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SPECIAL REPORT

Intrafamilial phenotypic distinction of hypophosphatasia with identical tissue nonspecific alkaline phosphatase gene mutation: a family report

Masaru Kato[1] · Toshiyuki Hattori[2] · Tomohiro Shimizu[3] · Keita Ninagawa[1] · Rimi Izumihara[1] · Hiroshi Nomoto[1] · Kazuhide Tanimura[2] · Tatsuya Atsumi[1]

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Abstract

Hypophosphatasia (HPP) is caused by mutations in the tissue nonspecific alkaline phosphatase (TNSALP) gene in an autosomal recessive or dominant manner and characterized by defective mineralization of bone and low serum ALP levels. In this report, we present a family with HPP mother (case 1) and HPP child (case 2) who have identical TNSALP gene mutation (c.1015G>A p.Gly339Arg heterozygous mutation) but distinct clinical phenotypes. Whereas case 1 appeared to be asymptomatic despite extremely low levels of serum ALP, case 2 had several HPP-related symptoms, such as tooth loss, fractures, short stature, with slightly decreased ALP levels. Upon the diagnosis of HPP, case 1 discontinued denosumab, which was used to treat her rheumatoid arthritis, concerning the risk of atypical femoral fractures. The clinical course of this family was suggestive in a genotype–phenotype imbalance in HPP, the underdiagnosis of HPP in adults, and the risk of atypical femoral fractures using bone resorption inhibitors.

Keywords Hypophosphatasia · TNSALP gene · Denosumab · Family report

Introduction

Hypophosphatasia (HPP) is caused by mutations in the tissue nonspecific alkaline phosphatase (TNSALP) gene and characterized by defective mineralization of bone and low serum ALP levels. HPP can result from either autosomal recessive or autosomal dominant inheritance. Due to its rarity as well as the varieties of its severity, the clinical characteristics of HPP, particularly those of adult cases, remains to be fully determined. Adult HPP is typically diagnosed based on musculoskeletal pain, bone fractures, dental abnormalities, and/or a childhood history of premature deciduous tooth loss [1, 2]. Since pain and fractures are nonspecific clinical features, there are cases who need to be differentiated from rheumatic diseases such as osteoporosis, rheumatoid arthritis (RA), spondyloarthritis, and fibromyalgia. The data regarding the correlation between HPP phenotype and TNSALP genotype also remain insufficient. Some genotype–phenotype correlations are seen in the perinatal lethal type of HPP, whereas different clinical features are associated with the same gene mutation in the non-lethal type. A German cohort of adult HPP has revealed that serum ALP levels and clinical symptoms vary strongly among individuals with the same mutation [3], suggesting HPP as a multifactorial disease. Moreover, an intrafamilial phenotypic variability of HPP was reported in a German family with heterozygous parents (maternal: p.Glu191Lys, paternal: p.Gly334Asp) and four children (two had compound heterozygous mutations) [4]. Therefore, more data are warranted to more precisely determine the genotype–phenotype correlations in patients with HPP. We herein report a Japanese family with HPP mother and HPP child who have identical heterozygous TNSALP gene missense mutation (p.Gly339Arg) but distinct clinical phenotypes. Whereas

✉ Masaru Kato
ktmasaru@med.hokudai.ac.jp

[1] Department of Rheumatology, Endocrinology and Nephrology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, N15W7, Kita-Ku, Sapporo 060-8638, Japan

[2] Hokkaido Medical Center for Rheumatic Diseases, Sapporo, Japan

[3] Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

the mother appeared to be asymptomatic despite extremely low levels of serum ALP, the child had several HPP-related symptoms, such as tooth loss, fractures, short stature, with slightly decreased ALP levels.

Family report

Mother (case 1)

