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Clinical and ALPL Gene Mutations Analysis in an Early Onset Chinese Odontohypophosphatasia Patient

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Abstract

Objective: To describe a Chinese case with novel frame shift ALPL gene mutation that results in infantile onset odontohypophosphatasia. **Methods**: Clinical data and genomic DNA of the patient and his parents were collected. Alkaline phosphatase gene (ALPL)of the patient and his parents were PCR following with sequencing. **Results:** The patient had premature exfoliation of primary teeth at 11 month, and showed no sign of development retardation or rickets in 3 years follow-up. Serum alkaline phosphatase was found significantly decreased. Sequence analysis of ALPL gene reveal a de novo heterozygous missense mutation in exon 10 c.1162T>C（p.Y371H）which has been reported previously. In addition, a heterozygous frame-shift mutation in exon 12 c.1532insC（p. L511Pfs*272) was found in the patient and his mother. This inserted base causes a frame shift voiding the original stop codon. As a consequence, the original protein composed of 524 amino acids is translated into a protein with 783 amino acids. **Conclusion:** We report an early onset Chinese Odontohypophosphatasia patient, and a novel frame-shift mutation in exon 12, c.1532insC, p.L511Pfs*272）

Keywords: odontohypophosphatasia; ALPL; gene mutation.

1. Introduction

Hypophosphatasia (HP, OMIM#146300) was first described by Rathbun (1948) [1]. It is a rare autosomal recessive or dominant disorder characterized by defective bone and tooth mineralization and deficiency of serum and bone alkaline phosphatase activity [2]. There are six types of HP according to the onset age and severity: lethal perinatal subtype, perinatal benign

form, infantile HP, childhood form, adult subtype and odontohypophosphatasia [2-4]. The manifestations can overlap among different subtypes of HP [4]. The prevalence of severe conditions has been estimated to be 1/100000 in Canada, while the incidence of moderate hypophosphatasia is remain unknown [3,5]. Low ALP level, increased urinary phosphoethanolamine (PEA), and increased serum pyridoxal-5′-phosphate (PLP) are the diagnostic markers of the disease [6, 7]. The treatment of this disease still keep to be challenge [7-9]. Currently there is no ideal method to cure this rare disease. Odontohypophosphatasia is a subtype with only dental problems, especially premature exfoliation of primary teeth on inferior alveolar, without abnormalities at skeleton [10]. Laboratory tests reveal low level of serum ALP without other abnormalities. Most cases of Odontohypophosphatasia are diagnosed by dentists. However, it is easily being misdiagnosed as dental disease. On the other hand, it is difficult to differentiate with moderate Hypophosphatemia [6].

HP is due to loss-of-function mutations in the alkaline phosphatase gene (ALPL), which encodes the tissue-nonspecific alkaline phosphatase isoenzyme (TNSALP). ALPL gene is composed of 12 exons and encodes 524 amino acid [3]. At least 260 mutations in ALPL gene have been reported [2, 11]. Until now there are few of clinical and genetic reports of Chinese odontohypophosphatasia patients [12, 13]. The purpose of this study is to describe the clinical manifestation and mutations of an early onset Chinese Odontohypophosphatasia patient.

2. Subjects and methods

This study was approved by the Ethics Committee of Beijing Children's Hospital**,** **C**apital Medical University. Patient's parents were informed about the study procedure and provided informed consent prior to the collection of peripheral blood samples.

2.1 Patient's case History

The proband was a 1 year and 2month old male at the time of study. He was full-term delivered with birth weight of 4000g. He was healthy in the neonatal period. His first tooth erupted at 6 months old. At 11 months, one of total 5 teeth was exfoliated. He was misdiagnosed as 'severe calcium deficiency' at a local hospital with the chief complaint of 'tooth losses". After 2 months calcium supplement treatment, the second tooth exfoliated. There were no medical history of night terror, hyperhidrosis, ostalgia or fracture. His developmental milestones were normal at that time. Physical examination show normal stature without any signs of skeletal deformity, rickets, as well as neural system irritability. There was no family history of similar complain. The parents denied for consanguineous marriage

In three years follow-up, the patient's mental and physical development kept in normal range. There were no growth retardation, delayed walking or a waddling gait. The only sign was lower incisor and canine teeth deficiency (see figure. 1.).

