［症例報告］Pulmonary Hypoplasia and Chondrocyte p21^<CIP1> Expression in Patients with Lethal Short-limbed Dwarfism

Pulmonary Hypoplasia and Chondrocyte p21^{CIP1} Expression in Patients with Lethal Short-limbed Dwarfism

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ABSTRACT

Lethal short-limbed dwarfism is a rare disorder associated with chondrodysplasia, micromelia, and severe pulmonary hypoplasia. Thus, most of the affected infants are stillborn or die shortly because of respiratory insufficiency. Two autopsy cases of lethal short-limbed drawfism are presented in this article. Both stillborn infants had markedly short extremities and small thorax. Pulmonary alveolar cells were immature and air apace was diminished. Chondrocytes of a long bone showed hypertrophy and irregular arrangement. Growth plate chondrocytes displayed a significant expression of the p21^{CIP1} protein. This is the systematic examination of the autopsy cases that highlight pulmonary hypoplasia and chondrocyte p21^{CIP1} expression in patients with lethal short-limbed dwarfism. *Ryukyu Med. J., 25(1,2) 41～46, 2006*

Key words: Lethal short-limbed dwarfism, pulmonary hypoplasia, p21^{CIP1}, thanatophoric dysplasia, hypophosphatasia

INTRODUCTION

Lethal short-limbed dwarfism (MIM 151210) is a complex group of diseases characterized by chondrodysplasia, micromelia and severe pulmonary hypoplasia. In most cases, the infants are usually stillborn or die in the first few days because of respiratory insufficiency. The incidence of this disorder is about 1: 19,000 live births[1)]. In 1936, Parenti first described the dwarfed infant who had congenital shortness of stature with severe micromelia[2)]. Fraccaro first used the term achondrogenesis for a type of congenital short-limbed dwarfism resulting in stillborn or death in the early hours or days of life[3)]. To date, eight disorders have been characterized[4)]. These disorders include thanatophoric dysplasia (TD) (MIM 187600), hypophosphatasia (HPH) (MIM 241500), osteogenesis imperfecta (OI) (MIM 166210), homozygous achondroplasia (ACH) (MIM 100800), campomelic dysplasia (CMPD) (MIM 114290), achondrogenesis (ACG) (MIM 200600), short-rib polydactyly syndrome (SRPS) (MIM 263510), and rhizomelic type of chondrodysplasia punctata (RCDP) (MIM 215100). Representative phenotype and affected genes of eight disorders of lethal short-limbed dwarfism are listed in Table 1.

In the present article, we describe the clinical, roentgenographic, macroscopic, and microscopic

Table 1 Representative phenotype and affected genes of lethal short-limbed dwarfism

	TD	HPH	OI	ACH	CMPD	ACG	SRPS	RCDP
MIM No.	187600	241500	166210	100800	114290	200600	263510	215100
Short-limbed dwarfism	+	+	+	+	+	+	+	+
Skull	Cloverleaf skull +/-	Absent or poor ossification vault	Poor ossification base	Well ossified with short base large skull	Normal or craniosynostosis Cloverleaf skull +/-	Poorly ossified membranous skull, normal or large skull	Normal	Normal Calcification in larynx and trachea less common
Trunk/supine	Normal trunk length Well ossification Markedly flattening bodies H-shaped vertebral bodies	Poor ossification	Ossified Flattening bodies	Short trunk	Square or slightly flat vertebral bodies	Short trunk Poor ossification Moderately flattening bodies	Square vertebral bodies	Coronal clefts of vertebral bodies
Hands and feet	Short and broad	Constriction hands	Osteoporotic	Short, metacarpals of equal length	Premature carpal ossification	Unossified	Pre or postaxial polydactyly Incomplete ossification of MC and MT bones	Stippling
Other features	Short ribs with wide-cupped anterior ends	Short thin ribs with cupping anterior ends Markedly decreased serum ALP activity	Spontaneous fractures, generalized osteoporosis	Most frequent form of short-limb dwarfism Flat nasal bridge, protuberant abdomen	Multiple cutaneous dimpling in the arms and legs Cleft palate, micrognathia, flat face, hypertelorism	Multiple fractures of ribs	Cleft lips, polycystic kidney, gastrointestinal or genitourinary atresia, transposition of great vessels	Cataract (72% of cases), Skin changes (27% of cases)
Presumably affected genes	*FGFR3*	*L/B/K ALP*	*COL1A1, COL1A2*	*FGFR3*	*SOX9*	Unknown	Unknown	*PEX7, RCDP*
Locus	4p16.3	1p36.1-p34	17q21.31-q22 7q22.1	4p16.3	17q24.3-q25.1			6q22-q24

