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MUTATION OF THE PROTON-COUPLED FOLATE TRANSPORTER GENE (PCFT-SLC46A1) IN TURKISH SIBLINGS WITH HEREDITARY FOLATE MALABSORPTION

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Abstract

Hereditary folate malabsorption (HFM) is a rare autosomal recessive disorder characterized by systemic and central nervous system folate deficiency. Turkish siblings are reported with the clinical syndrome of HFM, homozygous for deletion of 2 bases (c.204_205 delCC) within the first exon of the proton-coupled folate transporter (PCFT) gene, causing a frameshift. Low blood and cerebrospinal fluid folate levels were detected at ages 3.5 and 1 month. Treatment with parenteral 5-formyltetrahydrofolate resulted in normal development now at ages 3 and 1 year. Extending current knowledge on the phenotypic manifestations of HFM and the PCFT mutation spectrum will provide opportunities to define possible genotype-phenotype correlations and clarify the basis for the phenotypic variability that is characteristic of this disorder.

Keywords

folate deficiency; HCP1; hereditary folate malabsorption; HFM; megaloblastic anemia; *PCFT*; SLC46A1

Hereditary folate malabsorption (HFM) is a rare autosomal recessive disorder first described by Lubhy et al in 1961 [1]. Infants present in the first few months of life with failure to thrive, diarrhea, oral ulcers, megaloblastic anemia, infections, and, frequently, progressive neurologic deterioration. Early diagnosis and treatment with either low-dose parenteral, or high dose oral, 5-formyltetrahydrofolate (5-formylTHF) is critical. If the disease is untreated it can be fatal, and if treatment is delayed, there can be permanent neurologic damage [2].

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Recently, HFM was shown to be due to loss-of-function mutations in the proton-coupled folate transporter (PCFT-SLC46A1), a member of the super-family of facilitative carriers that mediates intestinal folate absorption within the acid microenvironment of the proximal small intestine and across the blood-choroid plexus-cerebrospinal fluid (CSF) barrier [3, 4]. Since then, additional patients with HFM and PCFT mutations have been documented [5-7]. In this report, we describe Turkish siblings with a clinical diagnosis of HFM. Genetic analysis revealed that these children have a novel homozygous mutation of the proton-coupled folate transporter (*PCFT*) gene.

CASE HISTORY

A 3.5-month-old Turkish boy was referred to the Tepecik Training and Research hospital with anemia and thrombocytopenia. His parents denied consanguinity. The patient was born at term weighing 3 Kg after an uncomplicated pregnancy. He was exclusively breast-fed. Moderate anemia was noted at the age of 2 months and he received blood transfusions when he was 3 months old. On admission the patient was alert and responsive. His weight was 5 kg (10–25th percentile); his height was 56 cm (10th percentile). His general physical and neurological examination was normal; no dysmorphic features were evident. Hemoglobin was 5.3 g/dL, white blood cell count was $4.6 \times 10^9/L$, and platelet count was $65 \times 10^9/L$, mean corpuscular volume (MCV) 83 fL, mean corpuscular hemoglobin (MCH) 29 pg, mean corpuscular hemoglobin concentration (MCHC) 35%. The peripheral blood smear revealed normocytic, hypochromic erythrocytes; the reticulocyte count was 2%. Serum iron, iron binding capacity, and ferritin levels were all within normal limits. Bone marrow aspirate was hypercellular with an excess of megaloblastic erythroblasts. Blood chemistries encompassing electrolytes and liver and renal functions were normal. Metabolic profiling including analyses of plasma amino acids and urinary organic acids was within normal limits. Cranial magnetic resonance imaging (MRI) showed no evidence of intracranial calcification or demyelination.

Serum folate was < 1 ng/mL (normal: 5–17 ng/mL); serum vitamin B₁₂ was within the normal range at 181 pg/mL (normal: 150–600 pg/mL). The CSF folate level was below 1 ng/mL (normal: >10 –25 ng/mL). Folic acid was started at 5 mg/day orally and increased progressively to 60 mg/day; however, serum folate remained low. A clinical diagnosis of HFM was made and the patient was treated with intramuscular 5-formylTHF at 1.5 mg/day. Serum folate was > 24 ng/mL at 1 hour, and 16.3 ng/mL at 24 hours, after the intramuscular dose; CSF folate was 3.6 ng/mL at 1 hour and 5.5 ng/mL at 24 hour. At the age of 5 months, the frequency of 5-formylTHF administration was decreased to 5 doses per week. The patient is now 3 years old and his hematological parameters and neurological development are normal. He is at the 25–50th percentile in height and weight.

The family had a second child when the patient was 2 years old. Because of the family history, the infant was evaluated at the age of 1 month. Her physical examination was normal. However serum and CSF folate levels were low, 1.82 and 3.64 ng/mL, respectively. Serum iron, iron binding capacity, and ferritin levels were within normal limits. She was started on intramuscular 5-formylTHF at 1.5 mg/day. She is now 1 year old and her hematological profile and neurological development are normal (50–75th percentile in height and weight).