Case 1 was a 60-year-old woman who exhibited an extremely low serum ALP level [33 IU/L (normal range 105–330 IU/L)] and was therefore suspected to have HPP. She had a diagnosis of RA 6 years ago based on polyarthritis, presence of rheumatoid factor [22 IU/mL (normal range <15 IU/mL)] and anti-cyclic citrullinated peptide antibody [8.6 U/mL (normal range <4.5 U/mL)], and ultrasound doppler signals in the synovium. Her RA remained in remission with the use of methotrexate (10 mg/week), iguratimod (50 mg/day), and denosumab (60 mg/6 months). Her serum ALP level before the use of antirheumatic drugs including denosumab was also low (59 IU/L). The diagnosis of HPP was confirmed with increased urinary phosphoethanolamine [330.5 μmol/g Cr (normal range 7–70 μmol/g Cr)] and the TNSALP gene mutation (c.1015G>A p.Gly339Arg heterozygous mutation). Her height and weight were 157 cm and 62 kg. She had no history of bone fracture, abnormal tooth loss, or gynecologic diseases. Her menopause occurred when she was 52 years old. Bone mineral densities were 96% and 78% of young adult mean, respectively, at the lumber spine and the femoral neck. Bone turnover markers, including serum levels of bone-specific ALP (BAP), procollagen type I N-terminal propeptide (PINP), and tartrate-resistant acid phosphatase 5b (TRACP-5b), were decreased (Table 1). Serum 25-hydroxy vitamin D level was also slightly decreased [19.3 ng/mL (normal range >20.0 ng/mL)]. She was unlikely to need treatment of HPP but discontinued denosumab concerning the risk of atypical femoral fractures (AFFs) [5–7], although femoral bowing and large femoral tibial angle were not found in X-ray on both sides. After discontinuing denosumab (12 months after the last injection), the serum level of PINP and that of TRACP-5b were increased to the upper range or more, whereas that of BAP remained around lower range (Table 1).

Child (case 2)

Case 2 was a 30-year-old woman, the daughter of case 1. She was born by vaginal delivery at 38 weeks of gestation. The birth weight, length, and head circumference were 2270 kg, 49 cm, and 33 cm, respectively. The Apgar score was 9 at 1 min and 7 at 5 min after birth. She required incubator care immediately upon birth due to meconium aspiration syndrome. She had cerebral palsy, mental retardation, and hearing loss from birth. Upon the diagnosis of HPP in her mother as described previously, she was also suspected to have HPP. Whereas her deciduous teeth were normal, the loss of permanent teeth started since she was 11 years old and, at the visit in our hospital, she had already lost most of her permanent teeth (Fig. 1). She has not ever experienced severe teeth caries, gingivitis, or alveolar pyorrhea that might cause early teeth loss. Her serum ALP level was decreased slightly (90 IU/L). She had the same TNSALP gene missense mutation as her mother. Her height and weight were 145 cm and 38 kg. She had a history of bone fractures on the fingers and toes. She had no history of seizures or intracranial hypertension.

Other family members

The family tree including case 1 and case 2 is presented in Fig. 2. The father and mother of case 1 had already died due to undifferentiated cancer and pancreatic cancer, respectively. The father had a diagnosis of RA but details, such as

Table 1 Bone turnover markers on the use of and after discontinuing (12 months after the last injection) denosumab in case 1

		On the use of denosumab	After discontinuing denosumab
BAP	[2.9–14.5] μg/L	1.9	4.2
PINP	[17.1–64.7] μg/L	7.7	64.7
TRACP-5b	[120–420] mU/dL	82	511
NTx	[7.5–16.5] nmolBCE/L	10.0	17.3
ALP	IU/L	33	59

BAP bone specific alkaline phosphatase, *PINP* procollagen type I N-terminal propeptide, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *NTx* serum cross-linked N-telopeptide of type I collagen, *[]* mean value ± 1.96 SD of healthy premenopausal women

