Clinical Pediatric Endocrinology

Vol.34 / No.1
January 2025
pp 60–65

Case Report

Idiopathic infantile hypercalcemia with a *CYP24A1* variant triggered by vitamin D supplementation in fortified milk: A case report

Sota Iwafuchi[1, 2], Nao Uchida[1], Naoya Saijo[1], Chisumi Sogi[1, 3], Miki Kamimura[1, 4], Jun Takayama[5–8], Gen Tamiya[5–8], Atsuo Kikuchi[1, 8], and Junko Kanno[1]

[1]*Department of Pediatrics, Tohoku University Graduate School of Medicine, Sendai, Japan*
[2]*Department of Pediatrics, Yamagata Prefectural Central Hospital, Yamagata, Japan*
[3]*Department of Pediatrics, Japan Community Health Care Organization Sendai Hospital, Sendai, Japan*
[4]*Department of Pediatrics, National Hospital Organization Sendai Medical Center, Sendai, Japan*
[5]*Department of AI and Innovative Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan*
[6]*Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan*
[7]*Statistical Genetics Team, RIKEN Center for Advanced Intelligence Project, Tokyo, Japan.*
[8]*Department of Rare Disease Genomics, Tohoku University Graduate School of Medicine, Sendai, Japan*

Highlights

- Vitamin D-fortified milk consumption in infants carrying a *CYP24A1* variant can cause hypercalcemia.
- *CYP24A1* genotypic analysis is useful in infants with unexplained hypercalcemia.

Abstract. Idiopathic infantile hypercalcemia (IIH) is characterized by hypercalcemia, nephrocalcinosis, vomiting, dehydration, and failure to thrive. It is caused by the presence of biallelic loss-of-function variants in the *CYP24A1* locus. Although hypercalcemia has been linked to the consumption of vitamin D-fortified milk, no reports have documented its role in triggering IIH in patients with *CYP24A1* variants. Herein, we describe a case of IIH triggered by vitamin D-fortified milk consumption in a 9-mo-old male patient carrying a *CYP24A1* variant. After BCG vaccination, the patient developed a facial rash, became anorexic, appeared to be in a bad mood, and began consuming vitamin D-fortified milk instead of baby food. Blood tests showed a marked hypercalcemia (18.5 mg/dL), high 1,25-$(OH)_2$D (98.7 pg/dL) levels, and low parathyroid hormone (PTH) (< 4.0 pg/dL) and PTHrP (< 1.0 pg/dL) levels. The calcium levels were successfully normalized after treatment with saline loading, furosemide, pamidronate, and a low-calcium milk diet. After discharge, blood calcium levels remained normal with no recurrence of symptomatic hypercalcemia, but circulating PTH levels were persistently suppressed. Renal ultrasonography at 8 yr of age revealed high medullary echogenicity and diffuse echogenic foci in both kidneys. Trio-based whole-genome sequencing identified the following biallelic pathogenic variants c.[464G>A];[1324C>T], p.[Trp155Ter];[Gln442Ter], in the *CYP24A1* (NM_000782.5) locus. Unexplained hypercalcemia in infants should raise suspicions of abnormal vitamin D metabolism and *CYP24A1* locus genotypic analysis can be informative in this regard.

Key words: hypercalcemia, *CYP24A1*, vitamin D metabolism, fortified milk

Received: July 9, 2024 Accepted: August 19, 2024 Advanced Epub: September 9, 2024
Corresponding Author: Junko Kanno, M.D., Ph.D., Department of Pediatrics, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan
E-mail: junkokan@ya2.so-net.ne.jp

