—Case Reports—

Prenatal Genetic Diagnosis of Severe Perinatal (Lethal) Hypophosphatasia

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Abstract

Hypophosphatasia is an inherited disorder characterized by defective bone mineralization and a deficiency in tissue-nonspecific alkaline phosphatase (TNSALP) activity. This disorder is caused by various mutations of the *TNSALP* gene. We report here the prenatal diagnosis of the perinatal (lethal) type of hypophosphatasia in a sibling of an affected infant. The infant had been found to have hypophosphatasia on the basis of both clinical and radiologic manifestations and the finding of a homozygous single T nucleotide deletion at 1559 (1559delT) of the *TNSALP* gene on molecular analysis. Both parents were carriers with a heterozygous mutation in the same position, although they were not consanguineous. After their next child had been conceived, fetal genomic DNA was extracted from cultured cells of amniotic fluid at 15 weeks' gestation. The fetus had a homozygous 1559delT mutation. An ultrasonography examination at 19 weeks' gestation showed marked hypomineralization of all bony structures. A prenatal genetic diagnosis for hypophosphatasia in combination with ultrasonography is thus considered to be useful for confirming the diagnosis of hypophosphatasia, which presents with a wide variety of phenotypes. As a result, prenatal genetic counseling for hypophosphatasia with collaboration between obstetricians and clinical genetics teams.
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Key words: Prenatal diagnosis, hypophosphatasia, genetic diagnosis, ultrasonography, genetic counseling

Introduction

Alkaline phosphatases (ALPs) are a group of ubiquitous, nonspecific enzymes that hydrolyze many types of monophosphate esters in most cells[1]. Hypophosphatasia is an autosomal recessive inherited systemic skeletal disorder caused by the defective function of a tissue-nonspecific alkaline phosphatase (TNSALP)[1]. Clinically, hypophosphatasia has been classified into at least five types according to severity and age at onset: perinatal or lethal,

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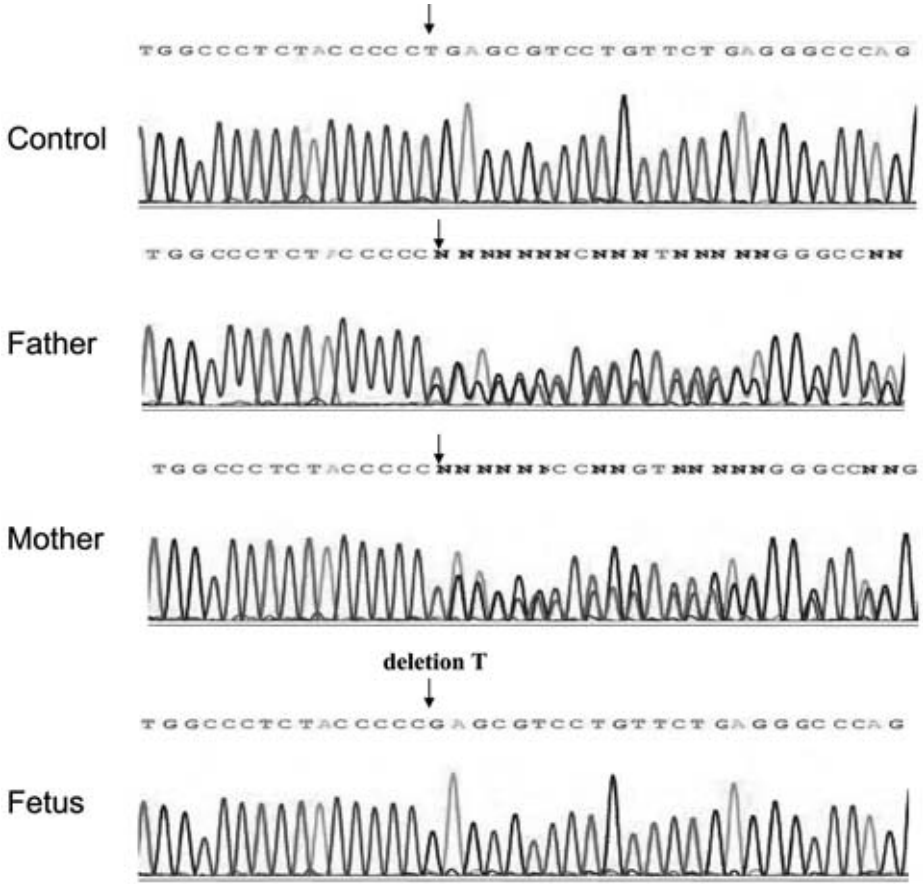

