Outcome of Perinatal Hypophosphatasia in Manitoba Mennonites: A Retrospective Cohort Analysis

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Abstract Hypophosphatasia (HPP) is the metabolic bone disease caused by loss-of-function mutation within the gene that encodes the “tissue nonspecific” isoenzyme of alkaline phosphatase (TNSALP). Perinatal HPP is usually fatal due to respiratory insufficiency, and infantile HPP often has a similar outcome although no formal study into the natural history of these severe forms of HPP has been undertaken. We reviewed our 80-year (1927–2007) cohort of 15 Canadian patients with perinatal HPP. All had Mennonite heritage. Family linkage studies indicated that nine were homozygous for a TNSALP disease allele, likely Gly334Asp. Three patients had parents who were carriers for the Gly334Asp allele by mutation analysis. One patient was confirmed by mutation analysis to be homozygous for the *TNSALP* Gly334Asp mutation. One patient who had only one Mennonite parent was a genetic compound for the Gly334Asp mutation and the Val382Ile mutation. This patient’s sibling was also affected. All 15 patients had profound skeletal hypomineralization, severe rickets, and respiratory insufficiency. All died by 9 months of age, usually soon after birth, from pulmonary failure.

Introduction

The first report of hypophosphatasia (HPP) in the English-language medical literature has been attributed by some to Dr. Bruce Chown of the University of Manitoba, Winnipeg. In 1936, Chown described two sisters of Welsh descent with what he called “renal rickets” (Chown 1936; Fraser 1957). The two infants had hypercalcemia and died at age 3 and 6 months, respectively, but Chown was unable to further define the biochemical nature of the suspected underlying inborn error of metabolism. Similar patients were subsequently identified with the identical clinical and radiographic phenotype and the biochemical hallmark was eventually proven to be decreased activity of the liver/bone/kidney “tissue nonspecific” isoenzyme of alkaline phosphatase (TNSALP) (Whyte 1994). HPP severity is generally inversely related to the age at onset (Whyte 1994). Now, HPP is classified when there is skeletal disease as “perinatal,” “infantile,” “childhood,” or “adult” based on age at presentation (Whyte 2013). Both autosomal recessive and autosomal dominant forms of the disease are described. All but odontohypophosphatasia manifest defective skeletal mineralization (Fraser 1957) and involve loss-of-function mutation of the *TNSALP* gene (Whyte 1994) which was characterized in 1988 (Weiss et al. 1988). To date, more than 260 mutations have been recorded in the TNSALP Gene Mutations Database (http://www.sesep.uvsq.fr/03_hypo_mutations.php).

In Canada, the prevalence in the general population of the most severe forms of HPP is estimated to be ~1:100,000 (Fraser 1957). In France, the prevalence for the severe forms is ~1:300,000 (Fraser 1957; Mornet et al. 2011). In perinatal HPP, there is profound hypomineralization of the skeleton, frequently with foreshortened and deformed limbs. Perinatal HPP has been reported in various case reports to be almost invariably fatal (Whyte 1994; Shohat

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et al. 1991). Death results from respiratory insufficiency due to pulmonary hypoplasia and biomechanical compromise due to rachitic disease of the chest. Radiographic examination reveals skeletal hypomineralization which in some cases may appear as almost total absence of bony structures (Fraser 1957; Cole 2008). Patients with infantile HPP may appear normal at birth, but then present within the first 6 months of life with failure to thrive and progressive respiratory failure. Seizures may occur and are typically due to pyridoxine deficiency in the central nervous system (Greenberg et al. 1990). When such seizures occur, the prognosis is grim: In a recent review of the literature, no patients with HPP and pyridoxine-responsive seizures survived beyond 18 months of age (Baumgartner-Sigl et al. 2007). The outcome of infantile HPP is less certain. The natural history of the perinatal and infantile forms of HPP has not been systematically reviewed or published. Recent reports of spontaneous postnatal improvement of skeletal deformities identified antenatally in some babies with “benign prenatal” HPP (Moore et al. 1999; Pauli et al. 1999; Wenkert et al. 2011) and also in 2012, the potential of enzyme replacement treatment for the most severe forms of HPP (Whyte et al. 2012) prompted review of our experience with this disease in Manitoba, Canada. We had previously reported that autosomal recessive HPP is especially prevalent in the Manitoba Mennonite population, and the molecular basis for the perinatal/early infantile form in the population is a founder mutation Gly334Asp in the *TNSALP* gene. In 1993, the carrier frequency for this mutation was estimated to be 1/25 in the Manitoba Mennonite population predicting the frequency of homozygous affected babies to be 1/2,500 births in this community, significantly higher than the general population (Greenberg et al. 1993).

Herein, we present our 80-year (1927–2007) retrospective review of 19 Manitoba patients with perinatal HPP.

Methods

We conducted a retrospective study of the 80-year experience with patients with perinatal or infantile HPP in the province of Manitoba, Canada (1927–2007). All patients with a diagnosis of HPP by 5 years of age were reviewed. Nineteen patients were identified from our Genetics clinic database and from previously published cases (Macpherson, Kroeker and Houston 1972, McGuire et al. 1987; Chodirker et al. 1990).