Introduction

Idiopathic infantile hypercalcemia (IIH) is caused by abnormalities in vitamin D metabolism, characterized by hypercalcemia, nephrocalcinosis, vomiting, dehydration, and failure to thrive (1, 2). Vitamin D is crucial for calcium homeostasis and bone health, and its deficiency leads to hypocalcemia and bone mineralization defects (3). Vitamin D supplementation (400 IU/d) is a well-established and effective preventive measure for rickets in infants and children (4). Vitamin D present in the skin and diet undergoes hydroxylation to its biologically active form, 1,25-$(OH)_2$D, in the liver and kidneys. Ultraviolet light converts 7-dehydrocholesterol to pre-vitamin D in the skin, and pre-vitamin D is isomerized to vitamin D at body temperature. In the liver, vitamin D is hydroxylated at position 25 by CYP2R1 and CYP27A1. In the kidneys, CYP27B1 hydroxylates vitamin D at the 1α-position to form 1α,25-dihydroxyvitamin D [1,25-$(OH)_2$D], the biologically active form of vitamin D. CYP24A1 inactivates vitamin D in multiple steps (5). The *CYP24A1* locus encodes CYP24A1, which regulates the catabolism of 1,25-$(OH)_2$D (6). Biallelic loss-of-function variants in the *CYP24A1* gene are associated with IIH, and this association was first identified in a cohort of familial cases in 2011 (2). Since that time, there have been additional case reports with similar genetic variants from various countries (7–9).

Vitamin D supplementation prevents rickets; however, excessive vitamin D intake induces hypercalcemia (10). Further, hypercalcemia due to overconsumption of vitamin D-fortified milk, rich in vitamin D and calcium, has also been reported (11).

Only a few detailed clinical case reports of IIH caused by *CYP24A1* variants exist (2, 7). While hypercalcemia linked to excessive consumption of vitamin D-fortified milk has been documented (11), our search did not yield any reports of IIH triggered by such intake in infants with *CYP24A1* variants. However, it is possible that patients with IIH may consume vitamin D-fortified milk, and the two conditions may coexist. Herein, we describe a case of IIH possibly triggered by vitamin D-fortified milk consumption in an infant with *CYP24A1* variants.

Case Report

The patient was the third child of healthy, nonconsanguineous Japanese parents, born via spontaneous delivery at 34 wk and 0 d of gestation, with Apgar scores of 8 and 9 at 1 and 5 min, respectively. His birth weight was 2,332 g (+0.67 SD), and his length was 45.5 cm (+0.65 SD). Blood tests at birth revealed normal serum Ca and P levels (Ca 9.1 mg/dL, P 5.6 mg/dL). No family history of IIH was reported. He exhibited normal development during the neonatal and infancy periods. He was fed a mixed diet of breast milk, formula milk, and baby food. He received his vaccinations as scheduled, including the BCG vaccination at 7 mo of age. Three weeks after the BCG vaccination, a rash appeared on his right cheek. At 9 mo of age, he became anorexic and in a bad mood, preferring vitamin D-fortified milk over baby food, and 1 wk later he was hospitalized. This coincided with a worsening of the rash, which spread to his face, arms, and legs. He also began vomiting daily. He was diagnosed with hypercalcemia (18.5 mg/dL) at a local hospital and was subsequently transferred to our hospital.

Upon assessment at our hospital, the patient had a body length of 69.5 cm (−0.87 SD) and a body weight of 7,380 g (–1.58 SD), thus exhibiting a weight loss of 1,200 g in 1 mo. His body temperature was 37.4°C, and his heart rate was 150/min. He was conscious but unwell. There was a blistering rash with flushing observed on the face, arms, and legs. Laboratory findings revealed marked hypercalcemia, elevated 1,25-$(OH)_2$D (98.7 pg/dL) levels, and suppressed levels of PTH (< 4.0 pg/dL), and parathyroid hormone-related peptide (PTHrP) (< 1.0 pg/dL) (**Table 1**). Hypercalcemia associated with malignancy was considered unlikely because PTHrP levels were low. Interleukin-2 receptor and angiotensin-converting enzyme levels were normal. A urinalysis done on the second day of admission, during saline loading and furosemide administration, revealed an elevated urinary calcium/creatinine ratio (Ca/Cr ratio; g/gCr), and a fractional excretion of calcium (FECa) of 3.0%. The skin biopsy results showed a conspicuous cellular infiltration in the dermis, primarily around blood vessels and appendages. The infiltrating cells were predominantly lymphocytes and plasma cells that were not atypical. The lymph node biopsy exhibited preexisting lymph node architecture, but histiocyte-like cells were observed in the interfollicular spaces. Tingible body macrophages were found to be common in the embryonic center. No granuloma or atypical cells were observed. Bone marrow biopsy revealed no signs of malignancy. Ophthalmologic examination revealed no evidence of uveitis. Neutrophil function tests were normal. After admission, the calcium levels were successfully normalized after saline loading, furosemide and pamidronate administration, and consumption of a low-calcium milk diet (**Fig. 1**). During hospitalization, the patient exhibited symptoms of papulonecrotic tuberculid as a side effect of BCG vaccination on two instances. However, all symptoms resolved spontaneously (**Fig. 1**). There were no findings consistent with sarcoidosis or malignant lymphoma. After discharge, low-calcium milk intake was continued, and serum calcium levels remained within the near-normal range, with no recurrence of symptomatic hypercalcemia. However, the PTH levels remained persistently suppressed (5.5–9.7 pg/dL). From infancy to school age, his growth was normal (**Fig. 2**). Renal echocardiography performed at 8 yr of age showed high medullary echogenicity and sporadic echogenic foci in both kidneys (**Figs. 3A, B**). Genetic testing was performed on the patient and both parents with their consent due to the suspicion of underlying calcium metabolic abnormalities.

