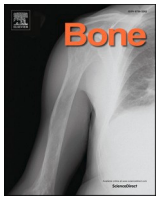
Bone 175 (2023) 116849

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone



Full Length Article

Zinc and vitamin D deficiency and supplementation in hypophosphatasia patients – A retrospective study

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ARTICLE INFO

Keywords:
Hypophosphatasia
Vitamin D
Zinc
Nutritional supplementation
Human

ABSTRACT

Hypophosphatasia (HPP) is characterized by severe skeletal symptoms including mineralization defects, insufficiency fractures, and delayed facture healing or non-unions. HPP is caused by mutations of the tissue non-specific alkaline phosphatase (TNSALP). Zinc is a cofactor of TNSALP and vitamin D an important regulator of bone matrix mineralization. Data from this retrospective study indicates that deficiencies in zinc or vitamin D occur in HPP patients with a similar frequency as in the general population. While guidelines for repletion of these micronutrients have been established for the general population, the transferability of the efficacy and safety of these regiments to HPP patients still needed to be determined. We filtered for variant classification (ACMG 3–5, non-benign) and data completeness from a total cohort of 263 HPP patients. 73.5 % of this subcohort were vitamin D deficient while 27.2 % were zinc deficient. We retrospectively evaluated the effect of supplementation according to general guidelines in 10 patients with zinc-deficiency and 38 patients with vitamin D-deficiency. The treatments significantly raised serum zinc or vitamin D levels respectively. All other assessed disease markers (alkaline phosphatase, pyrodoxal-5-phosphate) or bone turnover markers (phosphate, calcium, parathyroid hormone, bone specific alkaline phosphatase, creatinine, desoxypyridinoline) remained unchanged. These results highlight that general guidelines for zinc and vitamin D repletion can be successfully applied to HPP patients in order to prevent deficiency symptoms without exacerbating the disease burden or causing adverse effects due to changes in bone and calcium homeostasis.

1. Introduction

Hypophosphatasia (HPP) is a rare skeletal disorder caused by mutations of the *ALPL* gene encoding the tissue non-specific alkaline phosphatase (TNSALP, in short AP) [1]. There are >400 different variants known that can cause HPP with the clinical manifestation of this disorder depending on the exact variant and mode of inheritance. HPP is classified in six clinical subtypes (perinatal, benign prenatal, infantile, childhood, adult and odonto HPP) with perinatal HPP being the most severe form [2]. Perinatal HPP can be life-threatening due to chest deformities caused by low or absent skeletal mineralization, which leads to respiratory failure [2]. Patient survival has been markedly improved by the availability of a specific enzyme replacement therapy (Asfotase alfa) [3]. On the other end of the spectrum odonto HPP only manifests in dental tissues and is considered the mildest form of HPP [4].

Typically, the most prevalent symptoms of HPP are bone (i.e. osteomalacia) and dental mineralization defects resulting in increased fracture risk and skeletal deformities (Fig. 1A–F) [4]. Moreover, less specific symptoms such as musculoskeletal pain, muscular weakness, calcifications of tendons and joints as well as nephrocalcinosis and migraine are commonly reported [5]. All described mutations are considered to result in varying degrees of decreased AP enzymatic activity [4]. This results in the accumulation of substrates such as pyrophosphate and pyridoxal 5′-phosphate as well as the lack of products of the reactions catalyzed by AP [1], mostly phosphates, that serve highly relevant functions in the context of skeletal mineralization.

Deficiency symptoms, commonly found in the general population, are also observed in individuals with hypophosphatasia (HPP) and may exacerbate their disease burden. In the context of bone metabolism this is especially applicable to vitamin D, a cofactor essential for adequate

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https://doi.org/10.1016/j.bone.2023.116849
Received 2 June 2023; Received in revised form 7 July 2023; Accepted 14 July 2023
Available online 22 July 2023

