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A Case of Perinatal Lethal Form of Hypophosphatasia; and Review of Literatures

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Abstract Hypophosphatasia is a rare inborn error of metabolism. The disease is characterised by skeletal mineralisation and dentition defects. Mutations have been found in the *TNSALP* gene in various forms of hypophosphatasia. We report the first Chinese case of perinatal lethal form of hypophosphatasia due to previously reported pathogenic compound heterozygous mutation in the *TNSALP* gene; and successfully applied clinically in prenatal diagnostic testing. The authors emphasised the importance of accurate diagnosis of fetal presentation of skeletal dysplasia for genetic counselling, and discussed its differential diagnoses.

Key words Alkaline phosphatase; Hypophosphatasia; Perinatal lethal form of hypophosphatasia; *TNSALP* gene

Introduction

Hypophosphatasia is a rare inherited metabolic disorder of bone mineralisation in which the enzyme tissue non-specific alkaline phosphatase (*TNSALP*) is deficient. Alkaline phosphatase (ALP) is normally abundant in cartilages and in bones. It releases phosphate from sugars containing phosphate. Because it works well in alkaline environment, therefore it is called the ALP. ALP released phosphate in solutions within cartilage and bone tissue so that phosphate could combine with calcium to form crystals of calcium-phosphate (hydroxyapatite) to mineralise the skeleton.

Hypophosphatasia (meaning low ALP level, which is the biochemical hallmark of the disease) was first described in 1948 in an infant who died in infancy with severe rickets and seizures; and was found to have low level of ALP. It was soon found to recur in sibs, and thus realised it was not an infective disease in the 1950's. The oldest chemical "marker" for hypophosphatasia was phosphoethanolamine (PEA) which was found in the 1950's to be a substrate for ALP; and the level was high in plasma and urine of hypophosphatasia patients. Another important substrate was found in the 1960's namely inorganic pyrophosphate (PPi). It was known to inhibit the formation of calcium-phosphate crystals at high concentration and explained why hypophosphatasia patients do not mineralise their skeletons properly. The third natural substrate for ALP was found in 1980's called pyridoxal 5'-phosphate (PLP) which was a form of vitamin B6; and its level seems to be constant throughout childhood, adolescence, and adult life in hypophosphatasia patients, therefore reflecting the degree of severity regardless of the patient's age.

TNSALP is vital for skeletal mineralisation and dentition in human. Its deficiency causes defective skeletal

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mineralisation that manifests as rickets when young and osteomalacia in adults. In addition, premature loss of deciduous teeth is another major clinical feature of hypophosphatasia due to deficiency of the enzyme. Clinical presentation of the disease is remarkably variable. Rarely individuals with characteristic biochemical abnormalities may never become symptomatic.[1,2] In general, clinical and biochemical derangements are concordant.

Several forms of hypophosphatasia have been described; the perinatal lethal form, infantile form, late presentation form, odontohypophosphatasia and pseudohypophosphatasia; depending on the age at presentation or if only dental problems are present. There is a remarkable great range of severity from stillbirth to merely losing a few teeth in adult life. In general, the earlier the presentation, the more severe is the disease. Perinatal lethal and infantile forms are the commonest forms of hypophosphatasia, whereas late presentation form in adults are much less common in hypophosphatasia.

Perinatal lethal hypophosphatasia is the most severe form of hypophosphatasia. The disease is expressed in prenatal life and can result in stillbirth. The skeleton is characterised by extreme hypomineralisation with limbs shortening and deformity. There are rachitic changes in the ribs and osteochondrial spurs that may or may not protrude through the skin, or may just cause skin dimpling, in the forearms or the lower legs.

Radiographic study of the skeleton in Perinatal lethal hypophosphatasia is diagnostic with almost complete skeletal hypomineralisation; and irregular extension of radiolucency into metaphyses.