METHODS AND RESULTS

The *PCFT* gene was analyzed in blood specimens from family members in a protocol approved by the Albert Einstein College of Medicine's Clinical Committee of Investigation (CCI No. 2006-279). Informed consent was obtained in accordance with the Declaration of

Helsinki. Peripheral blood was collected and genomic DNA extracted with the Gentra Systems purification kit (Minneapolis, MN). The primers and conditions for the polymerase chain reaction (PCR) amplification were reported previously [3, 4]. *PCFT* genomic fragments that contained all 5 exons and flanking introns were purified on agarose gels and sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) in the Albert Einstein Cancer Center Genomics Shared Resource as reported previously [3, 4]. A deletion of 2 bases, positions 204 and 205 based on GenBank Accession Number NM_080669, was identified in the open reading frame of the first exon. This resulted in a frameshift at amino acid residue 68 within the first extracellular loop between the first and second transmembrane domains, which is a highly GC-rich region of 114 nucleotides; 65.8% of the bases are G+C. The siblings are homozygous, whereas the mother and father are both heterozygous, for this mutation (Figure 1).

DISCUSSION

HFM is a rare autosomal recessive disorder described to date in 19 families [2-8]. Since the molecular basis for this disorder was established [3, 4], loss-of-function mutations in the *PCFT* gene have been identified in 9 families with HFM [3-7]. The current study represents the 10th mutation reported in a family with HFM and the second family of Turkish nationality in which there are progeny with this disease. In the first family, there was a loss-of-function point mutation at R113S [4], the same residue that was subsequently found to be mutated, but to a different amino acid (R113C), in an Israeli Arab family [6]. This report also describes the youngest infant with HFM in which folate levels were documented at the age of 1 month, demonstrating systemic and CSF folate deficiency. Presumably, the time of onset of folate deficiency will depend on the level of folate reserves at birth, acquired by the fetus in utero, since the infant is unable to absorb folates from its mother's milk.

Infants with HFM can present with a variety of syndromes [2]. Commonly, failure to thrive with anemia is the prominent presenting feature, as in this case. Because of the hypogammaglobulinemia that accompanies severe folate deficiency in infants, the occurrence of infections such as *Pneumocystis jirovecii* pneumonia can confuse the clinical picture with the combined immunodeficiency syndrome [4, 7, 9]. The siblings reported here did not have a history of infections. Finally, neurological deficits with intractable seizures can be a major problem. HFM is associated with 2 defects: (i) Impaired intestinal folate absorption resulting in systemic folate deficiency. Despite administration of high doses of oral folic acid to this patient, the folate blood level remained very low, but the folate deficiency was rapidly corrected with parenteral 5-formylTHF. Others have utilized much higher doses of oral 5-formylTHF with apparent satisfactory results [2]. (ii) The neurological consequences of HFM are due to impaired folate transport from blood across the choroid plexus into the CSF [10, 11]. To achieve normal folate levels in the CSF, 2 to 3 times higher than the normal folate blood level, requires achieving folate blood levels far in excess of normal. Patients with HFM should not be treated with parenteral folic acid because of the potential for blocking folate receptor-mediated endocytosis, which appears to play a role in the delivery of folates across the choroid plexus [3, 10-12]. The dose of 5-formylTHF utilized to treat the patients in this report was based upon the successful outcome of treatment reported by Poncz and colleagues [5, 13, 14]. However, this regimen does not result in achievement of CSF folate levels in the normal range, which is now considered the optimal therapeutic endpoint [2]. Yet, with treatment the siblings reported here have developed normally.

To date, 2 other subjects with HFM from 2 different families have been reported with mutations in this highly GC-rich region that resulted in the loss of the PCFT protein. In one case, there was a frameshift mutation at residue 65; this patient had intractable seizures and

mental retardation [4, 9]. In the second case, a stop codon at residue 66 occurred in a patient who, now in her late 20s, has developed perfectly normally and completed higher education [5, 13, 14]. The basis for the difference in outcomes in subjects that lack the PCFT protein is unclear.

SLC46A1 was originally described as a low-affinity, pH-insensitive heme-iron carrier protein and designated as HCP1 [15]. However, subsequent studies demonstrated that this carrier has a very high affinity for folates, is pH dependent, and folate-proton coupled [3, 16-19]. It is unclear as to whether PCFT contributes at all to the intestinal absorption of heme-iron or the delivery of heme-iron into cells. In both patients in the current report, there was no evidence of iron deficiency, as has been the case in other subjects with HFM [3-5].

In conclusion, we report on a Turkish family with 2 children with HFM who have a novel homozygous frameshift mutation (c.204_205delCC) in the *PCFT* gene. Identification of the molecular defect in HFM makes possible rapid diagnosis and early treatment with 5-formylTHF; prenatal diagnosis is also possible in families that carry a mutated gene. Increased recognition of HFM and the identification of additional families and *PCFT* mutations will provide opportunities to define possible genotype-phenotype correlations and clarify the basis for the phenotypic variability that is characteristic of this condition.

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FIGURE 1.

(Top) Pedigree of the family. (Bottom) Chromatogram of the region in the first exon demonstrating deletion of 2 bases (CC, positions 300 and 301 based on NM_080669). The siblings are homozygous for this mutation. The mutated and wild-type alleles are detected in both parents, who are heterozygous.