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Case Report

No vascular calcification on cardiac computed tomography spanning asfotase alfa treatment for an elderly woman with hypophosphatasia☆,☆☆

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ABSTRACT

Hypophosphatasia (HPP) is the inborn-error-of-metabolism characterized enzymatically by insufficient activity of the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) and caused by either mono- or bi-allelic loss-of-function mutation(s) of the gene *ALPL* that encodes this cell surface phosphomonoester phosphohydrolase. In HPP, the natural substrates of TNSALP accumulate extracellularly and include inorganic pyrophosphate (PPi), a potent inhibitor of biomineralization. This PPi excess leads to rickets or osteomalacia in all but the most mild "odonto" form of the disease. Adults with HPP understandably often also manifest calcium PPi dihydrate deposition, whereas enthesopathy and calcific periarthritis from hydroxyapatite (HA) crystal deposition can seem paradoxical in face of the defective skeletal mineralization. In 2015, asfotase alfa (AA), a HA-targeted TNSALP, was approved multinationally as an enzyme replacement therapy for HPP. AA hydrolyzes extracellular PPi (ePPi) and in HPP enables HA crystals to grow and mineralize skeletal matrix. In direct contrast to HPP, deficiency of ePPi characterizes the inborn-errors-of-metabolism generalized arterial calcification of infancy (GACI) and pseudoxanthoma elasticum (PXE). In GACI and PXE, deficiency of ePPi leads to ectopic mineralization including vascular calcification (VC). Therefore, in HPP, ectopic mineralization including VC could hypothetically result from, or be exacerbated by, the persistently high circulating TNSALP activity that occurs during AA treatment. Herein, using a routine computed tomography (CT) method to quantitate coronary artery calcium, we found no ectopic mineralization in the heart of an elderly woman with HPP before or after 8 months of AA treatment. Subsequently, investigational high-resolution peripheral quantitative CT and dual-energy X-ray absorptiometry showed absence of peripheral artery and aortic calcium after further AA treatment. Investigation of additional adults with HPP could reveal if the superabundance of ePPi protects against VC, and whether long-term AA therapy causes or exacerbates any ectopic mineralization.

1. Introduction

Hypophosphatasia (HPP) is the inborn-error-of-metabolism caused by loss-of-function mutation(s) of the gene *ALPL* that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) [1]. In healthy individuals, this phosphomonester phosphohydrolase is expressed ubiquitously on cell surfaces and especially in bone and cartilage where it promotes skeletal mineralization by hydrolyzing extracellular inorganic pyrophosphate (ePPi), a potent inhibitor of biomineralization [2,3]. In HPP, TNSALP deficiency and the consequent superabundance of ePPi blocks hydroxyapatite (HA) crystal formation and growth, and thereby leads to tooth loss and often to rickets or osteomalacia [4]. Numerous typically missense *ALPL* mutations [5] alone or in combination largely account for the remarkably broad-ranging severity of HPP that spans perinatal lethality with an unmineralized skeleton to dental, skeletal, or arthritic complications first manifesting in adult life [1,6].

In direct contrast to HPP, generalized arterial calcification of

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infancy (GACI: OMIM #208000) [7] and pseudoxanthoma elasticum (PXE: OMIM #264800) [7] are inborn-errors-of-metabolism that feature low levels of ePPi. GACI and PXE are caused by autosomal recessive transmission of loss-of-function mutations in the genes that encode ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) and ATP-binding cassette transmembrane transporter sub-family C member 6 (*ABCC6*), respectively. In both disorders, the deficiency of ePPi leads to vascular calcification (VC) [8] that in GACI often kills in infancy [9].

In 2015, asfotase alfa (AA), a recombinant HA-targeted TNSALP replacement therapy [10], was approved multinationally typically for pediatric-onset HPP [11]. During AA treatment, patients of all ages with HPP have markedly elevated circulating "TNSALP" activity [12–15]. The clinical trials that evaluated AA (Strensiq™) were reassuring against the potential adverse effect of ectopic mineralization (EM), screened for using conventional radiographs, ophthalmologic examinations, and renal sonography [12–15]. However, computed tomography (CT) and dual-energy X-ray absorptiometry (DXA) have not been used to search for ectopic calcium (Ca^{++}) deposition in HPP patients, particularly the elderly. In this age group especially, it would be important to know if their superabundance of ePPi actually protects them against VC, and if AA treatment leads to or increases this common problem of aging [16] or the other types of ectopic mineralization including enthesopathy and calcific periarthritis.

