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Clinical Research Article

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24-Hydroxylase Deficiency Due to *CYP24A1* Sequence Variants: Comparison With Other Vitamin D–mediated Hypercalcemia Disorders

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Abbreviations: 1αH, 25(OH)D3 1-α hydroxylase; 24H, 24-hydroxylase; 24HD, 24-hydroxylase deficiency; EVT, exogenous vitamin D toxicity; IIH, idiopathic infantile hypercalcemia; IQR, interquartile range; NC, nephrocalcinosis; PTH, parathyroid hormone; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; uCa:Cr, urinary calcium-to-creatinine ratio.

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Abstract

Context: CYP24A1 encodes 24-hydroxylase, which converts 25(OH)D3 and $1,25(OH)_2D_3$ to inactive metabolites. Loss-of-function variants in CYP24A1 are associated with 24-hydroxylase deficiency (24HD), characterized by hypercalcemia, nephrolithiasis, and nephrocalcinosis. We retrospectively reviewed laboratory, imaging, and clinical characteristics of patients with suspected or confirmed 24HD and patients with other vitamin D–mediated hypercalcemia disorders: sarcoidosis, lymphoma, and exogenous vitamin D toxicity (EVT).

Objective: To identify features that differentiate 24HD from other vitamin D-mediated hypercalcemia disorders.

Methods: Patients seen at the Mayo Clinic (Rochester, MN) from January 1, 2008, to 31 December, 2016, with the following criteria were retrospectively identified: serum calcium ≥9.6 mg/dL, parathyroid hormone <30 pg/mL, and $1,25(OH)_2D_3$ >40 pg/mL. Patients were considered to have 24HD if they had (1) confirmed *CYP24A1* gene variant or (2) $25(OH)D_3$:$24,25(OH)_2D$ ratio ≥50. Patients with sarcoidosis, lymphoma, and EVT were also identified. Groups were compared using the Fisher exact test (categorical variables) or the Wilcoxon rank sum test (continuous variables).

Results: We identified 9 patients with 24HD and 28 with other vitamin D–mediated disorders. Patients with 24HD were younger at symptom onset (median 14 vs 63 years; P = .001) and had positive family history (88.9% vs 20.8%; P < .001), nephrocalcinosis (88.9% vs 6.3%; P < .001), lower lumbar spine Z-scores (median –0.50 vs 1.20; P = .01),

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higher peak serum phosphorus (% of peak reference range, median 107 vs 84; $P = .01$), and higher urinary calcium:creatinine ratios (median 0.24 vs 0.17; P = .047).
Conclusion: Patients with 24HD had clinical and laboratory findings that differed from other vitamin D–mediated hypercalcemia disorders. 24HD should be suspected in patients with hypercalcemia who present at younger age, have positive family history, and have nephrocalcinosis.

Key Words: Idiopathic infantile hypercalcemia, genetic, hypercalcemia, vitamin D, 24-hydroxylase, *CYP24A1*

Allelic variants in the human cytochrome P450 Family 24 Superfamily A member 1, or *CYP24A1*, were first shown to be associated with idiopathic infantile hypercalcemia (IIH) in 2011, when 10 patients with the disorder were described [1]. Most patients with IIH received medical attention for symptoms attributable to severe hypercalcemia, including lethargy, failure to grow and gain weight, dehydration, and hypotonia. Numerous reports established that loss-of-function mutations in *CYP24A1* were associated with a familial hypercalcemia syndrome attributable to 24-hydroxylase deficiency (24HD); this syndrome is characterized by intermittent or persistent hypercalcemia, nephrolithiasis, and nephrocalcinosis (NC) [2-22]. Although primary hyperparathyroidism and malignancy account for 80% to 90% of hypercalcemia cases, 24HD is nevertheless an important addition to the differential diagnosis of non-parathyroid hormone (PTH)–mediated hypercalcemia and high (or inappropriately normal) $1,25(OH)_2D_3$ [23]. Currently, however, the clinical and biochemical differences between 24HD and other disorders of vitamin D–mediated hypercalcemia are not well understood.