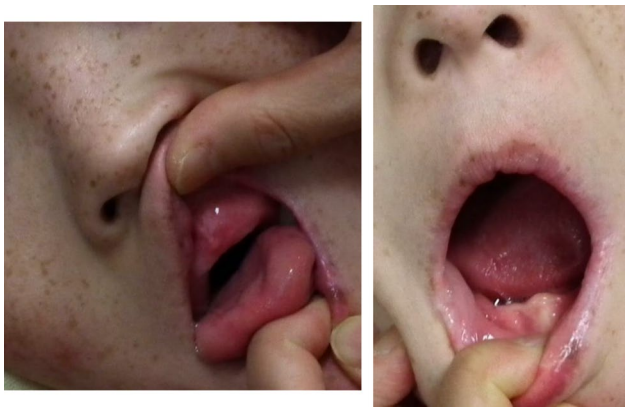

Fig. 1 The loss of permanent teeth in case 2

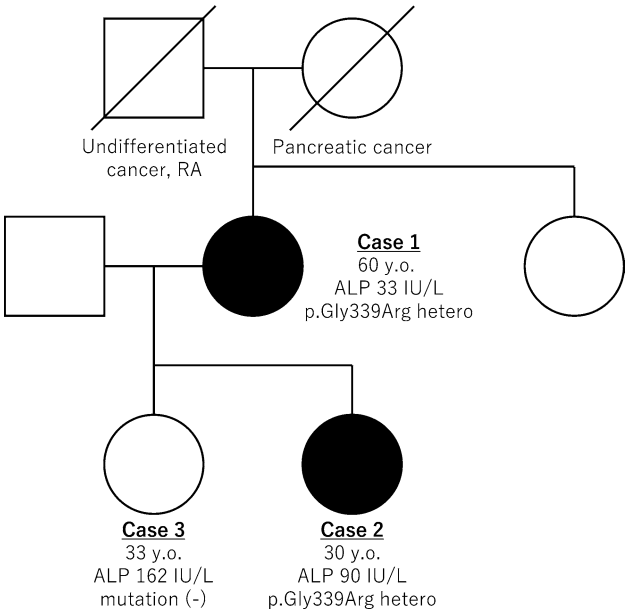

Fig. 2 Family tree including case 1, case 2, and case 3. *ALP* alkaline phosphatase, *RA* rheumatoid arthritis

autoantibody positivity and medications, were unknown. Her sister was healthy. Her husband was also healthy and had normal serum ALP levels (188 IU/L). We obtained informed consent from and performed genetic testing in another child of case 1, a 33-year-old healthy woman (case 3). She had no TNSALP gene mutations. Her serum ALP level was normal (162 IU/L). She was 21 cm taller (height: 164 cm, weight: 50 kg) than her sister, case 2. Besides the mutation, four single nucleotide polymorphisms (SNPs) in the TNSALP gene are observed in the family (Table 2). The carriage of these SNP alleles was same in case 2 and case 3.

Discussion

The current report describes an intrafamilial phenotypic distinction of HPP with identical heterozygous TNSALP gene missense mutation (p.Gly339Arg) in a Japanese family. Compared to the previous report presenting HPP phenotypic variability among children with compound heterozygous mutations (p.Glu191Lys and p.Gly334Asp) [4], our report presents that in parent and child with single heterozygous mutation. Whereas our first case (case 1) appeared to be asymptomatic, our second case (case 2) had several HPP-related symptoms, such as tooth loss, fractures, and short stature.

The TNSALP gene missense mutation c.1015G>A p.Gly339Arg is considered to have a small to intermediate dominant negative effect. It was detected in a compound heterozygous patient with perinatal HPP (the other mutation: c.526G>A p.Ala159Thr) [8] and in three single heterozygous patients with odonto-HPP [9], the least severe form of HPP lacking musculoskeletal abnormalities. By co-transfection of cells with wild-type and mutant TNSALP plasmids, the dominant negative effect of p.Gly339Arg was revealed to be 33.3% of wild type activity, whereas that of the most severe mutation (p.Gly456Trp) was 14.5% [9]. Another study showed a smaller dominant negative effect of p.Gly339Arg with wild-type/mutant activity of 45.8% [10]. Therefore, only some individuals carrying p.Gly339Arg single heterozygous mutation may develop HPP, particularly its mild form such as adult HPP or odonto-HPP. Conversely, case 2 in the current report had relatively severe symptoms from childhood, although cerebral palsy could affect her bone phenotype. It may be plausible but remains to be determined that other genetic or non-genetic factors modify the penetrance of a TNSALP gene mutation.

It remains controversial whether SNPs in the TNSALP gene affect HPP phenotype or have a dominant negative effect. Previous reports include some information regarding one of the TNSALP SNPs (rs3200254 c.787 T>C p.Tyr263His, minor allele frequency based on SNP database: 27%). Two members with premature deciduous tooth loss and low serum ALP levels in a Chinese family had a heterozygous substitution on rs3200254 without any other mutations causing amino acid substitution [11]. Conversely, a substitution on rs3200254 was associated with a high bone mineral density among postmenopausal Japanese women, thus suggesting a preventive role of this substitution against bone loss [12]. Given that case 1, case 2, and case 3 in the current report had homozygous, heterozygous, and heterozygous substitution of rs3200254 as

Table 2 TNSALP gene mutations and SNPs observed in the present family

			Case 1	Case 2	Case 3
c.330 T>C	p.Ser110Ser	rs1780316 (MAF 7%)	+, homo	+, homo	+, homo
c.787 T>C	p.Tyr263His	rs3200254 (MAF 27%)	+, homo	+, hetero	+, hetero
c.863-7 T>C	Intron variant	rs74063111 (MAF 26%)	+, homo	+, hetero	+, hetero
c.876A>G	p.Pro292Pro	rs3200255 (MAF 27%)	+, homo	+, hetero	+, hetero
c.1015G>A	p.Gly339Arg	Mutation	+, hetero	+, hetero	–

MAF minor allele frequency based on SNP database

shown in Table 2, rs3200254 was not likely to affect HPP phenotype in the family.

