

S-adenosylhomocysteine hydrolase deficiency: two siblings with fetal hydrops and fatal outcomes

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Received: 29 April 2010 / Revised: 17 June 2010 / Accepted: 5 July 2010
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Abstract This paper reports the clinical and metabolic findings in two sibling sisters born with fetal hydrops and eventually found to have deficient S-adenosylhomocysteine hydrolase (AHCY) activity due to compound heterozygosity for two novel mutations, c.145C>T; p.Arg49Cys and c.257A>G; p.Asp86Gly. Clinically, the major abnormalities in addition to fetal hydrops (very likely due to impaired synthetic liver function) were severe hypotonia/myopathy, feeding problems, and respiratory failure. Metabolic abnormalities included elevated plasma S-adenosylhomocysteine, S-adenosylmethionine, and methionine, with hypoalbuminemia, coagulopathies, and serum transaminase elevation. The older sister died at age 25 days, but the definitive diagnosis was made only retrospectively. The underlying genetic abnormality was

diagnosed in the second sister, but treatment by means of dietary methionine restriction and supplementation with phosphatidylcholine and creatine did not prevent her death at age 122 days. These cases extend the experience with AHCY deficiency in humans, based until now on only the four patients previously identified, and suggest that the deficiency in question may be a cause of fetal hydrops and developmental abnormalities of the brain.

Abbreviations

AdoHcy S-adenosylhomocysteine
AdoMet S-adenosylmethionine
AHCY S-adenosylhomocysteine hydrolase
tHcy total homocysteine

Communicated by: K. Michael Gibson

Competing interest: None declared.

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Introduction

This paper reports the case of two siblings with deficient *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1; OMIM # 180960; AHCY) activity characterized by fetal hydrops, impaired synthetic liver function, myopathy, respiratory insufficiency, and brain damage leading to early death. AHCY catalyzes the conversion of *S*-adenosylhomocysteine (AdoHcy) to homocysteine and adenosine, providing a metabolic means to remove AdoHcy, formed in each *S*-adenosylmethionine- (AdoMet)-dependent methyltransferase reaction. At least 60 such methyltransferases are known to occur in mammals (Brosnan and Brosnan 2006); in humans genes encoding them may comprise as much as 1% of the total genome (Katz et al. 2003). Genetically determined deficiency of AHCY activity has been reported to date in only four humans (Baric et al. 2004, 2005; Buist et al. 2006; Cuk et al. 2007). These patients were characterized clinically chiefly by myopathy and delayed development and, metabolically, by striking elevations of plasma AdoHcy and AdoMet. Methionine was usually very high but occasionally normal or close to normal. AdoHcy inhibits AdoMet-dependent methyltransferases (Clarke and Banfield 2001), and such inhibition is considered to play a major role in the pathophysiology of AHCY deficiency. Dietary methionine restriction to decrease the abnormal accumulation of AdoHcy and administration of creatine and phosphatidylcholine appeared to be clinically beneficial in two patients previously described (Baric et al. 2005). The two additional cases of AHCY deficiency in the siblings described here were each due to compound heterozygosity for two novel mutations in the AHCY gene, c.145C>T; p.Arg49Cys and c.257A>G; p.Asp86Gly (Vugrek et al. 2009). These babies were more severely clinically affected than the previously identified cases of AHCY deficiency. Both died within months of birth, even though the AHCY deficiency of the younger child was recognized and efforts were made to treat the condition.

