RESEARCH LETTER

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Distinctive Skeletal Phenotype in High Bone Mass Osteogenesis Imperfecta Due to a *COL1A2* Cleavage Site Mutation

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TO THE EDITOR:

Over 15 years ago, we encountered a family (six affected individuals) with a fragile bone disease inherited as an autosomal dominant trait. We reported the family as having a "new brittle bone disorder" in AJMG [Nishimura et al., 1999]. At that time, we presumed that the disorder differed from osteogenesis imperfecta (OI), because the clinical hallmark was bent bones rather than fractures.

We recently performed molecular analyses for the family (the proband, affected mother, and healthy father). After informed consent, the DNAs of the family members were obtained. Whole exome sequencing revealed a novel C-propeptide cleavage site mutation in *COL1A2* (c.3358G>A, p.Asp1120Asn) in the proband and affected mother, which was confirmed by subsequent Sanger sequencing (Fig. 1). We report here on the recent skeletal phenotype of the proband, and also discuss the radiological characteristics of patients with mutations that impair C-propeptide cleavage of type 1 procollagen.

As we previously reported, the phenotypic variations in this family were remarkable. Affected family members had varying degrees of bone fragility and abnormal bone mineralization. The proband was a boy who was most severely affected in this family. He presented with progressive symmetric bowing of the forearms and legs despite only a few episodes of fractures. The distal femora and distal ulnae showed sharp angular deformities associated

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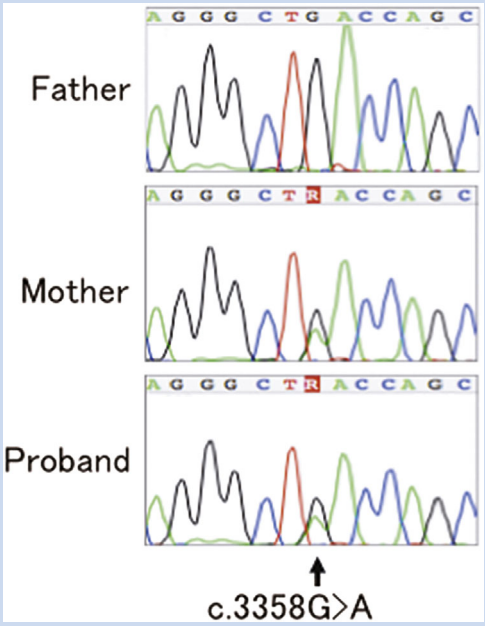

FIG. 1. Sanger sequencing for the novel C-propeptide cleavage site mutation of *COL1A2* (c.3358G>A) in the family. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

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with pseudo-fractures, the proximal fibulae were attenuated, and the right fibula showed a pseudo-fracture (Fig. 1A and B). The distal ulnae showed progressive shortening and constriction with increasing age. The bone trabecular pattern appeared coarse. He underwent corrective surgery for his leg and forearm deformities on a few occasions. His male cousin also had symmetric bowing of the forearms. Other affected individuals had very few or no fractures; yet, they all showed a coarse bone trabecular pattern.

At the last visit, the proband was 28 years old. No fracture occurred after adolescence. He never received bisphosphonate therapy. Height was 149.3 cm (−3.7 SD) and weight 57.9 kg (−0.5 SD). He presented with genu valgus deformities with patellar dislocation. The knee joints were sometimes painful, and knee flexion was mildly restricted. Forearm movement was mildly restricted as well. Radiological examination at age 19 years and 27 years displayed severe valgus deformities of the knee and irregular contours of the femora, tibiae and fibulae. The radii and ulnae were bowed, the proximal radii were dislocated, the distal ulnae were attenuated, and the proximal ulnae were mildly thickened. The radioulnar interosseous membranes exhibited mild ossification (Fig. 2). Radiographically the bone appeared dense, but unfortunately skeletal densitometry was not performed.

Recent investigations on OI have demonstrated that monoallelic C-propeptide cleavage site mutations in *COL1A1* and *COL1A2* and bi-allelic mutations in *BMP1* (the gene encoding a C-propetide cleavage enzyme) constitute a distinctive subgroup of OI. These mutations interfere with the removal of the global C-terminal propeptide from the type 1 pro-collagen chain in the endoplasmic reticulum, and cause incorporation of uncleaved C-propeptides into the extracellular matrices. The morphological consequences include increased osteoid and high bone mineral density; thus the phenotype is termed "high bone mass OI" [Lindahl et al., 2011; Asharani et al., 2012; Hoyer-Kuhn et al., 2013]. The pathogenesis of the increased osteoid and increased mineralization remains elusive; however, it is assumed that the former is attributed to delayed maturation of osteoid, while the latter is due to abundant mineralization in mature osteoid [Hoyer-Kuhn et al., 2013]. Osteoclastic activities are increased as well [Hoyer-Kuhn et al., 2013; Asharani et al., 2012]. Similarly, double knock-out mice of *Bmp1* and *Tll1* (another propeptide cleaving enzyme) show not only bone fragility but also histological evidence increased osteoid seams [Muir et al., 2014].

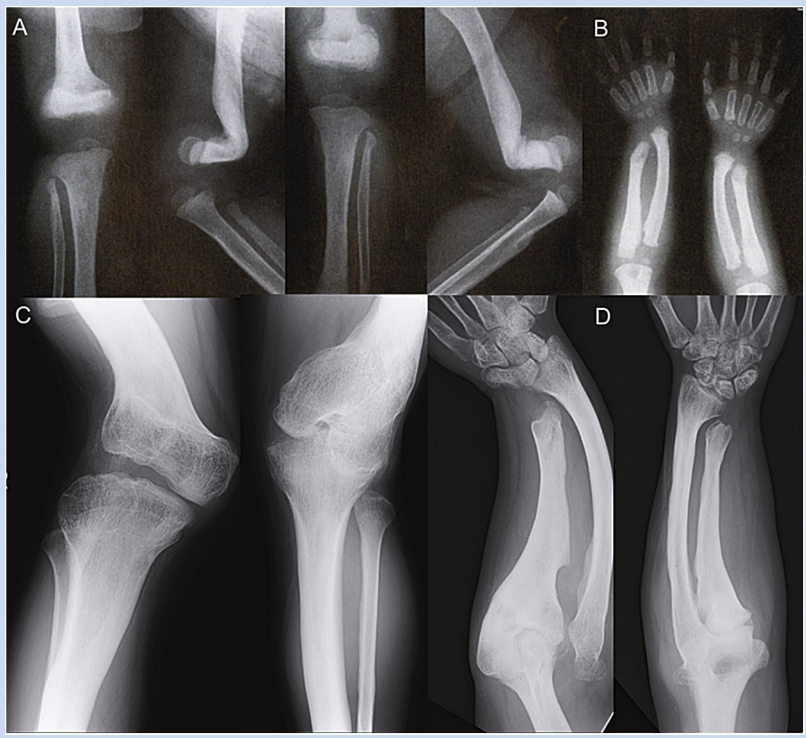

FIG. 2. A: Radiographs of the knees at age 22 months: the distal femora show sharp posterior angulation. The proximal fibulae are constricted. A pseudo-fracture is noted in the right proximal fibula. B: Radiographs of the forearms at age 3 3/12 years: The radii and ulnae are mildly bowed. The distal ulnae are constricted along with pseudo-fractures. C: Radiographs of the knees at age 19 years: Valgus deformity is remarkable. Irregular contours are seen in the left femur and right tibia. D: Radiographs of the forearms at age 27 years: The radii and ulnae are bowed, and the proximal radii are dislocated. The distal ulnae are narrow, while the proximal ulnae appear mildly thick. Mild ossification is seen in the radioulnar interosseous membranes. A and B are reproduced from AJMG 84:320–329 (1999) by permission of the publisher.

In the high bone mass OI, osteosclerosis is progressive with age, and may not be easily identifiable in younger patients. In the present patient, osteosclerosis did not initially come to our attention. In retrospect, however, sclerotic changes were discernible in the long bones, particularly the femora, radii and ulnae. His recent radiographs demonstrated more clearly the presence of osteosclerosis in the long bones (Fig. 2). His other skeletal hallmarks, including coarse trabeculae, pseudo-fractures, and metaphyseal constriction are quite similar to those of patients with *BMP1* mutations [Asharani et al., 2012; Cho et al., 2015]. Thus, these skeletal changes are likely to be an essential component in the high bone mass OI, which are probably related to hyperosteoidosis (increased osteoid) and/or increased osteoclastic activity.

From the clinical viewpoint, the high bone mass OI belongs to a benign form of OI spectrum [Lindahl et al., 2011; Asharani et al., 2012; Hoyer-Kuhn et al., 2013]. Fractures are not so frequent, deformities are not severely progressive, blue sclera and dentinogenesis imperfecta are absent, and short stature absent or mild, as was seen in the present patient. The benign clinical course is quite different from severe or lethal outcome in the other subset of dense bone OI due to C-propeptide mutations at the non-cleavage site [Pace et al., 2002; Takagi et al., 2011].

In conclusion, a phenotypically distinctive group of OI is caused by a mono-allelic *COL1* C-propeptide cleavage site mutations and biallelic *BMP1* mutations. The subgroup is characterized by high bone mass and other unique skeletal changes, such as coarse trabeculae, pseudo-fractures, and metaphyseal constriction.

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Case Report

A Case of Thanatophoric Dysplasia Type I with Fetal Hydrops in the First Trimester

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During a routine prenatal exam, a 36-year-old female in her third pregnancy was diagnosed with fetal hydrops at 11 weeks of gestation. The pregnancy was monitored with periodic ultrasounds; however, spontaneous resolution was not observed. Amniotic fluid examination at 16 weeks of gestation showed a normal karyotype; however, macrocephaly, a narrow thorax, and shortening of the long bones were observed on ultrasonography. With the strong suspicion of a fetal skeletal disease, specifically thanatophoric dysplasia (TD), and after extensive genetic counseling, termination of the pregnancy was performed per the parents' wishes with mechanical cervical dilation and gemeprost (PGE1) administration. Following delivery, the fetus was found to have macrocephaly, a narrow bell-shaped thorax, and a protuberant abdomen, as well as curved long bones, H-shaped platyspondyly, and curved clavicles on skeletal radiography. As a result, the fetus was diagnosed with TD type I. This case illustrates that although TD is a rare disease, an accurate prenatal diagnosis can be made with the use of ultrasonography.

1. Introduction

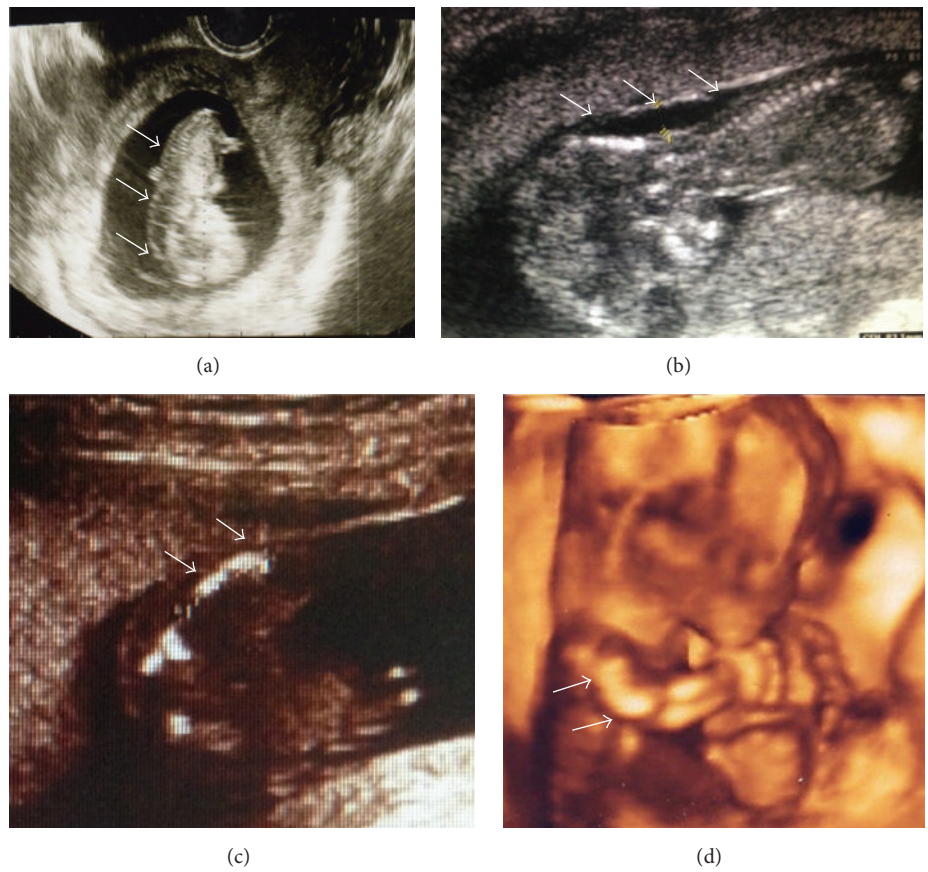
Thanatophoric dysplasia (TD) is a rare and lethal skeletal dysplasia with an estimated incidence of 1 in 20000 to 40000 births [1] and was first described by Maroteaux et al. in 1967 [2]. TD can be classified into two types: type I is characterized by micromelia with bowed femurs and, uncommonly, the presence of cloverleaf skull deformity; type II is characterized by micromelia with straight femurs and moderate to severe cloverleaf skull deformity [3]. A common feature to both types is the presence of a narrow thorax which causes respiratory failure shortly after birth [4, 5].

Fetal hydrops is defined as an abnormal fluid collection in two or more areas of the fetal body. It can be further classified as immune or nonimmune. Immune fetal hydrops develops due to fetal hemolysis secondary to incompatibility of maternal and fetal blood type. Nonimmune hydrops, on the other hand, can result from a large number of causes [6, 7]. However, 20–30% of hydrops is of unknown etiology.

Here, we report a case of TD type I with fetal hydrops diagnosed in the first trimester and thereafter shortening of the long bones and macrocephaly. Confirmation of the diagnosis was made by clinical examination and radiologic studies after delivery.

2. Case Presentation

A 36-year-old female in her third pregnancy came to our hospital at 8 weeks of gestation for a routine prenatal check. The couple and their previous children's past and family histories were unremarkable. Ultrasonographic examination at 8 weeks of gestation was unremarkable, with a crown-rump length (CRL) of 15.9 mm. At 11 weeks of gestation, although the CRL was 49.6 mm corresponding to gestational age, fetal hydrops was evident on ultrasonography (Figure 1(a)). Since screening for immune hydrops and congenital infections were negative, a transvaginal ultrasound was performed the following week for further evaluation. At 12 weeks of gestation, appropriate fetal growth was observed with a CRL of 60 mm; however, fetal hydrops was still present. The couple was counseled on the different etiologies of fetal hydrops; however, at this point the cause was not clear.



(a) (b) (c) (d)

Figure 1: Fetal ultrasound with skin edema in the posterior part of the head and vertebral column (→) (a). A longitudinal view of the head and the thorax demonstrating skin edema in the posterior part of the neck (→), macrocephaly, and narrow thorax (b). Fetal ultrasound showing short curved femur (→) (c). A three-dimensional image with a view of the upper limbs showing bowed humerus (→) (d).

At 13 weeks of gestation the biparietal diameter (BPD) was measured to be 27.4 mm, corresponding to the 95th percentile. From this week on macrocephaly was observed and a narrow thorax was suspected on subsequent ultrasonographies (Figure 1(b)). At 16 weeks of gestation, amniotic fluid examination showed a normal karyotype (46XX); however, a routine and a four-dimensional ultrasound revealed shortening and bowing of the long bones (femur length (FL) 11.6 mm) (Figures 1(c) and 1(d)) with no subsequent improvement. As a result, a fetal skeletal disease, specifically TD, was strongly suspected. At 20 weeks of gestation, the FL was 1.18 cm, compatible with less than the 5th percentile. Also, the BPD was 5.3 cm, corresponding to more than the 95th percentile. During genetic counseling, a molecular analysis was suggested to the parents in order to make an accurate prenatal diagnosis of TD; however, they opted not to pursue this exam and decided on termination of the pregnancy, instead. The patient was then hospitalized and underwent mechanical cervical dilation. The following day gemeprost (PGE1) was administered intravaginally every three hours. A 400 g female fetus was delivered dead at 20 weeks and 2 days of gestation (Figure 2). All limbs were noted to be extremely short with redundant skin folds. Macrocephaly was evident. A narrow bell-shaped thorax with short ribs and a protuberant abdomen were noticed; however, the skull and facial characteristics were within normal limits. Skeletal radiography showed telephone receiver-like curved femurs and humeri accompanied by irregular metaphyses, an H-shaped platyspondyly, and curved clavicles (Figure 3). No cloverleaf skull deformity was observed. These characteristics confirmed the diagnosis of TD type I.

3. Discussion

The incidence of immune hydrops has decreased due to routine screening and prophylaxis; however, the mortality rate of nonimmune hydrops, during either the fetal or the neonatal period, is up to 75.5% [8]. Although fetal hydrops is considered to be a nonspecific finding on obstetric ultrasounds, previous reports showed that an increased nuchal translucency (NT) and hydrops are common features of serious skeletal dysplasia [9]. Moreover, previous cases of TD reported a NT of 3.4–6.5 mm by 14 weeks of gestation which correlates with the 5.7 mm observed in this case [9].

As previous studies reported, 40–80% of TD cases can be correctly diagnosed by ultrasonography in the prenatal period [10]. Limb shortening in TD is also sonographically apparent from as early as 13 weeks of gestation. Similarly,

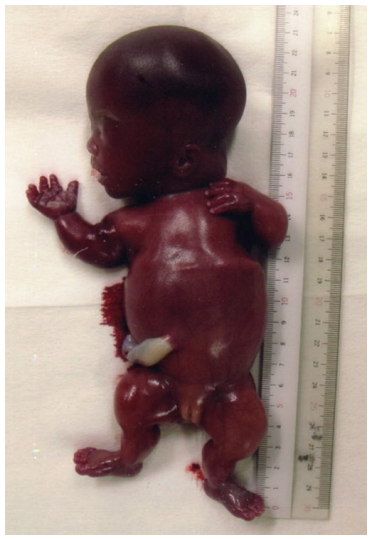


Figure 2: Female fetus of 20 weeks of pregnancy with short limbs and redundant skin folds. Also, macrocephaly, narrow bell-shaped thorax, and protuberant abdomen were noticed. Skull and facial characteristics were normal.

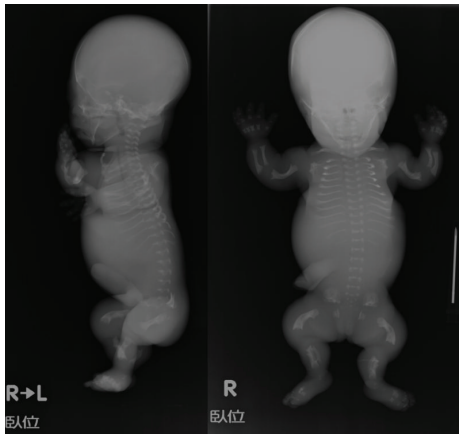


Figure 3: Anteroposterior and lateral radiographs of the fetus showing telephone receiver-like curved femurs and humeri with irregular metaphyses, H-shaped platyspondyly, and curved clavicles. No cloverleaf skull deformity was observed.

head circumference (HC) is increased throughout gestation, a feature that is present as early as the first trimester [10]. With the evidence of short limbs, a hypoplastic thorax, and macrocephaly, we strongly suspected a skeletal disease as TD.

Published reports have used a femur length/abdominal circumference (FL/AC) ratio <0.16 as a predictor for lethal skeletal dysplasia [11]. Also, a hypoplastic thorax is suspected with a thoracic circumference of less than 5% at the level of the four-chamber view of the heart or a thoracic-AC ratio of less than 0.79 [12]. In this case the FL/AC ratio was 0.103 (0.82 cm/7.9 cm) and 0.0769 (1.18 cm/15.34 cm) at 13 and 20 weeks of pregnancy, respectively. A narrow bell-shaped thorax was suspected as early as 13 weeks of pregnancy.

TD is the most frequent lethal skeletal dysplasia caused by mutation of the fibroblast growth factor 3 (FGFR3) gene [13, 14]. Although molecular analysis of fetal cells to detect Arg248Cys, Tyr373Cys, Lys650Glu, and other specific mutations enables an accurate prenatal diagnosis of TD [1], it was not performed in this case per the couple's wishes. However, since limb shortening is associated with a 2.7 relative risk for trisomy 21 [4] and a case of TD type I presented with trisomy 21 was reported previously [15], we considered an amniocentesis at 16 weeks of gestation important to perform. TD is an autosomal dominant genetic disease; however, it is almost always caused by a de novo mutation in FGFR3 [3]. As a result, a general empiric recurrence risk is estimated in only 2% [16]. This fact is important to consider in order to relieve parental anxiety over future pregnancies. After counseling, the patient had a fourth pregnancy without complications and delivered a normal female baby at term.

