Clinical Case Report

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Hereditary folate malabsorption with a novel mutation on *SLC46A1*

A case report

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Abstract

Rationale: Hereditary folate malabsorption (HFM) is characterized by folate deficiency with impaired intestinal folate absorption and impaired folate transport into the central nervous system. Its manifestations mainly include macrocytic anemia, recurrent infections, and neurological deficits. The neurological manifestations include progressive psychomotor retardation, behavioral disorders, and early-onset seizures.

Patient concerns: From early infancy, a Chinese boy had experienced macrocytic anemia, leukopenia, thrombocytopenia, recurrent pneumonia, diarrhea, and mouth ulcers. He also presented with progressive neurological symptoms.

Diagnosis: A novel mutation in the *SLC46A1* gene was identified, and HFM was diagnosed at 18 months of age.

Interventions: After the HFM diagnosis, the boy was treated with folinic acid.

Lessons: Folinic acid supplementation is effective and may offer life-changing therapy for patients with HFM.

Abbreviations: CNS = central nervous system, CSF = cerebrospinal fluid, HFM = hereditary folate malabsorption, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, PCFT = proton-coupled folate transporter, RBCs = red blood cells, WBCs = white blood cells.

Keywords: hereditary folate malabsorption, macrocytic anemia, proton-coupled folate transporter, seizure, *SLC46A1* gene

1. Introduction

Folates are essential nutrients for 1-carbon biosynthetic and epigenetic processes.[1] Hereditary folate malabsorption (HFM) is a rare autosomal recessive disorder characterized by proton-coupled folate transporter (PCFT) deficiencies that cause impaired intestinal folate absorption and impaired folate transport into the central nervous system (CNS).[2] It involves mutations of *SLC46A1*, which encodes a transmembrane PCFT that is essential for transporting folates across the choroid plexus.[3] The resulting PCFT defects reduce folate levels in serum and cerebrospinal fluid (CSF).

HFM has various manifestations that mainly include poor feeding, failure to thrive, macrocytic anemia, recurrent infections, and neurological deficits. It is often accompanied by leukopenia and thrombocytopenia.[2] Neurological deficits occur in approximately one-third to half of patients with HFM and include peripheral neuropathies, motor impairment, ataxia, behavioral disorders, and cognitive deficits.[2,4] Measurements of folate and 5-methyltetrahydrofolate in CSF are essential for diagnosing HFM, and folinic acid supplementation is therapeutically critical.

2. Case report

2.1. History, physical examination, and laboratory examinations

Our patient was a Chinese boy who was born at full term via cesarean section as the first-born child of non-consanguineous parents. During the pregnancy, his mother had taken folic acid for 1 month in the second trimester (0.4 mg, q.d.). She experienced cholestasis and recurrent abdominal pain without vaginal bleeding or asphyxia during the last days of pregnancy.

The boy was admitted to our hospital for pneumonia at 1 month old, and his complete blood count revealed anemia, leukopenia, and thrombocytopenia [white blood cells (WBCs): $3.93 \times 10^9\,L^{-1}$; platelets: $59 \times 10^9\,L^{-1}$; red blood cells (RBCs): $1.94 \times 10^{12}\,L^{-1}$; hemoglobin: 62 g/L; mean corpuscular volume (MCV): 98 fL; mean corpuscular hemoglobin (MCH): 34 pg]. Bone marrow aspiration revealed macrocytic anemia. Biochemical examinations revealed normal liver and renal functions and normal serum electrolyte levels, glucose levels, and coagulation function. His plasma folate and cobalamin levels were <1.45 nM and 77.85 pM, respectively, whereas healthy levels at 1 month old are >10.4 nM and 141 to 698 pM, respectively. He was therefore diagnosed as having macrocytic anemia. He was prescribed a 3-month course of folate tablets (5 mg, t.i.d.) and vitamin B_{12} (5 μg, t.i.d.) and showed a rapid reversal of anemia

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Written informed consent was obtained from the patient's parents.

The authors declare that they have no conflicts of interest in this work.