Patients and methods

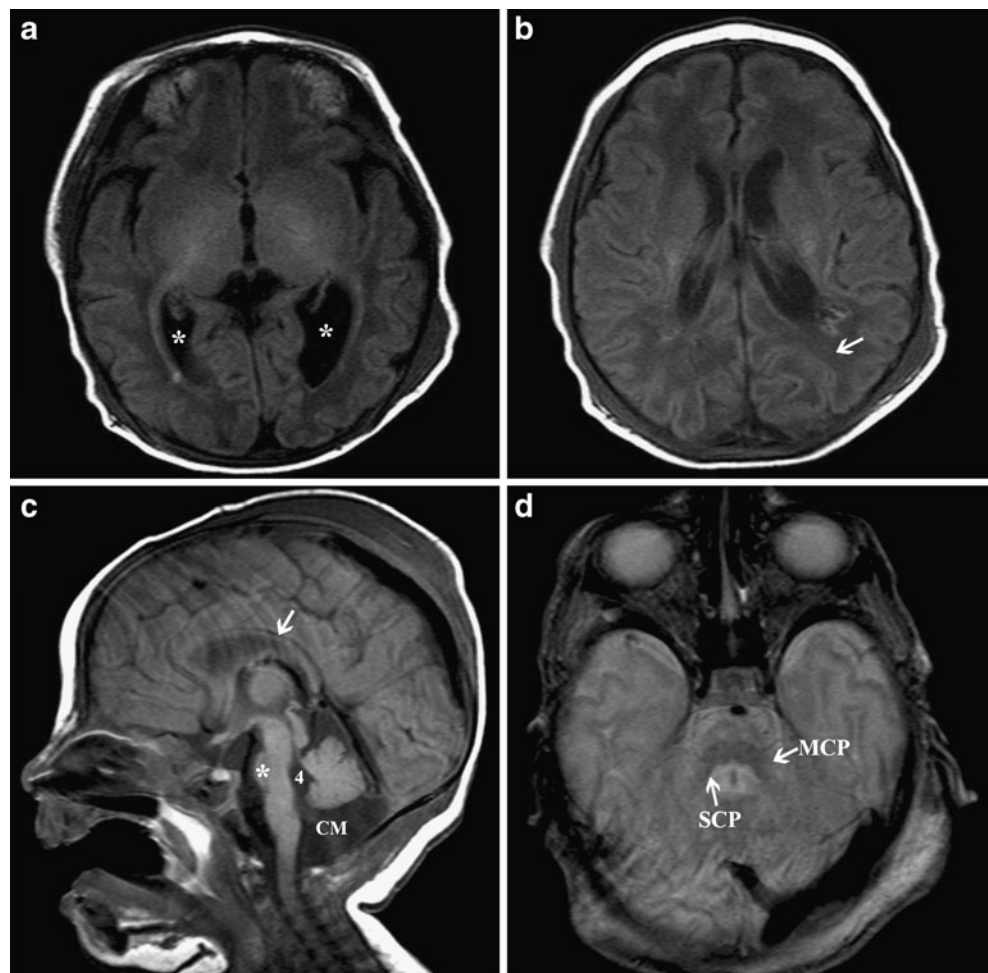
Assays of metabolites and AHCY activity were carried out as previously described (Baric et al. 2005). Extraction of genomic DNA from blood spots on filter paper cards was performed according to the GenElute Blood Genomic DNA kit (Sigma-Aldrich, St. Louis, USA). Appropriate amounts of eluted DNA were scheduled for polymerase chain reaction (PCR) for amplification and subsequent genomic analysis according to the protocol described previously (Vugrek et al. 2009).

Patient 1

This female infant, the first child of healthy, nonconsanguineous parents, was delivered by Caesarian section to a primigravid mother at 36 weeks gestation due to abnormal fetal heart rate decelerations. The pregnancy was complicated by decreased fetal movements and undiagnosed hydrops fetalis. At birth, the infant was apneic and bradycardic, requiring aggressive cardiopulmonary resuscitation. Apgar scores were 1, 3, and 4 at 1, 5, and 10 min. She was severely hydropic, requiring chest drainage and paracentesis to drain ascites shortly after delivery. Her pleural fluid was initially clear, but after initiation of breast-milk feedings, became turbid, consistent with chylothorax. She developed a progressively worsening coagulopathy from day 2 to 7, with increasing prothrombin time [International Normalized ratio (INR) 2.12], increasing activated partial thromboplastin time (>200 s, reference 22.4–37.5 s), and decreasing fibrinogen (<2.94 μ M, reference 5.88–11.8 μ M), and was treated with fresh frozen plasma. On day 3, platelet count was low at 89,000/ μ L. Albumin concentration at birth was low, at 13 g/L (reference 35–50 g/L), and total plasma protein concentration was 33 g/L (reference 46–74 g/L). She was treated aggressively with albumin and blood-product transfusions followed by diuretics, and had multiple chest tubes to drain recurrent pleural effusions. As she diuresed, and her mobility became less restricted from severe edema, a diffuse and marked hypotonia with contractures became apparent. Extubation failed due to hypoventilation. She also had *Pseudomonas* pneumonia. Despite treatment, respiratory failure persisted until her death at day 25.

Laboratory investigations revealed a normal hemoglobin and hematocrit at birth. She had normal electrolytes and calcium. Aminotransferases were mildly elevated. Urine protein in a random specimen was negative. Creatine kinase was not assayed. Toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, syphilis, and parvovirus B19 were excluded. On the day of birth, free thyroxine was normal, with low total thyroxine and elevated thyroid stimulating hormone consistent with sick euthyroid syndrome, and all had normalized on follow-up. Multiple abdominal ultrasounds were normal. Chest X-rays showed cardiomegaly, but two echocardiograms were normal. Early head ultrasounds showed only mild asymmetry of the lateral ventricles. Later ultrasounds showed cystic periventricular leukomalacia. Chromosomes were normal. DNA tests for myotonic dystrophy, Prader–Willi syndrome, and spinal muscular atrophy were negative. An electroencephalogram on day 22 was somewhat suppressed and of low amplitude, consistent with a diffuse encephalopathy. Magnetic resonance imaging (MRI) of the brain, performed on day 23, showed several prominent abnormalities (Fig. 1).