doi: 10.1297/cpe.2024-0049

Table 1. Laboratory test results of the patient at admission

Complete blood cell count		Biochemistry			Urinary biochemical	
				Reference range		
WBC (/μL)	21,100	AST (IU/L)	33		Cr (mg/dL)	3
Neut (%)	53.6	ALT (IU/L)	8		Na (mmol/L)	99
RBC ($\times 10^6$ /μL)	5.09	LDH (IU/L)	266		K (mmol/L)	14
Hb (g/dL)	12.9	ALP-JSCC (IU/L)	424		Ca (mg/dL)	5
Hct (%)	38.2	T-Bil (mg/dL)	0.3		P (mg/dL)	7
PLT ($\times 10^3$/μL)	510	BUN (mg/dL)	15		Ca/Cr ratio (g/gCr)	1.67
		Cr (mg/dL)	0.25			
		UA (mg/dL)	5.8		FENa (%)	5.4
		TP (g/dL)	6.9		FEK (%)	53.7
		ALB (g/dL)	3.9		FECa (%)	3
		Na (mEq/L)	141		%TRP (%)	97.7
		K (mEq/L)	4.5			
		Cl (mEq/L)	106			
		Ca (mg/dL)	18			
		IP (mg/dL)	4			
		CRP (mg/dL)	1.3			
		1,25-$(OH)_2$ Vit D (pg/dL)	98.7	(20–70)		
		whole PTH (pg/dL)	< 4.0	(9–39)		
		PTHrP intact (pg/dL)	< 1.0	(< 1.1)		
		IL-2R (U/mL)	761	(122–496)		
		ACE (U/L)	6	(8.3–21.4)		

FENa, fractional excretion of sodium (Na); FEK, fractional excretion of potassium (K); FECa, fractional excretion of calcium (Ca); TRP, tubular reabsorption of phosphate (P).

