Use of Next-generation Sequencing for Prenatal Diagnosis of Hypophosphatasia

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We report on a patient who had recurrent skeletal dysplasia in three of four of her pregnancies which all resulted in termination of pregnancies. The ultrasound and / or histological findings of her first and fourth pregnancy were suggestive of osteogenesis imperfecta while those in her second pregnancy were more suggestive of achondrogenesis. The exact diagnosis could not be made clinically at that time. With the emergence of next-generation sequencing (NGS), the stored placental tissue from her second pregnancy was retrieved for testing 4 years after the pregnancy was terminated. NGS detected two heterozygous pathogenic variants in the *ALPL* gene which were associated with autosomal recessive hypophosphatasia. This case demonstrated the usefulness of NGS in making an exact diagnosis on the type of skeletal dysplasia which was important in counselling the patients on the risk of recurrence, and offering prenatal diagnosis in their future pregnancies.
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Case Report

A patient, who enjoyed good past health, presented with hypophosphatasia. She and her husband were of average height and there was no family history of skeletal dysplasia. During her first pregnancy in 2010, when she aged 28 years, ultrasonography (USG) at 22 weeks of gestation revealed a fetus with severely shortened long bones with multiple fractures. The patient opted for termination of pregnancy. Babygram (Figure 1) and histological examination of the fetus showed that the upper and lower limbs were shortened with inward bowing and multiple fractures, including the ribs. A normal karyotype was found. The patient was counselled by the Clinical Genetics Service about a suspected diagnosis of osteogenesis imperfecta (OI) type 2. Although most cases are de novo, there is a risk of germline mosaicism with a consequent estimated risk of recurrence of 6%.

In her second pregnancy in 2011, USG at 17 weeks of gestation showed grossly shortened limb bones with poor bone mineralisation but no fractures (Figure 2). The patient underwent termination of pregnancy. Babygram and histological examination of the fetus showed markedly shortened limb bones but no fractures (Figure 3). Sections of the diaphysis and cortex showed lack of ossification. The clinical picture was more suggestive of achondrogenesis than OI. The patient was again referred to the Clinical Genetics Service and advised that her case was likely to be an autosomal recessive type of skeletal dysplasia, although the exact diagnosis was uncertain.

The patient had her third pregnancy in 2012. Serial USG scans showed normal growth of the fetus with normal long bone length. Subsequently she delivered a normal healthy baby.

She had her fourth pregnancy in 2013 and received no antenatal care until 32 weeks of gestation. USG at that time indicated that the bi-parietal diameter and abdominal circumference corresponded to the gestation, but again the long bones were severely shortened and corresponded to only around 17 weeks of gestation. There were multiple fractures and the thoracic chest wall was narrow and compressed. There was polyhydramnios with amniotic fluid index of 30 cm. The patient subsequently underwent termination of pregnancy in China.

Next-generation sequencing (NGS) was performed by the Clinical Genetics Service in 2015 on the placental tissue stored from the 2011 pregnancy. Two heterozygous pathogenic variants in exon 7 of *ALPL* gene were detected (c.650delinsCTAA and c.736A>T). These were reported to be associated with autosomal recessive hypophosphatasia.