Fig. 1 Magnetic resonance imaging (MRI) of the brain of patient 1 on day 23 of life. **a, b** Axial images showing mild dilation of the lateral ventricles (*asterisk*), with white matter volume loss (*arrow*) of the parietal and occipital lobes; **c** midsagittal T1-weighted image showing marked hypoplasia of the ventral pons (*asterisk*) and corpus callosum (*arrow*), widely opened fourth ventricle (*4*), and mega cisterna magna (*CM*); **d** axial view demonstrating hypoplasia of the superior (*SCP*) and middle (*MCP*) cerebellar peduncles



A metabolic disorder was suspected to unify the diagnoses of nonimmune hydrops and severe hypotonia. Several serum ammonia levels were normal. Quantitative serum amino acid chromatography on day 4 showed elevated levels of several amino acids that were not considered to indicate a specific metabolic disorder, including methionine (273 μM , reference 6–60), leucine (595 μM , reference 47–160), isoleucine (322 μM , reference 26–91), valine (591 μM , reference 64–336), lysine (638 μM , reference 92–325), arginine (457 μM , reference 6–140), and phenylalanine (247 μM , reference 38–137). On day 22, urine organic acids and a blood acylcarnitine profile did not indicate a specific metabolic disorder, and an assay of plasma amino acids in which the cutoff value for methionine elevation was set at 100 μM showed no abnormalities. However, in a repeat assay on day 23, methionine was elevated at 176 μM (reference <56 μM). Other amino acids were not. Total homocysteine (tHcy) was also normal. The significance of this methionine elevation was not recognized at the time. A muscle biopsy had been planned but was not obtained because her clinical condition deteriorated, presumably due to ventilator-associated pneu-

monia caused by *Pseudomonas aeruginosa*. The parents declined tracheostomy for long-term ventilator management and enacted a “Do Not Resuscitate” order. On day 25, the infant died. The parents declined an autopsy.

Patient 2

This younger sister of patient 1 was born one year later at 37 weeks of gestation by Caesarian section due to abnormal fetal heart rate decelerations and late hydrops fetalis detected on ultrasound just prior to delivery. She was severely hydropic at birth, with apnea and bradycardia that responded well to immediate intubation. Apgar scores were 1, 5, and 6 at 1, 5, and 10 min. It was not clear how much her severe edema from hydrops was restricting her and contributing to her hypotonia, limited range of motion, and lack of movements. She had high-arched palate. Breathing was coarse. Heart status, perfusion, liver and spleen size were normal.

The infant was not anemic and the Coombs test was negative, indicating the hydrops was non-immune. Total serum protein (43 g/L) and albumin (21 g/L) were low on day 1 but both gradually increased with bolus of 5% albumin and

furosemide. After diuresis, she was found to be severely hypotonic. She required mechanical ventilation assistance for almost 3 weeks. She had severe oral discoordination and symptomatic gastroesophageal reflux and, despite treatment with metoclopramide, had recurrent emesis and obstructive apnea. Ultimately, she required gastrostomy feeding and gastric fundoplication. Laboratory workup included normal electrolytes and urinary protein. Aminotransferases were normal on days 1 and 6 but elevated on day 13, with an AST of 247 U/L (reference 18–74 U/L) and an ALT of 324 U/L (reference 26–54 U/L), then trending back down to normal. There was mild prolongation of prothrombin time (16.6 s, reference 11.4–15.1 s), an INR (1.22), borderline elevation of activated partial thromboplastin time (38.3 s, reference 22.4–37.5 s), and a high D-dimer concentration (2.71 $\mu\text{g/ml}$, reference $<0.5 \mu\text{g/ml}$). Fibrinogen was normal on the day of birth. Creatine kinase on day 42, lactic acid, ammonia, thyroid stimulating hormone, free thyroxine, chromosomes, mucopolysaccharides, very long chain fatty acids, and sequencing of the entire mitochondrial DNA were all normal. Lysosomal enzyme assays were negative for the disorders associated with nonimmune hydrops. Organic acids, total carnitine, and acylcarnitine profile did not indicate a specific metabolic disorder.

With regard to methionine, for technical reasons, an accurate assay was not possible in a dried blood spot obtained on day 3. A repeat test on day 18 showed a methionine of 642 μM (normal $<54 \mu\text{M}$). Thereafter, on intakes consisting of various proportions of breast milk, milk formulas, and parenteral nutrition, quantitative amino acid analyses usually showed marked elevations of plasma methionine (Table 1). Plasma tHcy was normal. Urine amino acids were normal, including homocystine. In a sample drawn at age 30 days, AdoMet and AdoHcy were markedly raised, and at 42 days both plasma AdoHcy and AdoMet were again very high, with a mild increase of cystathionine (Table 1), findings indicative of AHCY deficiency. Deficient activity of AHCY was confirmed by assay of an extract of packed blood cells. The patient's activity was 1.16–1.22 nmol/h per milligram protein (range of 3 determinations), whereas previous assays had shown activity in control extracts ranging from 5.9–6.5 nmol/h per milligram protein (Baric et al. 2005; Buist et al. 2006).

MRI of the brain at age 26 days (Fig. 2) showed abnormalities similar to those in her sister. A muscle biopsy on day 42 revealed type 1 fiber atrophy/hypotrophy and significant myopathic changes consistent with a congenital dystrophic process (non-dystrophin-related), and ultrastructural sarcoplasmic masses suggestive of congenital myotonic dystrophy. However, CTG trinucleotide repeats of the myotonic protein kinase gene were within the normal range.

