Published in final edited form as:

Gene. 2013 September 25; 527(2): . doi:10.1016/j.gene.2013.06.039.

A novel deletion mutation in the proton-coupled folate transporter (PCFT; SLC46A1) in a Nicaraguan child with hereditary folate malabsorption

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Abstract

Hereditary Folate Malabsorption (OMIM 229050) is a rare autosomal recessive disorder caused by loss-of-function mutations in the proton-coupled folate transporter gene (*PCFT/SLC46A1*) resulting in impaired folate transport across the intestine and into the central nervous system. We report a novel, homozygous, deletion mutation in a child of Nicaraguan descent in exon 2 (c. 558-588del, ss778190447) at amino acid position I188S resulting in a frame shift with a premature stop.

Keywords

PCFT, proton-coupled folate transporter; HCP1, heme carrier protein; HFM, hereditary folate malabsorption; Foliates; Intestinal folate transport; Folate deficiency

1. Introduction

Hereditary Folate Malabsorption (HFM) is a rare autosomal recessive disorder identified in subjects of diverse ethnicities, world-wide, that have either homozygous or compound heterozygous loss-of-function mutations of the proton-coupled folate transporter (*PCFT*) gene [1]. The pathophysiological basis for the disorder is impaired intestinal folate absorption resulting in severe systemic folate deficiency and impaired transport of folates across the choroid plexus resulting in a marked deficiency of cerebrospinal fluid (CSF) folate. The manifestations of the disease are anemia, pancytopenia, hypogammaglobulinemia frequently associated with *Pneumocystis jiroveci* pneumonia, and neurological dysfunction that can result in, among others, developmental delays, cognitive impairment, and epilepsy. Consequences of the disorder may be prevented or reversed with

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early diagnosis and treatment with pharmacological oral doses of 5-formyltetrahydrofolate or, preferably, parenteral administration of lower doses. In either case, the endpoint for treatment is the achievement of physiological levels of CSF folate and resolution of clinical signs and symptoms. The properties of PCFT and the characteristics of HFM and its treatment have been the subject of recent reviews [2-4].

2. Methods

Isolation of genomic DNA from a patient with HFM

This study was approved by the Albert Einstein College of Medicine Institutional Review Board. Informed consent was obtained from the patient's parent and a sample of blood was collected at the Texas Children's Hospital, Houston, TX. Genomic DNA was isolated at the Albert Einstein College of Medicine Cancer Center Molecular Cytogenetic Core.

PCR amplification and sequencing of PCFT exons

All five exons of the *PCFT* gene including intronic flanking regions were amplified using published primers [1]. PCR products were run on 1% agarose gels, purified using Illustra™ GFX™ PCR DNA and the Gel Band Purification Kit (GE healthcare, Buckinghamshire, UK), and sequenced at the Albert Einstein College of Medicine Cancer Center Genomics Shared Resource.

3. Results

Case presentation

This male infant, currently 13 months of age, was born to cousins of Nicaraguan descent who share a maternal grandmother. The patient, a term birth without complications during gestation, presented at 5 months of age with pneumonia, respiratory failure, and a history of diarrhea. He had no neurological signs. His weight was at the 25th percentile. An influenza B infection was diagnosed along with severe anemia with a hemoglobin of 5.9 g/dL (nl 10.5-14.0), a hematocrit of 16.8% (nl 33-39), and an MCV of 96.6 fL (nl 76-90). The white blood and platelet counts were normal. His serum immunoglobulins were normal: IgG 791 mg/dL (nl 218-907), IgA 47 mg/dL (nl 10-85), IgM 102 mg/dL (nl 31-116). His total plasma homocysteine was 35.5 μM (nl 3.3-8.3). A bone marrow aspirate revealed changes consistent with megaloblastic anemia. The patient received four units of packed RBCs and was started on oral folic acid and parenteral vitamin B12 supplementation. Subsequently, the serum folate was 0.4 ng/mL (nl 5.4-40) while the vitamin B12 level was normal. When the child did not show clinical improvement and the blood folate level did not normalize, the patient was given IV leucovorin at 10 mg daily resulting in improved hematologic parameters (hemoglobin 12.7 g/dL, hematocrit 35.9%, MCV 85.7 fL, serum homocysteine 8 μM, and serum folate 48 ng/mL). The patient's pneumonia resolved and he was discharged on 15 mg/kg/day leucovorin p.o. At 9 months of age, there was some evidence of developmental delay; the patient was sitting but not crawling and was noted to have bilateral lower extremity hypotonia. He was last examined at 11 months of age. At that time, he was able to crawl and pull to a stand and cruise. He had developed a pincer grasp and had a vocabulary of two words. His lower extremity hypotonia had resolved. At that time his hemoglobin was 12.2 g/dL, hematocrit 35.5% and MCV 86.8 fL. His serum homocysteine level was still elevated at 15 μM. This patient has a younger full sibling, currently 2 months old, who was found to be heterozygous for the PCFT deletion.

Identification of a novel HFM patient mutation

Molecular genetic analysis demonstrated a homozygous deletion mutation (c.558-588, ss778190447) in exon 2 (Table 1) causing a frameshift starting at I188 and a premature stop resulting in a truncated, nonsense protein.