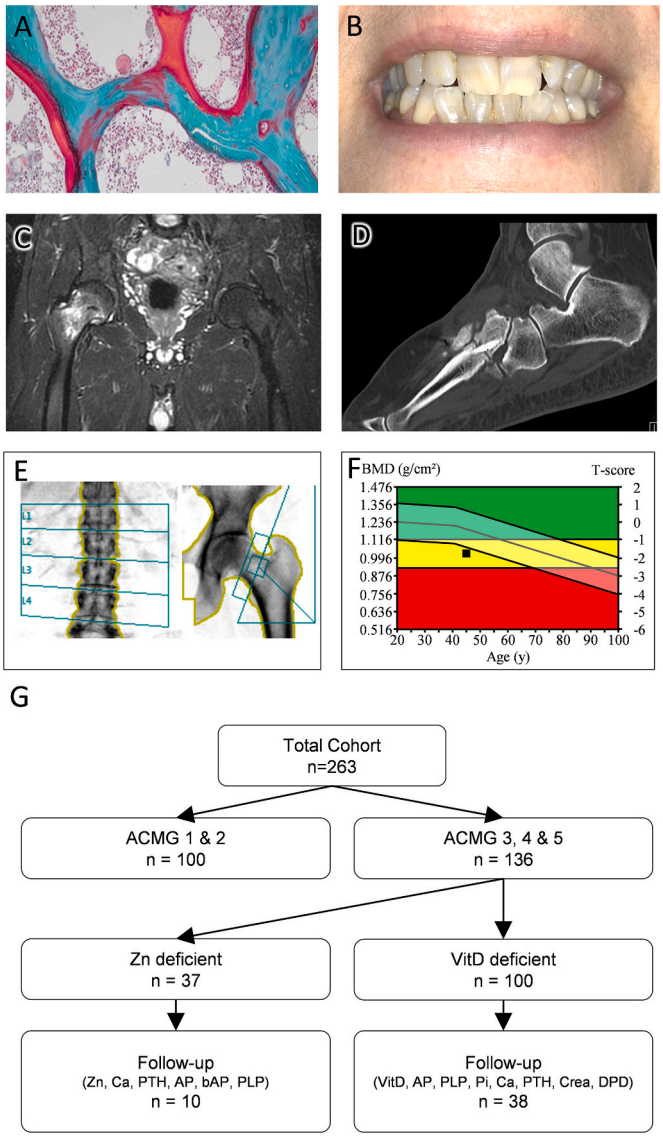

Fig. 1. Typical presentation of HPP patients. A) Histological bone section stained with Masson-Goldner trichrome shows pink areas representing unmineralized osteoid due to insufficient phosphate and high pyrophosphate levels caused by malfunctioning alkaline phosphatase (AP) enzyme and mineralized areas in green. B) Dental abnormalities are common in HPP patients due to impaired tooth mineralization by low activity of AP. C) MRI scan shows bone marrow edema in the femoral neck, due to the hampered bone health in HPP. D) HPP patients frequently suffer from recurrent fractures, delayed healing, and non-unions. E) Left depicts the lumbar spine and right the proximal femur from an DXA scan. This measurement often shows osteopenia (F) due to insufficient bone mineralization. G) Flow chart of the filtering and inclusion criteria for this retrospective study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nutritional calcium uptake among many other physiological functions. Considering that a hallmark symptom of HPP is osteomalacia, the potential impact of an additional calcium deficiency could be highly detrimental. It is estimated that 91.2 % of the female and 82.2 % of the male population in Germany have an inadequate vitamin D supply [6]. Indeed, another study showed that only 11.8 % of the population has vitamin D levels above the recommended threshold of 30 µg/l. [7] Furthermore, 30.2 % of the population suffer from severe vitamin D deficiency with levels below 12 µg/l. [7,8] These levels put the affected individuals at an elevated risk of developing reduced bone mineralization and osteomalacia. Inadequate nutritional intake of zinc, a known metal cofactor of numerous enzymes including AP, has been reported in approximately 19–30 % of the German population [6]. Indeed, zinc deficiency has been reported in 19 % of an elderly population cohort [9]. A similar prevalence of zinc deficiency is thus expected to occur in HPP patients. Generally, zinc deficiency can result in disturbed wound healing, increased susceptibility to infections, dermatitis and growth retardation among other symptoms [10]. Given the poor skeletal status of people suffering from HPP and their elevated fracture risk directly caused by this disorder, it would appear prudent to establish the best possible general conditions for skeletal metabolism in order to limit the severity of their disorder. However, it is also conceivable that modulation of the delicate balance of metabolites in these patients might exacerbate their disease burden, possibly by disrupting compensatory mechanisms or exerting a direct negative effect on bone cells or enzymatic activity. Indeed, to date it has not been reported if a) dietary supplementation of vitamin D or zinc is effective in normalizing these serum parameters in HPP patients and b) this normalization has no adverse effects on the calcium homeostasis and kidney function.

In this retrospective study we have investigated the prevalence of vitamin D and zinc deficiency in a cohort of 263 patients with suspected HPP and closely examined the effect in those patients that received dietary vitamin D or zinc supplementation to compensate for the measured deficiencies.

2. Material and methods

2.1. Patients & grouping

Data of 263 patients with signs and symptoms suggestive of HPP seeking medical attention at the University Medical Center Hamburg-Eppendorf Department of Osteology and Biomechanics outpatient clinic between February 2009 and September 2022 were anonymously collected in a retrospective approach. Patients were initially grouped by their American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) variant classification [11] for descriptive analysis. Variant classifications were 1: benign; 2: likely benign; 3: unknown significance; 4: likely pathogenic; 5: pathogenic. 136 patients with ACMG classes 3, 4 and 5 were included in comparative analyses. The inclusion of ACMG class 3 variants (unknown significance) in the same group with pathological variants was based on the observation that all patients in our cohort displayed symptoms and were thus suffering from HPP. After controlling for sufficient completeness of data 10 patients with zinc-deficiency and 38 patients with vitamin D-deficiency were included in the evaluation of the supplementation effects based on the following criteria: Either zinc levels below 700 µg/l or vitamin D deficiency (<30 µg/l), treatment with supplements and availability of data for the presented parameters at baseline and at least at one follow-up time point (Fig. 1G). All procedures performed in this study were in accordance with the declaration of Helsinki and local research protocols.