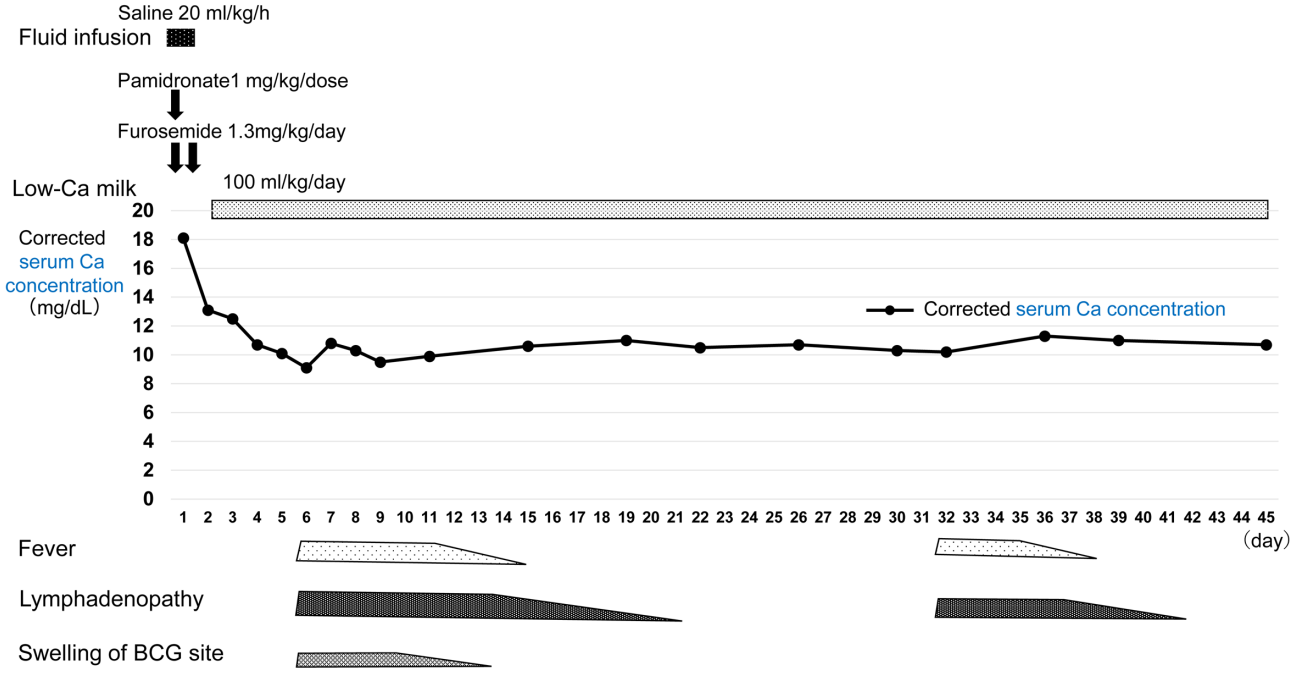

Fig. 1. Clinical course after admission. The line on the graph shows the trend in the corrected serum calcium concentration. The drugs administered and special milk introduced are listed at the top of the graph. The symptoms that appeared during hospitalization are shown at the bottom of the graph.

Genetic study

DNA was extracted from blood and saliva samples of the patient and his parents. Whole-genome sequencing using DNBSEQ T7 whole genome sequencer software (MGI Tech) in 150 bp paired-end mode and PCR-free libraries was conducted. The sequenced reads were mapped to the hg19 human reference genome using BWA MEM software (ver. 0.7.17-r1188). Single nucleotide variants (SNVs), short indels, and copy number

