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Case Report

A female case of 5,10-methenyltetrahydrofolate synthetase deficiency with novel neuro-imaging abnormalities

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Abstract

Background: Folate metabolism disorders can affect various organ systems, including the nervous system. 5,10-methenyltetrahydrofolate synthetase deficiency is a rare cerebral folate deficiency in which MTHFS activity is disrupted with low-normal cerebrospinal fluid (CSF) 5,10-methenyltetrahydrofolate levels, while peripheral folate levels are normal.

Case report: We present here a female patient with developmental delay, microcephaly, hypotonia, nystagmus, and seizure in which a distinct brain MRI and CT showed restricted diffusion in the bilateral parietal and occipital lobes, and calcifications of the bilateral putamen, globus pallidus, and caudate nucleus, and the bilateral parietal and occipital lobes. Laboratory tests revealed macrocytic anemia, increased homocysteine, low-normal CSF 5,10-methenyltetrahydrofolate, and low CSF folate, but normal serum vitamin B12 and folate levels. A whole exome sequencing analysis verified the diagnosis of 5,10-methenyltetrahydrofolate synthetase deficiency.

Conclusions: We have added novel knowledge which is nystagmus and hypotonia in the clinical findings, the involvement and restriction of bilateral putamen, globus pallidus, parietal and occipital lobes, and calcification of the bilateral putamen, globus pallidus, caudate nucleus, and parietal and occipital lobes in neuroimaging images and also low CSF folate in the metabolic investigation with the patient in 5,10-methenyltetrahydrofolate synthetase deficiency.

Keywords: 5,10-Methenyltetrahydrofolate synthetase deficiency; Neuroimaging; Macrocytic anemia; Folate

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1. Introduction

The folate metabolism pathway plays crucial roles in purine and thymidine monophosphate (dTMP) biosynthesis, amino acid metabolism, neurotransmitter synthesis, mitochondrial protein translation, and methionine regeneration, and also contributes to cell proliferation, mitochondrial respiration, and epigenetic regulation [1–3]. Disorders in this pathway can affect various systems in levels varying from mild to severe and can have also neurological involvement. 5,10-methenyltetrahydrofolate synthetase (MTHFS) deficiency is a rare folate metabolism disorder. We present here the case of a 27 month-old female who has been diagnosed MTHFS deficiency based on clinical, laboratory, and neuroimaging findings.

2. Case report

A 1-year-old female presented with poor head control, development delay, microcephaly, hypotonia, nystagmus, seizure, and cerebral hypomyelination (Fig. 1), with an unremarkable birth history of consanguineous parents. Metabolic investigations, including complete blood count, plasma/urine amino acid, urine organic acid, acylcarnitine profile, lactic acid, ammonia, pyruvate level, and thyroid function, were normal. An analysis of the proteolipid 1 *(PLP1)* gene was normal. By the age of 27 months, the patient had developed intractable seizures, and an examination revealed strabismus, poor head control, irritability, and normal deep tendon reflexes with flexor plantar responses. She had dysmorphic features including hypertelorism, broad nasal bridge, malar hypoplasia, prominent nasolabial folds, and a short and tent-shaped mouth. She was short in stature (74.5 cm, below the 1st percentile), low weight (7.5 kg, below the 1st percentile), microcephalic (44 cm, below the 1st percentile), and had feeding difficulties, requiring a gastric tube. An interictal electroencephalogram displayed a focal spike and slow waves in the left parietal region. The patient's seizures were controlled with levetiracetam, valproate, topiramate, and clonazepam. A repeat brain MRI revealed hyperintensity in the bilateral parietal and occipital lobes. Diffusion-weighted and apparent diffusion coefficient (ADC) MRI images revealed restricted diffusion in the bilateral putamen, globus pallidus, parietal and occipital lobes. Additionally, a cranial CT and susceptibility weighted imaging (SWI) in the MRI revealed calcification of the bilateral putamen, globus pallidus, caudate nucleus, and parietal and occipital lobes (Fig. 2). Repeated laboratory studies suggested macrocytic anemia (hemoglobin: 9.7 g/dL, mean corpuscular volume: 99.3 fL) and increased homocysteine [45.88–50.08 μmol/L (two measurements with an interval of one year), normal < 12], although her serum vitamin B12, folate, and thyroid function studies were normal. Following normal results in the initial metabolic studies, the tests, including cerebrospinal fluid (CSF) investigations, were repeated. 5-methyltetrahydrofolate (5-MTHF) was found to be at the lower end of the normal range at 41 nmol/l (normal:40–150 nmol/l), CSF folate was low at 1.7 ng/ml (control 15.2 ng/ml), and CSF neopterin and biopterin levels were normal. A whole-exome sequencing (WES) analysis demonstrated homozygosity for a predicted pathogenic frameshift mutation in exon 1 of the *MTFHS* gene (c.16_25delGTGAGCAGCG/p.Val6fs). The parents had the heterozygous variant. The study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval is not required as WES is performed for diagnostic purposes.

