CASE REPORT

Perinatal hypophosphatasia presenting as neonatal epileptic encephalopathy with abnormal neurotransmitter metabolism secondary to reduced co-factor pyridoxal-5′-phosphate availability

Shanti Balasubramaniam • Frank Bowling • Kevin Carpenter • John Earl • Jeffrey Chaitow • James Pitt • Etienne Mornet • David Sillence • Carolyn Ellaway

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Abstract We describe two neonates presenting with perinatal hypophosphatasia and severe epileptic encephalopathy resulting in death. Both had increased levels of urinary vanillactate, indicating functional deficiency of aromatic amino acid decarboxylase, a pyridoxal-5-phosphate (PLP) dependent enzyme required for dopamine and serotonin biosynthesis. Clinical findings and results of subsequent

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S. Balasubramaniam : C. Ellaway Genetic Metabolic Disorders Service, The Children's Hospital at Westmead, Sydney, Australia

F. Bowling Department of Biochemical Disease, Mater Health Services, Brisbane, Australia

K. Carpenter NSW Biochemical Genetics Service, The Children's Hospital at Westmead, Sydney, Australia

J. Earl Department of Clinical Chemistry, The Children's Hospital at Westmead, Sydney, Australia

J. Chaitow Department of Paediatric Rheumatology, The Children's Hospital at Westmead, Sydney, Australia

J. Pitt

Victorian Clinical Genetics Services, Murdoch Children's Research Institute, Royal Children's Hospital Melbourne, Melbourne, Australia

metabolic investigations were consistent with secondary pyridoxine-deficient encephalopathy. These patients highlight the importance of tissue non-specific alkaline phosphatase in the neuronal PLP-dependent metabolism of neurotransmitters. In addition, the disturbance of PLP metabolism appears to underlie the predominant neurological presentation in our patients. We recommend the

J. Pitt Department of Paediatrics, University of Melbourne, Melbourne, Australia

E. Mornet Laboratoire SESEP, Centre Hospitalier de Versailles, Le Chesnay, France

E. Mornet Equipe EA2493, Université de Versailles Saint-Quentin en Yvelines, Versailles, France

D. Sillence Department of Clinical Genetics, The Children's Hospital at Westmead, Sydney, Australia

K. Carpenter : D. Sillence : C. Ellaway Discipline of Genetic Medicine, University of Sydney, Sydney, Australia

C. Ellaway (\boxtimes) Western Sydney Genetics Program, The Children's Hospital at Westmead Clinical School, Locked Bag 4001, Westmead, NSW 2145, Australia e-mail: Carolyne@chw.edu.au

measurement of serum alkaline phosphatase (ALP) during the assessment of perinatal seizures.

Introduction

Hypophosphatasia is a rare inherited metabolic disorder characterised clinically by defective mineralisation of bone and teeth and, biochemically, by deficient activity of the tissue non-specific isoenzyme of alkaline phosphatase. The prevalence at birth of the severe forms of the disease has been estimated at 1:100,000 (Fraser [1957](#page-8-0)). Hypophosphatasia was initially described in a 9-week-old infant with severe rickets and very low alkaline phosphatase (ALP) activity in serum and tissues (Rathbun [1948](#page-8-0)). Based on the age at diagnosis, hypophosphatasia has been classified into six clinical subgroups: perinatal lethal, prenatal benign, infantile, childhood and adult onset forms, and odontohypophosphatasia. Clinical variability has been reported in all subgroups, and hypophosphatasia is best regarded as a spectrum disorder (Fedde et al. [1996](#page-8-0); Whyte et al. [1996\)](#page-8-0).