In summary, even though TD is a rare condition, ultrasonography can be used to obtain an accurate prenatal diagnosis and facilitate early parental counseling.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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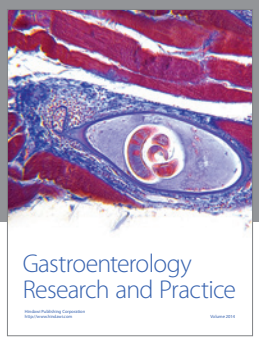

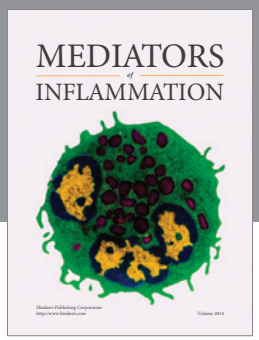

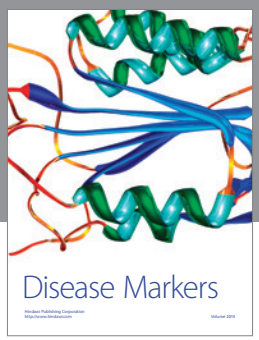

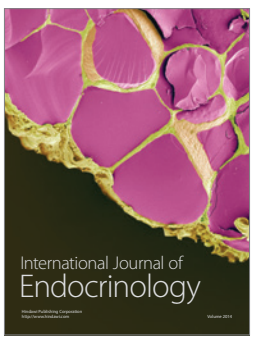

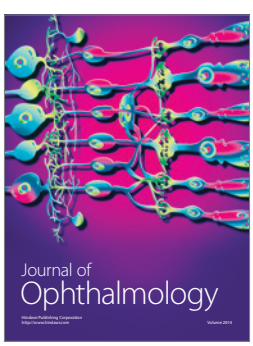

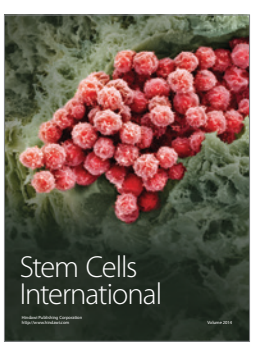

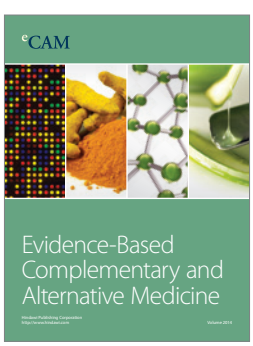

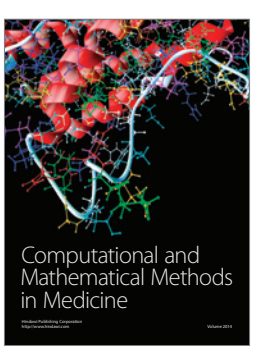

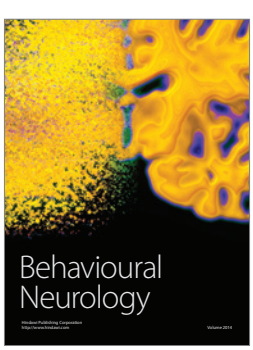

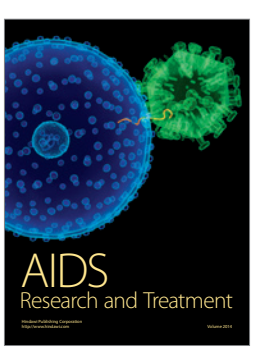

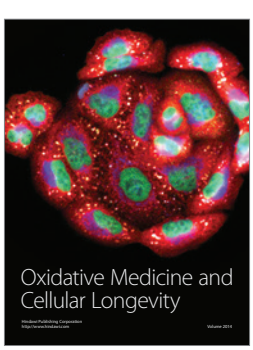

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ORIGINAL ARTICLE

Serum NT-proCNP levels increased after initiation of GH treatment in patients with achondroplasia/hypochondroplasia

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Summary

Objective Serum amino-terminal propeptide of C-type natriuretic peptide (NT-proCNP) levels have been proposed as a biomarker of linear growth in healthy children. The usefulness of NT-proCNP in patients with achondroplasia (ACH)/hypochondroplasia (HCH) remains to be elucidated. The objective was to study whether serum NT-proCNP level is a good biomarker for growth in ACH/HCH and other patients of short stature.

Design This was a longitudinal cohort study.

Patients Sixteen children with ACH (aged 0·4–4·3 years), six children with HCH (2·7–6·3 years), 23 children with idiopathic short stature (ISS) (2·2–9·0 years), eight short children with GH deficiency (GHD) (2·9–6·8 years) and five short children born small for gestational age (SGA) (2·0–6·6 years). Patients with ACH/HCH received GH treatment for 1 year.

Measurements Serum NT-proCNP levels and height were measured.

Results NT-proCNP levels positively correlated with height velocity in these short children ($P < 0{\cdot}05$, $r = 0{\cdot}27$). NT-proCNP levels inversely correlated with age in children with ISS alone ($P < 0{\cdot}01$, $r = -0{\cdot}55$). Serum NT-proCNP levels in patients with ACH/HCH were increased 3 months following the initiation of GH treatment ($P < 0{\cdot}05$). Height SDS gain during GH treatment for 1 year was positively correlated with the changes in NT-proCNP levels after the initiation of GH ($P < 0{\cdot}01$, $r = 0{\cdot}72$).

Conclusion Serum NT-proCNP levels may be a good biomarker to indicate the effect of GH treatment on growth in patients with ACH/HCH at least in the first year and height velocity in short stature patients.

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Introduction

C-type natriuretic peptide (CNP) has been shown to be a critical determinant of body height and length.[1,2] Studies in genetically modified mice have revealed that defects of the genes coding for CNP and natriuretic peptide receptor 2 (Npr2), the cognate receptor for CNP, result in dwarfism[3,4]: both the ligand and its receptor are expressed in the growth plate as well as in other tissues.[5] In humans, loss-of-function mutations in the *NPR2* gene cause an autosomal recessive skeletal dysplasia known as acromesomelic dysplasia, Maroteaux type (AMDM) (OMIM #602875), characterized by severe short stature with disproportionate shortened limbs. Heterozygous loss-of-function mutations in the gene have been recently identified in patients with idiopathic short stature (ISS).[6,7]

In contrast, overproduction of CNP in transgenic mice gives rise to excessive growth[8] and a similar phenotype is observed in patients with the overexpression of CNP due to chromosomal translocations.[9,10] We have established the disease entity of a heterozygous gain-of-function mutation in the *NPR2* gene in a family with tall stature (OMIM #615923), which was replicated in mice with overexpression of the activating mutation in cartilage.[11] Functional analyses demonstrated that the gain-of-function mutation increases maximal enzymatic velocity and blocks desensitization of NPR2.[12] Two additional families with tall stature have been described that harbour gain-of-function mutations in the *NPR2* gene.[13,14] These human and mouse genetic studies have shed light on the role of the CNP and NPR2 signalling pathway in determining growth and height.

Achondroplasia (ACH) (OMIM #100800) is the most common form of short-limb dwarfism in humans, affecting more than 250 000 individuals worldwide.[15] ACH and the milder form, hypochondroplasia (HCH) (OMIM #146000), occur because of constitutively active mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene, which influence the proliferation and differentiation of chondrocytes in growth plates. GH treatment improves height velocity at least during the first year and is approved in Japan for ACH and HCH patients with height standard deviation scores (SDS) below − 3·0 SD.[16–20] However, a biomarker to predict growth stimulated by GH treatment remains to be established.

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The amino-terminal propeptide of CNP (NT-proCNP) is an equimolar product of CNP biosynthesis[21] and is more easily measured in serum because of its longer half-life compared to CNP.[22,23] Serum NT-proCNP levels have been recently reported to correlate with height velocity and change with age in healthy children.[23,24] In addition, serum NT-proCNP levels have been proposed as a novel biomarker of growth during GH treatment in patients with ISS and GH deficiency (GHD),[25,26] although age-associated changes in these levels have not been proven in ISS patients. In the current study, we determined the association of serum NT-proCNP levels with age in ISS patients and the relationship between response to GH treatment and height gain in patients with ACH/HCH.

Subjects and methods

Subjects

This study included 58 prepubertal patients who attended Osaka University Hospital, Osaka, Japan, from January 2009 through December 2013 and who were diagnosed with ACH, HCH, ISS, GH-deficient short stature and short stature born small for gestational age (SGA). Patients treated with GH and other drugs related to growth before and at the first visit were excluded. The diagnostic criteria for ACH included short stature, shortened limbs, abnormal facial features (prominent forehead, nasal root depression, mid-face hypoplasia and mandibular protrusion), an enlarged head size, and trident hands as clinical signs, and thick and short long bones, metaphyseal cupping, bullet-shaped vertebra, fibula that are longer than the tibia, decreased distance of lumbar spine pedicles, a large cranial base yet with small facial bones, a small ischial notch and a small cavity of the lesser pelvis as radiographic signs.[27] An ACH patient with a traumatic acute epidural haematoma was excluded. Patients with HCH were diagnosed based on clinical and radiographic findings similar to but milder than those with ACH and the p.N540K mutation in the *FGFR3* gene on genetic analysis. The diagnostic criteria for GH-deficient short stature were the following: proportional short children with height SDS below −2·0, GH peak values less than 4·29 μg/l in two different provocation tests measured using a chemiluminescent enzyme immunoassay (Beckman Coulter) and no clinical and biochemical evidence of other disorders. The diagnostic criteria for short stature born SGA were the following: both birth length and weight for gestational age less than the 10th percentile, either birth length or weight SDS for gestational age less than −2·0, height SDS less than −2·0 at the age of 2 years and no clinical or biochemical findings of other disorders including GHD. Patients with ISS exhibited a proportional short stature with height SDS below −2·0 and lacked any features and findings of disorders with short stature, including GHD, other endocrine disorders, short stature born SGA, skeletal dysplasias, malformation syndromes and malnutrition.

Sixteen patients with ACH (8 boys, 8 girls) were included in the current study. Gene analysis in 13 of these patients revealed that all had p.G380R mutation in *FGFR3*. Median age and height SDS in patients with ACH was 3·1 years (minimum 0·4, 25th percentile 2·9, 75th percentile 3·4, maximum 4·3) and −5·2 (interquartile range: −5·7 to −4·2), respectively. Six patients with HCH (3 boys, 3 girls) were included: median age 3·2 years (2·7, 2·9, 4·1, 6·3) and height SDS −3·3 (−4·3 to −2·3). GH treatment is approved in Japan for both patients with ACH and HCH over the age of 3 with height SDS below −3·0, at a dose of 0·35 mg/kg/week (50 μg/kg/day). Fifteen of the ACH/HCH patients (11 ACH; 4 HCH) with GH treatment and 1-year follow-up were included where serum NT-proCNP levels were examined after the initiation of GH treatment. Mean height SDS increased significantly from −4·8 (1·0) [mean (SD)] to −4·3 (1·2) after GH treatment for 1 year. The remaining seven patients with ACH/HCH were not included for studies after the initiation of GH treatment due to the following reasons: two patients with ACH and two patients with HCH did not meet the criteria for GH treatment; two patients with ACH moved to a new location and were not followed up; one patient with ACH had GH treatment for a very short time. Twenty-three patients with ISS (14 boys, 9 girls) were included in this study: median age 5·4 years (2·2, 3·7, 6·7, 9·0) and height SDS −2·5 (−2·6 to −2·2). The study also included eight GH-deficient short patients (all boys) [median age 4·5 years (2·9, 4·0, 5·4, 6·8) and height SDS −2·9 (−3·1 to −2·5)] and five short stature born SGA patients (2 boys, 3 girls) [median age 3·0 years (2·0, 2·3, 5·8, 6·6) and height SDS −2·4 (−3·1 to −2·2)].

Measurements

Laboratory measurements included serum levels of alkaline phosphatase (AP) (reference range: 134–359 U/l for adults) and IGF-1 [for reference range see[28]]. Serum NT-proCNP levels were measured using an ELISA method (Biomedica, Austria). Measurement of serum NT-proCNP levels was approved by the Institutional Review Board at Osaka University Hospital, and written informed consent was obtained from parents or guardians of the patients.

Statistical analysis

Data were analysed by Wilcoxon rank–sum test, regression analysis, paired *t*-test or Smirnov–Grubbs' test using JMP (SAS Institute, Cary, NC, USA) and R (GNU General Public License, Free Software Foundation, Inc., Boston, MA, USA) statistical software.

Results

Serum NT-proCNP levels in patients with ACH/HCH, ISS, GHD and short stature born SGA

Serum NT-proCNP levels were positively correlated with height velocity in prepubertal patients with short stature (Fig. 1a), implying that the serum NT-proCNP level can be a biomarker of growth in short children independently of their pathogenesis. As the numbers of patients with GHD and short stature born SGA were small, we subsequently focused on patients with

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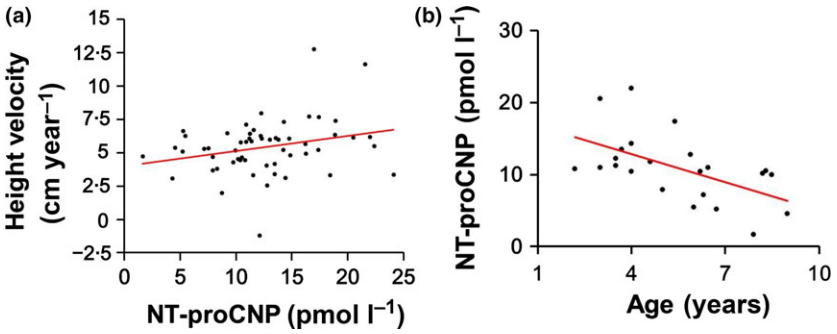

Fig. 1 Serum NT-proCNP levels in patients with short stature. (a) Height velocity positively correlates with serum NT-proCNP levels in prepubertal short children with ACH, HCH, ISS, GHD and SGA ($P < 0{\cdot}05$, $r = 0{\cdot}27$, $n = 58$). (b) Serum NT-proCNP levels were negatively correlated with age in ISS children ($P < 0{\cdot}01$, $r = -0{\cdot}55$, $n = 23$).

ACH/HCH and ISS. On division of ISS patients into two subgroups by the median age of the group, serum NT-proCNP levels were higher in those <5·4 years of age (median [interquartile range]: 12·0 [10·8–16·6]) compared to those >5·4 years of age (10·0 [5·2–10·5]). Consistently, serum NT-proCNP levels were negatively correlated with age in ISS children (Fig. 1b). Unlike in ISS patients, there was no obvious correlation between serum NT-proCNP levels and age in patients with ACH/HCH, probably due to small distribution of age (data not shown). In addition, serum NT-proCNP levels in patients with ACH/HCH ($n = 15$; median [interquartile range], 12·8 [9·2–16·3]) and ACH alone (11; 12·8 [9·2–16·3]) were similar to those in patients with ISS (5; 12·2 [11·1–17·0]) at 3 years of age, implying that CNP production is comparable between patients with ACH/HCH and ISS, at least at that age, even though there was a difference in height SDS between ACH/HCH or ACH alone and ISS.

Association of serum NT-proCNP levels with growth during GH treatment in patients with ACH/HCH

Serum NT-proCNP levels were assessed in patients with ACH/HCH before and after the initiation of GH treatment to determine the effect of GH on serum NT-proCNP levels. Serum NT-proCNP levels were increased in patients with ACH/HCH 3 months following the initiation of GH, which is consistent with increments in serum IGF-1 SDS and AP levels induced by GH (Fig. 2a–c). To test whether serum NT-proCNP levels can be a good biomarker of the effect of GH treatment on growth in patients with ACH/HCH, we examined the association of an increase in serum NT-proCNP levels following the initiation of GH treatment with height gain after 1 year of treatment. When patients with ACH/HCH were divided into subgroups with respect to their increase in serum NT-proCNP levels 3 months after the initiation of GH (lower and upper subgroups), the increase in height SDS was larger after 12 months' treatment in the subgroup with the higher increase in serum NT-proCNP levels (Figure 3a). In addition, the change in height SDS 1 year after GH treatment was positively correlated with fold change in NT-proCNP 3 months after the treatment (Fig. 3b). The association of NT-proCNP levels with height gain may differ between patients with ACH and HCH, as growth response to GH treatment in patients with HCH has been reported to be larger than that in patients with ACH.[29] We therefore examined this association in patients with ACH alone. The results in these patients were similar to those in patients with ACH/HCH; that is, the increase in serum NT-proCNP levels was positively correlated with height gain following the initiation of GH treatment (Fig. 4a, b). On the other hand, the association of height gain with an increment in serum IGF-1 SDS or AP levels following the initiation of GH treatment was not observed in patients with ACH/HCH (Figure 3c–f) or ACH alone (Fig. 4c–f), although these markers increased after GH treatment.

Discussion

Our study confirms that serum NT-proCNP levels are positively correlated with height velocity in children with short stature. In

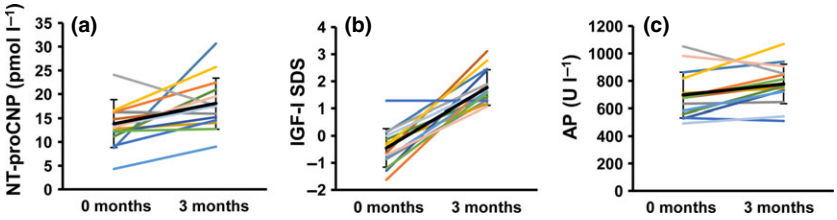

Fig. 2 Increase in serum NT-proCNP levels, IGF-1 SDS and AP levels of patients with ACH/HCH following the initiation of GH treatment. (a) NT-proCNP ($P < 0{\cdot}05$), (b) IGF-1 SDS ($P < 0{\cdot}01$), (c) and AP ($P < 0{\cdot}05$) levels were increased in patients with ACH ($n = 11$)/HCH ($n = 4$) 3 months after the initiation of GH treatment compared to prior to treatment. Bold lines show mean ± SD. ACH, achondroplasia; AP, alkaline phosphatase; HCH, hypochondroplasia; mo, month; NT-proCNP, amino-terminal propeptide of C-type natriuretic peptide; SDS, standard deviation score.

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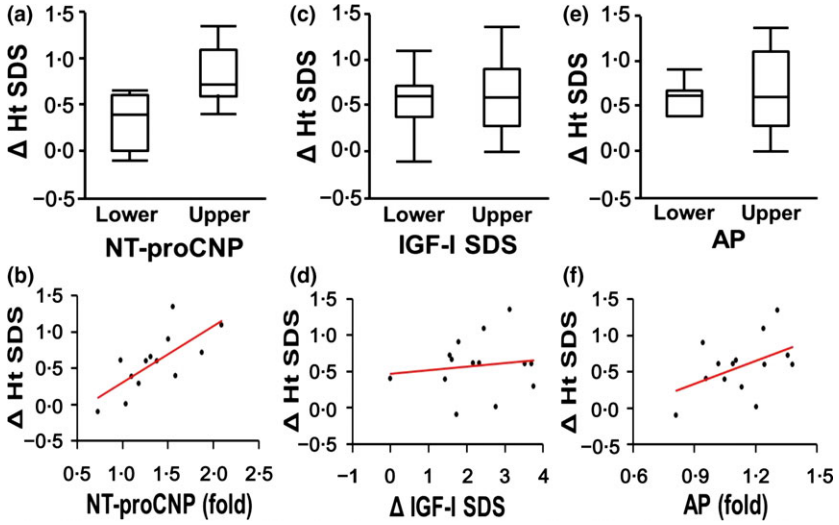

Fig. 3 Association of Δ Ht SDS with serum NT-proCNP level, IGF-1 SDS and AP level following the initiation of GH treatment in patients with ACH (n = 10)/HCH (n = 4). Upper panels show box plots and lower panels correlation. (a) When patients were divided into subgroups [lower (n = 7) and upper (n = 7)] according to a 35% cut-off increase in NT-proCNP level 3 months after starting GH treatment, Δ Ht SDS was significantly (P < 0·05) higher 1 year after GH treatment in the upper compared to the lower subgroup. (b) Δ Ht SDS 1 year after GH treatment was positively correlated with fold change of serum NT-proCNP 3 months after GH treatment (P < 0·01, r = 0·72, n = 13). (c) When patients were divided into subgroups [lower (n = 7) and upper (n = 7)] according to a 1·7 cut-off increase in IGF-1 SDS 3 months after starting GH treatment, Δ Ht SDS was comparable in the subgroups. (d) Δ Ht SDS 1 year after GH treatment was not correlated with Δ serum IGF-I SDS 3 months after GH treatment. (e) When patients were divided into subgroups [lower (n = 7) and upper (n = 7)] according to a 10% cut-off increase in serum AP 3 months after starting GH treatment, Δ Ht SDS was comparable in the subgroups. (f) Δ Ht SDS 1 year after GH treatment was not significantly correlated with fold change of serum AP 3 months after GH treatment. ACH, achondroplasia; AP, alkaline phosphatase; HCH, hypochondroplasia; NT-proCNP, amino-terminal propeptide of C-type natriuretic peptide; SDS, standard deviation score.

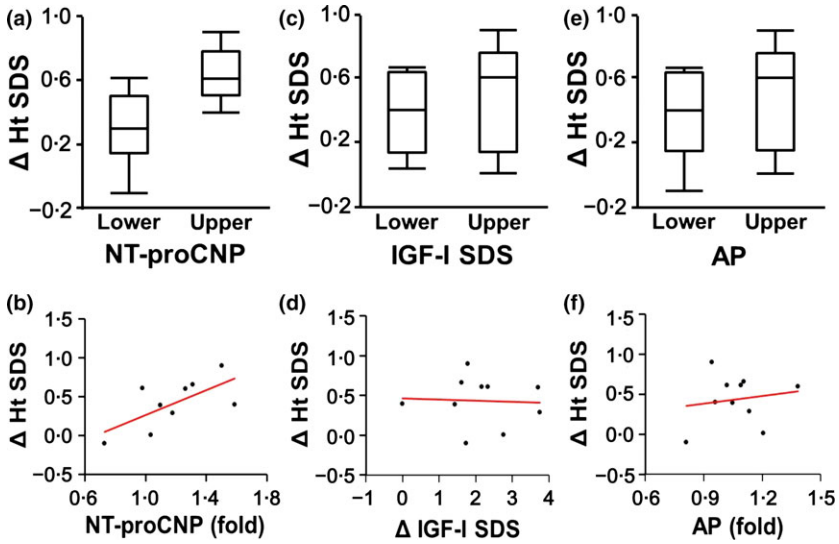

Fig. 4 Association of Δ Ht SDS with serum NT-proCNP level, IGF-1 SDS and AP level following the initiation of GH treatment in patients with ACH (n = 10). Upper panels show box plots and lower panels correlation. (a) When patients were divided into subgroups [lower (n = 5) and upper (n = 5)] according to a 20% cut-off increase in NT-proCNP level 3 months after starting GH treatment, Δ Ht SDS was significantly(P < 0·05) higher 1 year after GH treatment in the upper compared to the lower subgroup. (b) Δ Ht SDS 1 year after GH treatment was positively correlated with fold change of serum NT-proCNP 3 months after GH treatment (P < 0·05, r = 0·67, n = 10). (c) When patients were divided into subgroups [lower (n = 5) and upper (n = 5)] according to a 2·2 cut-off increase in IGF-1 SDS 3 months after starting GH treatment, Δ Ht SDS was comparable in the subgroups. (d) Δ Ht SDS 1 year after GH treatment was not correlated with Δ serum IGF-I SDS 3 months after GH treatment. (e) When patients were divided into subgroups [lower (n = 5) and upper (n = 5)] according to a 7% cut-off increase in serum AP 3 months after starting GH treatment, Δ Ht SDS was comparable in the subgroups. (f) Δ Ht SDS 1 year after GH treatment was not significantly correlated with fold change of serum AP 3 months after GH treatment. ACH, achondroplasia; AP, alkaline phosphatase; HCH, hypochondroplasia; NT-proCNP, amino-terminal propeptide of C-type natriuretic peptide; SDS, standard deviation score.

