Short communication

Elisabeth Steichen-Gersdorf*, Bettina Lorenz-Depiereux, Tim Matthias Strom and Nicholas J. Shaw

Early onset hearing loss in autosomal recessive hypophosphatemic rickets caused by loss of function mutation in *ENPP1*

Abstract: Autosomal recessive hypophosphatemic rickets 2 (ARHR2) is a rare form of renal tubular phosphate wasting disorder. Loss of function mutations of the ecto-nucleotide pyrophosphatase/pyrophosphodiesterase 1 gene (*ENPP1*) causes a wide spectrum of phenotypes, ranging from lethal generalized arterial calcification of infancy to hypophosphatemic rickets with hypertension. Hearing loss was not previously thought to be one of the features of the disease entities and was merely regarded as a complication rather than a part of the disease. We report two children who presented in mid to late childhood with progressive varus deformity of their legs due to hypophosphatemic rickets caused by mutations in the *ENPP1* gene. Both children had evidence of progressive hearing loss requiring the use of hearing aids. This report of two unrelated infants with compound heterozygous mutations in *ENPP1* and previously published cases confirms that mild to moderate hearing loss is frequently associated with ARHR2. Early onset conductive hearing loss may further distinguish the autosomal recessive *ENPP1* related type from other types of hypophosphatemia.

Keywords: autosomal recessive hypophosphatemic rickets; *ENPP1*; generalized arterial calcification of infancy; hearing loss.

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***Corresponding author: Elisabeth Steichen-Gersdorf,** Pediatrics I, Medical University of Innsbruck, Anichstraße 35, A-6020 Innsbruck, Austria, Phone: +43 51250423600, Fax: +43 51250424904, E-mail: elisabeth.steichen@i-med.ac.at
Bettina Lorenz-Depiereux and Tim Matthias Strom: Helmholz Centre, Munich, Germany
Nicholas J. Shaw: Department of Endocrinology and Diabetes, Birmingham Children's Hospital, Birmingham, UK

Introduction

Hypophosphatemic rickets (HR) is a heterogeneous group of renal phosphate wasting disorders caused by elevated circulating fibroblast growth factor 23 (FGF23), which inhibits tubular phosphate reabsorption and 1,25 dihydroxyvitamin D synthesis in the proximal renal tubules. Mutations in any of the four known genes, *PHEX* [XLH (MIM 307800)], *FGF23* [ADHR (MIM 193100)], *DMP1* [autosomal recessive hypophosphatemic rickets 1 (MIM 241520)] and ecto-nucleotide pyrophosphatase/pyrophosphodiesterase 1 (*ENPP1*) [autosomal recessive hypophosphatemic rickets 2 (MIM 613312)], cause hypophosphatemia (1–4).

Homozygous and combined heterozygous mutations in *ENPP1*, which encodes for an enzyme that generates inorganic pyrophosphate PP(i) are also known to cause the condition generalized arterial calcification of infancy (GACI). Patients demonstrate severe calcifications of the media of large and medium-sized arteries and stenosis due to myointimal proliferation within the first month of life, and severe hypertension and death in infancy due to congestive heart failure (5, 6). Milder variants with an intermediate phenotype between GACI and HR exist and response to treatment with bisphosphonates has been reported (7). Arterial calcification may disappear during childhood and hypertension improves. In the course of disease, some patients, however, have developed HR with persistent hypophosphatemia and a requirement for phosphate treatment.

Conductive hearing loss has been observed in X-linked hypophosphatemia due to PHEX inactivation in adults but it has not been reported in childhood. In contrast, sensorineural deafness has been reported in autosomal recessive HR due to dentin matrix protein-1 (DMP1) loss of function and was attributed to a narrowing of the internal auditory canals (8, 9).

Brachet et al. (10) recently reported that ENPP1 loss of function in autosomal recessive hypophosphatemic

rickets 2 (ARHR2) is sometimes associated with mixed hearing loss initially conductive and later on central. They concluded that early hearing loss should lead to consideration of rare recessive variants of HR related to ENPP1 or DMP1. We report on two more unrelated affected patients with childhood onset of ARHR due to *ENPP1* mutations and progressive hearing loss.