Fig. 1 PCR-direct sequencing analysis of genomic DNA from a healthy control, both parents, and the fetus. PCR was performed to amplify exon 12 of the *TNSALP* gene, and PCR products were sequenced directly. The sequence of the parents could not be determined in progress at cDNA number 1559 of the *TNSALP* gene. These results indicate both parents were heterogenous carriers for 1559delT of the *TNSALP* gene. The sequence of the fetus could be determined in progress containing the deletion of T at nucleotide 1559, which was different from that of a healthy control. This result indicates that the fetus is homozygous for a 1559delT of the *TNSALP* gene.

infantile, childhood, adult, and odontohypophosphatasia[1]. The perinatal or lethal type of hypophosphatasia exhibits the most severe symptoms, whereas other types tend to exhibit milder symptoms[1].

We herein describe our findings in a case of prenatally diagnosed perinatal hypophosphatasia which was homozygous for a deletion of T at nucleotide 1559 (1559delT) of the *TNSALP* gene.

Case Report

Hypophosphatasia was diagnosed in an infant on the basis of clinical and radiologic manifestations and laboratory findings after birth. Radiographs showed severe skeletal hypomineralization. Laboratory studies showed a deficiency in serum alkaline phosphatase. Direct sequencing of the *TNSALP* gene with the polymerase chain reaction (PCR) showed that this condition was caused by a homozygous single T nucleotide deletion at cDNA number 1559 (1559delT) (the first nucleotide (+1) corresponds to the A of the ATG initiation codon using the *TNSALP* cDNA number of the standard nomenclature[2]). Both parents were heterozygous carriers for the mutation at the same position (1559delT) of the *TNSALP* gene, but there was no evidence of consanguinity or clinical symptoms.

Hypophophatasia has an autosomal recessive pattern of inheritance with a 25% chance of

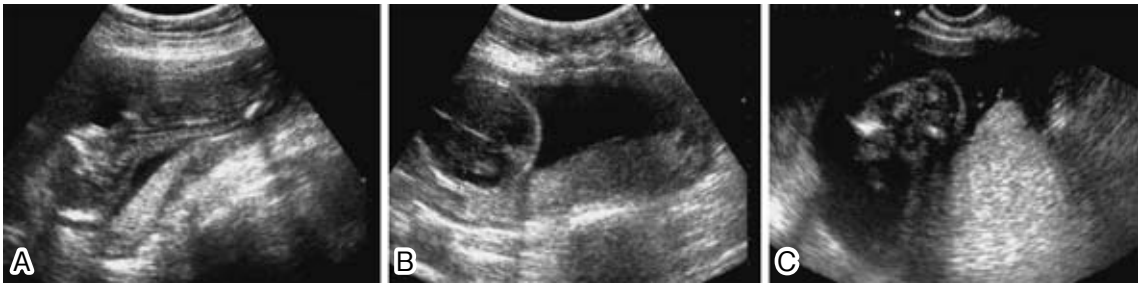

Fig. 2 Ultrasonography examination at 17 weeks' (A) and 19 weeks' (B, C) gestation of the fetus. A sagittal scan of the fetus at 17 weeks' gestation (A) showed no acoustic shadowing. The cranium at 19 weeks' gestation (B) was thin with marked hypomineralization. The femur at 19 weeks' gestation (C) was shortened with no evidence of fractures. These finding are consistent the perinatal (lethal) type of hypophosphatasia.

