Hydrocephalus presented as the prominent symptom of severe 5,10-methylenetetrahydrofolate reductase deficiency in an infant: A case report

YUAN DING, QIAO WANG and CHUN-XIU GONG

Department of Endocrinology, Genetics and Metabolism, Beijing Children's Hospital, Capital Medical University, National Centre for Children's Health, Beijing 100045, P.R. China

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Abstract. Hyperhomocysteinemia is a common medical condition observed in patients with aminoaciduria. Deficiency in cystathionine beta-synthase, metabolism of cobalamin associated C, peroxiredoxin 1, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, LMBR1 domain containing 1, 5-methyltetrahydrofolate-homocysteine methyltransferase or 5,10-methylenetetrahydrofolate reductase (*MTHFR*) all can result in an elevation in plasma homocysteine, which has been reported to be a risk factor of vascular events, such as atherosis, acute myocardial infarction and cerebral stroke. Hyperhomocysteinemia due to the deficiency of 5,10-methylenetetrahydrofolate reductase (MTHFR; also known as 5,10-methyl THR reductase) is an autosomal recessive rare disease caused by defects in the *MTHFR* gene. The clinical manifestations of this disorder are heterogeneous, ranging from asymptomatic to severe neurological disorders. However, hydrocephalus has seldomly been reported in patients with MTHFR deficiency. The present study thus describes a case of severe MTHFR deficiency in an infant, whose main manifestation was hydrocephalus. The clinical course and genotype of the patient were also examined. Specifically, a 4-month-old boy with hydrocephalus was admitted to hospital. Clinical examinations and genetic sequencing of the patient were performed to determine the probable causative factors. A physical examination revealed that the patient had developmental delay and progressive hydrocephalus. Amino acid analysis of the blood revealed an enhancement in serum homocysteine levels and a decrease in blood methionine and free carnitine levels. The organic acid levels in urine were normal. Therefore, he was diagnosed with hyperhomocysteinemia. Targeted next-generation sequencing was performed to determine the pathogenetic gene in this case. A paternal mutation c.1530G>A (p.K510K) and a maternal mutation c.233C>A (p.S78X) were identified. Previous experimental evidence indicated that these two mutations were all pathogenic; therefore, this patient was ultimately diagnosed with MTHFR deficiency. The patient in described herein study presented with severe progressive hydrocephalus in association with a delayed developmental milestone. According to the clinical and genetic tests, the patient was diagnosed with severe MTHFR deficiency. It thus is recommended that screening for metabolites and performing gene sequencing in infants presenting with undisclosed hydrocephalus.

Correspondence to: Professor Chun-Xiu Gong, Department of Endocrinology, Genetics and Metabolism, Beijing Children's Hospital, Capital Medical University, National Centre for Children's Health, Beijing 100045, P.R. China
E-mail: chunxiugong@163.com

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Introduction

Hyperhomocysteinemia (OMIM #603174) is a common metabolic error occurring in infants. A dysfunction in enzymes involving in the metabolism of methionine i.e., cystathionine β-synthase, methionine synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR; also known as 5,10-methyl THR reductase) can lead to an elevation in plasma homocysteine levels, which can cause vascular disorders (1). A deficiency in methionine synthetase and MTHFR caused by deteriorations of mecobalamin are the most common types (2). The clinical presentation of hyperhomocysteinemia is highly variable between individuals. Lens dislocation, vasculopathy, skeletal abnormality and psychomotor delay are the most common manifestations of MTHFR deficiency (1,3,4). Systemic abnormalities and mortality have also been reported (5). Therefore, the prognosis is also different among patients.

The MTHFR enzyme encoded by the *MTHFR* gene (OMIM #607093) is an essential enzyme which catalyzes the reduction of 5,10-methyletrahydrofolate (5,10-methyl THF) to 5-methyletrahydrofolate (5-methyl THF), which donates a methyl group to homocysteine, generating methionine. A deficiency in 5 MTHFR results in a decline in 5-methyl THF, which deteriorates the conversion of homocysteine to methionine, thereby inducing an increase in plasma homocysteine. To date, the vascular disorders caused by hyperhomocysteinemia

have been extensively explored (4). However, the neurological implications have been less investigated. The present study describes a case of a patient (infant) with MTHFR deficiency whose main symptom was hydrocephalus. To the best of our knowledge, this is the first case of early-onset 5 MTHFR deficiency in a patient presenting with hydrocephalus to be reported in China.