Methods

Mutational analysis was performed after informed consent was obtained. DNA from the patient and parents of case 1 was analyzed at the Helmholtz Centre in Munich. All 25 exons of *ENPP1*, including the intron boundaries, were amplified and sequenced according to standard techniques. The DNA of case 2 was analyzed at the molecular genetics unit of the Royal Devon and Exeter Hospital with sequence analysis of exons 1–25 and the flanking regions of the *ENPP1* gene.

Case report 1

This male patient was the first child born at term to healthy nonconsanguinous Austrian parents, with a birth weight of 3500 g and a length of 46 cm. Family history was unremarkable. A midline cleft palate affecting mainly the soft and partial hard palate was surgically repaired at the age of 9 months. Conductive hearing deficit was present since infancy and did not improve despite recurrent bilateral tympanostomy. A computed tomography scan of the middle and inner ear showed no structural abnormalities. As the conductive hearing loss deteriorated, the boy was supplied with hearing aids at the age of 6 years, when he started primary school. At the age of 10 years, the patient was referred with a progressive bowing of the legs with genu valgum deformity and bone pain. Medical examination at the hospital additionally detected arterial hypertension without myocardial hypertrophy, which was caused by hyperreninemia due to unilateral stenosis of the left renal artery following intimal proliferation. Biochemical investigation were consistent with hypophosphatemic rickets with decreased phosphate, 0.67 mmol/L (normal range: 0.95–1.65 mmol/L), due to reduced renal phosphate reabsorption of 81%, elevated alkaline phosphatase, 813 U/L (normal range: 74–390), elevated FGF23, 134 kRU/L (normal range: 26–110 kRU/L), and excessive hyperreninemia, 1053 ng/L (normal range: 3.5–65.6 ng/L). The patient was treated with phosphate substitution (Reducto spezial tablets®, Temmler, PHARMA, Marburg), 42 mg/kg/day, calcitriol, 10.2 ng/kg/day, and hypertension finally responded to a combination of aliskiren and metoprolole. Rickets in combination with arterial hypertension led us to investigate *ENPP1* after a negative mutation screening of *FGF23*. The affected patient was compound heterozygous for a non-synonymous sequence variation in exon 2 of the *ENPP1* gene and a splice site mutation in intron 21 of the *ENPP1* gene, NM_006208.2:c.[275G>A];[2230+1G>A], p.[Gly92Asp];[?], which has not been reported before. Parents were heterozygous for each of the mutations.

At the age of 16 years, his height was 163 cm (3rd percentile) and weight 48 kg (10th percentile). Axial bone deformity was corrected at the age of 16 years. His hearing deficit is moderate, mainly conductive with a mild sensorineural component. The boy attends normal school.

Case 2

This female patient was born at 34 weeks of gestation with a birth weight of 2.7 kg. There were some initial feeding difficulties but no significant problems and she was discharged after 1-week. There was concern about the shape of her legs from the age of 3 years with the development of a progressive genu valgum deformity. When first seen at the age of 4 years, there was a marked valgus deformity with an intermalleolar distance of 14 cm. A previous sibling born at 32 weeks of gestation who was reported to have kidney problems died in the neonatal period. Investigations were consistent with hypophosphatemia with a plasma phosphate of 0.77 mmol/L (normal range: 1.3–1.7 mmol/L), reduced tubular reabsorption of phosphate of 81%, and elevated alkaline phosphatase, 915 IU/L (normal range: 250–850 IU/L). Treatment was subsequently commenced with phosphate sandoz, 62.5 mg/kg/day, and alfacalcidol, 37.5 ng/kg/day. She required surgical correction of her valgus deformity. At the age of 7 years, there were concerns about hearing loss and investigations confirmed a bilateral conductive hearing loss that subsequently required the provision of hearing aids. Genetic investigations excluded mutations in the *PHEX* and *FGF23* genes but identified she was heterozygous for a nonsense mutation, a missense mutation, and a novel variant in the *ENPP1* gene, NM 006208.2:c.[2026C>T]; [2375 A>G];[.655G>A], p.[.Q676X];[N792S];.[G219R]. At the age of 13 years, her height was 143.6 cm (3rd percentile) and weight 35.6 kg (3rd–10th percentile).

