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Short Communication

A case of 5,10-methenyltetrahydrofolate synthetase deficiency due to biallelic null mutations with novel findings of elevated neopterin and macrocytic anemia

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ABSTRACT

We describe a case of 5,10-methenyltetrahydrofolate synthetase (MTHFS) deficiency characterized by microcephaly, global developmental delay, epilepsy, and cerebral hypomyelination. Whole exome sequencing (WES) demonstrated homozygosity for the R74X mutation in the *MTHFS* gene. The patient had the unexpected finding of elevated cerebrospinal fluid (CSF) neopterin. The novel finding of macrocytic anemia in this patient may provide a clue to the diagnosis of this rare neurometabolic disorder.

1. Introduction

Folate is involved in several aspects of cell division and homeostasis. Specifically, folate and its reduced metabolites act as coenzymes in nucleic acid synthesis, amino acid metabolism, methionine regeneration, neurotransmitter synthesis, and methylation [1]. MTHFS is one of the enzymes in the folate metabolism pathway and is encoded by the *MTHFS* gene located on chromosome 15q25.1. MTHFS is responsible for the conversion of 5-formyltetrahydrofolate (folinic acid) into 5,10-methenyltetrahydrofolate, metabolites of which are important for myelin stabilization and DNA synthesis [2,3].

Rodan et al. [4] described the first two cases of MTHFS deficiency due to heterozygous mutations in the *MTHFS* gene. This neurometabolic disorder is characterized by microcephaly, epilepsy, and cerebral hypomyelination. Our case of MTHFS deficiency is the third ever reported and has novel findings of biallelic null mutations, macrocytic anemia, and elevated CSF neopterin.

2. Case

This full-term female initially presented with microcephaly and diffuse hypertonia at 9 months old. She had developmental delay and spasticity. Outpatient brain magnetic resonance imaging (MRI) without contrast done at 10 months old demonstrated abnormal white matter with under-myelination of the internal capsules, relative under-myelination of the remainder of the subcortical white matter, and a thin corpus callosum (Fig. 1).

At 11 months old, she had a seizure during a hospitalization for respiratory failure secondary to viral bronchiolitis. Routine electroencephalogram (EEG) revealed periodic lateralized epileptiform discharges. Head computed tomography (CT) showed mild diffuse volume loss and faint hyperattenuation of the basal ganglia. CSF studies were negative for infections with a CSF cell count of 1 white blood cell, glucose of 82 mg/dL, and protein of 12 mg/dL. She was noted to have a macrocytic anemia with hemoglobin of 6.9 g/dL and MCV of 98.6 fL that required packed red blood cell transfusion (Table 1). A repeat CBC was done a little more than 2 weeks afterwards which showed a hemoglobin of 12.3 g/dL and MCV of 99.2 fL. Laboratory studies performed for anemia work-up during this hospitalization showed a normal serum total folate, normal serum vitamin B12, normal serum methylmalonic acid, and normal hemoglobin electrophoresis. She had unremarkable complete metabolic panel, serum lactate, free and total carnitine, creatine kinase, serum amino acids, very long chain fatty acids, urine organic acids, and CSF amino acids, including methionine (2.4 μmol/L, normal 1.3–14.7 μmol). CSF neurotransmitters were

Abbreviations: MTHFS, 5,10-methenyltetrahydrofolate synthetase; 5-MTHF, 5-methyl tetrahydrofolate; SAM, S-adenosylmethionine; 5-formyl THF, 5-formyl tetrahydrofolate; SHMT, serine hydroxymethyltransferase; AICARFT, phosphoribosylaminoimidazolecarboxamide formyltransferase; BH4, tetrahydrobiopterin

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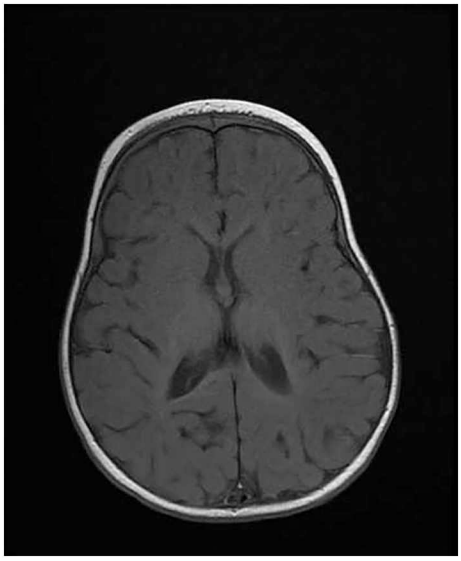

Fig. 1. Axial T1-weighted MRI of the brain at 10 months old showing under-myelination of the internal capsules, relative under-myelination of the remainder of the subcortical white matter, and a thin corpus callosum.

Table 1
Pertinent serum and CSF studies.

