Child Neurology: Hereditary Folate Malabsorption

Author(s):
Akshata Huddar, MD, DM; Shwetha Chiplunkar, MBBS, PhD; Madhu Nagappa, MD, DM; Periyasamy Govindaraj, PhD; Sanjib Sinha, MD, DM; Arun B. Taly, MD, DM; Bindu Parayil Sankaran, MD, DM, FRACP, PhD

Corresponding Author:
Bindu Parayil Sankaran
bindu.parayilsankaran@health.nsw.gov.au

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
Affiliation Information for All Authors: Akshata Huddar, Department of Neurology, NIMHANS, Bangalore; Shwetha Chiplunkar, Department of Clinical Neurosciences & Neuromuscular Lab-Neurobiology Research Centre NIMHANS, Bangalore, India; Madhu Nagappa, Department of Neurology & Neuromuscular Lab-Neurobiology Research Centre, NIMHANS, Bangalore, India; Periyasamy Govindaraj, Institute of Bioinformatics, International Tech Park, Bangalore, India & Manipal Academy of Higher Education, Manipal, India; Sanjib Sinha, Department of Neurology, NIMHANS, Bangalore, India; Arun B Taly, Department of Neurology & Neuromuscular Lab-Neurobiology Research Centre, NIMHANS, Bangalore, India; Bindu Parayil Sankaran, The Children Hospital at Westmead Clinical School, Sydney Medical School, The Faculty of Medicine and Health, The University of Sydney Australia.

Contributions:
Akshata Huddar: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Shwetha Chiplunkar: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Madhu Nagappa: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Periyasamy Govindaraj: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Sanjib Sinha: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Arun B. Taly: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.
Bindu Parayil Sankaran: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data; Additional contributions: Acquired funding for the study.

Number of characters in title: 48
Abstract Word count: 1498
References: 10
Figures: 1
Tables: 0


Study Funding: This study was supported by a grant from Indian council of Medical Research to Bindu Parayil Sankaran (Grant No. 54/9/2012-HUM-BMS)

Disclosures: Dr. Akshata Huddar reports no disclosures. Dr. Shwetha Chiplunkar reports no disclosures. Dr. Madhu Nagappa reports no disclosures. Dr. Periyasamy Govindaraj reports no disclosures. Dr. Sanjib Sinha reports no disclosures. Dr. Arun B Taly reports no disclosures. Dr. Bindu Parayil Sankaran reports receipt of the grant from Indian Council of Medical Research (Grant No. 54/9/2012-HUM-BMS)
Introduction

Hereditary Folate Malabsorption (HFM, Congenital Folate Malabsorption; OMIM#229050) is a rare potentially treatable autosomal recessive disorder with multisystem involvement. It is caused by homozygous or compound heterozygous mutations in the \textit{SLC46A1} resulting in loss of function of proton-coupled folate transporter (PCFT), required for intestinal absorption and transport of folate across choroid plexus. This leads to the deficiency of folate in serum and CSF, causing hematological, immunological gastrointestinal and neurological manifestations. Neuroimaging shows intracranial calcification. Diagnosis is confirmed by impaired absorption of an oral folate load, low cerebrospinal fluid (CSF) folate concentration (even after correction of the serum folate concentration); and/or the identification of pathogenic variants in \textit{SLC46A1} on molecular genetic testing. We describe the clinical, imaging, biochemical and genetic findings of a patient with HFM.

Clinical case

A 2-year-8-month-old girl, the second child of consanguineous parents, from the south Indian state of Karnataka, was referred to our centre for evaluation. She was born at term by normal delivery following an uneventful antenatal period with a birth weight of 3.5 kg. The perinatal period was uneventful. At 4 months of age, she presented to a peripheral hospital with fever, diarrhea, poor feeding, lethargy, and seizure. Evaluation showed hemoglobin of 3.4gms/dl, leucocyte count-6.9 x 10^9/L, platelet-16 x10^9/L suggesting pancytopenia. Bone marrow examination showed a megaloblastic picture. She received blood transfusion, antibiotics, vitamin B12 and folate supplements with a diagnosis of sepsis with pancytopenia. Hematological parameters improved, but she continued to have repeated respiratory and
gastrointestinal infections requiring ICU admissions. Seizures became refractory to multiple antiseizure drugs at optimal dosages (phenytoin, levetiracetam, carbamazepine, topiramate and clobazam). By 11 months she had generalized hyperpigmentation, tremors, and oral ulcers. Developmental delay was noted at 18 months of age with episodes of further regression following infections. The diagnosis of infantile tremor syndrome- a nutritional deficiency syndrome typically associated with vitamin B12 deficiency, characterized by pallor, developmental delay/regression, tremors, skin pigmentation, and brown scanty scalp hair was considered this stage. She received B12 supplementation. At 32 months, she was able to sit and stand with support, transfer objects, and say 2-3 meaningful words. There was a history of similar illness in elder sister with seizures, fever, vomiting, loose stools, and pancytopenia who died at 3 months of age.