Inclusion criteria required onset of signs and/or symptoms of HPP prior to 6 months of age. The diagnosis of HPP was made by the presence of radiographic evidence of HPP-related rickets and one or more of the following:

1. Two *TNSALP* alleles with previously reported HPP-causing mutation.
2. Both parents are carriers of at least one documented *TNSALP* allele with a previously reported HPP-causing mutation.
3. Confirmed or suspected homozygosity in the *TNSALP* gene based on the original family linkage study that contributed to the mapping of *TNSALP* gene to the Rh locus on chromosome 1p36.1 (Chodirker et al. 1987).

Survival information and other medical, surgical, and disease history data relevant to HPP of all patients was obtained from the medical records at primary, secondary, and tertiary care facilities, whenever necessary and feasible. We began *TNSALP* mutation analysis in 2000 in our center. Additional information previously reported concerning these patients in the medical/scientific literature could be included. This study was approved by the University of Manitoba Health Research Ethics Board.

Results

Diagnosis

Of the 19 patients whom we identified to be clinically diagnosed with perinatal or infantile HPP, four did not fulfill our study inclusion criteria because their medical records, including radiographic evidence of skeletal disease, were no longer available.

For the 15 patients who fulfilled our inclusion criteria, the parents of 13 patients were both Mennonites. Two patients were affected siblings from the same family where only one parent was Mennonite. HPP was confirmed with by *TNSALP* mutation analysis in two different families each with one affected patient. In one of these two families, the patient was shown to be homozygous for the Gly334Asp mutation. In the second family, the patient was a genetic compound for the founder Mennonite Gly334Asp mutation and a Val382Ile mutation which has previously been reported (Goseki-Sone et al. 1998). A sibling of this patient was similarly affected and their parents were shown to be carriers of either the Gly334Asp mutation or the Val382Ile mutation. The remaining 12 patients did not have a molecular diagnosis, but 3 sets of parents (all Mennonite) were shown to be carriers of the *TNSALP* Gly334Asp mutation and the other 9 sets of parents were shown to be carriers of a *TNSALP* mutation through linkage analysis that contributed to the mapping of HPP to the Rh locus chromosome 1p36.1 and subsequently to *TNSALP* gene identification (Greenberg et al. 1993; Chodirker et al. 1987, 1990).

Nine of the 15 patients had radiologic signs of HPP in utero, although reports were available for only 7 patients. Most abnormalities were detected using fetal ultrasound. The most common prenatal ultrasonographic findings were shortened limbs and decreased bone mineralization (Table 1).

Table 1 Summary of signs and symptoms of perinatal hypophosphatasia clinical cases

Point number	In utero signs?	Age at onset of HPP symptoms	Muscular hypotonia at presentation	Respiratory distress at presentation	Respiratory support	Seizures	First radiologic valuation	Age at death (days)
1	No	2 weeks	Yes	No	Unknown	Yes, Day 1	Date N/A, metaphyseal flaring	36
2	Yes; severe micromelia and undermineralized skull	At birth	No	Yes	No	No	N/A	30 min
3	Yes; fetal U/S, 29 wk GA, severe micromelia, undermineralized skull	At birth	No	Yes	No	No	N/A	10
4	Yes; fetal U/S 36 wk GA, micromelia, undermineralized skull, femoral fracture	At birth	No	Yes	Yes; at birth, intubated and ventilated	No	Day 1, osteopenia	1
5	Yes; fetal U/S, 25 wk GA, micromelia with bowing, bilateral forearm fractures	At birth	No	No	No	Yes, Day 36	Day 3, osteopenia; bilateral femoral bowing; left radial, left ulnar, and bilateral humeral fractures	263
6	Yes; fetal U/S, 21 wk GA, micromelia with bowing, demineralized skull	At birth	No	No	No	No	Prenatal X-ray (10 days before birth), osteopenia, thin gracile ribs, deformities of all long bones	3 h
7	Yes; fetal ultrasound, GA N/A, micromelia, demineralized skull	At birth	No	n.a.	n.a.	No	Day 1, report N/A	Stillborn
8	No	At birth	No	No	No	Yes, Day 1	Day 41, report N/A	281
9	Yes; report N/A	At birth	No	Yes	Yes; at Birth, intubated and ventilated	No	Day 2, osteopenia, marked metaphyseal irregularity of all tubular bones	1
10	No	At birth	Yes	Yes	Yes; at birth, supplemental oxygen	Yes, Day 1	Day 2, metaphyseal flaring	125
11	No	At birth	No	Yes	Yes; at birth, intubated and ventilated	No	Date N/A, metaphyseal flaring, rachitic chest	5
12	Yes; fetal X-ray, GA N/A, undermineralized bones	At birth	No	Yes	Yes; at birth, supplemental oxygen	No	Day 2, osteopenia, metaphyseal flaring	<1 h
13	Yes; fetal U/S, GA N/A, report N/A	At birth	No	n.a.	n.a.	No	Date N/A, absence of some bones	Stillborn
14	No	At birth	No	Yes	Yes; at birth, intubated and ventilated	No	Day 1, osteopenia, no ossification of forearm bones, deficient upper thoracic and cervical vertebrae	<1 day
15	No	At birth	No	No	Unknown	No	Date N/A, osteopenia, recent fracture	11

