Molecular Genetics and Metabolism 94 (2008) 431–434

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

PNPO deficiency: An under diagnosed inborn error of pyridoxine metabolism

Morad Khayat [a], Stanley H. Korman [b], Pnina Frankel [a], Zalman Weintraub [c,d], Sylvia Hershckowitz [c], Vered Fleisher Sheffer [c], Mordechai Ben Elisha [c], Ronald A. Wevers [e], Tzipora C. Falik-Zaccai [a,d,*]

[a] Institute of Medical Genetics, Western Galilee Hospital, Nahariya, P.O. Box 21, 22100 Nahariya, Israel
[b] Metabolic Diseases Unit, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
[c] Department of Neonatology, Western Galilee Hospital, Nahariya, Israel
[d] Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel
[e] Laboratory of Pediatrics and Neurology, Radboud University Nijmegen Medical Centre, The Netherlands

ARTICLE INFO

Article history:
Received 19 March 2008
Received in revised form 14 April 2008
Accepted 14 April 2008
Available online 15 May 2008

Keywords:
Neonatal seizures
Pyridoxamine
Pyridoxine 5′-phosphate oxidase (PNPO)
PNPO deficiency
Pyridoxal phosphate (PLP)

ABSTRACT

The rare autosomal recessive disorder pyridoxine 5′-phosphate oxidase (PNPO) deficiency is a recently described cause of neonatal and infantile seizures. Clinical evaluation, and biochemical and genetic testing, were performed on a neonate with intractable seizures who did not respond to anticonvulsant drugs and pyridoxine. Sequencing of the *PNPO* gene revealed a novel homozygous c.284G>A transition in exon 3, resulting in arginine to histidine substitution and reduced activity of the *PNPO* mutant to 18% relative to the wild type. This finding enabled molecular prenatal diagnosis in a subsequent pregnancy, accurate genetic counseling in the large inbred family, and population screening.

Introduction

Neonatal and infantile seizures present a common challenge in NICUs and pediatric wards. Diagnosis and treatment are complicated and many newborns suffer significant morbidity or die undiagnosed. Pyridox(am)ine-5′-phosphate oxidase (PNPO) deficiency is a rare, recently described autosomal recessive disorder that causes intractable seizures that are not responsive to anticonvulsant drugs and pyridoxine [1–7]. To date, 14 patients from eight kindreds have been reported with mutations in the *PNPO* gene [1–6].

PNPO is the rate-limiting enzyme in the synthesis of pyridoxal 5′-phosphate (PLP), the active form of vitamin B6. In conversion to PLP, all three ingested forms of this vitamin (pyridoxine, pyridoxamine, and pyridoxal) are phosphorylated by pyridoxal kinase. For pyridoxal this singular step suffices for conversion to PLP. The other two vitamers require, in addition, the catalytic activity of PNPO. When PLP synthesis is ineffective, exogenous pyridoxal/PLP becomes the only source of the active co-factor for the proper activity of aromatic L-amino acid decarboxylase (AADC), as well as for more than 100 additional enzymes [2,8]. Thus, PLP is involved in a wide range of biochemical reactions, including the metabolism of amino acids and glycogen, the synthesis of nucleic acids, hemoglobin, sphingomyelin and other sphingolipids, and the neurotransmitters serotonin, dopamine, norepinephrine, and gamma-aminobutyric acid (GABA).

The liver is the prime source of plasma PLP, though the muscle also contributes. The synthesis of PLP actually occurs twice during vitamin B6 metabolism. Not able to pass through, PLP is dephosphorylated at the external cell membrane surface by alkaline phosphatase, and then rephosphorylated within the cells. Proper activity of alkaline phosphatase is thus essential for maintenance of normal plasma PLP levels [9,10].

We sequenced the *PNPO* gene in a patient with clinical and biochemical findings suggestive of PNPO deficiency, and identified a novel homozygous missense mutation that markedly reduced PNPO activity. We conducted prenatal testing of a subsequent pregnancy, and population screening in individuals with the same ethnic background as the family reported here.

Methods

The patient

A 36-week gestation female was born spontaneously. With birth weight 2075 g (15th percentile), and head circumference 29.5 cm (5th percentile), she was transferred immediately to the NICU. On admission, she had mild respiratory distress and a grade 2/6 systolic heart murmur with weak femoral pulses. Otherwise, and with the exception of dysmorphic features such as low hairline, high nasal bridge, high arched plated, short neck, and hypoplastic nipples, the physical examination was normal. Medical history revealed an uneventful pregnancy except for low levels of beta-HCG determined at 17 weeks gestation.

* Corresponding author. Address: Institute of Medical Genetics, Western Galilee Hospital, Nahariya, P.O. Box 21, 22100 Nahariya, Israel. Fax: +972 4 9107 553.
E-mail address: Tzipora.Falik@naharia.health.gov.il (T.C. Falik-Zaccai).

1096-7192/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2008.04.008

Generalized myoclonic seizures began 1 h after birth. Seizures were initially controlled with a high dose of intravenous diazepam, but later recurred with increased frequency and severity. The dosage of the anti-epileptic therapy was increased, and intravenous midazolam and lidocaine were added gradually, with no response. A therapeutic trial with pyridoxine showed mild improvement for 2 days, but then seizures again became uncontrollable. Due to the severe status epilepticus, ventilation was initiated followed by induced barbiturate coma (I.V. pentothal, 5 mg/kg/h). Seizures were controlled for 10 days but then recurred.