Physiologically, *CYP24A1*-encoded 24-hydroxylase (24H) works in conjunction with *CYP27B1*-encoded $25(OH)D_3$ 1-α hydroxylase (1αH) to maintain appropriate vitamin D concentrations and calcium homeostasis [24, 25]. In response to low serum calcium, elevated PTH, or low serum phosphorus, 1αH converts $25(OH)D_3$ to its active metabolite, $1,25(OH)_2D_3$ [26]. $1,25(OH)_2D_3$ increases bone resorption, renal calcium and phosphorus reabsorption, and intestinal calcium and phosphorus absorption [27, 28]. When serum calcium is elevated, 24H converts $25(OH)D_3$ and $1,25(OH)_2D_3$ to inactive metabolites ($24,25[OH]_2D_3$ and $1,24,25[OH]_3D_3$, respectively) [24, 26, 29-31]. 24H is expressed in tissues containing the vitamin D receptor, including bone, kidney, and intestine, and its activity is increased by $1,25(OH)_2D_3$ and FGF23, but reduced by hypophosphatemia [24, 26, 32-35].

Other disorders associated with vitamin D–mediated hypercalcemia include sarcoidosis, lymphoma, exogenous vitamin D toxicity (EVT), and fungal infections; however, distinguishing among these disorders can be difficult because of their similar biochemical profiles [26]. We thus aimed to assess the clinical presentation, laboratory parameters, and imaging studies of patients with vitamin D–mediated hypercalcemic disorders to identify potential differentiating factors. Such data may expedite evaluation of patients with 24HD, facilitating faster diagnosis and avoiding unnecessary testing.

Materials and Methods

This study was approved by the Mayo Clinic Institutional Review Board. The reporting of this study is in compliance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement [36].

Patient Identification

A search was performed of an institutional patient database to identify those seen between January 1, 2008, and December 31, 2016, at the Mayo Clinic (Rochester, Minnesota) who had a single blood sample showing a serum calcium level of 9.6 mg/dL or higher, PTH less than 30 pg/mL, and $1,25(OH)_2D_3$ greater than 40 pg/mL. Though these laboratory parameters include values that are within the normal reference interval, they were chosen in order to include all patients with possible vitamin D–mediated hypercalcemia based on our review of our known patients with 24HD and recognition that, in these patients, these values sometimes fall in the normal range. We reviewed electronic health records to identify patients with confirmed or suspected 24HD, which was defined as at least 1 of the following: (1) genetic testing confirming a disease-associated *CYP24A1* sequence variant based on ACMG guidelines [37], (2) elevated ratio of $25(OH)D_3$:$24,25(OH)_2D_3$ (values >50), or (3) high serum calcium, low PTH, and a family history of 24HD. Four patients in our 24HD cohort were members of the family reported in Tebben et al [38]: proband II 3, III 1, III 2, and III 3. Patients with a diagnosis of sarcoidosis, lymphoma, EVT, or fungal infection were also identified through manual record review.

Data Collection

From each patient record, the following data were abstracted, if available: peak serum calcium (reference intervals 9.3-10.6 mg/dL for ages 1-17 and

8.6-10.0 mg/dL for ages 18-59); lowest serum PTH; serum calcium and phosphorus at the time of lowest PTH; peak serum phosphorus; alkaline phosphatase closest to peak calcium; peak serum $25(OH)D_3$; peak serum $1,25(OH)_2D_3$; $24,25(OH)_2D$; urinary calcium-to-creatinine ratio (uCa:Cr) from a spot urine test; 24-hour urine calcium, creatinine, and phosphorus; computed tomographic or ultrasonographic imaging evidence of urinary stones, NC, or cysts; bone density Z-score of the hip and spine; age at onset of symptoms attributable to stones, hypercalcemia, or hypercalciuria; family history of symptoms attributable to stones, hypercalcemia, or hypercalciuria; and other presenting features.

Hypercalciuria was defined by a uCa:Cr value exceeding the 95th percentile for the patient's age [39] or by a 24-hour urinary calcium excretion level exceeding 4 mg/kg per day [40]. Phosphorous at the time of lowest PTH, peak serum phosphorous, and alkaline phosphatase values were converted to percent of peak reference range before analysis because reference values differ significantly by age.

Statistical Analysis

Available data were summarized and reported as the median (interquartile range [IQR]) for continuous variables and number (percentage) for categorical variables. Comparisons between disease groups were evaluated with the Fisher exact test for categorical variables and the Kruskal–Wallis or Wilcoxon rank sum test for continuous variables, due to the skewed nature of the measurements. All calculated *P* values were 2-sided, and $P < .05$ was considered statistically significant. Data were analyzed with SAS version 9.4 software (SAS Institute Inc) and R 3.4.2 (The R Foundation).