3. Discussion

Inborn errors of cerebral folate deficiency mainly consist of deficiencies of the dihydrofolate reductase (DHFR), methylenetetrahydrofolate reductase (MTHFR), and MTHFS enzymes, and folate transport disorders [4]. Macrocytic anemia is a significant finding in DHFR and MTHFR deficiencies. Until now, Romero et al. [3] reported only a female case diagnosed MTHFS deficiency with macrocytic anemia, in which

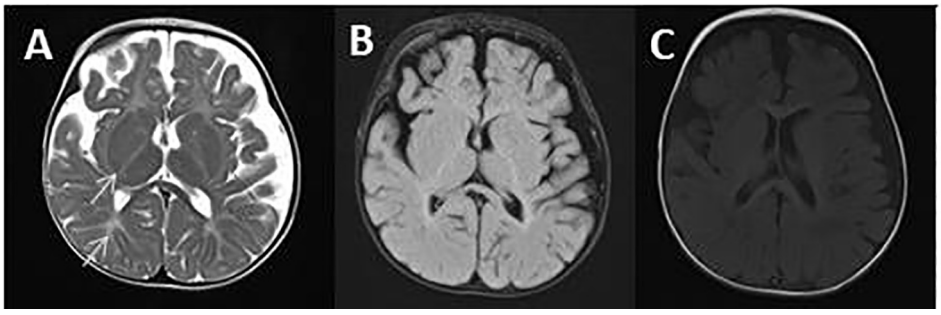

Fig. 1. (A-C) MRI findings at 12 months of age. Axial T2 weighted (A) and fluid attenuated inversion recovery (Flair) (B) images showed diffuse hypersignal of the hemispheric white matter and internal capsule (white arrow). Axial T1 (C) weighted image showed iso/hyposignal of the whole cerebral white matter.

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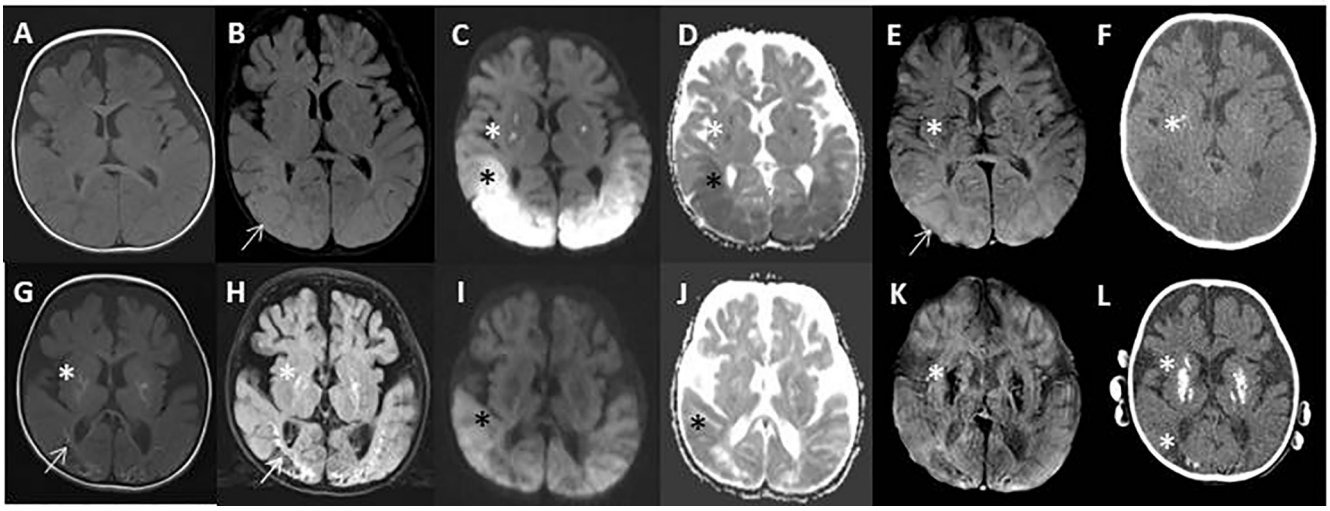

