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Case Report

Adult hypophosphatasia with a novel *ALPL* mutation: Report of an Indian kindred

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ABSTRACT

Hypophosphatasia is an inborn error in metabolism characterized by low serum alkaline phosphatase (ALP) activity resulting from deactivating mutations in *TNSALP* (also known as *ALPL*), the gene that encodes the 'tissue-specific' isoenzyme of ALP. The disease exhibits significant clinical heterogeneity that spans from death *in utero* to only dental complications in adult life. Herein, we report a 47-year-old woman presenting with fracture of shaft of left femur. She had been complaining of pain in both of her thighs for the past 3 years. In addition, she gave a history of premature loss of teeth. Review of old radiographs revealed pseudo-fractures involving the lateral cortices of the femora on both sides. Biochemical panel revealed hyperphosphatemia, persistently low total alkaline phosphatase (ALP) and low-normal bone turnover markers. Screening of her siblings revealed low ALP in her younger sister and brother who were otherwise free from any major dento-arthro-osseous complaints. Sanger sequencing showed a novel, heterozygous, missense mutation in exon 5 at position 311 (c.311a > g;p.104 Asn > Ser) of *ALPL* gene in the three members. The patient underwent open reduction and intramedullary nailing of left femur along with prophylactic nailing on right side. This case report represents the first genetically confirmed kindred of adult hypophosphatasia from the Indian subcontinent.

1. Introduction

Hypophosphatasia is an inborn error in metabolism characterized by low serum alkaline phosphatase activity resulting from deactivating mutations in *TNSALP* (also known as *ALPL*), the gene that encodes the 'tissue-specific' isoenzyme of alkaline phosphatase (TNSALP) (Whyte, 2016). In addition to serum, TNSALP is widely distributed in tissues, namely skeleton, liver, kidney and developing teeth (Millán, 2006). Lack of functional TNSALP leads to impaired mineralization of the skeletal matrix, manifesting as rickets in children and osteomalacia in adults (Whyte, 2008). The clinical severity of hypophosphatasia varies remarkably that generally reflects patient age at presentation with early onset forms being severe (and even lethal) and milder forms often presenting later in adult life or even going unnoticed (Orimo, 2016; Weinstein, 1981; Whyte, 2016).

Herein we report an Indian family of adult hypophosphatasia wherein the eldest sister had presented with fragility fracture of the shaft of left femur. Her serum total alkaline phosphatase (ALP) levels were low; Sanger sequencing revealed a novel, heterozygous, missense mutation in the *ALPL* gene in the proband and two siblings who were otherwise healthy.

2. Case presentation

A 47-year-old woman presented to the Emergency Department (ED) with severe pain in the left thigh and inability to bear weight on her left leg following a trivial fall onto the ground. On examination, her left lower limb appeared externally rotated. A plain radiograph of the pelvis and the left thigh showed a transverse infra-trochanteric fracture of the shaft of the left femur. She was put on analgesics for pain relief. She gave a history of persistent pain in her bilateral thighs for the past 3 years, although it had never been severe enough to impair her activities of daily living. She had visited multiple physicians for the same and had undergone serial radiographs of the pelvis; unfortunately, all were labeled as being 'normal' and following a magnetic resonance imaging of her lumbo-sacral spine that showed posteriorly prolapsed

