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The effects of cholecalciferol and afamelanotide on vitamin D levels in erythropoietic protoporphyria: a multicentre cohort study

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Abstract

Background Patients with erythropoietic protoporphyria experience lifelong painful photosensitivity resulting in a lack of sunlight exposure. Previous studies have shown that 47–63% of patients with EPP suffer from vitamin D deficiency and a high prevalence of osteoporosis. An effective treatment for EPP has been available since 2016: the α-melanocyte stimulating hormone analogue afamelanotide. So far, studies on vitamin D levels in EPP have only investigated patients who have not been treated with afamelanotide.

Objectives To investigate the effects of afamelanotide treatment on vitamin D levels in EPP.

Methods A multicentre observational cohort study in adults with EPP from the Erasmus Medical Centre, the Netherlands, and the University Hospital Düsseldorf, Germany, was carried out. Routinely collected vitamin D levels between 2005 and 2021 were used for analysis. Patient exposure to cholecalciferol or afamelanotide was categorized into four treatment groups: untreated, cholecalciferol, afamelanotide and combined treatment. A linear mixed model for longitudinal data was applied to measure the effect of the treatment groups compared with the untreated groups on vitamin D levels.

Results A total of 230 patients and 1774 vitamin D measurements were included. The prevalence of vitamin D deficiency and severe deficiency remained high despite afamelanotide treatment (< 50 nmol L^{-1} in 71.8% of patients and < 30 nmol L^{-1} in 48.1%, respectively). Afamelanotide treatment alone did not lead to a significant average increase in vitamin D levels [$\beta = 0.5$, 95% confidence interval (CI) −3.2 to 4.2]. In contrast, cholecalciferol and combined therapy with afamelanotide led to a significant increase in vitamin D levels [$\beta = 11.6$ (95% CI 7.2–15.9) and $\beta = 15.2$ (95% CI 12.3–18.1), respectively].

Conclusions Cholecalciferol remains essential for the treatment of vitamin D deficiency in EPP, irrespective of new treatment options like afamelanotide. Afamelanotide treatment did not affect vitamin D levels. We suggest that future guidelines include continuous monitoring of vitamin D and a prescription for cholecalciferol in all patients with EPP, including those treated with afamelanotide.

Lay summary

Erythropoietic protoporphyria (EPP) is a rare inherited condition. People with EPP experience severe pain after their skin has been exposed to sunlight. To avoid this severe pain, people with EPP avoid going out in the sun by limiting outdoor activities or by wearing protective clothing. As sunlight is needed for our skin to produce vitamin D, approximately half of people with EPP in Europe do not have enough of it. In 2016, a new treatment called afamelanotide (SCENESSE®) became available, which allows people with EPP to go outside and expose themselves to sunlight longer without pain.

In this study, we looked at how afamelanotide and vitamin D supplements affect vitamin D levels in people with EPP. We included information from patients treated in Rotterdam in the Netherlands and Düsseldorf in Germany and analysed levels of vitamin D in their blood. We also examined electronic patient files and collected questionnaires on the use of vitamin D supplements. In total, information from 230 patients was included.

We found that afamelanotide alone did not raise vitamin D levels, but in combination with vitamin D supplements, vitamin D levels did go up. Even though afamelanotide is now available, our findings suggest that people with EPP may need more time to adapt to an outdoor lifestyle, after being conditioned to avoid sunlight since their childhood.

Overall, our study demonstrates that vitamin D supplements remain crucial for people with EPP, with or without afamelanotide treatment.

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What is already known about this topic?

- Patients with erythropoietic protoporphyria (EPP) are significantly less exposed to (sun)light than healthy people.
- Observational studies in untreated patients with EPP have shown that 47–63% of European patients with EPP suffer from vitamin D deficiency.
- A high prevalence of osteopenia (36%) and osteoporosis (23%) has also been observed.

What does this study add?

- Following afamelanotide treatment, the prevalence of vitamin D deficiency remained remarkably high (72%).
- Both cholecalciferol alone and combined with afamelanotide resulted in an increase in vitamin D levels.
- Afamelanotide treatment did not lead to a rise in vitamin D levels.