findings in autopsy cases of TD and HPH, and also discuss the differential diagnosis. To further characterize the possible disease mechanism, we examined the features of hypoplastic lung and the level of expression of the $p21^{CIP1}$ protein in the growth plate chondrocytes.

CASES

Clinical summary

Case 1: A 29 year-old gravida 0, para 0, Japanese woman had a routine ultrasonographic checkup at 19 weeks gestation. The sonographic examination revealed that the fetus had markedly shortened extremities. The level of serum alkaline phophatase (ALP) of the fetus was normal. She delivered a male stillborn infant in a 20-week gestational age. The baby's mother was not exposed to any teratogenic drugs or radioactive agents before and throughout the pregnancy. There was no familial history of dwarfism or consanguineous marriage. Radiographic examination showed that the parietal region of the skull was relatively prominent and enlarged with normal ossification but the skull did not show a cloverleaf-like vault (Fig. 1A). The baby had H-shaped vertebrae and very short extremities. Ribs were short with wide-cupped anterior ends and thorax was narrow. Humeral, femoral, sacral, pubic and ischial bones were not clearly visible. The overall length of the trunk was not significantly reduced.

Case 2: A 23-year-old gravida 0, para 0, Japanese woman had a routine sonographic examination at 28 weeks gestation. The examination disclosed polyhydramnios. The course of pregnancy was uneventful except polyhydramnios. Magnetic resonance imaging (MRI) study showed short limbs with narrow thorax (Fig. 1B). The level of serum ALP of the fetus was markedly decreased (6 IU/l: normal range 55-120 IU/l). She delivered a female stillborn infant at 29 weeks gestation. Parents of the infant were healthy with normal stature. The infant's mother was not exposed to any teratogenic drugs or radioactive agents before and throughout

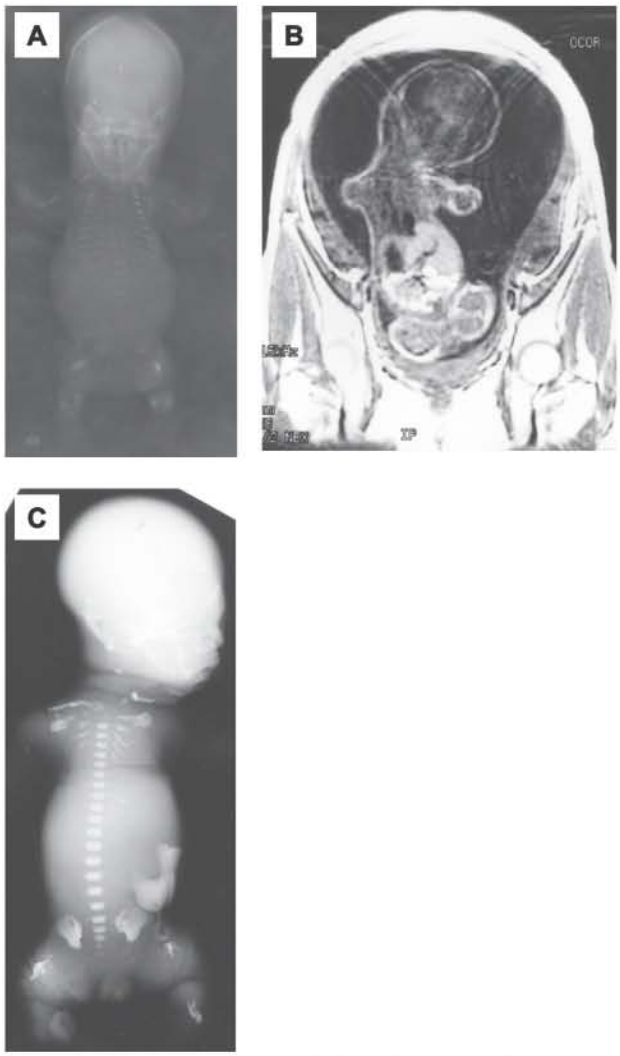

Fig. 1 A. Roentgenogram of TD. Note the shortness of ribs, flattened and H-shaped vertebral bodies, and well-ossified skull. B. MRI examination of HPH. Note extremely short limbs. C. Roentgenogram of HPH. Note absence of ossification in vault with abnormally short ribs.

