Teenaged Siblings with Progressive Neurocognitive Disease

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CASE DESCRIPTION

Two siblings were referred for workup for progressive neurological deterioration. The elder sibling was a 16-year-old boy who had been asymptomatic until 9 years of age when he developed walking difficulty that progressed to a bed-bound state followed by regression of cognitive function and generalized tonic clonic seizures. The younger sibling was a 14-year-old girl with onset of similar symptoms at the age of 6 years. The siblings were the eldest of 6 children from a family with no history of consanguinity. Both children principally ate a high-carbohydrate (maize)-based diet with sporadic access to fresh produce and animal protein. They had reached normal developmental milestones until the onset of symptoms. Both children had been treated unsuccessfully with sodium valproate. On examination, they demonstrated minimal communication skills and severe cognitive impairment. They had spastic paralysis of all extremities.

Electroencephalography in the elder sibling revealed generalized, highly potentially epileptogenic foci, and a brain computed tomography scan demonstrated marked cerebral atrophy with minimal white matter. Initial laboratory investigations, including a complete blood count, measurement of electrolytes and urea, and thyroid and liver function tests, were all within reference intervals. Syphilis serology was negative. Screening for inherited metabolic diseases included measurements of plasma amino acids and urine organic acids. Selected laboratory results of the elder boy are provided in Table 1.

QUESTIONS TO CONSIDER

1. What is the most common cause of a highly increased (>50 μmol/l) homocysteine?
2. Which nutrient deficiencies are associated with increased homocysteine concentrations?
3. What are the deleterious effects of increased plasma homocysteine concentrations?

DISCUSSION

HOMOCYSTEINE

Homocysteine is a nonessential, non–protein-forming, thiol-containing amino acid that is readily oxidized in the blood to homocystine and other disulfides. Only 1% is found in its free reduced form. The single source of homocysteine in humans is the demethylation of the essential amino acid, methionine, via 2 intermediate compounds, S-adenosylmethionine (SAM)3 and S-adenosylhomocysteine (SAH). Homocysteine can be regenerated to methionine via the remethylation pathway or irreversibly degraded to cysteine via transsulfuration.

Increased plasma homocysteine concentrations produce deleterious effects via several mechanisms (1). Homocysteine can react with molecular oxygen, generating homocysteine and reactive oxygen species. It can also form disulfides with proteins producing thiolated proteins. Extensive thiolation has been found to affect the function of proteins and enzymes. In addition, homocysteine at high concentrations can condense to form a thiolactone which may posttranslationally modify lysine residues, forming homocysteinylated proteins that are prone to multimerization, structural changes, and denaturation (2). Homocysteinylation of enzymes can also result in complete loss of activity.

HYPERHOMOCYSTEINEMIA

Hyperhomocysteinemia is fairly common, occurring in approximately 5% of the population. Most cases are acquired, and nutritional deficiencies of vitamin B12, vita-

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3 Nonstandard abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; MTHFR, methylenetetrahydrofolate reductase; methylTHF, methyltetrahydrofolate; NBS, newborn screening.
min B₆, or folate account for almost two-thirds of all cases (3). Folate is necessary for the remethylation of homocysteine (Fig. 1), and its insufficiency is the predominant cause of hyperhomocysteinemia. Vitamin B₁₂ and, to a lesser extent, vitamin B₆ insufficiency result in increased homocysteine concentrations because they are cofactors for methionine synthase and cystathionine synthase, respectively. Riboflavin is required for the flavin-dependent enzyme methylenetetrahydrofolate reductase (MTHFR), although its role in regulating homocysteine concent-

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine, mg/dL (µmol/L)</td>
<td>0.38 (34)</td>
<td>0.80–1.39 (71–123)</td>
</tr>
<tr>
<td>Plasma vitamin B₁₂, pg/mL (pmol/L)</td>
<td>255 (188)</td>
<td>196–863 (145–637)</td>
</tr>
<tr>
<td>Red cell folate, ng/mL (nmol/L)</td>
<td>995 (2256)</td>
<td>407–1472 (924–3337)</td>
</tr>
<tr>
<td>Plasma homocysteine, µmol/L</td>
<td>&gt;150</td>
<td>2.1–15.7</td>
</tr>
<tr>
<td>Plasma methionine, µmol/L</td>
<td>12</td>
<td>16–36</td>
</tr>
<tr>
<td>Plasma cystathionine, µmol/L</td>
<td>1.0</td>
<td>0–3</td>
</tr>
<tr>
<td>Urine methylmalonic acid, mmol/mol creatinine</td>
<td>0.54</td>
<td>&lt;3.6</td>
</tr>
<tr>
<td>Urine 2-methylcitrlic acid, mmol/mol creatinine</td>
<td>3.2</td>
<td>&lt;8.6</td>
</tr>
</tbody>
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Fig. 1. Overview of homocysteine metabolism with points of potential defects highlighted in grey. Laboratory findings are also shown. Ad-Cbl, adenosylcobalamin; B₆, pyridoxal phosphate; Cbl, cobalamin; CBS, cystathionine-β-synthase; CGL, cystathionine-γ-lyase; FAD, flavin adenine dinucleotide; Hcy, homocysteine; MC, macrocytosis; Met, methi-
onine; Met-Cbl, methylcobalamin; MMA, methylmalonic acid; MS, methionine synthase; THF, tetrahydrofolate; normal, within the reference interval; low-normal, near the lower limit of the reference interval.
tations appears less important. Other acquired causes of hyperhomocysteinemia include renal disease and drugs such as methotrexate, nitrous oxide, and certain antiepileptics.