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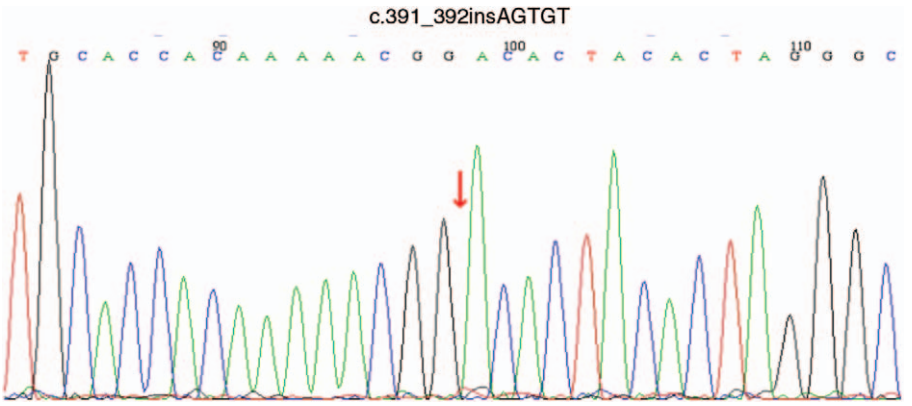

Figure 1. *SLC46A1* mutation (arrow) in the boy.

(WBCs: $8.84 \times 10^9 L^{-1}$; platelets: $136 \times 10^9 L^{-1}$; RBCs: $2.89 \times 10^{12} L^{-1}$; hemoglobin: 95 g/L; MCV: 92 fL; MCH: 32.7 pg), but his parents did not continue giving him the tablets regularly. During his first year of life, he developed recurrent pneumonia, diarrhea, and mouth ulcers.

At 10 months old, he began experiencing convulsions and exhibiting delayed motor and cognitive development. Electroencephalography revealed suspicious spikes in the right temporal lobe (T4, T6) during sleep and 4 to 5-Hz background activity during wakefulness. Magnetic resonance imaging revealed cerebellar atrophy and generalized broadening of the cerebral sulcus. The boy's seizures were treated with oxcarbazepine at doses that were gradually increased to 30 mg/kg/day, but the antiepileptic effect was suboptimal. We therefore added sodium valproate at a maximal dosage of 38 mg/kg/day, but he still experienced seizures to the point of status epilepticus.

At 18 months old, he underwent electroencephalography again due to recurrent seizures, and this revealed spikes and slow waves in the right temporal lobe (T4, T6) during sleep and 3 to 5-Hz background activity during wakefulness. His complete blood count, liver and renal functions, and serum electrolyte levels were normal. His plasma folate and cobalamin levels were >45.4 nM and 700.4 pM, respectively, whereas healthy levels at 18 months old are >8.83 nM and 141 to 698 pM, respectively. His CSF 5-methyltetrahydrofolate level was 14.88 nM, which is much lower than the healthy level of 70 to 210 nM. Moreover, neurological deficits were clearly observed. His head circumference was 45.5 cm (3–15th age-adjusted percentile), his bodyweight was 11 kg (50th age-adjusted percentile), and his height was 84 cm (85th age-adjusted percentile). His eyes were dull, and his hair was sparse. He could sit by himself, but he could not stand or walk unassisted due to gait instability. He could not vocalize anything except "pa-pa" and "ma-ma." We strongly suspected HFM and therefore conducted *SLC46A1* mutations analysis.

2.2. SLC46A1 analyses

Within the *SLC46A1* gene, we detected a c.391_392insAGTGT (p.S131_V132delinsX) mutation (Fig. 1) (see document 1, Supplemental Content, the original data on the mutation; http://links.lww.com/MD/B982) with next-generation sequencing. This mutation replaces a normal codon with a stop codon, which causes premature termination of PCFT protein synthesis and consequent PCFT deficiencies. It is so uncommon in the general population that it has not been reported in the Human Gene Mutation Database. Family verification analyses confirmed that both parents carry this novel mutation (Figs. 2 and 3) (see document 2–3, Supplemental Content, the original data on the mutation; http://links.lww.com/MD/B982), which supports the HFM diagnosis.