recurrence in the next pregnancy[1]. After the couple conceived another child, prenatal genetic diagnosis was performed by an obstetrician after both parents were given information by a clinical geneticist and gave informed consent and after permission was received from the Independent Ethics Committee of the Nippon Medical School Hospital. Psychological support by a nurse before and after testing was offered to the parents. We also held a clinical genetics conference to discuss this case and to provide suitable counseling to each client and to discuss the ethical, legal, and social issues regarding this case in the field of genetics.

Fetal genomic DNA was extracted from cultured cells of the amniotic fluid at 15 weeks' gestation. PCR was performed using primers of exons 12, as described previously[3], to amplify exon 12 of the *TNSALP* gene containing the position at cDNA number 1559. The mutation was confirmed by the direct-sequencing of PCR products from both parents and the fetus. The fetus was found to have a homozygous 1559delT mutation (**Fig. 1**). Ultrasonography showed no acoustic shadowing at 17 weeks' gestation but did show marked hypomineralization of all bony structures and a shortening of the tubular bones at 19 weeks' gestation (**Fig. 2**).

The clinical geneticist and the obstetrician provided this diagnostic information regarding the fetus and genetic counseling to the parents to enable them to make an informed decision by themselves. Genetic counseling was performed nondirectively, with an evaluation that enabled the clinical geneticist to see whether parents understood the information provided. The parents decided to terminate pregnancy 3 days after they were informed of the diagnosis. This waiting period allowed parents to deliberate after recovering from the initial shock of the diagnosis and reduced the possibility they would regret their decision. The parents had faced the grief process associated with unanticipated pregnancy loss since the pregnancy termination. Follow-up support after reaching this decision was offered to the parents, especially to the mother, by the obstetrician and the nurse. Six months after the pregnancy loss, the mother reported that she had stopped grieving.

Discussion

Hypophosphatasia is an inherited disorder characterized by defective bone mineralization and a defiiency of TNSALP activity[1]. It is classified into at least five types according to the severity and age at onset: perinatal or lethal, infantile (MIM [Mendelian Inheritance in Man[4]] number 241500), childhood (MIM number 241510), adult (MIM number 146300), and odontohypophosphatasia[1]. This disorder is caused by various mutations of the *TNSALP* gene[1]. The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database[5] currently includes 184 *TNSALP* gene mutations responsible for hypophosphatasia (update: July 2006). These mutations occur in a small number of patients in North America, Japan, and Europe, thus indicating that this the disease has a very strong allelic heterogeneity.

A deletion of T at 1559 in the *TNSALP* gene, which caused a frameshift downstream from leucine (Leu) at codon 503, resulted in the elimination of the termination codon at 508 and the addition of 80 amino acid residues at the C-terminus. The mutant protein caused by 1559delT formed an aggregate, was polyubiquitinated, and was then degraded in the proteome[6,7], thus allowing us to directly correlate the phenotype (perinatal type) and the genotype (1559delT). In this case, the parents had a heterozygous mutation at the same position (1559delT) of the *TNSALP* gene with no evidence of consanguinity. The mutation 1559delT appears to be common in the Japanese population because approximately 71% of reported Japanese patients with hypophosphatasia carry this deletion, corresponding to an allele frequency of approximately 36%[2].

Perinatal hypophosphatasia has been diagnosed in utero by ultrasonography performed with careful attention to the limbs and the skull[7]. In this case, the fetus exhibited typical signs of the most severe form of the disease, namely, perinatal hypophosphatasia: *in utero* impaired mineralization at 19 weeks' gestation. However, perinatal hypophosphatasia is occasionally not diagnosed with sonographic examination in the first trimester because incomplete ossification is a normal finding at this stage of development[8]. Our report also confirmed that the 1559delT mutation corresponds to an allele responsible for perinatal lethal hypophosphatasia. Prenatal genetic diagnosis for hypophosphatasia in combination with ultrasonography is thus considered useful for confirming a diagnosis of hypophosphatasia, which presents with a wide variety of phenotypes.

