workup revealed a suppressed PTH (6 pg/mL), an undetectable PTHrP (<2.8 pmol/L), a 25OHD of 45 ng/mL, and elevated $1,25(OH)_2D$ levels (165 and 195 pg/mL on postpartum days 1 and 5, respectively). A subsequent review of her outside medical records revealed the presence of hypercalcemia (12.3 mg/dL) at 12 weeks of gestation that had not been further evaluated.

In addition to altered mental status, the patient developed fevers, tachycardia, and epigastric pain. Acute pancreatitis was diagnosed on postpartum day 3 with a serum lipase level of 1416 U/L. Her triglyceride levels were 140 mg/dL. An abdominal CT scan revealed an edematous pancreas and moderate ascites but no evidence of cholelithiasis (Fig. 1). Also noted was extensive mesenteric and omental thickening that, along with an abrupt drop in serum calcium (Fig. 2), suggested saponification associated with pancreatitis (Fig. 1).

Before the pregnancy, the patient weighed 41.3 kg (BMI 17.8 kg/m^2) and did not receive regular medical care. She has had hypertension since childhood. Her medications during pregnancy consisted of a prenatal vitamin (discontinued postpartum) and labetalol (200 mg twice daily). She had no prior history of hypercalcemia, kidney stones, fractures, pancreatitis, or alcohol use. Renal ultrasound revealed simple cysts bilaterally but no nephrolithiasis or nephrocalcinosis. She was born in Mexico; her parents were from the same town but not known to be blood-related. There was no family history of calcium disorders, kidney stones, or pancreatitis.

A search for potential causes of the patient's vitamin D-mediated hypercalcemia revealed an angiotensin-converting enzyme level of 8 U/L (reference range 9 to 13 U/L) and a positive QuantiFERON-TB Gold In-Tube test (Cellestis Inc., Valencia, CA, USA) test. Her ascites fluid showed a serum-ascites albumin gradient of 0.3 g/dL and tested negative in bacterial and mycobacterial cultures as well as acid-fast bacilli (AFB) stains. Her chest radiograph revealed a calcified granuloma (5 mm) in the right upper lobe. This was redemonstrated by a chest CT, which unexpectedly also revealed multiple pulmonary emboli but no mediastinal lymphadenopathy, active pulmonary tuberculosis, sarcoidosis, or malignancy. A bone marrow biopsy revealed

Fig. 1. Abdominal CT scan showing an edematous pancreas, simple ascites, and renal cysts without evidence of nephrocalcinosis.

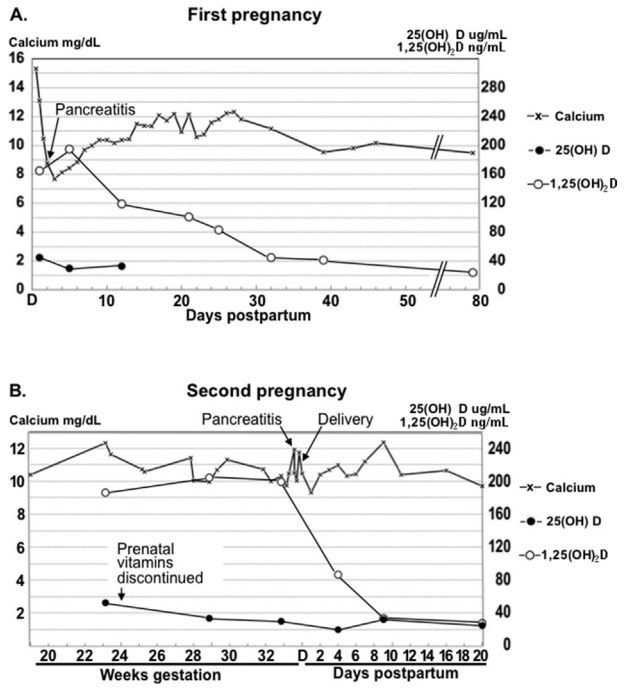

Fig. 2. Serum levels of calcium, 25OHD, and $1,25(OH)_2D$ during (*A*) first pregnancy and (*B*) second pregnancy.

normal cellularity and hematopoiesis, without evidence of lymphoma, granuloma, mycobacteria, or growth in AFB culture. Under the suspicion that sarcoidosis might be responsible for her hypercalcemia (12.0 mg/dL), she was given one 40-mg dose of prednisone on postpartum day 26. The next day, her serum calcium levels remained unchanged. She was discharged on postpartum day 28 with instructions to continue an anticoagulant and amlodipine. Between 1 and 3 months postpartum, her serum calcium and $1,25(OH)_2D$ levels decreased from 11.4 to 10.2 mg/dL and from 83 to 24 pg/mL, respectively. Over the ensuing 3 months, her serum calcium remained between 10.0 to 10.2 mg/dL and her 24-hour urine calcium was 277 mg.

