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RESEARCH ARTICLE

NEW MUTATION OF TISSUE NON SPECIFIC ALKALINE PHOSPHATASE IN HYPOPHOSPHATESIA

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ABSTRACT

Hypophosphatasia is a rare inborn error of metabolism characterized by defective bone and tooth mineralization with a deficiency of serum and tissue liver/bone/kidney tissue alkaline phosphatase (L/B/K ALP) activity. This study involves the identification and confirmation of this disorder in a new born infant subject by detecting the missing allele and is thought to be a X-linked disorder as the same allele is mutated in the mother's gene.

INTRODUCTION

Hypophosphatasia (MIM#s 146300; 241500; 241510) is an inherited disorder characterized by defective bone mineralization and a deficiency of serum and liver / bone / kidney tissue alkaline phosphatase (L/B/K ALP; orthophosphoric monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1) activity. The disease is highly variable in its clinical expression, which ranges from stillbirth without mineralized bone to pathologic fractures developing only late in adulthood (Whyte, 1994). The disease is due to mutations in the tissue-nonspecific alkaline phosphatase gene (ALPL; MIM# 171760). This gene, localized on chromosome 1p36.1-34 (Greenberg et al. 1990), consists of 12 exons distributed over 50 kb (Weiss et al. 1988). Mutations in the TNSALP gene have been found in North American, Japanese and European patients (Mornet, 2000). We now report the presence of X linked disorder in a newborn subject.

MATERIALS AND METHODS

A 1 year- old male child was admitted with a history of poor appetite, fatigue, poor systemic growth (in particular skeletal growth), bilateral testicular decent, unsteady gait (a condition involving a problem with walking in a coordinated manner), pigeon breast (the breastbone is pushed outward), mild lower extremity X type legs, bilateral thoracic depression**,** a little blue sclera, rickets, low ALP, hyperlipidemia and fallen tooth due to dental carries. Plain x-ray of the abdomen and abdomen ultrasound did not show any abnormalities. There was no fever or vomiting. The baby was born at 40th gestational week by cesarean section due to prelabour rupture of membrane, with a birth weight of 3.5kg, and no neonatal problems.

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The Patient was diagnosed with hypophosphatasia at the age of 1 yr on the basis of a low level of serum ALP activity. The patient had one sister aged 26 years from same father but different healthy mother with no symptoms of hypophosphatasia. There was no family history of consanguineous marriage. Genomic DNA from the family members was extracted from peripheral blood after written informed consent was obtained following agarose-gel electrophoresis. On examination, the baby's weight was 17.8 kg with height of 95 cm. He had normal color. His pulse, respiration, temperature and blood pressure were normal. There was mild general hypotonia, but reflexes were normal. Investigations showed hemoglobin of 11.3 g/dL, serum calcium was 3.50 mmol/L, phosphate 2.88 mmol/L, alkaline phosphatase 56 IU/L , urea 4.67 mmol/L, creatinine 62.3 umol/L, sodium 137.3 mmol/L, potassium 4.17 mmol/L, pH 7.372, bicarbonate 16.4 mmol/L, chloride 99.0 mmol/L and Mg 0.92 mmole/L.

The sequence analysis of the TNSALP gene was performed after the extraction of genomic DNA and total RNA from the peripheral mononuclear cells of patient and parents. The primers described in the previous report were used for the PCR; and we analysis the mutation of the TNSALP gene by RT-PCR and PCR with restriction enzyme digestion using primers corresponding to introns. The amplified fragments, after PCR and RT-PCR were digested by the restriction enzymes *Stu*I and *Dde*I to determine mutation analysis of tissue non specific ALP gene a missense mutations

RESULTS AND DISCUSSION

The results of electrophoresis PCR (Figure1A) and detection of mutations (Figure 1B) were done by digestion study. The

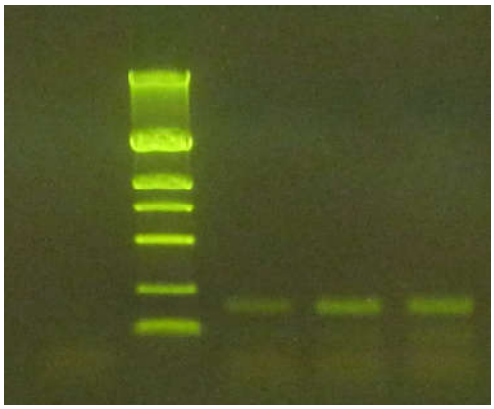
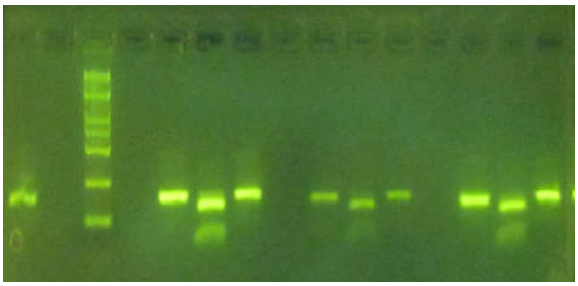