Infantile hypophosphatasia presents in first six months of life with failure to thrive and rickets. Radiographic features resemble that of perinatal lethal hypophosphatasia but less severe. The late presentation form in childhood and adult has an extremely variable clinical presentation that may include short stature, premature loss of teeth, rickets or osteomalacia, and stress fractures. Pseudo-hypophosphatasia is an extremely rare form of hypophosphatasia in which clinical presentation resemble that of infantile hypophosphatasia but without the characteristic biochemical abnormalities.

The inheritance of most forms of hypophosphatasia is primarily that of autosomal recessive, either homozygous or compound heterozygous in nature. This is particularly true for severe lethal forms; and even odontohypophosphatasia have been reported to be due to autosomal recessive inheritance.[3] Mild cases have been reported to be transmitted as autosomal dominant inheritance. However vertical transmission is less commonly seen in hypophosphatasia. Only rarely, in families where there was a mildly affected patient in one generation and a severely affected patient in their next generation, was it due to homozygosity of a dominant mutation. Mornet et al (2000) stated that same mutation in the *TNSALP* gene can cause both autosomal dominant or recessive hypophosphatasia, and that parents of severe recessive form may have undiagnosed mild symptoms of late onset adult hypophosphatasia or odontohypophosphatasia.[4]

Hypophosphatasia is said to be most prevalent in inbred Mennonite families from Manitoba, Canada. The incidence of severe forms in Toronto, Canada was said to be 1 per 100,000 live births.[3] Incidences for other ethnic groups are unknown but cases have been reported.[5] The human gene mapping symbol for *TNSALP* is called the ALPL (ALPL-liver). The *TNSALP* gene contains 12 exons and spanned more than 50 kb; and is located at chromosome 1p36.1-34.[6] Majority (80%) of mutations are missense.[7] Other mutations reported include nonsense, splice-site and frameshift; and they are all loss of function mutations. There is no mutational hotspot in the gene; clinical diagnosis is confirmed by extensive analysis of the gene.

Biochemical ascertainment of hypophosphatasia carriers using the three markers as previously described is not straight forward.[8] Molecular testing in parents of affected would promptly reveal their carrier status.

We here report the first Chinese case of perinatal lethal form of hypophosphatasia confirmed by mutational analysis, and discuss its differential diagnoses. The authors emphasised the importance of correct diagnosis for proper genetic counselling that include counselling on the nature of the condition, inheritance, recurrence risk and the choice of prenatal molecular testing available in order for them to make informed decisions.

Subjects, Methods and Results

Consultand and her husband were both of Chinese descent and were non-consanguineous couple. They were both University graduates and enjoyed good health all along. They both had normal full sets of teeth; and with no past history of fractures. Consultand measured 155 cm in height (25-50%) and her husband measured 160 cm in height (3-10%). Both sides of the family had no family history of fetal anomaly or fetal death.

In her past history, it was noted that her first pregnancy in year 2000 ended in first trimester spontaneous

miscarriage. In 2001, she gave birth to a healthy boy. In 2003, fetal anomaly was detected at 22 weeks gestation. Termination of pregnancy was opted.

Consultand presented to Clinical Genetic Service in her fourth pregnancy at 14th week gestation when she was referred in 2004. At that time, based on the babygram of the third pregnancy abortus taken in 2003, the presumptive diagnosis of the third pregnancy was the perinatal lethal form of hypophosphatasia.

The features of the babygram of the third pregnancy abortus (Figure 1) were summarised as below:

1) Absence of ossification of the calvaria, defective ossification in the facial bones and base of skull.
2) Defective ossification in the paravertebral neural arches.
3) Small triangular scapulae and small irregular pelvic bones.
4) Short thin ribs and tubular bones. Metaphyseal ossification defects extending into diaphyses of tubular bones were noted.

