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The clinical course and genetic defect in the PCFT gene in a 27-year old woman with hereditary folate malabsorption

Sang Hee Min, MD¹, Sun Young Oh, MD¹, George I. Karp, MD³, Mortimer Poncz, MD⁴, Rongbao Zhao, PhD^{1,2}, and I. David Goldman, MD^{1,2}

¹Department of Medicine, Albert Einstein College of Medicine, Bronx, NY

²Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY

³Central Jersey Oncology Center and the Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

⁴Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA

Abstract

Two sequential homozygous mutations are demonstrated in the recently cloned proton-coupled folate transporter (PCFT) gene resulting in the absence of this protein in a 27 year old woman with hereditary folate malabsorption, who is normal in all respects and has completed higher education, following treatment with parenteral 5-formyltetrahydrofolate since infancy.

Keywords

Intestinal folate absorption; folate transport; low pH folate transporter; proton-coupled folate transporter; RFC; reduced folate carrier

Hereditary folate malabsorption (HFM; Online Mendelian Inheritance in Man [OMIM 229050]) is a rare autosomal recessive disorder caused by impaired intestinal folate absorption and impaired folate transport into the central nervous system. Infants are normal at birth but within a few months develop folate deficiency anemia often accompanied by immunoglobulin deficiency, infections, and diarrhea. Seizures and neurodevelopmental defects are frequent. With rapid diagnosis, this disorder can be managed by high-doses of oral or parenteral folate ^{1,2}. However, because of the rarity of HFM, there is little information on the long-term outcome of treatment. Recently, HFM was shown to be due to loss-of-function mutations in a proton-coupled folate transporter (PCFT) ^{3,4} allowing the possibility of rapid diagnosis and correlations among specific mutations, clinical manifestations, and outcome. This study focuses on a patient with HFM, begun on therapy at two months of age, who was the subject of two previous reports prior to the elucidation of

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Correspondence: I. David Goldman, Departments of Medicine, and Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY, 10461; igoldman@aecom.yu.edu; phone number: 718-430-2302; fax number: 718-430-8550.

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the molecular basis for this disorder^{5,6}. This patient is now shown to have a complete absence of the PCFT protein but has had an excellent outcome.

Methods

This study was approved by the Albert Einstein College of Medicine Clinical Committee of Investigation (CCI). The patient and her parents provided Informed Consent and peripheral blood was collected. Genomic DNA extraction, polymerase chain reaction, sequencing analysis, site-directed mutagenesis, transient transfection, Northern and Western blots, and [³H]5-methyltetrahydrofolate transport analyses were performed as reported previously^{3,4}.

Results

The patient was first reported in 1981 when she presented with severe anemia (Hb 6 g/dL), a serum folate of 0.9 ng/mL (nl, 4 – 20 ng/mL), a megaloblastic bone marrow and multiple organ failure⁵. Her Portuguese parents denied consanguinity and were asymptomatic; a male sibling died at age 3 months with diarrhea, lethargy and pallor (Figure A). After failing to respond to oral folic acid, the patient was placed on IM folic acid (5 mg every 2–3 weeks). Her blood folate and hematological measurements normalized. At age 6 months, IM 5-formyltetrahydrofolate was substituted (5 mg biweekly) largely because CSF folate while on folic acid was 2.8 ng/mL (nl, 17 – 40 ng/mL) with a serum folate of 18 ng/mL; on IM 5-formyltetrahydrofolate, CSF folate was 5.0–5.5 ng/mL. At 14 months, the regimen was changed to 1.5 mg 5-formyltetrahydrofolate/d based in part on the CSF folate half-life; this was decreased to 5 times/week at age 6. In a follow-up report at age 16, the patient was completely normal, had reached menarche at age 12 and was at the 75th percentile in weight and 50th percentile in height⁶. She ranked in the top 10% of her high school class with a combined SAT score of 1210. Her hemoglobin was normal and serum folate ranged from 2.5 to 8.3 ng/mL. In her teens, 5-formyltetrahydrofolate administration was decreased to 1.5 mg, IM, 3 times/week; this has been continued to date.