Fig. 2. Axial T1 weighted (A, G) fluid attenuated inversion recovery (Flair) (B,H), diffusion-weighted and apparent diffusion coefficient (ADC) (C, D, I, J), susceptibility weighted imaging (SWI) (E, K) and cranial CT (F, L) of the patient at 15 (A-F) and 28 (G-L) months of age. Axial T1 (A) weighted image showed iso/hyposignal. Abnormal signals revealed in axial T1 weighted (G), flair (B, H) and SWI (E, K) images in bilateral putamen, globus pallidus (white *), parietal and occipital lobes (white arrow). Diffusion-weighted and ADC MRI images (C, D, I, J) showed restricted diffusion in bilateral putamen, globus pallidus (white *), parietal and occipital lobes (black *). Second SWI (K) image showed more significant hypointensity in bilateral putamen, globus pallidus (white *) than first SWI (E) image. Second cranial CT (L) demonstrated calcification more severe in bilateral putamen, globus pallidus, parietal and occipital lobes (white *) than first cranial CT (F).

thymidylate and purine deficiency was said to have inhibited red blood cell precursors. Additionally, reduced MTHFS activity produces increased 5-formyl tetrahydrofolate (5-formyl THF), leading to the hindrance of serine hydroxymethyltransferase (SHMT) and phosphoribosylaminoimidazolecarboxamide formyltransferase (AICARFT). Moreover, decreased SHMT and AICARFT activity compose less thymidylate and purine synthesis, respectively [3]. Zheng et al. attributed the source of megaloblastic anemia to sustained cell growth without division in the process of red blood cell production resulting from deranged DNA synthesis and leading to chromosome breakage [1]. Furthermore, microcephaly may result from damaged cell growth and division, and also the deficiency of purine and thymidylate metabolism.

The pathophysiology of MTHFS deficiency is still poorly understood. Intracellular folate deficiencies may be responsible for the clinical manifestation. Although it could be attributed to borderline-low 5-MTHF levels, our patient was also recorded with a low folate CSF level. Decreasing the level of S-adenosylmethionine (SAM) providing the methylation of the myelin basic protein, and reducing central nervous system choline and phosphatidylcholine levels may contribute to abnormal myelination and disruptions of the myelin architecture [2,5]. It can help to better understand the hypomyelination of brain MRI. Secondary mitochondrial dysfunction originating from an intramitochondrial folate deficiency is another proposed pathophysiology and has been associated with intramitochondrial translation by needing mitochondrial methionyl-tRNA [2].

The present case differs from previously reported cases of MTHFS deficiency in its novel clinical and MRI findings (Table 1). Nystagmus and hypotonia have been peculiar manifestations in the other patients with MTHFS deficiency. Cerebral hypomyelination is one of the most common neuroradiological features in MRI findings. The differential diagnosis of nystagmus and cerebral hypomyelination should include primarily Pelizaeus-Merzbacher disease, and so we first investigated alterations in the *PLP1* gene. There was no identified changes. On the neuroradiological follow-up. Cranial CT revealed calcification of the bilateral putamen, globus pallidus, caudate nucleus, and parietal and occipital lobes. Therefore, a wide differential diagnoses of bilateral deep brain calcification include like TORCH (toxoplasma, rubella, cytomegalovirus, herpes simplex, and others) syndrome, hypoparathyroidism, mitochondriopathy, carboanhydrase II deficiency, Neurofibromatosis type 1, Coat's disease, GM1 gangliosidosis, Fahr's disease and some neurodegenerative disease like Cockayne syndrome and Aicardi-Goutières syndrome [6]. However, the differential diagnosis list can be narrowed to cobalamin/folate pathway and transport disorders according to MRI findings of restricted diffusion in the bilateral parietal and occipital lobes, and macrocytic anemia in addition to calcifications. Among these disorders, dihydrofolate reductase deficiency, cerebral folate transport deficiency, hereditary folate malabsorption (HFM), and methylcobalamin deficiency

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Table 1
Clinical summary of patients with MTHFS variants.