Herein, for an elderly woman with HPP given AA treatment, we used two types of CT as well as DXA to search for ectopic Ca^{++} in her heart, limb vasculature, and aorta.

2. Materials and methods

2.1. Patient

This 68-year-old nurse recalled weakness while walking during childhood considered a sequela of polio. In 1995, we reported that her biochemical characteristics of HPP included serum ALP of 8 IU/L (Nl 35–100) and elevated plasma pyridoxal 5′-phosphate (PLP) at 624 nM (Nl 30–110) [17]. Later, we found that she and her two sons with the childhood form of HPP, all three sufficiently affected to be given AA treatment, were heterozygous for the *ALPL* mutation (i.e., pathogenic variant) c.1133A > T, p.Asp378Val, which causes the most common autosomal dominant form of HPP in the United States (see below) [18]. She had suffered tooth loss and multiple rib fractures and undergone intramedullary fixation of broken femurs; all complications typical of HPP in adults [19,20]. Therefore, in March 2017, she began AA treatment by injecting 1 mg per kg body weight of AA subcutaneously daily (i.e., a total of 7 mg/kg/week; the package insert dose is 6 mg/kg/wk). Baseline serum ALP activity was reported as < 20 U/L (Nl 40–130); i.e., too low for accurate quantitation, and electrolytes, calcium, magnesium, creatinine, and glucose were normal. At Mayo Clinic Laboratories (Rochester, MN, USA), serum 25-hydroxyvitamin D was 45 ng/dL (Nl 30–100), PLP 128 μg/L (Nl 5–50), and osteocalcin 12 ng/mL (Nl 9–42). After eight months of AA therapy, serum ALP activity was recorded at 8100 U/L.

The patient and her two sisters, who were older, had no history of cardiovascular or cerebrovascular disease. One sister wore leg braces during childhood, and then at age 60 years required intramedullary rodding of her femurs and was diagnosed with HPP. Her serum ALP activity was 11 IU/L. The other sister had no skeletal problems. Their mother, whose serum ALP was 47 IU/L (Nl 25–100) had been well lifelong until a stroke at age 92 years. Their father, who died at age 95 years, had weak legs, all teeth removed by age 20 years, serum ALP of 16 IU/L (Nl 25–100), and plasma PLP of 1359 nM (Nl 30–110).

2.2. Coronary artery Ca^{++} computed tomography

We chose, as a precaution for the patient's treatment with AA, to perform routine coronary artery Ca^{++} scoring CT before the therapy began, and then scheduled an identical follow-up study eight months later using the same equipment and procedures; i.e., sooner than the several years that would be conventional for follow-up of positive findings from atherosclerotic disease. Image acquisition for Ca^{++} scoring came from established clinical and research techniques to calculate an Agaston Score, as well as Ca^{++} volume, while minimizing X-radiation exposure [21–23].

The images were acquired using a 64-detector CT scanner (Sensation 64; Siemens Medical Systems, Forchiem, Germany) together with a prospective electrocardiogram (ECG)-gated method. Scan range was limited to the heart to minimize X-radiation exposure, and extended from the carina to the level of the hemidiaphragm. Images were obtained in the mid-diastolic phase (65% of the ECG RR interval). The scan parameters included a 370 ms gantry rotation time, 32 × 0.6 mm collimation, and 120 kVp with a reference 40 mA. Images were reconstructed with a 3 mm slice thickness utilizing a b30 kernel (smooth). The estimated X-radiation exposure or scan was approximately 30 mGy*cm.

Ca^{++} scoring was performed from commercially available Vitrea Cardiac Software (Vitrea®, Vital Images, Inc., Minnetonka, MN, USA) designed for clinical and research purposes and using the Agaston method [24], as well as calculating Ca^{++} volume. Ca^{++} is determined by having three contiguous Voxels with a Hounsfield unit of > 130. Regions of interest are drawn for each coronary artery distribution to calculate the Ca^{++} score for that vessel, as well as for the coronary artery vascular bed. Patient-specific calibration was not performed, as it has been shown not to impact Ca^{++} scoring [25].

2.3. Peripheral artery Ca^{++} computed tomography

Reassured by the cardiac CT findings (see Results), we then searched for peripheral VC. Following informed written consent, investigational high-resolution peripheral quantitative CT (HR-pQCT: XtremeCT II, Scanco Medical, Zurich, Switzerland) was used to study the patient's non-dominant forearm and foreleg after 14 months of AA treatment.