Discussion

Since the first report (5) of homozygous and compound heterozygous mutations in *ENPP1*, more than 50 cases have been reported. Hearing loss was mentioned in a minority of cases and attributed to arterial calcification and reduced perfusion of arteries supplying the inner ear or stapedovestibular calcification but was not considered as a frequent feature in ENPP1 related disorders.

Functional inactivating mutations in homozygous or compound heterozygous state have been scattered over the whole coding region and the spectrum of phenotypes is wide, ranging from extensive arterial calcification in infancy and early death to widespread arterial stenosis or hypophosphatemia and rickets. Except for a lethal mutation (Pro305Thr), the clinical presentation appeared independent of the mutation and even siblings with the identical genotype had a variable clinical course postulating additional modifiers to influence the severity of the disease (11).

Brachet et al. (10) recently focused on hearing loss as part of the clinical picture of *ENPP1* loss of function

mutations. There is an existing knockout mouse model with decreased expression of ENPP1, which has significantly reduced brainstem-auditory-evoked potential without ectopic calcification and deafness that supports the theory that ENPP1 plays an important role in the inner ear development and function. Originally, Rutsch and Nitschke (5, 11, 12) mentioned conductive, as well as sensorineural, hearing loss in children aged 3–12 years with phenotypes of GACI and HR, which was mainly regarded as a complication of the disease. The increasing reports of mildly affected patients with rickets and hearing problems lead the focus on the abnormal pyrophosphate metabolism per se.

These two patients with compound heterozygous mutations in the *ENPP1* gene presented with HR, in addition, one patient presented with hypertension, without severe signs of arterial calcification in infancy. Hearing problems in case 1 were initially related to cleft palate, with frequent occurring ventilation problems of the middle ear and flat tympanograms. The progression of hearing problems and the mixed sensorineural and conductive components suggested a different explanation. Bilateral conductive hearing loss in an unrelated girl with ARHR2 and *ENPP1* mutation and concomitant decreased enzyme activity are convincing evidence of a disease inherent dysfunction of the ear. According to Brachet et al., and previous reports, it is a rare finding. The true incidence of deafness, however, may be much higher than estimated from previous reports. In fact, hearing loss could be a major sign to help discriminate between the various forms of HR. In the most common X-linked form of hypophosphatemia (XLH), caused by inactivating mutations in the *PHEX* gene, hearing loss is frequently observed, but is usually of adulthood onset (13–15). The youngest patient reported with hearing impairment was 17 years of age with a positive family history (16). The mechanism seems to be different as hearing in XLH mainly affects the high frequencies and it is attributed to a cochlear source on the basis of osteosclerosis of the petrous bone. The relative frequency is age dependent and up to 50% of patients will be affected throughout their lives (17).

The true incidence of hearing loss in ARHR2 will be difficult to evaluate because it is a rare disorder with a wide spectrum of manifestations and high mortality in infancy in the case of GACI. Mild hearing problems may have been missed or have not been reported in patients, and are therefore, probably underreported in the literature. According to recent reports, the performance of complete hearing studies in *ENPP1* related disorders has to be stressed.

In conclusion, we report two children with autosomal recessive HR with genetically confirmed compound heterozygous *ENPP1* mutations with childhood onset of mainly conductive hearing impairment. On reviewing the literature, hearing loss would appear to be a major feature of the disease and qualifies to delineate this rare type from other FGF23 dependent forms of hypophosphatemic rickets.

Conflict of interest statement: The authors have no relevant conflict of interest to disclose.

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