

Historical Vignette

Hypophosphatasia: Molecular Diagnosis of Rathbun's Original Case

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ABSTRACT

In 1948, Dr. John Campbell Rathbun characterized the disorder “hypophosphatasia” when he reported paradoxically low levels of alkaline phosphatase (ALP) activity in blood and in several tissues from an infant who died with rickets and epilepsy, which seemed to reflect “a new developmental anomaly.” Hypophosphatasia is now recognized to be an inborn error of metabolism featuring deficient activity of the tissue-nonspecific isoenzyme of ALP (*TNSALP*) caused by deactivating mutations in *TNSALP*. Here, we show, more than 50 years after Rathbun's case report, that analysis of the parental DNA indicates compound heterozygosity involving two missense mutations (G340A and A881C) in *TNSALP* caused the death of Rathbun's patient. (*J Bone Miner Res* 2001;16:1724–1727)

Key words: alkaline phosphatase, rickets, osteoblast, inborn error of metabolism, mutation

INTRODUCTION

IN 1946, Dr. John Campbell Rathbun (Fig. 1), then a fellow in pediatrics at The Hospital for Sick Children, Toronto, Canada, was referred a 3-week-old boy who seemed well at birth but then lost weight, developed epileptic seizures and, when held, cried as though in pain. Radiographs showed severe osteopenia, fractures, and metaphyseal irregularities, but serum calcium and inorganic phosphate levels were repeatedly normal or elevated, and serum alkaline phosphatase (ALP) activity was paradoxically low. Despite a variety of attempted treatments, including vitamin D supplementation, his patient died at 2 months of age shortly after a series of tonic seizures. Several tissues obtained at autopsy, including specimens of bone that revealed histopathologic changes consistent with rickets, also were deficient in ALP activity. Accordingly, in 1948 Rath-

bun reported this new “developmental anomaly” as “hypophosphatasia” (Fig. 2).⁽¹⁾

We now appreciate that Rathbun's publication represents the discovery of hypophosphatasia.⁽²⁾ More than one-half of a century later, approximately 300 cases of this inborn error of metabolism have been reported.⁽²⁾ Rathbun's patient is an example of the infantile form of the disease with an incidence of approximately 1 per 100,000 live births and a lethal outcome in approximately 50% of such patients.⁽³⁾ In hypophosphatasia, defective skeletal mineralization seems to reflect endogenous accumulation of inorganic pyrophosphate (PPi), an inhibitor of skeletal mineralization.⁽⁴⁾ Epilepsy occurs in severely affected infants possibly because of disturbed vitamin B₆ metabolism.^(4,5) There is no established medical treatment,⁽²⁾ but preliminary reports indicate that marrow cell transplantation rescued one severely affected girl⁽⁶⁾ and seemed to ameliorate the skeletal disease

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FIG. 1. Dr. John Campbell Rathbun (1915–1972) circa his discovery of hypophosphatasia in 1948 (reproduced with permission from MP Whyte 1994, Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. *Endocr Rev* 15:439–461).

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"HYPOPHOSPHATASIA"

A New Developmental Anomaly

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FIG. 2. Rathbun's report in 1948 characterizing hypophosphatasia as a new developmental anomaly.

of tissue-nonspecific isoenzyme of ALP (*TNSALP*) knock-out mice⁽⁷⁾ that recapitulate the infantile form of hypophosphatasia.⁽⁵⁾

Despite a remarkable range of disease severity, all individuals with hypophosphatasia studied to date have had a mutation(s) in the gene that encodes *TNSALP* ("liver/bone/kidney ALP").^(8,9) No other gene or epigenetic factor has been implicated. Molecular studies have shown at least 65

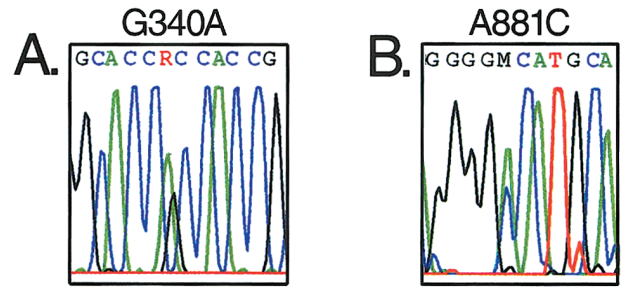


FIG. 3. DNA sequence profiles showing the *TNSALP* missense mutations in each parent of Rathbun's patient. (A) Maternal G340A mutation in exon 5 identified by the "R" because of overlap of the G and A peaks. (B) Paternal A881C mutation in exon 9 identified by the "M" because of overlap of the A and C peaks. Because of a change in nomenclature, the A881C mutation was previously reported as A1057C. The new nomenclature designates base number one as the first base in the coding sequence (i.e., the A in the ATG start codon).

distinctive deactivating *TNSALP* defects among hypophosphatasia patients worldwide.^(8,9)

Here, we report the molecular pathology of Rathbun's case using DNA acquired from the elderly parents 50 years after their son succumbed to hypophosphatasia.

