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CLINICAL REPORT

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Monoallelic *FGFR3* and Biallelic *ALPL* mutations in a Thai girl with hypochondroplasia and hypophosphatasia

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Skeletal dysplasias are a complex group of more than 350 disorders with phenotypic and genotypic heterogeneity affecting bone and cartilage growth. We studied a 2-year-old girl and her 21-year-old mother with disproportionate short stature. In addition to typical features of hypochondroplasia found in both patients, the child had deformities of the extremity bones, metaphyseal flares, and bilateral transverse (Bowdler) fibular spurs with overlying skin dimples detected at birth. Intravenous pamidronate was started in the child since the age of 17 days, and then every two months. Exome sequencing revealed that the girl was heterozygous for a missense mutation (c.1651A>G, p.Ile538Val) in exon 13 of *FGFR3*, a known mutation for hypochondroplasia, inherited from her mother. Interestingly, the child also harbored compound heterozygous missense mutations in exon 12 of *ALPL*, c.1460C>T (p.Ala487Val) inherited from her mother and c.1479C>A (p.Asn493Lys) inherited from her healthy father. The former mutation was previously reported in perinatal hypophosphatasia while the latter was novel. Constantly reduced serum alkaline phosphatase levels including the one before the pamidronate administration and a substantially elevated level of plasma pyridoxal 5′-phosphate detected at age 28 months supported the diagnosis of hypophosphatasia. After a definite diagnosis was achieved, pamidronate was withdrawn at the age of 28 months. No adverse events were observed during pamidronate therapy. In conclusion, we describe a unique case with monoallelic *FGFR3* and biallelic *ALPL* mutations leading to features of both hypochondroplasia and hypophosphatasia.

KEYWORDS
dento-osseous, dual diagnosis, exome sequencing, metabolic bone disease, two mendelian diseases

1 | INTRODUCTION

Skeletal dysplasias are a heterogeneous group of more than 350 disorders affecting bone and cartilage growth with vast phenotypic and genotypic variability. Hypochondroplasia (HCH; OMIM #146000) is an autosomal dominant skeletal dysplasia characterized by short stature, increased upper-to-lower segment ratio, disproportionately short arms and legs, short and broad hands and feet, and relative macrocephaly (Bober, Bellus, Nikkel, & Tiller, 1999). Radiographic features include shortening of long bones with mild metaphyseal flaring, decreased interpedicular distances between lumbar vertebrae L1 and L5, short femoral neck, lumbar lordosis, and square ilia (Hall & Spranger, 1979; Bober et al., 1999). HCH is caused by gain-of-function mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene

(Le Merrer et al., 1994). Hypophosphatasia (HPP; OMIM # 46300, 241500, 241510) is a rare autosomal dominant or autosomal recessive dento-osseous metabolic bone disease with a wide-ranging severity spanning from life-threatening extensive bone deformities at birth to dental complications without bone abnormalities presenting at any age (Millan & Whyte, 2016; Whyte, 2016). It is caused by mutations in the alkaline phosphatase (*ALPL*) gene which encodes tissue-nonspecific alkaline phosphatase (TNSALP).

Here, we report a Thai girl with clinical and radiological features of HCH and HPP. Exome sequencing was used to identify a monoallelic mutation in *FGFR3* and biallelic mutations in *ALPL*. To our knowledge, this is the first reported case of an individual with both HCH and HPP.

2 | MATERIALS AND METHODS

The study was exempted by the institutional review board (IRB) of Faculty of Medicine, Chulalongkorn University, Thailand. After informed consent, blood samples from the proband and her parents were collected. The proband's genomic DNA was extracted from peripheral blood leukocytes and sent to Macrogen, Inc. (Seoul, Korea). The DNA sample was prepared as an Illumina sequencing library. The sequencing libraries were enriched by SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA) and sequenced onto Hiseq 4000 (Illumina, San Diego, CA). The raw data per exome were mapped to the human reference genome hg19 using Burrows–Wheeler Alignment software (bio-bwa.sourceforge.net/). Variant calling was performed using GATK with HaplotypeCaller. Finally, SNVs and Indels were annotated by using SnpEff and annotation databases, dbpSNP142, 1000 Genome, ClinVar, and ESP. All SNVs and Indels were filtered by genes causing skeletal dysplasias. The remaining variants were subsequently filtered out if they were present in our in-house database of 200 unrelated Thai exomes. The most likely pathogenic variants were confirmed by PCR and Sanger sequencing. DNA of her parents was also Sanger sequenced to determine the presence of the mutations.