Results

Demographic Characteristics

We identified 222 patients that fit the aforementioned criteria (serum calcium level ≥9.6 mg/dL, PTH <30 pg/mL, and $1,25(OH)_2D_3$ >40 pg/mL); of these, 6 had genetic testing confirming 24HD and 3 had an elevated $25(OH)D_3$:$24,25(OH)_2D$ ratio without genetic verification available (ie, 9 patients had confirmed or suspected 24HD). *CYP24A1*-specific genetic and laboratory parameters in our 24HD cohort are presented in Table 1. We identified 37 patients with other disorders associated with vitamin D–mediated hypercalcemia: 6 with EVT, 7 with lymphoma, 15 with sarcoidosis, and 9 with fungal infection. We excluded patients with fungal infection from the subsequent analyses because nearly all had superficial infections without systemic involvement upon further review.

Characteristics at Presentation for Patients with Vitamin D–mediated Hypercalcemia

Presenting characteristics of patients with 24HD, sarcoidosis, lymphoma, and EVT are shown in Table 2. Among patients with 24HD, 2 (22%) had IIH and 5 (56%) had urinary stones. For the 2 patients with IIH, symptoms included poor sleep, difficulty feeding, and weight loss.

Table 1. Genetic characteristics and laboratory parameters of patients with 24-hydroxylase deficiency (n = 9)

Genetic variant or laboratory parameter	
***CYP24A1* (24-hydroxylase) variant, n(%)**[a]	
c.1226T>C (p.Leu409Ser) (homozygous)	1 (11.1)
c.999_106del (p.Ser334Valfs*9) and c.1186C > T (p.Arg396Trp)	1 (11.1)
IVS5 + 1G>A and IVS6-2A > G (N/A)	1 (11.1)
IVS6-2A>G (N/A) (single mutation)	2 (22.2)
IVS5 + 1G>A (N/A) (single mutation)	1 (11.1)
Unknown	3 (33.3)
$24,25(OH)_2D_3$, ng/mL, median (IQR)	0.20 (0.15, 0.23)
$25(OH)D_3$:$24,25(OH)_2D$ ratio, median (IQR)	353 (336, 460)
Family history of 24-hydroxylase variant	
Yes	4 (44.4)
Unknown	5 (55.6)

Summary statistics are presented as median (IQR) for continuous variables and n (%) for categorical variables.
Abbreviations: IQR, interquartile range; N/A, not applicable.
[a]Protein alteration, if applicable, is shown parenthetically after the variant.

Table 2. Characteristics at presentation for patients with vitamin d–mediated hypercalcemia, stratified by disease group

Feature, n(%)	24HD (n = 9)	Sarcoidosis (n = 15)	Lymphoma (n = 7)	EVT (n = 6)
Urinary stones	5 (55%)	4 (27%)	0 (0%)	0 (0%)
Severe symptoms of hypercalcemia[a]	3 (33%)	6 (40%)	6 (86%)	3 (50%)
Palpitation	1 (11%)	0 (0%)	0 (0%)	1 (17%)
Weight loss	2 (22%)	3 (20%)	0 (0%)	2 (33%)
Pulmonary symptoms[b]	0 (0%)	3 (20%)	2 (29%)	0 (0%)
Asymptomatic	2 (22%)	3 (20%)	1 (14%)	3 (50%)

Abbreviations: 24HD, 24-hydroxylase deficiency; EVT, exogenous vitamin D toxicity.
[a]Symptoms included loss of appetite, nausea, vomiting, abdominal pain, constipation, polyuria, polydipsia, dehydration, constitutional symptoms (fatigue, weakness, muscle pain), altered mental status, or poor feeding or failure to thrive (in neonates).
[b]Symptoms included cough or shortness of breath.

Comparison of 24HD With Other Hypercalcemia Disorders

We compared clinical and biochemical parameters of patients with 24HD (n = 9) and the combined group of patients with sarcoidosis, lymphoma, or EVT (n = 28) (Table 3). Patients with 24HD were significantly younger at symptom onset (median [IQR], 14 [1-35] vs 63 [56-79] years; $P = .001$) and more commonly had a family history of symptoms (88.9% vs 20.8%; $P < .001$) and NC on imaging (88.9% vs 6.3%, respectively; $P < .001$) than other hypercalcemic disorders. Patients with 24HD also had lower Z-scores for total lumbar spine (median –0.50 [–0.80, 0.70] vs 1.20 [0.80, 2.10], respectively; $P = .01$), higher peak serum phosphorus levels (% of peak reference range, median 107 [98, 108] vs 84 [81, 91]; $P = .01$), and higher uCa:Cr ratios (median 0.24 [0.21, 1.70] vs 0.17 [0.14, 0.18]; $P = .047$).