The patient is currently pregnant in 2017. The

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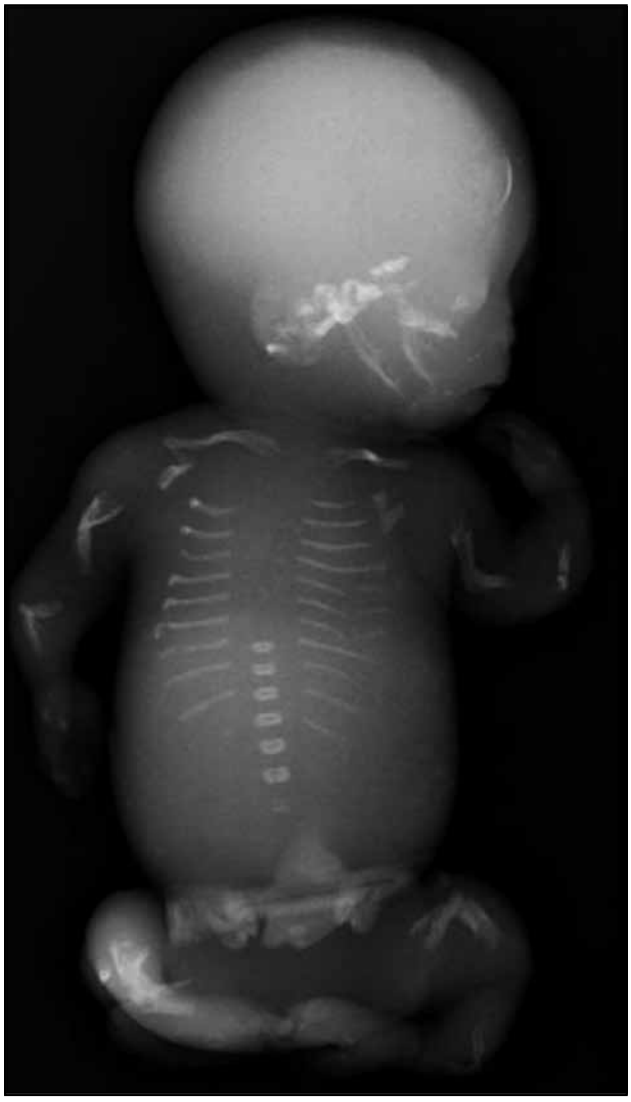

Figure 1. Babygram of the first pregnancy in 2010 shows grossly shortened long bones of the appendicular with multiple fractures

couple have been advised that they are both likely to be carriers of the hypophosphatasia gene with an associated 25% risk of fetal hypophosphatasia in this pregnancy. They have been offered blood tests to confirm their carrier status. If status is confirmed, prenatal diagnosis by genetic testing of the *ALPL* gene can be offered. As of March 2017, serial USG scans at 14, 17, and 20 weeks of gestation revealed a fetus with normal long bones, indicating a likely unaffected pregnancy.

Discussion

Hypophosphatasia is caused by missense mutations in the *TNSALP* gene on chromosome 1p36.1-p34, and it impairs bone mineralisation[1]. It is subdivided into six clinical types according to the age of onset: perinatal, prenatal benign, infantile, childhood, adult, and odontohypophosphatasia. The perinatal type is fatal and is inherited in an autosomal recessive manner, affecting 1 in 100,000 pregnancies[2].

Traditional diagnosis of hypophosphatasia was made by USG, features of which include severe micromelia, decreased thoracic circumference, and bone demineralisation[2]. The demineralisation of the long bones may be patchy or generalised with bowing of the bones, and fractures are occasionally seen. The skull size is typically normal but the demineralised calvarium causes the brain to be more easily visualised on USG scan and the cranial vault may be compressible under gentle transducer pressure. Skin-covered osteochondral spurs (Bowdler spurs) protruding from the midshaft or around the elbow or knee joints are specific features diagnostic of hypophosphatasia[2,3].