the pregnancy. There was no familial history of relatives of short stature or consanguineous marriage. Roentgenogram of the skull disclosed that bone formation of the vault was absent except the base of the skull (Fig. 1C). Cervical vertebrae, long bones of upper extremities, pubic and sacral bones had no visible bone minerals. Thoracic and lumbar vertebral bones were visible and showed a round shape. Vertebral column was straight. Clavicula, scapula, femoral and tibial bones had thin and irregular epiphysis. Ribs were very short with poor mineralization, and thorax was small.

Macroscopic findings

Case 1 (TD): The infant had short limbs with small chest and showed the typical features of TD with prominent soft tissues, a narrow thorax, a distended abdomen, and markedly shortened extremities. The infant had a normally ossified skull with relative enlargement. Brain appeared normal but slight dilatation of the third ventricle was noted. Face appeared normal and the skin was systemically edematous. Lung was small and atelectatic. There was no abnormality in pulmonary lobe. The measurements (normal range) were as follows (normal range): body weight 300 g (361±132 g), crown-heel length 19 cm (26.1±3.5 cm), head circumference 18.5 cm, thoracic circumference 11 cm, the greatest abdominal circumference 14.5 cm, distance from acromion to the tip of the thumb 5.5 cm, distance from iliac crest to the heel 6.5 cm, brain weight 59 g (53.2±25.3 g), both lungs weight 3.6 g (10.7±5.9 g). The ratio of lung weight (LW): body weight (BW) was 0.012. This value was proposed as a criterion of pulmonary hypoplasia[5]. Fetus with pulmonary hypoplasia shows LW: BW ratio of < 0.012 at all gestational ages or < 0.016 at less than 32-week gestation. The foramen ovale and ductus arteriosus were patent. The liver weighed 14 g (20.4 ± 9.5 g) and both kidneys weighted 3 g (3.8±2.5 g). No anomalies of digestive and genital organs were noted.

Case 2 (HPH): The infant had markedly short and curved limbs with poorly ossified skull. The skull could be pressed easily. Hydrocephalus was not present. Skin was covered with thick folds. Small and atelectatic lungs were noted. There was no abnormality in pulmonary lobe. The measurements (normal range) were as follows: body weight 1310 g (1230±340 g), crown-heel length 32 cm (37.8±3.7 cm), head circumference 28.5 cm, thoracic circumference 20 cm, the greatest abdominal circumference 23.5 cm, distance from acromion to the tip of the thumb 7 cm, distance from iliac crest to the heel 8.5 cm, brain weight 160 g (166±55 g), both lungs weight 6 g (28±11 g). LW: BW ratio was 0.005. Heart weighed 10 g (9.3±3.3 g). Patent ductus arteriosus was present. The weight of liver was 56 g (53± 19 g) and combined weight of kidneys was 14 g (12.3±3.9 g). These major organs had no abnormal appearance. Digestive and genital organs revealed no deformities.