We also compared characteristics across each individual disease subgroup (24HD, lymphoma, sarcoidosis, EVT) (Table 3 and Fig. 1). Compared with the lymphoma and sarcoidosis groups, patients with 24HD were significantly younger at symptom onset (median 14 [1, 35] vs 80 [78, 88] and 59 [56, 67] years, respectively; $P < .05$ for both) and more commonly had a family history of symptoms (88.9% vs 0% and 7.7%, respectively; $P < .05$ for both), NC on imaging (88.9% vs 0% and 0%, respectively; $P < .05$ for both), and higher peak serum phosphorus levels (% of peak reference range, 107 [98, 108] vs 82 [78, 91] and 84 [76, 89], respectively; $P < .05$ for both). Compared with the lymphoma group, patients with 24HD had lower peak serum calcium levels (10.9 [10.6, 11.4] vs 13.6 [11.3, 15.4]; $P = .01$). Additionally, patients with 24HD had significantly higher peak $25(OH)D_3$ levels than the lymphoma and sarcoidosis groups (56 [54, 61] vs 39 [35, 44] and 31 [28, 37], respectively; $P < .05$ for both) but lower levels than the EVT group (223 [109, 371]; $P = .002$). Aside from having the highest peak $25(OH)D_3$ levels of any group, the EVT group did not reach statistical difference in any other observed category.

Discussion

This study identifies features that may help distinguish patients with 24HD from those with other disorders of vitamin D–mediated hypercalcemia. In 2011, Schlingmann et al. [1] first associated *CYP24A1* variants with IIH. With the subsequent recognition of late-onset disease in adults (characterized by hypercalcemia, NC, nephrolithiasis, or a combination) [3, 7, 9, 12, 14, 21, 38, 41] and studies that showed only select cases of IIH were associated with *CYP24A1* sequence variants [4], it is important to characterize this disorder as 24HD, which in its most severe form may cause IIH. This distinction is important because not all patients with 24HD will have IIH and vice versa. Schlingmann et al. also reported variants in *SLC34A1* encoding NaPi2a present in the renal proximal tubule and responsible for phosphate reabsorption to be another cause of IIH [42]. As in patients with *CYP24A1* variants, the underlying cause of hypercalcemia and hypercalciuria is elevated $1,25(OH)_2D_3$. However, in contrast to patients with *CYP24A1* variants, those with *SLC34A1* variants exhibit renal phosphate wasting with low FGF23 concentrations.

Hypercalcemia is common in the inpatient and outpatient settings and has a broad differential diagnosis [23]. Discovery of the abnormality often prompts an extensive and costly evaluation, particularly when non-PTH mediated. Although much about the prevalence and disease course of 24HD still remains unknown, a recent study from several European university centers reported 35% of patients with hypercalcemia (serum calcium >2.6 mmol/L) and low serum PTH (<20 pg/mL) harbored variants in *CYP24A1* [15]. Additionally, Nesterova et al. [17] estimated that the prevalence of biallelic *CYP24A1* variants in the general population is as high as 0.4% to 2%. Thus,

Table 3. Comparison of clinical and biochemical parameters for patients with 24HD or other hypercalcemia disorders