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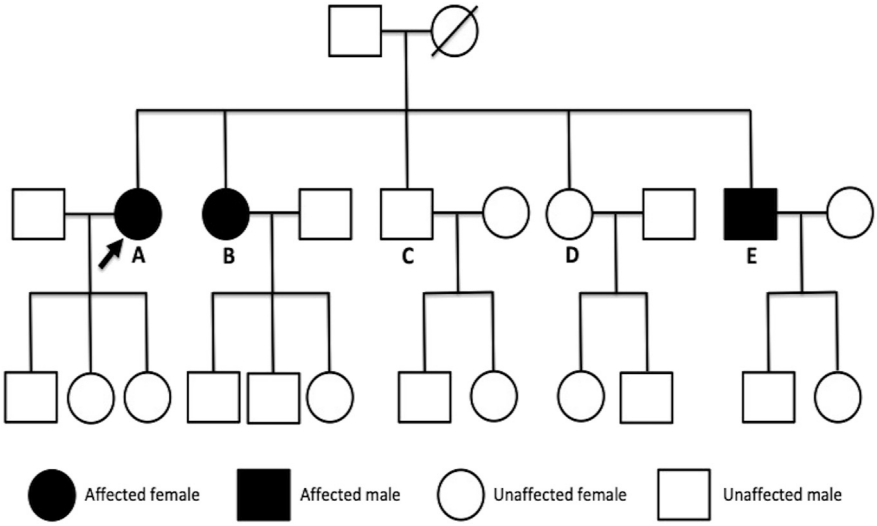

Fig. 1. Family pedigree of the reported kindred. The proband (A) is marked with a black arrow. B, C, D and E represent the siblings of the proband.

intervertebral disc at the L_2-L_3 level (with unaltered signal intensity of the spinal cord), her pain was designated as 'radicular' in origin. In addition, she gave a history of premature loss of teeth. She was a mother of three healthy children and was presently eumenorrhoeic. She was not any medications and had never been treated with anti-resorptive drugs. Family history was significant in that her mother had sustained a low-trauma fracture of the right femur at the age of 40 years and had succumbed 12 years later to breast carcinoma. All her siblings, nieces and nephews were apparently healthy (family pedigree depicted in Fig. 1).

Investigations revealed normal hemoglobin level, normal renal function, normal thyroid function, normoglycemia, normocalcemia and hyperphosphatemia [serum inorganic phosphate 5.5 mg/dl (reference range: 2.7–4.5)]. Of note, her serum total alkaline phosphatase (ALP) levels were persistently low ranging from 22.4 IU/l to 31.7 IU/l (reference range: 42–128 IU/l) even in the presence of fracture. She was vitamin D sufficient (25-hydroxyvitamin D 32.1 ng/ml) with intact parathyroid hormone of 26 pg/ml (range: 15–65). Her bone turnover markers were low-normal [serum P1NP 21 ng/ml (reference range: 15–58) and serum CTX 25 pg/ml (reference range: 25–573)]. Celiac serology was negative and arterial blood-gas analysis was normal. Review of the old radiographs revealed pseudo-fractures involving the lateral cortices of the femora on both sides (Fig. 2, marked in arrows).

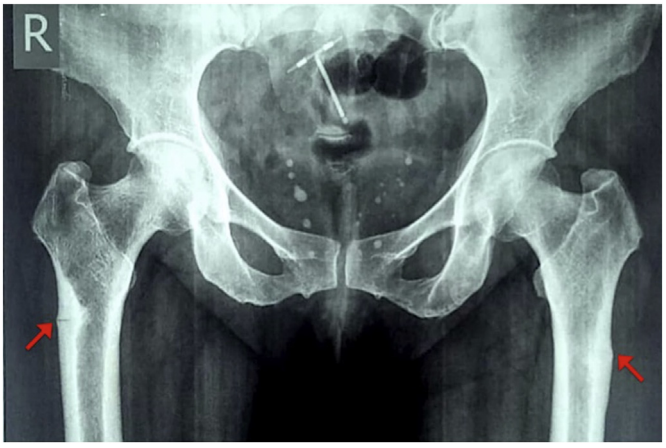

Fig. 2. Plain radiograph showing pseudo-fractures involving the lateral cortices of the femora on both sides (marked in red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