The enzymatic deficiency results from mutations in the liver/bone/kidney alkaline phosphatase gene (ALPL; OMIM 171760). Laboratory diagnosis is confirmed by reduced levels of serum alkaline phosphatase, raised levels of urinary phospho-ethanolamine (PEA) and DNA sequencing of the ALPL gene, that detects approximately 95% of mutations in the severe (perinatal and infantile) forms (Mornet [2007\)](#page-8-0). The ALPL gene is located on chromosome 1p36.1-34 (Greenberg et al. [1990\)](#page-8-0) and consists of 12 exons spanning 50 kb (Weiss et al. [1986](#page-8-0)). More than 190 distinct mutations have been described, most of which are missense mutations (79%) [\(http://www.](http://www.sesep.uvsq.fr/Database.html) [sesep.uvsq.fr/Database.html\)](http://www.sesep.uvsq.fr/Database.html) that result in variable clinical expression.

Patients with the perinatal lethal form present with complications of markedly impaired mineralisation in utero and usually succumb to respiratory distress due to hypoplastic lungs and rachitic deformities of the chest. Skincovered osteochondral spurs (Bowdler spurs) protruding from the forearms or legs are often diagnostic of hypophosphatasia. Typical radiographic features include reduced ossification with unusually dense, round, flattened and butterfly shaped vertebral bodies and generalised smaller bones. Other clinical features include apnoea, seizures and significant shortening of the long bones (Whyte [1994](#page-8-0)). The prenatal benign form may show spontaneous improvement of the skeletal defects. Progressive ultrasonography has revealed resolution of the skeletal deformities and improved mineralisation during the third trimester of pregnancy (Pauli et al. [1999;](#page-8-0) Wenkert et al. [2007\)](#page-8-0).

In this report, we describe two neonates with perinatal hypophosphatasia and epileptic encephalopathy who demonstrated secondary abnormalities in intermediary metabolism consistent with pyridoxine-responsive seizures (PRSs).

Case reports

Patient 1

The patient was a full-term girl born at 39 weeks' gestation, with good Apgar scores after an uncomplicated pregnancy. Her birth weight was 2.59 kg (2nd percentile) and head circumference was 33 cm (2nd percentile). Her parents were non-consanguineous Australians of European ancestry. She was active and fed well for her initial 4 days of life. On day 5 she had a right-sided clonic seizure that lasted approximately 10 min. The seizure was managed intravenously with phenobarbitone, and she required supplemental oxygen. She was examined and treated for presumed sepsis. The initial sepsis work up, including a lumbar puncture, showed no evidence of infection. A cranial ultrasound was normal.

She had a further seizure on day 7 of life. Despite commencement of pyridoxine orally, 25 mg daily, the seizures increased in frequency when she was 3 weeks of age, and a loading dose of phenytoin was administered intravenously. She was noted to have a mild metabolic acidosis with lactic acidaemia, (lactate 10.4 mmol/l, reference range 0.0–2.0 mmol/l). She progressed to respiratory failure necessitating intubation and ventilation and was transferred to the neonatal intensive care unit. An electroencephalogram (EEG) was abnormal, with a moderate burst-suppression pattern and multifocal epileptiform discharges.

Clinical examination showed the patient to have wide anterior and posterior fontanelles that were continuous in the midline, but she had no other dysmorphic features. She had generalised hypotonia and muscle weakness, with limited antigravity movements of her limbs. Her deep tendon reflexes were difficult to elicit. A chest X-ray showed lytic lesions of both proximal humeri and 11 ribs bilaterally that were short and narrow but of normal density (Fig. [1](#page-2-0)). A modified skeletal survey, which did not include skull radiographs, confirmed the punched-out lucencies in the proximal humeri, and they were also present in the femora, tibiae and the distal ulnar metaphyses (Fig. [2\)](#page-2-0). There was cupping of the long bone metaphyses. Her blood lactate levels normalised.