3 | RESULTS

3.1 | Clinical report

A 9-day-old girl was referred to our hospital for abnormally short upper and lower limbs. The patient was the only daughter of non-consanguineous Thai parents. The parents had no history of bone pain, fracture, or early tooth loss. The mother had short stature and disproportionately short arms and legs. The girl was delivered naturally at 41 weeks of gestation. Her birth weight was 2,260 g (−3 SD); length 40 cm (<−3 SD); head circumference 33 cm (0 SD). Low serum alkaline phosphatase (36 U/L; 110–320 U/L) was detected at 16 days of age. The patient exhibited short upper and lower extremities, relatively long trunk, narrow thorax, bilaterally curved legs, pretibial skin dimples, prominent forehead, and midfacial hypoplasia. Blue sclerae were not present (Figure 1a). Radiographs showed abnormal curvature of long bone extremities, metaphyseal flares, and transverse (Bowdler) bone spurs at the midshafts of fibulae underlying a skin dimple (Figure 1b,c). Physical data, and values of bone scan and serum biochemistry are shown in Supplementary Table S1. Because of several deformities of long bones, the patient was given intravenous pamidronate at a dose of 7.2 mg/kg/year, started at the age of 17 days, and given regularly every 2 months. At age 1 year, the radiographs demonstrated bilateral bending of humeri, radii, and ulnae with mild metaphyseal flaring (Figure 1d,e). AP pelvis revealed squared ilia and short femoral neck (Figure 1f). Lower extremities showed bilateral bowing and sclerotic diaphyses of femora, tibiae, and fibulae (Figure 1f–h). The zebra stripe sign of pamidronate therapy was noted at the iliac crests and metaphyses of long bones (Figure 1d–h). The skull was normal. No wormian bones and heterotopic ossification were visible (Figure 1i,j). The left mandibular canine was absent (Figure 1j,k). At 28 months, she developed hyperlordosis and abnormal gait without any complaints of bone or joint pain (Figure 2a). Chest radiographs revealed an enlarged anterior portion of the ribs (Figure 2b). The alignment of thoracolumbar spine, pedicles, and the interpedicular distance were within normal limits (Figure 2c). Radiographs of long bones revealed "tongues" of radiolucency projecting from growth plates into metaphyses (Figure 2d–h). Short and broad femoral necks and square ilia were present (Figure 2g). Radiographs of knees and wrist revealed mild fraying and cupping of metaphyses and irregularity of the growth plates (Figure 2h,i). Skull radiographs revealed widening of sagittal and coronal sutures with the presence of wormian bones, and reduced alveolar bone (Figure 2j,k). Her primary mandibular right central incisor was exfoliated with intact root at age 26 months (Figure 2l). Spine radiographs showed loss of the normal widening of the interpedicular distance proceeding down the lumbar spine from L1 to L5 (Figure 2m).

3.2 | Variant identification and bioinformatics analysis

Exome sequencing of the proband revealed a heterozygous missense mutation c.1612A>G (p.Ile538Val) in exon 12 of the *FGFR3* gene (NM_000142.4). Sanger sequencing showed that her mother also harbored the mutation, while her father had only the wild-type allele. In addition, compound heterozygous missense mutations in the *ALPL* gene (NM_000478.5), c.1460C>T (p.Ala487Val), and c.1479C>A (p.Asn493Lys) were observed. Sanger sequencing showed that her mother was heterozygous for the c.1460C>T (p.Ala487Val) while her father was heterozygous for the c.1479C>A (p.Asn493Lys). Both altered amino acid positions are highly conserved among species. The p.Asn493Lys mutation has never been previously reported in patients with HPP. It was found in one of 121,132 alleles in the ExAC database (Lek et al., 2016) and predicted to be deleterious by PolyPhen-2 (Adzhubei et al., 2010) (Supplementary Figure S1).

4 | DISCUSSION

We report a girl having combined physical and radiographic features of HCH and HPP. Genetic testing confirmed the diagnosis of HCH and autosomal recessive HPP. The known mutation p.Ile538Val found in