Erythropoietic protoporphyria (EPP) is a rare inherited disorder in the haem biosynthesis pathway, characterized by lifelong painful photosensitivity, resulting in vitamin D deficiency and osteoporosis.[1,2] Without treatment, patients resort to sun-protective clothing and sun-avoiding behaviour. A previous study has shown that patients with EPP are significantly less exposed to (sun)light compared with healthy controls.[3] When taking protective clothing into consideration, even less cutaneous light exposure is to be expected. This lack of sunlight exposure is a known risk factor for vitamin D deficiency. During sunlight exposure, 7-dehydrocholesterol in the skin absorbs ultraviolet B (UVB) and is converted to previtamin D3, which isomerizes into vitamin D3.[4,5] This cutaneous synthesis of vitamin D in response to sunlight is the main source of vitamin D production.[6] In 2016, afamelanotide, a potent α-melanocyte-stimulating hormone analogue, was approved. With this treatment, patients with EPP can spend more time outside, bear more light exposure and experience less severe phototoxic reactions.[3,7]

Observational studies in untreated patients with EPP have shown that 47–63% suffer from vitamin D deficiency.[1,2,8–10] A high prevalence of osteopenia (36%) and osteoporosis (23%) has also been observed.[1] Vitamin D deficiency is a known risk factor for early-onset osteoporosis.[11,12] Osteoporosis increases the risk of fractures, especially with increasing age and fragility, leading to pain and disability.[13] Recent findings show that vitamin D supplementation and monitoring leads to increased serum 25-hydroxy-vitamin D (vitamin D levels) in patients with EPP, but the levels are still below those of the healthy general population.[2] So far, research has been concentrated on cohorts without afamelanotide treatment. The effect of afamelanotide treatment on vitamin D levels has yet to be investigated.

In this multicentre cohort study, we aimed to provide insights into the prevalence of vitamin D deficiency in patients with EPP receiving afamelanotide. Furthermore, we investigated the effects of afamelanotide on vitamin D levels with and without cholecalciferol supplementation. Using real-world data, we investigated whether there is an ongoing need for intensive monitoring and supplementation of vitamin D in patients treated with afamelanotide. We hypothesized that patients receiving afamelanotide, who have been proven to endure more sunlight exposure,[3] have increased cutaneous vitamin D production and an improved vitamin D status.

Patients and methods

In this multicentre ambispective observational cohort, all adult patients with EPP attending the Porphyria Centre Rotterdam, Erasmus MC, the Netherlands, and those attending the Porphyria Centre of the Department of Dermatology at the University Hospital of Düsseldorf, Germany, were eligible for inclusion. The inclusion criteria were age $\geq$ 18 years, a confirmed diagnosis of EPP based on phototoxic symptoms and increased erythrocyte protoporphyrin IX levels ($>$ 4 μmol L^{-1} in erythrocytes).

The following data were collected retrospectively from medical records: date of birth, sex, vitamin D levels (with measurement dates) and prescription data. The two-time questionnaire was prospectively collected in the Dutch cohort: the first was completed before starting afamelanotide treatment and the second during a follow-up in 2022. Questionnaires inquired about medication use, specifically cholecalciferol, calcium and multivitamins, including start and stop dates, dosage and frequency.

Vitamin D levels in the Netherlands were monitored from 2005 to 2021, while in Germany, monitoring was done throughout 2021. Patients were routinely followed up as part of regular care. The frequency of vitamin D levels measurements varied over time and depended on the frequency of visits. In general, patients who do not receive afamelanotide treatment are routinely invited once a year, whereas those receiving afamelanotide visit, on average, 3.4 (range 1–4) times a year.[7]

Vitamin D deficiency was defined as a vitamin D level $<$ 50 nmol L^{-1} ($<$ 20 ng mL^{-1}) according to the clinical practice guidelines of the Endocrine Society Taskforce.[14] Severe vitamin D deficiency was defined as $<$ 30 nmol L^{-1} ($<$ 12 ng mL^{-1}), according to the international vitamin D standardization programme.[6,15,16] Vitamin D levels in the Netherlands from 2005 to 2017 were assessed using radioimmunoassays (IDS®, Newcastle, UK). From November 2018 onward, levels were assessed with a Lumipulse® G1200 platform (Fujirebio, Tokyo, Japan). In Germany, levels were determined by immunoassay using electrochemiluminescence technology on a Cobas 8000® (model e801; Roche, Basel, Switzerland). For levels obtained from Germany, values were converted from ng mL^{-1} to nmol L^{-1} by applying a conversion factor of 2.5.

Starting in 2016, adults with EPP could choose to receive treatment with afamelanotide. Patients could select

dates for afamelanotide administration for a maximum of four times a year and a minimum of 60-day intervals. Cholecalciferol was prescribed to all vitamin D-deficient patients with EPP, starting in 2005 for the Netherlands and in 2021 for Germany. In the Netherlands, either an 800–1000 IU daily dose or a 50 000 IU monthly dose of cholecalciferol was standard. In the case of remaining deficiency, the dose was increased and compliance was discussed. In Germany, either a daily dose of 500–2000 IU or a weekly dose of 20 000 IU was prescribed.