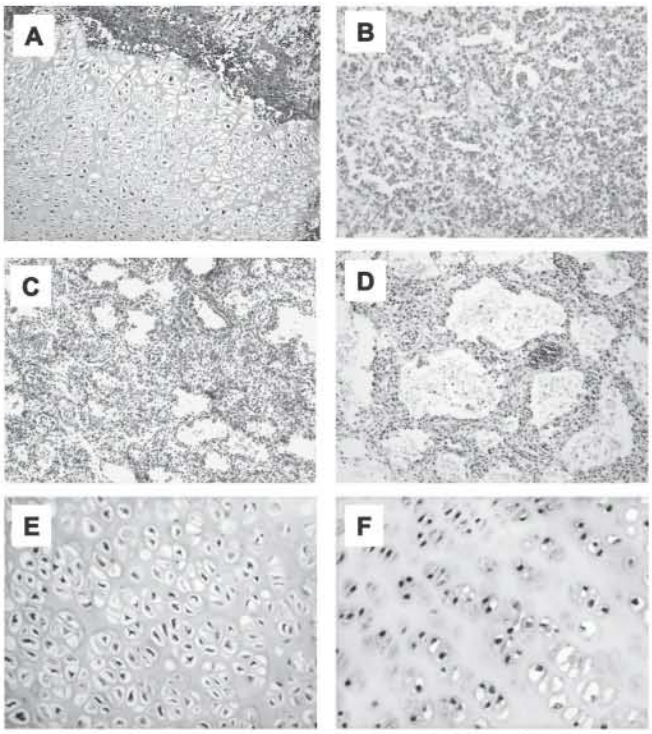

Fig. 2 A. Irregular growth plate and lack of columnar formation (TD). B, C. Hypoplastic pulmonary tissues (TD (B) and HPH(C)). Note immature alveolar cells and diminished air space. D. Control lung (29 weeks gestation). (hematoxilin and eosin staining) E, F. Immunohistocemical staining of the $p21^{CIP1}$ protein. Note $p21^{CIP1}$-positive nuclei of chondrocytes (TD (E) and HPH (F)).

Microscopic findings

In both cases, the epiphysial growth plate of the femoral bone showed irregular arrangement with normal resting cartilage. Chondrocytes appeared variable-sized hypertrophy with abnormal columnar arrangement (Fig. 2A). There was no evidence of bone destruction. In the TD case, combined lung weighed 3.6 g. This weight corresponds to 14 to 16-week maturation of lung. Lung of a normal 20-week-gestation fetus is usually in the canalicular phase[6], but the lung mainly appeared pseudoglandular phase in this case (Fig. 2B). The acinar epithelium displayed a pseudostratified appearance but most cells were not completely differentiated. In the HPH case, both lungs weighed 6 g, corresponding to 15 to 16-week maturation. Pulmonary alveolar cells appeared in the pseudoglandular and canalicular phases (Fig. 2C). However, lung of a 29-week-gestation fetus is usually in the saccular or alveolar phases[6]. Alveolar cells in both cases were decreased in number and immature, and air space was diminished, when compared to the 29-week-gestation fetal lung (Fig. 2D). Considering these features the lung was hypoplastic. However, other major organs showed normal appearance in both cases.

Immunohistochemical staining

We examined the level of expression of the $p21^{CIP1}$ protein in the growth plate chnodrocytes. These assays were done with an established method with slight modification by using an anti-$p21^{CIP1}$ (1: 200 dilution, sc-817, Santa Cruz Biotechnology, Santa Cruz, CA) antibody by immunohistochemical staining. Staining intensity of the $p21^{CIP1}$ protein was scored as follows; 0, no positive cells; 1+, <10% of positive cells; 2+, 10-30% positive cells; 3+, 31-60% positive cells; 4+, 61-100% positive cells. The $p21^{CIP1}$ staining intensity of growth plate chondrocytes in both TD and HPH cases was 3+ (Figs. 2E and F).

DISCUSSION

TD was first distinguished from achondroplasia in 1967 by Maroteaux *et al*[7]. The incidence is estimated about 1:35,000 births[8]. It is thought that TD may have an autosomal recessive inheritance since several cases occurred in siblings and one case was associated with a consanguineous marriage[9,10]. Most cases are sporadic thus it may arise from a new dominant mutation but the mode of inheritance is not yet precisely known. A previous literature reported that some drug might involved in the induction of this disease[9]. However, an exact etiology still remains unclear. Two conditions of TD may coexist[11]. An affected individual with curved and short femurs is designated TD type 1. While, patients who have straight and relatively long femurs with a cloverleaf skull anomaly are called TD type 2. Prenatal diagnosis of this disorder can be done by ultrasonographic and/or MRI examinations[12]. The pregnancy is frequently complicated with polyhydramnios[4,8,9]. A precise differential diagnosis should be made from ACH and ACG because clinical features of these two disorders resemble those of TD. In this respect, radiological features are very useful. In the radiographical examination of the TD cases, the baby has very short extremities, shortened ribs, and a narrow thorax. The skull is reported to be enlarged or normal. Especially, TD type 2 cases have a cloverleaf skull