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addition, we showed the increase in NT-proCNP following the initiation of GH treatment in patients with ACH/HCH was significantly correlated with the gain in height velocity. A similar study has reported that changes in NT-proCNP levels are positively correlated with growth velocity in the first year of GH treatment in ISS patients.[25] Therefore, CNP production in response to GH treatment may be maintained even in chondrocytes carrying the *FGFR3* mutation.

Serum NT-proCNP levels were increased 3 months following the initiation of GH treatment in patients with ACH/HCH, as well as serum IGF-1 SDS and AP levels, indicating that CNP is produced in response to GH and/or IGF-1 stimulation in these patients. This is in agreement with previous reports on the effect of GH treatment in ISS and GHD patients.[25,26] However, the mechanisms by which GH and/or IGF-1 stimulate CNP expression are unclear. Although CNP expression *in vitro* is regulated by TGF-β and receptor tyrosine kinase growth factors in a cell/tissue-specific and sometimes species-specific manner, crucial details of the regulatory pathways that lead to suppression or induction of CNP transcription remain unknown.[30] Our *in vivo* data suggest that CNP production may be upregulated, at least in part, by the endocrine GH and IGF-1 system.

We also found that an increase in serum NT-proCNP levels by GH treatment is positively correlated with the increase in height velocity during treatment in patients with ACH/HCH. Serum IGF-1 SDS and AP levels were also increased but they were not correlated, which is consistent with the results of ISS patients in previous studies in terms of serum IGF-1 levels.[25,31] A previous report did not show a correlation in patients with ACH.[17] With respect to serum levels of AP, a marker of bone formation, a study of patients with HCH treated with GH found no correlation between height gain and increase in osteocalcin, another bone formation marker,[19] which is consistent with the AP data in the current study. These results indicate that serum NT-proCNP levels may be a useful marker to indicate the effect of GH treatment on growth in patients with ACH/HCH. Moreover, the positive correlation between height velocity and NT-proCNP after GH treatment may indicate that CNP production promotes height gain in patients with ACH/HCH, which has been reported in a mouse ACH model.[32]

Serum NT-proCNP levels were negatively correlated with age in prepubertal children with ISS and positively correlated with height velocity in short children with ACH/HCH, ISS, GHD and short stature born SGA. The correlation of NT-proCNP levels with height velocity in each of the conditions was not proven, probably due to the small numbers in each group (data not shown). Regarding ISS patients, serum NT-proCNP levels are likely to be positively correlated with height velocity due to the decline of height velocity with age in prepubertal children. These results are consistent with those in healthy children and patients with ACH.[24,33] This indicates that serum NT-proCNP levels may be a biomarker of growth velocity in patients with ISS, and presumably other short children with ACH, HCH, GHD and short stature born SGA.

Serum NT-proCNP levels were comparable in ISS patients to those in patients with ACH/HCH and ACH alone at 3 of years age despite a difference in height SDS. This was not studied at other ages because GH treatment is generally initiated in most patients with ACH/HCH at 3 years of age. Furthermore, comparable NT-proCNP SDS has been described for patients with ACH and HCH despite a difference in height SDS.[33] These results indicate that NT-proCNP levels might not reflect height SDS at the time of measurement. On the other hand, NT-proCNP SDS has been reported to be elevated in patients with ACH and HCH compared to the reference population.[33] The difference in NT-proCNP levels in ACH may be due to a difference in NT-proCNP assay and/or a difference in the age of patients with ACH. Whatever, it is noteworthy that CNP production is not reduced in patients with ACH even though chondrocytes carry the *FGFR3* mutation and develop in an abnormal pattern.

Our study has some limitations. First, the sample size is small. However, our results indicate that serum NT-proCNP levels are a biomarker of growth velocity in patients with ACH/HCH following the initiation of GH treatment. Secondly, we did not have a control group composed of healthy infants, as sampling from healthy children was not permitted from an ethical point of view.

In summary, our study revealed that serum NT-proCNP level is a biomarker of growth velocity in patients with ACH/HCH following the initiation of GH treatment. It is also suggested that serum NT-proCNP levels might be a biomarker of growth in ISS patients.

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Disclosure summary

The authors have no conflicts to disclose.

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Safety and Efficacy of Treatment with Asfotase Alfa in Patients with Hypophosphatasia: Results from a Japanese Clinical Trial

Short title: Clinical Trial of Asfotase Alfa

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Summary

Objective Hypophosphatasia (HPP) is a rare skeletal disease characterized by hypomineralization and low alkaline phosphatase activity. Asfotase alfa (AA) has been recently developed to treat HPP complications. This study evaluated its safety and efficacy in Japan.

Design Open-label, multicenter, prospective trial. Patients were enrolled in 11 hospitals from June 2014 to July 2015.

Patients Thirteen patients (9 females, 4 males) ages 0 days to 34 years at baseline were enrolled and treated with AA (2 mg/kg three times weekly subcutaneously in all but one patient). All had *ALPL* gene mutations. HPP forms were perinatal (n = 6), infantile (n = 5), childhood (n = 1), and adult (n = 1).

Measurements Safety determined from adverse events (AEs) and laboratory data was the primary outcome measure. Efficacy was assessed as a secondary outcome measure from overall survival, respiratory status, rickets severity, and gross motor development.

Results Injection site reactions were the most frequent AEs. Serious AEs possibly related to treatment were convulsion and hypocalcemia observed in a patient with the perinatal form. In addition, hypercalcemia and/or hyperphosphatemia was observed in three patients with the infantile form and a low-calcium and/or low-phosphate formula was given to these patients.

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With respect to efficacy, all patients survived and the radiographic findings, developmental milestones, and respiratory function improved.

Conclusion AA therapy improved skeletal, respiratory, and physical symptoms with a few serious AEs in patients with HPP. Our results add support to the safety and efficacy of AA therapy for HPP patients.

Introduction

Hypophosphatasia (HPP) is a rare skeletal disease caused by defects of the gene *ALPL*, which encodes tissue-non-specific alkaline phosphatase (TNSALP). More than 300 mutations are registered on the website edited by Prof. Monet E (http://www.sesep.uvsq.fr/03_hypo_mutations.php). Impaired bone mineralization due to low levels of alkaline phosphatase (ALP) activity is characteristic of HPP.[1,2] However, the phenotype of HPP varies and is usually classified into six forms based on age at onset and severity of clinical features: perinatal, benign pre-natal, infantile, childhood, adult, and odonto forms.[1–7] The mutations leading to a complete loss of ALP enzyme activity are generally associated with severe forms. In Japanese patients, c.1559delT is the most frequent mutation and is often associated with severe forms.[8–11]

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The prognosis associated with the most severe form of HPP is very poor, because such patients suffer from respiratory failure due to marked hypomineralization of bones. Advances in neonatal intensive care including respiratory and circulatory support have improved prognosis. However, patients with the severe form of HPP require highly effective therapy to survive because its associated survival rate has been reported to be 42% and 27% at the age of 1 and 5 years, respectively.[12] Enzyme replacement therapy using bone-targeting recombinant alkaline phosphatase (asfotase alfa: AA) has been developed and is expected to improve prognosis. A clinical trial of AA has reported it to be safe and effective for 10 patients with perinatal, infantile, and childhood HPP.[13,14] Moreover, a long-term study of AA demonstrated the improvement of mineralization, respiratory function, and survival rate in 37 patients with perinatal and infantile HPP compared to 48 historical controls.[12] However, more experience is needed to better establish its safety and efficacy. We therefore conducted an investigator-initiated clinical trial of AA in Japan to further evaluate its safety and efficacy.

Methods

Study design

The study design was devised by the authors at Osaka University with the assistance of Dr. Kiyoshi Okada (Medical Center for Translational and Clinical Research, Department of

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Medical Innovation, Osaka University Hospital, Osaka, Japan). Dr. Keiichi Ozono acted as the coordinating investigator. Data on the safety and efficacy and the statistical analyses were monitored by Translational Research Informatics Center (Kobe, Japan). The protocol was approved by the local institutional review boards of the participating institutions. The study was conducted following Good Clinical Practice guidelines and adhered to the Declaration of Helsinki. Written informed consent was obtained from the parents of the patients except for an adult patient who gave informed consent himself. The trial was registered (NCT02456038, UMIN000014816, and HPPJEAP-01).

We studied the safety and efficacy of treatment with repeated subcutaneous (SC) injections of AA in patients with HPP in an open-label, single-arm, multicenter, investigator-initiated clinical study. The primary outcome measure was the safety of AA. Secondary outcome measures included overall survival, respiratory status, rickets severity, physical growth, and gross motor development. Patients were enrolled in 11 hospitals from June 2014 to July 2015 when AA received marketing approval in Japan.

Eligible patients included those of any age who had been already diagnosed with HPP, who had been already treated with AA outside of this clinical trial, or who had one or more features of HPP such as serum ALP activity below age-adjusted lower limits of normal, HPP related-findings, or positive genetic testing (*ALPL* mutations). All of the patients enrolled in the study, in fact, had mutations of the *ALPL* gene. Exclusion criteria included treatable forms

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of rickets, hypocalcemia, hypophosphatemia, or treatment with bisphosphonates. Detailed inclusion/exclusion criteria are provided as Supplementary Information (Supplementary Methods).

Clinical assessments including physical growth, vital signs, and laboratory data were collected at baseline, every 4 weeks until 24 weeks, and every 12 weeks thereafter. **All laboratory data, including serum levels of ALP, calcium (Ca), and phosphate (P), were measured in the laboratory of each institute. Abnormal levels of ALP, Ca and P were based on the age-matched reference range.** Renal ultrasonographic and ophthalmologic examinations were performed at baseline and every 24 weeks. In patients who had been already treated by AA outside of this clinical trial, data at the initiation of that treatment were defined as baseline.

Treatment

All of the patients except one received AA by SC injection three times weekly at a dose of 2 mg/kg and the maximum volume of a single injection was 1 mL (40 or 80 mg/ml concentration). Patient 004-001, with the adult form, received AA at a dose of 1·17 mg/kg (1 mL SC at a concentration of 80 mg/ml) because that volume was the maximum permitted for a single injection. The mean ± SD duration of the treatment was 363·6 ± 275·3 days (median 251, range 49–868).

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Safety

Adverse events (AEs) including injection-associated reactions and injection site reactions (ISRs) were graded by the investigator as mild, moderate, or severe, and whether or not related to treatment. **ISRs are defined as related AEs that are localized to the area surrounding the injection occurring either during or at any time point after administration of the study drug injection.** Safety was also assessed from change of vital signs and laboratory data, renal ultrasonographic findings, ophthalmologic findings, and drugs used concomitantly during the study. Regarding laboratory data, changes from baseline after treatment were calculated at every visit.

Efficacy

Overall survival was defined as the time from birth to death during the study. Ventilator-free survival for patients who were not mechanically ventilated at the time of enrollment was defined as the percentage of patients who were alive and ventilator-free at the end of the study after treatment.

Respiratory function in patients with respiratory support was evaluated by ventilator status, time on respiratory support (including time on ventilator or supplemental oxygen), ventilator rate, oxygen volume, ventilator pressures, and fraction of inspired oxygen.

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Radiological findings of the wrists and knees were evaluated according to Thacher and colleagues.[15] This scoring method was developed to assess nutritional rickets. Radiographs were evaluated and scored on the rickets severity scale (RSS) every 12 weeks and changes from baseline were calculated.

The effect of AA treatment on physical growth was measured from height, weight, arm span, head circumference, and chest circumference. Height and weight data were transformed to age- and sex-specific z-scores from the average results for the reference Japanese population.[16] Changes of all measurements from baseline were calculated at every visit.

Assessment of changes in gross motor development was based on developmental milestones using a gross motor milestone checklist for this study **(see Supplementary Methods)**. The checklist consisted of four sections assessing each postural position: supine, prone, sitting, and standing. Each section had different numbers of items for gross motor skills: supine (n = 9), prone (n = 10), sitting (n = 6), and standing (n = 16). **Examiner checked skills, "yes" or "no" depending on if the patient was able to perform each activity.** The number of skills that had been achieved was scored and totaled.

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Statistical analysis

Paired *t*-tests were used to assess changes from baseline in measurements. A *P*-value of <0·05 was considered as significant. Statistical analyses were performed using SAS (version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

Patient baseline characteristics

Baseline patient characteristics are summarized in Table 1. Thirteen patients (9 females and 4 males) were enrolled in this study. Their median age was 91 days (range 0 days to 34 years). Ethnically, all were Japanese except for two patients (007-001 and 008-001). The father of patient 007-001 was American and his mother was Japanese. Patient 008-001 was Chinese. Ten hospitals each enrolled single patients and one hospital three patients. HPP form was: perinatal (*n* = 6), infantile (*n* = 5), childhood (*n* = 1), and adult (*n* = 1). **No patients with benign pre-natal and odonto form were included.** All patients had low levels of serum ALP at 0–240 IU/l (median 39·0) (see Supplementary Information Table S1 for reference range). Five of the 13 patients had already been treated with AA prior to this study. Mean height z-score was –2·5 ± 1·8 SD and mean weight z-score was –1·6 ± 1·5 SD.

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The medical history of the patients at baseline is summarized in Table 2. In patients with perinatal and infantile forms, the main symptoms at the onset of HPP were poor weight gain (30·7%), dyspnea (30·7%), and hypomineralization of bone (30·7%). Four patients (30·7%) had family members with HPP. While there were no patients with pseudo-fracture, three patients (23·1%) had a history of bone fractures. Four patients (30·7%) had muscle weakness, and two of these (004-001 and 007-001) had difficulty walking and an unusual gait· Furthermore, patient 004-001 had impaired activities of daily living caused by myalgia and arthralgia. The clinical course of two patients had already been reported before treatment with AA (007-001) or on the compassionate use of AA (003-001) before recruitment into the study.[17,18]

Mean serum Ca was 9·93 ± 0·59 mg/dl, serum P 5·73 ± 1·32 mg/dl, and spot urine calcium/creatinine ratio (Ca/Cr) 0·45 ± 0·50 g/gCr (Supplementary Information Fig. S1). Serum Ca and P levels were within the reference range at baseline (see Supplementary Information Table S2 for reference range). All other laboratory data were normal at baseline.

Safety

A total of 195 AEs was reported: mild 177 (91%), moderate 14 (7%), and severe 4 (2%). Of these, 104 AEs (53%) were assessed by the investigators as related to AA. The most common treatment-related AEs were injection site reactions (total 88 events in 7 patients), which

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consisted of mild, localized, and transient erythema that was sometimes associated with induration and pruritus. No generalized injection-associated reactions, including anaphylactic shock or hypotension, were observed in any of the patients (see Supplementary Information Tables S3–S7 for additional AE data).

A total of six serious AEs was reported in two patients (003-001 and 006-001) [Table 3]. Two of these serious AEs (convulsion and hypocalcemia) were possibly related to the treatment in patient 003-001. The convulsion occurred on day 28 and was attributed to hypocalcemia that appeared from day 23. Serum Ca level was improved from 4·7 mg/dl on day 28 to 9·7 mg/dl on day 33 by increasing the dose of Ca administration. No convulsion was observed after the serum Ca level had been stabilized. The other four serious AEs were considered unrelated to treatment. Two of them were associated with infections. **One of 4 serious AEs was mixed deafness in case 003-001.** None of the serious AEs necessitated a change in the doses of the study drug.

Renal ultrasonographic examinations detected nephrocalcinosis (23%) in three patients (001-001, 002-001, and 007-001). These findings were not considered by the investigators as AEs because of the lack of exacerbation during treatment. No patients had ophthalmologic findings, including papilledema and ectopic calcification.

With respect to laboratory data, all measurements except ALP, Ca, and P remained within the normal range (see Supplementary Information Tables S1 and S2 for age-specific reference

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ranges for ALP, Ca, and P). For all patients, mean serum ALP level increased rapidly and markedly from 79 ± 86 IU/l at the baseline to 24 407 ± 12 701 IU/l 4 weeks after starting AA treatment. High levels of serum ALP persisted during AA treatment. Serum P levels were transiently or continuously increased above the upper limit of normal (ULN) in 11 patients (84·6%) during treatment. The highest level of serum P (9·7 mg/dl) was observed in patient 003-001 at 4 weeks concomitantly with hypocalcemia (Ca 4·7 mg/dl), as previously described. At a later time point, a high serum P level was also observed in the same patient (8·3 mg/d at 120 weeks). All patients had normal renal function.

In eight patients (61·5%), serum Ca levels were transiently or continuously elevated above the ULN during treatment. The highest serum Ca level during the study was 11·9 mg/dl, observed in patient 009-001 at 16 weeks. In addition, the highest Ca/Cr was observed in patient 001-001 at 8 weeks (3·05 g/gCr), while using a low-calcium formula as additional management for hypercalcemia. Transient or continuous elevations of serum Ca and P levels during AA treatment were observed in three patients (001-001, 005-001, and 008-001). They used low-calcium formula and/or a low-phosphorus formula (see Supplementary Information for details of additional management in these patients).

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Efficacy

The survival rate was 100% (95% CI 75·3–100·0) at the end of the study (median 24, range 4–120 weeks).

Five patients (004-001, 005-001, 007-001, 008-001, and 010-001) did not require mechanical respiratory support at baseline nor during the study. Ventilator-free survival among them was 100% at the end of the study (median 24, range 12–60 weeks).

A total of eight patients (61.5%) required some form of respiratory support and three (37·5%) of them were managed with non-invasive respiratory support. Five patients (62·5%) [3 perinatal and 2 infantile form] required invasive mechanical ventilation (IMV) support. Since three perinatal patients had respiratory failure at birth, mainly due to bronchomalacia and hypomineralized ribs, they received IMV support from day 0 and continued after baseline. On the other hand, two infantile patients did not show apparent respiratory failure at birth. However, they had failure to thrive and showed a gradually deteriorating respiratory condition during infancy. At baseline, they had poorly mineralized ribs on radiological findings and received respiratory support (Table 4). Four patients also received tracheostomy to maintain their quality of life during mechanical ventilation support (see Supplementary Information Results for detail).

Respiratory status and mineralization of ribs improved in all patients during AA treatment (see Supplementary Information Results **and Fig. S3** for details). Three patients (37·5%) did

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not require respiratory support and two patients were starting to wean off support during the first month of treatment. At the end of the study, one patient was on mechanical ventilation and two received supplementary oxygen, making a total of three patients requiring some form of respiratory support at the end of the study.

Baseline radiographs showed undermineralized metaphyses as hypophosphatasia-associated skeletal lesions in all except two patients with a closed growth plate (004-001 and 007-001). Mineralization gradually improved in all patients. At week 24, these metaphyses were mineralized with distinct provisional zones of calcification, and the metacarpals had wider widths, better-defined cortexes, and no lytic areas at their proximal ends. Mean RSS score decreased during treatment and the changes from the baseline to 12 and 24 weeks were –3·4 ± 3·4 (P = 0·016) and –5·4 ± 2·7 (P = 0·002), respectively (Fig. 1).

Weight was assessed in all patients and height were evaluated in 11 patients. Height data at baseline were not obtained for two patients who had already been treated with AA prior to this study. Mean weight z-score did not markedly change from –1·6 ± 1·5 SD at the baseline to –1·6 ± 1·7 SD at the end of the study (P = 0·881). Mean height z-score, also, did not change significantly from –2·5 ± 1·9 SD at the baseline to –2·0 ± 1·4 SD at the end of the study (P = 0·139) [Fig. 2]. Additional data on physical growth are provided in Supplementary Information (Results).

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Gross motor milestones were evaluated in 10 patients. Five infants were assessed at baseline, of whom four were evaluated at 12 weeks. Mean total score for gross motor milestones was significantly increased at 12 weeks compared to baseline (P = 0·003). Although no baseline data were obtained in five patients who had already been treated with AA prior to this study, the total score of four infants gradually increased during treatment (see Supplementary Information Fig. S2).

Discussion

The safety and efficacy of AA have been reported in patients with HPP in international, multicenter clinical trials, following which SC treatment with AA was approved in several countries including Japan and the USA.[19] Treatment with the recombinant enzyme is generally safe, although some AEs have to be carefully monitored. The most frequent AE is a local reaction to the injection. Hypotensive shock due to anaphylaxis was not observed in our trial. Our clinical study provides evidence supporting the tolerability of AA treatment at the recommended dose.

An important AE is hypocalcemia on AA treatment. The recombinant enzyme stimulates mineralization in bone and this process requires calcium deposition in bone. This may account for hypocalcemia during treatment. Newborns or infants with HPP sometimes show

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hypercalcemia and receive low-calcium formula, which may facilitate the development of hypocalcemia during AA treatment. We recommend ordinary milk when starting AA treatment and monitoring serum calcium levels during treatment. Administration of calcium and vitamin D may be necessary to prevent or resolve hypocalcemia.

Hyperphosphatemia can also be an AE. However, serum phosphate levels were not particularly high in our study: often less than 8 mg/dl but sometimes over 8 mg/dl up to 9·7 mg/dl. The elevation of serum phosphate levels may aggravate ectopic calcification. Thus, careful observation is necessary and hyperphosphatemia may require the restriction of phosphorus intake. While ectopic calcification in the kidney and retina was not observed, craniosynostosis **that is one of the complications of HPP** occurred in this clinical trial. These potential AEs should be carefully monitored during treatment. **Mixed deafness was considered a serious AE in case 003-001 by doctors who treated the patient. Sensorineural hearing loss was indicated by auditory brainstem response test, and conductive deafness was suggested by deformities of the middle ear ossicles detected by computed tomography. They might be due to their hypomineralization in HPP. In addition, deafness is reported to be one of clinical features in HPP.[11] Thus, mixed deafness in this case might be one of the complications in HPP.**

Convulsion was observed after the initiation of treatment in one patient. Indeed, vitamin B6-dependent convulsion is one of the complications of HPP.[1] However, the convulsion in

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the patient was due to hypocalcemia. Thus, it is necessary to consider both vitamin B6-dependent and hypocalcemia-induced convulsions during treatment.

AA clearly stimulated bone formation. Patients with severe HPP showed marked hypomineralization in all bones, especially the skull, long bones, and ribs. The long bones and ribs thickened during treatment. The effect was observed at 1 month after starting treatment. Lung function was improved after bone formation became evident. Thus, supportive therapy for the respiratory and circulatory functions is essential even after the initiation of treatment with AA. The survival rate and respiratory function improved in the trial. Six patients suffered from perinatal HPP, of whom five required mechanical ventilation and two underwent tracheostomy. **Although AA improved respiratory status, periods to the end of respiratory support were different among the patients with perinatal HPP (Table 4). Patients with high-frequency oscillatory ventilation or synchronized intermittent mandatory ventilation at baseline required longer respiratory support than those with nasal continuous positive airway pressure or supplemental oxygen, suggesting that respiratory status at baseline predicts periods of respiratory support after the initiation of AA. In agreement with our study, the previous report describes that severe or long-standing pulmonary impairment at baseline might influence the improvement period of respiratory status.**[12] The six patients with perinatal HPP were all

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alive at the final visit (median 48, range 4–120 weeks) and four of them were free from respiratory support including oxygen supply.