Based on the patient's clinical and radiological features and the biochemical findings of low serum alkaline phosphatase activity and grossly elevated levels of urinary PEA (Table [1](#page-3-0)), a provisional diagnosis of perinatal hypophosphatasia was made. Urinary organic acid analysis of a urine sample obtained prior to the commencement of

Fig. 1 Chest X-ray of patient 1, showing lytic lesions of the proximal humeri. The ribs numbering 11 bilaterally are short and reduced in width but are otherwise of normal density

pyridoxine administration and while the patient was not on catecholamine support revealed the presence of vanillactate, compatible with secondary pyridoxine deficiency and consequent aromatic amino acid decarboxylase deficiency. Levels of other dopa-metabolites [homovanillic acid (HVA), vanillylmandelic acid (VMA), 3,4-dihyroxyphenylacetate] were normal, therefore excluding common secondary causes of increased vanillactate excretion such as L-dopa or catecholamine treatment and neonatal tyrosinaemia. There was normal excretion of 2-amino-adipic semi-aldehyde and pipecolic acid, which excluded amino-adipic semi-aldehyde dehydrogenase deficiency secondary to mutations in the PNPO gene as a cause of seizures. An EEG obtained 2 weeks after commencement of pyridoxine therapy showed some improvement, with no burst suppressions, but it remained abnormal, with an excess of sharp waves for her age. Her condition remained clinically unstable, with frequent desaturations and wide fluctuations in her blood pressure, ranging from mean arterial pressures of 110 mmHg to 30 mmHg. Her neurological status deteriorated. She became more obtunded 6 days after ventilation, with ongoing episodes of desaturation, tachycardia and hypertension, but no overt seizures. The pyridoxine dose was increased to 100 mg daily orally, and then to 100 mg intravenously. She succumbed to her illness at 5-weeks of life.

Cerebrospinal fluid (CSF) analysis, performed in the peri-mortem period, revealed elevated levels of PEA, while levels of other amino acids were normal and that of 5 hydroxyindole-acetic acid (5-HIAA) was mildly reduced (Table [2\)](#page-3-0). Biopterin was borderline low, and there were additional peaks from pyridoxine metabolites, due to the pharmacological doses administered. Both parents were clinically asymptomatic. Their serum ALP levels were low, and their urinary PEA levels were normal (see Table [1\)](#page-3-0). These biochemical findings were consistent with parental heterozygosity.

Patient 2

This patient was a boy born to healthy non-consanguineous parents. At birth he was found to have a malformed rib cage (Fig. [3\)](#page-4-0), multiple skeletal (Fig. [4](#page-4-0)) anomalies and rhizomelic shortening of the limbs. The radiographs showed bowing of some long bones and slender and demineralised ribs. The skeletal survey was consistent with changes seen in infantile rather than perinatal lethal hypophosphatasia. Ineffective respiratory effort compounded by refractory seizures from day 1 of life necessitated mechanical ventilation. Seizure control was initially not achieved with phenobarbitone and clonazepam. The EEG showed a burstsuppression pattern. Pyridoxine was administered intravenously at 50 mg daily, which led to the cessation of seizure activities. A cranial ultrasound was normal. A maintenance dose of pyridoxine, (10 mg/day) was continued, while all other anticonvulsants were ceased. An EEG performed several days after the commencement of pyridoxine treatment showed the disappearance of the burstsuppression pattern. The ALP level was low ≤ 5 IU/l, reference range 100–400 IU/l) (Table [1](#page-3-0)). Urinary levels of PEA and vanillactate were elevated, CSF 5-HIAA and HVA were reduced, and 3-O-methyldopa level was elevated (Table [2\)](#page-3-0). The baby died on day 7 of life. The mother had poor dentition, with badly decayed teeth. She had no

Fig. 2 a, b Radiographs of both lower limbs of patient 2, showing punched-out metaphyseal lucencies in the proximal and distal femora and tibiae

Family members	Age	ALP (IU/l)	Reference ranges (IU/l)	Urinary phospho-ethanolamine	Reference ranges (µmol/mmol creatinine)
Patient 1	3 weeks	14	160–400	1,300	$10 - 20$
Father	38 years	46	$80 - 355$	9	$5 - 18$
Mother	30 years	53	$80 - 355$	17	$5 - 18$
Patient ₂	week	≤ 5	$100 - 400$	1015	$0 - 70$
Mother	39 years	16	$35 - 110$	39	$5 - 20$
Father	NA	NA	NA	NA	NA

Table 1 Serum levels of ALP activity and urinary phospho-ethanolamine in the patients and their parents. Abbreviation: NA, not available

history of fractures. She had low levels of serum ALP and mildly elevated urinary PEA (Table 1). We were unable to obtain blood or urine samples from the father.