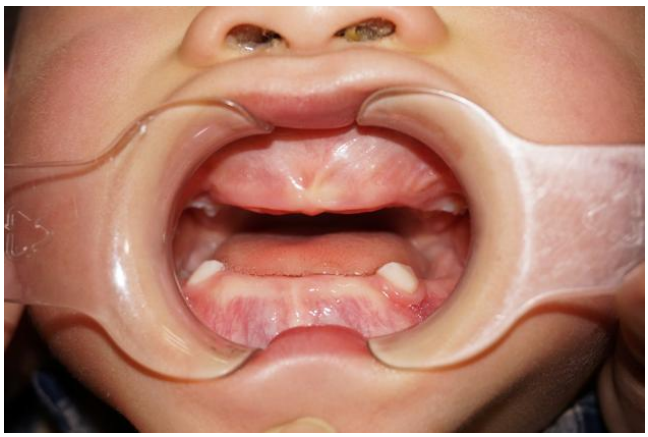

Figure. 1. The patient is short of incisor and canine teeth at the time of 4 year old. The picture shows premature exfoliation of teeth.

2.2 Laboratory investigation

Serum total calcium and Phosphorus were normal, which ranged from 2.45 to 2.65mol/L, and 2.0 to 2.12mol/L respectively. Urea nitrogen, creatinine, blood gas analysis and parathyroid hormone (PTH) levels were normal. Alkaline phosphatase ranged from 8 to 13.0 IU/L (normal range 20-220IU/L). Physical and radiology study did not reveal any new malformation in 3 years follow-up.

2.3 Mutation analysis:

Genome DNA was extracted from whole blood of the patient and his parents according to the manufacturer's instruction of BloodGen Midi Kit (CWBIO, China) at Joy Orient Translational Medicine Research Center Co., Ltd. China. Primers were designed to amplify ALPL gene according to Mornet et al. [3,4,14] and other researches [12, 15] (see Table 1). All 12 exons and the exon-intron boundaries of the ALPL gene were amplified and directly sequenced.

Tab1 .The primer sequences and PCR conditions

Primer Name	Primersequence (from 5' to 3' end)	Annealing temperature(℃)	Amplification product size（bps）
ALPL-1-F	CTGGTCTGTAATAGGTGCTCAC	56	421
ALPL-1-R	GTTTCCTGCTCTGAACACTGT		
ALPL-2-F	CTCCAAGTTCAGGCATTCCAG	60	880
ALPL-2-R	CGCAAGCAGGTACAGTGATG		
ALPL-3-F	TAGGTAGTCCTGTGGCTCTGG	61	1520
ALPL-3-R	CCTGGATGCCTGGTTCTTGG		
ALPL-4-F	GGAAGCCAAGTAAGGTAAGTTATC	58	656
ALPL-4-R	CTCAATGTCCACGCAGGTTAT		
ALPL-5-F	CATTAGAACATCACCTCCACCAG	59	521
ALPL-5-R	GCCTAATTCCAGGAACCAGAAC		
ALPL-6-F	GTCACAGCCTCTCAGCATCC	59	321
ALPL-6-R	CTCCTTCCACAACCTATTCTCCT		
ALPL-7-F	CAGGTTGAATGGCTGCCTAA	59	1279
ALPL-7-R	CTGCTAGATTGTAGAAGGCGATT		
ALPL-8-F	CAGGCTCAGGTTCAAATCCC	60	795
ALPL-8-R	AATGTTCCACGGAGGCTTCA		

3. Result

The sequencing of patient's ALPL gene revealed a de novo heterozygous mutation in exon 10, c.1162T>C （ p.Y371H ） . The mutation was not identified in his parents. Sequencing also revealed a novel frame-shift mutation caused by an insertion of cytosine in location 511, exon 12 of the patient and his mother as well (figure. 2.).

4. Discussion

The patient had premature exfoliation of primary teeth and showed no sign of development retardation or skeletal deformation which is common in rickets. Serum alkaline phosphatase significantly decreased. So he was tentatively diagnosed as odontohypophosphatasia. Belma et, al. [6] reported an infant with teeth loss and low ALP levels as first clinical signs. The patient was diagnosed as odontohypophosphatasia initially. As the patient growing up, they found a mild bone defect, osteopenia, and short stature, which implied the patient was a mild infantile hypophosphatasia. So, we followed up our patient carefully in three years. There were no any other change besides premature exfoliation.

A missense mutation in exon 10 c. 1162T>C （p.Y371H) was first reported at 2010[16], the patient in the article has a heterozygous mutations in exon 10 c.1162T>C and is suffered from HP. It implies that odontohypophosphatasia is related to the missense mutation in exon 10 c.1162T>C.