Brain CT showed an impression of diminished sulci, mildly enlarged Sylvian fissure, and hypodense white matter. Heterotopia or schizencephaly were suspected. Brain MRI showed abnormalities of the white matter with decreased myelinization, presence of tiny sub cortical foci, simplified sulcation and gyration, and abnormal diffused high signal in the basal ganglia.

On day 45, the infant developed high temperature and exacerbation of her uncontrolled seizures. *Klebsiella pneumoniae* was isolated from blood cultures. Despite antibiotic therapy, and other life-supportive management, the baby died on the 47th day of her life. The parents refused post mortem but agreed to CSF tap and biopsies from skin, liver, and muscle for further investigations.

Family history

The patient was the result of a third pregnancy. The parents are first-degree cousins of Arab Moslem origin and belong to a four-generation extended, consanguineous family (Fig. 1).

Mutation analysis of the PNPO gene

Sequence analysis of the *PNPO* gene extracted from genomic DNA of patient IV_3 (Fig. 1) was applied to all 7 exons and the flanking splice junction consensus sequences, using the Big dye terminator kit, and analyzed using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK). Mutation analysis was performed on the patient's parents and other family members (Fig. 1).

Expression analysis

Site-directed mutagenesis was carried out using the QuikChangew XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) as described by Mills et al. [1].

Expression studies in CHO cells were transfected with wild-type construct, mutant cDNA constructs, or plasmids without construct (Mock) using Lipofectamine™ transfection agent (Invitrogen Ltd, Paisley, UK) as described by Mills et al. [1].

Five microliters of each lysate supernatant from transfected CHO cells with *PNPO* wild-type construct, mutant cDNA constructs, and Mock were electrophoresed on 10% SDS–polyacrylamide gel under reducing conditions. Immunodetection was performed using anti-human PNPO polyclonal antibody (Abnova Corporation, Taipei, Taiwan) according to the manufacturer's instructions.

Prenatal diagnosis and population screening

DNA was isolated from amniocyte cell culture and direct sequencing was performed to test for the mutation in a subsequent pregnancy. PCR based restriction analysis, as described above, was used to screen 80 healthy individuals (160 chromosomes) of Arab Moslem origin (the same ethnic background as the patient), for the presence of the c.284G>A mutation.

This research was performed under the approval of the Supreme Helsinki Committee of the Israeli Ministry of Health, and after obtaining informed consent from participants.

Results

Metabolic and genetic investigations

Urine organic acid analysis (by gas chromatography–mass spectrometry) revealed elevated excretion of homovanillic acid (HVA) and a prominent peak of vanillactic acid (VLA, normally undetectable or trace only). Amino acid determination (Table 1) showed elevated glycine and reduced arginine concentrations in both CSF and plasma, and slightly elevated plasma threonine. Analysis of a CSF sample taken around the time of death (Table 1), showed massively elevated VLA in CSF, markedly increased L-DOPA, and mildly increased 3-methoxytyrosine, consistent with a defect in PNPO. Unexpectedly, HVA was increased and most amino acids levels extremely elevated (data not shown), suggesting a peri-mortem effect. During the week prior to death, alkaline phosphatase levels fluctuated from a normal value of 25 U/L to 245 and 455 U/L.

Mutation analysis of the PNPO gene

Sequence analysis of the *PNPO* gene revealed a homozygous missense G>A transition at nucleotide 284 in exon 3 (c.284G>A), resulting in arginine to histidine substitution at position 95 (R95H). Both parents were heterozygotes for this mutation.

Site-directed mutagenesis and expression studies

Transfection with DNA construct containing the R95H mutation resulted in a reduced level of PNPO activity of ~18% (17, 18%; $n=2$) compared to that of the wild type.

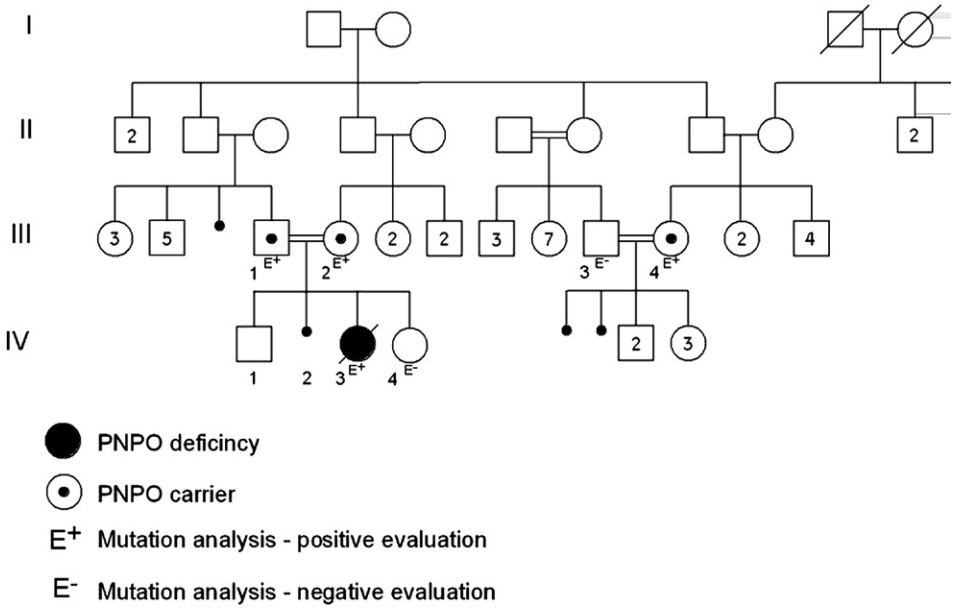

Fig. 1. Pedigree of the Arab Moslem kindred with PNPO deficiency from an isolated village in northern Israel.