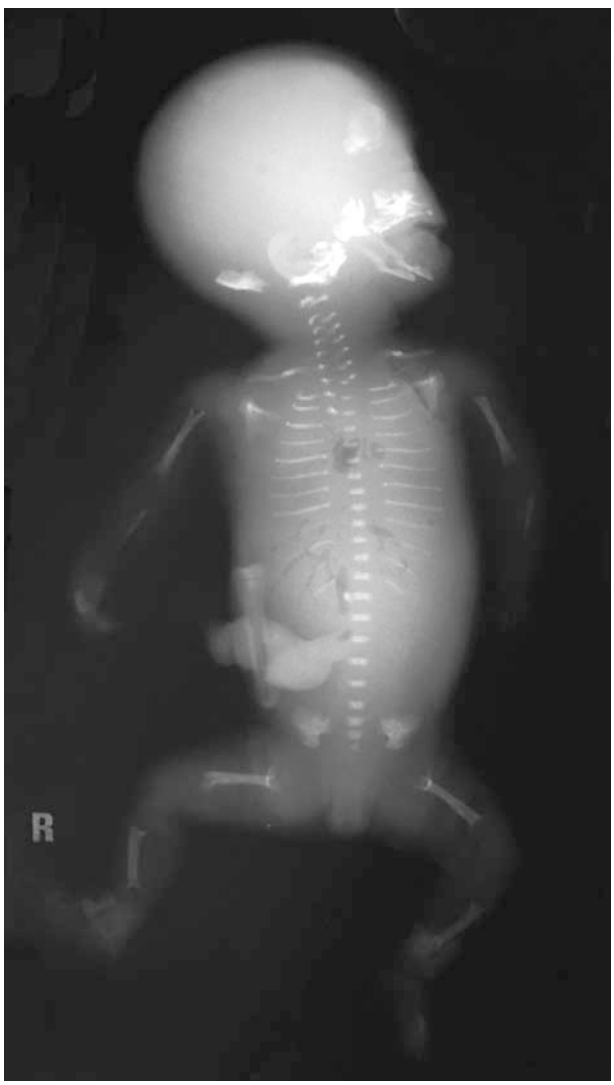

Figure 1 Babygram of abortus.

Both couples had low levels of serum ALP. Father's ALP level was 29U/L(49-138U/L), Consultand's ALP level was 29U/L(34-104U/L).

Molecular study for the couple showed that the consultand carried 1154 del CTT,[9] while her husband carried the ala94thr mutation[4] of the *TNSALP* (ALPL) gene. Both mutations were published pathogenic mutations.

Amniocentesis was performed for the fourth pregnancy at 18 weeks gestation in 2004 for *TNSALP* mutational analysis.

All coding exons and flanking introns of the *TNSALP* (ALPL) gene were amplified by PCR. PCR products were purified by Microspin S-300 HR columns (GE Healthcare, Uppsala, Sweden) and both strands were sequenced by BigDyeDeoxy terminator cycle sequencing reagents according to the manufacturer's instructions (Applied Biosystems). Products of sequencing reactions were purified by Auto-Seq G-50 columns (GE Healthcare). Purified sequencing fragments were separated by capillary electrophoresis and detected by laser induced fluorescence on an ABI Prism 3100 genetic analyzer (Applied Biosystems).

Mutational analysis of cultured amniocytes showed that the fetus carried the maternal mutation alone. Thus, the fetus was a carrier of hypophosphatasia, like mother.

Subsequent prenatal ultrasound examination at 21 weeks gestation of the fourth pregnancy showed normal fetal growth and no gross fetal abnormality. The fetus was born at terms, and was informed by obstetrician to be normal and well.

Parental consent for publication that included photos and radiographs were kindly given and signed.

Discussion

Clinical presentation of hypophosphatasia is remarkably variable. This may reflect the degree of residual enzyme activity in individual *TNSALP* mutation found. Zurutuza et al (1999) found correlation between genotype and phenotype in which severe missense mutation mostly located at active sites whereas milder phenotype were not found in the active sites of the enzyme.[10] The tentative

classification of hypophosphatasia based on age of presentation and radiographic appearance is commonly used.