2.2. Diagnostics

Laboratory investigations were performed as part of the routine treatment and follow-up of included patients and were spaced 6–12 months apart. The following parameters were determined according to standard protocols by a routine diagnostics laboratory: Zinc (Zn), vitamin D_3 (VitD), alkaline phosphatase (AP), bone specific alkaline phosphatase (bAP), pyrodoxylphosphate (PLP), calcium (Ca), parathyroid hormone (PTH), creatinine, desoxypyridinoline crosslinks (DPD), phosphate (P_i). Patients with proven deficiency in either vitamin D or zinc had received substitution according to national guidelines for zinc [12] and vitamin D supplementation according to the Endocrine Society clinical practice guideline [13]. Deficiency was defined as <700 µg/l for zinc and <30 µg/l for vitamin D [13]. The substitution was adapted to weight, age and gender with 1000–3000 IU vitamin D/d, 10-

15 mg/d zinc orally and further adapted after blood sampling three month after start of substitution, if values did not sufficiently rise.

Genetic sequencing of diagnostically relevant genes was performed in accordance to national laws and regulations and reported as categories suggested in the current standards and guidelines by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [11].

2.3. Statistical analysis

Datasets were tested for normality by the Shapiro-Wilk algorithm and consecutively analyzed via either paired Student's *t*-test (parametric datasets) or Welch's *t*-test (non-parametric datasets) for paired comparisons (before and after substitution) or unpaired Student's t-test (parametric datasets) or Mann-Whitney t-test (non-parametric datasets) for unpaired comparisons (sub-cohorts). All statistical tests were two-sided with a level of significance of 5 %. Mean differences are shown with 95 % confidence interval (95 % CI). Calculations were carried out using GraphPad Software Inc. Prism® 7, Version 7.04.

3. Results

263 patients with suspected HPP were included in this retrospective study with a mean age of 44.12 ± 19.91 years, mean weight of 67.86 ± 22.13 kg, mean height of 166 ± 17 cm and a mean BMI of 24.34 ± 5.68 kg/m^2 (Table 1). Of 263 patients with suspected HPP, 160 (61.6 %) presented with vitamin D levels below 30 µg/l whereas zinc deficiency (<700 µg/l) was observed in 63 patients (23.9 %). 9.1 % of all patients suffered from a combined vitamin D and zinc deficiency (Fig. 2A). Furthermore, closer inspection of all individual zinc values revealed that even non-deficient HPP patients had serum zinc levels that were in the lower half of the physiological range (Fig. 2B). The distribution of ACMG-AMP variant classes as a surrogate marker for disease burden was similar between the total cohort, zinc deficient and vitamin D deficient patients (Fig. 2A). 136 of the 263 patients had a non-benign ACMG class 3–5 *ALPL* variant (Fig. 3A). For this sub-cohort the mean age was 43.51 ± 20.54 years, mean body weight 68.22 ± 24.31 kg, mean body height 163.5 ± 19.8 cm, and accordingly BMI was 25.11 ± 5.80 kg/m^2 (Table 1).

Only patients with variant classes 3, 4 and 5 (non-benign) according to ACMG guidelines and sufficient data completeness were included for further analyses. This filter was applied in order to emphasize the effect of zinc and vitamin D supplementation in cases with distinct disease burden where improvements or deteriorations could be conclusively evaluated. In this subgroup of patients, vitamin D and zinc deficiency was observed in 73.5 % and 27.2 % respectively with 11 % of these patients presenting with a combined vitamin D and zinc deficiency (Fig. 3A, B). The overall prevalence of these deficiencies was slightly higher (89.7 % vs. 76.4 %) than in the total cohort, highlighting the necessity for supplementation in this patient subgroup that is potentially afflicted by a more severe disease burden. However, mean serum vitamin D and zinc levels did not differ significantly between the subgroup of patients with ACMG class 1 or 2 and patients with class 3, 4 or 5 variants. In fact, none of the measured laboratory parameters was significantly different between the class 1 or 2 subgroup and the class 3, 4 or 5 subgroup of patients with the notable exception of AP and PLP (Fig. 3C). Reflecting the higher disease burden, serum AP activity was reduced and PLP serum levels were significantly higher in patients with ACMG class 3, 4 or 5 variants. When excluding ACMG class 3 variants and comparing only the data from patients with established pathological *ALPL* variants (ACMG class 4 or 5) against the class 1 or 2 subgroup, we observed similar results with the notable exception of significantly increased calcium and DPD levels and reduced serum PTH (Suppl. Fig. 1).

