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Perinatal hypophosphatasia caused by uniparental isodisomy

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ABSTRACT

Hypophosphatasia (HPP) is an inherited disorder characterized by defective bone mineralization caused by mutations in the alkaline phosphatase gene (*ALPL*). Clinically, the disease spans a great continuum of disease severity and six forms can be distinguished according to the age of onset. The most severe is the autosomal recessive perinatal form, a major prenatal skeletal dysplasia in Japan. The *ALPL* mutation c.1559delT causes perinatal HPP and occurs frequently in the Japanese. Most patients with perinatal HPP in Japan are homozygous for c.1559delT, and their parents are usually heterozygous with no evidence of consanguinity.

Here we identified a fetus with perinatal HPP resulting from an unusual mechanism known as paternal uniparental isodisomy (UPD) of chromosome 1. Sequence analysis of *ALPL* in the patient revealed the presence of the homozygous mutation c.1559delT. We suspected UPD because the father and mother were heterozygous and wild type, respectively. Analysis of polymorphic microsatellite markers spanning chromosome 1 and whole-genome arrays revealed a uniparental inheritance from the father and excluded deletions or *de novo* mutations.

This is the first description of perinatal HPP caused by UPD. This report also emphasizes the low recurrence risk of a non-Mendelian inheritance pattern in UPD and the value of determining parental genotypes with homozygous mutations in a patient to confirm whether the condition is caused by UPD or not, even when the mutation is detected as a hot spot, as described in the literature.

1. Introduction

Hypophosphatasia (HPP) is an inherited disorder characterized by defective bone mineralization and low alkaline phosphatase (ALP; EC 3.1.3.1) activity [1]. HPP spans a great continuum of disease severity and is classified into six forms according to severity and age of onset: perinatal, benign prenatal, infantile (Mendelian Inheritance in Man (MIM) # 241500), childhood (MIM# 241510), adult (MIM# 146300), and odonto [2–4]. All HPP forms display decreased unfractionated serum ALP activity and presence of either one or two pathological mutations in the liver/bone/kidney alkaline phosphatase gene (*ALPL*, MIM# 171760) located on chromosome 1p36.1-p34, which encodes tissue-nonspecific ALP isoenzyme (TNSALP) [1].

Perinatal HPP is the most severe form and is inherited as an autosomal recessive trait; both parents of most patients are *ALPL* heterozygotes. Patients with perinatal HPP show markedly impaired mineralization *in utero*. Perinatal HPP is a major cause of prenatal skeletal dysplasia in Japan. Moreover, the c.1559delT mutation in *ALPL* is commonly associated with perinatal HPP and has only been detected in patients of heritage [5,6].

Here we report the first case of uniparental iso-disomy (UPD) in HPP in the fetus of a non-carrier mother. The homozygosity (c.1559delT) occurred because of paternal isodisomy of entire chromosome 1.

1.1. Clinical case

A 33-year-old Japanese primigravida and her 42-year-old husband were referred for evaluation after a fetal anomaly was suspected on sonography at 19 weeks of gestation. Sonography *in utero* at 19 weeks of gestation showed a fracture of the left radius (Fig. 1A) and angulation of the right humerus (Fig. 1B). Mineralization of calvarium and spine, particularly in the thoracic segment, was markedly decreased. The findings were consistent with lethal skeletal dysplasia. Differential diagnosis included osteogenesis imperfecta because serum ALP activity of the parents was normal (father: 250 IU/L, mother: 160 IU/L; normal range: 115–359 IU/L for adults). Neither parents had clinical symptoms of skeletal dysplasia including no history for premature loss of deciduous teeth. The marriage was not consanguineous, and there was no family history of skeletal dysplasia. Pregnancy was terminated at 20 weeks of gestation after genetic counseling, and the fetus died at birth. Radiographic examination at post termination showed markedly decreased mineralization in the thoracic segment (Fig. 1C) and long bone (Fig. 1D) segment. The classical clinical and radiographic signs of perinatal HPP were present on examination of the abortus (proband), and ALP activity was below 4 IU/L (normal range: 400–1450 IU/L for neonates)