It is strongly suggested that AA treatment of patients with HPP improves prognosis.[13,14] The treatment has been called transformative.[5] Thus, the early diagnosis of HPP is important in this situation. A treatment algorithm for HPP has been proposed.[20,21] Other therapies including teriparatide, bone marrow transplantation, and gene induction have been reported.[22–24] We hope to develop a guide on how to manage patients with HPP in terms of diagnosis and treatment in the near future. In summary, asfotase alfa was safe and effective for the treatment of 13 patients with HPP including perinatal and childhood forms.

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Figure legends

Fig. 1 A) Change of rickets severity scale (RSS) score during treatment. Mean RSS score decreased from 8·0 ± 2·9 at baseline to 4·9 ± 3·8 at 12 weeks (n = 10) and 3·0 ± 2·8 at 24 weeks (n = 9). **B) Change of the wrist and knee radiographs during treatment. Patient 011-001, with the perinatal form, is shown. Undermineralized methaphyses were improved at week 12. RSS, Rickets Severity Score.**

Fig. 2 Change in (A) height and (B) weight z-scores during treatment. Mean weight z-score did not markedly changed from –1·6 ± 1·5 SD at baseline to –1·6 ± 1·7 SD at the end of the study (P = 0·881). Mean height z-score did not change significantly from –2·5 ± 1·9 SD at baseline to –2·0 ± 1·4 SD at the end of the study (P = 0·139).

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Table 1. Baseline patient characteristics

Case no.	Sex	Age	HPP form	Mutations of *ALPL*	ALP (U/l)*	Height z-score (cm)	Weight z-score (kg)	Final visit (week)
001-001	F	113 days	I	p.M295I/c.1559delT	70	51·0 (–4·1)	4·25 (–2·4)	36
002-001	F	230 days	I	p.H336R/c.1559delT	21	61·0 (–2·8)	4·80 (–4·2)	120
002-002	M	16 days	P	c.1559delT/c.1559delT	23	51·0 (1·0)	2·89 (–0·3)	84
002-003	F	0 days	P	c.1559delT/c.1559delT	0	41·0 (–3·6)	2·43 (–1·2)	4
003-001	F	1 day	P	p.G491R/p.G491R	1	42·8 (–2·7)	2·68 (–0·6)	120
004-001	M	34 years	A	c.1559delT heterozygous	51	159·4 (–2·3)	68·6 (–0·0)	24
005-001	F	158 days	I	p.E191G/c.1559delT	144	57·0 (–3·4)	6·06 (–1·1)	24
006-001	F	54 days	P	c.1559delT/c.1559delT	15	46·6 (–2·8)	2·93 (–2·3)	24
007-001	M	17 years	I	p.K224E/p.G426C	195	148·8 (–3·7)	40·0 (–3·9)	24
008-001†	F	36 days	I	p.R136C/p.F327L	240	57·0 (2·1)	4·70 (0·9)	60
009-001	F	0 days	P	p.R450C/c.1559delT	39	41·0 (–3·6)	2·30 (–1·6)	72
010-001	M	10 years	C	p.F327L/p.G339R	209	119·2 (-3·1)	24·4 (–1·7)	12
011-001	F	91 days	P	c.1559delT/c.1559delT	23	52·0 (–3·6)	4·68 (–1·7)	12

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A, adult; ALP, alkaline phosphatase; C, childhood; F, female; HPP, hypophosphatemia; I, infantile; M, male; P, perinatal.

* Reference range at 0–1 months: 530–1620 U/l.

†Height measured at 3 days after the baseline.

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Table 2. Baseline patient medical history

Case no.	HPP form	Age at onset	Symptoms at HPP onset	Family members with HPP	Fracture history (age detected)	Orthopedic findings	Respiratory status	Serum calcium and phosphate levels, and nephrocalcinosis
001-001	I	2m	Poor weight gain (poor feeding)	Elder brother*	Rib (at infancy)	Chest deformity, cranial deformity	Pneumonia	Nephrocalcinosis, hypercalcemia
002-001	I	1m	Poor weight gain			Chest deformity, cranial deformity, muscle weakness	Dyspnea	Nephrocalcinosis, hypercalcemia
002-002	P	0m	Seizure, dyspnea, hypercalcemia, hyperphosphatemia				Dyspnea	Hypercalcemia, hyperphosphatemia
002-003	P	0m	Chest deformity, dyspnea, short extremities	Elder brother (002-002)			Dyspnea	
003-001	P	0m	Hypomineralization of bone (pre-natal)			Chest deformity	Dyspnea	
004-001†	A	24y	Muscle pain, muscle weakness	Child	Rt. humerus (6y) Lt. clavicle (23y)	Gait disturbance, arthralgia		

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005-001	I	0m	Bowed legs		Genu varum, forearm curvature		Hypercalcemia
006-001	P	0m	Short extremities, hypomineralization of bone, chest deformity, dyspnea	Lt. radius (1m) Rt. ulna (1m)	Genu varum, muscle weakness	Dyspnea, pneumonia	
007-001‡	I	2m	Poor weight gain, bad mood		Delayed walking, chest deformity, cranial deformity, bone pain, arthralgia, gait disturbance, muscle weakness	Dyspnea, pneumonia	Nephrocalcinosis, hypercalcemia
008-001	I	0m	Hypomineralization of bones (metaphyseal flaring)				Hyperphosphatemia
009-001	P	0m	Short extremities, hypomineralization of bones		Genu varum		
010-001	C	3y	Premature tooth loss		Gait disturbance, arthralgia		
011-001	P	1m	Dyspnea, poor weight			Dyspnea	Hypercalcemia

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gain

A, adult; C, childhood; HPP, hypophosphatasia; I, infantile; Lt, left; m, month; P, perinatal; Rt, right; y, year

*Brother of 001-001, died of respiratory dysfunction from pneumonia at the age of 3 months, diagnosed with HPP by genetic testing.

†Patient 004-001 had internal plate fixation of humerus fracture and muscle biopsy for muscle weakness.

‡Patient 007-001 underwent surgery for craniosynostosis at the age of 1 year.

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Table 3. Serious adverse events

Case no.	Age at AE onset	Preferred term	Severity	Causality	Outcome	AE duration
003-001	28 days	Convulsion	Severe	Probable	Resolved	16 days
	23 days	Hypocalcemia	Severe	Probable	Resolved	21 days
	7·7 months	Mixed deafness*	Severe	Unrelated	Unresolved	
	1·3 years	Upper respiratory tract infection†	Moderate	Unrelated	Resolved	5 days
006-001	3·4 months	Staphylococcal infection‡	Severe	Unrelated	Resolved	20 days
	5·3 months	Convulsion§	Moderate	Unrelated	Resolved	2 days

AE, adverse event.

*Mixed deafness was based on auditory brainstem response. Deformities of the middle ear ossicles were detected using computed tomography.

†Upper respiratory tract infection resolved by intravenous fluid therapy for 5 days while hospitalized.

‡Staphylococcal infection caused by catheter infection and cured by antibacterial therapy.

§Convulsion was a withdrawal symptom from benzodiazepine given for sedation during respiratory management.

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Table 4. Respiratory support

Case no.	HPP form	Respiratory status at the baseline	Period from baseline to weaning off mechanical ventilation	Period from baseline to the end of respiratory support*
001-001	I	SIMV (started at 8 days before baseline)	121 days (tracheostomy at 9 days from baseline: SIMV for 23 days and CPAP for 98 days.)	Ongoing supplemental oxygen at the final visit (36 weeks)
002-001	I	BiPAP (SIMV for 32 days before BiPAP)	205 days	272 days
002-002	P	Nasal CPAP	No mechanical ventilation support	23 days
002-003	P	Nasal CPAP	No mechanical ventilation support	34 days (nasal CPAP for 3 days and nasal high flow for 31 days)
003-001	P	HFO (with inhaled nitric oxide)	225 days (tracheostomy at 78 days from baseline. HFO control for 108 days; SIMV for 7 days and then gradually tapered)	1·8 years
006-001	P	HFO	110 days (tracheostomy at 90 days from baseline. HFO control for 35 days; A/C mode for 51 days and then SIMV control.)	Ongoing supplemental oxygen at the final visit (24 weeks)
009-001	P	Supplemental oxygen	No mechanical ventilation support	3 days
011-001	P	SIMV (tracheostomy at 14 days before baseline)	Ongoing SIMV control	Ongoing SIMV control at the final visit (12 weeks)

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A/C, assist control ventilation; BiPAP, biphasic positive airway pressure; CPAP, continuous positive airway pressure; HFO, high-frequency oscillatory ventilation; I, infantile; P, perinatal; SIMV, synchronized intermittent mandatory ventilation.

*Respiratory support including oxygen supply.

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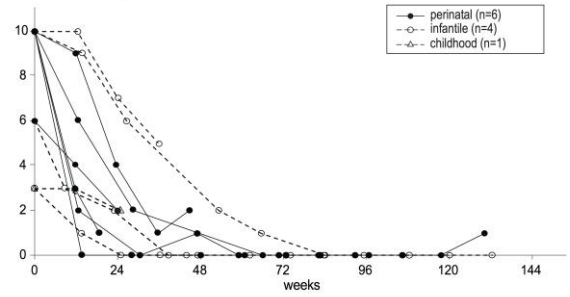
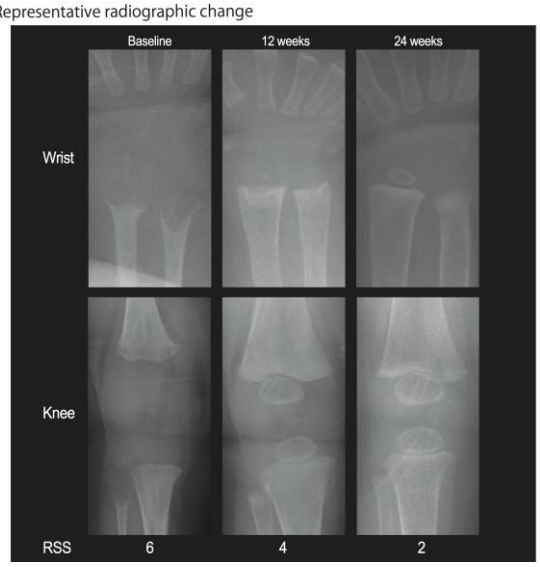
A. Rickets severity scale



B. Representative radiographic change



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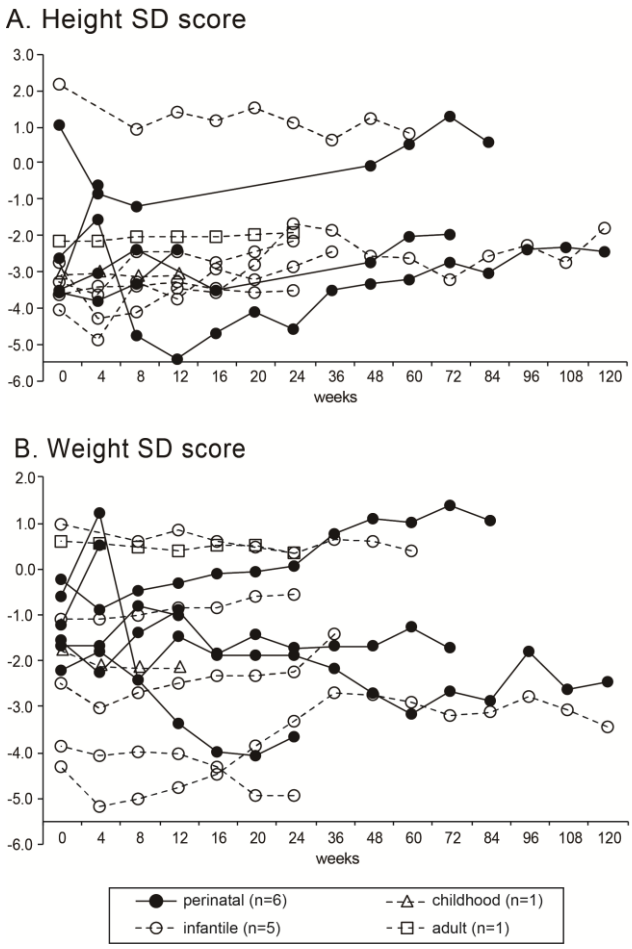

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ORIGINAL ARTICLE

Characteristic calcaneal ossification: an additional early radiographic finding in infants with fibrodysplasia ossificans progressiva

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Abstract
Background We have clinically encountered children with fibrodysplasia ossificans progressiva who had abnormal calcaneal ossification.
Objective To evaluate whether calcaneal ossification variants are significant radiographic findings in children with fibrodysplasia ossificans progressiva.
Materials and methods Lateral feet radiographs in nine children who fulfilled the diagnostic criteria of fibrodysplasia ossificans progressiva were reviewed. The studies were obtained during infancy or early childhood.
Results Fourteen lateral foot radiographs of fibrodysplasia ossificans progressiva were available for this study (ages at examination: 1-104 months). Four children ages 2 months to 11 months showed double calcaneal ossification centers; 7 children had plantar calcaneal spurs that decreased in size with age. Overall, eight of nine children with fibrodysplasia ossificans progressiva demonstrated double calcaneal ossifications and/or plantar calcaneal spurs in infancy or childhood.
Conclusion Double calcaneal ossification centers in early infancy and plantar calcaneal spurs in childhood are frequently seen in children with fibrodysplasia ossificans progressiva and may be a useful radiologic indicator for early diagnosis.

Keywords Calcaneal spur · Children · Double calcaneal ossification center · Early diagnosis · Fibrodysplasia ossificans progressiva · Radiography

Introduction

Fibrodysplasia ossificans progressiva is a genetic disorder of the connective tissues caused by activating mutations in the gene-encoding activin receptor IA/activin-like kinase 2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor [1]. Most affected individuals have a common mutation (c.617G > A, p.R206H). The clinical hallmark of fibrodysplasia ossificans progressiva is progressive heterotopic ossification of soft tissues, such as muscles, ligaments, tendons, fasciae and aponeuroses, which causes significant physical morbidity and may lead to early mortality. Traumatic injury and surgical intervention induce explosive heterotopic ossification in patients with fibrodysplasia ossificans progressiva. Early diagnosis is necessary to prevent additional trauma or iatrogenic harm [2].

Fibrodysplasia ossificans progressiva is associated with a variety of bone malformations as well as variant ossification. Awareness of the bone anomalies may facilitate prompt diagnosis. Malformations of the great toes are well-known and are the most prevalent indicators of this disorder [3]. Other anomalies include shortening of the first metacarpal bones and

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hypertrophy of the posterior element of the cervical spine [4]. Quantitative analyses of bone changes in the hand and cervical spine are also available [5]. Recently, we encountered two children with distinctive double ossification centers and plantar spurs in the calcaneus. Search of the database identified seven additional children with fibrodysplasia ossificans progressiva. We evaluate whether the presence of calcaneal changes may be commonly seen in patients with fibrodysplasia ossificans progressiva.

Materials and methods

Two children (cases 2 and 3 in Table 1) were seen clinically and subsequently presented and discussed in web case consultations. The online discussion enabled us to collect clinical data and foot radiographs from members of the Research Committee on Fibrodysplasia Ossificans Progressiva (Japan), who provide pediatric orthopedic services in four hospitals across Japan. Six Japanese children and one Indian child (5 males and 2 females) who underwent lateral feet radiography in infancy or early childhood were thus additionally enrolled. All nine children fulfilled the diagnostic criteria of fibrodysplasia ossificans progressiva, including deformities of the great toes, extraskeletal heterotrophic ossification, joint contractures, fusion of the cervical spine, broad femoral necks and osteochondroma-like lesions. Seven of the nine children underwent molecular testing, showing the common ACVR1/ALK2 mutation within the glycine/serine-rich regulatory (GS) domain (c.617G > A, p.R206H). All radiographs were examined by a single reader (G.N.) with 30 years' clinical experience in the field of skeletal dysplasias and specifically focused on calcaneal configurations.

Results

Fourteen lateral foot radiographs obtained between the ages of 1 month to 104 months were available for this study. Three children (cases 1, 2 and 4) underwent serial radiographic examinations in infancy and early childhood. We found two distinctive findings: 1) double calcaneal ossification centers and 2) plantar calcaneal spurs. These findings were bilateral and symmetrical. The radiologic findings and pertaining clinical information are summarized in Table 1.

Double calcaneal ossification centers

Four children (cases 1, 2, 3 and 4) showed double ossification centers in radiographs obtained in infancy (Figs. 1, 2, 3 and 4). Three of them (cases 2, 3 and 4) had a cleft separating the anterior two-thirds from the posterior third in the calcaneus. The posterior ossification centers were small, intermediate and large in size at ages 2, 4 and 10 months, respectively, partially fused with the anterior ossification centers at ages 4 and 10 months, and completely incorporated into the anterior ossifications at ages 12 months and 22 months. The remaining child (case 1) had a cleft separating the anterior third from the posterior two-thirds in the calcaneus. The anterior ossification center manifested as punctate multiple ossifications in early infancy, which evolved into double ossifications at age 11 months and completely coalesced at age 3 years.

Plantar calcaneal spur

Seven children showed a small spur from the plantar aspect of the posterior calcaneal body (Figs. 2, 3, 4 and 5). The spurs are pedunculated and projected posteriorly from the posterior

Table 1 Demographic, clinical, genetic and radiologic summaries of nine children with fibrodysplasia ossificans progressive

Case number	1				2		3	4		5	6	7	8	9
Sex	male				male		female	male		male	female	male	male	female
Current age	4Y8M				6Y		2Y9M	3Y2M		2Y	3Y5M	4Y6M	7Y7M	8Y8M
Ethnic background	Japanese				Caucasian		Turkish	Japanese		Indian	Japanese	Japanese	Japanese	Japanese
ACVR1 mutation	R206H				R206H		ND	R206H		R206H	ND	R206H	R206H	R206H
Great toe abnormalities	+				+		+	+		+	+	+	+	+
Other radiological findings of FOP	+				NA		NA	+		+	+	+	+	+
Ectopic ossification	+				-		-	-		-	-	-	+	+
Age at X-ray (months)	1	3	11	36	2	12	4	10	22	15	18	33	70	104
Double calcaneal ossification centers	punctate	punctate	+	-	+	-	+	+	-	-	-	-	-	-
Plantar calcaneal spur	-	-	-	-	+	+	+	+	+	+	-	+	+	+

NA not available , *ND* not determined

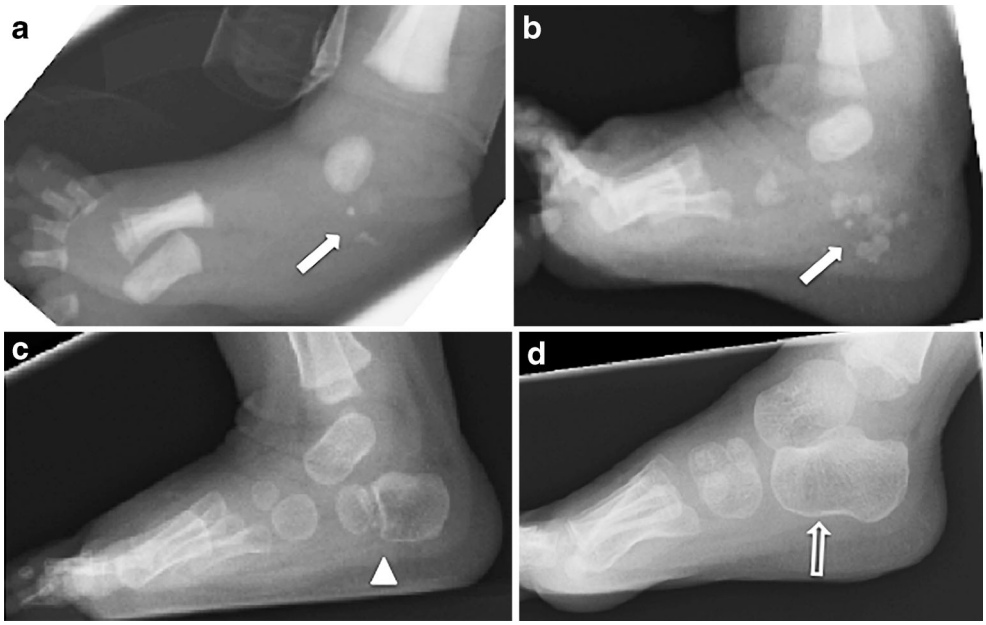

Fig. 1 Lateral foot radiographs in a boy with fibrodysplasia ossificans progressiva (case 1 in Table 1). Sequential radiographs at 1 months of age (**a**), 3 months of age (**b**), 11 months of age (**c**), and 3 years of age (**d**) demonstrate punctate multiple ossifications in early infancy (*solid arrows*), double ossification centers with a cleft separating the anterior third from the posterior two-thirds of the calcaneus (*arrowhead*), and normal calcaneal configuration after complete fusion of the ossification centers (*open arrow*)

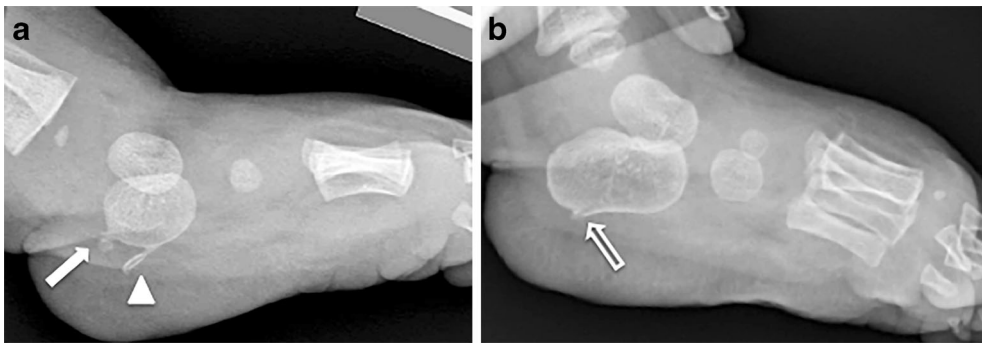

Fig. 2 Lateral foot radiographs in a boy with fibrodysplasia ossificans progressiva (case 2 in Table 1). Sequential radiographs at 2 months of age (**a**) and 12 months of age (**b**) show a small posterior ossification (*solid arrow*) and posteriorly pedunculated calcaneal spur (*arrowhead*), which became smaller in size with age (*open arrow*)

aspect of the anterior calcaneal ossification in infancy. They became sessile and projected inferiorly with age. In patient 2, the spur became smaller in size with age.