2.4. Dual-energy X-ray absorptiometry

After 18 months of AA therapy, we used the vertebral fracture assessment (VFA) method of DXA (Horizon A; Hologic Inc., Waltham, MA, USA) to look for VC in our patient's abdominal aorta [26]. We elected not to add for this purpose a "cross-table" lateral radiograph [27].

2.5. Mutation analyses

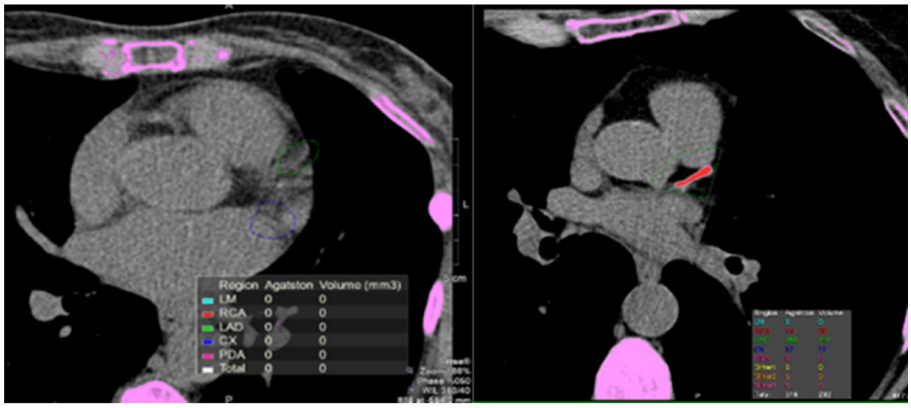
Using genomic DNA isolated from the patient's blood leukocytes, *ALPL* exon 10 (where her sons' mutation had been identified) was PCR-amplified and Sanger-sequenced. Her DNA was then sequenced using Ion Torrent next generation sequencing (Thermo Fisher Scientific, Waltham, MA, USA) for 15 genes involved in PPi and phosphate metabolism and/or genetic forms of rickets (*ABCC6*, *ALPL* (*TNSALP*), *ANKH*, *CYP27B1*, *DMP1*, *ENPP1*, *FGF23*, *GALNT3*, *KL* (*KLOTHO*), *MEPE*, *PHEX*, *PHOSPHO1*, *SLC34A3*, *SPP1* (*OPN*), *VDR*).

3. Results

3.1. Coronary artery Ca^{++} computed tomography

The coronary artery Ca^{++} score before and after eight months of AA therapy was zero. Both the distance scores and the Ca^{++} volumes remained zero. No Ca^{++} was detected visually throughout the coronary artery tree at either time point (Fig. 1).

A) Before asfotase alfa therapy:



B) After eight months of asfotase alfa therapy:

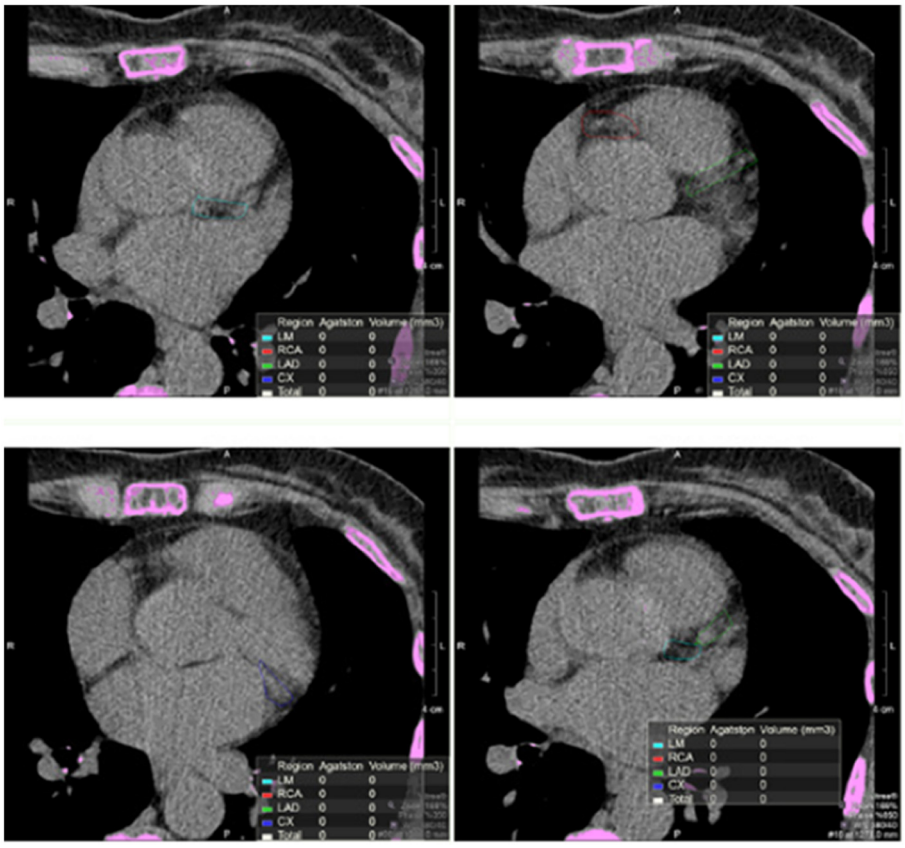


Fig. 1. A) Before asfotase alfa therapy: Left panel: Representative image from the coronary Ca^{++} scoring CT shows no Ca^{++} in the coronary artery tree, either visually or calculated by drawing a region of interest. Right panel: In contrast, a VC-positive "control" patient demonstrates extensive coronary artery Ca^{++}. The bright red streak is the Ca^{++} within the region of interest. B) After eight months of asfotase alfa therapy: Follow-up CT after AA treatment demonstrates no coronary artery Ca^{++} in any of these four areas (i.e. panels). There is no red color in the regions of interest that would indicate Ca^{++}. Pink color is, as anticipated, seen due to skeletal Ca^{++} perhaps reflecting our patient's healing osteomalacia. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Peripheral artery Ca^{++} HR-pQCT

HR-pQCT of the patient's distal forearm and distal leg after 14 months of AA treatment showed no evidence of peripheral artery Ca^{++}, clearly detected instead in a "positive control" individual who did not have HPP (Fig. 2).

3.3. Aortic Ca^{++} lateral DXA

No aortic Ca^{++} was apparent in our patient using DXA VFA imaging of her lateral spine (Fig. 3).

3.4. Mutation analyses

A single heterozygous missense mutation c.1133A > T, p.Asp378Val was identified in exon10 of the patient's *ALPL* gene and verified using the Ion Torrent method. No mutation was identified in the other examined genes, some involved in ePPi metabolism (*ABCC6*, *ANKH*, and *ENPP1*).

4. Discussion

Arterial calcification usually involves intimal Ca^{++} deposition from aging, atherosclerosis, or diabetes [16]. When predominantly medial, VC is typically from chronic renal failure [28,29] and seems to involve multiple factors acting as nucleation sites [30–32]. In either region of an artery, the deposited Ca^{++} is both extracellular and intracellular, includes HA crystals [28], and sometimes represents bone [33].

Supraphysiological concentrations of ePPi impair skeletal mineralization [34,35], and in adults can manifest as calcium pyrophosphate