The vitamin D level measurements were assigned to one of four groups: untreated, afamelanotide, cholecalciferol and combined treatment (afamelanotide + cholecalciferol). All measurements after the initial afamelanotide implant were labelled as being in the afamelanotide group. All measurements after the first prescription and – if applicable – before stopping the prescription of cholecalciferol were labelled as being in the cholecalciferol group. If the use of cholecalciferol was indicated in the questionnaire at the time of measurement, this was also labelled as a cholecalciferol treatment. All measurements that were concurrently eligible for both cholecalciferol and afamelanotide treatment were placed in the combined treatment group. Measurements obtained prior to the first afamelanotide implantation, when no cholecalciferol use was indicated in the questionnaires, and if they were taken before the initial cholecalciferol prescription, were placed in the untreated group.

Follow-up duration (in months) was calculated using the calendar date of each measurement. This involved determining the time difference between the initial measurement of each patient in each treatment group and the subsequent observations. Additionally, the calendar date of each measurement was used to ascertain the meteorological season, starting on the first day of the month, and the age of the patient at that specific time.

Dutch data from clinical records and questionnaires were entered and saved in a database program called Castor (version 2022.5.2.0) from 2021 to 2022. All Dutch cohort vitamin D levels were exported from electronic patient files and imported directly into Castor. A database validation check was performed for 10% of all data, chosen at random by a fellow investigator; an error percentage of $< 5\%$ was accepted. German data were collected from medical records and inputted into Microsoft Excel and shared with Dutch investigators.

Statistical tests with *P*-values were two-sided with a significance level of 0.05. For descriptive analyses, data were summarized using mean (SD) for parametric data, median [interquartile range (IQR)] for nonparametric data, and frequencies and percentages for categorical data. Depending on the distribution, a Student's *t*-test or Wilcoxon signed ranked test was used to compare two groups. Furthermore, an ANOVA or Kruskal–Wallis test was applied when comparing more than two groups.

To investigate the association between treatment and the course of vitamin D levels over time, a multivariable multivariate linear mixed model was used with random intercepts, random slopes and an unstructured covariance matrix. The fixed effects in the mixed model included age, sex, season and cohort, and the random effects included a random intercept and linear random slopes of time. Nonlinearity of the association between continuous determinants and vitamin D levels was assessed by splines with two knots. Residual plots were made for all associations; these did not show heteroscedasticity. The percentage of missing values was $< 2\%$ for all determinants. We applied multivariate imputation by chained equations (5 imputations, 10 iterations) to impute missing values. However, complete case analyses based on nonimputed data showed similar results.

Multiple subgroup analyses were conducted, including treatment-specific analysis and subgroup analysis by season, sex, follow-up duration and age. Not all subgroup analysis models could fit a random slope. In such cases, a model with a random intercept and a fixed slope was employed. For the stratification of follow-up duration, the inclusion of the cohort as a covariate was excluded for the three upper quantiles of follow-up time, resulting in the exclusion of this covariate from the model. All statistical analyses were performed using RStudio version 2021.09.2 (R Foundation for Statistical Computing, Vienna, Austria). The 'lme4' package was used to fit the mixed model.[17]

Results

The final cohort consisted of 230 patients, 138 from the Netherlands and 92 from Germany. Thirteen patients were excluded from the analysis owing to missing data or lack of informed consent (Figure S1; see Supporting Information). A total of 1774 vitamin D measurements were included. These measurements were placed into one of four treatment groups: untreated ($n = 235$); afamelanotide ($n = 331$); cholecalciferol ($n = 104$); combined treatment group ($n = 1104$). An overview of baseline characteristics is provided in Table 1, categorized by cohort and treatment group.

The median vitamin D level for all untreated measurements was 48.0 (IQR 33.0–64.5) nmol L^{-1}. Cholecalciferol alone and combined with afamelanotide treatment resulted in higher vitamin D levels [60.0 nmol L^{-1} (IQR 40.8–72.3) and 62.0 nmol L^{-1} (IQR 47.0–75.0), respectively]. In contrast, afamelanotide treatment alone did not result in higher levels than in the untreated group [median 50.0 nmol L^{-1} (IQR 29.5–71.5); Table S1 (see Supporting Information)].

