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Repeatedly low plasma alkaline phosphatase in a 56-year-old woman. A case of hypophosphatasia diagnosed in adulthood☆

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In the clinic, alkaline phosphatase (ALP) is primarily measured to detect high activities of the enzyme. A number of clinical conditions are associated with low level of ALP, including the rare bone mineralization disease hypophosphatasia (HPP) [1]. HPP has an extremely broad clinical spectrum and should be considered when persistently low activities of ALP are found.

1. Case description

A 56-year-old woman had blood samples taken regularly to monitor lithium therapy for bipolar affective disorder. Plasma alkaline phosphatase (P-ALP) was repeatedly < 10 IU/L and the consultant psychiatrist (OBS) contacted the laboratory to exclude erroneous measurements. P-ALP was analyzed on a Dimension Vista® (Siemens Healthineers, Ballerup, Denmark) routinely calibrated and quality assured with certified internal and external quality control material. Over the previous five years, only this patient had more than one measurement of P-ALP < 10 IU/L and faulty results were not suspected.

Medical causes of hypophosphatasemia were excluded and possible explanations according to the algorithm suggested by Fraser were considered [2]. The patient was not anemic, did not take estrogen supplements, bisphosphonate, clofibrate, omeprazole, or lansoprazole. Nor did she follow any specific diet. She was previously diagnosed with hypothyroidism, and was well-supplemented with levothyroxine. There was no history of cardiac surgery or cardiopulmonary bypass [1]. Repeat laboratory tests on plasma revealed consistently low P-ALP activity, increased pyridoxal 5´-phosphate (PLP) (the major form of vitamin B6 [3]) to more than ten times the upper reference level, and low phosphate concentration. Thyrotropin (TSH) and zinc were within the respective reference ranges (Table 1). Vitamin D was not measured, though it would have been relevant.

The patient had a history of bilateral metatarsal fracture. In both cases the fractures were caused by minor traumas. When the patient was 50 years old, she slipped on stairs and sustained a fracture of the distal left fourth metatarsal bone. One year later, she was wearing odd sized clogs and broke the right fifth metatarsal bone (Jones fracture). Both fractures were confirmed on X-ray and so was

Abbreviations: HPP, hypophosphatasia; ALP, alkaline phosphatase; P-ALP, plasma alkaline phosphatase; TNSALP, the gene encoding the tissue-nonspecific isoenzyme of alkaline phosphatase; ALPL, synonym to TNSALP; PPi, inorganic pyrophosphate; PLP, pyridoxal 5´-phosphate

☆ A case of hypophosphatasia diagnosed in adulthood.

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Table 1
Biochemical parameters in plasma of the patient.

Analyte (units)	Concentration	Reference interval
Alkaline phosphatase (U/L)	< 10	35–105
Phosphate (mmol/L)	0.55–0.67	0.76–1.41
Pyridoxal 5-Phosphate (nmol/L)	1590	20–120
Ascorbate (μmol/L)	58.4	26.1–84.6
Zinc (μmol/L)	12.7	10.0–19.0
Cobalamin (pmol/L)	697	150–800
Folate (nmol/L)	> 45	> 6
Thyrotropin (TSH) (mIU/L)	2.8	0.3–4.0

the prolonged healing. The fractures were treated conservatively. Both during the years prior to radiological confirmation of the metatarsal fractures and subsequently, the patient suffered from persistent pain in both feet, diffuse but distally located and provoked when stepping on e.g. pebbles. Also, she reported bilateral knee and hip discomfort. The patient was of average height and did not experience premature loss of deciduous teeth. There was no history of additional fractures. When questioned about her childhood, the patient reported not being capable of participating in any sports (running, jumping, roller-skating etc.), due to discomfort or pain in both feet. The take-off/pronation phase of the gait cycle was especially painful. Doctors explained her symptoms as rickets and "weak bones" and she was not further examined.

1.1. Testing for hypophosphatasia

In view of the laboratory tests and the clinical history, HPP was suspected. The patient agreed to genetic testing and sequencing of the ALPL gene was performed at Centogene AG (Rostock, Germany), revealing two heterozygous variants in the ALPL gene. These two mutations were located in exon 6: c.571G > A (p. Glu191Lys), previously described as disease-causing [4], and c.512A > G (p. His171Arg) a so far unpublished but known variant (Fig. 1). Both mutations are listed in The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database [5]. Testing of relevant family members was recommended.