The radiographic signs in the perinatal lethal type of hypophosphatasia are diagnostic; and should be differentiated from other skeletal dysplasia that present similarly in the prenatal period. Achondrogenesis and hypophosphatasia both have severely retarded ossification of the vertebrae. However, the two can be distinguished from hypophosphatasia in that there is defective ossification of neural arches but well visualized vertebral bodies in hypophosphatasia. Achondrogenesis, on the other hand, has defective ossification of vertebral bodies, giving a "zipper" appearance. In hypophosphatasia, tubular bones are formed but defective; while in achondrogenesis, in particular type 1, tubular bones are seen as clumps of disorganised bone tissues with arms and legs looked flipper-like.

Pauli et al in 1999 suggested a new addition to the spectrum of hypophosphatasia disorder; and a new differential diagnosis to antenatally presented bowed limbs, in reporting a case in which they named "the benign prenatal form of hypophosphatasia".[11] The fetus had "marked bowing of long bone" that resembled fractures in Osteogenesis Imperfecta, but did not have the skull, ribs and vertebral changes that were characteristic of the lethal types of Osteogenesis Imperfecta or the perinatal lethal type of hypophosphatasia. The authors commented that the condition "spontaneously improved" because the limbs deformity was subjectively looked "lessened" at 31 5/7 gestation; and that it was not lethal, hence the name "benign". In postnatal life, severe multiple limbs deformities were noted, bone mineralisation and skull ossification however were normal. "Minimal osteopenia...limb foreshortening and bowing persisted" when they last followed up the patient. There was no family history of limbs bowing or premature loss of teeth. Borderline biochemical abnormalities were found in the proband and her phenotypically normal mother. The authors then suggested that inheritance could be due to either imprinting defect or maternal autosomal dominant inheritance. However it would be interesting to see in long term follow-up if proband had complete resolution of all her deformities by adulthood, presumably like her phenotypically normal mother, who in turn did not have history of deformity; or whether limb deformities persist; or whether maternal inheritance in the family is confirmed by molecular testing. Is it really maternal inheritance as suggested by the authors? Or is it a variable presentation of existing forms; infantile form or pseudohypophosphatasia? Or is it a completely new form of hypophosphatasia, a transient hypophosphatasia that presented as severe form in the antenatal period but completely resolved without sequelae, giving a normal phenotype in adulthood? All these questions await future answers with great interest.

Moore et al (1999) similarly reported four pregnancies in two families with mild hypophosphatasia inherited in an apparent dominant trait. They presented in the prenatal period as severe long bone bowing; again with no skull, ribs and vertebral changes that were characteristic of the lethal types of Osteogenesis Imperfecta or the perinatal lethal type of hypophosphatasia. Bone mineralisation and skull ossification were commented as normal.[12] In family 1, II-6, the fetus had symmetrical bowing of arms and legs. Mineralisation of the whole skeleton was normal. At age 5, height was below third percentile with minimal femoral bowing. No additional clinical information was given. In family 1, II-7, the fetus also had symmetrical bowing of arms and legs but mineralisation of the whole skeleton was also normal. At 16 months of age, she required palliative operation for severe lower limbs bowing. At age 4, height was below the third percentile. Except for persistent femoral bowing and premature loss of lower incisors, no clinical details were given. In family 1, II-10, this fetus was the most severely affected and was the only one who had decreased ossification of calvaria and spine; in addition to, "bowing and shortening of limbs...femora and humeri most severely affected". Parents elected termination of pregnancy. Postmortem radiographics were interpreted as perinatal form of hypophosphatasia by Cedars-Sinai medical Center.

An elder sister (family 1, II-3) who had history of premature loss of teeth and lower limb bowing when young, made a remarkable recovery with improvement in height from the 3rd centile at 5 years to between the 50-75th centile at 10 years. No other details were given.