Results

The patient became pregnant again 6 months postpartum and resumed prenatal vitamin supplementation. At 14 weeks of gestation, her calcium was 10.0 mg/dL (albumin 3.5 g/dL) and her $1,25(OH)_2D$ level exceeded 200 pg/mL. To establish the diagnosis of CYP24A1 deficiency, we found her $24,25(OH)_2D$ level (both D_2 and D_3) to be below the detection limit of 2 ng/mL, whereas a concurrent 25OHD level was 53 ng/mL. Exon sequencing of the *CYP24A1* gene in peripheral leucocytes revealed a homozygous, 8-nucleotide (nt) deletion (c.999_1006del, p.Ser334Valfs*9) in exon 8, causing an S334V substitution and premature termination due to a frame shift (Fig. 3).

Upon confirmation of CYP24A1 deficiency at 22 weeks of gestation, the patient was advised to discontinue prenatal vitamins and to minimize sun exposure and calcium-rich foods. To reduce intestinal calcium absorption, she was prescribed omeprazole (20 mg daily) and phosphate (250 mg of K-Phos Neutral with each meal, Beach Pharmaceuticals, Tampa, FL).

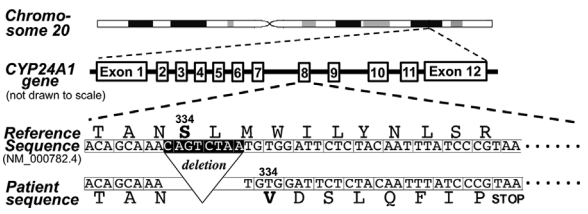

Fig. 3. The intron-exon structure of the *CYP24A1* locus on chromosome 20. Deletion of the 8 nucleotides highlighted in exon 8 (c.999_1006del) causes a valine substitution of Ser334 followed by a premature stop codon (p.Ser334Valfs*9). This frame shift deletion corresponds to a minor *CYP24A1* allele (SNP ID: rs770055617, http://www.ncbi.nlm.nih.gov/snp) with an apparent allele frequency of <0.0005. *CYP24A1* homozygosity in the patient was confirmed by additional deletion/duplication analyses (data not shown).

Despite these measures, her calcium levels rose to 11.5 mg/dL (albumin 3.5 g/dL) at 33 weeks of gestation, when severe epigastralgia led to the diagnosis of acute pancreatitis. Her pancreas was edematous and enlarged on ultrasound. Her amylase and lipase levels peaked at 1688 U/L and 4440 U/L, respectively. Because neither cholelithiasis nor dilatation of the biliary tree was found on ultrasound or magnetic resonance cholangiopancreatography, we attributed the pancreatitis to hypercalcemia.

The patient received aggressive saline infusion and one dose of subcutaneous calcitonin for her pancreatitis and hypercalcemia. Because of worsening hypertension, labor was induced at 34 weeks. The male newborn weighed 1786 g (14.5th percentile), had a cord blood calcium level of 9.2 mg/dL, and showed no signs of hypercalcemia or hypocalcemia during the 12 days of hospitalization. Comparisons of maternal serum (8 days antepartum) with cord blood showed a transplacental gradient of 30 to 14 ng/mL for 25OHD, and 199 to 39 pg/mL for 1,25$(OH)_2$D. The patient was discharged on postpartum day 7 with a serum calcium level of 10.7 mg/dL and 1,25$(OH)_2$D level of 87 pg/mL. She self-injected calcitonin (100 μg) on postpartum days 10, 13, and 18, while her calcium decreased from 12 to 9.8 mg/dL. Her calcium level was 10.0 mg/dL (albumin 4.5 g/dL) 6 months postpartum.

Discussion

CYP24A1 mutations can cause hypercalcemia, nephrolithiasis, and nephrocalcinosis that manifest during infancy[4,9] or adulthood.[5,7,8,10] This case report describes a CYP24A1-deficient young woman with gestational hypercalcemia during two consecutive pregnancies, each confounded by acute pancreatitis, a complication not previously attributed to *CYP24A1* mutations. The pathogenic 8-nt deletion (c.999_1006del) has not been reported in CYP24A1-related hypercalcemia. This deletion is expected to abolish the 24-hydroxylase activity of CYP24A1 because the resulting premature stop codon is predicted to trigger degradation of the mRNA[11] and the deletion removes the heme-binding domain (aa 455–464) critical to enzymatic activity.[12]

A plausible explanation for the limited penetrance of our patient's biallelic *CYP24A1* mutation is that she has consistently avoided sun exposure since age 4 years, when the exposure was noted to cause a rash. Alternatively, she might have been protected from vitamin D-mediated hypercalcemia by compensatory mechanisms that downregulate 1,25$(OH)_2$D production or upregulate its degradation through CYP24A1-independent pathways that have been proposed by mouse models of CYP24A1 deficiency.[13]

During pregnancy and peripartum, several factors could synergize to unmask the hypercalcemic effect of CYP24A1 deficiency. These include increased calciferol intake from prenatal vitamins, increased activation of 25OHD by the placenta and maternal kidney, the cessation of transplacental calcium flux at delivery,[14,15] and the production of PTHrP by the placenta[16] and lactating mammary gland.[17] We expect these gestation-specific hypercalcemic factors to be poorly tolerated in the CYP24A1-deficient state, where the already-suppressed PTH levels offer no room for further suppression.