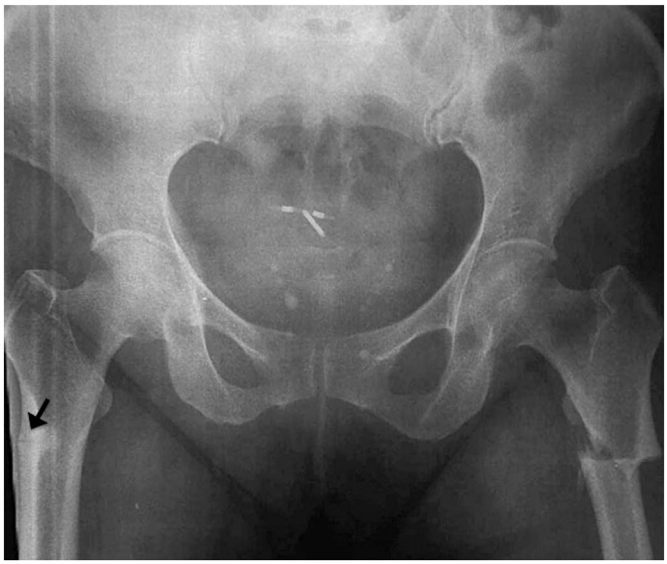

Fig. 3. Plain radiograph showing a transverse infra-trochanteric fracture of the shaft of the left femur and a pseudo-fracture involving the lateral cortex of the shaft of the right femur (marked in black arrow).

When carefully examined, the present radiograph also showed a similar pseudo-fracture involving the apparently unaffected right femur (Fig. 3). ^{99m}Tc-MDP bone scintigraphy showed increased uptake at the site of fracture (on left side) and pseudo-fracture (on the right side). There was no uptake in any other part of the body. Dual-energy X-ray absorptiometry (DEXA) of the lumbar spine (L_1–L_4) showed a *Z*-score of −1.8. Putting together the history of femoral pseudo-fractures, premature loss of permanent teeth, family history of low-trauma fracture in mother and biochemical finding of low ALP, a clinical possibility of adult hypophosphatasia arose. All her family members were clinically screened, however, none had any major dental complaints, neither any history of any significant bone/joint pain or any history of pathological fractures. Serum total ALP levels of all her siblings were estimated (Table 1).

Genomic DNA obtained from peripheral blood leukocytes of the index case and her siblings were subjected to PCR amplification of the *ALPL* gene followed by Sanger sequencing at the IGMM Sequencing Service (Edinburg, UK). It revealed a novel, heterozygous, missense mutation in exon 5 at position 311 (c.311a > g;p.104 Asn > Ser) of

Table 1
Table showing serum total alkaline phosphatase activity and *ALPL* mutation status in the proband and her siblings.

Member	Serum total ALP (Reference range: 42–128 IU/l)	*ALPL* mutation status
A (Proband)	22.4–31.7	Present
B	32.8	Present
C	48.7	Absent
D	85.0	Absent
E	35.6	Present

the *ALPL* gene (Fig. 4) in the proband and two siblings (B and E). *In-silico* analysis predicted it to be pathogenic, confirming our diagnosis of hypophosphatasia (Supplementary Table 1). Clustal Omega analysis showed that the mutation was located in a highly conserved area of the protein. The patient underwent intramedullary nailing of the left femur and prophylactic nailing on the contralateral side. At 6 months post-surgery, she is able to bear weight on her own and repeat radiograph has shown excess callus formation, although the fracture has not completely healed.