Parameter	24HD (n = 9)	All non-24HD disorders (n = 28)	*P* value[a]	EVT (n = 6)	*P* value[a]	Lymphoma (n = 7)	*P* value[a]	Sarcoidosis (n = 15)	*P* value[a]	Global *P* value[b]
Age at onset of symptoms, years	14 (1, 35)	63 (56, 79)	**.001**	52 (30, 56)	.09	80 (78, 88)	**.005**	59 (56, 67)	**.004**	**<.001**
Family history of symptoms, n(%)	8 (88.9)	5/24 (20.8)	**<.001**	4 (66.7)	.53	0/5 (0)	**.003**	1/13 (7.7)	**<.001**	**<.001**
Imaging findings, n(%)										
Stones, n(%)	5 (55.6)	6/15 (40.0)	.68	0/2 (0)	…	2/5 (40.0)	…	4/8 (50.0)	…	.73
Nephrocalcinosis, n(%)	8 (88.9)	1/16 (6.3)	**<.001**	1/2 (50.0)	.35	0/6 (0)	**.001**	0/8 (0)	**<.001**	**<.001**
Cysts, n(%)	7 (77.8)	7/15 (46.7)	.21	0/2 (0)	…	4/5 (80.0)	…	3/8 (37.5)	…	.11
Z-score										
Left hip	−0.60 (−1.70, 0.60)	−0.25 (−0.70, 1.30)	.47	0.45 (−0.90, 1.80)	…	−0.70 ()	…	0.20 (−0.70, 1.30)	…	.81
Right hip	−0.70 (−1.70, 0.40)	0.80 (−0.30, 1.85)	.34	0.75 (−0.80, 2.30)	…	…	…	0.80 (0.20, 1.40)	…	.56
Spine (total lumbar)	−0.50 (−0.80, 0.70)	1.20 (0.80, 2.10)	**.01**	1.10 (0.70, 1.50)	…	0.80 ()	…	2.10 (0.90, 2.30)	…	.06
Peak serum calcium, mg/dL[d]	10.9 (10.6, 11.4)	11.5 (10.9, 13.0)	.11	10.9 (10.6, 11.2)	.95	13.6 (11.3, 15.4)	**.01**	11.6 (10.8, 12.7)	.19	**.03**
Lowest PTH, pg/mL	6.6 (6.0, 14.0)	13.0 (8.6, 18.0)	.08	15.0 (12.0, 19.0)	…	12.0 (7.9, 15.0)	…	14.0 (7.9, 20.0)	…	.25
Calcium at lowest PTH, mg/dL	10.6 (10.4, 10.8)	11.0 (10.4, 11.8)	.13	10.8 (10.6, 11.0)	…	11.3 (11.0, 14.9)	…	10.9 (10.1, 12.0)	…	.15
Phosphorous at lowest PTH, %PRR	96 (71, 96)	80 (71, 89)	.43	90 (80, 94)	…	71 (67, 91)	…	80 (73, 84)	…	.25
Peak serum phosphorous, %PRR	107 (98, 108)	84 (81, 91)	**.01**	90 (89, 94)	.11	82 (78, 91)	**.04**	84 (76, 89)	**.02**	**.03**
Alkaline phosphatase[c], %PRR	55 (30, 70)	62 (51, 72)	.48	64 (48, 126)	…	52 (48, 76)	…	63 (56, 69)	…	.66
Peak 25(OH)D_3, ng/mL	56 (54, 61)	37 (29, 47)	.09	223 (109, 371)	**.002**	39 (35, 44)	**.01**	31 (28, 37)	**.002**	**<.001**
Peak 1,25$(OH)_2D_3$, pg/mL	145 (114, 149)	87 (69, 133)	.05	67 (46, 80)	…	90 (82, 143)	…	95 (70, 150)	…	.09
Urinary spot test, mg/L										
Calcium, mg/L	207 (93, 325)	214 (144, 288)	.78	160 ()	…	…	…	267 (127, 308)	…	.81
Creatinine, mg/L	1111 (370, 1525)	870 (848, 1,679)	.52	625 (380, 870)	…	…	…	1679 (848, 2048)	…	.26
Calcium:creatinine ratio	0.24 (0.21, 1.70)	0.17 (0.14, 0.18)	**.047**	0.18 ()	…	…	…	0.15 (0.13, 0.18)	…	.10
24-Hour urinary test										
Calcium, mg per 24 h	248 (72, 407)	385 (226, 521)	.23	237 (226, 399)	…	385 (375, 536)	…	391 (221, 521)	…	.47
Creatinine, mg per 24 h	1285 (351, 2219)	1122 (832, 1536)	>.99	984 (811, 1157)	…	1184 (832, 1536)	…	1122 (1102, 1541)	…	.97

Table 3. Continued

Parameter	24HD (n = 9)	All non-24HD disorders (n = 28)	*P* value[a]	EVT (n = 6)	*P* value[a]	Lymphoma (n = 7)	*P* value[a]	Sarcoidosis (n = 15)	*P* value[a]	Global *P* value[b]
Phosphorus, mg per 24 h	1118 (311, 1332)	956 (757, 1154)	>.99	757 ()	…	…	…	1154 ()	…	.67
Calcium excretion, mg/kg per h	4.47 (3.52, 5.17)	4.53 (2.67, 5.73)	>.99	4.53 (2.99, 7.33)	…	5.27 (4.47, 7.20)	…	4.24 (2.50, 5.25)	…	.53
Hypercalciuria, n(%)	8 (88.9)	10/18 (55.6)	.19	2/4 (50.0)	…	3/3 (100)	…	5/11 (45.5)	…	.12

Reported statistics are median (IQR) for continuous variables and n (%) for categorical variables. Results followed by empty brackets indicate that only 1 sample was available for analysis. Laboratory measures taken closest to diagnosis. *P* values in bold indicate statistical significance at the 0.05 alpha level.