To evaluate the effect of compensatory supplementation of Vitamin D and Zinc, we compared the serum parameters of patients before beginning supplementation to the values measured after 250.62 ± 191.88 days of supplementation with 1000–3000 IU of Vitamin D/day and/or 10–15 mg zinc/day orally. Oral zinc supplementation succeeded in significantly raising serum zinc levels by 27.04 % (Fig. 4A, Table 1) without affecting bone metabolism markers (calcium, PTH) (Fig. 4B) or disease progression markers (AP, bAP, PLP) (Fig. 4C) positively or negatively. Similarly, vitamin D supplementation resulted in significantly increased serum vitamin D levels by 31.14 % (Fig. 5A, Table 1) while all other investigated serum parameters relating to disease severity (AP, PLP, phosphate) (Fig. 5B) or bone metabolism (calcium, PTH, urinary DPD crosslinks) and kidney function (creatinine) (Fig. 5C) remained unaltered. When evaluating the effects of supplementation only in patients with pathological *ALPL* variants (ACMG class 4 or 5), we observed comparable effects (Suppl. Figs. 2 & 3). However, the sample number was severely reduced, thus limiting the validity of any conclusions drawn from this smaller dataset.

4. Discussion

In this study we demonstrated that the frequency of zinc- and vitamin D-deficiency in hypophosphatasia (HPP) patients is comparable to the general population. Furthermore, supplementation of zinc or vitamin D in patients with non-benign variants of ALPL was effective in raising the respective micronutrient without affecting disease severity or bone turnover markers.

HPP is a relatively rare metabolic disorder caused by mutations in the *ALPL* gene coding for the enzyme TNSALP (in short AP) that results in skeletal mineralization defects among other skeletal and extra skeletal symptoms. The severity of this disorder can vary greatly and is predominantly determined by the exact position and type of *ALPL* variant present in the patients. The enzymatic activity of AP is dependent on cofactors such as zinc ions and reduced availability of these ions can potentially lead to further impairment of the enzymatic function [14]. This is of particular interest, given the already challenging skeletal condition of HPP patients. Indeed, zinc deficiencies can lead to impaired growth, impaired immune function, delayed wound healing, skin

Table 1
Characterization of analyzed patient cohorts and sub-cohorts. (BL: baseline, post: after supplementation).

	Unit	All		ACMG 1–2		ACMG 3–5		ACMG 3–5 Zn deficient		ACMG 3–5 Vit D deficient	
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Age	Years	46.22	20.59	49.93	19,02	43.94	21.18	41.73	20.71	37.62	19.93
Height	m	1.65	0.14	1.67	0.13	1.64	0.40	1.69	0.11	1.64	0.16
Weight	kg	64.64	18.34	63.86	17.34	65.26	19.08	69.13	23.04	65.65	21.26
BMI	kg/m^2	23.58	5.16	22.34	4.58	24.52	5.36	25.91	3.68	24.32	6.23
Zn BL	µg/l	747.38	134.05	718.38	132.11	465.23	132.11	609.82	56.14	792.88	133.16
Zn post	µg/l	–	–	–	–	–	–	777.60	129.94	–	–
Vit D BL	µg/l	30.20	15.65	31.86	13.00	29.16	17.02	26.99	11.42	19.55	5.32
Vit D post	µg/l	–	–	–	–	–	–	–	–	25.06	8.57