Upon discharge home on day 48, the patient's edema had resolved, with no apparent dysmorphic features, but she

continued to have decreased muscle tone at the neck, shoulders, trunk, and hips, with decreased range of motion due to contractures at the elbows, knees, and ankles. At that time, the etiology of her hydrops and hypotonia remained uncertain, and the significance of her hypermethioninemia remained unclear until the reports of the elevations of AdoMet and AdoHcy became available. At that point, she was referred to the outpatient metabolic clinic of the University of Texas Southwestern Medical Center for follow-up. Her plasma methionine on day 76 was 617 μM . A methionine-restricted diet was started –50% methionine-free formula (Hominex[®]) and 50% Pregestimil[®], providing initially 27 mg/kg per day of methionine. As in the regimen reported by Baric and colleagues (2005), supplements of creatine (3 g/day) and phosphatidylcholine (1,200 mg/day) were added. The next day, she received her first immunizations (diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type B, hepatitis B, and inactivated polio), following which she appeared more irritable with periodic breathing. Two days later, respiratory arrest required emergency intubation, and on day-of-life 79, she was admitted with right upper lobe pneumonia. She improved and was extubated a day later. During this hospitalization, fasting plasma methionines were 6–9 μM (Table 1) and an hour after feeding increased to 27 μM . A second MRI of the brain at 85 days showed abnormalities similar to those in the earlier MRI (Fig. 3). She became more alert, moved well, and was discharged from the hospital on day 87. Two weeks later, she was readmitted due to another apnea that followed a 2-day history of mixed irritability and lethargy, with no fever but dyspnea shortly preceding the apnea. Chest radiograph showed a small right pleural effusion and patchy opacities consistent with atelectasis rather than infiltrate. Complete blood count was normal, blood cultures were negative, and a tracheal aspirate culture showed only light growth of *Streptococcus pneumoniae*. Treatment with ceftriaxone was initiated. Respiratory viruses were not detected on nasopharyngeal wash. Electroencephalogram did not demonstrate evidence of seizure activity. She remained very hypotonic and lethargic. Plasma methionine was 25 μM . She was hypoalbuminemic (18 g/L) and had a prolonged prothrombin time (INR 2.0). Because of concern that low methionine might be limiting protein synthesis, the methionine intake was raised to 60 mg/kg per day and creatine was increased to 4.5 g/day. Variations of plasma methionine due to efforts to optimize methionine intake between days 103 and 119 are shown in Table 1. On day 121, plasma levels of AdoHcy and AdoMet were lower than previously, but remained markedly elevated (Table 1).

A neurological consultant suggested her myopathy was likely contributing to her episodes of respiratory failure. A cardiologic evaluation showed only qualitative septal and

Table 1 Patient 2: methionine intakes and metabolite concentrations in plasma

Metabolite	Age (day-of-life, taking DOB as 1)												
	18	21	22	30	42	76	83–90	97	100	104–106	108–117	119	121
Methionine (13.3–42.7 μ M)	642	1158	757	420	17	617	6–9 (4) ^a	12	25	57–103 (3) ^a	7–17 (4) ^a	21	
AdoMet (93 \pm 16 nM)				2,179 ^b	1,197	3,727		2,459					315
AdoHcy (28 \pm 8 nM)				2,276 ^b	627	5,596		5,872					735
tHcy (5.1–13.9 μ M) ^c				7.3									
Cystathionine (44–342 nM)					395								
Tyrosine (28–134 μ M)				61									
Creatine kinase (< 232 U/L)					147	1028							
Methionine intake (mg/kg/day)	136	139	125	115	48	^d	27	27	27	60	30	15	15

^a Number of assays on different days during the specified period.

^b Assay carried out by Dr Terry Bottiglieri

^c Range for subjects age 18–65 years. Means for children age <10 years in papers cited by Baric et al. (2004) were 4.7–8.3 μ M.

^d Accurate estimate not available because patient was on ad lib volume diet of Lactofree formula

right ventricular apical hypertrophy and trivial patent foramen ovale shunting from left to right, i.e., no evidence of a cardiogenic etiology for her apnea or hypoxemic episodes. A sleep study showed sleep apnea. She continued to deteriorate, with persistent hypoxia and progressively worsening hypoventilation, and died on day 122.

Postmortem examination revealed cardiomegaly with biventricular hypertrophy, but no signs of storage disease, necrosis, or an inflammatory condition. The lungs showed pulmonary hypertensive vasculopathy, organized thrombi of the pulmonary arterioles, and alveolar hypoplasia (possibly attributable to neuromuscular weakness and/or the mechanical effects of hydrops). There was lymphangiectasia of the lungs, with pleural and peritoneal effusions. It was unclear whether these represented a primary lymphatic maldevelopment or were secondary to lymphatic obstruction. The liver showed hepatomegaly with hepatocellular and canalicular cholestasis. There was myopathic degeneration of the diaphragm and psoas muscles. Examination of the brain revealed hypoplasia of the ventral pons and markedly diminished myelination of cerebro-pontocerebellar pathways, with extensive gliosis of the cerebrum, cerebellum, and brainstem (Fig. 4).