The patient had no children, one brother and no nieces or nephews. Her father passed away at the age of 57 years due to myocardial infarction and had no known symptoms possibly attributable to HPP. Her brother (born 1967) was not interested in genetic testing. According to the patient, her brother was diagnosed with HPP as an infant without this leading to diagnosis of his sister. The mother had a spontaneous abortion early in pregnancy, between the birth of the patient and her brother. The mother suffered from arthritis and agreed to be tested for the same mutations as demonstrated in the index patient. She had a normal level of P-ALP and was found to be a carrier the c.571G > A (p. Glu191Lys) mutation.

The c.571G > A (p. Glu191Lys) mutation, found in both the index patient and her mother, has been described as a moderate (not severe) mutation and is frequently seen in HPP patients of European ancestry. Fauvert et al. [6] stated that many cases of moderate HPP are compound heterozygous for a moderate and a severe allele or heterozygous for a missense mutation with a dominant negative effect. In the same publication the authors reported that many cases of HPP are found in patients who are either compound heterozygotes or carry two missense mutations with a dominant negative effect [6]. The patient described here is a compound

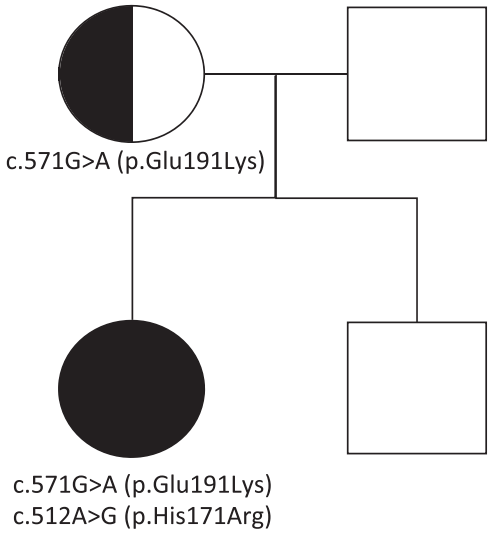

Fig. 1. Genetic pedigree. Compound heterozygosity was found in the patient (black circle). Her mother (black and white semi-circles) was a carrier of the c.571G > A (p.Glu191Lys)-mutation.

heterozygote for a known pathological allele and an allele of unknown significance (according to the laboratory performing the sequencing). The fact that the mother is a carrier of the c.571G > A (p. Glu191Lys) mutation and exhibits a normal level of P-ALK suggests a pathological influence of the c.512A > G (p. His171Arg)-mutation. Otherwise we would expect the index patient to express a normal level of P-ALP, as her mother did. We did not validate or examine the possible pathogenic effect of the second mutation further.

2. Discussion

The ALP enzyme was discovered in 1923 and HPP was registered as a disease fifteen years later [3]. ALP is widespread in the body, particularly in the skeleton, liver, kidneys and developing teeth [3], and catalyzes hydrolysis of a range of substrates [7]. The bone isoform of alkaline phosphatase hydrolyzes inorganic pyrophosphate (PPi) into phosphate, involved in the formation of hydroxyapatite and normal bone formation [8]. As already mentioned, in clinical practice little attention is paid to low P-ALP activities [1]. This case highlights that P-ALP activities below the lower limit of the reference interval are of potential clinical importance.

HPP is a highly heterogeneous genetic disease caused by loss-of-function mutations in the gene encoding the tissue non-specific (liver/bone/kidney) isoenzyme or ALP – the ALPL or TNSALP gene [5]. Phenotypically the presentations range from a condition fatal *in utero* to milder forms of osteopenia and poor fracture healing. HPP can be classified into various forms: adult; mild childhood; severe childhood; infantile; perinatal, as well as odontological HPP. Previously, prior to genetic confirmation of the disease, HPP was considered a syndrome of persistently low P-ALP, a relevant medical history and paraclinical (radiological and additional biochemical) findings. Thirty years ago, in 1988 the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) gene was sequenced and more than 300 genetic changes are now detected [3].