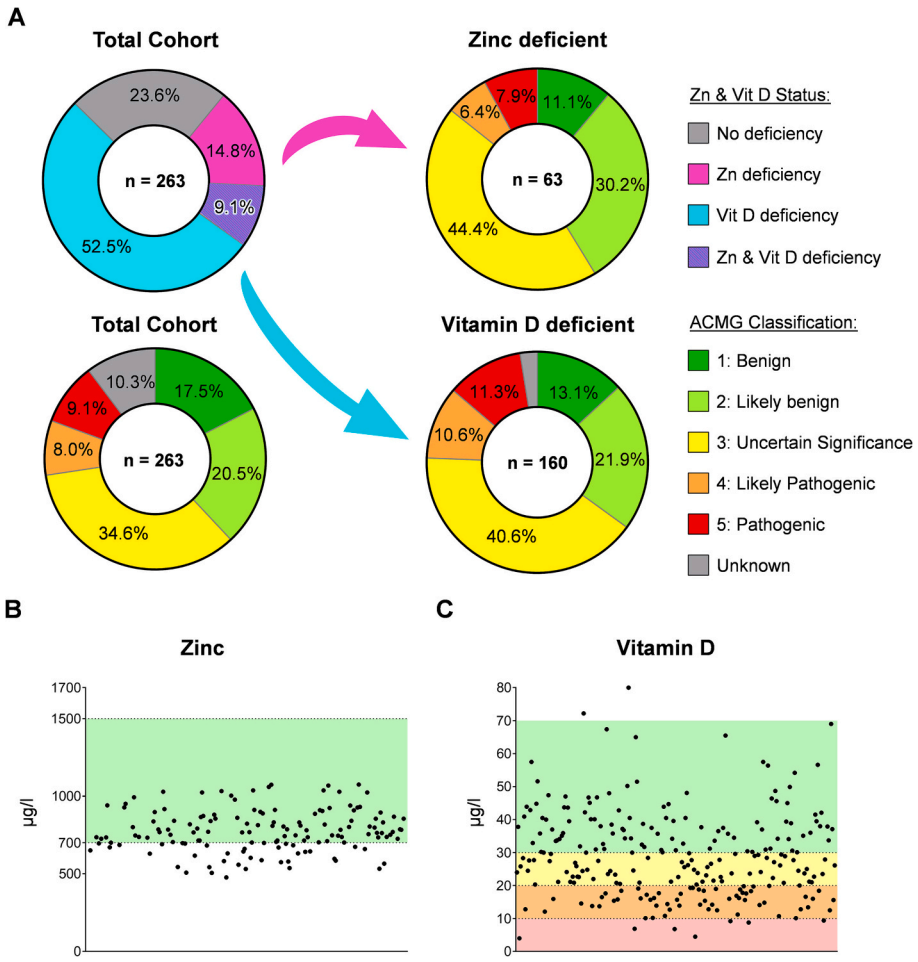

Fig. 2. Basic characterization of the total patient cohort. A) Visual representation of the proportions of the deficiency status for Zinc and Vitamin D (upper left pie chart) and ACMG classifications (lower left pie chart) of the included patient cohort. Upper right pie chart: Proportion of ACMG classifications in patients with zinc deficiency. Lower right pie chart: Proportion of ACMG classifications in patients with vitamin D deficiency. Total number of included patients is indicated in the center of the pie charts. B) Scatter plot showing serum zinc concentrations for all patients of the cohort. Each dot represents an individual value. The normal range of serum concentration as defined by the laboratory is shown in green. C) Scatter plot showing serum vitamin D concentrations for all patients of the cohort. Each dot represents an individual value. The normal range of serum concentration as defined by the laboratory is shown in green. The range for mild vitamin D deficiency is indicated in yellow (30–20 µg/l, for moderate vitamin D deficiency in orange (20–10 µg/l) and for severe vitamin D deficiency in red (<10 µg/l). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

changes, changes in taste and smell, diarrhea, loss of appetite, as well as cognitive and behavioral changes [15,16]. Zinc deficiency and HPP share similarities in their effects on bone health. Both conditions can lead to impaired bone growth and mineralization, which can cause skeletal deformities and fractures [1,16,17]. Therefore, zinc deficiencies may increase the disease burden endured by HPP patients [14]. Likewise, vitamin D is an essential factor for calcium uptake and subsequently bone matrix mineralization. Therefore, inadequate vitamin D supply, a very common occurrence in western cultures [13], might further exacerbate the skeletal mineralization defects observed in HPP patients. Both zinc and vitamin D deficiencies can easily be improved by oral supplementation [15] and previous studies have shown that daily dosing of zinc can effectively raise levels [18] and improve deficiency symptoms in otherwise healthy individuals [19–21]. With regard to zinc or vitamin D supplementation in HPP patients, there is currently no data available. Finally, it needs to be considered that any intervention might theoretically upset the critical metabolic balance in HPP patients and interfere with the calcium homeostasis and kidney function. Given the lack of clear data, the aim of our retrospective study was to determine if zinc and vitamin D supplementations are effective and feasible in HPP patients.

When evaluating the prevalence of zinc and vitamin D deficiencies in our cohort of HPP patients, we observed a similarly high percentage of persons with deficiencies as were previously described for the general population [6,9]. The high prevalence of these deficiencies highlights the need for reliable data on the efficacy and potential adverse effects of zinc and vitamin D supplementation in HPP patients in order to provide optimal care.

Especially zinc metabolism appears to be an issue in HPP patients as our data show that even those patients with normal zinc levels are all displaying values at the lower end of the physiological range, even though a prior study has established a reasonably balanced zinc intake in HPP patients. [22] Since zinc is an established cofactor for AP [23], it is conceivable that this may influence enzymatic activity and thus the severity of HPP [14]. A positive correlation of AP and rising zinc levels has already be shown [14] and may be advantageous especially for HPP patients carrying AP variants with especially low residual enzymatic activity. Furthermore, this finding may indicate a potential link between AP activity and zinc homeostasis regulation although no such mechanism has been described to date and evaluating the relevance of this hypothesis will require further research. With regard to vitamin D concentrations we did not observe any unusual patterns in our patient data.