Methods

Urine phospho-ethanolamine quantitation

Urinary PEA was quantitated by amino acid analysis using ion exchange chromatography and post-column ninhydrin detection.

Urine vanillactate detection

Urinary vanillactate was detected by standard organic acid analysis using gas chromatography–mass spectrometry following ethyl acetate extraction and formation of trimethylsilyl derivatives.

Urinary 2-amino-adipic semi-aldehyde and pipecolic acid detection

Urinary 2-amino-adipic semi-aldehyde and pipecolic acid were measured by gas chromatography–mass spectrometry after derivatisation with ethoxyamine and propylchloroformate (Pitt and Eggington [2007\)](#page-8-0).

CSF neurotransmitter analysis

Cerebrospinal fluid analysis for 5-HIAA, HVA and 3-Omethyldopa were measured by high performance liquid chromatography (HPLC) with electrochemical detection.

ALPL gene mutation detection by DNA sequencing analysis

Primer sequences were designed from the intronic sequences obtained from Genbank (accession numbers D87874 to D87888), Orimo et al. [1997,](#page-8-0) and from Mornet et al. [1998\)](#page-8-0). These primers allowed analysis of the entire coding region of the ALPL gene. Polymerase chain reaction (PCR) was performed in a final volume of 50 µl with 30 pmol of each primer, 250 µM of each deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP) and deoxythymidine triphosphate (dTTP), 1 unit of gold Taq DNA polymerase (Perkin-Elmer, Foster City, CA, USA] and 1–3 mM magnesium chloride $(MgCl₂)$, depending on the exon amplified. The mixtures were heated at 95°C for 10 min and subjected to 30 cycles of 1 min at 95°C, 1 min at the annealing temperatures and 40 s at 72°C. The PCR products were purified and sequenced by the direct sequencing method using the ABI Prism Dye Terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS (Perkin-Elmer) and

Table 2 Cerebrospinal fluid levels of specific catecholamines and amino acids. Abbreviation: NA, not available

Fig. 3 Chest X-ray of patient 2. The ribs are slender, reduced in width and flared anteriorly

migrated on to an ABI Prism 310 electrophoresis system (Applied Biosystems, USA).

Results

DNA from patient 1 was not available, but her parents were both heterozygotes for mutations of the ALPL gene. The father was heterozygous for a frameshift mutation that resulted in a premature stop codon, c.891C>A (p.Y297X). This mutation has not been previously described in hypophosphatasia. The mother was heterozygous for the mutation, c.1471G>A (p.G491R), which has been previously reported in the severe perinatal lethal form. The combination of a truncating mutation and a severe mutation in the parents was consistent with the inheritance of both these alleles in patient 1 and the diagnosis of perinatal lethal hypophosphatasia. The second patient was found to have a pathogenic mutation, c.318G>C (p.Q106H) that involves the homodimer interface, a critical domain. In addition, the mutation may create an exon splicing enhancer (ESE), which may result in alternative splicing. The results were compatible with homozygosity for the p.Q106H mutation or double heterozygosity, which could occur in association with a deletion in the second allele. The patient's parents needed to be tested so that we could distinguish homozygosity from hemizygosity. Unfortunately, the father was not available for testing.

Discussion

Hypophosphatasia is a rare inherited skeletal dysplasia with highly variable clinical expression. It is caused by muta-

tions in the liver/bone/kidney alkaline phosphatase gene (ALPL; OMIM 171760), which encodes tissue non-specific alkaline phosphatase (TNAP).