… Indicates that the global *P* value was not statistically significant using the Kruskal–Wallis test, so no further between-group tests were performed.

Abbreviations: 24HD, 24-hydroxylase deficiency; EVT, exogenous vitamin D toxicity; IQR, interquartile range; PTH, parathyroid hormone. %PRR = % of peak reference range.

[a]Comparison with 24HD values.

[b]Global *P* value (comparison across 4 disease groups).

[c]Lab taken closest to peak calcium.

[d]Reference intervals for serum calcium are 9.3-10.6 mg/dL for ages 1-17 and 8.6-10.0 mg/dL for ages 18-59.

despite limited data, 24HD is an important addition to the differential diagnosis of a patient with vitamin D–mediated hypercalcemia.

Recent studies have reported that the ratio of 25(OH)D_3:24,25$(OH)_2$D, as determined by liquid chromatography with tandem mass spectrometry, may sensitively identify patients with 24HD [15, 43]; ratios exceeding 50 (usually >80) are considered compatible with the disorder (reference range, 7-23) [44]. Thus, we used the ratio of 50 or higher in patients with clinical findings consistent with 24HD as an inclusion criterion for our study. More than 65% of our 24HD cohort had genetic testing that confirmed the condition. The average 24,25$(OH)_2D_3$ concentration for patients with 24HD in our cohort was 0.19 (range, 0.1-0.28), and all patient values were below the reference range for vitamin D–replete healthy individuals (>1.7 ng/mL) [44].

Our results indicate that presenting features overlap considerably for different forms of vitamin D–mediated hypercalcemia (Table 2). However, 55% of our cohort with 24HD initially presented with urinary stones and 22% with IIH, suggesting that stones may be a more common finding compared to other causes of vitamin D mediated hypercalcemia. Although the current cohort was small, our observation was consistent with findings of a recent literature review by Jobst-Schwan et al. [12]. Features of patients with sarcoidosis were less specific, although pulmonary symptoms may be a differentiating factor. Severe hypercalcemia symptoms were most common at presentation for patients with lymphoma (86% of cases).

Comparison of patients with 24HD with those having other forms of vitamin D–mediated hypercalcemia revealed several significant differences (Table 3). Given the genetic basis of the disease and the previously reported characteristics, we had suspected that patients with 24HD would be significantly younger at symptom onset and would be more likely to have a positive family history and NC than all other groups. However, the disease-specific subgroup analysis showed that patients with 24HD were younger and more likely to have a positive family history and NC than the lymphoma and sarcoidosis groups only (not EVT). The lack of significant difference between 24HD and EVT was likely driven by a few families that were included in an already small cohort: we had 1 family of 4 individuals in our 24HD group (n = 9) and 3 siblings in our EVT group (n = 6) who all received supplements with a very high vitamin D content. Furthermore, we had imaging studies (to assess for NC) available for only 2 individuals in the EVT group. Further studies of a larger cohort would be needed to adequately compare these 2 groups.

The effect of the *CYP24A1* variant on bone density is also unclear, with previous studies reporting inconsistent results that ranged from low [17, 38] to normal [14, 41,