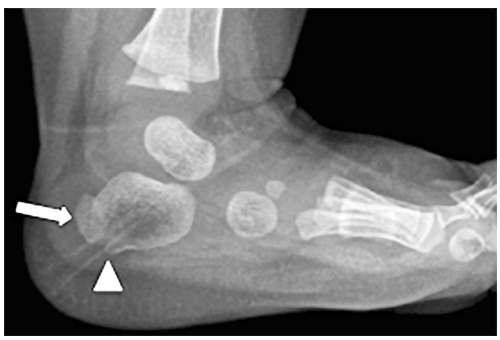

Fig. 3 Lateral foot radiograph in a girl with fibrodysplasia ossificans progressiva (case 3 in Table 1) at 4 months of age demonstrates double ossification centers (*arrow*) and plantar spur (*arrowhead*) of the calcaneus

Discussion

Our findings suggest that abnormal/variant calcaneal ossification, double calcaneal ossification and plantar spurs may be of diagnostic significance in patients with fibrodysplasia ossificans progressiva. These are congenital anomalies, which are perceptible in early infancy and can be a clue to the early diagnosis of this disorder, as are malformations of the great toes, shortening of the thumb and hypertrophy of the posterior element of the cervical spine. This finding is not exclusive of fibrodysplasia ossificans progressiva, as double calcaneal ossifications and plantar spurs are also described as rare developmental variations in normal children [6–10].

The normal double calcaneal ossification is sometimes referred to as bifid os calcis [8–10]. This variation has been reported in children with mild foot deformities, such as pes planovalgus, metatarsus varus and mild talipes equinovalgus. Bifid os calcis shows complete coalescence and remodeling of

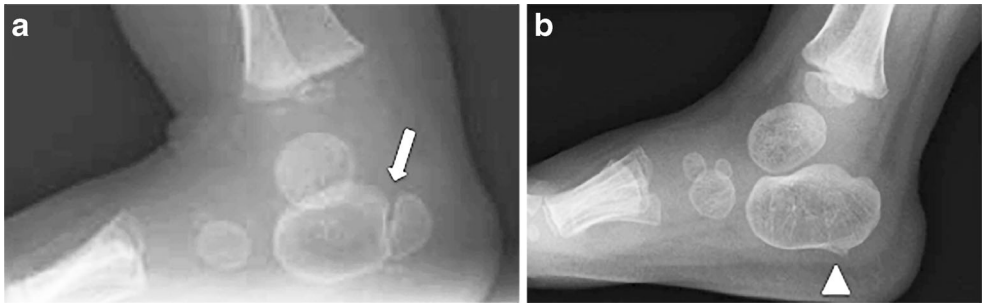

Fig. 4 Lateral foot radiographs in a boy with fibrodysplasia ossificans progressiva (case 4 in Table 1). Sequential radiographs at 10 months of age (**a**) and 22 months of age (**b**) reveal double ossification centers with a cleft separating the anterior two-thirds from the posterior third in the calcaneus (*arrow*) and an inferiorly projected small spur in the plantar calcaneus (*arrowhead*)

two separate ossifications during early childhood and typically shows a cleft separating the anterior third from the posterior two-thirds of the calcaneus. A patient with bifid os calcis reported by Ogden [9] showed punctate ossification centers in the anterior portion. In three children in our series, double calcaneal ossifications were associated with a cleft separating the anterior two-thirds from the posterior third of the calcaneus. However, the manifestation in the remaining one was similar to that in Ogden's case.

A duplicate/triplicate calcaneus was observed in specific skeletal dysplasias, including chondrodysplasia punctata, thanatophoric dysplasia and short rib polydactyly syndromes [11]. Double calcaneal ossifications with a cleft separating the anterior two-thirds from the posterior third of the calcaneus resemble those commonly seen in an infant with Larsen syndrome. Larsen syndrome is caused by heterozygous mutations in filamin B gene (*FLNB*). Filamen B is a cytoskeletal protein involved in a multicellular process [12]. Zheng et al. [13] demonstrated that *FLNB* mutant mice display ectopic mineralization in various cartilaginous elements, including carpal and tarsal bones, and this mutant phenotype is rescued by removing Runx2 through TGFβ-Smad pathway. Overexpression of the R206H mutant ACVR1, on the other hand, enhances Smad1/5 signaling. Molecular interactions between filamin B and Smad signaling in skeletal morphogenesis may lead to similar phenotypes of ossifications in the calcaneal region in Larsen syndrome and fibrodysplasia ossificans progressiva.

The normal plantar calcaneal spur is seen at the posterior two-thirds of the bone, tends to be bilateral and symmetrical, may point anteriorly, posteriorly or inferiorly, and disappears by 1 year of age. The normal calcaneal spur is morphologically indistinguishable from the late manifestation of the calcaneal spur in fibrodysplasia ossificans progressiva. However, the early pedunculated appearance in fibrodysplasia ossificans progressiva is not seen in the normal spur. In addition, the spur in fibrodysplasia ossificans progressiva persists in childhood.

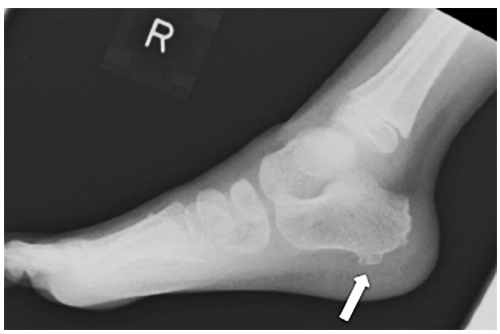

Fig. 5 Lateral foot radiograph in a boy with fibrodysplasia ossificans progressiva (case 8 in Table 1) at 5 years, 10 months of age shows a small spur from the plantar aspect of the posterior calcaneal body (*arrow*)

Conclusion

Double calcaneal ossification centers in early infancy and plantar calcaneal spurs in childhood may be significant radiologic findings useful for early diagnosis of fibrodysplasia ossificans progressiva.

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Compliance with ethical standards

Conflicts of interest None

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ORIGINAL ARTICLE

Criteria for radiologic diagnosis of hypochondroplasia in neonates

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Abstract
Background A radiologic diagnosis of hypochondroplasia is hampered by the absence of age-dependent radiologic criteria, particularly in the neonatal period.
Objective To establish radiologic criteria and scoring system for identifying neonates with fibroblast growth factor receptor 3 (FGFR3)-associated hypochondroplasia.
Materials and methods This retrospective study included 7 hypochondroplastic neonates and 30 controls. All subjects underwent radiologic examination within 28 days after birth. We evaluated parameters reflecting the presence of (1) short ilia, (2) squared ilia, (3) short greater sciatic notch, (4) horizontal acetabula, (5) short femora, (6) broad femora, (7) metaphyseal flaring, (8) lumbosacral interpedicular distance narrowing and (9) ovoid radiolucency of the proximal femora.
Results Only parameters 1, 3, 4, 5 and 6 were statistically different between the two groups. Parameters 3, 5 and 6 did not overlap between the groups, while parameters 1 and 4 did. Based on these results, we propose a scoring system for hypochondroplasia. Two major criteria (parameters 3 and 6) were assigned scores of 2, whereas 4 minor criteria (parameters 1, 4, 5 and 9) were assigned scores of 1. All neonates with hypochondroplasia in our material scored ≥6.
Conclusion Our set of diagnostic radiologic criteria might be useful for early identification of hypochondroplastic neonates.

Keywords Achondroplasia · FGFR3 · Hypochondroplasia · Neonate · Radiography · Radiologic diagnosis · Scoring system

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Introduction

Hypochondroplasia is the mildest form of fibroblast growth factor receptor 3 (FGFR3)-associated skeletal dysplasia with an incidence of about 1 in 50,000 [1]. Affected individuals usually present after 2 years of age and seek medical help at preschool age because of mild body disproportion and short stature. A diagnosis of hypochondroplasia rests on the presence of several distinctive radiologic findings, such as broad long bones, lumbosacral interpedicular distance narrowing, short femoral necks and elongation of the fibula [2, 3]. However, the diagnosis of hypochondroplasia is hampered by the absence of age-dependent radiologic criteria, particularly in the neonatal period. It has been reported that younger affected children are not definitively diagnosed with hypochondroplasia [4].

We previously reported two cases of hypochondroplasia in children with FGFR3 mutations, focusing on prenatal ultrasonography findings in the third trimester and postnatal radiologic findings [5]. These children had short femora with increased biparietal diameter in utero; however, they were not diagnosed with hypochondroplasia in the neonatal period. The final diagnosis was made at the age of 3 years, when they visited our clinic because of short stature. Upon retrospective radiologic review, we learned that the radiologic findings relevant to hypochondroplasia were apparent in the neonatal period and that radiologic diagnosis may have been even easier in the neonatal period than in early childhood. The manifestations related to the ilia and proximal femora were particularly useful. The identification of short, squared ilia with short greater sciatic notches and horizontal acetabula along with the ovoid radiolucency of the proximal femora mimicking that of achondroplasia warranted the diagnosis.

The present study is dedicated to radiologic features in hypochondroplastic neonates with FGFR3 mutations and quantitative measurements that facilitate definitive diagnosis. We propose radiologic criteria for the identification of hypochondroplasia in the neonatal period.

Materials and methods

Subjects included seven hypochondroplasia neonates with FGFR3 mutations, three term neonates with nonsyndromic fetal growth restriction, and 30 term control subjects with available results of radiologic examination within 28 days after birth. All hypochondroplasia neonates underwent radiologic examination in the neonatal period, such as partial skeletal survey or chest and abdominal radiographs, because of short femoral length on fetal ultrasonography or clinically suspected disproportionate micromelia. Control subjects and individuals with nonsyndromic growth restriction were hospitalized from 2010 to 2014 and were born after 36 weeks of gestation. They did not have major congenital anomalies, and they underwent radiologic examination with extension position of hip joint and knee joint because of transient tachypnea of the newborn, meconium aspiration syndrome or suspected neonatal infection. We hypothesized that skeletal changes in the pelvic bones, femora and lumbar spine, which were seen in achondroplasia, were most useful for the diagnosis of hypochondroplasia. Accordingly, we calculated eight parameters and monitored one radiologic sign: (1) ratio of maximal transverse diameter of the ilia to its maximal longitudinal diameter (assessment of short ilia), (2) iliac angle (squared ilia), (3) length of the greater sciatic notches (short greater sciatic notch), (4) acetabular angle (horizontal acetabula), (5) ratio of femoral length (FL) to body length (femoral shortening), (6) ratio of diameter of the femoral mid-shaft to femoral length (broad femora), (7) ratio of width of the distal femoral metaphysis to femoral length (metaphyseal flaring), (8) ratio of interpedicular distance of the L1 vertebra to that of L4 (lumbosacral interpedicular distance narrowing) and (9) presence or absence of ovoid radiolucency of the proximal femora. Measurement procedures are illustrated in Fig. 1.

The open-source OsiriX software dedicated to the analysis of Digital Imaging and Communications in Medicine (DICOM) images (http://homepage.mac.com/rossetantoine/osirix) was

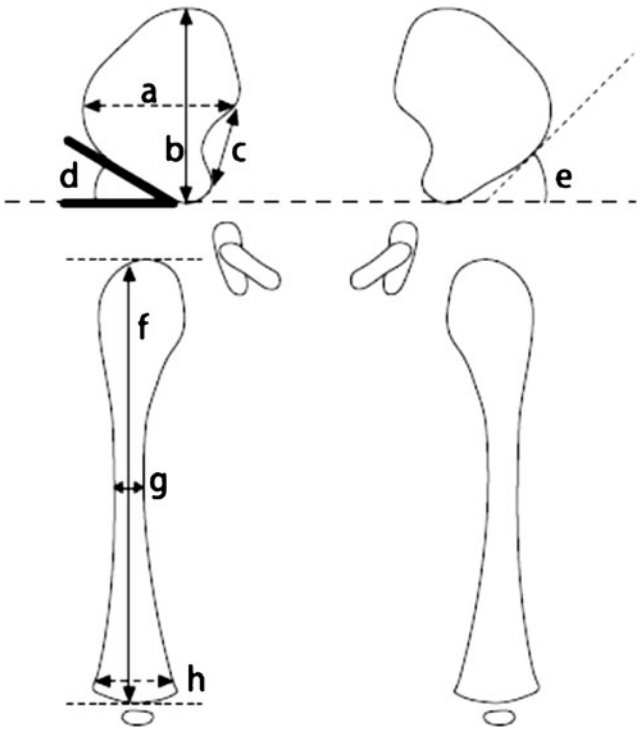

Fig. 1 Diagrams illustrate the measurements based on radiologic findings. The dotted line connects the bottom ends of the ilia: **a** maximal transverse iliac diameter, **b** maximal longitudinal iliac diameter, **c** greater sciatic notch, **d** acetabular roof angle, **e** iliac angle (formed by the tangent line of iliac wing with dotted line), **f** femur length, **g** mid-femur width, **h** maximal distal width of the femur

used for performing measurements. Only radiographs taken with the hip and knee joints extended and without significant joint rotation were analyzed.

Statistical significance of differences between control subjects and hypochondroplasia subjects was analyzed with the Mann–Whitney *U* test. A *P*-value<0.01 was considered significant. All analyses were performed with JMP, version 10.0 (SAS Institute Inc., Cary, NC, USA).

This study was approved by the Institutional Review Board Committee at Niigata University School of Medicine, and informed consent was given by the parents or guardians of the patients with hypochondroplasia.

Results

Clinical manifestations of hypochondroplasia are summarized in Table 1. All the subjects with hypochondroplasia showed low femoral length and biparietal diameter at or above the higher limit of the normal range on prenatal ultrasonography. The results of the Shapiro-Wilk W test showed that all the measurement parameters in the control group followed the Gaussian distribution. The measurement parameters for short ilia, short greater sciatic notch, horizontal acetabula, short femora and broad femora (parameters 1, 3, 4, 5 and 6) were statistically different between the hypochondroplasia and control groups ($P<0.01$), while the remaining parameters were not. Parameters 3, 5 and 6 did not overlap between the 2 groups, while parameters 1 and 4 did (Fig. 2). To distinguish subjects with hypochondroplasia from control subjects, we defined the following cut-off values based on the differences of at least 2 standard deviations from the average values in the control group: >0.80 for parameter 1, <7.5 mm for parameter 3, <22° for parameter 4, <0.14 for parameter 5 and >0.10 for parameter 6. Although assessment of ovoid radiolucency of the proximal femora was somewhat subjective, careful interpretation confirmed its presence in 6 out of 7 children with hypochondroplasia (Fig. 3). There were no abnormalities in other bones.

Based on these results, we defined a tentative scoring system for the diagnosis of hypochondroplasia (Fig. 4). The 2 major criteria (parameters 3 and 6 – short greater sciatic notch and broad femora) were assigned scores of 2. In addition, 4 minor criteria (parameters 1, 4, 5 and 9) were assigned scores of 1 for the following reasons: (a) femoral shortening (parameter 5) was a nonspecific finding; (b) short ilia and acetabular angle (parameters 1 and 4) showed overlaps between the hypochondroplasia neonates and normal controls and (c) the results of the assessment of ovoid radiolucency (parameter 9) were interpreter-dependent. Because all 7 neonates with hypochondroplasia showed combined scores of 6 points or more (Table 2), we presumed that a total score of 6 points or higher warrants thinking about a diagnosis of FGFR3-associated hypochondroplasia. We applied this scoring system to 30 control subjects and the 3 neonates with nonsyndromic growth restriction. The corresponding total scores were less than two in all these cases.

Discussion

It was previously believed that the diagnosis of hypochondroplasia was difficult to establish in infancy. However, recent in utero identification of short femora on prenatal ultrasonography has led to several reports on the early diagnosis of hypochondroplasia [6–10]. It has been found that discrepancy in growth between femoral length and biparietal diameter in the third trimester is highly indicative of this disease [5, 9, 11]. The final diagnosis of hypochondroplasia is established based on the molecular analysis of the FGFR3 gene. This test, however, is relatively expensive and a reliable radiology-based scoring system would be highly beneficial.

Table 1 Genetic and clinical manifestations in 7 children with hypochondroplasia

Child	1	2	3	4	5	6	7
FGFR3 mutation	L324V	N540K	N540K	N540K	S351C	N540K	N540K
Femur length standard deviation score in last trimester	−2.1	−2	-	−3.3	−3.3	−3.5	−2.7
Biparietal diameter standard deviation score in last trimester	0.3	1.3	-	3.3	0.3	3	1.8
Gestational age (weeks) at birth	38	40	38	38	39	38	39
Birth weight (g)	2,780	3,270	2,603	3,102	3,146	2,936	3,228
Birth length (cm)	45.5	49	44.5	49	47	46	45.5
Sex	M	M	F	F	M	F	M
Age at diagnosis[a]	3y 6 m	3y 6 m	1 m	2y	1y 7 m	1 m	1 m

M male, *F* female

[a] The diagnosis was based on the radiologic findings

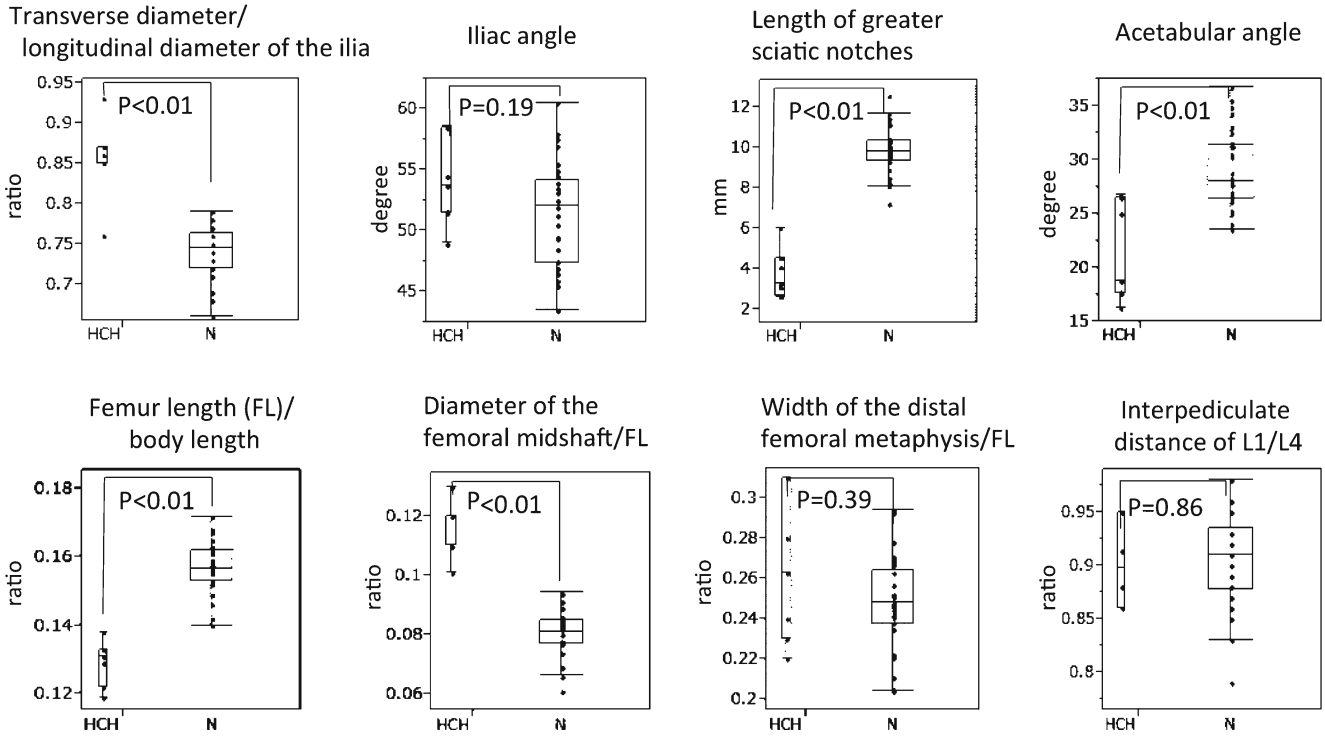

Fig. 2 Results of the measurements in 30 controls and in 7 children with hypochondroplasia. The bottoms and tops of the boxes correspond to the first and third quartiles, respectively, and the horizontal lines inside the boxes indicate the median values. Boxes show as follows: (1) Ratio of maximal transverse diameter to maximal longitudinal diameter of the ilia, (2) iliac angle, (3) length of the greater sciatic notches, (4) acetabular angle, (5) ratio of femoral length to body length, (6) ratio of diameter of the femoral mid-shaft to femoral length, (7) ratio of width of the distal femoral metaphysis to femoral length and (8) ratio of interpediculate distance of L1 to L4. Parameters 1, 3, 4, 5 and 6 were significantly different between the hypochondroplasia and control groups ($P<0.01$). *HCH* hypochondroplasia, *N* control group

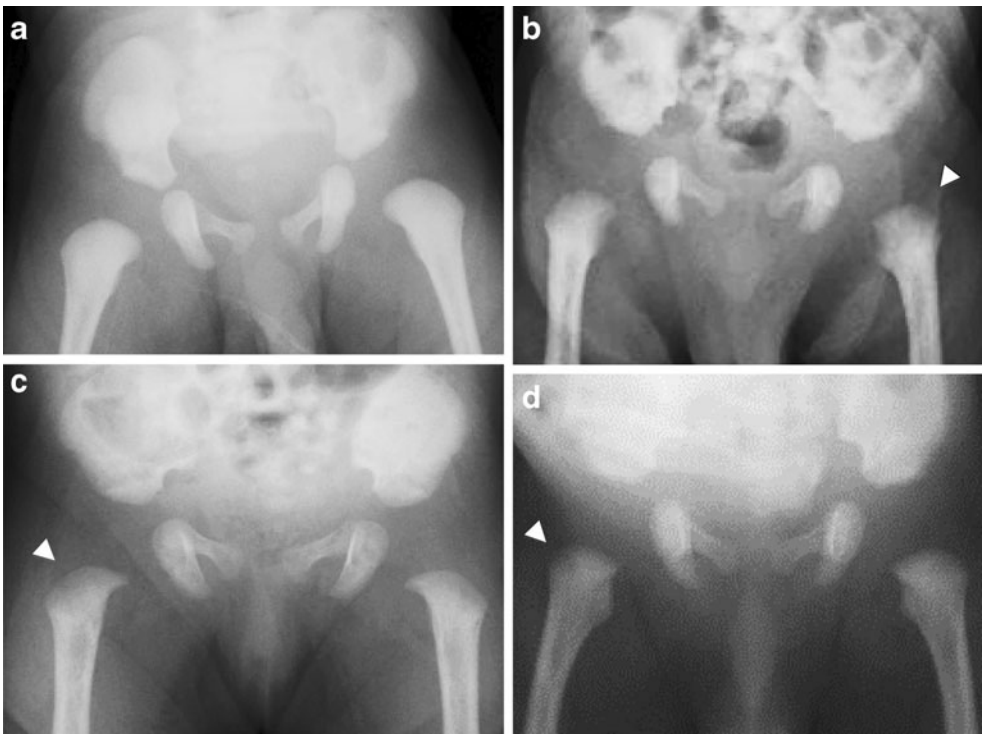

Fig. 3 Ovoid radiolucency of the femoral neck in anteroposterior radiographs. **a** A child in the control group; **b** Child 5 in the hypochondroplasia group (male neonate); **c** Child 6 in the hypochondroplasia group (female neonate); **d** Child 7 in the hypochondroplasia group (male neonate). An ovoid lucency (*arrowheads* in **b-d**) is seen in the femoral neck of the children with hypochodroplasia. All the subjects underwent radiologic examination in the neonatal period.