Discussion

Diagnoses of AHCY deficiency

Patient 1 died within a few months of the publication describing the first identified human case of AHCY deficiency, and no definitive diagnosis was made during her life. For patient 2, the metabolic abnormalities indicated she had hypermethioninemia accompanied by extremely elevated

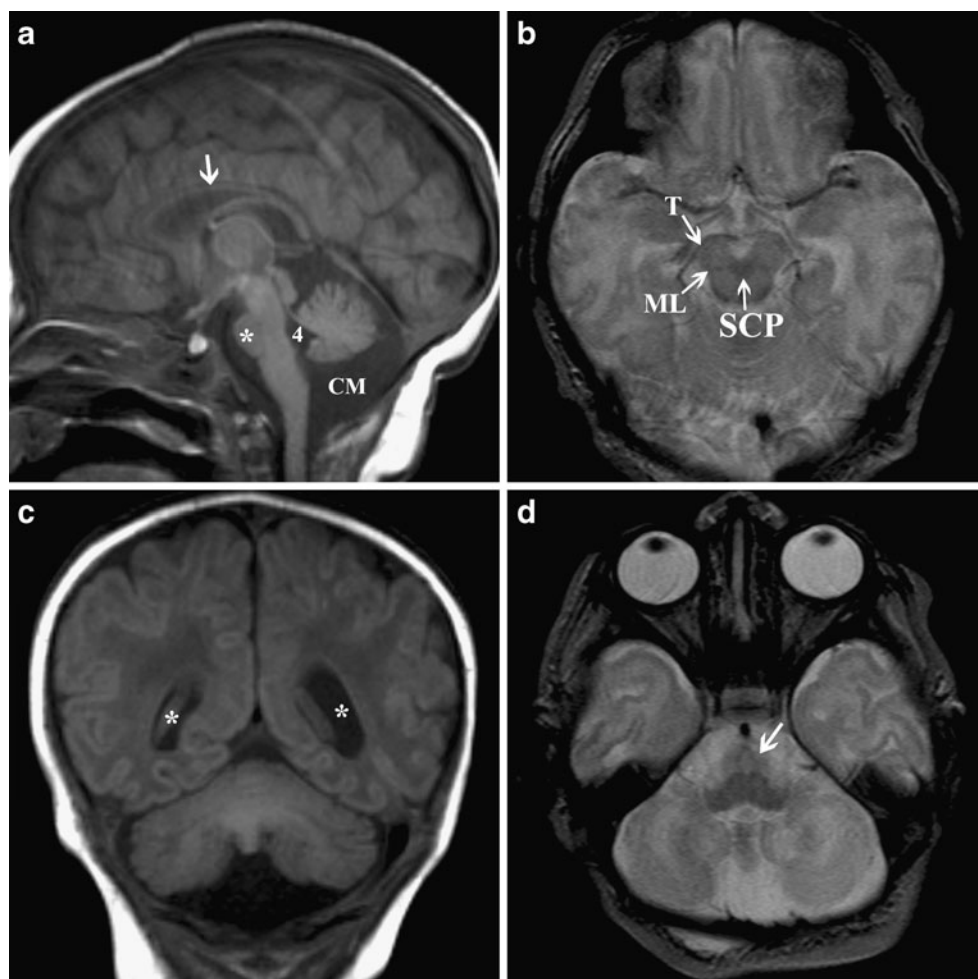
plasma AdoHcy, found, to date, only in AHCY deficiency. AHCY deficiency was confirmed by the findings of a lower-than-normal activity of that enzyme in an extract of packed blood cells and by the demonstration that she possessed two severely inactivating AHCY mutations, p.Arg49Cys and p.Asp86Gly (Vugrek et al. 2009). In the second patient, as in those previously reported, plasma AdoHcy elevation was clearly secondary to the deficient activity of AHCY (Baric et al. 2004). AdoMet elevations were attributed to inhibition by AdoHcy of the methyltransferases that utilize AdoMet (Baric et al. 2004). Methionine elevations are thought to be due to feedback down-regulation by AdoMet of the methionine to AdoMet flux (Baric et al. 2004), as occurs also in glycine *N*-methyltransferase deficiency secondary to AdoMet accumulation (Mudd et al. 2001; Augoustides-Savvopoulou et al. 2003).

For patient 1, the diagnosis was established retrospectively by family history, methionine elevations, and presence of the same two inactivating AHCY mutations as in her sibling. Because the latter finding was made only retrospectively from DNA retrieved from dried blood spots, assays of plasma AdoHcy and AdoMet were not done, nor were tissues available to assay AHCY activity. Unfortunately, the significance of the methionine elevation to 273 μ M in serum from day 4 was masked by elevations of several additional amino acids, so that further investigations of her hypermethioninemia were not carried out during her life.

Clinical features

Clinically, these patients shared important features with the previously reported cases of AHCY deficiency. All had severe muscular weakness associated with elevated creatine kinase