doi: 10.1297/cpe.2024-0049

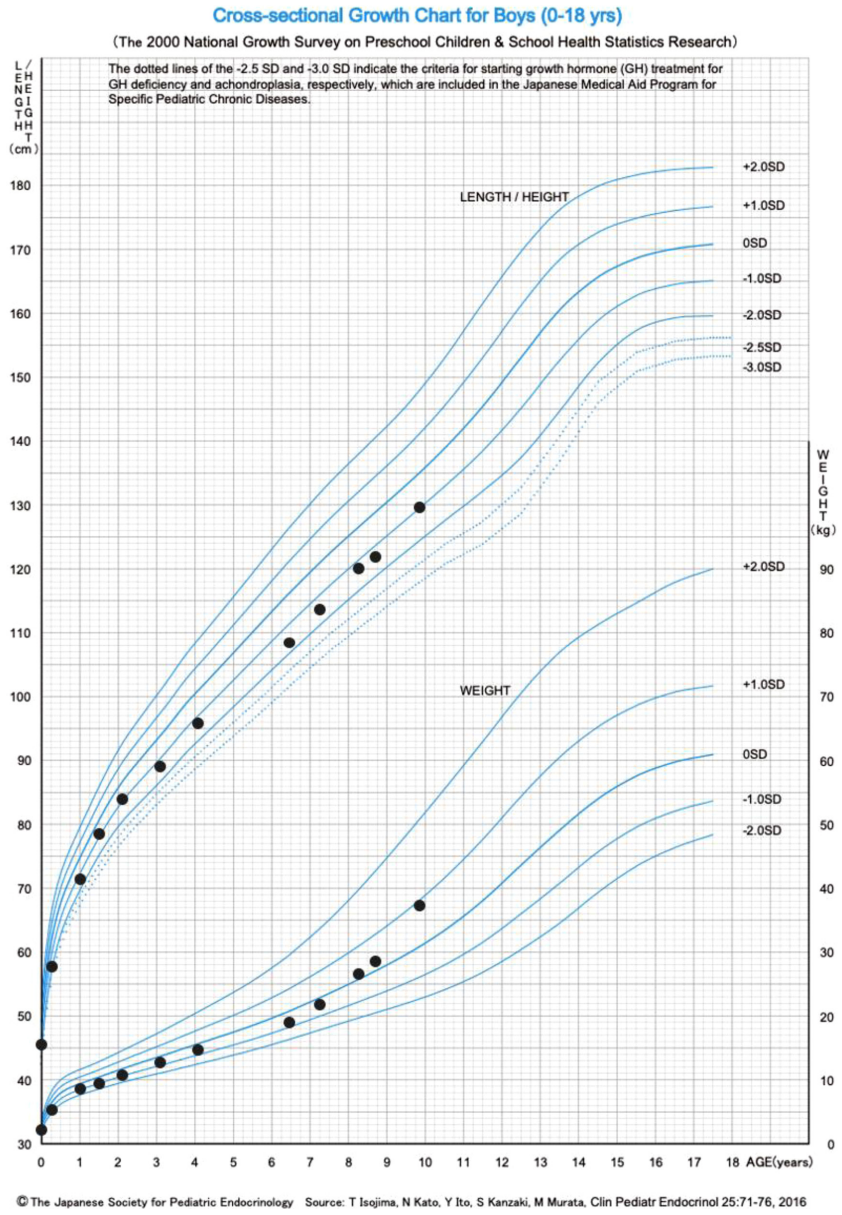

Fig. 2. Height and body weight growth curves of the patient in our case report.

variants (CNVs) were identified using the Genome Analysis Toolkit software ver.I 4.2.6.1, and structural variants were detected using Smoove software (ver. 0.2.8). After quality filtering, variants were annotated using SnpEff software (ver. 5.1). Introns ± 100 bp from exons, CNVs, and structural variants were included in the analysis. Trio-based whole genome sequencing revealed biallelic pathogenic variants, namely c.[464G>A];[1324C>T] p.[Trp155Ter];[Gln442Ter], in the *CYP24A1* (NM_000782.5) locus. One variant, c.464G>A, p. Trp155Ter, inherited from the father, has been previously identified as a causative variant of IIH (9). The other variant, c.1324C>T, p.Gln442Ter, inherited from the mother, was a novel nonsense variant classified as pathogenic in the ACMG guidelines (PVS1+PM2+PM3+PP3).

Discussion

We present a rare case of IIH associated with a *CYP24A1* variant and exacerbated by intake of vitamin D-fortified milk. In this patient, the loss-of-function variants of the *CYP24A1* locus impaired the inactivation of active vitamin D. In addition, our patient consumed a high volume of vitamin D-fortified milk, which has been linked with hypercalcemia due to excessive vitamin D and calcium consumption (11). To our knowledge, there are no prior reports of these conditions coexisting in a patient with hypercalcemia.

After BCG vaccination, our patient developed a generalized papulonecrotic tuberculoid rash, became anorexic and in a bad mood, and began drinking vitamin D-fortified milk rather than baby food, leading to excessive intake of vitamin D and calcium. Normally,