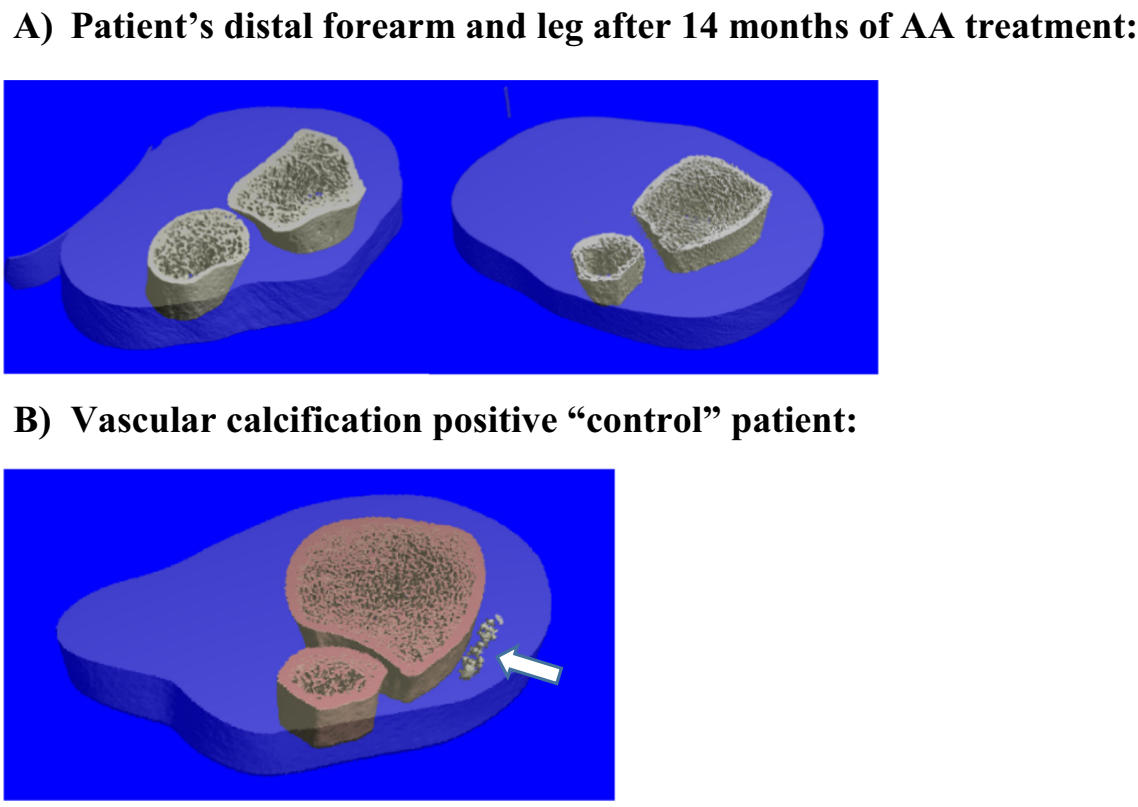


Fig. 2. A) Patient's distal forearm and leg after 14 months of AA treatment: No VC is apparent in the patient's distal left forearm (left image) or distal left leg (right image). B) Vascular calcification positive "control" patient: VC (arrow) near the tibia is apparent in this "positive control" individual without HPP.

dihydrate (CPPD) deposition [36], but also be accompanied by what might seem paradoxical; enthesopathy [36], calcific periarthritis featuring HA crystal deposition [37], and conjunctival calcification [38]. Screening for EM due to the persistently high circulating TNSALP activity potentially causing low ePPi was a safety assessment in the clinical trials of AA for HPP [12–15]. In fact, disturbances in mineral homeostasis that might predispose to EM are not uncommon in this metabolic bone disease [1,12,39,40]. The block in skeletal mineralization engendered by superabundant ePPi is especially severe in the perinatal and infantile forms of HPP, and often leads to hypercalcemia and hypercalciuria that can cause nephrocalcinosis with renal compromise [12]. In the more mild childhood and adult forms of HPP,

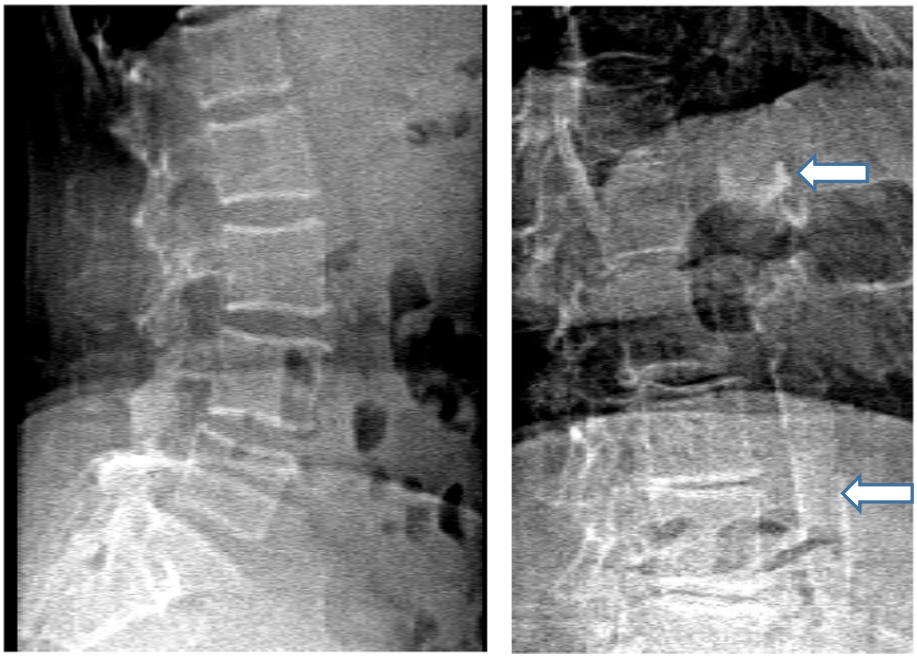


Fig. 3. Patient after 18 months of AA treatment: Left panel: No VC is apparent in our patient's abdominal aorta. Right panel: In a 90-year-old "positive control" patient without HPP, VC is readily apparent (arrows).