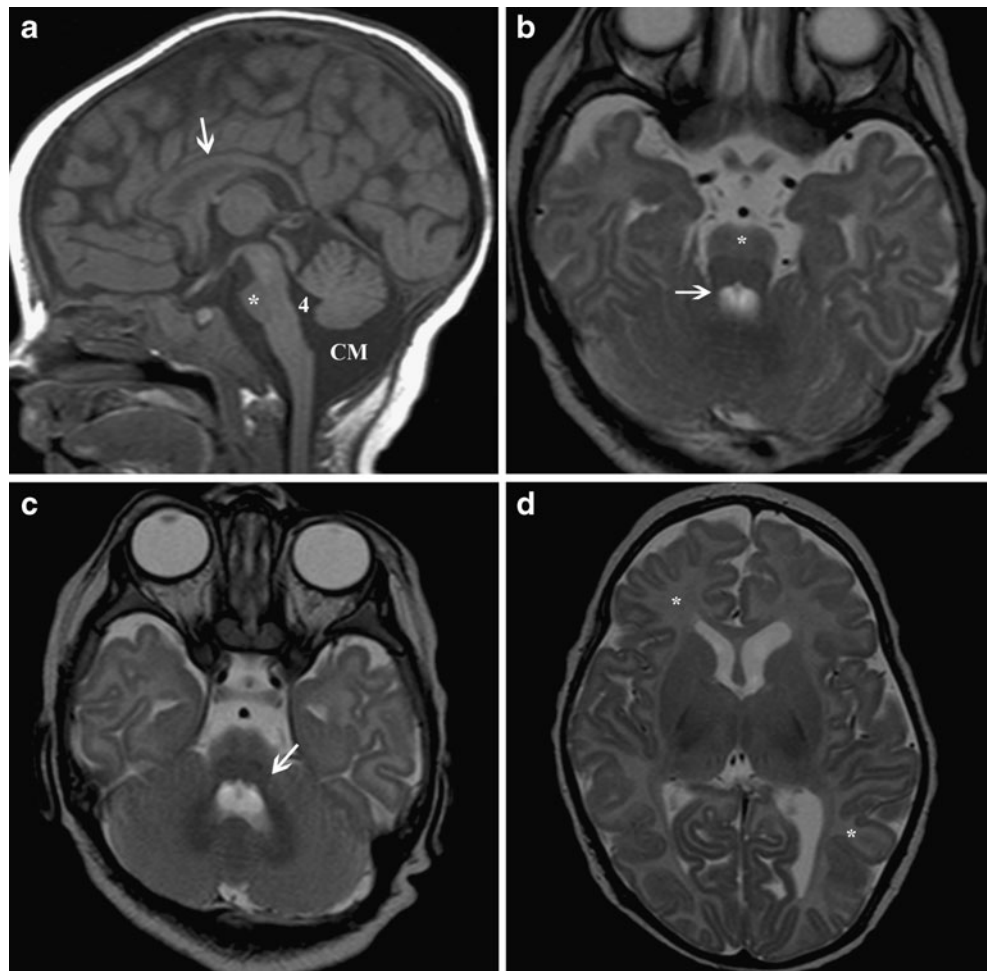
Fig. 2 Magnetic resonance imaging (MRI) of the brain of patient 2 at age 26 days: **a** Midsagittal postcontrast T1-weighted image showing similar findings to those in her sister (patient 1), namely, marked hypoplasia of the ventral pons (*asterisk*) and corpus callosum (*arrow*), widely open fourth ventricle (*4*), and mega cisterna magna (*CM*); **b** axial view showing hypomyelination of the medial lemniscus (*ML*), corticospinal and corticobulbar tracts (*T*), as well as hypoplasia of the superior cerebellar peduncles (*SCP*); **c** coronal image showing mild dilation of the lateral ventricles (*asterisk*). **d** axial view showing hypomyelination of the corticospinal tracts (*arrow*)



levels, indicating muscle damage or breakdown. Creatine kinase elevations were present in some cases in the first postnatal days (Baric et al. 2004, 2005; Cuk et al. 2007; Buist et al. 2006). In patient 2, creatine kinase was elevated on day-of-life 75 (Table 1). For reasons that are not clear but that might include reduced muscle mass, limited activity, or a laboratory mistake, in a sample from day-of-life 42 creatine kinase measured normal. The pathophysiologic reasons for this myopathy have not been established, although deficiency of phosphatidylcholine and—less likely—creatine may play a role. The tendency of patient 2 to develop hypoalbuminemia and prolonged prothrombin time were present also in the initial patient (Baric et al. 2004). MRIs of the brains of both patients showed signs indicative of hypomyelination, a feature seen in the initial two patients described (Baric et al. 2005) but not in the third, an older man who had been treated with methionine restriction at an early age (Buist et al. 2006). Autopsy of patient 2 revealed markedly diminished myelination. A novel feature, not previously described in AHCY-deficient patients, was the presence of developmental brain anomalies in the posterior

fossa—mega cisterna magna and hypoplasia of ventral pons and cerebellar components—abnormalities that could contribute to the clinical course. Although MRI images of the brain might be interpreted as indicating an abnormally small vermis (see, for example, Fig. 3), Robinson and Goldstein point out that, given the coexisting cisterna magna, such an interpretation would not be justified (Robinson and Goldstein 2007). Indeed, on postmortem examination, the vermis size was normal. Delayed physical and mental development have been features of all previously described AHCY-deficient patients, but, because the patients reported in this study died so early, it is difficult to judge the extent to which they would have been developmentally delayed. Given the severity of their hypotonia/myopathy, the abnormalities in brain images, and the developmental delays already seen in patient 2, significant neurodevelopmental impairment could reasonably be expected. Both patients were born with fetal hydrops, a finding not present in previously described cases, suggesting a cause-and-effect relationship between that condition and their deficient AHCY activities.