In addition to low ALP, a rise in PLP is regularly seen in patients with HPP and the concentration often correlates to the severity of the disease [3]. The patient described in this paper had a PLP concentration more than ten times the upper limit. She had no history of premature loss of deciduous teeth, but her dental status was poor. Her osteogenic symptoms had been life-long but moderate compared to more severe HPP phenotypes. In The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database [5] a case with similar mutations is listed as suffering from adult HPP. The case is unpublished and the phenotype is not specified. Interestingly, the patient described in this case has had symptoms attributable to HPP since childhood.

HPP is the last form of osteomalacia to be medically treated [3]. In 2015 the human recombinant TNSALP replacement therapy asfotase alfa (Stensiq®, Alexion) was approved by the US Food and Drug Administration and the European Medicines Agency for pediatric-onset HPP [9,10]. Asfotase alfa has also been approved for treatment of adults with symptoms of HPP in childhood. However, guidelines on treatment of adults with asfotase alfa are not available [11]. Bisphosphonates are relatively contraindicated in HPP as the treatment is believed to worsen the unbalanced condition with elevated PPi [12]. Adults with HPP may present with fractures and low bone mass. In patients with these symptoms and findings as well as low P-ALP, HPP must be excluded before initiating bisphosphonate therapy [13]. The patient described in this case has now received genetic counselling and will be referred for further orthopedic and endocrinology consultation. The patient is menopausal but for younger HPP patients family planning is relevant.

2.1. *In conclusion,*

we report a case of HPP diagnosed in adulthood, due to awareness of low P-ALP activity. This case exemplifies the importance of a critical and curious approach to common clinical biochemical laboratory tests.

The patient and her mother gave signed written informed consent to the publication of this clinical case study.

Disclosures

The authors report no conflicts of interest.

References

[1] G. Lum, Significance of low serum alkaline phosphatase activity in a predominantly adult male population, Clin. Chem. 41 (1995) 515–518.
[2] W.D. Fraser, Bone and mineral metabolism, in: N. Rifai, A.R. Horvath, C.T. Wittwer (Eds.), Tietz Textbook of Clinical Chemistry and Molecular Diagnosis, 6th ed., Elsevier, St. Louis, MO, USA, 2018, pp. 1422–1480.
[3] M.P. Whyte, Hypophosphatasia: an overview for 2017, Bone 102 (2017) 15–25.
[4] P.S. Henthorn, M. Raducha, K.N. Fedde, M.A. Lafferty, M.P. Whyte, Different missense mutations at the tissue-nonspecific alkaline phosphatase gene locus in autosomal recessively inherited forms of mild and severe hypophosphatasia, Proc. Natl. Acad. Sci. USA 89 (1992) 9924–9928.
[5] The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database Versailles, France: University of Versailles Saint-Quentin-en-Yvelines; [updated 04-08-2017. Available from: 〈http://www.sesep.uvsq.fr/03_hypo_mutations.php〉].
[6] D. Fauvert, I. Brun-Heath, A.S. Lia-Baldini, L. Bellazi, A. Taillandier, J.L. Serre, et al., Mild forms of hypophosphatasia mostly result from dominant negative effect of severe alleles or from compound heterozygosity for severe and moderate alleles, BMC Med. Genet. 10 (2009) 51.
[7] M. Panteghini, R. Bais, Serum enzymes, in: C.A.A. Burtis, E.R. Ashwood, D.E. Bruns (Eds.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th ed., Elsevier Saunders, St. Louis, MO, USA, 2012, pp. 565–598.
[8] J. Risteli, W.E. Winter, M. Kleerekoper, L. Risteli, Bone and mineral metabolism, in: C.A.A. Burtis, E.R. Ashwood, D.E. Bruns (Eds.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th ed., Elsevier Saunders, St. Louis, MO, USA, 2012, pp. 1781–1782.
[9] FDA News Release - FDA approves new treatment for rare metabolic disorder [press release]. U.S. Food & Drug Administration, U.S. Department of Health and Human Services, 10-23, 2015.

[10] First treatment recommended for rare bone disease [press release]. European Medicines Agency, European Union, 06-26, 2015.
[11] J.R. Shapiro, E.M. Lewiecki, Hypophosphatasia in adults: clinical assessment and treatment considerations, J. Bone Miner. Res. 32 (2017) 1977–1980.
[12] E. Mornet, Hypophosphatasia, Orphanet J. Rare Dis. 2 (2007) 40.
[13] N. Bishop, Clinical management of hypophosphatasia, Clin. Cases Miner. Bone Metab. 12 (2015) 170–173.