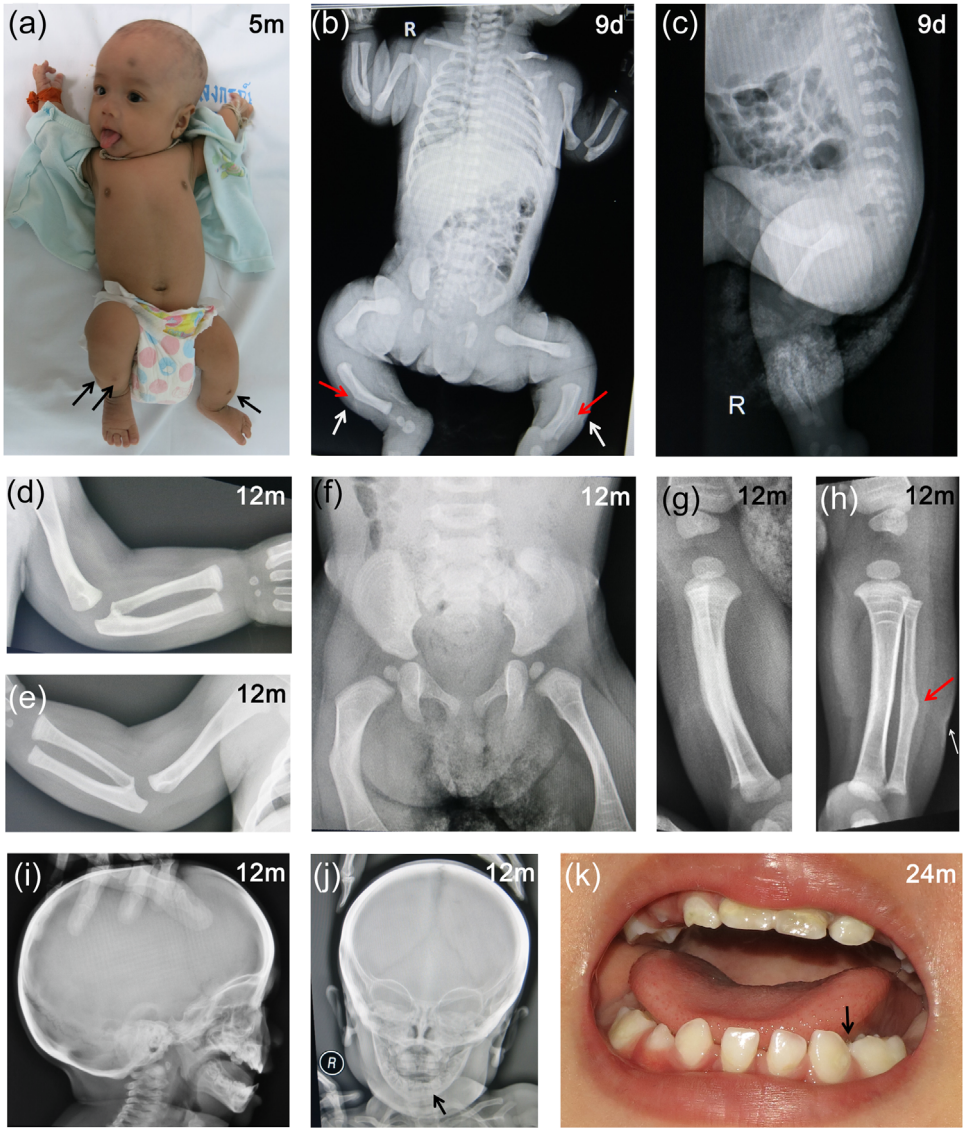

FIGURE 1 Clinical and radiographic findings of the proband. Photographs taken at 5 months of age showed relatively long trunk, short limbs, and pretibial skin dimples on both sides of legs (a, arrows). The radiographs at age 9 days showed bending of bilateral femora, tibiae, fibulae, humeri, radii, and ulnae, metaphyseal flaring, and Bowdler spurs at midshafts of the fibulae (red arrows) underneath skin dimples (white arrows) (b,c). Radiographs at age 1 year exhibited bending of bilateral humeri, radii, and ulnae with mild metaphyseal flaring (d,e), squared ilia, short femoral necks, bowing and sclerotic diaphyses of bilateral femora, tibiae, and fibulae, and metaphyseal cupping, fraying, and widening of metaphyses (f–h). Bowdler spur (red arrow) and skin dimple (white arrow) were present (h). The zebra stripe sign of pamidronate therapy was demonstrated at the iliac crests and metaphyses of long bones (d–h). No abnormalities of bone ossification were observed on skull radiographs (i,j). The mandibular left canine was absent (j,k, arrows). [Color figure can be viewed at wileyonlinelibrary.com]