Clinical expression of hypophosphatasia ranges from stillbirth with demineralised bone to pathological fractures in adult life (Whyte [1994](#page-8-0); Mornet [2007\)](#page-8-0). The autosomal recessive perinatal lethal form is the most severe and is characterised by impaired bone mineralisation in utero, shortening of the long bones, respiratory distress, apnoea and seizures (Mornet [2007](#page-8-0)). Pauli and colleagues reported in 1999 a sixth form of hypophosphatasia; a prenatal benign form that demonstrates spontaneous postnatal improvement. This "bent but not broken" phenotype (Pauli et al. [1999](#page-8-0)) has a postnatal course that is much less severe than either the perinatal lethal or infantile forms. The inheritance of the prenatal benign phenotype is consistent with autosomal dominant inheritance due to the dominantnegative effect of ALPL gene mutations on TNAP catalytic activity (Lia Baldini et al. [2001](#page-8-0); Brun-Heath et al. [2008\)](#page-8-0). The skeletons of both patients in this study were assessed as being less severely affected than in typical perinatal lethal cases, patient 2 even more so than patient 1. Therefore, the impact of the neonatal encephalopathy was surprising.

At least four genes encode the isoenzymes of ALP in humans (Harris [1990\)](#page-8-0). Three of these genes, clustered on chromosome 2q34-47, direct synthesis of the tissue-specific ALP isoenzymes of the intestine, placenta and germ cells. The fourth gene, ALPL, located at chromosome 1p36.1-34, has expression that is more widespread than originally recognised, including the developing brain (Fonta et al. [2005](#page-8-0)). The enzyme cleaves extracellular substrates such as pyridoxal-5-phosphate (PLP), PEA, and inorganic pyrophosphates (Whyte [1994](#page-8-0)). TNAP is normally abundant in osteoblasts and chondrocytes, where it is coupled to the cell surface and functions as an ectoenzyme. In bone, inorganic

Fig. 4 Left forearm of patient 2, showing bowing of the proximal ulna

pyrophosphate retards the growth of nascent hydroxyapatite crystals present in bone matrix vesicles, which are the initial sites of mineral deposition (Anderson et al. [2004](#page-7-0); Cole [2008\)](#page-8-0). Intracellular accumulation of inorganic pyrophosphates accounts for impaired skeletal mineralisation (Whyte et al. [1996](#page-8-0)). Human TNAP also plays an important role in vitamin B6 metabolism, by dephosphorylating PLP to pyridoxal (PL) and is necessary for cellular uptake. This is indicated by the increased plasma concentration of PLP (Iqbal et al. [1998](#page-8-0); Litmanovitz et al. [2002](#page-8-0)) and reduced plasma concentration of pyridoxal in severe forms of hypophosphatasia (Whyte et al. [1988\)](#page-8-0).

Vitamin B6 is a water-soluble vitamin that is present in the body as six vitamers: pyridoxine, pyridoxamine, pyridoxal and their 5′-phosphorylated esters, with pyridoxal being the only vitamer with co-factor activity (Fig. 5). Animal-derived vitamin B6 consists primarily of phosphorylated pyridoxal and pyridoxamine, whilst the plant-derived forms consist largely of free and bound pyridoxine (Surtees et al. [2006\)](#page-8-0). Intestinal alkaline phosphatases convert phosphorylated B6 vitamers to their bases, which are subsequently absorbed from the upper small intestine by a carrier-mediated system into the portal circulation and liver (Said [2004;](#page-8-0) Surtees et al. [2006](#page-8-0)). In the liver, pyridoxal kinase phosphorylates pyridoxine, pyridoxamine and pyridoxal to their 5′-phosphate esters, whilst pyridoxamine-5′ phosphate oxidase (PNPO) oxidises pyridoxine phosphate and pyridoxamine phosphate to form PLP. Albumin-bound PLP is released into the circulation and forms approximately 60% of circulating vitamin B6. Human TNAP dephosphorylates circulating PLP at the choroid plexus, releasing pyridoxal for transportation into the cerebrospinal fluid by an active transport mechanism (Surtees et al. [2006](#page-8-0)). PL is the form of vitamin B6 that crosses cell plasma membranes to be rephosphorylated to PLP in the neurons. Pyridoxine kinase catalyses the phosphorylation of pyridoxal, pyridoxine and pyridoxamine, whilst pyridoxine and pyridoxamine phosphate are then oxidised by PNPO to form PLP (Kang et al. [2004](#page-8-0); Surtees et al. [2006](#page-8-0)). PLP is the active metabolite of vitamin B6 and is an essential coenzyme for synthesis of various neurotransmitters and biogenic amines (Whyte et al. [1988\)](#page-8-0). It acts as a co-factor for more than 100 apoenzymes. The corresponding holoenzymes catalyse diverse reactions such as transamination, decarboxylation, racemisation, elimination and replacement. In the brain, PLP-dependant enzymes [aromatic amino acid decarboxylase, branched-chain amino acid 2-oxoglutarate aminotransferase, gamma-aminobutyric acid (GABA) transaminase, glutamate decarboxylase, glycine cleavage enzyme, kynureninase, kynurenine aminotransferase and L-serine race-