Case report

The patient was a 4-month-old boy who was admitted to the Department of Endocrinology and Metabolism at Beijing Children's Hospital (Beijing, China) with the chief complaint of 'developmental delay for 4 months with frequent cyanosis induced by crying for 14 days'. He was the third child of a non-consanguineous family. The first two children of this family died of unexplained severe hydrocephalus within their first 2 months of life. The patient in question was born by cesarean delivery at full-term with a birth weight of 3.2 kg. Weak crying and cyanosis were observed at birth, which improved following oxygen inhalation and upper respiratory tract clearance. However, on the 9th day after birth, he was admitted to the local hospital again due to feeding difficulties and high-pitched crying. The results of magnetic resonance imaging (MRI) results indicated ischemic-anoxic changes and a hemorrhage at the dura mater and left occipital lobe. Therefore, mouse nerve growth factor, creatine phosphate sodium and ganglioside were administered; however, no improvement had been observed. The patient was then transferred to Peking University First Hospital due to recurrent seizures at the age of 1 month. Ventricle dilation was observed by MRI this time; thus, hypoxic-ischemic encephalopathy was again considered. However, mouse nerve growth factor combined with hyperbaric oxygen therapy still failed to lead to any responses. At the age of 4 months, the boy presented with recurrent transient crying-induced cyanosis, which autonomously remitted in 2-3 min. Feeding problems and vomiting were also reported. Delayed developmental milestones were also manifested. The patient could neither hold his head up nor roll over. He was also unable to gaze or follow along. He also did not acquire the ability to smile or recognize individuals. A cranial CT scan revealed profound hydrocephalus this time. Therefore, the patient was referred to the Department of Endocrinology and Metabolism at Beijing Children's Hospital.

Physical examination. The weight of the patient was 4 kg (<-3 SD), his height was 60 cm (-2 SD) and his head circumference was 41 cm (0±1 SD) upon hospital admission. Apathy, dry skin and acrocyanosis were observed upon an examination. The patient also presented with respiratory distress and head bobbing. However, no obvious abnormalities were found in the heart, lungs, spleen and liver. Although the patient had no nystagmus or evident abnormalities in the eyes, a sluggish response to light was still identified. Hyperreflexia was found in the lower limbs, whereas muscular tension was normal. Ankle clonus and the Babinski sign were positive. The patient had no neck stiffness. Neither Kernig's sign nor Brudzinski's sign was presented. Transverse palmar crease was found in the right palm.

Laboratory examination. Routine blood and urine tests were conducted to evaluated liver and renal functions, as well as serum electrolyte, glucose, lactate and pyruvate levels. The analysis of blood amino acids and acylcarnitines was performed using an Applied Biosystes API 3200 MS/MS analyzer and ChemoView software. Urinary organic acids were analyzed using gas chromatography-mass spectrometry (GC/MS) using the Shimadzu GC/MS QP2010 analyzer (Shimadzu Corporation) and the Inborn Errors of Metabolism screening system software for the differential diagnosis of organic acidurias. A cranial MRI was also evaluated. No abnormalities were found in the blood and urine routine examination. A cerebrospinal fluid (CSF) routine and biochemical test both revealed normal results. The biochemical blood tests revealed that the alanine aminotransferase level was 101 U/l (reference value, 5-40 U/l); the aspartate aminotransferase level was 48.7 U/l (normal range, 5-40 U/l); the alkaline phosphatase level was 384 U/l (reference range, 5-350 U/l); and the creatine kinase-muscle/brain (CK-MB) level was 77 U/l (reference, 0-25 U/l). The patient had no viral infections according to the serum TORCH (*Toxoplasma gondii*, rubella virus, cytomegalovirus and herpes simplex virus) IgM and IgG antibody test results. Gas chromatography-mass spectrometry analysis of the blood revealed that the blood methionine level was 8.12 μmol/l (reference, 11.0-54.0 μmol/l); the free carnitine level was 11.06 μmol/l (reference, 20.0-60.0 μmol/l); and the serum homocysteine level was >50 μmol/l (reference, 1.6-16.0 μmol/l). Urine organic acid analysis did not reveal any abnormalities (Table SI).

Imaging analysis. The cranial MRI result revealed that the patient had severe progressive hydrocephalus (Fig. 1). The echocardiogram revealed asymmetric septal hypertrophy, pulmonary arterial hypertension, patent foramen ovale (2 mm) and mild tricuspid regurgitation. A chest X-ray examination revealed increased lung markings with blurred margins. A video electroencephalography test demonstrated hypsarrhythmia, dissociation and epileptic discharges in the brain. Intermittent rhythmic disorders were also found in the fast waves over posterior head regions, especially at the left part.