our patient is located in the highly conserved tyrosine kinase domain of FGFR3 (Supplementary Figure S1). This mutation has been previously reported in a Swedish family with typical HCH (Grigelioniene et al., 1998). The p.Ile538Val mutation in the *FGFR3* gene was expected to cause weak FGFR3 activation and aberrant RAS/ERK pathway (Krejci, 2014; Ornitz & Legeai-Mallet, 2017). These could lead to the partial inhibition of proliferation and differentiation of the chondrocytes in the growth plate cartilage, reduced matrix production, and disrupted endochondral ossification processes affecting the growth of long bones (Krejci, 2014; Ornitz & Legeai-Mallet, 2017). The culture of *Fgfr3* (G369C/+) cells (mimicking human ACH) showed the up-regulation of ALP activity, expression of osteoblast marker genes, and reduced bone matrix mineralization (Su et al., 2010). It is therefore possible that the reduced FGFR3 concentration might affect the activity of ALPL and vice versa, but their interactions have not yet been clarified. Rachitic abnormalities of the growth plate were observed in HPP (Whyte, 2016) whereas reduced chondrocyte proliferation, decreased numbers of hypertrophic chondrocytes, and decreased height of the hypertrophic zone of the growth plate were shown in the HCH growth plate (Lui, Nilsson, & Baron, 2014). These demonstrate that both FGFR3 and