Note: Arrows in red indicate biochemical findings in AADC deficiency

5-HTP=hydroxytryptophan; 3-*O*-MD= 3 - *O*- methyldopa; AADC= aromatic L- amino acid decarboxylase; DA=dopamine; L-DOPA= L-dopamine; NA=noradrenaline; EPI=epinephrine; 5-HIAA= 5-hydroxyindole acetic acid; HVA= homovanillic acid; MHPG= 3-methoxy-4 hydroxyphenylglycol; VMA= vanillylmandelic acid; PL= pyridoxine; PLP= prridoxal-5-phosphate; TNAP=Tissue Non Specific Alkaline Phosphatase.

Fig. 5 Monoamine metabolism pathway and secondary aromatic amino acid decarboxylase (AADC) deficiency

mase] are involved in the metabolism of dopamine, serotonin, glutamate, glycine, GABA, D-serine and taurine (Surtees et al. [2006](#page-8-0)). Hence, inadequate levels of PLP in the neurons may lead to neurological dysfunction, particularly epilepsy.

Neonatal seizures have previously been described in hypophosphatasia and were believed to be related to cranial deformity, haemorrhage or hypoxia (Whyte et al. [1988](#page-8-0)). These complications were excluded in both our patients. Takahashi and colleagues suggested in 1984 that high endogenous concentrations of PEA, a natural substrate of TNAP, may also be epileptogenic. The association of pyridoxine-responsive seizures (PRSs) with perinatal hypophosphatasia was first described in 1967 (Bethenod et al. [1967\)](#page-8-0), with seven similar cases of PRSs with hypophosphatasia (HPP) subsequently reported (Whyte et al. [1988](#page-8-0); Bethenod et al. [1967](#page-8-0); Litmanovitz et al. [2002](#page-8-0)). Reports of at least ten additional hypophosphatasia patients with neonatal seizures have been published, mostly as abstracts, where pyridoxine or PLP were not administered. Whether seizures in these patients were pyridoxine- or PLPresponsive/dependent is unclear, as the anticonvulsive action of vitamin B6 was not known at that time or it was not given (Baumgartner-Sigl et al. [2009](#page-7-0)). The pathophysiology of PRSs in hypophosphatasia appears to be reduced brain PLP concentrations, decreasing the seizure threshold. Baumgartner-Sigl et al. [2009](#page-7-0) described a 7-month-old girl with infantile hypophosphatasia, who uniquely presented as a neonate with PRSs but did not have skeletal abnormalities. One other report briefly describes infantile hypophosphatasia presenting with PRSs, which was lethal when the patient was aged 3 months but had relatively mild bone disease (Posen et al. [1997\)](#page-8-0). Like our patient, all reported HPP patients with neonatal seizures died within 18 months.