SLC46A1, which is localized on chromosome 17q11.2 and consists of 5 exons,[5] is the only known HFM-associated gene. Most HFM-associated mutations are distributed between p. 65

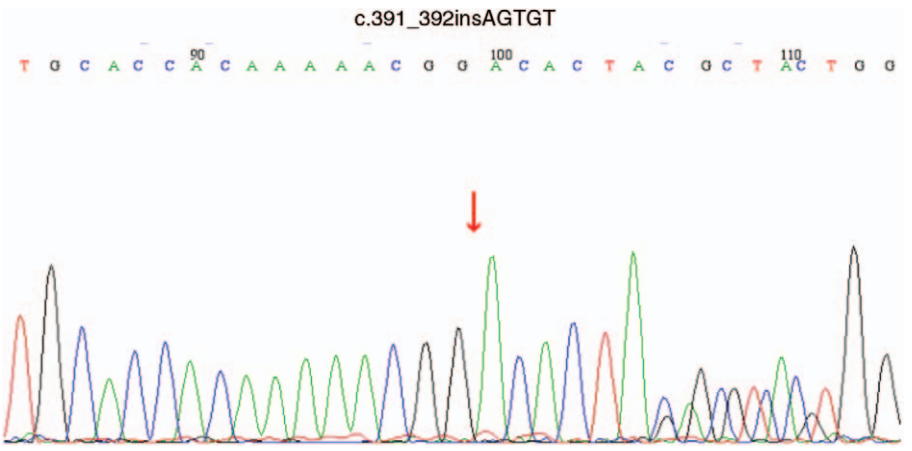

Figure 2. *SLC46A1* mutation (arrow) in the father.

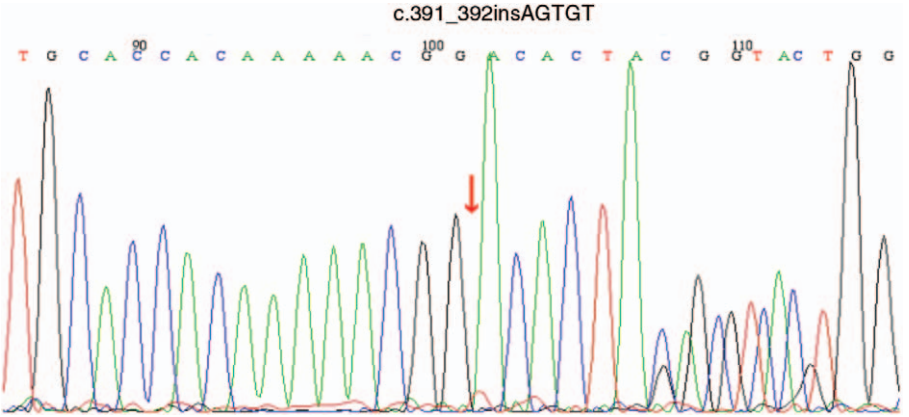

Figure 3. *SLC46A1* mutation (arrow) in the mother.

and p. 68 (c.194-c.204) and are mainly insertion- or deletion-related frame shifts or stop codon generations.[6] This gene encodes a transmembrane PCFT that facilitates the movement of folate and antifolate substrates across cell membranes and functions optimally at acidic pH values.[4] PCFTs are expressed in apical brush-border membranes in the jejunum, duodenum, and choroid plexus.[3,5]*SLC46A1* mutations cause malabsorption of oral folate loads and low folate levels in serum and CSF.

2.3. Treatments and follow-up

After receiving the diagnosis of HFM at 18 months old, the boy was prescribed folinic acid at an initial oral dosage of 30 mg/day. To our surprise, his seizures were eliminated within a month despite having been refractory to oxcarbazepine and sodium valproate. However, past reviews revealed that only high folinic acid doses can normalize CSF folate levels and alleviate psychomotor retardation,[2,4] so we increased the dosage to 60 mg/day after 1 month despite his seizure-free status. After he had received folinic acid for 6 months, we rechecked his CSF 5-methyltetrahydrofolate levels to guide adjustments to his folinic acid dosage, and the observed value of 12.28 nM was still far below normal levels despite his plasma folate level being far above normal levels. He had also made little progress in psychomotor development, still could not stand or walk unassisted, and could not vocalize anything except "pa-pa" and "ma-ma." We therefore plan to increase his folinic acid dosage to at least 90 mg/day to improve his psychomotor development. We will also perform long-term follow-up evaluations and taper off the antiepileptic drugs.