3. Discussion

First described by Canadian physician John C. Rathbun in 1948, hypophosphatasia represents a rare clinical condition characterized by loss-of-function mutations in the *TNSALP* (*ALPL*) gene that encodes for tissue-nonspecific alkaline phosphastase. TNSALP is widely expressed in bone and developing teeth where it hydrolyses extracellular pyrophosphate and promotes deposition of hydroxyapatite crystals in the unmineralized bone matrix (Weinstein, 1981). Lack of TNSALP therefore impairs mineralization of the osteoid matrix manifesting as rickets in pediatric population or osteomalacia in adults (Whyte, 2008). However, there exists significant clinical heterogeneity with hypophosphatasia probably having the broadest expressivity amongst all skeletal diseases. Hitherto, seven clinical forms of hypophosphatasia exist spanning across the entire range of disease severity and includes 'odontohypophosphatasia', 'perinatal', 'benign prenatal', 'infantile', 'childhood', 'adult' and 'pseudohypophoaphatasia' (Whyte, 2016). The marked variability in presentation depends primarily on the nature of the mutation affecting the *TNSALP* gene. Severe forms of hypophosphatasia are transmitted as an autosomal recessive trait and usually result from missense mutations that results in a mutant protein that fails to translocate to the cell membrane; instead it accumulates in the *cis*-golgi apparatus and is degraded by the ubiquitin-proteasome pathway. Milder forms, on the other hand, have an autosomal dominant mode of inheritance and result from missense mutations that produce a defective protein, which, in part, can correctly localize to the cell membrane (Mornet, 2007).

Adult hypophosphatasia usually presents during middle age. The initial complaint is often foot pain, resulting from metatarsal pseudo-fracture (Khandwala et al., 2006). Thigh pain, a consequence of femoral pseudo-fractures could be the primary complaint, as in our patient. Unlike most causes of osteomalacia, the pseudo-fractures in hypophosphatasia tend to affect the lateral aspect of the subtrochanteric region of the femoral shaft rather than involving the femoral neck (Whyte, 2009). Accordingly, these fractures resemble the prodromal lesions of 'atypical femoral fractures' seen in patients on long-term bisphosphonate therapy (Shane et al., 2014). They often have a predilection for chondrocalcinosis and debilitating polyarthropathy resulting from periarticular deposition of calcium pyrophosphate dihydrate (CPPD) and/or hydroxyapatite crystals (Braunstein, 2016; Mornet, 2007; Whyte, 2016). In addition, ossification of ligaments, nephrocalcinosis, recurring headaches and psychiatric symptoms are frequent (Galeano-