Urinary levels of vanillactate were elevated in both our patients. This metabolite indicates functional deficiency of aromatic amino acid decarboxylase (AADC), a PLPdependent enzyme involved in dopamine and serotonin metabolism (Fig. [5](#page-5-0)). Patient 1 was therefore examined for amino-adipic semi-aldehyde dehydrogenase deficiency, which causes secondary pyridoxine deficiency. The results of these investigations were essentially normal, as evidenced by normal excretions of 2-amino-adipic semialdehyde. Levels of urinary vanillactate are also increased in primary AADC deficiency, due to mutations in the gene encoding this enzyme. Analysis of the CSF of both patients showed reduced 5-HIAA levels. Reduced HVA and elevated 3- methoxytyrosine (3-O-methyl-dopa), and mildly raised levels of glycine, were observed in the second patient. These abnormal metabolites may have been indicators of deficient flux through multiple pathways involving PLP-dependent enzymes, including AADC (Clayton [2006](#page-8-0)). If AADC activity is measured in plasma,

it may be normal, as it is a secondary dysfunction resulting from co-factor inadequacies, rather than a true enzymatic deficiency, in which case the HVA and 5-HIAA are more markedly reduced. Mutation analysis of the AADC gene can be performed to confirm the diagnosis of primary AADC and distinguish it from secondary dysfunction. Recessively inherited deficiency of AADC (OMIM 608643) results in a severe neurometabolic disorder with developmental delay, movement disorder with truncal hypotonia, limb hypertonia, dystonia, athetosis and hypokinesia, oculogyric crises and vegetative symptoms such as hypersalivation, nasal congestion, excessive sweating and gastro-oesophageal reflux disease. Patients display a typical pattern of neurotransmitters in CSF, with a marked reduction of the stable degradation products of dopamine and serotonin pathways, HVA and 5-HIAA, as well as elevation of the precursors of dopamine and serotonin, laevodopa, 5-hydroxytryptophan and 3-O-methyldopa (3- OMD) resulting from methylation of accumulating laevodopa (Manegold et al. [2009](#page-8-0)). A similar pattern was recently described in patients with mutations in the PNPO gene (Mills et al. [2005](#page-8-0)). These patients have a secondary AADC deficiency due to a defect in the synthesis of pyridoxal phosphate. In contrast to our patients, there are only few reported AADC patient with epileptic seizures (Ito et al. [2008](#page-8-0); Swoboda et al. [2003](#page-8-0); Manegold et al. [2009](#page-8-0)). More often, the paroxysmal movement disorder in AADC deficiency is misinterpreted as epileptic seizures. Plasma and CSF concentrations of PLP and pyridoxal were not measured simultaneously in either patient, but these would be expected to show increased plasma levels of PLP and reduced or undetectable amounts of pyridoxal. Both our patients showed disappearance of baseline burst suppression after commencement of pyridoxine therapy. The improvements in the EEG, however, was more evident in the second patient, and this could perhaps be attributed to the higher dose of intravenously administered pyridoxine that was used, in contrast to a smaller dose of pyridoxine given orally to the first patient. We also speculated that the Q106H mutation is possibly milder than the G491R mutation.

Carriers of the severe forms of hypophosphatasia are usually clinically unaffected. They may, however, show modestly reduced levels of serum ALP, as did the parents of our patients, and increased urinary PEA levels, observed in the mother of the second patient (Mornet [2007](#page-8-0)). Important in our understanding of hypophosphatasia is the temporal variability of the bone disease (Whyte et al. [2006](#page-8-0); Cole [2008](#page-8-0)). Progressive improvements in limb shortening, long bone bowing and mineralisation may occur during the third trimester of pregnancy (Pauli et al. [1999;](#page-8-0) Wenkert et al. [2007\)](#page-8-0). Recognition of this possibility is crucial and emphasises the need for expert assessment and careful