Genetic analysis. The genomic DNA of the patient and his parents were isolated from peripheral leukocytes using standard methods. Inherited metabolic disease-related gene analysis of the patient was performed using gene capture and high-throughput (next-generation) genomic sequencing (performed by Beijing Kangso Medical Inspection). The patient was found to carry a paternal mutation c.1530G>A (p.K510K) and a maternal mutation c.233C>A (p.S78X) of the *MTHFR* gene. These two mutations have all been previously reported (as mentioned in the Discussion section below). According to the ACMG guidelines, these two mutations can all be classified as likely pathogenic and pathogenic, respectively (Fig. 2).

Treatment and follow-up examinations. A shunt system was surgically inserted to help divert the CSF. Vitamin B12 (1,000 μg per day) were intramuscularly injected. A combination of L-carnitine (0.5 g per day), folate (10 mg per day), betaine (500 mg per day) and vitamin B6 (30 mg per day) was

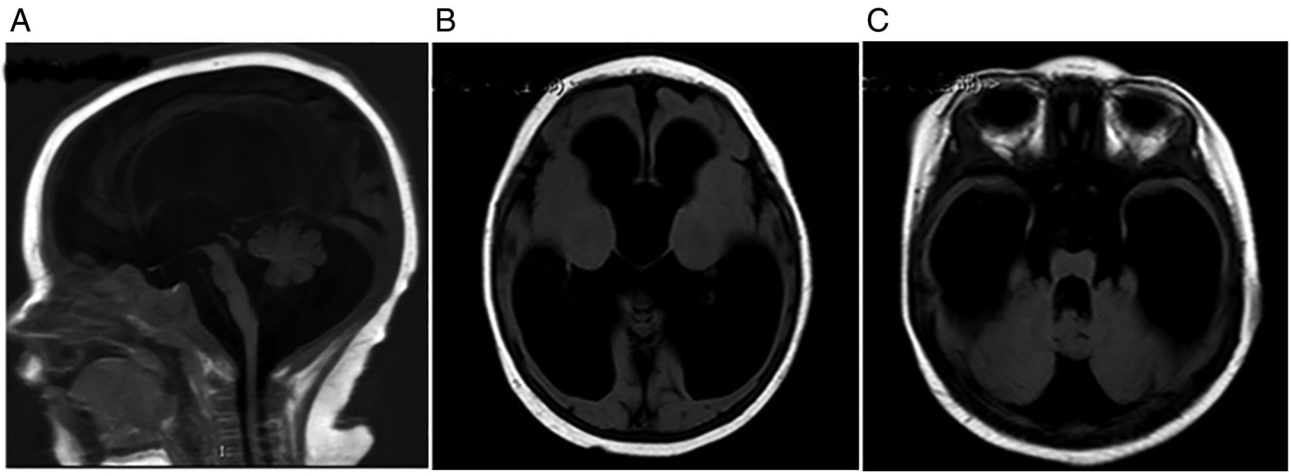

Figure 1. Brain magnetic resonance imaging result of the patient at 4 months of age. (A) Sagittal T1-weighted image showing dilation in the cisterna magna and vallecula of the cerebellum, as well as atrophy in bilateral cerebellar hemispheres. Hypoplasia in the brain stem could also be observed. (B and C) Axial T1-weighted image revealed dilated ventricular system and the loss of white matter in cerebral hemispheres. Enlarged sulci could also be observed.

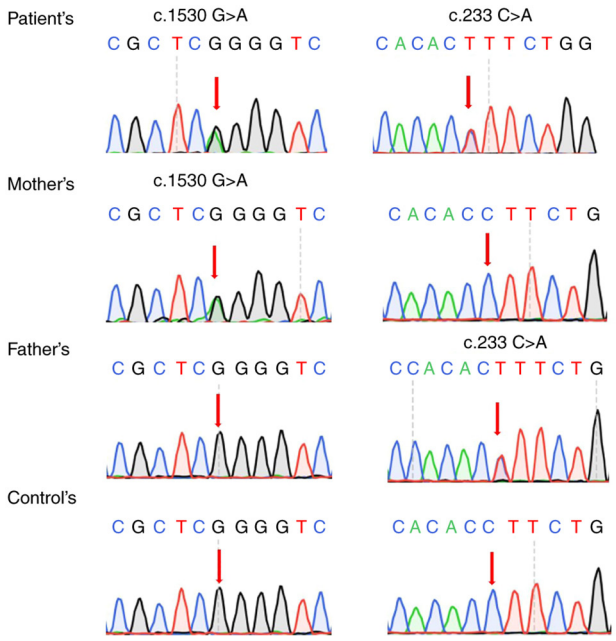

Figure 2. Partial MTHFR gene sequencing of the patient with the deficiency of MTHFR and his parents. In the MTHFR gene of the patient, two mutations, a paternal mutation c.1530G>A (p.K510K) and a maternal mutation c.233C>A (p.S78X), were found. The control shown in the figure represents in-house data for controls belonging to the company which performed the sequencing (Beijing Kangso Medical Inspection).