We next evaluated if the distribution of AP variants according to ACMG classifications was different between patients deficient in either zinc or vitamin D. Here we observed that variants of all ACMG classifications were similarly distributed in the zinc or vitamin D deficient sub-cohorts, indicating that deficiencies of these two molecules manifest independently of the variant class. However, we did note a slightly higher prevalence of vitamin D deficiency in patients with non-benign ACMG class 3–5 variants in comparison to the total cohort. Nonetheless, absolute zinc and vitamin D serum levels did not differ significantly between the ACMG 1–2 and the ACMG 3–5 sub-cohorts. In fact, of all measured parameters, only AP activity and pyridoxal phosphate (PLP) levels, both well-established indicators and potential severity markers of HPP [4], were significantly altered in patients with ACMG class 3–5 variants in comparison to carriers with ACMG class 1–2 variants. This is especially relevant since we have focused all further investigations on

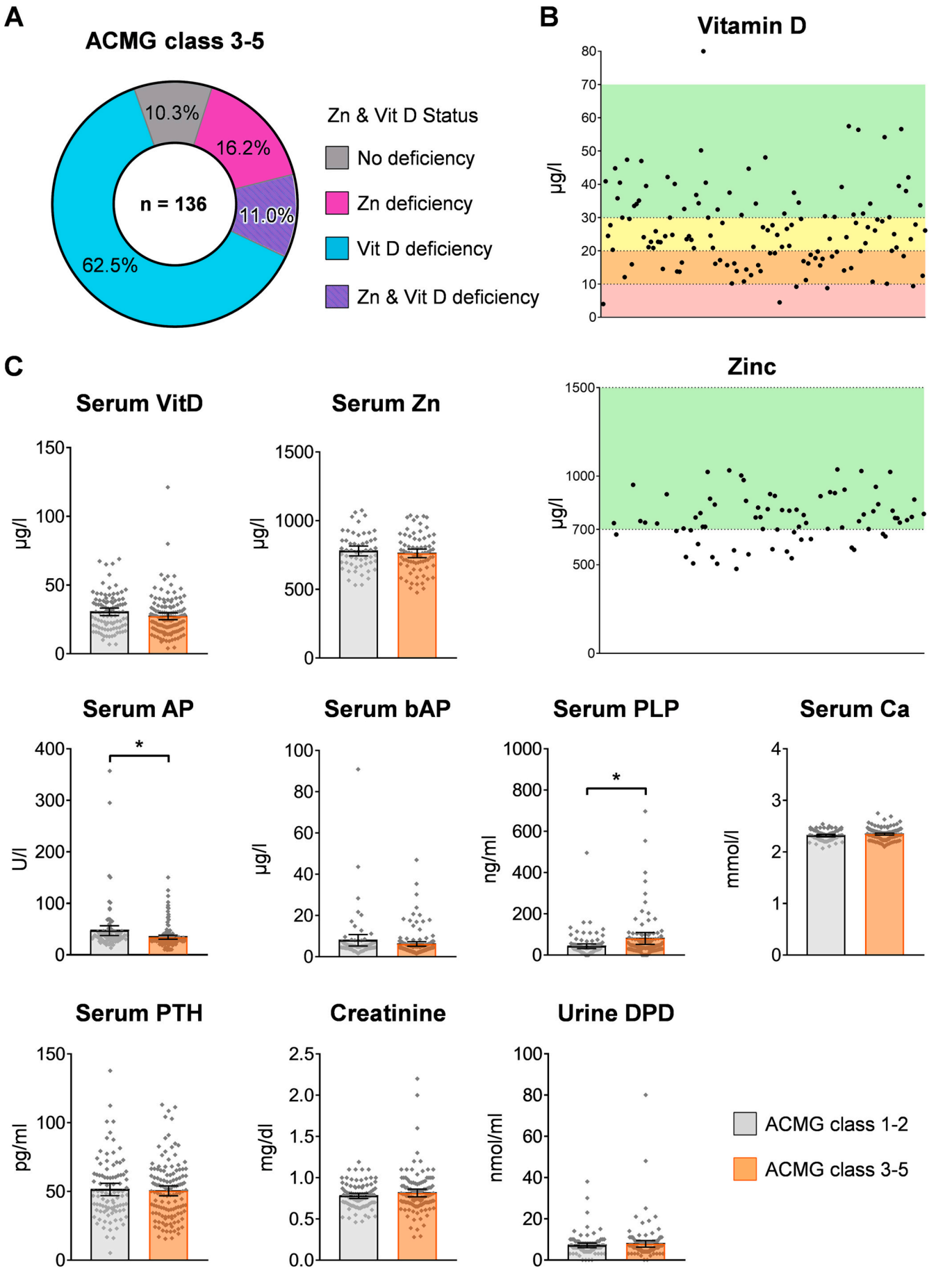

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Fig. 3. Laboratory diagnostic characteristics of patients with ACMG classification 3, 4 and 5 non-benign *ALPL* variants (ACMG class 3–5 sub-cohort). A) Visual representation of the proportions of the deficiency status for zinc and vitamin D. The total number of included patients is indicated in the center of the pie chart. B) Scatter plot showing serum vitamin D (upper chart) and zinc (lower chart) concentrations for all patients of this sub-cohort. Each dot represents an individual value. The normal range of serum concentration as defined by the laboratory is shown in green. The range for mild vitamin D deficiency is indicated in yellow (30–20 μg/l, for moderate vitamin D deficiency in orange (20–10 μg/l) and for severe vitamin D deficiency in red (<10 μg/l). C) Relevant HPP disease and bone turnover markers (vitamin D, zinc, alkaline phosphatase, pyridoxal phosphate, calcium, bone specific alkaline phosphatase, parathyroid hormone, creatinine and urine desoxypyridinolin) in ACMG 3–5 sub-cohort compared to patients with ACMG classification 1–2. Bars represent mean values ±95 % CI. Dots represent individual values. Data were analyzed by Student's *t*-test or Mann-Whitney t-test depending on normality. $*p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

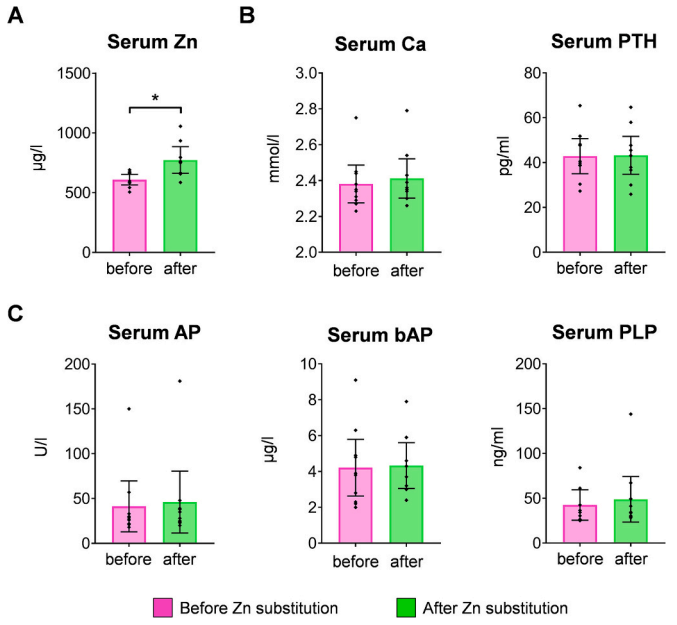