The prevalence of vitamin D deficiency ($\leq$ 50 nmol L^{-1}) in patients with EPP, based on the lowest value measured per participant, was 79% with 55% experiencing a severe deficiency ($\leq$ 30 nmol L^{-1}). This prevalence remained high in patients receiving afamelanotide, with 71% suffering from deficiency and 48% from severe deficiency. Prevalence decreased in the group receiving cholecalciferol (from 71% to 46% in those suffering from deficiency and from 48% to 19% in those with a severe vitamin D deficiency) (Figure 1).

The effect of treatments on vitamin D levels, after adjusting for age, sex, season and cohort, is presented in Table 2 and Figure 2(a). Compared with the untreated group, afamelanotide exhibited a nonsignificant average increase of 0.5 nmol L^{-1} [95% confidence interval (CI) –3.2 to 4.2] over time. Both cholecalciferol and combined treatment demonstrated a statistically significant average increase over time compared with no treatment. Cholecalciferol treatment led to an increase of 11.6 nmol L^{-1} (95% CI 7.2–16) and combined treatment to an increase of 15.2 nmol L^{-1} (95% CI 12.3–18.1). The course of vitamin D levels over time for each treatment group can be observed in Figure 2(b–d).

Table 1 Baseline characteristics of patients with erythropoietic protoporphyria by cohort and treatment group

		Cohorts		Treatment groups			
Variable	**All**	**The Netherlands**	**Germany**	**Untreated**	**Afamelanotide**	**Cholecalciferol**	**Combined[a]**
Patients (*n*)	230	138	92	125	133	57	176
The Netherlands (%)	60.0	100.0	0.0	99.1	45.9	96.5	68.8
Germany (%)	40.0	0.0	100.0	0.9	54.1	3.5	31.2
Age (years), mean (SD)	39.1 (15.3)	38.7 (15.4)	39.7 (15.1)	38.0 (15.3)	41.3 (15.8)	42.1 (14.9)	40.4 (15.1)
Sex (%)							
Male	53.0	48.6	59.8	50.4	54.1	47.4	51.7
Female	46.0	51.4	40.2	49.6	45.9	52.6	48.3
Vitamin D measurements (*n*)	1774	1466	308	235	331	104	1104
Measurements per patient (average, *n*)	7.7	10.7	3.4	1.9	2.5	1.8	6.3
The Netherlands (%)	82.6	100.0	0.0	99.1	53.2	98.1	86.5
Germany (%)	17.4	0.0	100.0	0.9	46.8	1.9	13.5
Follow-up duration (months), median (IQR)	8.0 (3.0–34.0)	18.0 (8.0–48.0)	3.0 (1.0–5.0)	14.0 (6.0–37.0)	3.0 (2.0–8.0)	4.0 (3.0–21.0)	24.0 (4.0–45.0)
Interval between vitamin D measurements (months), median (IQR)	2.0 (1.0–6.0)	2.0 (1.0–7.0)	1.0 (1.0–3.0)	2.0 (1.0–7.0)	1.0 (1.0–3.0)	0.5 (4.0–7.0)	2.0 (1.0–6.0)

All data are reported as percentages for categorical values, as mean (SD) for parametric continuous variables and as median [interquartile range (IQR)] for nonparametric continuous variables. [a]Combined treatment was afamelanotide plus cholecalciferol.

Follow-up time

Subgroup analysis was conducted to assess the impact of follow-up time on treatment effects [Table 2; Figure S2 (see Supporting Information)]. Cholecalciferol and combined treatment demonstrated its biggest effects immediately after being prescribed. This led to an increase of 14.1 nmol L^{-1} (95% CI 6.6–21.6) for cholecalciferol and an increase of 17.8 nmol L^{-1} (95% CI 11.8–23.8) for combined treatment. In contrast, the effect of afamelanotide was only statistically significant after 30–60 months of follow-up, with a relevant increase of 17.5 nmol L^{-1} (95% CI 1.5–33.5) vs. no treatment.