longstanding hyperphosphatemia without hypercalcemia is common, and explained by increased renal reclamation of inorganic phosphate (Pi) possibly due to deficiency of TNSALP in the kidney itself, low or inappropriately normal circulating levels of phosphatonins, and/or the high urinary levels of PPi [39]. It may be that this hyperphosphatemia explains the asymptomatic punctate corneal and conjunctival calcifications that sometimes occur in untreated HPP [38], and that perhaps became more prevalent during AA treatment [13,14]. Although the pathogenesis is uncertain and their presence might seem paradoxical in face of elevated ePPi levels, adults with HPP often have ectopic calcification: ossification of spinal ligaments (e.g., Forrestier disease), other enthesopathies, osteophytosis, and calcific periarthritis from HA deposition [36,37].

During AA treatment, patients of all ages with HPP have persistently elevated circulating ALP activity that may be 5000–10,000 U/L (Nl 30–120 U/L) [12–15]. Thus, hypothetically, this might cause or increase ectopic calcification, including VC, if ePPi levels were excessively lowered at susceptible sites [8,9]. In fact, circulating bone-specific ALP activity has been found to correlate positively with abdominal aortic calcification in maintenance hemodialysis patients [27]. Accordingly, pharmacokinetic and pharmacodynamic studies of AA dosing in HPP have included assays of circulating levels of the TNSALP substrates PPi and PLP [12–15], and low levels were uncommon during the clinical trials of AA for HPP [12–15]. Blood collection tubes for those studies contained levamisole, a noncompetitive inhibitor of TNSALP activity [41], to prevent potential pre-assay PLP or PPi hydrolysis and falsely low results. However, such tubes are currently not routinely available, and thus the very high serum TNSALP activity during AA treatment might hydrolyze these TNSALP substrates during prolonged preparation of laboratory specimens. Commercial assays for circulating PLP are available, but PPi quantitation remains a research procedure.

In the general population, coronary artery Ca^{++} (and therefore the Ca^{++} score assessed using CT) increases with age [16]. Men typically show more of this Ca^{++} than women, yet only ~11% of women older than age 67 years have a Ca^{++} score of zero. Thus, the precise likelihood of no VC in our patient, who was relatively young for the above age range, is unknown, but likely > 11%. Perhaps her baseline absence of VC by CT was explained by protective superabundance of ePPi from her HPP. However, other explanations are possible, yet her family's longevity is notable and includes additional individuals with HPP.

We now appreciate that > 365 *ALPL* mutations cause HPP worldwide [5]. Some, with a dominant-negative effect, account for relatively mild multigenerational HPP, whereas homozygosity and compound heterozygosity involving two defective *ALPL* alleles typically explain severe HPP presenting in the first year-of-life [6]. The heterozygous *ALPL* mutation (c.1133A > T, p.Asp378Val) carried by our patient is the most common explanation for multigenerational HPP in the United States [6,18]. Thus, our findings might be particularly relevant to individuals harboring this defect. However, all patients, especially adults, with HPP seem worthy of study to understand any relationship with VC [42]. In 2018, Lewis et al. [26] assessed abdominal aorta calcification in elderly woman, but without HPP, using DXA lateral spine VFA. Investigation of adults with HPP for VC using this generally available methodology would decrease patient X-radiation exposure and is now part of our surveillance of these patients. Detection of VC during AA treatment for HPP would seem to merit follow-up radiological imaging, but the timing of reassessment will require future investigation.

In summary, our observations from cardiac and peripheral CT and DXA imaging provide some reassurance against VC occurring during AA treatment. However, this experience reflects just one elderly patient, and a relatively brief period of therapy. Investigation of additional patients with HPP with baseline assessments and longer follow-up will be necessary to determine if their superabundant ePPi protects against VC and if AA treatment causes or exacerbates any type of ectopic mineralization.

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Author roles

All authors helped write and approved the submitted manuscript. MPW treated the patient, ordered the screening studies for vascular calcification, and drafted and finalized the manuscript. WHM and AJB interpreted and illustrated the computed tomography findings. SM performed the mutation analyses.

Disclosures

The authors have nothing to disclose.

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