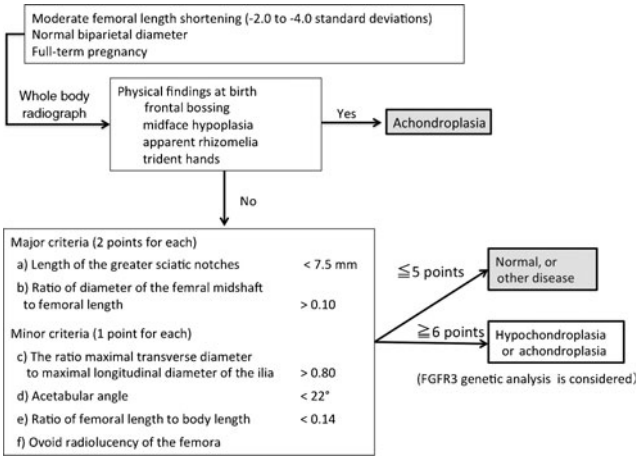

Fig. 4 Proposed flow chart for diagnosis of hypochondroplasia in the neonatal period

In this study, we used radiologic measurements of the ilia and femora to verify that hypochondroplastic neonates had short ilia with short greater sciatic notches and short, broad long bones. Furthermore, ovoid radiolucency of the proximal femora, which reflects the scooped-out appearance of the proximal femoral metaphysis typical of achondroplasia, was always discernible. Horizontal acetabula were also evident, but their presence was inconsistent among the hypochondroplasia neonates. In contrast, although lumbosacral interpedicular distance narrowing is an important diagnostic sign in childhood, it was not useful in our neonatal patients.

Identification of cases with mildly shortened femoral length has become more common with the widespread utilization of fetal ultrasonography. Such cases indicate the presence of mild bone dysplasia exemplified by hypochondroplasia, chromosome disorders such as trisomy 21, and nonsyndromic or syndromic fetal growth retardation (FGR). Although still tentative, our diagnostic criteria might be useful for the differentiation between hypochondroplasia and nonsyndromic growth restriction. Moreover, the radiologic changes in neonates with hypochondroplasia are relatively mild, and their identification may be difficult for nonexperts in bone dysplasias. This emphasizes the potential value of the measurement parameters proposed in the present study.

However, our scoring system is based on nonspecific skeletal changes, such as iliac hypoplasia, a scooped-out appearance of the proximal femora and short, broad femora; thus, it does not enable one to distinguish hypochondroplasia from other skeletal dysplasias, including mild achondroplasia [12]. The final radiologic diagnosis should depend on the overall pattern recognition and other distinctive skeletal changes. For example, cartilage hair hypoplasia causes a diagnostic difficulty in the neonatal period, as does hypochondroplasia [13]. However, mild femoral bowing and round distal femoral epiphyseal ossification warrant a diagnosis of cartilage hair hypoplasia. Molecular diagnoses are essential in difficult cases. To be essential, our scoring system would be utilized as screening for mild neonatal skeletal dysplasias.

The relatively small number of subjects is also a limitation of this study. Furthermore, all measurements were obtained in term neonates; it is currently unknown whether these data are applicable to premature neonates. Finally, correct positioning (extended hip and knee joints without joint rotation) is essential for obtaining interpretable measurements. Further studies in a larger population of hypochondroplasia, including premature neonates, are warranted to validate these criteria for the diagnosis of hypochondroplasia.

Conclusion

We propose a set of diagnostic radiologic criteria that can be useful for early identification of hypochondroplastic neonates.

Table 2 Application of the new scoring system to 7 neonates with hypochondroplasia

Parameter	Child	1	2	3	4	5	6	7
3	Short greater sciatic notches	2	2	2	2	2	2	2
6	Broad femora	2	2	2	2	2	2	2
1	Short ilia	1	1	1	1	1	1	0
4	Horizontal acetabula	1	1	0	1	0	1	0
5	Femoral shortening	1	1	1	1	1	1	1
9	Ovoid radiolucency of the femoral neck	1	0	1	1	1	1	1
Total score		8/8	7/8	7/8	8/8	7/8	8/8	6/8

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Compliance with ethical standards

Conflict of interest None

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Survey of prenatal testing for genetic disorders in Japan: Recent report

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Abstract

Aim: In order to investigate the current status of prenatal testing for genetic disorders, we conducted a multicenter retrospective questionnaire survey.

Methods: The questionnaire was sent to 105 facilities with genetic counseling systems. The questionnaire consisted of two parts: (i) the number of prenatal tests conducted for genetic disorders from January 2010 to December 2012, whether the laboratory was combined with the counseling facility or separate, the sampling procedure method, the testing results, and the outcomes of the affected fetus in addition to treatment; and (ii) a survey of personal comments regarding prenatal testing for genetic disorders.

Results: We received responses from 69 of the 105 facilities (65.7%), and genetic testing was performed at 26 of these facilities. Nucleic acid sequential testing was performed on 45 disorders and 252 cases during a three-year period. There were 67 cases of affected fetuses. Six cases continued pregnancy and were treated. The comment survey highlighted difficulties in locating a laboratory to assess prenatal samples, as well as inadequate counseling and preparation for genetic disorders.

Conclusions: Our study revealed that a number of prenatal testing for genetic disorders are conducted in Japan; however, it is difficult for counselors to locate a laboratory capable of testing for specific genetic disorders. Inadequate counseling and healthcare providers' lack of knowledge is a current problem. A well-established system of prenatal testing for genetic disorders and the further education of general physicians is required.

Key words: counseling, genetic disorder, prenatal testing.

Introduction

The advances in molecular genetics enable us to analyze gene mutations in patients with genetic disorders. Genetic information remains constant over a lifetime; therefore, it requires careful scrutiny via preclinical and prenatal genetic testing. The Japanese Association of Medical Sciences and the Japan Society of Obstetrics and Gynecology (JSOG) developed guidelines and opinions for appropriate genetic counseling prior to prenatal genetic testing.[2,3] Based on this, pregnant carriers of genetic disorders in the neonatal or childhood periods can obtain adequate genetic counseling before and after prenatal testing for the genetic disorder.

Several methods for the prenatal testing of genetic disorders are available. The majority test for chromosomal abnormalities, such as aneuploidy, by means of a maternal serum blood test, ultrasound examination, and noninvasive prenatal genetic testing.[4] Recent advances in molecular biology have made major contributions to

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prenatal diagnosis. An increasing number of genetic disorders have been identified in the prenatal period using fetal cells derived from invasive testing.[5–9]

In some genetic disorders, such as ornithine transcarbamylase deficiency, prenatal genetic testing is useful for neonatal treatment.[10] However, general obstetricians are not familiar with the requirements for prenatal testing and lack current information regarding genetic testing and neonatal treatment. Additionally, in Japan, private companies that provide DNA testing do not offer the prenatal service, and almost all prenatal molecular testing is performed in non-commercial research laboratories. Therefore, it is difficult to find an appropriate laboratory for the prenatal testing of genetic disorders.

In order to investigate the current status of prenatal testing for genetic disorders, we, as members of the subcommittee in Perinatology Committee of the JSOG, conducted a multicenter retrospective questionnaire survey.

Methods

The questionnaire was sent to 105 facilities, including Miyagi Children's Hospital and 104 other facilities, which belong to Japan's National Liaison Council for Clinical Sections of Medical Genetics and contain genetic counseling systems.

The questionnaires consisted of two parts: (i) the number of prenatal testing for genetic disorders evaluated from January 2010 to December 2012, whether the laboratory that received the samples contained a counseling facility or the testing was outsourced domestically or abroad, the sampling procedure, the testing results and the outcome of the affected fetus and treatment; and (ii) a survey of personal comments for prenatal genetic testing. We allowed for five options and space for a description.

Results

We received responses from 69 of the 105 facilities (65.7%). Prenatal testing for genetic disorders was conducted on 46 disorders and 277 cases during a three-year period. Testing was performed at 26 facilities (Table 1). Nucleic acid sequential testing was performed on 45 disorders and 252 cases. Only gender testing was performed on nine disorders and 22 cases, while only enzymatic activity testing was performed on two disorders and two cases. Genetic testing was not performed for a chromosome abnormality in one case.

The number of prenatal tests conducted for each disorder and the testing laboratory is presented in Table 2. The testing laboratory and the counseling facilities were the same for 32 disorders and 142 cases (56.3%). The testing was outsourced within Japan for 26 disorders and 107 cases (42.5%). Among them, 13 disorders were tested at another facility that responded to the questionnaire. The remaining disorders were assessed at unknown facilities. Genetic testing was conducted abroad for three disorders and three cases (1.2%); however, all three diseases could have been assessed at one of the domestic facilities that responded to the questionnaire.

Table 1 The number of prenatal tests for genetic disorders conducted at each facility during a three-year period

Number of genetic tests	Number of facilities
1–3	12
4–9	7
More than 9	7

Ninety-four genetic tests were conducted at the National Center for Child Health and Development, 64 at Tokyo Women's Medical University Hospital, 16 at Osaka University Hospital, 12 at Tottori University Hospital, 10 at Hokkaido University Hospital, 10 at Hiroshima University Hospital and 10 at Miyagi Children's Hospital.

In regard to the type of sampling procedure, chorionic villi sampling (CVS) was conducted on 203 cases (73%), amniocentesis on 70 cases (25%), and the other four cases (2%) entailed fetal gender testing via maternal blood examination. There were 67 cases of affected fetuses. Among autosomal dominant disorders, four of 12 cases (33.3%) were affected, while 63 of 240 cases (26.2%) were affected by recessive disorders. Two cases were undefined as a result of contamination.

The outcomes of the affected fetuses are presented in Table 3. In the six cases in which the pregnancy was continued, the disorders were treated. One infant affected with the Wiskott-Aldrich syndrome was treated with an unknown medication after birth. In two cases of a female fetus affected with 21-hydroxylase-deficient congenital adrenal hyperplasia (21-OHD CAH), the mothers continued glucocorticoid intake to prevent neonatal masculinization of genitalia. In a case of a male fetus affected with 21-OHD CAH, the mother discontinued glucocorticoid intake after being apprised of the fetal gender. In another case of a female fetus affected with 21-OHD CAH, the prenatal diagnosis suggested an atypical outcome of 'not affected'. Because of the incorrect prenatal diagnosis, the mother discontinued glucocorticoid intake and the infant exhibited masculinization of genitalia at birth. In a case of myotonic dystrophy, prenatal testing showed that the fetus was not affected; however, the infant was floppy, and was diagnosed with congenital myotonic dystrophy.

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Table 2 The number of genetic disorders identified and destinations of the testing samples

Disease	Number of genetic tests	Number of cases/facilities (Same)†	Number of cases/facilities (Domestic)‡	Number of cases/facilities (Abroad)§
Neuromuscular Diseases				
Fukuyama-type congenital muscular dystrophy	52	24/4	28/7	ND
Spinal muscular atrophy	46	19/1	27/9	ND
Duchenne muscular dystrophy	38	28/6	10/2	1/1
Myotonic dystrophy	9	1/1	8/5	ND
X-linked hydrocephalus	6	2/2	4/4	ND
Myotubular myopathy	5	3/2	2/2	ND
Ryanodine receptor disorders	5	2/2	3/1	ND
Pelizaeus-Merzbacher disease	4	3/1	1/1	ND
Adrenoleukodystrophy	1	ND	1/1	ND
Lissencephaly	1	ND	1/1	ND
Joubert syndrome	1	ND	1/1	ND
Metabolic Diseases				
Ornithine transcarbamylase deficiency	7	6/3	1/1	ND
Hunter syndrome	6	5/1	1/1	ND
Carbamoyl phosphate synthetase deficiency	4	3/2	1/1	ND
Gaucher disease	4	2/1	2/2	ND
Methylmalonic acidemia	3	3/3	ND	ND
Mucolipidosis II	3	3/2	ND	ND
MCT8 gene disorder	3	ND	3/1	ND
Menkes disease	2	2/1	ND	ND
Krabbe disease	2	2/1	ND	ND
Zellweger syndrome	2	ND	2/2	ND
Pompe disease	1	1/1	ND	ND
Pyruvate dehydrogenase complex deficiency	1	1/1	ND	ND
Glutaric acidemia	1	1/1	ND	ND
Very-long-chain acyl-CoA dehydrogenase deficiency	1	1/1	ND	ND
Trifunctional protein deficiency	1	1/1	ND	ND
Metachromatic leukodystrophy	1	1/1	ND	ND
Tay-Sachs disease	1	1/1	ND	ND
Holocarboxylase synthetase deficiency	1	ND	1/1	ND
Niemann-Pick disease	1	ND	1/1	ND
Endocrine Diseases				
21-Hydroxylase-deficient congenital adrenal hyperplasia	17	13/4	4/3	ND
Skin Diseases				
Xeroderma pigmentosum	3	3/1	ND	ND
Congenital ichthyosis	3	ND	3/3	ND
Bone Diseases				
Hypophosphatasia	3	3/2	ND	ND
Osteogenesis imperfecta II	1	ND	1/1	ND
Achondroplasia	1	1/1	ND	ND
Urinary Tract Disorders				
Lowe syndrome	2	1/1	ND	1/1
Autosomal recessive polycystic kidney	1	1/1	ND	ND
Congenital nephrotic syndrome of the Finnish type	1	ND	1/1	ND
Immunodeficiency Diseases				
X-linked severe combined immunodeficiency	2	2/1	ND	ND
Wiskott-Aldrich syndrome	2	1/1	ND	1/1
Chronic granulomatous disease	1	1/1	ND	ND
Other				
Juvenile polyposis syndrome	1	ND	1/1	ND
Bannayan-Riley-Ruvalcaba syndrome	1	ND	1/1	ND

†Same: the laboratory that conducted the genetic testing was the same facility. ‡Domestic: the laboratory that conducted the genetic testing was another domestic facility (in Japan). §Abroad: the laboratory that conducted the genetic testing was abroad (outside Japan). ND, no data.

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Table 3 Outcomes of affected fetuses

Outcome of affected fetuses	Number
Termination of pregnancy	57 (85%)
Continuation of pregnancy	6 (9%)
Unknown	4 (6%)

The percentage of respondents who provided comments is presented in Table 4. Representative descriptions included: 'It is necessary to locate an appropriate laboratory following each instance of genetic counseling, and the requirements for genetic testing differ for each laboratory'; and 'The incomplete system for genetic testing in domestic laboratories forces an increasing number of samples from patients to be sent abroad'.

Discussion

In this report, we presented the current status of prenatal testing for genetic disorders in Japan. Forty-five disorders and 252 cases of prenatal genetic nuclear acid sequencing testing were conducted during a three-year period. To the best of our knowledge, this is the first nationwide report regarding prenatal testing for genetic disorders in Japan. However, this survey had some limitations.

Any single facility could not conduct all types of genetic testing. The testable genetic disorders differed at each facility. Therefore, it was difficult to ascertain how many samples were submitted to outsourced laboratories. In this survey, 13 genetic disorders could not be assessed at the responding facilities. This indicated that a facility not surveyed or that did not respond to the questionnaire actually conducted a number of prenatal tests for genetic disorders.

Table 4 Comments for prenatal testing for genetic disorders

Comments	Agreement† (%)
There are no commercial laboratories to treat prenatal genetic samples in Japan.	78.3
Patient consultation was too late for appropriate evaluation (e.g. after pregnancy).	58.0
Case lacks genetic testing of the proband.	52.2
Approval by ethical review board is required in each case.	37.7
Genetic counseling was inadequate.	20.3

†Agreement denotes the percentage of respondents who made the comment.

In the comment survey for prenatal genetic testing, 78% of responding facilities advised that no commercial laboratory was available to assess prenatal genetic samples. This might indicate frustration with suboptimal systems for the prenatal testing of genetic disorders in Japan. Some respondents were unsure of the accuracy of the testing, and misinterpretation could lead to an inappropriate choice. Furthermore, the individuals in charge of the prenatal testing carried a heavy responsibility in terms of the results. It is difficult for counselors to obtain appropriate prenatal testing in Japan. Even if a domestic laboratory could have conducted the prenatal testing for a genetic disorder, the counselors may have thought that it would be easier to send the samples abroad. Testing abroad may often be an expensive and time-consuming process; thus, a well-established system of prenatal genetic testing within Japan is needed.

In the comment survey, 58% of the responding facilities stated that the consultation for prenatal testing was too late for appropriate evaluation (e.g. after establishment of next pregnancy); and 52% of the respondents commented that some cases lacked genetic testing of the proband. Furthermore, some respondents also noted that previous counseling was inadequate, which impacted the counseling quality for subsequent pregnancy. These indicated that general physicians and patients were unfamiliar with the preparation required for appropriate genetic testing in subsequent pregnancy. Therefore, education of general physicians regarding prenatal testing for genetic disorders would be beneficial.

In conclusion, our study revealed that a number of prenatal testing for genetic disorders were conducted in Japan; however, it was difficult for counselors to locate an appropriate laboratory that could test for a specific genetic disorder. Inadequate counseling and testing for genetic disorders is a current problem. Therefore, in Japan, a well-established system of prenatal testing for genetic disorders and further educational programs for general physicians is required.

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Disclosure

The authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

Parental serum alkaline phosphatase activity as an auxiliary tool for prenatal diagnosis of hypophosphatasia

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ABSTRACT

Objective The objective of this study is to clarify the usefulness of parental alkaline phosphatase (ALP) for prenatal diagnosis of hypophosphatasia (HPP).

Methods Maternal (m) and paternal (p) ALP values were measured in 77 cases from a multicenter cohort (fetal skeletal dysplasia forum in Japan) of cases with short limbs on ultrasonography during pregnancy. After birth, X-rays, cord blood ALP, and gene analysis were evaluated to achieve an exact diagnosis. The screening usefulness of ALP was examined retrospectively.

Results Seventeen cases were eventually diagnosed as HPP and 60 as not HPP; the overall mean m-ALP and p-ALP (standard deviation) values were 133.4 (53) versus 197 (69) IU/L and 149.6 (71.8) versus 231 (61.4) IU/L ($p < 0.001$). Receiver operating characteristic curve analysis showed that the optimal m-ALP and p-ALP cutoff values were 123 and 165 IU/L, respectively. Presence of at least one of the m-ALP or p-ALP values abnormally low had a sensitivity, specificity, and positive predictive values of 82% (14/17), 93%, and 78%, respectively, for the diagnosis of HPP.

Conclusion Parental ALP measurement might be an auxiliary tool to hone in the prenatal diagnosis of fetal HPP.
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INTRODUCTION

Hypophosphatasia (HPP) is a well-known metabolic bone disease caused by a defect involving impaired mineralization with thin limbs and long bone bowing. Short limbs characterize the condition especially in the Japanese population. The perinatal type is defined as a life-threatening fetal disease.[1–3] Recently, new enzyme-replacement therapy after birth has been reported as a possible and promising therapy. Thus, some babies could be saved by this therapy without major sequelae.[4]

After birth, exact diagnosis of HPP can be performed by radiographic examination, enzyme activities, and gene analysis,[5–7] and, recently in Japan, clinical diagnosis by imaging after birth, gene analysis, and successful enzyme-replacement therapy has been reported.[8] As for the prenatal differential diagnosis of fetal skeletal dysplasia, ultrasonographic imaging is used first as a screening tool.[9,10] However, a definitive imaging diagnosis of HPP has not yet been established. Wenkert *et al.*[11] reported 15 different prenatal diagnoses, including type II osteogenesis imperfecta (OI) and campomelic dysplasia among 17 cases of benign-type HPP confirmed after birth. Given the new medical interventions available after birth, the focus has been on a more precise prenatal diagnosis. A definitive prenatal

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diagnosis of HPP can be attained with tools other than imaging, such as parental and fetal gene analysis or measurement of cord blood alkaline phosphatase (ALP) activity. However, both methods are invasive and not cost-effective, given the large number of fetal skeletal dysplasias.

We analyzed the usefulness of parental blood ALP[12,13] as an auxiliary tool for the differential diagnosis of HPP from the many other fetal skeletal dysplasias and causes of short-limb fetuses in order to narrow down the differential diagnosis prior to proceeding to the diagnostic step, such as cord blood analysis of ALP, gene examination, and preparation of neonatal enzyme-replacement therapy. ALP activity increases gradually during pregnancy particularly in the third trimester, because of the contribution of the placental isoenzyme.[13] Thus, we have also assessed the effect of the trimesters of pregnancy on the screening efficacy of ALP measurement.

MATERIALS AND METHODS

This was a retrospective analysis of all cases submitted to the Fetal Skeletal Dysplasia Forum in Japan from 2007 to 2016. Such forum consists of a panel of voluntary experts in prenatal diagnosis.

Final diagnoses were made by radiographic analysis and gene analysis after birth, when indicated, to attain the final diagnoses of thanatophoric dysplasia (TD), OI, HPP, campomelic dysplasia, spondyloepiphyseal dysplasia congenita, and others. Maternal skeletal diseases with clear phenotype were not included.