doi: 10.1297/cpe.2024-0049

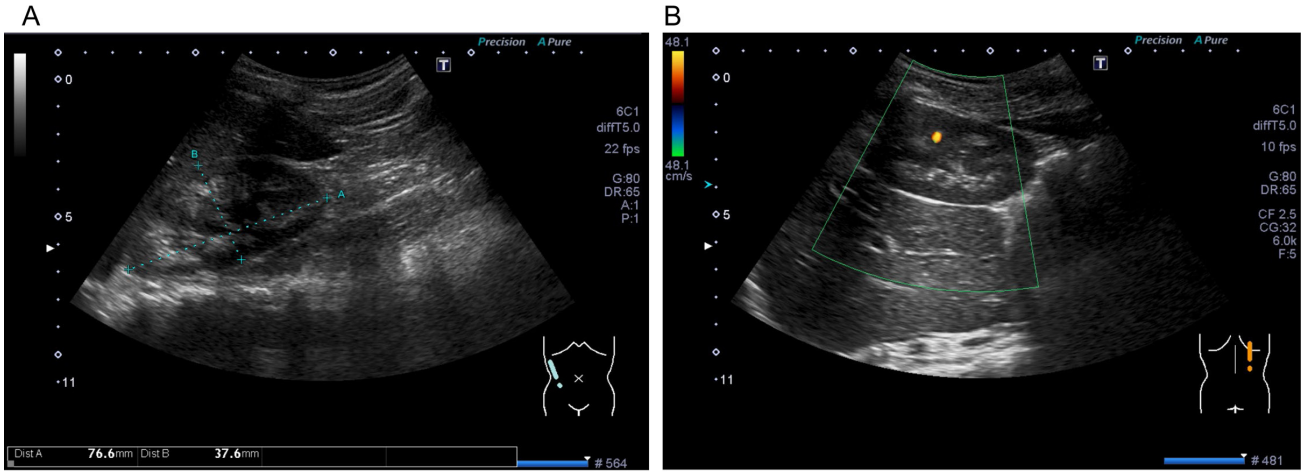

Fig. 3. Renal ultrasonography radiographs of the patient acquired at 8 yr of age. (A) The right kidney view from ventral side. (B) The right kidney view from the dorsal side. Ultrasonography showed high medullary echogenicity and sporadic echogenic foci in both kidneys, which were classified as grade 1 in the nephrocalcinosis grading system (15).

vitamin D supplementation is effective in preventing and curing infants and children (4), but those with pathological *CYP24A1* variants are at risk of developing symptomatic hypercalcemia (7). Vitamin D-fortified milk is a rich source of iron, vitamin D, and calcium, which are often insufficient in breast milk nutrition alone (12). The calcium content of vitamin D-fortified milk is about five times that of breast milk and twice that of artificial milk (13). Therefore, if a 9-mo-old infant consumes more than 1,000 mL of vitamin D-fortified milk daily, this can lead to an excess intake of at least 1,000 mg of calcium and 7.0 μg of vitamin D. In 2019, a consensus statement on infant beverages led by the American Academy of Pediatrics discouraged infant consumption of vitamin D-fortified milk (14). In Japan, there are two reported cases of hypercalcemia linked to excessive consumption of vitamin D-fortified milk (11). In our case, the loss-of-function variants of *CYP24A1* suppressed the normal inactivation of active vitamin D, resulting in dysregulated serum calcium levels, which likely led to symptomatic hypercalcemia.

A 2022 systematic literature review identified 154 cases of *CYP24A1* variants, 39 of which had an infantile onset (8). Infantile onset IIH cases typically manifest with nephrocalcinosis (84.6%), dehydration (46.1%), and developmental delay (41.0%), and these outcomes are consistent with the clinical presentation of our patient except for developmental delay. Shen *et al.* demonstrated an association between low vitamin D metabolite levels and cardiovascular disease risk (9). Early detection of IIH is crucial for long-term prognosis because there are several complications in cases of IIH associated with *CYP24A1* variants. Thus, in the evaluation of infants with unexplained hypercalcemia, it is important to consider abnormal vitamin D metabolism and accordingly perform *CYP24A1* genotypic analysis.

However, the coexistence of IHH and excessive vitamin D intake that we have documented here is a rare condition, and it took us 8 years to make a definitive diagnosis. We followed this patient closely because, although he presented with marked hypercalcemia at the onset, we were unable to identify the cause at that time. Based on the mild but persistent suppression of PTH levels and the findings of calcification on ultrasonography of the kidneys, we considered the presence of some genetic alteration and ultimately reached a definitive diagnosis.