prescribed. The dose of vitamin B12 was reduced to 500 μg every other day. A follow-up examination after 2 months revealed that the proband could support his head and follow objects, but could still not roll over. A physical examination revealed a weight of 6 kg. The blood carnitine level was 85.93 μmol/l and the methionine level was 12 μmol/l, which was elevated following treatment. The serum homocysteine level decreased to 78.1 μmol/l, although it was still above the normal range. A re-examination of a head CT scan suggested that the symptoms of cranial hypertension had improved and the ventricle was slightly retracted. At that time, the patient was 4 years and 4months old, and he could not speak, did not recognize individuals and could not walk; however, his parents have been lost to follow-up (Table SII).

Discussion

An abnormal elevation in homocysteine levels was first reported as homocystinuria in 1962 (6,7). A deficiency in vitamin B12 metabolism was first identified as a cause of homocystinuria and hyperhomocysteinemia (8,9). Subsequently, hyperhomocysteinemia, including its pathogenesis, mechanisms, clinical presentations, genotype, diagnosis, screening and the prevention of the disease have been further researched. The genes that cause hyperhomocysteinemia include cystathionine beta-synthase, peroxiredoxin 1, metabolism of cobalamin associated C (also known as C2orf25), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, LMBR1 domain containing 1,5-methyltetrahydrofolate-homocysteine methyltransferase and MTHFR. A deficiency in the *MTHFR* gene can cause a dysfunction in MTHFR. The deficiency in 5 MTHFR destroys the metabolism of homocysteine, leading to hyperhomocysteinemia. The clinical presentations of MTHFR deficiency are heterogeneous. However, hydrocephalus has been rarely observed in patients with MTHFR deficiency (10). To the best of our knowledge, the present study presents the first case of MTHFR deficiency in a patient in China, whose main manifestation was hydrocephalus. The *MTHFR* gene is located on 1p36.22 and encodes MTHFR, a 74.6 kD enzyme composed of 656 amino acids. This protein contains a methylenetetrahydrofolate reductase domain (amino acid 38-337) and can catalyze the reduction of 5,10-methyl THF to 5-methyl THF. In this reaction, the cofactor flavin adenine dinucleotide functions as a transient carrier of electrons, which accepts the reducing equivalents from NAD(P)H and donates it to 5,10-methyl THR. In humans, 5,10-methyl THF reductase functions as a homodimer (11,12).

A MTHFR deficiency is an autosomal recessive disorder. Only the homozygous carrier or biallelic mutations carrier may have the disease. To date, there are 131 known disease-causing

mutations in *MTHFR* listed in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). Of these mutations, >60% are missense. The majority of the pathogenic mutations are located in exon 5-6 of the gene. This may be due to the fact that the loci encoding the catalytic domain is located within the 5th and the 6th exon. A destruction in this domain may affect the affinity between the enzyme and its substrates (13). In the present study, a synonymous mutation c.1530G>A (p.K510K) and a nonsense mutation c.233C>A (p.S78X) were identified. The synonymous mutation was validated to be a paternal mutation, while the nonsense mutation was validated to be a maternal mutation by Sanger sequencing. Therefore, the patient carried a pair of compound-heterozygous variants and this was in accordance with the inheritance pattern of MTHFR deficiency. The synonymous mutation c.1530G>A (rs765586205) has been previously reported. It locates at the consensus splice site (the last nucleotide) of exon 9. It has been demonstrated that this alteration can affect splicing, resulting in an actual frame-shift mutation by skipping of exon 8, to produce a premature termination of the protein (PS3) (14,15). This mutation is present in population databases with an extremely low frequency (PM2). In addition, this mutation is also the most common variant in MTHFR deficiency and has been reported in various affected patients or families (PP1 in ACMG but upgrading to moderate evidence due to increasing sample size) (15-19). Therefore, this mutation is considered a likely pathogenic mutation. For the nonsense mutation, p.S78X (PVS1), and has been previously reported (PP5) (20). Therefore, this variant is also interpreted as a pathogenic mutation. The biallelic mutations would result in less catalytic efficiency of the enzyme, thus causing MTHFR deficiency.