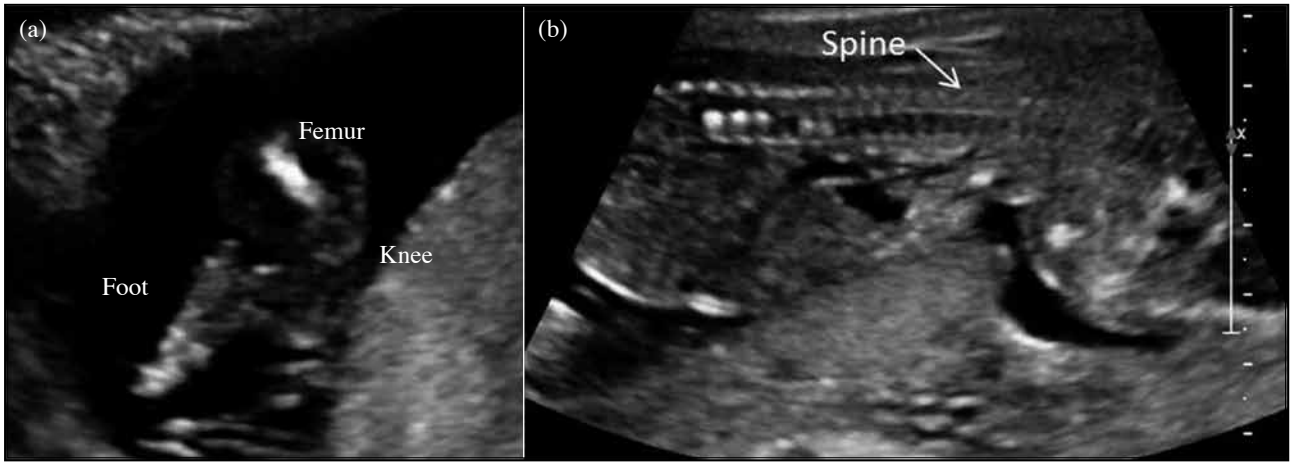

Figure 2. Ultrasonogram at 17 weeks of gestation in the second pregnancy in 2011. (a) The femur is grossly shortened with underdevelopment of the tibia and fibula. (b) Poor mineralisation of the vertebral column is shown

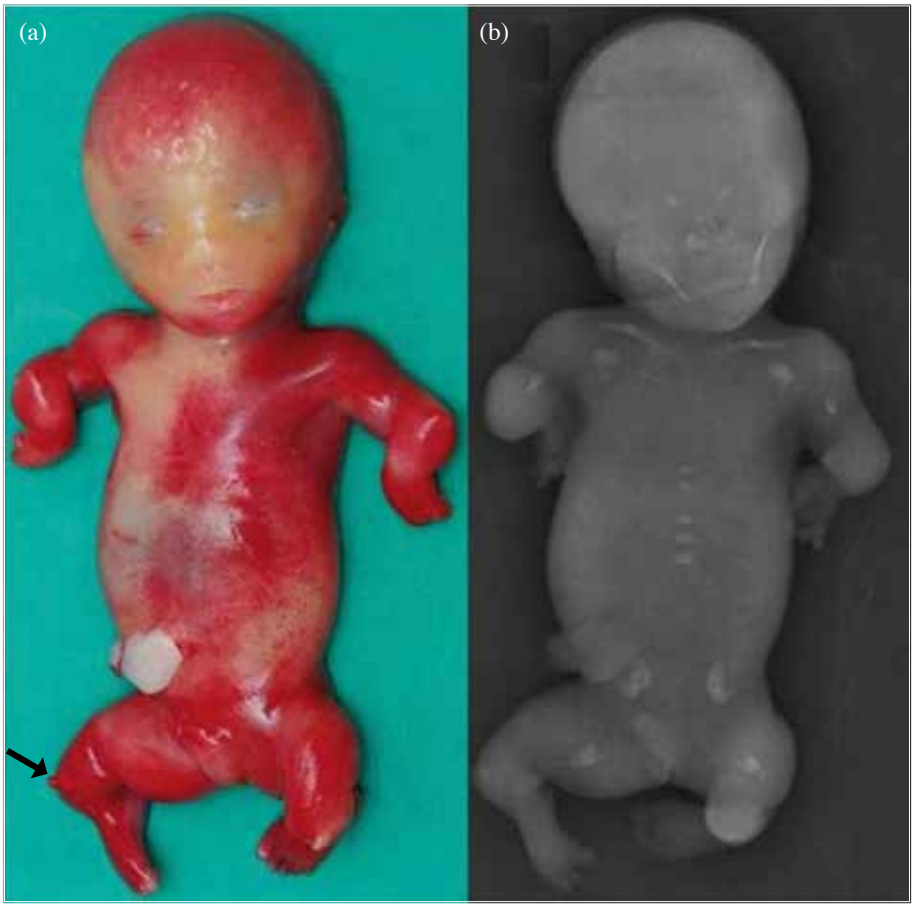

Figure 3. (a) Abortus of second pregnancy in 2011; bone spur in the right knee is shown (arrow). (b) Babygram showing poor mineralisation of the calvarial bones, most part of the vertebral column, rib cage, and lack of ossification of the sacrum and ischial bones