Mother in family 1 on the other hand, despite a completely normal phenotype clinically had biochemical abnormalities suggestive of hypophosphatasia. Similar to the case reported by Pauli et al (1999), it was unfortunate that the mutation was not found in the *TNSALP* gene in this family, as this mutation would be of great research interest in its functional role in producing a vast spectrum of severity from the most severe classic presentation, to transient intermediate presentation with eventual remarkable catch-up growth, and complete recovery to a complete normal phenotype right from birth to adulthood.

In family 2 reported by Moore et al, "ossification of skull and ribs appeared normal...symmetric bowing of upper and

lower limbs". At last follow-up aged 1 year, the authors commented that there was "mild residual femoral bowing". Clinical examination and molecular study confirmed autosomal dominant type of mild hypophosphatasia in the family. There were no detailed information on parental health and family histories. Again, long-term follow-up of this variant type with complete clinical (but not biochemical) recovery of a transient antenatal and infantile presentation, as seen in relatives or parents of the affected, would be of interest.

Spontaneous complete resolution of hypophosphatasia has rarely been reported since the publication of these, two papers. Whyte et al (June 2006) (a co-author of the above two papers) reported three cases of infantile hypophosphatasia, all due to the same homozygous *TNSALP* missense mutation.[13] These three cases were previously reported by Moore et al in 1990.[5] The proband was the only one in the family who manifested transient disease correction following subcutaneous injections of bovine parathyroid hormones, oral prednisone and infusions of normal human plasma. However, he eventually passed away, as was his affected younger brother who died at aged 11 months but did not show transient correction despite subcutaneous injections of synthetic salmon calcitonin and intravenous infusions of Paget plasma.[5] Their affected cousin also carried the same homozygous mutation, had infantile hypophosphatasia, did not manifest transient correction (not sure whether she had received same treatment), but survived. The authors could not explain the transient effect in the proband but suggested that epigenetic or non-genetic effect may account for the more benign course in the cousin with identical homozygous *TNSALP* mutation.[13]

Apart from osteogenesis imperfecta being an important differential diagnosis of multiple bowed long bones that present antenatally, bulging calvaria, delayed ossification of the skull, teeth, and extremities; clavicular hypoplasia or agenesis, and vertebral malformation bring cleidocranial dysplasia (CCD) into the differential diagnosis.

Morava et al reported in 2002 a mother and daughter with CCD who also had biochemical abnormalities of hypophosphatasia, including decreased levels of alkaline phosphatase.[14] Both patients had a heterozygous mutation in the *RUNX2* gene, and the authors concluded that this specific mutation caused secondary features of hypophosphatasia. Unger et al reported in 2002 a similar patient with CCD and osteopenia with decreased serum alkaline phosphatase, and concluded that osteopenia, osteoporosis, and decreased alkaline phosphatase may be underemphasised findings in CCD.[15]

Hypophosphatasia may present with rickets when young and osteomalacia in later life. It is important to differentiate from vitamin D-deficiency rickets as it may rarely present in the neonatal period and be confused with perinatal or infantile forms of hypophosphatasia. Vitamin D-deficiency rickets or osteomalacia from causes other than hypophosphatasia do not have low ALP level. On the other hand, rickets or osteomalacia in hypophosphatasia have low ALP levels, and is a disorder of bone matrix rather than vitamin D metabolism. Therefore vitamin D therapy is not useful, and may do more harm than good in the patient.

The path to correct diagnosis is further complicated by the fact that features of hypophosphatasia may not be accompanied by biochemical abnormalities as in pseudohypophosphatasia in which the exact underlying mechanism is not known. A recently proposed, but has not been formally classified as a new form, "Benign" variant in which spontaneous complete resolution of bent bones was suggested and anticipated by the phenotype of relatives of affected, requires further supportive evidence since its last reports in 1999. These patients had bent but not broken bones, and did not have the signs of severe hypophosphatasia of generalised skeleton mineralisation defect. Whether this "benign" variant represents variant of mild types of hypophosphatasia or compound heterozygosity of mild dominant mutations inherited from their parent(s) will hopefully be solved in future reports.