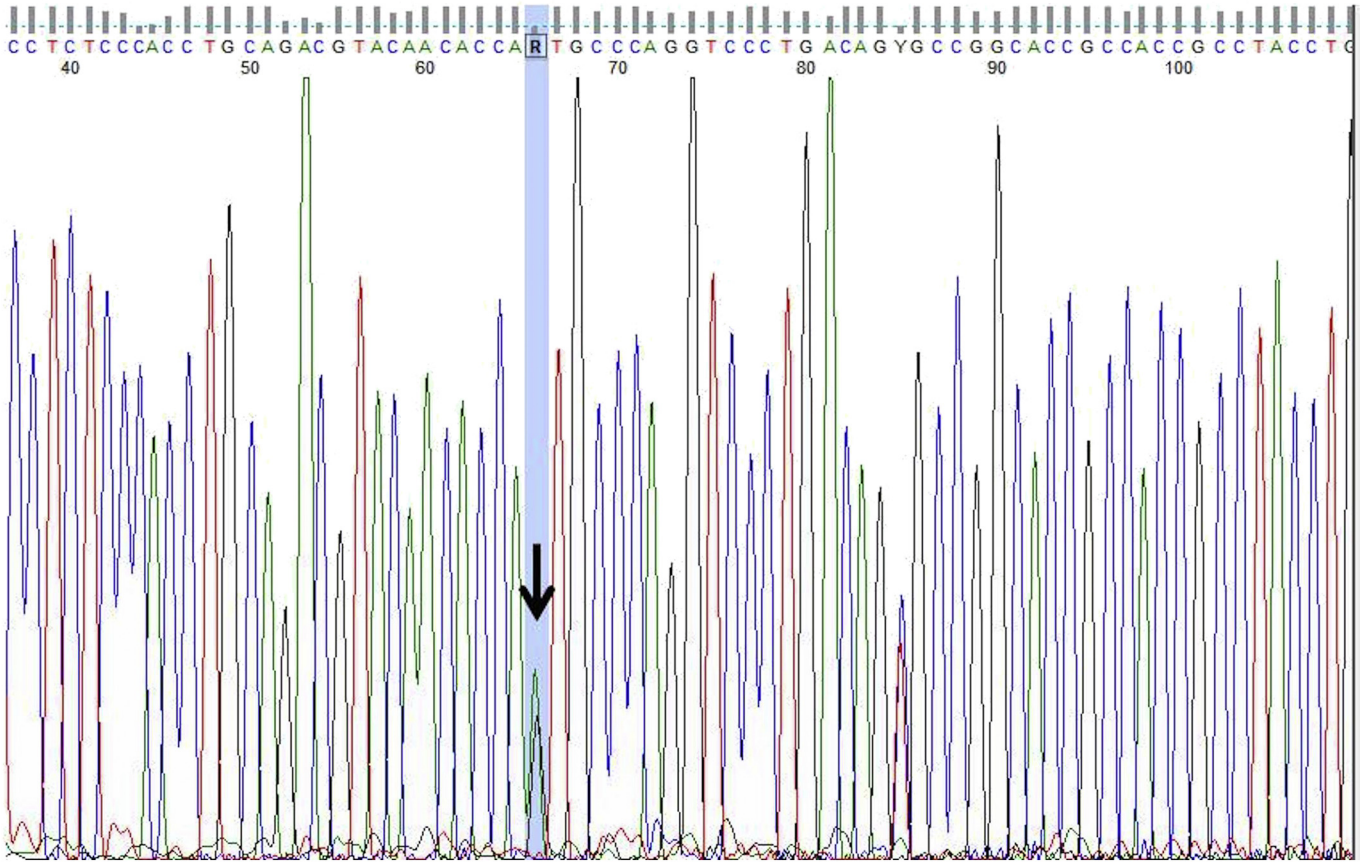

Fig. 4. Electropherogram showing a heterozygous, missense mutation (marked in black arrow) in exon 5 at position 311 of *ALPL* gene on chromosome 1.

Valle et al., 2019). In-depth history may reveal that such patients may have had premature loss of deciduous and/or adult teeth (Whyte et al., 1979), as was forthcoming in our patient. In a cohort of 22 adults with hypophosphatasia diagnosed at the Mayo Clinic between 1976 and 2008, most of the patients (68%) were symptomatic at presentation. A history of fracture and musculoskeletal pain was present in 54% and 41% of the cases, respectively (Berkseth et al., 2013). In another group of 125 adult patients with hypophosphatasia, pain, fractures, muscle weakness and unusual gait were reported in 95%, 86%, 62% and 52% of the participants, respectively (Weber et al., 2016). In addition, ovarian pathology or other gynaecological abnormalities are more common in women with hypophosphatasia (Dahir et al., 2018).