U/S ultrasound, wk weeks, GA gestational age, N/A not available, n.a. not applicable

Birth History and Congenital Anomalies

Two patients were born prematurely at 30 and 33 weeks while 2 others were born early at 36 weeks gestation. The remaining 11 patients were born at term. Birth weights were available for 6 patients and ranged from 2.3 kg (<5 percentile) to 3.3 kg (25–50 percentile). Length and head circumference measurements were available for three patients at birth. Length ranged from 40 cm (<5 percentile) to 51 cm (50–75 percentile). Head circumference ranged from 32.5 cm (5–10 percentile) to 33.5 cm (10–25 percentile).

At birth, 12 of the 15 patients had sufficient documentation for evaluation of skeletal anomalies. Ten patients had obvious skeletal anomalies while two did not. Of the ten with documented abnormalities, nine had severe shortening/bowing of limbs at birth, six had skull deformities, and two had rachitic chest deformities.

Disease Presentation and Progression

Two patients were stillborn, one at 30 weeks, another at term. Eleven patients presented with HPP at birth whereas the remaining patient presented at 2 weeks of life, consistent with perinatal HPP. Initial symptoms included respiratory distress (9 patients); abnormal skull shape (4 patients); rachitic chest deformity, hypotonia, fractures (2 patients each); and failure to thrive and seizures (1 patient each).

Six patients required respiratory support at birth (two initially with supplemental oxygen and four required intubation and ventilation). All four ventilated patients eventually succumbed to respiratory failure within the first 5 days of life.

Four patients developed seizures. One patient was treated with phenobarbital alone and another patient was treated with phenobarbital, phenytoin, and pyridoxine but there was insufficient documentation to determine whether either patient responded to his/her treatment. The remaining two patients responded to pyridoxine therapy. For all four patients, there was no further documentation of medically intractable seizures. However, all four patients succumbed to respiratory failure.

Radiographic Evaluation

Twelve patients had radiographic examinations shortly after birth although another patient had a prenatal X-ray 10 days before birth. Unfortunately, two patients' postnatal films and reports were not available for review. Common documented radiographic abnormalities included hypomineralization/osteopenia (7 patients), recent fractures (4 patients), metaphyseal flaring (4 patients), and apparent

Fig. 1 Skeletal radiograph of Patient 11: Rachitic chest, metaphyseal flaring, and significant hypomineralization of bones to a point of near absence and absence of skeletal mineralization

absence of some bones (2 patients). There was no improvement radiographically in any of the 6 patients who survived up to 281 days (Fig. 1).

Survival Data

For the 15 patients who fulfilled our study inclusion criteria for severe HPP, 9 (including the stillbirth) did not survive beyond 1 day of life (4 died despite respiratory support). The remaining patients died at days 4, 11, 36, 125, 263, 281 of life, respectively (Fig. 2). The cause of death was not available for five patients, one of whom died in utero shortly before birth. One patient died after a series of apneic, breath-holding spells, whereas the remaining nine patients died from respiratory complications.

Discussion

Our cohort of perinatal HPP patients in Manitoba, Canada, had major morbidity apparent in the neonatal period. All died within first 9 months of life. All were confirmed by mutation analysis to have autosomal recessive HPP or were presumed to have autosomal recessive HPP because both

Fig. 2 Kaplan-Meier survival data for Manitoba perinatal hypophosphatasia cohort

parents were confirmed or presumed to be *TNSALP* Gly334Asp mutation carriers through linkage analysis. Hence, in our Mennonite population, the Gly334Asp mutation is a founder mutation, and appears to predict a lethal phenotype when homozygous or when compound heterozygous with the Val382Ile mutation.

For four patients who developed seizures, regardless of their anticonvulsant therapy regimen, their survival was longer than 1 month, unlike the rest of our cohort. It is unclear whether this is a coincidence due to the small number of patients or whether the development of seizures is a prognostic factor for longer survival.

In our cohort of patients who had prenatal ultrasonographic or prenatal radiographic evaluation, all seven reports described either shortened limbs or decreased bone mineralization. None appeared to have the long bone features of fetuses of spontaneous improvement postnatally who were later diagnosed with benign prenatal HPP (Wenkert et al. 2011) and our patients all followed a lethal course.

Most attempts to treat severe HPP have been unsuccessful. Two unrelated patients with worsening infantile HPP received marrow cell transplantations and survived a likely lethal outcome (Cahill et al. 2007; Whyte et al. 2003). Now, enzyme replacement therapy for severe HPP using human recombinant bone-targeted alkaline phosphatase provides promise for improved outcome (Whyte et al. 2012).

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Competing Interest

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Synopsis

Perinatal hypophosphatasia in Manitoba Mennonites has been uniformly fatal.

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