Our patient shared several gestational complications with pregnant women who have PTH-mediated hypercalcemia. These include worsening hypertension, peripartum hypercalcemic crisis, pancreatitis (both pregnancies), and fetal demise (first pregnancy).[14,18] Although not previously linked to CYP24A1 deficiency, the pancreatitis in our patient was likely attributable to hypercalcemia because she did not have the usual risk factors such as cholelithiasis, hypertriglyceridemia, or alcohol use.

Maternal PTH-mediated hypercalcemia is known to cause transient neonatal hypocalcemia.[19] Our patient's baby was free from hypocalcemia presumably because of our multipronged approach to minimize gestational hypercalcemia. The baby was also free from hypercalcemia, in line with the typically recessive nature of CYP24A1-related hypercalcemia.[4] The normal 1,25$(OH)_2$D level in the cord blood (39 ng/mL) despite a maternal level of 199 ng/mL was also in keeping with the limited placental transfer of this vitamin.[20]

The calcemic effect of breastfeeding has not been investigated in CYP24A1-deficient mothers. By stimulating mammary production of PTHrP, lactation often induces mild hypercalcemia[21] and occasionally causes symptomatic hypercalcemia.[22] We advised our patient to stop breastfeeding after colostrum production because of the concern that lactation-induced hypercalcemia might exacerbate ongoing hypercalcemia and acute pancreatitis. Indeed, her PTHrP level on postpartum day 4 was found to be 3.4 pmol/L, the upper limit of the normal range.

It is noteworthy that during the resolution of hypercalcemia after each pregnancy, our patient transitioned through a phase when hypercalcemia was accompanied by 1,25$(OH)_2$D levels that fell within the reference range (20 to 79 pg/mL) (Fig. 2). An analogous transitional phase has been observed in sarcoid patients with hypercalcemia, where glucocorticoid treatment normalizes 1,25$(OH)_2$D levels approximately 1 week before the resolution of hypercalcemia (3 to 7 versus 12 to 15 days).[23] This illustrates the caveat that normal levels of 1,25$(OH)_2$D do not exonerate this hormone as the cause of hypercalcemia.

Our patient received one 40-mg dose of prednisone just before leaving the hospital following the first pregnancy, when sarcoidosis was presumed to cause her hypercalcemia. Although this trial of glucocorticoids was of inadequate duration to demonstrate a treatment effect, or lack thereof, available literature suggests that CYP24A1-associated hypercalcemia does not respond to glucocorticoids.[10,24]

Historically, the cause of 1,25$(OH)_2$D-mediated hypercalcemia remains unidentified in up to 18% of cases.[25] Given the recent realization that many of these cases are caused by *CYP24A1*

mutations,[26] measurement of plasma 24,25$(OH)_2$D level and its ratio over 25OHD should be included in the evaluation of vitamin D-mediated hypercalcemia. A low ratio ($<$0.04 in our case) suggests impaired vitamin D catabolism and may warrant sequencing of the *CYP24A1* gene. As reported previously[7,8] and herein, hypercalcemia from CYP24A1 deficiency may be unmasked by the physiological changes of pregnancy. It is therefore important to establish a pregnancy- or trimester-specific reference range for 24,25$(OH)_2$D and its ratio over 25OHD.

In summary, hypercalcemia caused by CYP24A1 deficiency may manifest only during pregnancy in women without baseline hypercalcemia or nephrocalcinosis. Potential complications in pregnancy may include maternal hypertension, acute pancreatitis, and fetal demise. CYP24A1 deficiency should be considered in patients with otherwise unexplained vitamin D-mediated hypercalcemia, including those with "inappropriately normal" 1,25$(OH)_2$D levels or subnormal levels of 24,25$(OH)_2$D. Behavioral management of CYP24A1-related hypercalcemia includes the avoidance of sun exposure, vitamin D, and dietary calcium. Future studies will investigate the indications and modalities of pharmacological management during pregnancy and the safety of breastfeeding.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Data analysis: AS, HG, NJC, NWC, and RLF. Data interpretation: GNW and NWC. Manuscript preparation and revision: GNW and NWC. Approving final version of manuscript: All authors.

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