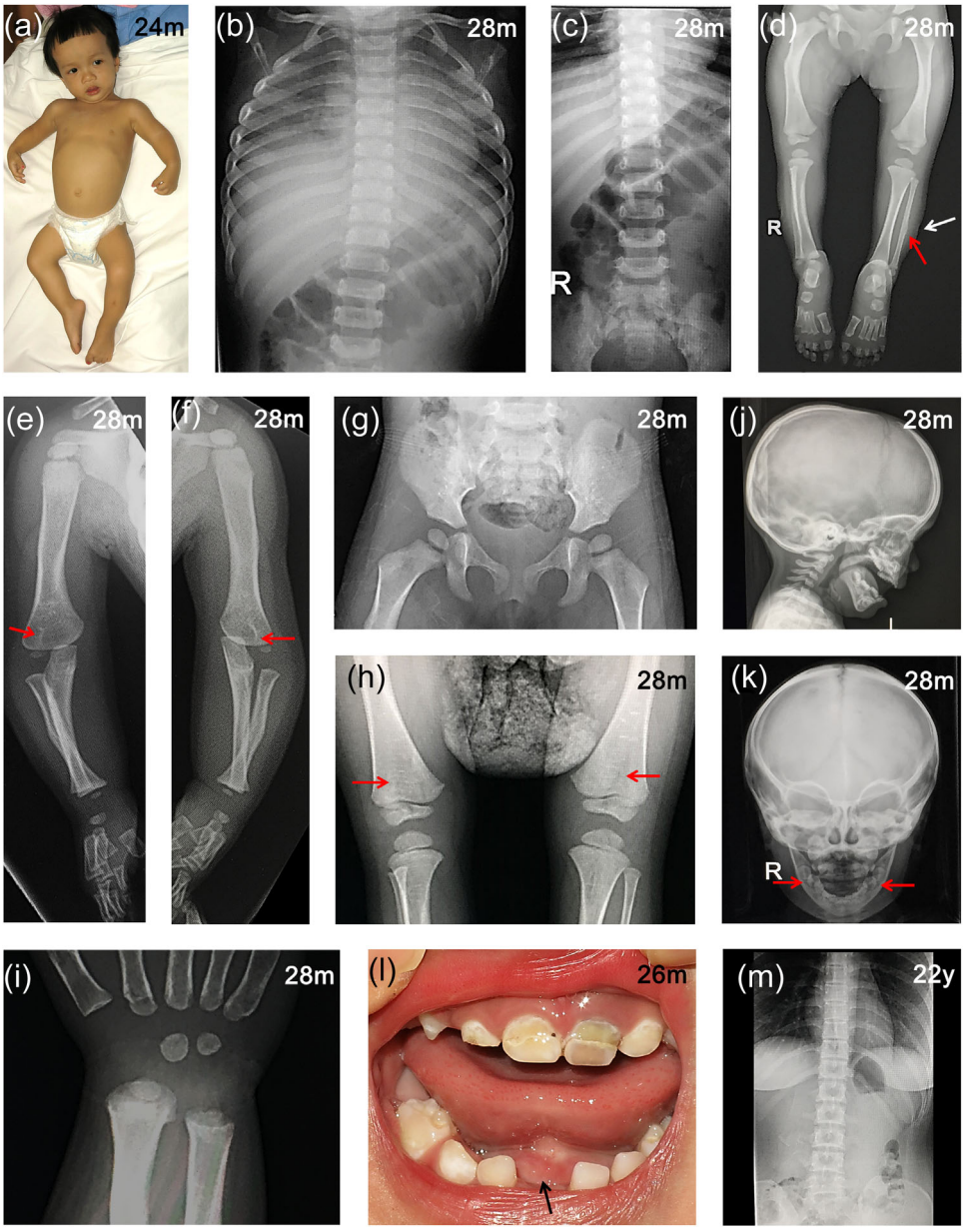

FIGURE 2 Clinical and radiographic findings of the proband and the mother. Photographs taken at age 2 years exhibited midfacial hypoplasia, prominent forehead, pretibial skin dimples, small stature, and relative shortening and abnormal curvatures of upper and lower extremities (a). An enlarged anterior portion of the ribs was observed on the chest radiograph (b). Spine radiograph revealed normal alignment of the thoracolumbar spine, pedicles, and the interpedicular distance (c). Bowing of long bones (d–f), a fibular spur (red arrow), and a dimpling of the overlying soft tissue (white arrow) were observed (d). The tongue of lucency projecting from growth plates into metaphyses was detected at the elbows and knees (e,f,h). Pelvic radiograph illustrated short and broad femoral necks and square ilia (g). The zebra stripe sign of pamidronate therapy was demonstrated at the iliac crests and metaphyses of long bones (e–h). Radiographs of the knees and wrist showed mild fraying and cupping of metaphyses and irregularity of the growth plates (h,i). Skull radiograph showed widening of the sagittal and coronal sutures with the presence of wormian bones, and alveolar bone loss (j,k). Oral photo at 26 months showed generalized enamel hypoplasia, multiple caries, absence of mandibular left canine, and early exfoliation of the primary mandibular right central incisor (l, arrow). Loss of the normal widening of inferior lumbar interpedicular distance was present in the mother (m). [Color figure can be viewed at wileyonlinelibrary.com]

ALPL are important for cartilage and bone development in different, possibly overlapping, aspects.

The *ALPL* amino acid change p.Ala487Val in combination with p.Phe328del has been previously reported in a fetus diagnosed with severe perinatal HPP and subsequent pregnancy interruption (Taillandier et al., 2015). The mutation p.Phe328del might lead to more severe phenotypes compared to our proband, as seen in this fetus. The *ALPL* amino acid change p.Asn493Lys is located in the

disulfide bonds of ALPL which are important in maintaining the structure and bioactivity of the protein including substrate hydrolysis, proteolysis, and oligomer formation (Hogg, 2003). This second mutation has never been reported in HPP. The parents who were heterozygous for each mutation in *ALPL* had normal levels of PLP and ALP without any bone and dental problems. The phenotype and the mutational status indicate autosomal recessive HPP in our patient. The prenatal bowing of long bones in the proband suggests a severe bone dysplasia, but long bone angulation and fibular spurs have shown favorable postnatal improvement. These might imply that the proband had a benign form of prenatal HPP (Matsushita, Kitoh, Michigami, Tachikawa, & Ishiguro, 2014; Whyte, 2016).

The proband was given pamidronate starting at a very young age. It acts against osteoclast-mediated bone loss and slows skeletal remodeling which could reduce ALP activity. This might potentially aggravate the severity of the underlying HPP. Interestingly, the bone phenotype at 1 and 2 years of age was milder with reasonably shaped metaphyses and relatively less changes in the mineralization. Taken together, besides the possibility of the proband having a benign form of prenatal HPP, this could be a result of HCH that somewhat ameliorates the HPP phenotype.

A modified human TNSALP, asfotase alfa (Strensiq™; Alexion Pharmaceuticals, Inc., New Haven, CT) was the first approved enzyme replacement therapy for pediatric-onset HPP (Whyte et al., 2012). The therapy has been shown to improve bone mineralization as well as survival, and has substantial and sustained efficacy with a good safety profile for juvenile-onset HPP (Scott, 2016; Whyte, 2016). However, alfotase alfa has not been available in Thailand. A concern of bisphosphonate treatment in HPP patients has been raised based on its structure which is similar to inorganic pyrophosphate, the natural substrate of TNSALP (Sutton, Mumm, Coburn, Ericson, & Whyte, 2012). A long-term exposure to bisphosphonates has been linked with atypical subtrochanteric femoral fractures in adult HPP (Sutton et al., 2012). However, no adverse reactions have been reported in children with HPP. Cyclic administration of pamidronate has been started in our patient since the neonatal period before a definite diagnosis could be achieved. During 28 months of regular treatment, neither fractures nor adverse events were noticed. After the diagnosis of HCH and HPP was definite, the parents were informed. They decided to discontinue pamidronate treatment for their child.

In conclusion, we describe the patient who has both HCH and HPP. Our patient harbors mutations in both *FGFR3* and *ALPL* genes. We demonstrate that exome sequencing is a useful diagnostic tool to identify causative mutations in combined disorders including skeletal dysplasias and metabolic bone disorders.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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