counselling on the natural history of hypophosphatasia in each case of perinatal diagnosis (Cole [2008](#page-8-0)). The progressive improvements of affected patients from infancy to adulthood may be attributable to epigenetic factors involved in the variable expression of the disease or the temporal requirements for ALP during development (Mornet [2007](#page-8-0)). An alternative explanation for the spontaneous remission of skeletal deformities in the prenatal benign form is that maternal ALP compensates, to a degree, via foetal– maternal exchanges, but this is primarily observed when the mother is heterozygous for a mutation with a dominant negative effect (Pauli et al. [1999;](#page-8-0) Moore et al. [1999](#page-8-0); Mornet [2007\)](#page-8-0). It has been reported that maternal serum ALP activity increases during approximately the last trimester of pregnancy, when the placental ALP isoenzyme appears in the maternal circulation. This increase could mask the hypophosphatasaemia in the mother (Whyte [1995\)](#page-8-0). On the other hand, if the mutation is inherited from the father, normal maternal ALP levels will prevent manifestation of the disorder in the foetus.

Currently, there is no specific treatment for hypophosphatasia. Management is directed towards preventing or correcting the symptoms and complications. Good responses to treatment with pyridoxine and pyridoxal phosphate have been described for the control of neonatal convulsions with burst suppressions on EEG or infantile spasms with hypsarrhythmia (Clayton [2006](#page-8-0)). The wide clinical heterogeneity and the rarity of the disease render controlled clinical trials almost impossible. Preliminary results have suggested that dietary phosphate restriction could be beneficial, because patients are hyperphosphataemic and inorganic phosphate is a natural inhibitor of TNAP (Cole [2008;](#page-8-0) Wenkert et al. [2007\)](#page-8-0). Treatments with zinc and magnesium, which act as catalytic ions of the enzyme, showed inconsistent results.

Bone marrow transplantation has been performed in the more severe infantile cases, in a small number of patients. The results suggested that the effects may be transient, with bone lesions possibly recurring 6 months after transplantation (Whyte et al. [2003\)](#page-8-0). Donor bone fragments and marrow may provide precursor cells for distribution and engraftment in the skeletal microenvironment that form TNAP-replete osteoblasts and may improve mineralisation (Whyte et al. [2003\)](#page-8-0). Further trials with improved transplant protocols may enable the establishment of this treatment modality (Cole [2008\)](#page-8-0).

Finally, enzyme replacement therapy appears to be the most promising potential therapy in the near future. The first successful treatment of TNAP knockout mice, using exogenous administration of recombinant ALP that targeted mineralised tissues, has been reported (Millan et al. [2007\)](#page-8-0). Human studies will clarify the efficacy of enzyme replacement therapy in substituting

endogenously deficient TNAP and potentially ameliorating this disorder.

Conclusion

We have described two patients with autosomal recessive perinatal hypophosphatasia with epileptic encephalopathy and central hypoventilation, and autonomic instability in one of them. Of interest, both patients had relatively mild defects in skeletal mineralisation, in comparison with the severe extraskeletal features. In both patients progressive encephalopathy, refractory to therapy, resulted in perinatal death.

Deactivating mutations in the ALPL gene that encodes TNAP may lead to secondary AADC deficiency, due to reduced co-factor pyridoxal-5-phosphate availability. Both patients had raised levels of urinary vanillactate and reduced levels of CSF 5-HIAA, whilst reduced HVA and raised 3-O-methyldopa levels were also documented in the second case. In addition, both patients exhibited burst suppressions on EEG that responded to pyridoxine therapy.

We recommend that assessment of any neonate suffering PRSs should include the measurement of serum ALP. Further detailed metabolic studies comprising measurements of plasma and CSF PL and PLP levels in cases of hypophosphatasia should be conducted. Accurate clinical diagnosis is crucial. Neurological complications should be anticipated and, if present, we suggest the early institution of treatment with pyridoxine or pyridoxal phosphate, as it may prevent or ameliorate seizures. Furthermore, we emphasise the importance of a treatment trial of pyridoxine and/or pyridoxal phosphate in neonates with intractable seizures. Further studies involving complementary approaches corroborating clinical data evaluation, sitedirected mutagenesis and computer–assisted threedimensional modelling of the ALPL gene may provide a more profound elucidation of genotype–phenotype correlation. Greater emphasis should be placed on detailed molecular characterisation to guide clinical management, genetic counselling of the family and prenatal testing for future pregnancies.

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