Examination revealed microcephaly (HC:45 cm, <3rd centile), weight (10.5 kg, <3rd centile). She was alert, fixed, and followed light and sound. She had normal extraocular, facial, palatal and tongue movements. She had generalized hypotonia, normal limb movements and brisk deep tendon reflexes. She had choreoathetosis involving distal extremities. There was no organomegaly. Fundus showed normal retina and optic discs.

Investigations revealed anemia (Hemoglobin-10.4 gms/dl, ref=13-17 gms/dl) with MCV on higher end of normal (96.6 fl, ref.=83-101 fl), normal leukocyte and platelet count. Renal and hepatic function tests were normal. Serum folate (1.52ng/mL, ref=3.1-19.9 ng/mL) and Vitamin B12 (54 pg/mL, ref=180-914 pg/ml) were low, while homocysteine was high (27.8 umol/L, ref.=<8 umol/L). Metabolic investigations revealed normal lactate, mildly elevated ammonia, normal plasma amino acids, acylcarnitine profile and urinary organic acid profile.
Cranial CT scan done at 12 months of age (Fig. 1A) was normal. MRI Brain done at 13 months showed mild cerebral atrophy and hypointensity involving the basal ganglia and left parieto-occipital region on T1 weighted images (Fig. 1B) with the corresponding hyperintensity on T2 weighted sequences (Fig. 1C). Repeat neuroimaging at 32 months of age showed progressive changes, including calcification in bilateral basal ganglia and thalamus on CT scan (Fig. 1D). MRI Brain also done at 32 months showed diffuse cerebral and cerebellar atrophy, T2/FLAIR hyperintensities of periventricular and deep white matter and mixed intensity lesions in bilateral basal ganglia (Fig. 1E). EEG showed focal epileptiform activity across the left temporal region.

Constellation of megaloblastic anemia, oral ulcers, recurrent infections, and neurological features with positive family history, biochemical and imaging findings led to the suspicion of hereditary folate deficiency. CSF folate estimation was not done. Clinical exome sequencing showed a pathogenic homozygous single base pair duplication in exon 2 of the \textit{SLC46A1} (chr17:26732094_26732095dupC) c.620dupG variation resulting in a frameshift and premature truncation of the protein 25 amino acids downstream to codon 208 (p. Tyr208LeufsTer25; ENST00000440501) confirming the diagnosis of HFM. Both parents were heterozygous carriers.

The patient received oral folic acid from four months of age prior to presentation to our facility. Hematological parameters and hyperpigmentation improved, but neurological features worsened, and she continued to have recurrent infections. After the genetic diagnosis, she was treated with intramuscular injection of folic acid (120mg/day), leading to the cessation of seizures. After one-week parenteral folic acid was replaced
by oral folinic acid (150mg/day, 15mg/kg/day) due to financial constraints and
continued along with anticonvulsants. At 2 months follow-up, she was seizure-free, and
development had stabilized. However, she was non-compliant to treatment and
presented multiple times with recurrent seizures, anemia and infections which
responded to parenteral folinic acid. On follow up at 7 years, she was able to stand with
support, speak 5-10 words and, feed herself. Though there was a reduction in seizure
frequency, she continued to have daily seizures requiring multiple anticonvulsants.

Discussion:
Hereditary folate malabsorption (HFM) was first described in 1961 in an infant
with relapsing megaloblastic anemia. Patients with HFM are normal at birth as
they have in utero stores of folate and develop symptoms within 2-6 months of
age due to impaired absorption and transport of folate. Folates mediate many
important biological processes. In HFM, there is both systemic and cerebral
deficiency of folate and 5-MTHF (5-methylenetetrahydrofolate, the active form of
folate) leading to multisystem involvement; (1) hematological: megaloblastic
anemia, pancytopenia (2) immunological: recurrent infections, stomatitis, failure
to thrive, reduced immunoglobulins (3) gastrointestinal: diarrhea, stomatitis,
failure to thrive and (4) neurological: epilepsy, movement disorder, ataxia,
peripheral neuropathy, acquired microcephaly with progressive encephalopathy.
The neurological manifestations could be part of the initial presentation or can
appear later in the disease course.