4. Discussion

To date, there have been sixteen different *PCFT* mutations reported in subjects with HFM [4] which include five different deletion/insertion mutations, ten point mutations in the coding region and one mutation in the splice acceptor site at the intron 2/exon 3 interface. We now report a new deletion mutation of 31 base pairs in exon 2 leading to a nonsense truncated protein (Table 1). Sixty-eight percent of the 31 base pairs in this region represent GC. This is similar to the GC content in the first extracellular loop which represents the most frequent site of *PCFT* mutations with 4 deletion/insertion mutations found between residues G65 and N68. Of the mutations associated with HFM reported to date, most are private. However, the c.1082-1G>A p.Y362_G389 del mutation, resulting in deletion of exon 3, has been identified in subjects of Puerto Rican heritage with HFM and in a screen of newborns from various regions of the island suggesting the presence of a founder mutation in Central Puerto Rico [5].

Normalization of the blood folate level is easily achieved in subjects with HFM using pharmacological doses of oral folate, or much lower doses of parenteral folate. However, blood levels far above normal are required to achieve physiological levels of CSF folate in subjects with HFM which is an endpoint for treatment [4,6-8]. However, it is not always possible to obtain CSF folate levels, as has been the case to date with the patient in this report. In adults, CSF folate levels are 2 to 3 fold higher than the blood level. In infants and young children serum folate levels are higher than in adults [9,10] as are CSF levels, the latter ranging from 100 to 150 nM in the first year of life, to 70-90 nM by age 5, and to ~65 nM by age 19 [9-11]. This likely reflects the need for folate during brain development. The preferred folate for the treatment of HFM is the natural isomer of 5-formyltetrahydrofolate, levoleucovorin either in its oral or injectable forms. Although an oral preparation of 5-methyltetrahydrofolate, the physiological blood folate, is now available, the dose in the current formulation is too low for use in HFM and a parenteral form is not available.

With the family-specific mutation now known, molecular genetic testing is possible at minimal expense on at-risk siblings, as was done on a recently born sister who was found to be heterozygous for the mutation. In the absence of an *in vitro* analysis, infants can be provided with folate supplementation after birth until the molecular analysis can be performed.

References

- [1]. Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, Sandoval C, Zhao R, Akabas MH, Goldman ID. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell*. 2006; 127:917–928. [PubMed: 17129779]
- [2]. Zhao R, Matherly LH, Goldman ID. Membrane transporters and folate homeostasis: intestinal absorption and transport into systemic compartments and tissues. *Expert. Rev. Mol. Med*. 2009; 11:e4. [PubMed: 19173758]
- [3]. Zhao R, Diop-Bove N, Visentin M, Goldman ID. Mechanisms of Membrane Transport of Folates into Cells and Across Epithelia. *Annu. Rev. Nutr*. 2011; 31:177–201. [PubMed: 21568705]
- [4]. Diop-Bove, N.; Kronn, D.; Goldman, ID. Hereditary Folate Malabsorption. In: Pagon, RA.; Bird, TD.; Dolan, CR.; Stephens, K., editors. *GeneReviews* [Internet]. University of Washington; Seattle, Seattle (WA): 2011.

- [5]. Mahadeo K, Diop-Bove N, Ramirez SI, Cadilla CL, Rivera E, Martin M, Lerner NB, DiAntonio L, Duva S, Santiago-Borrero PJ, Goldman ID. Prevalence of a loss-of-function mutation in the proton-coupled folate transporter gene (PCFT-SLC46A1) causing hereditary folate malabsorption in Puerto Rico. *J. Pediatr.* 2011; 159:623–627. [PubMed: 21489556]
- [6]. Poncz M, Colman N, Herbert V, Schwartz E, Cohen AR. Therapy of congenital folate malabsorption. *J. Pediatr.* 1981; 98:76–79. [PubMed: 6969796]
- [7]. Corbeel L, Van den BG, Jaeken J, Van Tornout J, Eeckels R. Congenital folate malabsorption. *Eur. J. Pediatr.* 1985; 143:284–290. [PubMed: 3987728]
- [8]. Malatack JJ, Moran MM, Moughan B. Isolated congenital malabsorption of folic acid in a male infant: insights into treatment and mechanism of defect. *Pediatrics.* 1999; 104:1133–1137. [PubMed: 10545560]
- [9]. Opladen T, Ramaekers VT, Heimann G, Blau N. Analysis of 5-methyltetrahydrofolate in serum of healthy children. *Mol. Genet. Metab.* 2006; 87:61–65. [PubMed: 16310391]
- [10]. Ormazabal A, Garcia-Cazorla A, Perez-Duenas B, Gonzalez V, Fernandez-Alvarez E, Pineda M, Campistol J, Artuch R. Determination of 5-methyltetrahydrofolate in cerebrospinal fluid of paediatric patients: reference values for a paediatric population. *Clin. Chim. Acta.* 2006; 371:159–162. [PubMed: 16624264]
- [11]. Verbeek MM, Blom AM, Wevers RA, Lagerwerf AJ, van de GJ, Willemsen MA. Technical and biochemical factors affecting cerebrospinal fluid 5-MTHF, biopterin and neopterin concentrations. *Mol Genet. Metab.* 2008; 95:127–132. [PubMed: 18722797]

Highlights

- A novel mutation in the *pcft* gene causing hereditary folate malabsorption
- First report of hereditary folate malabsorption in a subject of Nicaraguan ancestry
- A *pcft* deletion causing a frameshift and premature stop in a subject with HFM

Table 1

The normal and mutated *PCFT* gene sequences are shown. 31 base pairs are missing in exon 2 in an HFM patient of Nicaraguan ancestry (c.558-588 del, ss778190447; p.I188Sfs)

Mutated	GAAGC-----CTCCT
Normal	GAAGCCAGCATCGGGGTGGCTGGGATGCTGGCAAGCCTCCT