Bone resorption inhibitors, including bisphosphonates and denosumab, lower the serum level of ALP, particularly that of the bone-specific isoform of ALP. This is mainly due to decreased bone formation secondary to the inhibition of bone resorption. In patients with HPP, the decrease of bone formation following the use of bone resorption inhibitors may further undermine the defective mineralization and therefore precipitate AFFs, as reported in some cases [5–7]. In addition, bisphosphonates, but not denosumab, inhibit ALP activity by competing for binding to divalent metal ions, such as zinc or magnesium, which is vital for ALP to exert a mineralization of bone [13]. Although there has so far been no reported case of HPP and AFFs following exposure to denosumab without the past use of bisphosphonates [14], it remains unknown whether the use of denosumab, compared with that of bisphosphonates, is less related to the risk of AFFs in patients with HPP. Given the persistent low BAP levels even after discontinuing denosumab as well as the relatively low 25-hydroxy vitamin D level, case 1 in the current report should be carefully observed and periodically undergo the risk evaluation for AAFs.

The serum levels of ALP are increased in patients with RA [15, 16], possibly reflecting periarticular and systemic high turnover osteoporosis. Given the low serum ALP level before the use of antirheumatic drugs including denosumab in Case 1 in the current report, it can be speculated that increased bone resorption, such as post menopause and RA, is hardly compensated by bone formation in patients with HPP.

Among extra-musculoskeletal manifestations of HPP, neurological symptoms, such as headache, sleep disturbance, gait change, vertigo, depression, anxiety, neuropathy, and hearing loss, occur commonly [17]. In addition to those symptoms, seizure, attention deficit hyperactivity disorder, and mental retardation are observed in patients with pediatric onset HPP [17, 18]. In case 2 in the current report, mental retardation and hearing loss from birth could be due to both cerebral palsy and HPP. More reports are desired to determine whether the less severe TNSALP gene mutation can be a cause of neurological symptoms from birth or childhood.

Another aspect of the current report is that the TNSALP gene missense mutation c.1015G>A p.Gly339Arg has not previously been reported in the Japanese population. In a nationwide clinical survey of Japanese pediatric patients with HPP, the first and second most frequent TNSALP gene mutations were c.1559delT and c.979 T>C p.Phe327Leu, respectively, whereas c.1015G>A p.Gly339Arg was not observed in any patients [18]. All patients carrying c.1559delT homozygous mutation had the perinatal lethal phenotype with respiratory failure. In a more recent study on 98 Japanese patients with HPP, c.1015G>A p.Gly339Arg was also absent [19]. These data suggest that carriers of the less severe TNSALP gene mutation may be underdiagnosed or misdiagnosed as other diseases such as osteoporosis and fibromyalgia. It was also pointed out that some patients developing femoral pseudo fractures or AFFs would have the TNSALP gene mutation even if they had used bone resorption inhibitors [5].

Conclusion

The current family report describes a genotype–phenotype imbalance in HPP, the clinical courses of asymptomatic mother and symptomatic child with the identical single heterozygous TNSALP gene mutation, and would indicate the underdiagnosis of HPP in individuals using bone resorption inhibitors. More cases of HPP, particularly those of mild HPP, need to be reported to fully determine the variability of HPP phenotypes. It would be plausible that the more knowledge about HPP characteristics could help to prevent the development of AFFs by avoiding inappropriate use of bone resorption inhibitors.

Compliance with ethical standards

Conflicts of interest Masaru Kato has received research grants from AbbVie, Actelion, and GlaxoSmithKline and speaking fees from Eli Lilly. Tatsuya Atsumi has received research grants from Astellas, Takeda, Mitsubishi Tanabe, Chugai, Daiichi-Sankyo, Otsuka, Pfize, Alexion, Bayer, Otsuka, Chugai, Takeda, Eisai, Bristol-Myers Squibb, Daiichi Sankyo, Mitsubishi Tanabe and AsahiKasei, consultant fees from Ono, Sanofi, Daiichi Sankyo and Pfizer and speaking fees from Mitsubishi Tanabe, Chugai, Astellas, Takeda, Pfizer, Daiichi Sankyo, Bristol-Myers Squibb and Eli Lilly. Other authors have nothing to declare.

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