A correct diagnosis is crucial for genetic counselling for affected families. Explaining the nature of the disease, the inheritance, recurrence risks, and the availability of prenatal molecular testing would allow better understanding of the disease and the options available to families concerned, thus providing them basis to make informed decisions regarding in particular, family planning.

References

1. Whyte MP, Murphy WA, Fallon MD. Adult hypophosphatasia with chondrocalcinosis and arthropathy. Variable penetrance of hypophosphatasemia in a large Oklahoma kindred. Am J Med 1982;72:631-41.
2. Whyte MP, Teitelbaum SL, Murphy WA, Bergfeld MA, Avioli LV. Adult hypophosphatasia. Clinical, laboratory, and genetic investigation of a large kindred with review of the literature. Medicine (Baltimore) 1979;58:329-47.
3. Whyte M. The metabolic and molecular basis of inherited disease. 8th Edition. Scriver CR et al. New York : McGraw-Hill, 2001;207:5313-29.
4. Mornet E. Hypophosphatasia: the mutations in the tissue-

nonspecific alkaline phosphatase gene. Hum Mutat 2000; 15:309-15.
5. Moore CA, Ward JC, Rivas ML, Magill HL, Whyte MP. Infantile hypophosphatasia: autosomal recessive transmission to two related sibships. Am J Med Genet 1990; 36:15-22.
6. Sergi C, Mornet E, Troeger J, Voigtlaender T. Perinatal hypophosphatasia: radiology, pathology and molecular biology studies in a family harboring a splicing mutation (648+1A) and a novel missense mutation (N400S) in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. Am J Med Genet 2001;103:235-40.
7. Herasse M, Spentchian M, Taillandier A, et al. Molecular study of three cases of odontohypophosphatasia resulting from heterozygosity for mutations in the tissue non-specific alkaline phosphatase gene. J Med Genet 2003;40:605-9.
8. Gehring B, Mornet E, Plath H, Hansmann M, Bartmann P, Brenner RE. Perinatal hypophosphatasia: diagnosis and detection of heterozygote carriers within the family. Clin Genet 1999;56:313-7.
9. Orimo H, Goseki-Sone M, Sato S, Shimada T. Detection of deletion 1154-1156 hypophosphatasia mutation using TNSALP exon amplification. Genomics 1997;42:364-6.
10. Zurutuza L, Muller F, Gibrat JF, et al. Correlations of genotype and phenotype in hypophosphatasia. Hum Mol Genet 1999;8:1039-46.
11. Pauli RM, Modaff P, Sipes SL, Whyte MP. Mild hypophosphatasia mimicking severe osteogenesis imperfecta in utero: bent but not broken. Am J Med Genet 1999;86:434-8.
12. Moore CA, Curry CJ, Henthorn PS, et al. Mild autosomal dominant hypophosphatasia: in utero presentation in two families. Am J Med Genet 1999;86:410-5.
13. Whyte MP, Essmyer K, Geimer M, Mumm S. Homozygosity for TNSALP mutation 1348c>T (Arg433Cys) causes infantile hypophosphatasia manifesting transient disease correction and variably lethal outcome in a kindred of black ancestry. J Pediatr 2006;148:753-8.
14. Morava E, Karteszi J, Weisenbach J, Caliebe A, Mundlos S, Mehes K. Cleidocranial dysplasia with decreased bone density and biochemical findings of hypophosphatasia. Eur J Pediatr 2002;161:619-22.
15. Unger S, Mornet E, Mundlos S, Blaser S, Cole DE. Severe cleidocranial dysplasia can mimic hypophosphatasia. Eur J Pediatr 2002;161:623-6.