Fig. 3 Magnetic resonance imaging (MRI) of the brain of patient 2 at age 85 days: **a** midsagittal postcontrast T1-weighted image showing similar findings to those on the previous imaging (see Fig. 2), namely, marked hypoplasia of the ventral pons (*asterisk*) and corpus callosum (*arrow*), widely open fourth ventricle (4), and mega cisterna magna (CM); **b** axial view showing hypoplasia of the ventral pons (*asterisk*) and superior cerebellar peduncles (*arrow*); **c** axial image demonstrating hypoplasia of the middle cerebellar peduncles (*arrow*); **d** axial view showing that, in addition to the hypomyelination of the hindbrain, there is now hypomyelination of the supratentorial white matter (*asterisks*)



Inheritance is Mendelian recessive

Although each parent of the patients reported here was heterozygous for one of the AHCY mutations found in their children (Vugrek et al. 2009), they, as well as the parents of previously described children with AHCY activity deficiency (Baric et al. 2004; Buist et al. 2006) had normal plasma values of both AdoHcy and AdoMet

Remaining clinical questions

The early deaths of these siblings suggest they may have had more severe AHCY activity deficiency than the other known deficient individuals. AHCY activity in an extract of blood cells from patient 2 was far below the mean reference value, but there was still some activity, not clearly different from the activities reported in other deficient patients (Baric et al. 2004, 2005; Buist et al. 2006). This activity may have been due to the patients' mutated AHCY proteins because: (a) human AHCY is encoded by a single structural locus (Arredondo-Vega et al. 1989); (b) other proteins that might

contribute AHCY activity, for example, the recently discovered group of AHCY-like proteins (Dekker et al. 2002), have not been found to have such activity (Ando et al. 2003) (Gomi et al. 2008); (c) expressed in *Escherichia coli*, protein carrying p.Arg49Cys has 6–7% of wild-type activity in the presence of dithiothreitol, and that with p.Asp86Gly transiently has 16% activity in vitro (Vugrek et al. 2009). In the first reported patient, the relative activity in the liver was lower than in red blood cells or fibroblasts (Baric et al. 2004). Thus, the hepatic activity may not be accurately reflected by that in blood cells, and the deficits of AHCY activity in the siblings reported upon here may well have been severe enough to jeopardize survival.

A striking abnormality present in both these patients, but not in the previously reported AHCY-deficient patients, was nonimmune hydrops fetalis. Given the absence of significant anemia, cardiac failure, or kidney disease, severe hypoalbuminemia, the beneficial effect of albumin, and the disturbed coagulation in both sibs, impaired synthetic liver function (noticed also in some previously reported AHCY-deficient patients) appears to be the most likely cause of fetal hydrops.