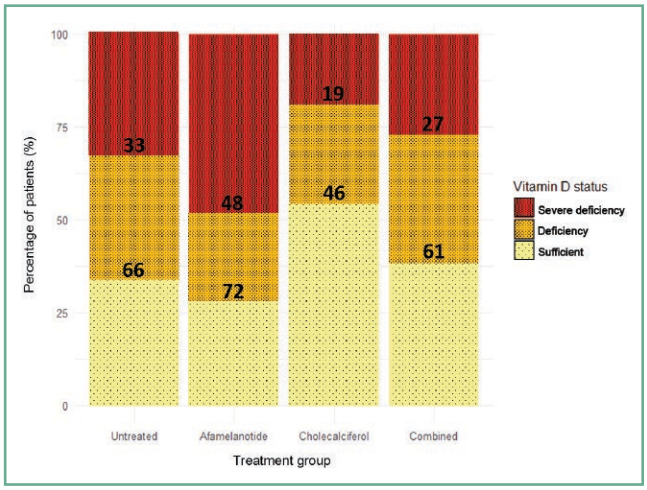

Figure 1 Prevalence of vitamin D deficiency and severe deficiency across treatment categories. The prevalence of vitamin D deficiency (< 50 nmol L^{-1}) and severe deficiency (< 30 nmol L^{-1}) in patients with erythropoietic protoporphyria (EPP), based on their respective treatments. All vitamin D measurements were grouped according to four treatments. The data represent the lowest measurement per patient within each treatment group. Combined treatment included both afamelanotide and cholecalciferol.

Season

In the main analysis, the influence of season on vitamin D levels was examined, using summer as the reference group. The results indicated that the three remaining seasons (spring, autumn and winter) resulted in lower average vitamin D levels than summer (Table 2).

The effect of treatments was assessed per season. The season subgroup analysis in Table 2 highlights that cholecalciferol was found to be most effective during the winter months. Across all seasons, combination treatment led to higher vitamin D levels vs. no treatment. The effect of afamelanotide was not statistically significant across the seasons.

Sex

Women with EPP had higher vitamin D levels than men with EPP, with an average difference of 8.0 nmol L^{-1} (95% CI 3.6–12.4). Subgroup analyses conducted separately for women and men revealed that all three treatment groups demonstrated a more favourable effect in women (Table 2).

Age

Increasing age was associated with higher levels of vitamin D, with an average increase of 0.2 nmol L^{-1} (95% CI 0.1–0.4) per year. Subgroup analyses indicated that all treatments had a more pronounced effect on vitamin D levels in patients older than 50 years of age (Table 2).

Discussion

This study aimed to report the prevalence of vitamin D deficiency in patients with EPP treated with afamelanotide

Table 2 Multivariable linear mixed models for the effect of each treatment on vitamin D levels over time

Variable	Levels	Estimate (β)	(95% CI)
Main model[a]			
Treatment	Untreated	Ref.	Ref.
	Afamelanotide	0.5	–3.2, 4.2
	Cholecalciferol	11.6	7.2, 16.0[b]
	Combined[c]	15.2	12.3, 18.1[b]
Sex	Male	Ref.	Ref.
	Female	8.0	3.6, 12.4[b]
Age (years)		0.2	0.1, 0.4[b]
Cohort	The Netherlands	Ref.	Ref.
	Germany	7.6	2.4, 12.7[b]
Season	Summer	Ref.	Ref.
	Spring	–13.8	–15.9, –11.6[b]
	Autumn	–4.6	–7.1, –2.2[b]
	Winter	–11.9	–14.4, –9.4[b]
Subgroup analysis			
Sex[d]			
Female	Untreated	Ref.	Ref.
	Afamelanotide	1.2	–4.5, 6.8
	Cholecalciferol	15.2	8.6, 21.6[b]
	Combined[c]	16.8	12.3, 21.2[b]
Male	Untreated	Ref.	Ref.
	Afamelanotide	–0.3	–5.1, 4.6
	Cholecalciferol	7.4	1.6, 13.2[b]
	Combined[c]	13.6	9.9, 17.4[b]
Age (years)[e]			
18–33	Untreated	Ref.	Ref.
	Afamelanotide	–4.2	–11.6, 3.2
	Cholecalciferol	7.5	–2.2, 17.0
	Combined[c]	11.6	6.1, 17.0[b]
33–50	Untreated	Ref.	Ref.
	Afamelanotide	6.2	–0.7, 13.0
	Cholecalciferol	15.0	8.3, 21.7[b]
	Combined[c]	19.2	14.0, 24.3[b]
50–82	Untreated	Ref.	Ref.
	Afamelanotide	6.1	0.9, 11.3[b]
	Cholecalciferol	17.2	9.9, 24.5[b]
	Combined[c]	20.5	16.1, 24.9[b]
Season[f]			
Winter	Untreated	Ref.	Ref.
	Afamelanotide	–2.1	–11.0, 6.9
	Cholecalciferol	16.8	7.3, 26.3[b]
	Combined[c]	12.0	5.2, 18.7[b]
Spring	Untreated	Ref.	Ref.
	Afamelanotide	1.6	–5.6, 8.6
	Cholecalciferol	12.6	3.0, 22.1[b]
	Combined[c]	14.7	8.5, 20.7[b]
Summer	Untreated	Ref.	Ref.
	Afamelanotide	0.9	–5.7, 7.6
	Cholecalciferol	5.7	–2.2, 13.7
	Combined[c]	9.1	4.4, 13.8[b]
Autumn	Untreated	Ref.	Ref.
	Afamelanotide	5.8	–1.6, 13.3
	Cholecalciferol	11.8	2.8, 20.6[b]
	Combined[c]	22.3	16.3, 28.3[b]
Follow-up duration[g]			
Baseline	Untreated	Ref.	Ref.
	Afamelanotide	2.3	–4.2, 8.9
	Cholecalciferol	14.1	6.6, 21.6[b]
	Combined[c]	17.8	11.8, 23.8[b]
0–12 months	Untreated	Ref.	Ref.
	Afamelanotide	2.1	–3.9, 8.1
	Cholecalciferol	10.3	1.2, 19.4[b]
	Combined[c]	11.7	6.9, 16.4[b]
12–30 months	Untreated	Ref.	Ref.
	Afamelanotide	3.9	–6.3, 14.2
	Cholecalciferol	9.0	–0.1, 18.2
	Combined[c]	10.7	5.2, 16.2[b]
30–60 months	Untreated	Ref.	Ref.
	Afamelanotide	17.5	1.5, 33.5[b]
	Cholecalciferol	4.8	–10.7, 20.3
	Combined[c]	14.7	6.6, 22.8[b]