Results	Patient data
Remarkable laboratory studies	Serum - macrocytic anemia: hgb 6.9 g/dL (normal 10.4–15.6 g/dL) MCV 98.6 fL (normal 78–102 fL) CSF - elevated neopterin: 138 nmol/L (normal 7–65 nmol/L) - decreased 5-MTHF: 33 nmol/L (normal 43–187 nmol/L) - normal BH4: 47 nmol/L (normal 18–58 nmol/L)

notable for elevated neopterin, normal tetrahydrobiopterin (BH4), and decreased 5-methyltetrahydrofolate (5-MTHF) (33 nmol/L, normal 40–187 nmol/L) (Table 1). The patient was started on levetiracetam for seizures and on baclofen for spasticity. Over the following years, she did not develop recurrent seizures and was eventually weaned off of levetiracetam.

At 4 years old, the physical exam was remarkable for microcephaly, low weight (13.7 kg, fifth percentile), short stature (99.1 cm, tenth percentile), strabismus, and high arched palate. Her head circumference was persistently below the third percentile up until the last measurement done at 29 months of age. Given that her head circumference was 33 cm at 1-month-old, this placed her below the third percentile for her age. As a result, her microcephaly was likely congenital. She also was nonverbal and nonambulatory with significant spasticity.

Chromosomal microarray showed 2 copy number variations of unknown significance and a long region of homozygosity on chromosome 15. DNA methylation study was negative. WES demonstrated homozygosity for a predicted pathogenic null mutation in exon 2 of the MTFHS gene (c.220C > T, p.R74X). Parents were nonconsanguineous but from the same town in Haiti; they were both heterozygous for the same mutation.

3. Discussion

MTHFS deficiency presents with cerebral hypomyelination in association with neurodevelopmental delay. The process by which cerebral folate deficiency disrupts myelination during development involves several mechanisms [5]. 5-MTHF deficiency leads to a downstream deficiency in S-adenosylmethionine (SAM), an important methyl donor. When there is a deficiency of SAM production through the folate metabolism pathway, SAM can be generated through an alternate pathway involving choline oxidation. Choline is necessary for production of phosphatidylcholine and sphingomyelin which are both important for the appropriate composition of myelin. Therefore, a defect in MTHFS activity will shunt choline away from myelin production. Furthermore, methylation of myelin basic protein by SAM is necessary to maintain stability of CNS myelin [5], so a defect in MTHFS activity will also lead to decreased stability of myelin. Lastly, a recent study suggests that folate promotes oligodendrocyte maturation and survival in vitro and in vivo [6].

As mentioned above, defects in folate metabolism lead to thymidylate and purine deficiency, which hinder normal division of red blood cell precursors, leading to macrocytic anemia [1]. Furthermore, it has been shown in silico that decreased MTHFS activity will lead to increased 5-formyl tetrahydrofolate (5-formyl THF), an intracellular storage form of folate, which will go on to inhibit serine hydroxymethyltransferase (SHMT) and phosphoribosylaminoimidazolecarboxamide formyltransferase (AICARFT). These are both enzymes in the folic acid metabolism pathway. With decreased SHMT activity, less of the intermediate needed for thymidylate synthesis will be made. Similarly, with decreased AICARFT activity, less de novo purine synthesis will occur. Therefore, both of these effects will greatly impair DNA synthesis, affecting processes such as hematopoiesis [7]. Laboratory levels of serum total folate measure several folate metabolites including 5-MTHF, which is the largest contributor to these measurements, unmetabolized folic acid, and other folic acid metabolites [8]. An NHANES study has shown that ingestion of foods fortified with folate or folate supplements leads to higher concentrations of serum folate metabolites [8]. Our patient had a normal serum total folate level, yet a macrocytic anemia, likely related to the aforementioned reason. In addition, the low CSF 5-MTHF may be a result of her MTHFS deficiency and 5-formyl THF accumulation leading to inhibition of SHMT. Both MTHFS and SHMT are involved in producing 5-MTHF. Although folate supplementation via the diet may have contributed to a normal serum folate level, it is possible that this value is actually representative of significantly elevated 5-formyl THF in our patient given her deficiency.

We describe elevated CSF neopterin in this patient in conjunction with low 5-MTHF and normal BH4 levels. One would expect low CSF neopterin in the setting of deficiency in cerebral folate metabolites due to low 5-MTHF and subsequent low BH4 production. However, there are other pathways that lead to production of BH4 that are independent of 5-MTHF [9]. Thus, BH4 production and downstream neopterin production can be spared even in the presence of low 5-MTHF. Additionally, elevated neopterin levels have been described in the setting of febrile convulsions [10], and our patient's levels were measured in that context which could explain this paradoxical finding.

In conclusion, we report a third case of MTHFS deficiency and it's clinical and radiological characteristics. This case report expands on that published by Rodan et al. [4] with the added novel finding of macrocytic anemia. The elevated neopterin in our patient was in some ways a red herring that should not prevent consideration of this diagnosis for other patients with a similar phenotype.

Declaration of Competing Interest

We, J. Romero, I. Abdelmoumen, D. Hasbani, D. Khurana, and M. Schneider, declare that we have no conflicts of interest.

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