	Our study	Rodan et al. [2]		Romero et al. [3]
	Patient	Patient 1	Patient 2	Patient
MTHFS variant	c.16_25delGTGAGCAGCG/p.Val6fs frameshift Homozygous	c.434G>A (p.R145Q) c.107T>C (p.L36P) Compound heterozygous	p.Q162X (c.484C>T) p.R145Q (c.434G>A) Compound heterozygous	c.220C>T (p.R74X) null Homozygous
Age/sex	F/27 months	M/8 years	M/11years	F/11 months
Consanguinity	+	-	*N/A*	*same town*
Microcephaly	+	+	+	+
Clinical features				
Development delay	+	+	+	+
Strabismus	+	*N/A*	*N/A*	+
Nystagmus	+	*N/A*	*N/A*	-
Dysmorfic features	+	-	+	+
Epilepsy	+	+	+	+
Spasticity/hypertonia	-	+	+	+
Hypotonia	+	-	-	-
Short stature	+	+	+	+
Weight	Below the 1^{th} percentile	5^{th}–10^{th} percentiles	*N/A*	*5^{th} percentile*
Brain MRI				
Cerebral hypomyelination	+	+	+	+
Lateral ventriculomegaly	-	+	-	-
Cerebellar atrophy	+	-	+	-
Restricted diffusion	+	-	-	-
Calcification	+	-	-	-
Laboratory studies				
• **Serum**				
macrocytic anemia	+	-	-	+
• **CSF**				
Neopterin	N	N	N	↑
5-MTHF	LENR	LENR	LENR	↓
BH4	*N/A*	N	N	N
Folic acid	↓	*N/A*	*N/A*	*N/A*

LENR: lower end of the normal range, CSF: cerebrospinal fluid, 5-MTHF: 5-methyltetrahydrofolate, BH4: tetrahydrobiopterin, N/A: Not available.

suggest cerebral hypomyelination in MRI images [7]. Calcifications in the cortex or basal ganglia were reported with HFM [8] and so at this point, the borderline-low 5-MTHF level, low CSF folate, normal serum vitamin B12, and folate but increased homocysteine levels helped to distinguish MTHFS deficiency from other diagnoses. Finally, a WES analysis revealed a homozygosity mutation, confirming the diagnosis of MTHFS deficiency. Although other reported cases of MTHFS deficiency did not reveal calcification in neuroimaging, Romero et al. noted mild hyperattenuation in the brain CT [3]. However, bilateral deep brain calcification is firstly descripted in the present case among reported MTHFS deficiency. Therefore, we can not interpret that calcification is a common or rare finding. As the number of cases increases, this issue will become clearer.

Despite the small number of case reports with MTHFS deficiency, a different phenotype between reported male and female patients including the present case can be suggested. The female cases (our patient and the patient reported by Romero et. al) share some common findings like strabismus and macrocytic anemia unlike males. Additionally, the genetic background may contribute to this gender phenotype or the genotype mainly causes the phenotype because of the female patients carry homozygotes mutations. In the homozygotes mutations, frameshift mutations may be associated with broad spectrum neuroimaging findings like our patient.

In the female case of MTHFS deficiency with clinical, laboratory, and neuroimaging manifestations reported here, adding to the findings of previous reports, we identified new manifestations of nystagmus and hypotonia in the clinical spectrum, the involvement and restriction of bilateral putamen, globus pallidus, parietal and occipital lobes, and calcification of the bilateral putamen, globus pallidus, caudate nucleus, and parietal and occipital lobes in neuroimaging images, and low CSF folate in the metabolic investigation.

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Patient consent

Written informed consent was obtained from the patient's parents for publication of this case report.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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We thank the patient and families for their generous cooperation.

Authors' contribution

DC conceptualized this study after having investigating clinical and genetics data of the patient, following her up until the final diagnosis. MK and EE analyzed clinical data and metabolic tests. DC wrote the draft, under the guidance, supervision and methodology of NOD and KA in the light of the literature. ME identified the mutations in homozygosity, hence extending the analysis to the patient's parents. DC and KA analyzed MRI findings and made the differential diagnosis with other neurometabolic disorders together with PG. DC, NOD and KA discussed its peculiar features. DC and NOD reviewed the final version of the paper.

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