Subgroup analysis for treatment effects across sex, age, season and follow-up duration. All linear mixed models (LMMs) included a random slope and intercept unless otherwise specified. CI, confidence interval. [a]LMM adjusted for age, sex, follow-up duration, season and cohort. [b]Statistically significant. [c]Treatment with both afamelanotide and cholecalciferol. [d]LMM for each sex, adjusted for age, cohort and season. [e]LMM for each age group adjusted for sex, cohort, age and season. [f]LMM for each season, with random intercept and fixed slope adjusted for age, sex and cohort. [g]LMM for each follow-up time group, with random intercept and fixed slope adjusted for sex, age, cohort and season.

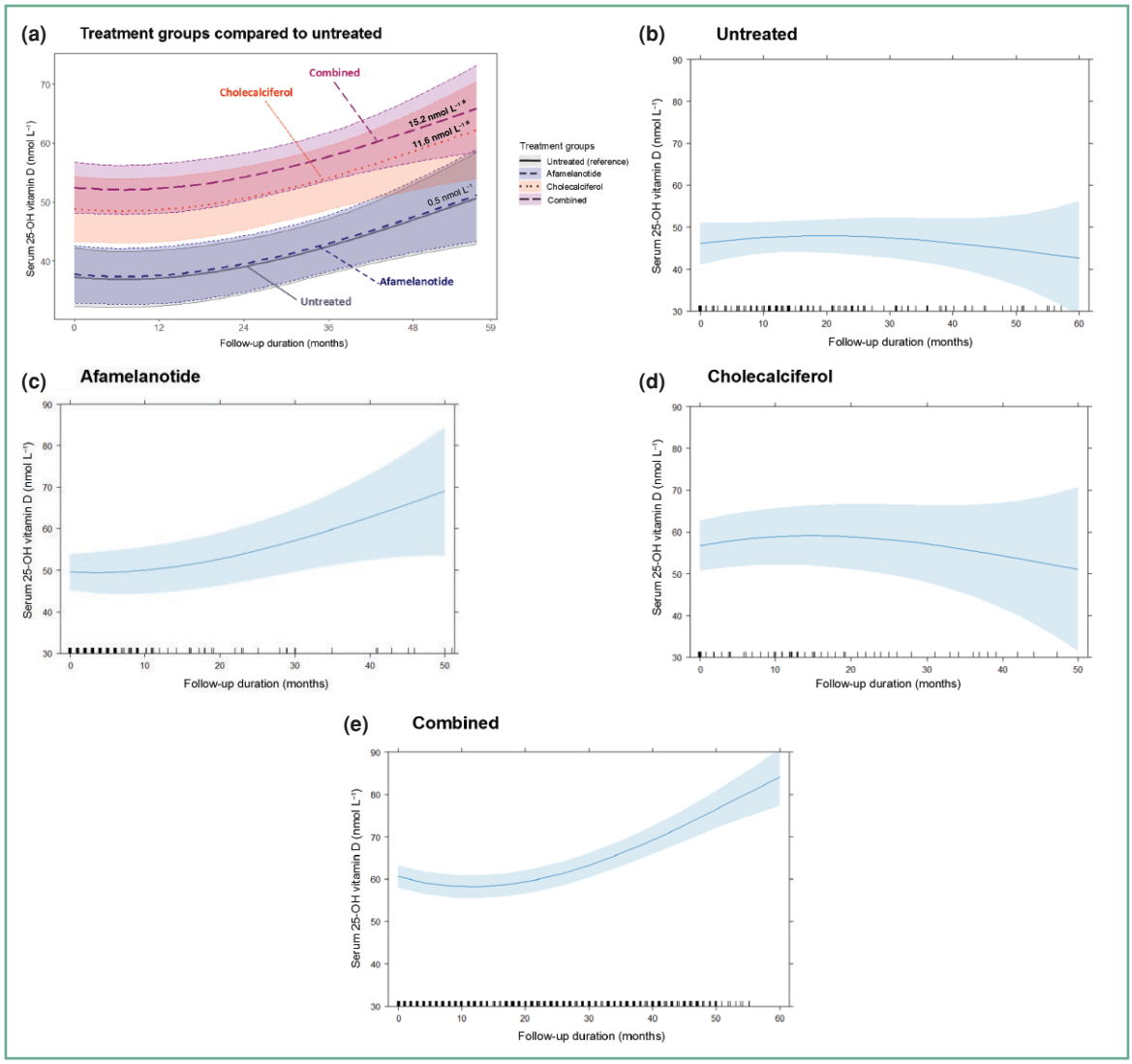

Figure 2 Effect of treatment groups on vitamin D levels over time. Lines represent the average vitamin D levels over time. The averages were corrected for sex, age, season and cohort. The 95% confidence intervals are depicted by the colour shadings. In (b–e) tics on the *x*-axis represent the number of observations recorded at each time point. (a) average vitamin D levels for treatment groups vs. untreated patients (reference group). Estimates (concentrations) denoted in the figure represent the average difference vs. the untreated group. (b) Course of vitamin D levels labelled as untreated over time. (c) Course of vitamin D levels labelled as cholecalciferol over time. (d) Course of vitamin D levels labelled as afamelanotide over time. (e) Course of vitamin D levels labelled as combined treatment (afamelanotide plus cholecalciferol) over time. *Statistically significant.

and to assess the treatment's effects on vitamin D levels. Following afamelanotide treatment, the prevalence of vitamin D deficiency remained remarkably high (72%). Afamelanotide treatment did not lead to an increase in vitamin D. Both cholecalciferol alone and in combination with afamelanotide resulted in an increase in vitamin D levels. These results emphasize the ongoing need for vitamin D monitoring and cholecalciferol prescription in the EPP population treated with afamelanotide.