A prenatal genetic diagnosis gives a couple important information about the fetus[9]. A prenatal diagnosis should be provided in a supportive, noncoercive atmosphere that allows the couple to make informed choices regarding what is are best for them in view of their values and parenting goals. Genetic counseling is particularly important before prenatal diagnosis to enable parents to make an informed choice. Counseling before testing makes counseling after testing (for those with an affected fetus) less difficult because prospective parents are better prepared[10]. In view of the psychological distress that the choice of abortion presents for women, follow-up is required for all women who receive prenatal diagnostic results showing the presence of a genetic condition, whatever their decision[9]. Careful counseling regarding if and how to inform the parents about the child can help to overcome this potential problem. A prenatal genetic diagnosis may also help the professional team to prepare for a difficult delivery[10]. Nippon Medical School established a genetics clinic in 1998[11] and a division of clinical genetics as one of its central service departments in May 2003. We hold a clinical genetics conference once a month to discuss each case to provide suitable counseling to each client and to discuss the ethical, legal, and social issues in the field of genetics. This prenatal genetic diagnosis is, therefore, considered to be helpful for parents based on close collaboration between the obstetricians and the clinical genetics teams.

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References

1. Whyte MP: Hypophosphatasia. In The Metabolic and Molecular Bases of Inherited Disease, vol III, 8th edn. (Scriver CR, Beaudet AL, Sly WS, Valle D, eds) 2001; pp 5313–5329, McGraw-Hill, New York.
2. Orimo H, Goseki-Sone M, Inoue M, Tsubakio Y, Sakiyama T, Shimada T: Importance of deletion of T at nucleotide 1559 in the tissue-nonspecific alkaline phosphatase gene in Japanese patients with hypophosphatasia. J Bone Miner Metab 2002; 20: 28–33.
3. Orimo H, Goseki-Sone M, Sato S, Shimada T: Detection of deletion 1154-1156 hypophosphatasia mutation using TNSALP exon amplification. Genomics 1997; 42: 364–366.
4. Mendelian Inheritance in Man. [OMIM: Online Mendelian Inheritance in Man site] available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&itool=toolbar. Accessed July 24, 2006.
5. The Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database. Available at http://www.sesep.uvsq.fr/Database.html. Accessed July 24, 2006.
6. Goseki-Sone M, Orimo H, Iimura T, et al.: Expression of the mutant (1735T-DEL) tissue-nonspecific alkaline phosphatase gene from hypophosphatasia patients. J Bone Miner Res 1998; 13: 1827–1834.
7. Komaru K, Ishida Y, Amaya Y, Goseki-Sone M, Orimo H, Oda K: Novel aggregate formation of a

frame-shift mutant protein of tissue-nonspecific alkaline phosphatase is ascribed to three cysteine residues in the C-terminal extension. Retarded secretion and proteasomal degradation. FEBS J 2005; 272: 1704–1717.
8. Tongsong T, Pongsatha S: Early prenatal sonographic diagnosis of congenital hypophosphatasia. Ultrasound Obstet Gynecol 2000; 15: 252–255.
9. WHO (World Health Organization) Meeting on Ethical Issues in Medical Genetics: WHO Proposed International Guidelines on Ethical Issues in Medical Genetics and Genetic Services, 1998; Available at http://www.who.int/genomics/publications/en/. Accessed July 24, 2006.
10. Genetic Medicine Related Societies in Japan: Guidelines for genetic testing. 2004; Available at http://jshg.jp/Accessed July 24, 2006.
11. Watanabe A, Migita M, Watanabe Y, Shimada T: Perspective of clinical genetics. Nippon Ika Daigaku Zasshi 1999; 66: 340–342. Japanese.

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