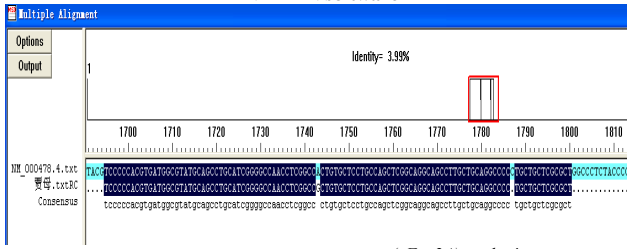
Fig. (1A). Results of electrophoresis PCR



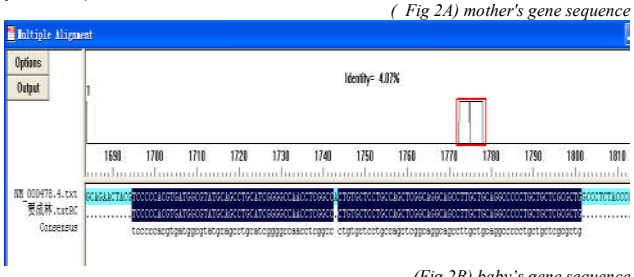
1 Baby PCR Product+DdeI buffer(without Enzyme)
2 Baby PCR Product+DdeI buffer +DdeI
3 Baby PCR Product+StuI buffer +StuI
4 Father PCR Product +DdeI buffer (without Enzyme)
5 Father PCR Product +DdeI buffer +DdeI
6 Father PCR Product +StuI buffer +StuI
7 Mother PCR Product+DdeI buffe（withoutEnzyme）
8 Mother PCR Product +DdeI buffer +DdeI
9 Mother PCR Product +StuI buffer +StuI
0 Baby PCR Product

Fig. (1B). Detection of mutations by digestion study with restriction enzymes StuI and DdeI

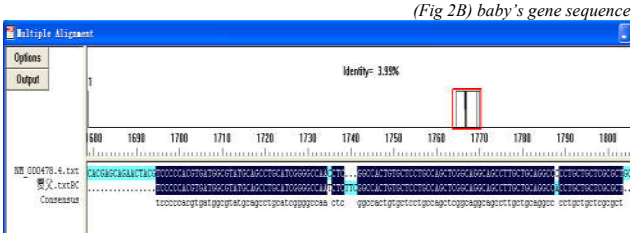
Sequence and measured on NM-000478.4 NCBI site comparison by using DNAMEN software



(Fig 2A) mother's gene sequence



(Fig 2B) baby's gene sequence



(Fig. 2C). Father's gene sequence

family members gene sequence were compared with gene database to interpret the mutation(Figure 2A, 2B, 2C).

Mutation analysis of the TNSALP gene

Detection of mutations by digestion study, the amplified fragments, after PCR or RT-PCR were digested by *Stu*I and *Dde*I to determine the nucleotide conversion of (A → G in position 1743); and deletion of (C at nucleotide 1787) in mathore's gene sequence in figure (2A); and also there is a deletion of (A at nucleotide 1743) was occurred in the baby's gene sequence in figure (2B); while the father's gene sequence in figure (2C) there are two conversion of (C → T) & (C → A) at nucleotide 1735 and 1788 respectively; and inserted a triple codon (TTC occurred at nucleotide 1738). Patients with childhood form should have regular follow-up appointments with their orthopedist. Refer all patients with any form of hypophosphatasia to a dental specialist. Children particularly benefit from skilled dental care, as early tooth loss can cause malnutrition and inhibit speech development. Patients who have good relationships with their doctors tend to receive better care and are more satisfied with the care they receive.

In our case, the alkaline phosphatase was persistently low but the serum calcium was upper normal, and there was clinical and radiological evidence of florid rickets in additional abnormalities in dentition, so our case fits into the milder form of childhood type HP. The prognosis associated with hypophosphatasia is directly related to the severity of the disease, childhood disease is associated with skeletal deformities in some cases, and symptoms may improve, however, during adolescence only to occasionally reappear in adulthood. Patients with childhood form should have regular follow-up appointments with their orthopedist. Refer all patients with any form of hypophosphatasia to a dental specialist. Children particularly benefit from skilled dental care, as early tooth loss can cause malnutrition and inhibit speech development.

In conclusion, the missing allele of the patient is thought to be a X-linked disorder as the same allele is mutated in the mother's gene and it is autosomal dominant genetic disorder Such an inheritance is also known as autosomal dominant pattern of inheritance. Our patient was not given any specific treatment. The course and prognosis of the disease were explained to the parents, and the baby was discharged from the hospital with further follow-up arrangements in our outpatient clinic.

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