Fig. 4. Effect of zinc substitution in zinc deficient HPP patients of the non-benign ACMG 3–5 sub-cohort. A) Serum zinc levels before and after substitution. B) Calcium homeostasis markers (calcium, parathyroid hormone) before and after zinc substitution. C) Serum HPP markers (alkaline phosphatase, bone specific alkaline phosphatase & pyridoxal phosphate) before and after zinc substitution. Bars represent mean values ±95 % CI. Dots represent individual values, $n = 10$. Data were analyzed by Student's t-test or Welch's test depending on normality. $* p \leq 0.05$.

the ACMG 3–5 sub-cohort with non-benign variants in order to conclusively evaluate the effect of zinc and vitamin D supplementation in cases with distinct disease burden.

Evaluating the outcome of zinc substitution in HPP patients affected by zinc deficiency, we were able to demonstrate that serum zinc levels were significantly raised (Table 1) after on average 250.62 days of substitution and reached mean values at the lower threshold of the normal range (700 μg/l) although not all patients achieved this level. With regard to disease and mineral metabolism markers we did not observe any significant changes after substitution. The lack of positive change might indicate that serum zinc is not directly interacting with the HPP pathomechanism at the concentrations examined in this study. Furthermore, potential influences of zinc on HPP and AP activity may be varying depending on the specific mutation and are thus not reflected in our evaluation. Most importantly however, these results show that zinc supplementation was effective and may prevent patients from experiencing HPP-independent zinc deficiency symptoms while not negatively impacting the HPP disease situation.

Similarly to zinc substitution, vitamin D supplements led to a significant increase of serum vitamin D levels (Table 1), although on average the patients did not fully reach the desired threshold of the normal range (30 μg/l). Furthermore, as previously observed for the zinc substitution, vitamin D substitution did not lead to any significant changes in the determined HPP, kidney function and bone metabolism serum markers. Indeed, even serum calcium and especially PTH levels were not affected by this treatment. Therefore, it can be concluded that vitamin D has no detectable influence on the HPP pathomechanism in this patient cohort and with regard to the determined parameters. On the other hand, vitamin D supplementation clearly has no negative influence on measured disease markers. Thus, we recommend vitamin D supplementation in HPP patients in order to prevent further worsening of their bone matrix mineralization due to vitamin D deficiency and lack of calcium uptake, especially considering the already prevalent occurrence of osteomalacia in HPP.