The protein encoded by ALPL contains 5 important domains [17, 18]: activation domain, N-terminal coiled region, homodimer, crown domain and calcium binding site. Mutations around these sites may affect the activity of the protein. The crown domain is composed of the amino acid residues from 370 to 435 which is a highly conservative region forming an antenna-like structure. The missense mutation in exon 10 c.1162T>C（p.Y371H）in this patient is close to the crown structure, which is likely to change the activity of the enzyme. In order to study the effect of the mutation on protein function. Yang et, al. [12] described a patient with mutation of p.Y371H who showed symptom of tooth loss at 18 months old. The enzyme activity study showed the mutant protein had very low ALP activity [12].

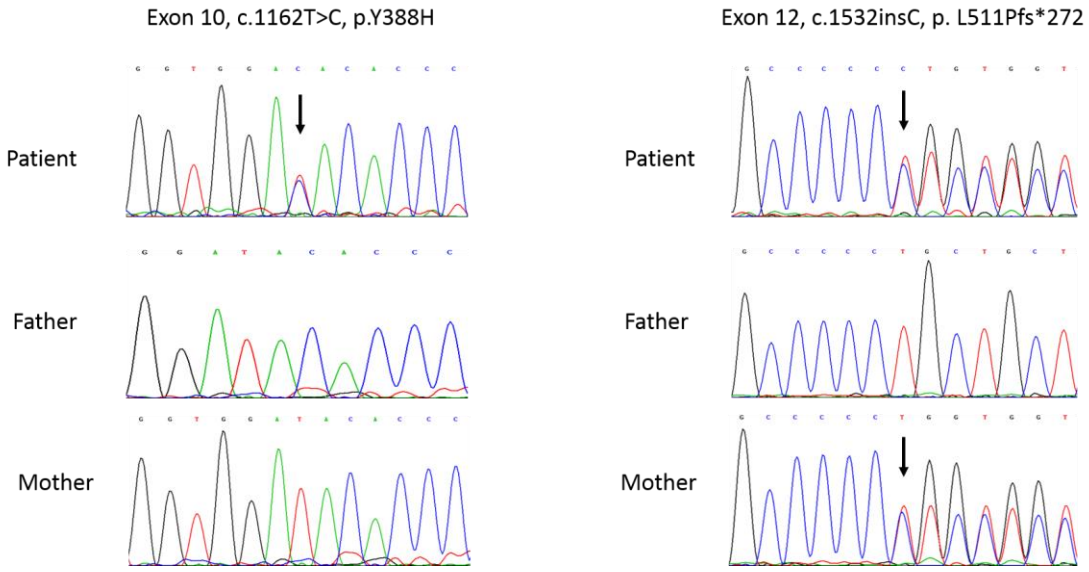

Figure. 2. The sequencing results of the ALPL gene in the patient and his parents. The patients were compound heterozygous carriers and the Mother was carrier of heterozygous mutation. Arrows indicated the mutated sites.

A frame-shift mutation in exon 12 c.1532insC（p. L511Pfs*272) of ALPL gene was found in the patient and his mother, and this mutation has not been reported before. The inserted base causes a frame-shift mutation and the original termination codon disappears. As a result, the protein sequence composed of an additional 259 amino acids. Since this frame-shift mutation lead the propeptide changed, it may disturb the final maturation of the protein by modifying the recognition site of proteases involved in the final maturation process [16, 19]. In fact the patient's mother carries a single mutation do not has the clinical manifestations of Odontohypophosphatasia indicates this novel frame-shift gene mutation is recessive inherited.

The missense mutation in exon 10 c.1162T>C（p.Y371H）and the novel frame-shift mutation in exon12 c.1532insC（p. L511Pfs*272) of the boy theoretically can lead to odontohypophosphatasia. Whether it is recessive pathogenic factor still requires experiment to be confirmed. How the novel frame-shift mutation in exon 12 c.1532insC（p. L511Pfs*272) contributes to the disease presentation remain being illustrated by further study.

In summary, HP is characterized by defective bone mineralization, rickets and fracture. In severe cases, it may be life-threatening for breath failure. We report an early onset Chinese odontohypophosphatasia patient with a novel frame-shift mutation. Careful follow-up should be perform in this kind of patient to differentiate moderate form. Currently, there is no medical therapy for the disease [2, 20]. Specific enzyme replacement is a possible way to

manage HP [8, 9]. ALPL gene analysis is crucial for the diagnosis of HP, and is essential for genetic counseling and prenatal diagnosis.

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