Inherited hyperhomocysteinemia occurs in mild (<50 μmol/L) or severe (>50 μmol/L) forms, based on plasma total homocysteine concentrations. Causes of the mild form include 677C>T (Ala222Val) and 1298>A>C (Glu429Ala) mutations of the methylenetetrahydrofolate reductase (NAD(P)H) ([MTHFR]4 gene, causing partial MTHFR deficiency. The 677C>T mutation produces a thermolabile protein with a 70% reduction in enzyme activity in homozygotes.

The most common cause of severe inherited hyperhomocysteinemia is cystathionine-β-synthase deficiency (classical homocystinuria, type I), which has a prevalence of between 1 in 200,000 and 1 in 335,000 (4). Less common causes are MTHFR deficiency, defects of methionine synthase (involving mutations in 5-methyltetrahydrofolate-homocysteine methyltransferase [MTR, also known as CblG]), and defects in vitamin B12 metabolism, such as mutations in methionine synthase reductase [5-methyltetrahydrofolate-homocysteine methyltransferase reductase ([MTRR], also known as CblE)] and the CblD-variant-1 defect of the methylmalonic aciduria (cobalamin deficiency) [cobalamin deficiency (CblD type, with homocystinuria ([MMADHC]) gene.

PATIENT FOLLOW-UP
The clinical presentation of juvenile-onset progressive neurocognitive disease in 2 adolescent siblings suggested a high probability of an inherited metabolic disorder. The finding of severe hyperhomocysteinemia suggested a disorder of homocysteine metabolism. Cystathionine-β-synthase deficiency was considered unlikely because the typical musculoskeletal abnormalities (long-bone overgrowth, high arched palate, crowded dentition) and lens subluxation were absent (4). A vitamin deficiency was excluded by the findings of concentrations within reference intervals for red cell folate, serum vitamin B12, and plasma cystathionine, together with the absence of macrocytosis. In addition, urine methylmalonic acid and 2-methylcitrate concentrations within reference intervals made vitamin B12 deficiency, together with a number of disorders of vitamin B12 metabolism, unlikely. Of the remaining rare inherited causes of severe hyperhomocysteinemia, only deficiency of MTHFR was consistent with the laboratory and clinical findings.

MTHFR activity was determined in cultured skin fibroblasts. In both patients, MTHFR activity was <5% of controls. Gene sequencing revealed homozygosity for a novel c.760C>T (Pro254Ser) mutation in the MTHFR gene. Familial screening confirmed both parents as carriers of the same mutation, and an additional, as yet asymptomatic, homozygous 6-year-old sibling was also identified.

The finding of a novel homozygous mutation for a rare recessive disorder in a nonconsanguineous family was surprising. Although an additional 182 unrelated DNA samples from individuals of the same ancestry tested negative for the mutation, a recently introduced localized founder effect remains a possibility.

Both affected siblings were started on high-dose (20 mg daily) folate, and within 3 months seizures had resolved to the point where the sodium valproate could be withdrawn. Cognitive function improved mildly in both cases but unfortunately spasticity remained.

MTHFR DEFICIENCY
MTHFR is a cytoplasmic enzyme that catalyzes the reduction of methylenetetrahydrofolate to methyltetrahydrofolate ([methylTHF]). MTHFR deficiency (MIM 236250), an autosomal recessive disorder, is the most common inborn error of folate metabolism, presenting with a range of neurological and vascular complications. Over 150 patients and more than 50 disease-causing mutations have been reported (5, 6). The severity of the clinical course correlates well with age of onset and residual enzyme activity, and three forms have been described (7). The infantile-onset form is rapidly progressive and presents in the first year of life with hypotonia, lethargy, poor feeding, apnea, seizures, and microcephaly. The childhood-onset form affects children from 1–10 years of age, and typically presents with developmental delay, gait disturbance, ataxia, and seizures. Weakness, spastic paresis, pyramidal signs combined with dorsal column findings, sensory changes, and speech defects are more variably present, and thrombotic events and lens dislocation are rare. The adult form has a similar presentation to the childhood form but peripheral neuropathy and psychiatric symptoms are also evident.