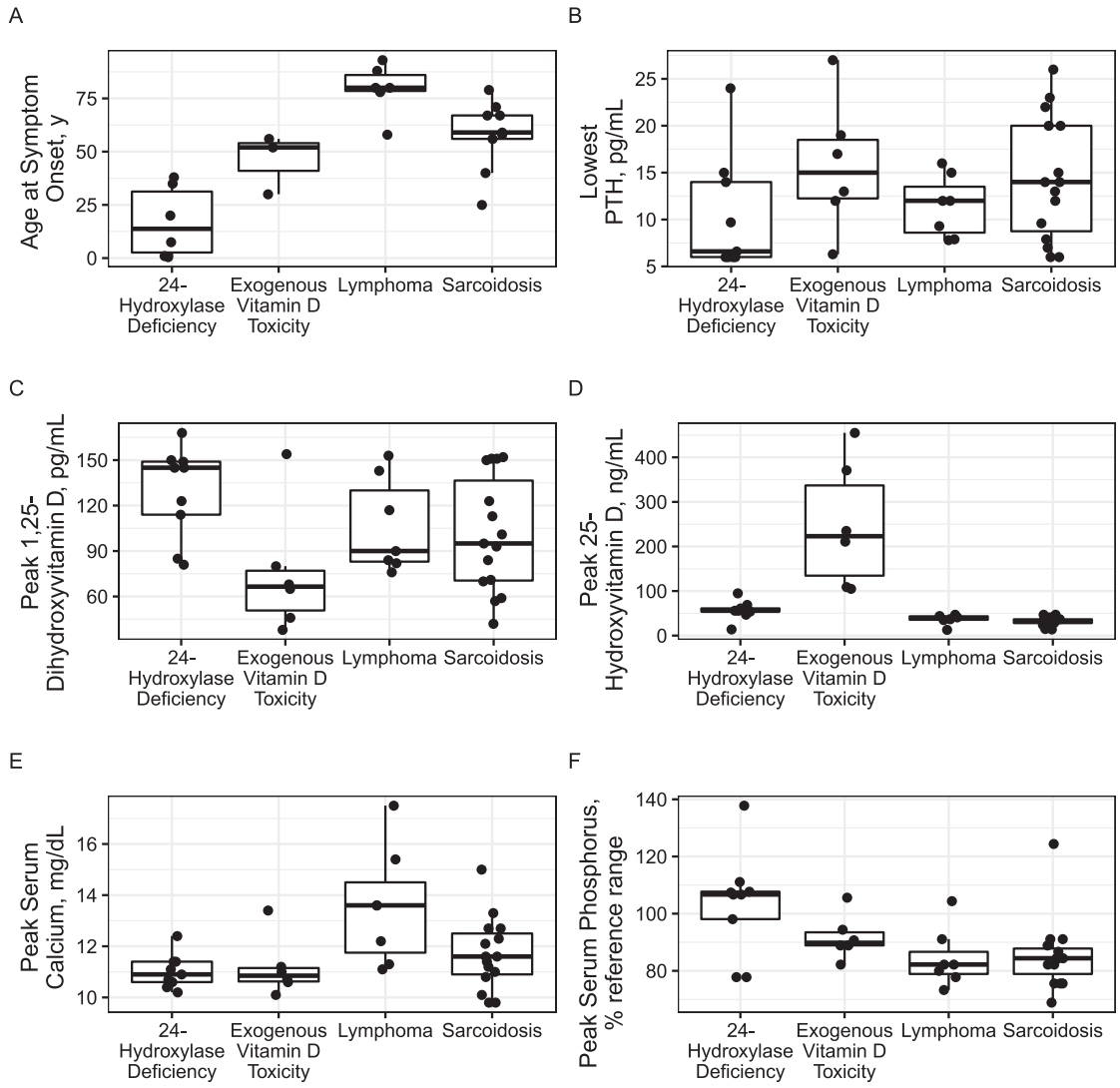

Figure 1. Boxplots show comparisons across disease groups.The heavy line represents the median, boxes represent the first and third quartiles, and whiskers represent 1.5 × interquartile range values. (A) Age at symptom onset. (B) Lowest parathyroid hormone (PTH). (C) Peak 1,25 dihydroxyvitamin D_3. (D) Peak 25-hydroxyvitamin D_3. (E) Peak serum calcium. (F) Peak serum phosphorus.

45] to increased [3] bone density. We observed significantly lower lumbar spine Z-scores in patients with 24HD, including some members of the family reported by Tebben et al. [38] plus 5 unrelated patients with suspected or confirmed 24HD. Of note, we did not observe significant differences in hip Z-scores when comparing 24HD with all other groups, nor did we observe differences in hip or lumbar spine Z-scores when comparing 24HD to individual groups, likely related to the relatively small groups sizes. Decreased bone density in 24HD would theoretically be caused by chronic high or high-normal $1,25(OH)_2D_3$ levels resulting in increased osteoclastic resorption from bone. Our results from this small cohort suggest that 24HD may decrease bone density and affect trabecular bone to a greater degree than cortical bone. Nevertheless, with such varied results across the literature, the effects of 24HD on bone density remains incompletely understood and additional studies are needed for elucidation.