In all cases, maternal ALP activity and paternal ALP activity if possible were measured. Maternal ALP activity was measured through the prenatal period just after the initial preliminary diagnosis of fetal bone disease. After final diagnosis, the parental ALP values and their accuracy for the exact diagnosis of fetal HPP were evaluated. ALP activity assays were performed in a few different ways. The value of the Japanese standard method, such as that of the Japanese Society of Clinical Chemistry,[14] was used, and the adult normal range has been established as 110–350 IU/L in every institute.

In our population, three major skeletal diseases are found more frequently: HPP, TD, and OI. Thus, the possibility of differential diagnosis was evaluated particularly focusing on these three diseases. Furthermore, the impact of gestational age on the accuracy of prenatal diagnosis was analyzed.

Statistical analysis was performed using SPSS version 20 (IBM Inc., Armonk, NY, USA) and PRISM version 5 (MDF Inc., Tokyo, Japan). A p value below 0.05 was considered significant. Receiver operating characteristic curve analysis was used to establish optimal screening thresholds of ALP values in maternal and paternal blood samples. All patients who registered with the fetal skeletal dysplasia forum provided written informed consent to participate in this study. The protocol was approved by the Institutional Review Board of Nagara Medical Center with regard to human rights and privacy issues.

RESULTS

There were 77 cases in the cohort, including 17 of HPP (Table 1), 6 of achondrodysplasia, 18 of TD, 24 of OI, 2 of spondyloepiphyseal dysplasia congenita, 2 of campomelic dysplasia, 3 of fetal growth restriction, and 5 other skeletal dysplasias (Figure 1). The mean ± standard deviation gestational age at measurement of maternal ALP was not significantly different between the HPP and non-HPP groups (26.7 ± 5.7 vs 25.7 ± 6.8 weeks, respectively; $p > 0.05$). Overall,

Table 1 **Parental serum ALP value and background of HPP cases**

Case	GW measure	Maternal ALP	Paternal ALP	Umbilical ALP	Prognosis	Genetic analysis
1	17	81	54	4	Still birth	
2[15]	18	160	250	2	Still birth	Paternal 1559delT, UPD
3	19	122	155	8	Still birth	1559delT, parent hetero
4	21	79	127	3	Still birth	
5	21	80	No data	No data	Still birth	
6	24	76	227	6	ND 37 weeks, 2119 g	
7	26	165	No data	No data	Alive, benign type	
8	27	62	No data	No data	Alive	
9	28	117	No data	6	ID 4 months	1559delT
10	29	165	121	7	Still birth 32 weeks, 1794 g	
11[8]	29	110	No data	0	Alive, ERT	exon12c 1471G >A
12	31	170	No data	3	ND, 31 weeks, perinatal lethal	1559delT
13	31	121	No data	0	ID 4 months	1559delT, parent hetero
14	32	240	110	82	Alive, ERT	
15	33	183	134	33	Alive, ERT	1559delT
16	33	108	300	205	Alive, benign type	exon7, hetero
17	35	229	118	9	ND 39 weeks, 2710 g	

ND, neonatal death; ID, infant death; ERT, enzyme-replacement therapy; hetero, heterozygous mutant; ALP, alkaline phosphatase; HPP, hypophosphatasia; GW, gestational weeks; UPD, uniparental disomy.

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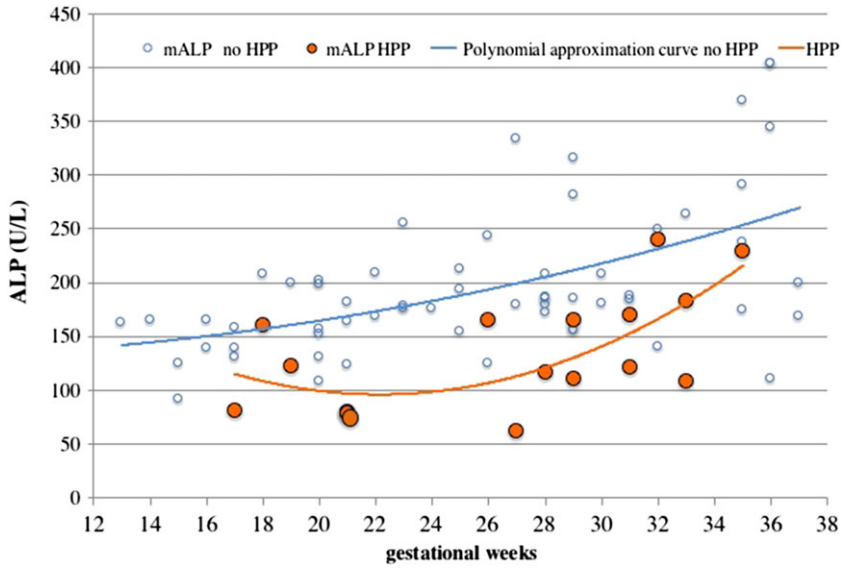

Figure 1 **All maternal alkaline phosphatase (mALP) values for short-limb cases including fetal hypophosphatasia (HPP) and other skeletal disease measured during pregnancy**

maternal ALP values were significantly lower in HPP than in non-HPP cases (133.4 ± 53 vs 197.4 ± 69 IU/L, $p < 0.001$). Significant differences were seen before 28 weeks of gestation (103.5 ± 40 vs 172.8 ± 46.6 IU/L, $p < 0.001$) and after 28 weeks (160.3 ± 50 vs 227.3 ± 80.5 IU/L, $p = 0.0016$) (Table 2).

Paternal ALP values were measured in 37 cases, and ALP values were significantly lower in HPP ($n = 10$) than in non-HPP cases (149.6 ± 72 vs 231 ± 61 IU/L, $p = 0.0016$).

Focusing on the three major skeletal dysplasias (HPP, OI, and TD), maternal ALP values showed significant differences between HPP and OI ($p < 0.001$, multiple comparison, post-hoc Tukey test). Importantly, during the second trimester, significantly different ALP values were noted between both HPP and OI ($p < 0.001$) and HPP and TD ($p < 0.05$). As for paternal ALP values, significant differences were noted between HPP and OI ($p < 0.05$) and between HPP and TD ($p < 0.001$) (Tables 3 and 4).

The optimal maternal ALP cutoff value for diagnosis was established by receiver operating characteristic curve analysis as 123 IU/L (any time during pregnancy), and the optimal paternal ALP value as 165 IU/L (Figure 2). When using 'at least one low value' diagnostic criterion based on these maternal and paternal cutoff values, the sensitivities throughout the entire pregnancy period, before 28 weeks of gestation, and after 28 weeks of gestation until 37 weeks of gestation were 82.4%, 93.3%, and 77.8%. The corresponding specificities were 75%, 93.9%, and 75%, with positive predictive values of 80%, 96.2%, and 88.9%, respectively (Table 5). Although the numbers were small, the paternal low ALP activity had high

Table 2 **Parental ALP values for HPP and non-HPP groups**

	Maternal ALP HPP all period	Maternal ALP no HPP all period	Statistics
n	17	60	
Mean (SD)	133.4 (53)	197 (69)	0.0008
Range	62–240	91–404	
	Maternal ALP HPP <28 weeks	Maternal ALP no HPP <28 weeks	
n	8	33	
Mean (SD)	103.5 (40.2)	172.8 (46.6)	0.0009
Range	62–165	91–333	
	Maternal ALP HPP 28–37 weeks	Maternal ALP no HPP 28–37 weeks	
n	9	27	
Mean (SD)	160.3 (50.48)	227.3 (80.5)	0.0167
Range	108–240	110–404	
	Paternal ALP HPP	Paternal ALP no HPP	
n	10	27	
Mean (SD)	149.6 (71.8)	231 (61.4)	0.0016
Range	54–300	130–429	

ALP, alkaline phosphatase; HPP, hypophosphatasia; SD, standard deviation.

Table 3 **Serum ALP value among three major diseases such as HPP, OI, and TD**

	n	Mean	SD	Median	Range
OI all period	24	216	91	192.5	91–404
TD all period	18	174	38.6	178.5	108–263
HPP all period	17	133.6	53	121	62–240
OI <28 weeks	14	179.5	62.8	161	91–333
TD <28 weeks	14	163.7	33.4	173	108–209
HPP <28 weeks	8	103.5	40.1	81	62–165
Paternal ALP OI	14	223.9	79.2	196	178–269
Paternal ALP TD	9	248.7	37.9	232	220–329
Paternal ALP HPP	10	179.6	72	127	54–300

HPP, hypophosphatasia; OI, osteogenesis imperfecta; TD, thanatophoric dysplasia; SD, standard deviation; ALP, alkaline phosphatase.

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Table 4 **Multiple comparison (post-hoc Tukey test) of serum ALP value among three major diseases such as HPP, OI, and TD**

Factors (Tukey)	Mean difference	*q*	Significance	95% CI of difference
All period HPP vs TD	−40.3	2.46	ns	−96.12 to 15.52
All period HPP vs OI	−83.25	5.422	$p < 0.001$	−135.6 to −30.93
All period TD vs OI	−42.94	2.844	ns	−94.41 to 8.516
HPP <28 weeks vs TD <28 weeks	−60.21	3.975	$p < 0.05$	−112.8 to −7.609
HPP <28 weeks vs OI <28 weeks	−76	5.017	$p < 0.001$	−128.6 to −23.39
TD <28 weeks vs OI <28 weeks	−15.79	1.222	ns	−60.65 to 29.08
Paternal HPP vs paternal TD	−99.07	4.47	$p < 0.001$	−176.3 to −21.81
Paternal HPP vs paternal OI	−74.26	3.718	$p < 0.05$	−143.9 to −4.639
Paternal TD vs paternal OI	24.81	1.204	ns	−47.03 to 96.65

ALP, alkaline phosphatase; HPP, hypophosphatasia; OI, osteogenesis imperfecta; TD, thanatophoric dysplasia; CI, confidence interval; ns, not significant.

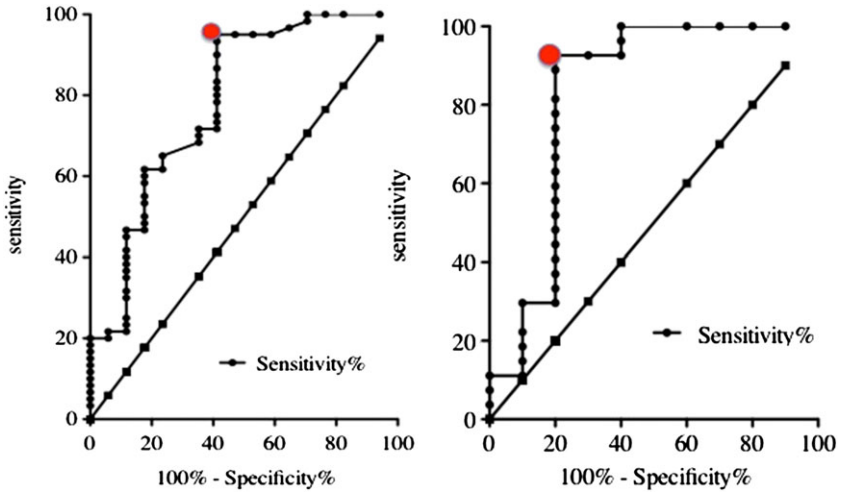

Figure 2 **Receiver operating characteristic curve of alkaline phosphatase (ALP) for the diagnosis of hypophosphatasia, with an optimal maternal ALP cutoff value of 123 and paternal ALP cutoff value of 165 [Colour figure can be viewed at wileyonlinelibrary.com]**

sensitivity, specificity, and positive predictive values of 80%, 92.6%, and 80%.

DISCUSSION

Recent medical developments such as enzyme-replacement therapy for HPP have increased survival even without sequelae.[4] It is thus important to reach a precise prenatal diagnosis of HPP. However, because of the many variations and limitations of fetal imaging, exact imaging criteria for the diagnosis for HPP have not yet been established. Although cord blood sampling for fetal ALP and parental gene analysis can allow accurate prenatal diagnosis, both methods are not easy to perform as first routine screening tests in cases of short fetal limbs because of their cost and invasiveness. We have found that parental blood ALP values provide a supportive tool to identify cases which may benefit from further diagnostic examination and preparation for enzyme-replacement therapy soon after delivery.

Because of the natural increase in maternal ALP values in late pregnancy both in normal and fetal HPP cases[13] due to ALP secretion from the placental isoenzyme, many investigators have abandoned using this method for prenatal diagnosis of the condition. However, the present results show the effectiveness of the 'at least one low' screening criterion (i.e. at least maternal and/or paternal ALP, cutoff below 123 and/or 165 IU/L, respectively). During the period of this study, 17 HPP cases were seen: paternal ALP activity was measured in 10 cases, and the positive rate of 'at least one low' was 82% (14/17 cases). Three cases showed both low maternal and paternal ALP values. Because paternal ALP is not affected by placental secretion of ALP, it can be measured at any time during the pregnancy. Indeed in our series, after 28 weeks, only 3/8 cases would have been detected by low maternal ALP measurement, whereas 4 cases would have been diagnosed using low paternal values. Thus, overall 7/8 cases would have been screened positive using 'at least one low' criterion.

An additional benefit of ALP is that the cost of performing this measurement is low as it is commonly used in daily clinical medicine. Only one case previously reported[15] showed normal ALP activity criteria for both parents. This case was found to have a paternal genomic abnormality of 1559 del and showed uniparental disomy.

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Table 5 Diagnosis accuracy of fetal HPP of parental serum ALP activity

	HPP		No HPP		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPA (%) (95% CI)	NPA (%) (95% CI)
	Low	Normal	Low	Normal				
At least 1 low in all period	14	3	4	56	82.4 (59–106)	93.3 (85–101)	77.8 (53–102)	94.9 (88–102)
Maternal low in all period	10	7	3	57	58.8 (29–89)	94.9 (88–102)	76.9 (48–106)	88.9 (79–98)
Paternal low in all period	8	2	2	25	80 (48–112)	92.6 (80–105)	80 (48–112)	92.6 (80–105)
At least 1 low <28 weeks	6	2	2	31	75 (37–113)	93.9 (84–104)	75 (37–113)	93.9 (84–104)
Maternal low <28 weeks	6	2	2	31	75 (37–113)	93.9 (84–104)	75 (37–113)	93.9 (84–104)
Paternal low <28 weeks	4	1	0	19	80 (35–125)	100 (1–1)	100 (1–1)	95 (83–107)
At least 1 low 28–37 weeks	8	2	1	25	80 (48–112)	96.2 (87–106)	88.9 (63–115)	92.6 (80–105)
Maternal low 28–37 weeks	14	3	5	26	82.4 (59–106)	83.9 (67–100)	73.7 (48–99)	89.7 (76–104)
Paternal low 28–37 weeks	8	1	2	25	88.9 (63–115)	92.6 (80–105)	80 (48–112)	96.2 (87–106)

At least 1 low, lower ALP either mother (ALP < 123) or father (ALP <165) using ROC cutoff, respectively.
PPA, positive predict accuracy; NPA, negative predict accuracy; ROC, receiver operating characteristic; ALP, alkaline phosphatase; HPP, hypophosphatasia; CI, confidence interval.

In considering the genetics of HPP, the mother, the father, or both can be carriers, as the condition follows either an autosomal recessive or – less commonly – an autosomal dominant inheritance. The carrier frequency is estimated to be 1/480 in Japan, based on genetic analyses.[16] Among all of the skeletal diseases, the Japanese population seems to have a higher incidence of fetal HPP. Mulivor *et al.*[1] noted that carriers apparently heterozygous for HPP had enzyme activity levels substantially lower than controls. A heterozygous carrier for HPP might show 50% ALP enzyme activity. So measurement of ALP activities in both parents would be useful for screening the carrier state and the HPP fetus.

Recently, a benign perinatal type of HPP has been diagnosed. Fetal diagnosis by imaging is also difficult for such mild phenotype cases.[12] Thus, ALP as a test is helpful after diagnosis of fetal skeletal dysplasia or bone disease by prenatal screening imaging. We successfully detected a case of benign type by measuring a maternal ALP value of 108 IU/L at 33 weeks of gestation (Table 2, case 16) although the fetus showed only slight bowing of the femur. Our method may contribute to detect benign perinatal types of HPP.

In light of our findings, a practical and cost-effective screening flow chart for HPP would include: (1) short-limb and thin head bone (clear brain imaging) on prenatal imaging and (2) ALP measurements of the parents with a low value of at least one (maternal and/or paternal). Cases fulfilling such criteria would benefit from more invasive or expensive prenatal diagnostic tests.

A limitation of this analysis is that a nomogram of ALP activity during the pregnant period has not been established in all countries outside of Japan. Additional studies are needed to confirm the value of parental ALP in the screening of HPP outside of Japan.

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The authors would like to express their special gratitude to the other members of the fetal skeletal dyplasia forum in Japan such as consisting of volunteer member of experts for prenatal diagnosis established in 2007.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Prenatal diagnosis of fetal HPP is difficult to achieve.
- An accurate prenatal diagnosis is desirable because enzyme-replacement therapy after birth is beneficial.

WHAT DOES THIS STUDY ADD?

- In addition to fetal imaging, measuring parental ALP activity is useful to distinguish HPP from osteogenesis imperfecta and other fetal short-limb conditions throughout the whole period of pregnancy.
- Documentation of at least one parent with a low level of ALP seems to be a useful criterion to hone in the diagnosis of fetal HPP.
- ALP assay is not invasive, is inexpensive, and is easy to use.

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Follow-Up Study on Fetal CT Radiation Dose in Japan: Validating the Decrease in Radiation Dose

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OBJECTIVE. In 2011, we collected data on fetal CT radiation dose to determine the diagnostic reference level (DRL); however, continuous evaluation of the DRL is necessary. The hypothesis of this study is that the fetal CT radiation dose has decreased, and we predict a widespread use of iterative reconstruction (IR). We also predict that the national decrease in exposure is because of the DRL reported as a result of the previous national study.

MATERIALS AND METHODS. Various testing protocols from each site were summarized as part of the study results. The minimum, one-fourth (25th percentile), median, three-fourths (75th percentile), and maximum values were obtained for volume CT dose index ($CTDI_{vol}$), dose-length product (DLP), and scan length of 120 fetal CT examinations. The trends for IR usage and tube voltage were also investigated.

RESULTS. Compared to the results of the 2011 study (n = 119), the minimum, 25th percentile, median, and 75th percentile values for $CTDI_{vol}$ and DLP have decreased for the tabulated results in 2015 (n = 120). The 75th percentile value for $CTDI_{vol}$ was 4.9 mGy, which is 43% of the previous value. IR was used in 70% of the sites. The radiation dose was significantly lower among groups that used IR.

CONCLUSION. Four years passed between our initial survey on DRL and the present follow-up survey, and it appears that the previous report sufficiently fulfilled its objective and role in contributing to the decrease in DRL observed in this follow-up study.

Keywords: CT, diagnostic reference level, fetus, prenatal diagnosis, skeletal dysplasia

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According to an international classification issued in 2015 [1], there are 436 disease names, classified into 42 disease groups, and 364 responsible genes associated with genetic disease skeletal dysplasia. During the neonatal period, these diseases are found at a rate of two in 10,000 births, and half are lethal [2]. The recent widespread use of fetal ultrasound testing and improvement in diagnostic accuracy has led to the detection of short limbs and more cases of suspected skeletal dysplasia [3]. The advantage of ultrasound is that it can be used for pregnant women without involving x-ray irradiation. However, it is extremely difficult to detect, evaluate, and interpret the abnormal findings of skeletal structures using ultrasound, and a specific diagnosis cannot be made by the ultrasound operator without vast knowledge and experience in making such diagnoses. Unless the region that may contain the abnormalities is searched in detail during the scan, sufficient material for retrospective diagnosis cannot be obtained. This is the disadvantage of ultrasound testing that is based on real-time diagnosis.

However, the effectiveness of prenatal 3D CT for detecting fetal skeletal dysplasia has been previously reported, and 93% of pathognomonic abnormal findings confirmed after birth with simple x-ray were diagnosable with fetal skeletal CT. It was reported that the diagnosis determined by ultrasound was revised for about 60% of the cases after fetal CT [4].

Prenatal diagnosis using CT provides visual and clinical information to facilitate planning for a birthing method and selection of treatment by the various people involved, including the parents, family members, obstetrician, and perinatal care staff. It also allows the parents of the fetus to prepare themselves psychologically. Although there are many advantages, the risk of radiation exposure to the fetus and mother cannot be avoided because CT uses x-rays.

In 2011, we surveyed medical institutions in Japan that conduct fetal CT and collected data on CT conditions and radiation exposure; that study was published in 2014 [5].

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From the data, we reported the estimated diagnostic reference level (DRL). To our knowledge, that report was the first and only fetal CT DRL study in the world. We decided to investigate DRL again in 2015 because 3 years and 9 months had passed since the initial survey was conducted. Periodic assessment of DRL every few years at each medical institution or at the national level is recommended [6]. Since the first study was conducted, CT equipment has seen further progress in the number of detectors, and high-performance CT equipment with 64 or more detectors are almost in common use. In addition, there are some reports in the pediatric radiology field that show an increase in popularity for using an iterative reconstruction (IR) protocol to reduce the radiation dose [7].

The hypothesis of this study is that the fetal CT radiation dose has decreased throughout the country compared with the nationwide study conducted in Japan in 2011 [5], and we predict the widespread usage of fetal CT with IR. We also predict that the national decrease in exposure is because of the DRL reported as a result of the previous national study.

Materials and Methods

Selection of Target Medical Institutions

This study was approved by the ethics board of the National Center for Child Health and Development and was conducted after approval of the institutional review board.

The 16 sites that were studied in the 2011 survey [5] are major facilities performing fetal CT in Japan and were included in this study. In addition, an Internet forum for fetal skeletal dysplasia based in Japan, the Japan Forum of Fetal Skeletal Dysplasia (with 53 registered users), was used to call for participation by medical institutions that did not participate in the previous study. Medical institutions that consulted the radiologist and obstetrician members of the forum were also selected as candidates. Furthermore, medical institutions that have presented results in past society meetings in Japan and overseas, or submitted journal papers, were sought and considered as candidates. As a result, 25 medical institutions were selected as study sites. These study sites were mailed a CD-ROM with the questionnaire sheet, together with a letter inquiring about their interest regarding participation. The following 22 medical institutions expressed interest: Hokkaido University Hospital; Aomori Prefectural Central Hospital; Miyagi Children's Hospital; Tohoku University Hospital; Yamagata University Hospital; Chiba Kaihin Municipal Hospital; Juntendo University Urayasu Hospital; National Center for Child Health and Development; Jikei University School of Medicine Hospital; Tokyo Women's Medical University Hospital; Fujita Health University Hospital; Nagara Medical Center; University Hospital, Kyoto Prefectural University of Medicine; Osaka Medical Center and Research Institute for Maternal and Child Health; Hyogo College of Medicine Hospital; Shikoku Medical Center for Children and Adults; Perinatal Medical Center, Ehime Prefectural Central Hospital; Kochi Health Sciences Center; Tokushima University Hospital; Hiroshima University Hospital; Perinatal Medical Center, Yamaguchi Prefectural Grand Medical Center; and Kurume University Hospital.