MTHFR deficiency (OMIM #236250) is one of the most prevalence congenital disorders of folate metabolism characterized by hyperhomocysteinemia. The clinical manifestations are heterogeneous, ranging from asymptomatic to severe neurological symptoms. The onset age also varies from neonate to adolescent even to adult. Clinical presentations, including neurodegeneration, psychomotor delay, seizures, psychiatric disorders, vascular diseases and peripheral neurological disorders have all been observed. Some patients also have respiratory failure, thus leading to mortality (15). Early-onset MTHFR deficiency presenting with neurological symptoms has been described as severe MTHFR. Patients with late-onset MTHFR deficiency with severe neurological symptoms have seldom been observed, apart from one case reported in 2014 (21). The typical manifestations of neonatal patients with severe MTHFR deficiency include hypotonia, feeding problems, drowsiness, sleep apnea and epilepsy. Patients with epilepsy usually first present with myoclonic seizures which progress to head nodding syndrome or complex focal seizures (22). Patients with MTHFR deficiency with hydrocephalus have rarely been reported (23).

The main manifestation of the present case was increased intracranial pressure and hydrocephalus when admitted to hospital at the age of 4 months. Mild developmental delay with recurrent seizures was reported when the medical history of the patient was further inquired upon. The patient did not present vomiting or other symptoms related to increased intracranial pressure or ventricle dilation until he was 4 months old. The MRI indicated that the patient had severe hydrocephalus without atrophy. The basal ganglia of the patient were not affected. No invasion or calcification were found. Brain tumor, intraventricular hemorrhage, head injury and infection-induced meningitis were all excluded. In addition, the patient also presented with a decreased blood methionine level and an enhanced homocysteine level, which is in accordance with the typical presentations of MTHFR deficiency.

The mechanisms of hydrocephalus in MTHFR deficiency in patients remain unclarified. However, hyperhomocysteinemia has been reported to cause vascular disease (1). With its thrombotic tendency, hyperhomocysteinemia can also cause thrombi in cerebral capillaries, which may impair the absorption of CSF, causing hydrocephalus (24). When the CSF flows into the subarachnoid space, it is diffused and absorbed into capillaries through intracranial arterial pulsation. The diffusion and absorption of CSF protects the balance of arterial pulsation in the brain. However, in metabolic disorders, such as MTHFR deficiency, the deleterious metabolites can destroy the vessel wall and affect the diffusion and absorption of the CSF, causing increased intracranial pressure and hydrocephalus (25).

MTHFR deficiency is clinically characterized by hyperhomocysteinemia, hypomethioninemia and a decreased level of folate in serum, erythrocytes and CSF (26). Hypomethioninemia may be considered as an important indicator for diagnosis. However, biochemical indexes combined with genetic testing are required for the final diagnosis (27).

Betaine (trimethylglycine) is an amino acid derivate which functions as a methyl donor in the alternative pathway of methionine synthesis. The supplementation of betaine can accelerate the synthesis of methionine by increasing the substrate concentration. Clinical trials also support the efficiency of betaine in preventing disease progression (24,28). Vitamins B6 and B12 are the essential cofactors in methionine metabolism. Combined treatment with betaine, and vitamins B6 and B12 can effectively reduce the deleterious effects of homocysteine accumulation. The patient described herein also exhibited a notable improvement following drug administration.

In conclusion, the typical clinical presentation of MTHFR deficiency includes neurological deterioration and vascular disorders. The severity also varies, depending on the type of mutation and the extent of enzyme activity defects. To the best of our knowledge, the present study is the first to report hydrocephalus as the main manifestation of MTHFR deficiency. These findings not only expand the mutation spectrum of severe MTHFR deficiency, but also highlight the necessity of newborn metabolic screening and genetic consulting. The majority of the sequelae can be prevented if patients are diagnosed at an early stage and are effectively treated. Genetic testing can also provide prenatal guidance for couples who have a relevant family history.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The original data have been submitted to the NCBI-SRA database with the submission no. PRJNA801899 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA801899).

Authors' contributions

YD, QW and CXG contributed to the acquisition, analysis and interpretation of the data; they made substantial contributions to the conception and design of the work, and supervised and substantially revised this work. All authors had equal contributions, equal participation and same rights to this article. QW and CXG confirm the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The Ethics Committee of Beijing Children's Hospital, Capital Medical University, National Centre for Children's Health granted approval for this study with the decision no. 2020-k-180 from 20. 07. 2021.

Patient consent for publication

Consent for participation was granted by the patient's parents and is part of the personal observation sheet.

Competing interests

The authors declare that they have no competing interests.

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