When compared with the healthy European population,[15] patients with EPP have a substantially higher prevalence of vitamin D deficiency. Seventy-nine per cent of patients with EPP suffer from deficiency vs. 40% in the healthy population. Moreover, the prevalence of severe deficiency is also higher (55% vs. 13%).[6,15,16] Median vitamin D levels in the general Dutch population (63.7 nmol L^{-1})[15] far exceed vitamin D levels in untreated patients with EPP (48.0 nmol L^{-1}). Cholecalciferol (60.0 nmol L^{-1}) and combined treatment (62.0 nmol L^{-1}) led to increased vitamin D levels, although they were still lower than in the general population.

Afamelanotide treatment did not have the hypothesized effect on vitamin D levels. There are five arguments for this finding. Firstly, although patients most probably spend more time outdoors when treated,[3,7] most are still restricted and continue to wear protective clothing,[7] limiting cutaneous synthesis of vitamin D. Secondly, vitamin D synthesis is complex and influenced by various factors such as the time of day, latitude, altitude, air pollution, skin pigmentation and sunscreen use.[4] This suggests that more time spent outdoors does not guarantee enhanced vitamin D synthesis. Thirdly, patients are limited to four implants annually, with 60-day intervals. However, these 60-day intervals are not based on effectiveness studies and seem to be insufficient for the majority of patients.[18] This limitation results in unmet needs, including during the

spring and summer. Fourthly, afamelanotide stimulates eumelanogenesis, and eumelanin provides photoprotection against ultraviolet light,[4] thereby reducing UVB light penetration, which stimulates the conversion of 7-dehydrocholesterol to pre-vitamin D3.[4,5] Finally, patients with EPP only began afamelanotide treatment in 2016–17. We hypothesize that patients need more time to adapt to an outdoor lifestyle because they have been conditioned to avoid sunlight since childhood. It is possible that they have not yet discovered the full potential of afamelanotide, limiting themselves out of a fear of pain. This is supported by our subgroup analysis, which demonstrated that afamelanotide has an effect on vitamin D levels after a longer duration of treatment (30–60 months).