PATHOPHYSIOLOGY
Severe hyperhomocysteinemia is associated with early atherosclerosis and both arterial and venous thrombosis. The main causes of morbidity and mortality are thromboembolism, cerebrovascular accidents, peripheral arterial thrombosis, and myocardial infarction. Low methionine and high homocysteine concentrations also result in a high SAH-to-SAM ratio, which

4 Human genes: MTHFR, methylenetetrahydrofolate reductase (NAD(P)H); MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; MTRR, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; MMADHC, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase.
inhibits over 115 different methylation reactions, including synthesis of neurotransmitters, posttranslational methylation of myelin basic protein, and DNA methylation, which is essential for the epigenetic regulation of gene expression (8). Cerebral atrophy and white-matter changes occur secondarily to demyelination and vascular pathology. All these factors are proposed to contribute to the neuropathology of this disorder. In addition, among the natural folates, only methylTHF crosses the blood–brain barrier in significant quantities, resulting in functionally low brain folate concentrations (9).

TREATMENT
Early diagnosis and intervention may modify the poor neurological outcome associated with severe MTHFR deficiency (5). The goal of treatment is to bypass the remethylation defect, thereby correcting the biochemical parameters and ensuring normal neurological development. This is achieved by replacing methylTHF and methionine, resulting in correction of methionine deficiency and a reduction of plasma homocysteine. Pyridoxine and cobalamin are supplemented to enhance homocysteine clearance, and riboflavin is administered to boost any residual MTHFR activity. Carnitine supplementation has been advocated because SAM is required for its de novo synthesis. Homocysteine can also be methylated to methionine via the alternate methyl donor, betaine. This occurs through the enzyme betaine-homocysteine methyltransferase, which is present only in the liver and kidney.

Early treatment may have a favorable outcome in terms of developmental recovery and prevention of further neurological deterioration, but the benefits are considerably more modest when treatment is started later, as in this case.

NEWBORN SCREENING
Although evidence clearly supports the benefits of early diagnosis and treatment of complete MTHFR deficiency, newborn screening (NBS) for this disorder is rarely performed. Current screening for homocystinuria is based on the identification of increased plasma methionine and will not detect nonclassical homocystinuria. Incorrect timing of sample collection and low methionine concentrations in breast milk reduce the diagnostic sensitivity of this test. Cases of classic homocystinuria with methionine concentrations within reference intervals detected by NBS have also been described. To address this issue, measurement of homocysteine in NBS protocols has recently been tested (10). Although generally successful, uncertainties remain regarding the stability of homocysteine and variations in its concentration during the newborn period. Until this type of screening becomes a mainstream assessment procedure, clinicians should be aware that MTHFR deficiency is one of the treatable metabolic disorders not identified by current NBS and, therefore, plasma total homocysteine should be considered in the laboratory workup of an infant or child with progressive neurocognitive disease.

Table: POINTS TO REMEMBER

- The most common cause of severe inherited hyperhomocysteinemia is cystathionine β-synthase deficiency (homocystinuria type I), but other inherited causes should be considered, such as MTHFR deficiency and MTR (CblG) and MTRR (CblE) mutations.
- The causes of hyperhomocysteinemia may be assessed by measuring homocysteine, methionine, methymalonic acid, and red cell mean corpuscular volume.
- Early diagnosis and treatment of complete MTHFR deficiency may halt disease progression and improve neurological outcome.
- MTHFR deficiency can cause severe hyperhomocysteinemia that is not identified by current NBS and, therefore, should be included in the differential diagnosis of an infant or child with progressive neurocognitive disease.

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Commentary

Ivo Barić¹,²*

This case report describes one of the countless examples of a tragic outcome for an inherited metabolic disorder because of late diagnosis. This fact is additionally painful because the cooccurrence in two siblings of similar symptoms lasting for years did not lead to the correct diagnosis in time.

The story underlines the necessity of including the measurement of total homocysteine in all patients with unexplained neurological symptoms, particularly because metabolic disorders associated with hyperhomocysteinemia are treatable. Because of the low incidence of inherited metabolic diseases and the related limited experience with them by the majority of physicians, physicians often consider metabolic diseases at the end of the list of differential diagnoses. There are other classic reasons for the delayed diagnosis of diseases associated with hyperhomocysteinemia. There is limited knowledge that homocysteine, as a marker of several diseases, should be included in metabolic screenings (as done with amino acids and organic acids), and ignorance that total homocysteine is not measured by standard amino acid analyses but must be requested separately. These reasons and the fact that only total homocysteine adequately represents the homocysteine body pool could be better stressed in the article.

While discussing the differential diagnosis of hyperhomocysteinemia, the authors mentioned that cystathionine γ-synthase deficiency was considered unlikely owing to the lack of classic clinical signs of this disease (long-bone overgrowth, lens dislocation). However, readers should be warned that this disease may present with only vascular problems. Several other points can be made related to Fig. 1. First, macrocytosis is not reliable for distinguishing causes of hyperhomocysteinemia, because mean corpuscular volume depends on many factors. Second, in patients with inherited disorders of cobalamin metabolism CblC and CblF, methionine can be low. Third, the defect CblJ has recently been discovered, with findings similar to those observed in CblF.

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