These spurs are often unossified and difficult to detect on USG imaging, but they may be evident on histological examination. On close scrutiny of our patient, such spurs were present in the abortus of the second pregnancy (Figure 3; spur on right knee of the abortus). A low serum alkaline phosphatase (ALP) level and a high pyridoxal-5-phosphate level are helpful in the detection of heterozygous hypophosphatasia carriers[4]. Retrospective review of liver function tests of our patient revealed that serum ALP at 18 weeks of gestation in her second pregnancy was below normal at 21 IU/L (reference range, 35-104 IU/L), and in her current pregnancy it was at the lower normal range (35 IU/L) at 24 weeks of gestation. This is compatible with a maternal carrier of hypophosphatasia with a lower-than-normal serum ALP. As gestation advances, however, there will be a physiological increase in maternal ALP level due to placental ALP and this may mask the previous abnormal findings[4]. Therefore, in the prenatal diagnosis of suspected fetal hypophosphatasia detected by USG scan at or after mid-second trimester, analysis of these biochemical markers in maternal blood may not be useful. Confirmation can probably only be made after the pregnancy. Paternal blood should be analysed in parallel to determine carrier status of the father.

Hypophosphatasia has considerably overlapping clinical features with other types of skeletal dysplasia such as OI type 2 and achondrogenesis[2,3,5,6]. As these types of skeletal dysplasia all show severe micromelia and decreased thoracic circumference, a specific diagnosis based on radiological / pathological findings alone is achievable in only 55% of patients[2]. Furthermore, long bone fractures are sometimes present in hypophosphatasia that mimicks OI type 2 as in the first pregnancy of our patient. Both hypophosphatasia and achondrogenesis show bone demineralisation on radiological and histological examination that makes diagnosis of a specific skeletal dysplasia difficult. OI type 2 is inherited in an autosomal dominant pattern. Almost all cases are due to de-novo mutations. The risk of recurrence in a future pregnancy should be very low. Allowing for the possibility of a germ-line mutation, risk is usually estimated to be approximately 6%[6]. On the contrary, hypophosphatasia is inherited in an autosomal recessive fashion with a recurrence risk of 25%

in future pregnancy. Since the recurrence risk of these two conditions is so different, an accurate diagnosis is essential so that genetic counselling can provide precise information about risk of recurrence in future pregnancy.

Traditionally, genetic diagnosis of hypophosphatasia is performed by sequencing the *TNSALP* gene for mutations, of which over 300 have been reported[7]. Previous studies have reported a 95% detection rate of these mutations for hypophosphatasia by sequencing the 12 exons and intron/exons borders in the *ALPL* gene by Sanger method[8]. The remaining undetected mutations probably affect intronic or regulatory sequences, or correspond to large deletions partly detected by quantitative polymerase chain reaction (PCR) or semi-quantitative methodologies like quantitative multiplex PCR of short fluorescent fragments[9]. However, the Sanger method supplemented by PCR methods is expensive and time-consuming[7]. In addition, since OI, campomelic dysplasia, and various other skeletal dysplastic conditions are often the main differential diagnoses, in the presence of a negative result for *ALPL* mutations, these specific conditions would have to be analysed and excluded sequentially, lengthening the time before diagnosis. Advances in NGS technology now enable one-time sequencing of several genes (targeted NGS) or all the coding sequences of genes (exome sequencing) or the full genome. An NGS 'bone panel' can now be used to establish the diagnosis in these cases. Typically, the panel can include the *ALPL* gene, genes of differential diagnosis *COL1A1* and *COL1A2* that represent 90% of OI cases, *SOX9* responsible for campomelic dysplasia, as well as an additional 8 to 10 potential modifier genes of hypophosphatasia[7]. Molecular genetic analysis using such a comprehensive panel will allow us to diagnose a specific type of skeletal dysplasia as in our patient in a single sequencing run. The carrier status of the couple can then be checked by NGS and prenatal diagnosis can be offered. Such NGS techniques are therefore particularly useful in prenatal diagnosis when time is of the essence. Recent studies have also supported the use of NGS in diagnosing specific types of skeletal dysplasia to help in counselling couples about the recurrence risk and to provide prenatal diagnosis for future pregnancies[7-10]. With technological advances, pre-implantation genetic diagnosis by NGS for carrier couples will likely be feasible in the near future.

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Declaration

The authors have disclosed no conflicts of interest.

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