Caution is warranted when interpreting the effect of afamelanotide on vitamin D levels after 30–60 months vs. a shorter treatment duration. This comparison was based on a limited number of measurements. Furthermore, misclassification of patients may have occurred owing to incomplete information regarding over-the-counter cholecalciferol use. Lastly, it is potentially susceptible to confounding by indication, as patients may not require cholecalciferol owing to the absence of vitamin D deficiency.

Combined treatment had a better effect than cholecalciferol by itself. Patients in the combined treatment group were mainly from the Dutch cohort, which had a longer follow-up time and more intensive monitoring of vitamin D levels.

The higher vitamin D levels observed in summer are in line with previous findings.[10] Cholecalciferol treatment leads to the most substantial increase in vitamin D levels during winter, when cutaneous synthesis is at a minimum.

Women and older patients exhibited higher vitamin D levels, and both afamelanotide and cholecalciferol demonstrated the greatest effect in this patient group. Previous research has also found a trend towards female patients having higher vitamin D levels.[9] One possible explanation for this could be better adherence to cholecalciferol. In previous studies, the effect of afamelanotide itself was also more pronounced in females and increased with age.[7]

The limitations of this study included the imbalanced distribution of the two cohorts across treatment groups, and very limited measurements of untreated patients from the German cohort. In some subgroup analysis, there were a limited number of measurements. Patients transitioned between treatment groups, resulting in variation in care duration when entering a new treatment group. This variation in care duration, follow-up time and monitoring between treatment groups could have introduced confounding. Monitoring vitamin D levels was part of routine care, potentially introducing indication bias by repeatedly measuring levels in patients with a deficiency. This was probably not a large effect, as vitamin D levels were obtained at least once a year during routine care, irrespective of outcome. Statistical analysis using linear mixed modelling adjusted for the cohort and the follow-up duration was chosen to mitigate these limitations.

Furthermore, patients with sufficient vitamin D were not given cholecalciferol, leading to potential confounding by indication. There was also a possibility of misclassification bias, as some patients might have self-administered cholecalciferol. This issue was partly addressed in the Netherlands through use of a questionnaire. However, data were absent for some Dutch participants and entirely lacking for the German cohort. Another shortcoming was possible noncompliance with cholecalciferol prescription. Furthermore, we did not consider the differences in the number of annual afamelanotide implants or the intervals between implants. These differences could potentially influence the treatment effects of afamelanotide. The generalizability of the findings may be influenced by the sample population and contextual factors such as the Northern European diet and weather. Despite these limitations, the study offers valuable insights into real-world circumstances, acknowledging that nonadherence is a potential limitation in any clinical trial.

This study had a number of strengths. We used real-world evidence to evaluate the effectiveness of treatment strategies in routine clinical practice. The inclusion of a substantial number of patients with EPP from two European countries improved the reliability and generalizability of our findings. Our large sample size allowed us to allocate sufficient participants across four treatment groups, considering confounders and within-subject correlation. The large sample size facilitated subgroup analyses to explore treatment effects. The extended 16-year follow-up period allowed us to assess how treatment effects evolved over time, while accounting for real-world adherence.

In conclusion, our study highlights the continued importance of cholecalciferol as part of supportive care for patients with EPP, even with the availability of afamelanotide. Afamelanotide treatment did not affect vitamin D levels. Future research should focus on patients with EPP taking afamelanotide for extended periods, to assess its impact on vitamin D levels. Based on these findings, we recommend that future guidelines include cholecalciferol prescription and regular monitoring of vitamin D levels in patients with EPP, including those receiving afamelanotide.

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Conflicts of interest

J.G.L. participates in contract studies for clinical trials with Ultragenyx Pharmaceutical, Clinuvel Pharmaceuticals Ltd and Alnylam® Pharmaceuticals (paid to institution). M.A.E.M.W. participates in contract studies for clinical trials with Moderna (paid to institution). B.H. participates in contract studies for clinical trials with Galderma, Trevi, Celldex, Amgen and

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Data availability

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available as they contain information that could compromise the privacy of participants.

Ethics statement

All participants provided written informed consent for inclusion before taking part in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. Data collected in the Netherlands were under the accordance of the protocol of the Dutch biobank for rare metabolic disease (PRISM), with approval from the Ethics Committee of Erasmus MC (MEC-2011-525). Data collected in Germany were under the accordance of the biobank (Characterization of the effects of the alpha-MSH analogue Afamelanotide in the therapy of EPP patients) and approved by the ethics committee at the medical faculty of the Heinrich-Heine-University Düsseldorf (2020-1189).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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