Since then, the patient completed college, where she did well in mathematics and statistics, and went on to receive an MBA. The patient, now 27 years old, is employed full-time and married. Current laboratory data include Hb of 12.9 g/dL, WBC $3.7 \times 10^9/L$ (nl, $3.4\text{--}9.8 \times 10^9/L$), platelets $271 \times 10^9/L$ (nl, $137\text{--}331 \times 10^9/L$), serum folate 4.3 ng/mL (nl, > 5.4 ng/mL) and homocysteine 4 $\mu\text{mol/L}$ (nl, $5.4\text{--}11.9 \mu\text{mol/L}$). Serum iron was 141 $\mu\text{g/dL}$ (nl, $140\text{--}175 \mu\text{g/dL}$), total iron binding capacity 390 $\mu\text{g/dL}$ (nl, $250\text{--}400 \mu\text{g/dL}$), and ferritin 78 ng/mL (nl, $10\text{--}154$ ng/mL).

This patient was found to have two sequential, homozygous, mutations (GC>AA) at positions 197, 198 of PCFT cDNA (starting from the ATG initiation codon - Figure B) located to exon 1 (Figure C), resulting in a TGC(cysteine) → TAA(stop) substitution at amino acid 66(C66X). Transient transfection of cDNA constructs into a reduced folate carrier- and PCFT- deficient Hela cell line⁷ demonstrated the lack of [³H]5-methyltetrahydrofolate uptake mediated by the mutated carrier as compared with wild-type PCFT (Figure D). PCFT mRNA expression was comparable in mutated and wild-type transfectants (Figure E). However, consistent with the proximal position of the inserted stop codon in the first exon, PCFT protein was not detected by Western blot in cells transfected with the mutant PCFT (Figure F).

Discussion

With a regimen of mostly parenteral 5-formyltetrahydrofolate for over 26 years, this patient is normal in all respects. Of particular interest is her mathematical ability, an intellectual

function particularly compromised in children treated with intrathecal methotrexate who have survived acute lymphoblastic leukemia⁸. Hence, the prognosis in HFM can be excellent with early diagnosis and appropriate treatment even when PCFT protein is entirely absent. Residual PCFT function is not required to achieve an optimal outcome. Further, the patient did well despite a decrease in frequency of 5-formyltetrahydrofolate administration over time, likely due to a decreased folate requirement with maturation of the central nervous system. Although this patient's blood folate level was normalized with parenteral folate treatment, the normal CSF:blood folate ratio of 2–3:1 was not achieved, as is usually the case⁵, likely due to a role for PCFT in folate receptor-mediated folate transport across the choroid plexus^{3,4,9–11}. Despite this, the level of folate supplementation was sufficient for normal brain development. Hence, the subject's low CSF folate level may not have reflected what were presumably higher folate levels within the cerebral cortical parenchyma.

Studies in this patient suggested that parenteral 5-formyltetrahydrofolate produced higher blood and CSF folate levels than did folic acid⁵. However, there have been good outcomes in other children using high-dose oral 5-formyltetrahydrofolate, although the duration of follow-up has not been as long as in this case¹. The absence of controlled studies due to the rarity of HFM, and the long duration of follow-up required, preclude clear definition of optimal therapy. Considering that the highest level of intellectual development is a critical therapeutic objective, a case could be made for parenteral treatment with a reduced folate, either 5-methyltetrahydrofolate (MetafolinTM), the physiological blood folate, or 5-formyltetrahydrofolate (LeucovorinTM).

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Figure. Genetic and Functional analysis of PCFT mutation

(A) Pedigree of the family with HFM. The filled symbols indicate the patient with both PCFT alleles mutated. The half-filled symbols indicate the parents with one normal and one mutated PCFT allele.

(B) Chromatograms of sequenced PCFT genomic DNA showing the homozygous GC>AA mutations in the patient and the heterozygous GC>AA mutations in her parents.

(C) Genomic organization of PCFT showing the location of the GC>AA mutations.

(D) Functional analysis of the patient's mutated and wild-type PCFT cDNA transiently transfected into a HeLa cell line that lack both genomic reduced folate carrier and PCFT expression⁴. Uptake of 0.5 μ M [3 H]5-methyltetrahydrofolate was assessed over two minutes at pH 5.5 as previously described⁴.

(E) Northern blot analysis of mRNA obtained from the HeLa cells transiently transfected with the patient's mutated or wild-type PCFT mRNA.

(F) Western Blot analysis of whole cell lysate from the cells described in Figure E. PCFT proteins were tagged with hemagglutinin epitope inserted in the C-terminus and the blot was probed with anti-hemagglutinin antibody.