There are several limitations to our present study that we would like to address: Only a relatively small number of patients was included in the final analysis due to stringent inclusion criteria especially with regard to data availability in the context of this retrospective study. When analyzing only the data from patients with pathological ALPL variants (ACMG class 4 or 5) that may provide a more accurate insight into the effects of zinc and vitamin D supplementation in the context of HPP, due to potentially higher disease burden, we generally observed similar results. However, the number of patients was even further reduced, thus severely limiting any attempt to draw well-founded conclusions from this dataset. The retrospective nature of this study further introduces variations due to different supplementation regiments for individual patients with regards to supplement concentration and time between analyses. Furthermore, we opted to use ACMG classifications as a readily available surrogate parameter for disease burden that may not be fully reflecting the actual symptoms experienced by the patients. An evaluation of the quality of life is missing but was not feasible in the context of a retrospective study. Finally, all different variants within our analysis groups were treated equally. However, from a molecular and biochemical viewpoint it is very likely that specific variants (e.g. at the metal binding site of AP) may be more susceptible to deficiency effects than others. Nonetheless, we are convinced that our results are highly relevant and valuable as a general evaluation of zinc and vitamin D supplementation in HPP patients.

In conclusion, our results show that supplementation of both vitamin D and zinc is feasible and does not have adverse effects in HPP patients with non-benign *ALPL* variants. Serum levels of both vitamin D and zinc could be raised significantly by oral supplementation and biomarkers for HPP severity remained unaltered. While we did not observe positive effects on HPP markers or bone turnover markers in general, we nonetheless believe that attempts should be made to normalize serum levels of vitamin D and zinc in HPP patients, if necessary, in order to reduce the risk of potential adverse effects caused by these deficiencies and improve the overall condition of the patients. Based on our findings, it can be assumed reasonable to advice practitioners that substitution of zinc and/or vitamin D in deficient patients affected by HPP can be safely and effectively carried out according to clinical best practice and existing guidelines aimed at the general population.

Grant supporters

This study was performed without external funding.

CRediT authorship contribution statement

Philip Wiedemann: Writing – review & editing, Writing – original

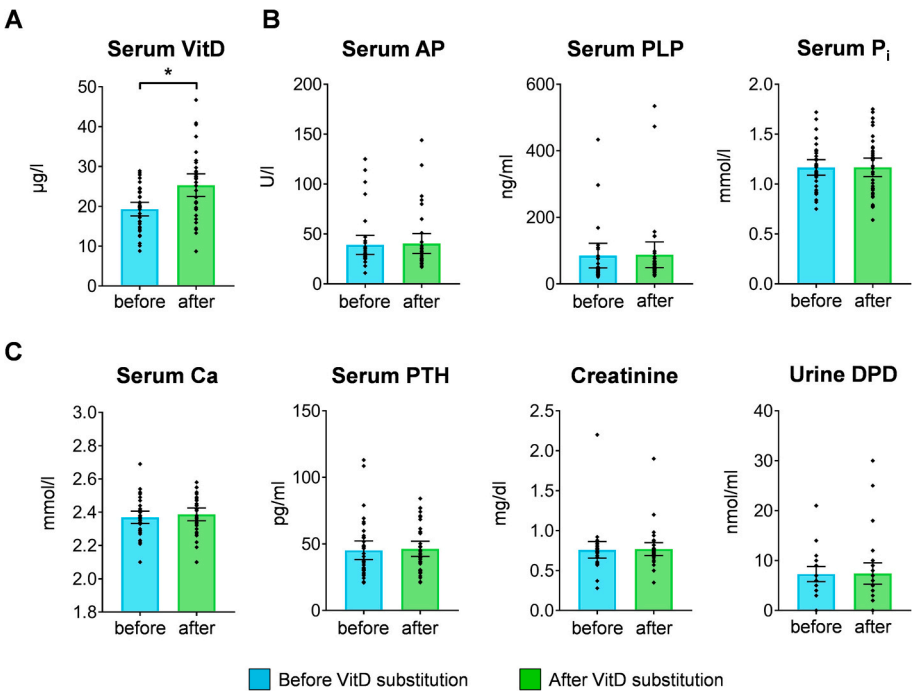

Fig. 5. Effect of vitamin D substitution in vitamin D deficient HPP patients of the non-benign ACMG 3–5 sub-cohort. A) Serum vitamin D levels before and after substitution. B) Serum HPP markers (alkaline phosphatase, pyridoxal phosphate & phosphate) before and after vitamin D substitution. C) Bone homeostasis and kidney markers (calcium, parathyroid hormone, creatinine and urine desoxypyridinolin) before and after vitamin D substitution. Bars represent mean values ±95 % CI. Dots represent individual values, $n = 38$. Data were analyzed by Student's t-test or Welch's test depending on normality. * $p \leq 0.05$.

draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Felix N. Schmidt:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Michael Amling:** Writing – review & editing, Resources, Conceptualization. **Timur A. Yorgan:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation. **Florian Barvencik:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

PW, FNS, MA and TAY declare no conflict of interest. FB received speaker and consultant fees from Alexion and DiaSorin and research funding from Alexion.

Data availability

Data is available upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2023.116849.

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