anomaly[8,10]. The vertebrae represent H- or U-shaped appearance because of flattened vertebral bodies with protruded out pedicles[10,13]. In TD cases, the overall length of the trunk was not significantly reduced, while ACH or ACG cases represent a shortened trunk[4]. These features were quite different from those of ACH or ACG. In addition, ACG patients have a poorly ossified or membranous skull[4]. In contrast, TD patients have a well-ossified skull[4]. In the present case, the limbs were very short but the overall length of the trunk was not reduced. The skull was well ossified and did not show a cloverleaf appearance. Infants bearing ACH have parents who are both bearing ACH since homozygous ACH is inherited as a doubly dominant trait[4]. Histopathologically, in the present TD case the most striking abnormality is the irregular arrangement of the growth plate of long bones and pulmonary hypoplasia1[3]. These features are in accordance with those of TD cases that have been described before1[3]. In view of these findings the present case 1 was diagnosed with TD type 1.

TD types 1 and 2 have recently been shown to carry missense or nonsense mutations in the fibroblast growth factor receptor (*FGFR*) 3 gene[14]. Abnormal expression in the FGFR3 protein was also found in cartilage of TD patients[15]. These genetic alterations may cause modification of the ligand binding between the mutated and wild-type FGFR proteins. This may affect the subsequent alteration of the downstream signaling pathway(s) and then results in mutation-specific phenotypes[16]. It has also been demonstrated that overexpression of the $p21^{CIP1}$ protein in chondrocytes correlates with phenotypic severity and defective chondrocyte differentiation in patients with FGFR3-related chondrodysplasias[17]. In the present two cases, we found that the $p21^{CIP1}$ protein was significantly expressed in chondrocytes of the growth plate (Figs. 2E and F). These findings suggest that the $p21^{CIP1}$ protein plays a critical role in progression of chondrogenic differentiation.

HPH is an inherited rickets-like disorder characterized by defective bone mineralization, decreased activities of serum and tissue liver/bone/kidney ALP (L/B/K ALP) and an increased urinary excretion of phosphoethanolamine (PEA)[18]. This disease was first described by Rathbun in 1948[19]. The incidence is approximately 1: 100,000 live births[19]. Three forms have been defined: a lethal perinatal type, childhood type and adult type[18]. The severe forms of the disease are transmitted in an autosomal recessive fashion[18]. The ultrasonographic and/or MRI studies are useful to confirm prenatal diagnosis. A fetus showed shortened and strangely curved limbs. Hydramnios was sometimes observed[19]. Radiological appearances demonstrated poor ossification of whole bones and ribs were usually short and thin with cupped anterior ends[18,19]. These features are inportant diagnostic tools. However, histological findings resemble severe rickets[19]. The metaphysial growth plate reveals irregular without regular columnar arrangements. Degenerating cartilage with marked hypertrophy has failure of calcification[18,19]. Furthermore, pulmonary hypoplasia is present[18]. In the present case 2, macroscopic and microscopic findings were consistent with those of above-described features. The markedly decreased serum ALP activity appeared to be the most significant finding. We therefore diagnosed this case with HPH.

ALP has been thought to be implicated in bone mineralization, but the precise nature of its function is not known. However, a missense mutation and small deletion in the L/B/K ALP gene were found in patients with HPH, suggesting that HPH might be caused by mutation in this gene[20]. These mutations or levels of mRNA expression of this gene may reflect heterogeneity of HPH or different degrees of clinical severity.

Pulmonary hypoplasia has been found during postmortem examination in neonates who died with congenital abnormalities[5]. In fact, the present TD and HPH cases displayed pulmonary hypoplasia. However, the precise reason why pulmonary hypoplasia occurs in such neonates is not known. High level of cell proliferation was seen in normal embryonic lung at 13.5 to 15.5 days' gestation of Sprague-Dawley rats[21]. Myogenin null mouse embryo exhibited a significant decrease in lung cell proliferation, which may contribute to pulmonary hypoplasia[22]. Furthermore, it has been demonstrated that aberrant expression of $p21^{CIP1}$ during alveolization may be associated with the lung hypoplasia seen in a premature baboon model[23]. These findings suggest that pulmonary cell proliferation and $p21^{CIP1}$ expression may play a role in the development of fetal lung.

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