Diagnosis of hypophosphatasia relies upon consistent documentation of subnormal total serum ALP. ALP levels should be interpreted cautiously with respect to the age and sex of the patient (Mornet, 2007). Serum ALP, although sensitive, has low specificity for the diagnosis of hypophosphatasia as hypophosphatasaemia is seen in multiple other conditions, namely, profound anemia, celiac disease, hypothyroidism, multiple myeloma, starvation and clofibrate therapy (Khandwala et al., 2006; Whyte, 2016). On the contrary, conditions that increase serum ALP, most notably pregnancy, hepatobiliary disease and fracture can confound the diagnosis. The index case had persistently low serum ALP levels even in the presence of a femoral fracture, thereby strengthening the case. Other biochemical markers that may help in diagnosis include serum pyridoxal 5′-phosphate and urinary phosphoethanolamine, levels of which are elevated in patients with hypophosphatasia (Mornet, 2007). Skeletal radiographs, although pathognomonic in patients with perinatal, infantile and childhood hypophosphatasia, are non-specific in adult-onset cases showing osteopenia, metatarsal stress fractures, pseudo-fractures involving the lateral subtrochanteric regions of the femurs, periarticular CPPD deposition and arthropathy (Whyte, 2016). Bone scan provides a complete skeletal survey and can help detect subclinical pseudo-fractures in one single study. *Z*-scores measured by DEXA are only slightly reduced in most adult hypophosphatasia patients, hence, low bone mass is usually not a feature (Schmidt et al., 2017). Histomorphometry of un-decalcified bone specimens, obtained after tetracycline labeling from patients with hypophosphatasia (except odontohypophosphatasia) shows impaired mineralization. Although not a pre-requisite, documentation of a mutation in the *ALPL* gene aids in confirming the diagnosis. As of 2nd October 2019, 397 different *ALPL* mutations have been compiled in the *ALPL* gene mutations database (http://www.sesep.uvsq.fr/03_hypo_mutations.php). Most of them are missense mutations (Mornet et al., 2014; Tenorio et al., 2017). The index case had a novel, heterozygous, missense mutation in exon 5 at position 311 of the *ALPL* gene that resulted in a substitution of asparagine with serine. The same mutation was identified in two of her siblings, who were otherwise clinically 'disease-free'. The mutation was most probably inherited from her mother who had a similar history of fragility fracture at a middle age; although the possibility of paternal transmission cannot be ruled out. The mutation is inherited in an autosomal dominant pattern concordant with the mild adult form of hypophosphatasia. Although we do not have any report of bone histology/histomorphometry to confirm the diagnosis of osteomalacia in the index case, however, considering the genetically confirmed diagnosis of hypophosphatasia, absence of history of use of any anti-resorptive medications (like bisphosphonates), past history of long standing bilateral femoral pseudo-fractures and presentation with typical infratrochanteric left femoral fracture, we can assume that the woman had underlying osteomalacia.

Treatment of adult hypophosphatasia has evolved from symptomatic management to 'off-label' use of teriparatide to asfotase alfa. Teriparatide use has been reported in a handful number of anecdotal case reports with results being variable (Camacho et al., 2016; Laroche, 2012; Schalin-Jäntti et al., 2010). A phase IIA trial with monoclonal anti-sclerostin antibody (BPS804) showed improvement in lumbar spine BMD in 8 adult patients with hypophosphatasia (Seefried et al., 2017). Asfotase alfa, an enzyme replacement therapy, although FDA approved for treatment of perinatal, infantile and childhood hypophosphatasia, is yet to receive approval for management of the adult form of the disease. A recent study has evaluated the five-year efficacy and safety of asfotase alfa therapy in adults and adolescents with hypophosphatasia, however, all the recruited patients had pediatric onset of disease and hence cannot be strictly labeled as adult hypophosphatasia (Kishnani et al., 2019). Hence, at present, there exist no guidelines for selecting adult patients for treatment, for evaluating the response to treatment or ascertaining the optimal duration of treatment (Shapiro and Lewiecki, 2017). Of importance, caution should be exercised while prescribing medications commonly used in the treatment of osteoporosis, as they might be harmful in hypophosphatasia. Inadvertent use of anti-resorptive drugs like bisphosphonate and denosumab would lead to suppression of bone turnover and further lowering of serum ALP (Rassie et al., 2019).

In conclusion, we have reported a kindred of adult hypophosphatasia wherein the eldest family member had presented with fragility fracture of the left femur. The biochemical finding of low serum ALP was corroborated by the documentation of a missense mutation in the *ALPL* gene. The same mutation was identified in two of her siblings who were otherwise free from any dento-arthro-osseous complaints. Apart from being a novel mutation, this case report represents the first genetically confirmed family of hypophosphatasia from the Indian subcontinent.

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Transparency document

The Transparency document associated with this article can be found, in online version.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Data availability statement

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Patient consent

Obtained.

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