Our patient presented with multisystem involvement. Her elder sibling also had
multisystem involvement with a severe phenotype and died undiagnosed without
adequate healthcare at the age of three months. This illustrates the fatal outcome in the absence of appropriate treatment. Untreated, the intracranial changes are progressive as depicted in our patient. Intracranial calcification involving the cortex and basal ganglia has been reported in HFM and can be prevented by folinic acid therapy\(^3,7\). Therefore children with neurological manifestations and intracranial calcification should be evaluated for disorders of folate metabolism including \(SLC46A1\) mutations\(^8\). Other reported imaging abnormalities include cerebral and cerebellar atrophy\(^3,7\). The intracranial calcifications are not specific to HFM and can also be seen in other genetic diseases including Aicardi-Goutieres Syndrome, RNASET2, Collagen IV related disorders, Cockayne syndrome and other DNA repair disorders among others\(^8\).

The biochemical markers of HFM include low levels of serum folate, immunoglobulins and CSF 5-MTHF, high level of serum homocysteine and failure to raise serum folate levels after oral folate load\(^1\). The diagnosis is confirmed by the presence of biallelic pathogenic variants in \(SLC46A1\), including nonsense, missense, frameshifts and splice site variants.\(^2\) There is no clear phenotype-genotype correlations\(^1\). The Tyr208 residue is located in the highly conserved region of extracellular loop 3 of the PCFT, leading to an unstable protein or protein with reduced function.

The differential diagnoses of HFM include acquired folate and B12 deficiency due to nutritional and pharmacological causes, immunodeficiency diseases, inborn errors of metabolism, mitochondrial disorders, and malignancy like erythroleukemia\(^1\). Folate metabolism disorders present with either neurological manifestations alone or a combination of both hematological and neurological features\(^6\). Hematological
manifestations are seen in HFM (SLC46A1), 5,10-methylenetetrahydrofolate dehydrogenase deficiency (MTHFD1) and dihydrofolate reductase deficiency (DHFR)\textsuperscript{6}.

Cerebral folate deficiency (CFD) is characterized by low CSF 5-MTHF levels but normal serum folate levels and is caused by loss of function mutations in folate receptor - α (FRα)\textsuperscript{9}. This is an autosomal recessive disorder and differs from HFM. While HFM presents within a few months of birth, patients with CFD present later with developmental delay/regression, ataxia, motor disorders, hypotonia, and seizures\textsuperscript{2,9}.

HFM is one of the very few treatable inborn errors of metabolism\textsuperscript{6}. Oral or parenteral folinic acid/levofolinate leads to improved systemic features and serum folate levels over 1-2 months. However, CSF 5-MTHF levels remain low for a longer period of time even with higher doses, and there is a partial response concerning neurological symptoms\textsuperscript{7}. Intramuscular folinic/levofolinic acid in a dose of 1-2mg/kg/day has led to normalization of CSF levels by 9-12 months in few cases\textsuperscript{7,10}. Preventive treatment with intramuscular folinic acid has been reported in one patient with HFM whose elder sibling had severe illness and treatment commenced on day 2 of life. The patient remained symptom-free till 6 months of age, at last follow up\textsuperscript{7}. Oral folate should not be used as it competes with 5-MTHF for cerebral folate receptor and impairs transport across choroid plexus resulting in worsening neurologic symptoms as in our patient\textsuperscript{10}. Higher doses of folinic acid, parenteral in preference to oral folinic acid, early intervention and compliance are required to achieve good neurological outcome\textsuperscript{7}. Genetic counselling with prenatal/early screening of other
siblings is also warranted¹.

References


**Title and legends to the figures:**

**Figure 1:** Serial imaging of Brain.

(A) CT scan at 1 year of age shows no evidence of basal ganglia calcification.

(B-C) Brain MRI one month later. T1W axial view (B) shows cerebral atrophy and hypointensity involving left temporo-occipital region. T2W axial view (C) shows cerebral atrophy, basal ganglia signal changes and left sided parieto occipital hyperintensity.

(D-F): Neuroimaging at 2 years & 8 months. (D) CT Brain demonstrates bilateral basal ganglia and thalamic and parietooccipital calcification. (E) MRI Brain T1W axial sections show cerebral and cerebellar atrophy and patches of hyperintensity. (F) MRI Brain T2Weighted axial image shows mixed intensity signal changes in basal ganglia, thalamus and white matter.