In conclusion, in cases of unexplained marked hypercalcemia in infants, it is important to consider some genetic alteration and its triggers, and to follow up carefully with biochemical tests and renal ultrasonography for an early diagnosis and optimal management.

Conclusion

We report a case of IIH triggered by intake of vitamin D-fortified milk in a Japanese infant carrying pathogenic *CYP24A1* variants. We conclude that in cases of unexplained marked hypercalcemia in infants, it is important to consider the genetic background of the patient and its triggers, and to follow up carefully with biochemical tests and renal ultrasonography for an early diagnosis and optimal management.

Conflict of interests: Jun Takayama, Gen Tamiya, and Atsuo Kikuchi received a research grant from Astellas Pharma. The other authors have no conflicts of interest to declare.

Acknowledgements

We thank the patient and his family for participating in our study. Finally, we thank Masato Nihei and Naonori Kumagai for their diagnostic support.

References

1. Marcinowska-Suchowierska E, Kupisz-Urbańska M, Łukaszkiewicz J, Płudowski P, Jones G. Vitamin D toxicity – A clinical perspective. Front Endocrinol (Lausanne) 2018;9: 550. [Medline] [CrossRef]
2. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, *et al*. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. N Engl J Med 2011;365: 410–21. [Medline] [CrossRef]
3. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol 2005;289: F8–28. [Medline] [CrossRef]
4. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, *et al*. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab 2016;101: 394–415. [Medline] [CrossRef]
5. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev 1998;78: 1193–231. [Medline] [CrossRef]
6. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21: 319–29. [Medline] [CrossRef]
7. Molin A, Baudoin R, Kaufmann M, Souberbielle JC, Ryckewaert A, Vantyghem MC, *et al*. CYP24A1 mutations in a cohort of hypercalcemic patients: Evidence for a recessive trait. J Clin Endocrinol Metab 2015;100: E1343–52. [Medline] [CrossRef]
8. Zheng Z, Wu Y, Wu H, Jin J, Luo Y, Cao S, *et al*. Clinical heterogeneity and therapeutic options for idiopathic infantile hypercalcemia caused by *CYP24A1* pathogenic variant. J Pediatr Endocrinol Metab 2023;36: 999–1011. [Medline] [CrossRef]
9. Shen H, Bielak LF, Ferguson JF, Streeten EA, Yerges-Armstrong LM, Liu J, *et al*. Association of the vitamin D metabolism gene CYP24A1 with coronary artery calcification. Arterioscler Thromb Vasc Biol 2010;30: 2648–54. [Medline] [CrossRef]
10. Lightwood R. Idiopathic hypercalcaemia with failure to thrive: Nephrocalcinosis. Proc R Soc Med 1952;45: 401.
11. Hirota S, Isho I, Hitoshi H, Keiichi M, Fujiko F, Masahiro T, *et al*. Hypercalcemia and nephrocalcinosis induced by long-term surplus intake of follow-up formula in a child with Down syndrome. Jpn J Pediatr Gastroenterol Hepatol Nutr 2016;30: 14–9.
12. Itkonen ST, Erkkola M, Lamberg-Allardt CJE. Vitamin D fortification of fluid milk products and their contribution to vitamin D intake and vitamin D status in observational studies – A review. Nutrients 2018;10: 1054. [Medline] [CrossRef]
13. Cormick G, Betran AP, Romero IB, Cormick MS, Belizán JM, Bardach A, *et al*. Effect of calcium-fortified foods on health outcomes: A systematic review and meta-analysis. Nutrients 2021;13: 316. [Medline] [CrossRef]
14. Lott M, Callahan E, Welker Duffy E, Story M, Daniels S. Healthy Beverage Consumption in Early Childhood: Recommendations from Key National Health and Nutrition Organizations. Consensus Statement. Durham, NC: Healthy Eating Research, 2019. Available at http://healthyeatingresearch.org.
15. Dick PT, Shuckett BM, Tang B, Daneman A, Kooh SW. Observer reliability in grading nephrocalcinosis on ultrasound examinations in children. Pediatr Radiol 1999;29: 68–72. [Medline] [CrossRef]

doi: 10.1297/cpe.2024-0049