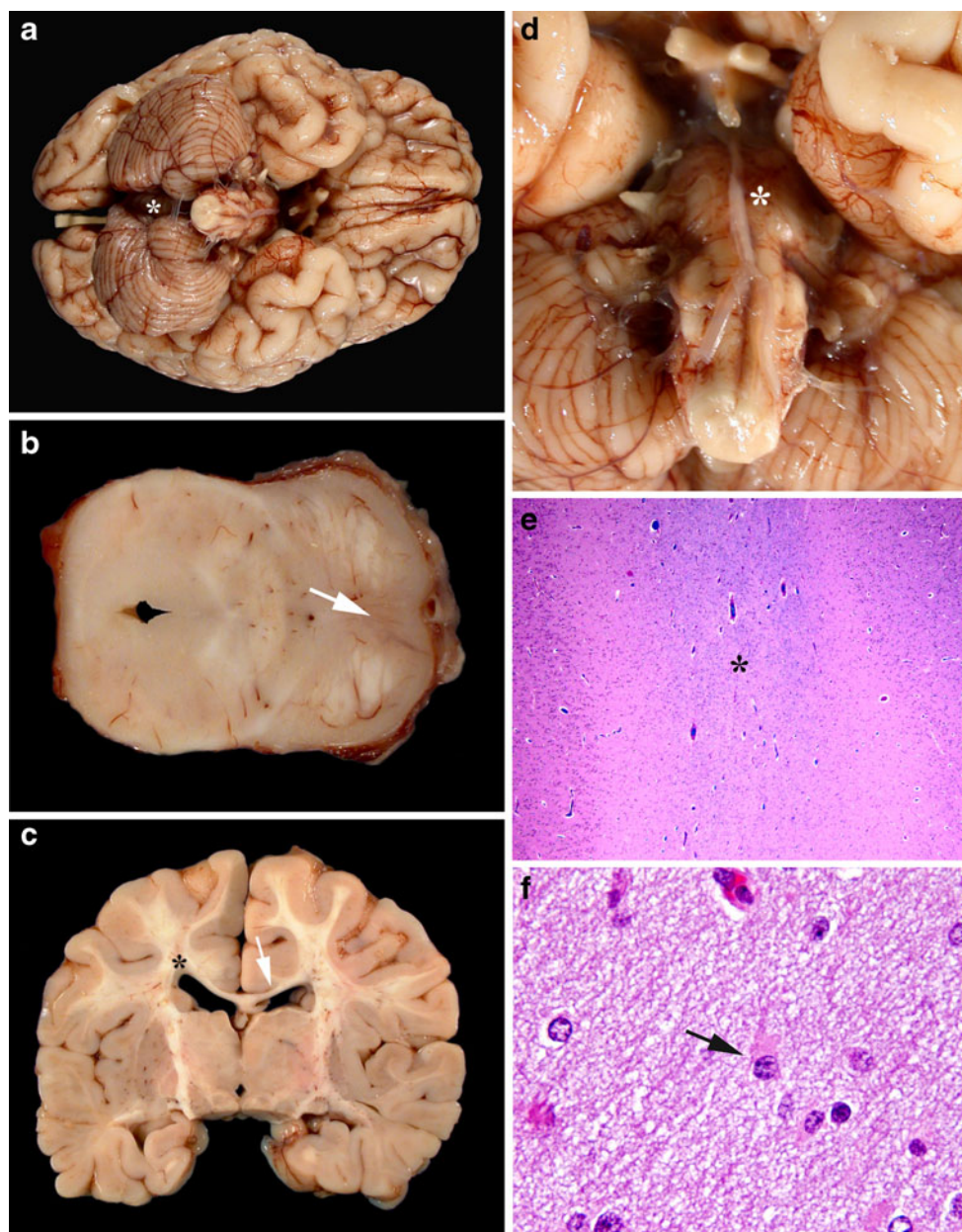


Fig. 4 Postmortem findings in the brain of patient 2: **a** Ventral view showing symmetric but small cerebellar hemispheres. The cerebellar vermis (*asterisk*) is normally formed; **b** Cross-section of the pons demonstrating marked hypoplasia of the basis pontis (ventral pons, *arrow*). The pontine tegmentum (dorsal pons) is symmetric and of normal size; **c** Coronal section of the parietal and temporal lobes (at the level of the red nucleus) showing marked thinning of the corpus callosum (*arrow*) and gray discoloration of the periventricular white matter, accentuated at the angles of the lateral ventricles (*asterisks*); **d** Ventral aspect of the hindbrain showing marked hypoplasia of the ventral pons (*asterisk*). The medullary pyramids and olivary bodies

are normally formed and symmetric; **e** Low-power ($\times 20$) view of a section from the frontal lobe, stained for myelin with luxol-fast blue/periodic acid-Schiff/hematoxylin (LFB/PAS/H), demonstrating a striking lack of myelin in the subcortical white matter (*asterisk*); **f** High-power ($\times 400$) view of a section from the subcortical white matter of the frontal lobe stained with hematoxylin and eosin (H&E), showing astrocytes (*arrow*) characterized by abundant eosinophilic cytoplasm with peripheral fibrillar processes, slightly enlarged nuclei with smooth nuclear contours, and irregularly dispersed chromatin—features consistent with reactive astrocytosis

No studies of a knockout mouse lacking only AHCY activity have been reported. However, mice with a deletion that includes the AHCY gene undergo embryonic death (Miller et al. 1994). The hypotonia/myopathy of all previ-

ously described AHCY-deficient patients (Baric et al. 2004, 2005; Buist et al. 2006; Cuk et al. 2007) as well as in the patients reported in this study, was manifest at birth. These lines of evidence, as well as fetal hydrops and congenital

brain abnormalities of our two patients, suggest that the most severe forms of AHCY deficiency may be lethal in utero.

How is optimal management of AHCY deficiency achieved? Dietary methionine restriction has produced decreases in abnormally high AdoHcy, presumably relieving to some extent inhibitions of AdoMet-dependent methyltransferase reactions (Baric et al. 2004, 2005). With patient 2, when methionine intake was reduced to 27 mg/kg per day, plasma methionine decreased to 6–25 μ M, but plasma AdoHcy remained as high as 5,600–5,900 nM (Table 1). Limitation of methionine intake (to 15 mg/kg per day, the lower limit of the intake recommended by Acosta and Elsas for infants 0 to <3 months of age with cystathionine β -synthase deficiency (Acosta and Elsas 1992)) lowered plasma AdoHcy to 735 nM (Table 1). For previous patients, methionine restriction during infancy to 15–20 mg/kg per day led to plasma methionine concentrations of 16 ± 18 and 7 ± 2 μ M and AdoHcy concentrations of 912 ± 903 and 56 ± 38 , respectively. These children continued to grow and improved clinically (Baric et al. 2004, 2005). For future cases, a possible strategy would be to lower methionine intake to maintain plasma methionine at about 10 μ M, hoping to lower AdoHcy to a level that is not too adverse. Careful monitoring is mandatory to ensure optimal balance between too high and too low methionine/protein intakes. Phosphatidylcholine and cysteine supplementation seem justified, and creatine theoretically may be useful (Baric et al. 2004, 2005).

Acknowledgements The authors thank Dr. Teodoro Bottiglieri for performing retrospectively the assays listed in Table 1 for day-of-life 30 and Dr. Neil Buist for advice on managing patient 2. This work was supported by grants 098-0000000-2463 (to OV) and 108-1081870-1885 (to IB) of the Ministry of Science, Education, and Sports, Republic of Croatia

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