3. Discussion

The known clinical syndrome of HFM highlights the gastrointestinal, hematologic, immunologic, and neurological consequences of folate deficiency from the newborn period onward.[7] Impaired oral folate absorption and low CSF folate levels underlie HFM,[2,4,6] so folate and 5-methylterahydrofolate levels in serum and CSF should be examined.

The goal of treatment is to prevent hematologic, immunologic, and neurologic deficits and to optimize the cognitive development of children with HFM. However, HFM is a rare disorder, and there have been no controlled studies to establish the optimal treatment. Many studies have reported that the safest approach is parenteral folate administration in order to normalize CSF folate levels as much as possible.[8] Folic acid should not be used to treat HFM because it tightly binds and inactivates folate receptors in the choroid plexus[4] that are necessary for transporting 5-methyltetrahydrofolate from blood to CSF.[9] The preferred drug is therefore 5-methyltetrahydrofolate itself, which is the major physiological form of folate found in blood and tissues.[10,11] Parenteral delivery of 5-methyltetrahydrofolate is unavailable, but racemic 5-formyltetrahydrofolate is readily available and often parenterally administered to treat HFM.

It is easy to completely reverse the anemia, immune dysfunction, and gastrointestinal signs that folate deficiency causes,[4,8,12] but correcting the neurological consequences is more difficult. The major challenge is achieving CSF folate levels that can alleviate psychomotor retardation, as this requires careful monitoring of CSF levels until a satisfactory concentration is reached. CSF 5-methyltetrahydrofolate levels of 18 to 46 nM may be sufficient to eradicate CNS-related symptoms.[13] The oral dose required to achieve adequate blood folate levels is much higher than the parenteral dose because it must overcome the loss of PCFT-mediated intestinal folate absorption.[2] The reported oral folic acid dose associated with satisfactory outcomes is approximately 150 to 200 mg/day,[2] whereas the necessary intramuscular injection dose of 5-formyltetrahydrofolate is approximately 0.5 to 1.0 mg/day. HFM-associated neurological deficits can be somewhat improved when adequate CSF 5-methyltetrahydrofolate levels are finally achieved, but this requires much higher folate doses.[13]

From his early infancy, the boy described here exhibited macrocytic anemia, recurrent pneumonia, diarrhea, and mouth ulcers. More worrisomely, progressive neurological symptoms were observed soon afterwards. Low plasma folate levels, very low CSF 5-methyltetrahydrofolate levels, and a *SLC46A1* gene mutation supported the diagnosis of HFM.

His anemia, immune dysfunction, and gastrointestinal signs were easily cured with 15 mg/day oral folate doses. His epilepsy was refractory even to high doses of oxcarbazepine and sodium valproate, but his seizures stopped within a month of increasing the folate dose to 30 mg/day. It is frustrating that his CSF 5-methyltetrahydrofolate levels remained very low despite his plasma folate levels being far above normal levels, and he made little progress in psychomotor development even when his folate dose was increased to 60 mg/day. The major cause may be that this is still a low folate dosage. We will gradually increase the dosage to normalize his CSF 5-methyltetrahydrofolate levels.

4. Conclusion

HFM is a rare autosomal recessive disorder characterized by folate deficiency, impaired intestinal folate absorption, and impaired folate transport into the CNS. Folinic acid supplementation can offer life-changing therapy in patients with HFM and should be initiated as early as possible. It is easy to reverse the systemic consequences of folate deficiency, but it is more difficult to correct the neurologic consequences. There may be some amelioration of HFM-associated neurological deficits when adequate CSF 5-methyltetrahydrofolate levels are achieved, so CSF investigations are critical. The intramuscular and oral folate doses necessary to ameliorate HFM-associated neurological disorders remain undetermined.

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