MATERIALS AND METHODS

In 1996, 50 years after the birth of her son, the mother of Rathbun's patient wrote to us to learn what was known about hypophosphatasia and to find out if carrier detection was possible for her family. The half-century of progress was reviewed, and a unique opportunity was recognized. After obtaining informed written consent, whole blood was obtained from her and her husband to extract leukocyte DNA for analysis of their *TNSALP* alleles. Denaturing gradient gel electrophoresis (DGGE) is established in our laboratory to investigate the molecular pathology of hypophosphatasia.⁽¹⁰⁾

Genomic DNA was isolated from lymphocytes using the Puregene kit (Gentra Systems, Inc., Minneapolis, MN, USA). Analysis of genomic DNA in leukocytes from both elderly individuals included polymerase chain reaction (PCR) amplification of all 11 coding exons of *TNSALP*, and DGGE was used to identify abnormalities.⁽¹⁰⁾ PCR products for exons containing mutations/polymorphisms were reamplified and sequenced using Big-Dye terminators on an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA).

RESULTS

In the mother, a missense mutation was identified in exon 5, a G → A change at nucleotide number 340 (G340A) causing an amino acid change (alanine → threonine) at position 97 (Ala97Thr; Fig. 3). This mutation has not been reported previously.^(8,9,11–13) In fact, in approximately 100 hypophosphatasia patients that we have screened thus far,

we have detected this point mutation in only one other individual who suffers from the adult form of hypophosphatasia. To show that this change was “disease-causing” and not a polymorphism, we looked for the G340A change in 84 individuals unaffected by hypophosphatasia, using an allele-specific oligonucleotide (ASO) assay.⁽¹⁴⁾ The G340A change was not detected in the 168 alleles and, therefore, was not a polymorphism.⁽¹⁵⁾ In fact, alanine at position 97 is conserved in human TNSALP, human placental ALP, and *Escherichia coli* ALP proteins, suggesting that this amino acid has a critical role in ALP function.⁽¹¹⁾

In the father, a missense mutation was detected in exon 9, an A → C change at nucleotide number 881 (A881C) causing an amino acid change (aspartic acid → alanine) at position 277 (Asp277Ala). This alteration has been reported in several cases of hypophosphatasia and may represent one of the most common mutations (because of a change in nomenclature, establishing the first base of the start codon as base number one, the A881C mutation was previously called A1057C⁽¹²⁾).^(7,8) The father also showed a common polymorphism in exon 12 (T1565C; Val505Ala) that causes an amino acid change, but is not considered detrimental to ALP activity or contributory to the disease.⁽⁹⁾

To date, our cumulative preliminary findings involving 20 probands⁽¹⁰⁾ agree with published reports^(8,9) that lethal hypophosphatasia is always inherited as an autosomal recessive trait involving deactivating mutations in *TNSALP*. In Rathbun’s patient, a different *TNSALP* mutation was discovered in each parent. Their son presumably inherited both defects and was a compound heterozygote with two defective *TNSALP* alleles. Unfortunately, we have had no success amplifying DNA contained in archived patient liver obtained at autopsy. We learned that in 1947 these tissues had undergone Zenker fixation, which contains mercury that can interfere with molecular testing.

DISCUSSION

Although more than one-half of a century has elapsed since the death of Rathbun’s patient,⁽¹⁾ we believe that we have identified the *TNSALP* defects that caused this infant’s death from hypophosphatasia. Ironically, in his case report, Rathbun records that he was studying his patient on December 19, 1946—the day one of us (M.P.W.) was born.⁽¹⁾

Rathbun’s patient seems to have been a compound heterozygote for deactivating *TNSALP* missense mutations. In fact, all patients reported to date with either the perinatal or infantile form of hypophosphatasia are either homozygotes or compound heterozygotes for *TNSALP* defects.^(8,9) We know of no case of hypophosphatasia in which there has been uniparental disomy or evidence of involvement of another gene. The defect inherited from the father of Rathbun’s patient is among the most common of the >65 known *TNSALP* mutations.^(8,9) We have encountered the defect (G340A) found in the mother in only one other individual, a middle-aged woman with the adult form of hypophosphatasia who also has a C577G mutation causing a Pro176Ala amino acid change.

Archival tissue specimens could enable a diagnosis of hypophosphatasia. This approach would be useful for an unexplained lethal skeletal “dysplasia.” Apparently, because of the fixation method used at the time Rathbun studied his patient, we were unsuccessful in amplifying the DNA in tissue obtained at autopsy. Our findings instead illustrate that the molecular basis for severe forms of hypophosphatasia can be deduced by studying the *TNSALP* genes in parents or other family members. Our work is now focused on a molecular nosology for hypophosphatasia, hoping that this endeavor will improve both our understanding of the physiological role of *TNSALP* and prognostication for this inborn error of metabolism that manifests with striking variable severity and sometimes unpredictable lethality.

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