We also noted significantly higher age-adjusted peak serum phosphorus levels in patients with 24HD than in all other groups (Table 2 and Fig. 1F). Phosphorus levels are often not reported in previously published papers regarding 24HD and reviewing the literature reveals that, among those reporting serum phosphorus, there is variability, with some reporting normal levels, some elevated, and some low [3, 37, 38, 46]. The higher serum phosphorus concentration could be related to chronic elevation of $1,25(OH)_2D_3$ increasing intestinal phosphate absorption. Additionally, suppressed PTH would decrease renal phosphate loss, though elevated FGF23 would be expected to

induce phosphaturia [14]. Unfortunately, we do not have data regarding FGF23 for this cohort and it is difficult to predict with certainty whether FGF23 would be elevated due to higher serum phosphorus and $1,25(OH)_2D_3$ or decreased due to low PTH, while acknowledging that the impact on FGF23 of 24H and $24,25(OH)_2D$, remain unknown. Further investigation into phosphate homeostasis including FGF23 analysis in this population is needed.

Hypercalciuria has been noted in patients with 24HD [3, 4, 7, 22]. In our cohort, more 24HD patients presented with hypercalciuria compared with all other groups (88.9% vs 55.6%). However, the difference was not statistically significant and hypercalciuria therefore does not appear to be a differentiating factor. We did observed significantly higher uCa:Cr in patients with 24HD. This result is challenging to interpret because patients in the 24HD group were younger at symptom onset, and normal uCa:Cr values are higher in infants and slowly decline during the first few years of life [39]. Thus, age alone may be the primary reason for this result. Weight-adjusted 24-hour urinary calcium excretion values, which are less influenced by age variation, were not significantly different, and uCa:Cr ratios were not significantly different when comparing individual groups. In summary, urine studies likely do not meaningfully contribute to differentiation of 24HD from other disorders of vitamin D–mediated hypercalcemia.

Comparison of individual groups showed that patients with 24HD, while hypercalcemic, had significantly lower serum calcium levels than patients with lymphoma (Table 3 and Fig. 1E). Markedly elevated calcium levels are a hallmark of hypercalcemia of malignancy, with multiple mechanisms implicated beyond elevated $1,25(OH)_2D_3$, including PTH-related peptide and osteolytic metastases [47]. Thus, marked hypercalcemia with severe symptoms, in the absence of strong 24HD-differentiating factors (eg, family history, NC, younger age) should prompt investigation for malignancy.

Comparisons among individual disease groups also showed that patients with 24HD had significantly higher $25(OH)D_3$ concentrations than those with lymphoma or sarcoidosis but significantly lower concentrations than patients with EVT. $25(OH)D_3$ concentration exceeding at least 80 ng/mL (and usually much higher) is typically required to produce hypercalcemia from EVT [48]. Thus, high-normal $25(OH)D_3$ levels may prompt suspicion for 24HD, but values >80 ng/mL may be more consistent with an exogenous source. Furthermore, high $1,25(OH)_2D_3$ levels are anticipated in 24HD and have been documented in multiple patients [2, 3, 38, 41]. Although patients in our cohort with 24HD did have relatively higher $1,25(OH)_2D_3$ values, the difference was not statistically different compared with other vitamin D–mediated causes of hypercalcemia. Figure 1C shows the high-normal range of $1,25(OH)_2D_3$ values for patients with 24HD. Thus, as previously reported, $1,25(OH)_2D_3$ concentrations within the reference range should not exclude the diagnosis [1, 4, 9]. Dauber et al. [4] explained this phenomenon with the hypothesis that serum $1,25(OH)_2D_3$ concentrations were not indicative of tissue levels.

Though generally perceived as an autosomal recessive disease, we described a kindred that included several affected members with a single allele variant, suggesting dominant inheritance in some is possible [38]. Although it may be that a second pathogenic mutation was not identified, the milder phenotype described in this cohort suggests that those with single allele variants have reduced penetrance. Nonetheless, given that the vast majority of publications regarding patients with 24HD report autosomal recessive inheritance, further evidence from nuanced genotype–phenotype studies will be required to support single allele disease causation in this condition.

Limitations of this study include the retrospective study design, which affected the availability of data for each disease group. Additionally, our cohort size was small and included a family in the 24HD subgroup (4 individuals) and another in the EVT subgroup (3 individuals), which may bias findings due to genetic overlap. Future prospective studies with larger nonrelated cohorts are needed to further clarify the defining characteristics of this disease relative to other vitamin D–mediated hypercalcemia disorders.

Conclusions

Our study identified disease characteristics that may help differentiate 24HD from other disorders of vitamin D–mediated hypercalcemia, and thus may assist in the clinical evaluation of these patients. 24HD should be considered early in the diagnostic process, especially in younger patients, those with a positive family history of hypercalcemia or hypercalciuria, and those with nephrocalcinosis.

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Additional Information

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