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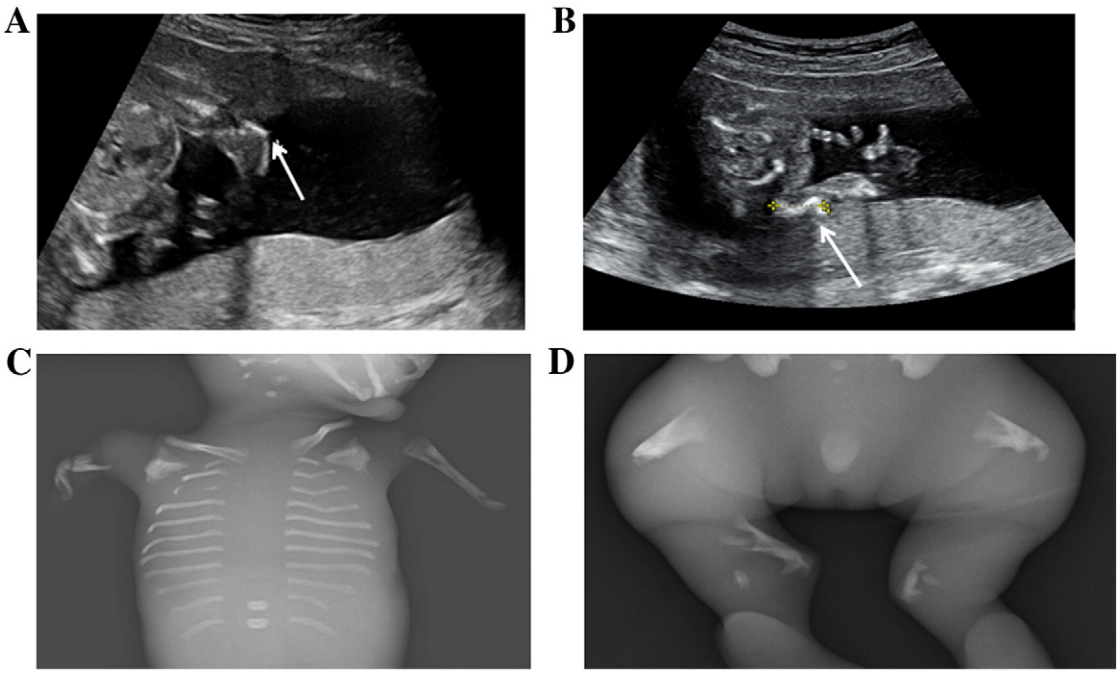

Fig. 1. Sonographic and radiographic findings. Sonographic images *in utero* at 19 weeks of gestation (A, B). A: Fracture of the left radius (white arrow). B: Angulation of the right humerus. Radiographs taken after delivery at 20 weeks of gestation. (C, D) Typical radiographic features of perinatal HPP as follows: C: Absence of ossification of thoracic vertebrae, and incomplete ossification of the ribs; D: Incomplete of ossification of long bones and severe shortening of long bones with deep metaphyseal cupping.

in the cord blood. HPP was diagnosed on the basis of imaging data and low ALP activity in the cord blood. To confirm the family's genetic status, in hopes of also estimating recurrence risks for them, genetic testing was offered and performed after obtaining informed consent. Genomic DNA was extracted from blood samples obtained from both parents and from 2 mL of umbilical cord blood of the proband for further molecular analysis in the research laboratory at Nippon Medical School (Tokyo, Japan).

2. Material and methods

2.1. ALPL analysis

Exons 2–12, including all coding regions and flanking intronic regions of *ALPL*, were amplified from genomic DNA of the proband and parents using the polymerase chain reaction (PCR). Amplicons were bidirectionally sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit and an ABI Prism 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA). To analyze each allele, the amplicons containing single nucleotide polymorphism(SNP)s spanning intron 11–exon 12, including the mutation c.1559delT, were cloned using the pGEM-T Easy Vector (Promega, Madison, WI, USA).

2.2. Haplotype analysis

Seven microsatellite markers on 1p and two on 1q, spanning chromosome 1, were amplified from genomic DNA of the proband and parents using the ABI PRISM Linkage Mapping Set v2.5 (Life Technologies). Fragments were analyzed using an ABI Prism 3130 Genetic Analyzer with the denaturing POP7 polymer. Electropherograms were analyzed using GeneMapper software (Life Technologies).

2.3. Whole-genome array cytogenetics

Genomic DNA of the proband was analyzed using the CytoScan HD Array (Affymetrix, Santa Clara, CA, USA) including 1.9 million nonpolymorphic copy number probes and 750,000 SNP probes. The overall average inter-probe distance is approximately 1150 base pairs. Genomic coordinates are based on genome build 37/hg19 (2009). The hybridized arrays were washed using an Affymetrix GeneChip Fluidics 450 and scanned with a GeneChip Scanner 3000 7G, and the data were analyzed using Affymetrix Chromosome Analysis Suite (ChAS) Software.

3. Results

3.1. ALPL mutation analysis

The proband was diagnosed with perinatal HPP and carried a homozygous mutation, c.1559delT, in *ALPL*. Sequence analysis identified the father as a heterozygous carrier. The mother was wild type (Figs. 2A,B), suggesting a complete deletion of one allele in the mother, a maternal allelic *de novo* mutation, or paternal UPD.

3.2. ALPL SNPs

The proband was homozygous for all other SNPs from *ALPL* exons 7 to 12 surrounding c.1559delT. Heterozygous maternal SNPs excluded a complete deletion. One informative SNP in the proband, c.1640T, indicated uniparental (paternal) inheritance, because no maternal allele was inherited (Fig. 2C). Next, we performed haplotype analysis and found that the proband was homozygous for all paternal *ALPL* SNPs (Fig. 2D).

3.3. Loss of heterozygosity (LOH) on chromosome 1

We performed haplotype analysis with nine polymorphic microsatellite markers spanning chromosome 1 to investigate the possibility of UPD (Fig. 3). The proband was homozygous for each marker, and analysis of microsatellite markers did not show typical Mendelian inheritance in the alleles of the proband derived from the paternal and maternal alleles. Five informative loci indicated that both copies were uniparentally (paternal) inherited, while analysis of markers on another chromosome showed normal biparental inheritance. Genotyping of the