Preparation of the Work Sheet

The first page of the work sheet was to be entered by the obstetrician of the hospital, and the remaining pages were to be entered by the radiologic technologist or radiologist. Eight questions were listed on the first page. First, what is the category of your hospital (choice of three options: university hospital, perinatal medical center, or regional core hospital)? Second, was specific written consent for fetal CT obtained? Third, how many fetal CT examinations have been conducted in the past 3 years and 9 months? Fourth, at what range of fetal stage (weeks) was CT conducted and what was the mean number of weeks? Fifth, on the basis of the 2011 study [5], have there been any changes to the protocol? Sixth, if the protocol has been changed, has the diagnostic ability decreased as a result of lowering the dose? Seventh, is sedation used on the fetus when conducting fetal CT? Finally, do you know the approximate fetal CT radiation dose for your hospital?

The CT survey from the second page onward had entry sheets for four manufacturers: Toshiba, GE Healthcare, Siemens Healthcare, and Philips Healthcare. The sheet for the manufacturer of the equipment used for testing at each site was also filled out. If multiple pieces of CT equipment were used at one site, we requested that each piece was entered into separate work sheets. Because parameters are different depending on the manufacturer, the work sheet entry items tailored for each CT manufacturer were chosen by a subgroup of seven radiologic technologists selected from sites that frequently perform fetal CT. The staff in charge of setting imaging protocols for each manufacturer at each of the four CT manufacturers was requested to check for the appropriateness of the chosen work sheet items, and the revised items were distributed. The content of the work sheet differs for each company, but basic items include tube voltage, tube current, scan time, pitch, scan FOV, scan length, volume CT dose index ($CTDI_{vol}$), dose-length product (DLP), whether IR is used for scanning and 3D imaging, and the name and degree of IR protocol used. This questionnaire was prepared using Excel software (version 2013, Microsoft) and was distributed to each site.

The study started when the survey was sent on December 5, 2014, and results were collected by January 31, 2015. The study implementation period was 58 days.

Summary of the Collected Data on Fetal CT From the Sites

The frequency of usage for each piece of CT equipment and the protocol was summarized from the survey results. The CT parameters used at the sites were also compared among the results using the same protocol. The trend in results for the protocol that used the lowest dose and the protocol that used the highest dose was studied.

CT Exposure Evaluation Method

The minimum, one-fourth (25th percentile), median, three-fourths (75th percentile), and maximum values were obtained for the $CTDI_{vol}$, DLP, and scan length from the 139 fetal CT examinations collected in this 2015 study. The results were compared with the previous results in 2011 [5], and the changes over the 4 years were evaluated. In addition, the 75th percentile values of $CTDI_{vol}$ and DLP in this study were determined as the new DRL. The median value was compared for scan length.

Regarding usage of IR, the frequency of usage was studied and the difference in $CTDI_{vol}$ value was compared among cases with and without IR use.

Regarding tube voltage, the frequency of usage of 80, 100, and 120 kV was studied. The results were compared with those of the 2011 study [5]. In addition, the $CTDI_{vol}$ values for the frequently used voltages, 100 and 120 kV, were compared among the different protocols used. For statistical analysis of the data, an unpaired *t* test was used to test for significance (Excel version 2013, Microsoft).

Results

Response to Questions Addressed to the Obstetrician

Category of your hospital—The breakdown of the 22 sites was 13 university hospitals (59%), followed by six perinatal centers (27%), and three regional core hospitals (14%). However, there were inconsistencies in CT dose data on two sites. These sites were excluded and a total of 20 sites were used for analysis.

TABLE 1: Summary of 32 Fetal CT Protocols for Scanners Used at Each Survey Site

Manufacturer	Brand Name	Tube Voltage (kV)	Rotation Time (s)	Helical Pitch	Volume CT Dose Index (mGy)	Dose-Length Product (mGy·cm)	Iterative Reconstruction
GE Healthcare	Discovery 750HD (*n* = 3)	100 (*n* = 4)	0.4–1	0.5–1.4	0.5–9	18–353	ASiR (*n* = 3)
	LightSpeed 16 (*n* = 1)	120 (*n* = 1)					Veo (*n* = 2)
	LightSpeed VCT (*n* = 1)						
Siemens Healthcare	Sensation (*n* = 2)	120 (*n* = 10)	0.28–0.5	0.6–1.4	1.6–13	52–514	SAFIRE (*n* = 4)
	Definition (*n* = 8)						IRIS (*n* = 1)
Toshiba	Aquilion 16 (*n* = 4)	80 (*n* = 1)	0.3–0.75	0.8–1.5	1.8–27.5	55–943	AIDR3D (*n* = 6)
	Aquilion 64 (*n* = 4)	100 (*n* = 4)					
	Aquilion other (*n* = 4)	120 (*n* = 12)					QDS (*n* = 1)
	Aquilion ONE (*n* = 3)						
Philips Healthcare	iCT Elite (*n* = 1)	80 (*n* = 1)	0.4–0.75	0.6–0.9	1.6–7.9	70–281	IMR SoftTissue (*n* = 1)
	iCT (*n* = 1)	100 (*n* = 1)					iDose 4 (*n* = 1)
Summary of fetal CT protocols	*n* = 32	120 (*n* = 23)	0.28–0.75	0.5–1.5	0.5–27.5	18–943	Hybrid iterative reconstruction (*n* = 17)
		100 (*n* = 9)					Full iterative reconstruction (*n* = 2)

Note—ASiR = adaptive statistical iterative reconstruction, SAFIRE = sinogram-affirmed iterative reconstruction, IRIS = iterative reconstruction in image space, AIDR3D = adaptive iterative dose reduction 3D, QDS = quantum denoising software, IMR = iterative model reconstruction.

Specific written consent for fetal CT—Sixteen sites (80%) obtained specific written consent for conducting fetal CT.

Number of fetal CT examinations conducted in the past 3 years and 9 months—The number of cases per site during this time period ranged from one to 24 and there were 139 cases in total.

The range of and mean gestational week when CT was conducted—The range was 17–36 weeks' gestation, and the mean (± SD) was 30.1 ± 3.1 weeks.

Knowledge of the previous study report (2011), and whether the protocol has been changed—Six sites changed their protocol on the basis of the 2011 DRL (30%). Seven sites (35%) each answered that the study results did not affect their protocol or they did not know about the 2011 DRL.

Whether diagnostic ability decreased because of protocol change—No sites that changed their protocol answered that diagnostic ability decreased by lowering the dose.

Use of sedation on fetus during fetal CT—Two sites (10%) used sedation when conducting fetal CT, but the remaining 18 sites (90%) did not use sedation. One site that used sedation answered "walking, use of Diazepam in some cases," and the other site did not give a detailed response.

Knowledge of an approximate fetal CT radiation dose at own site—Obstetricians at 17 sites (85%) answered that they knew the approximate fetal CT radiation dose at their own site.

Summary of Collected Data on Fetal CT From Each Site

Table 1 summarizes the survey results about the 32 CT protocols used by the sites for each scanner manufacturer. The most common scanner manufacturer among the survey sites was Toshiba; however, no obvious difference of parameter setting was identified among CT vendors.

There was a wide range of radiation doses as measured by $CTDI_{vol}$ and DLP, and the lowest scanning condition was a $CTDI_{vol}$ of 0.5 mGy with the full IR method (Veo, GE Healthcare), even though full IR was used for only 10% of the protocols. This is somewhat surprising, because the maximum values of $CTDI_{vol}$ and DLP (27.5 mGy and 943 mGy·cm, respectively) were 50 times as large as the minimum settings (0.5 mGy and 18 mGy·cm, respectively). Approximately 68% of CT protocols were performed using a tube voltage of 120 kV.

CT Exposure Evaluation

Among the 139 fetal CT examinations performed at the study sites during the study period, inquiries were made to the staff in charge of setting imaging protocols for each manufacturer regarding incomplete data submitted from the sites to recover and ensure consistency in the data. However, inconsistencies in data on $CTDI_{vol}$ and DLP for 19 cases could still not be resolved. These data were excluded, and a total of 120 cases were used for analysis.

Evaluation and Change of Volume CT Dose Index, Dose-Length Product, and Scan Length

The comparison of values for $CTDI_{vol}$, DLP, and scan length in 2011 and 2015 is

TABLE 2: Comparison of Volume CT Dose Index ($CTDI_{vol}$), Dose-Length Product (DLP), and Scan Length Between 2011 and 2015

	$CTDI_{vol}$ (mGy)[a]		DLP (mGy·cm)[a]		Scan Length (mm)[b]	
Measurement	2011	2015	2011	2015	2011	2015
Maximum	23.1	27.5	1025.6	943.5	476	520
75th percentile	11.3	4.9	382.6	176.4	356	341
Median	7.7	3.2	276.8	104.3	319	313
25th percentile	3.7	2.4	122.3	84.8	295	287
Minimum	2.1	0.5	69	18.3	190	133

[a] $p < 0.01$.
[b] $p > 0.05$.

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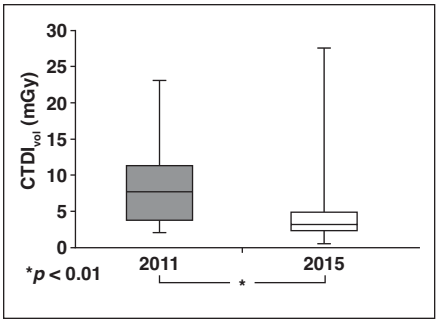

Fig. 1—Boxplot of volume CT dose index ($CTDI_{vol}$) values in 2011 and 2015. Boxes denote ranges, lines in boxes denote medians, and vertical lines and whiskers denote 95% CIs.

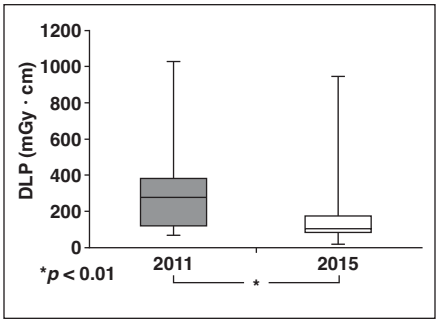

Fig. 2—Boxplot of dose-length product (DLP) values in 2011 and 2015. Boxes denote ranges, lines in boxes denote medians, and vertical lines and whiskers denote 95% CIs.

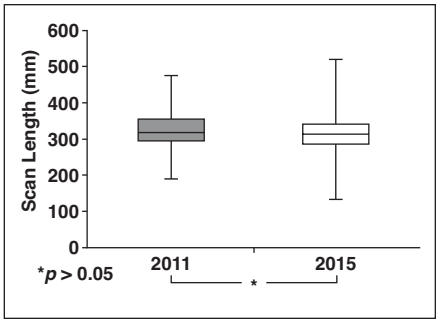

Fig. 3—Boxplot of scan length values in 2011 and 2015. Boxes denote ranges, lines in boxes denote medians, and vertical lines and whiskers denote 95% CIs.

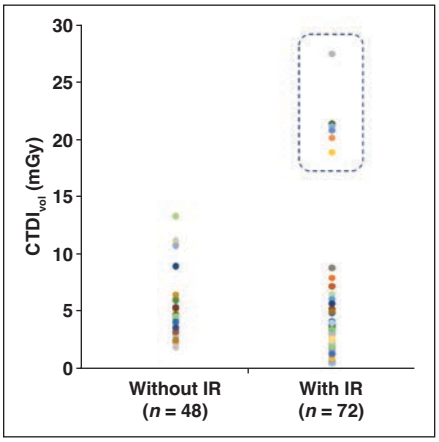

Fig. 4—Comparison of median volume CT dose index ($CTDI_{vol}$) between CT performed with and without iterative reconstruction (IR). If sites surrounded by dashed line are excluded, $CTDI_{vol}$ values for sites using IR are lower than for those that do not use IR, with statistically significant difference between groups ($p < 0.01$).

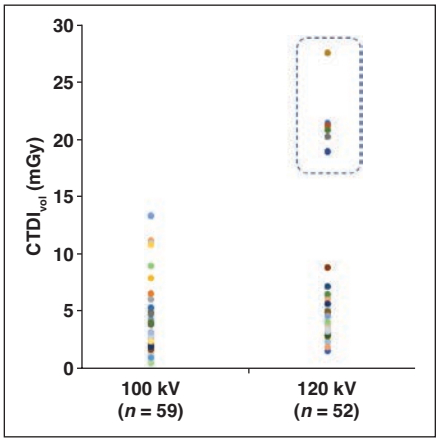

Fig. 5—Comparison of median volume CT dose index ($CTDI_{vol}$) values at 120 and 100 kV. In contrast to results for iterative reconstruction, results for tube current did not show significant difference in $CTDI_{vol}$ even when sites surrounded by dashed line were excluded.

shown in Table 2, and boxplots are shown in Figures 1–3. Compared with the study in 2011 (n = 119), the minimum, median, 75th percentile, and 25th percentile values of $CTDI_{vol}$ and DLP for the tabulated results in 2015 (n = 120) were lower, although some sites had a higher maximum value than that of the previous study. The 75th percentile value of $CTDI_{vol}$ that is the DRL for this study was 4.9 mGy, and this was 43% of the previous value of 11.3 mGy. During this period, a 57% reduction in the radiation dose was found (Fig. 1). Similar to $CTDI_{vol}$, the 75th percentile value for DLP was 176.4 mGy·cm, and this was 46% (about half) of the previous value of 382.4 mGy·cm. The differences were statistically significant ($p < 0.01$) (Fig. 2). On the other hand, the scan range was almost the same as in the previous study, and although the data included over 100 cases, the median was different by only 6 mm, and the data were considered to be accurate. It was found that the decrease in DLP was not caused by a shortening of the length (Fig. 3).

In this study, 14 of 20 sites (70%) used IR. Among the 14 sites that used IR, six (43%) did not use the method at the beginning of the study period but started to incorporate it during the study period. As shown in Figure 4, a comparison of $CTDI_{vol}$ between CT with IR (n = 72) and CT without IR (n = 48) shows that it is lower in the group that uses IR than the group that does not use IR, although one site had set a significantly higher radiation dose than the others (section surrounded by the dashed line in Fig. 4). If the site surrounded by the dashed line is excluded, the $CTDI_{vol}$ for sites using IR compared with those that do not use IR is statistically significantly lower ($p < 0.01$). The group that uses IR had a significantly lower radiation dose setting compared with the group that did not (Table 3).

Tube voltage was 120 kV in 52 cases, 100 kV in 59 cases, and 80 kV in nine cases, with 100 kV the most frequently used voltage. Six sites had the voltage fixed at 120 kV, whereas the other 14 sites used a tube voltage lower than 120 kV.

As with IR, comparison of $CTDI_{vol}$ values by tube current (120 vs 100 kV) showed that the median $CTDI_{vol}$ values for 120 and 100 kV were 3.3 and 2.5 mGy, respectively. As shown in Figure 5, the tube voltage was set at a significantly higher radiation dose in one

TABLE 3: Relationships Between Volume CT Dose Index ($CTDI_{vol}$) and Iterative Reconstruction (IR) and Between $CTDI_{vol}$ and Tube Voltage

	$CTDI_{vol}$ With and Without IR (mGy)[a]		$CTDI_{vol}$ at Different Tube Voltages (mGy)[b]	
Measurement	With IR	Without IR	120 kV	100 kV
Maximum	8.8	13.4	8.8	13.4
75th percentile	3.5	5	4.8	4.1
Median	2.5	3.3	3.3	2.5
25th percentile	2	3.2	3.2	2
Minimum	0.5	1.8	1.6	0.5

[a] $p < 0.01$.
[b] $p > 0.05$.

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site in the group that used IR (section surrounded by a dashed line in Fig. 5). In contrast to the results for IR, the results for tube current did not show a significant difference in $CTDI_{vol}$ even when the site surrounded with the dashed line was excluded.

Discussion

From the response to the questions answered by the obstetricians, the following situation was revealed. In Japan, 86% of fetal CT examinations were performed at university hospitals or public perinatal centers that act as the center for medical care in that region. It is assumed that obstetricians and gynecologists at local clinics who detect short limbs by fetal ultrasound send a referral to obstetricians or fetal examination specialists for retesting by ultrasound with higher accuracy, and then the case is indicated for CT if necessary.

In Japan, 80% of the sites obtained specific written consent for fetal CT with informed consent from the parents regarding fetal radiation exposure.

The mean timing for the CT was 30.1 ± 3.1 weeks' gestation. This is thought to be the result of a recommendation by the leading academic research group on fetal CT in Japan, the Japan Forum of Fetal Skeletal Dysplasia, which recommends testing at around 30 weeks. This recommendation is based on several reasons, including the fact that the effect of radiation on the CNS is less of a concern during the third trimester of pregnancy [8] and that depiction of the skeletal structure is clearer compared with the early stages of pregnancy because of fetal growth. The result was almost the same as the previous study [5] (mean, 30.2 ± 2.6 weeks) with essentially no change. It is understandable that the sites that perform CT are actually following the recommendations of the Japan Forum of Fetal Skeletal Dysplasia.

Thirteen sites (65%) were aware of the DRL results from the 2011 study [5], and six sites (30%) had changed their protocol on the basis of the 2011 DRL. On the other hand, seven sites (35%) answered that they were not aware of the study results. DRL was proposed in the 1980s to optimize simple x-ray radiation dose and it was introduced as a method for optimizing CT radiation dose in the 1990s [7, 9]. It is considered that there is high awareness of DRL in the sites performing fetal CT, and DRL is correctly functioning as a regulatory pressure on the entire community as intended. No sites responded that a lower dose resulting from protocol changes led to decreased diagnostic ability, and this further reinforces that these scanning conditions are appropriate.

It was revealed that 90% of sites did not sedate the fetus for fetal CT. In the previous survey, one site used pancuronium (Mioblock, MSD) to suppress fetal movement, but this response was not found in the current study. It is considered that sedation is not necessary for fetal CT.

Obstetricians at 17 sites (85%) knew the approximate fetal CT radiation dose of their site when they order the scan. According to a literature search, it has been reported in a past survey that only 16% of residents in a radiology department knew the radiation dose for abdominal CT [10]. The obstetricians at sites performing fetal CT may be aware of the dose because they are required to explain the dose to patients. This may be inaccurate because the specific number was not asked, but compared with past reports, obstetricians who are involved in fetal CT are considered to have a fairly high awareness of CT exposure.

This study has revealed that since the 2011 study [5], the DRL for fetal CT in Japan has been reduced by half, a result that was statistically significant. This may be due to three factors. One is the first-ever proposal of fetal CT DRL in Japan from the study results in 2011. The results were widely reported at fetal CT forums and research groups that were active in performing fetal CT. A total of 65% of the sites studied in this analysis answered that they were aware of the results, and, furthermore, 30% of sites responded that they had lowered their dose setting on the basis of the DRL of the previous study. This is the result of DRL fulfilling its correct function, and it could be evaluated as an extremely significant result for managing CT radiation exposure in Japan. It is considered that DRL should be studied every few years and, indeed, this study was conducted at the appropriate time. We should conduct another national survey in 3–4 years and confirm the decrease in DRL as a community.

The next factor is the decrease in dose due to widespread use of IR. In the previous 2011 study [5], only one site of 16 (6%) used IR, but in this study, there was a significant increase with 14 of 20 sites (70%) using IR. This is considered to be due to the recent recognition of IR in all CT scans, including those of children [11] and adults, and the experience of the scanner, who can easily enhance image quality by changing the console setting. Therefore, among the 14 sites that used IR, the fact that six sites (43%) did not use IR at the beginning of the study but introduced IR later during the study period is considered to be the result of recognizing IR's usefulness in fetal CT.

The site that performs scans with the lowest exposure ($CTDI_{vol}$, 0.5 mGy) in this study does not use hybrid type IR but has introduced full IR (Veo, GE Healthcare) to aim for lower exposure. There are only a few reports on using full IR for fetal CT [12], but a decrease in fetal CT radiation dose would be possible if the full IR method becomes widespread, leading to a further decrease in DRL throughout Japan.

Similarly, this study found that many sites used low tube voltage. No significant difference in $CTDI_{vol}$ was found between different protocols using 120 or 100 kV, but the use of low tube voltage at these sites may be the result of awareness of lowering exposure and an adjustment to avoid an increase in unnecessary tube current. This is thought to be due to a change in techniques and awareness among the technicians in charge of CT machines in the clinical field.

In this way, radiation exposure of fetal CT has been optimized, but as shown in Figures 4 and 5, there are still sites with conditions leading to high radiation doses, and further education on DRL is required.

On the other hand, a limitation of this and previous studies was that only the radiation dose to set DRL was studied, and we did not collect data on image quality. Focusing on decreasing the dose may lead to a failure to maintain sufficient diagnostic quality for images. Goske et al. [13] have published their thoughts regarding the diagnostic reference range as a method to maintain both an appropriate radiation dose management for CT and diagnostic image quality. They suggest that the upper limit for radiation exposure should be the 75th percentile value and the lower limit should be the 25th percentile value to maintain image quality, and the appropriate scan condition should fall within this range. If this is applied to the results of this study, the appropriate dose for fetal CT would be a $CTDI_{vol}$ between 2.5 and 5 mGy (median, 3 mGy). In cases without full IR, this range for diagnostic reference range is considered to be appropriate.

A significant reduction in radiation exposure is possible if fetal CT is used only for observation of skeletal structure of the fetus, but it is necessary for each medical institution to consider the characteristics of IR for their fa-

cility's CT equipment and to perform a CT examination with sufficient quality for diagnosis.

In summary, since the previous DRL survey was conducted [5], the original objective and role for DRL that the International Commission on Radiological Protection [14] had proposed has been sufficiently reached. In addition, widespread use of IR and low tube voltage has helped in managing the radiation dose throughout Japan.

The objective of CT is to deliver an accurate diagnosis when skeletal dysplasia is suspected after careful fetal ultrasound testing and to determine the strategy for perinatal patient care. From this study, it was heartening to find that 85% of obstetricians who order CT scans were aware of the approximate CT radiation dose at their site.

We intend to conduct another survey in 4 years to confirm whether there is a further decrease in DRL. In addition, compared with 2011, there is concern that image quality may have been affected by the decrease in dose. Ideally, the next study would also include an evaluation of image quality.

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