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ORIGINAL ARTICLE

S-Adenosylhomocysteine hydrolase deficiency in a 26-year-old man

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Summary This paper reports the third proven human case of deficient *S*-adenosylhomocysteine (AdoHcy) hydrolase activity. The patient is similar to the only two previously reported cases with this disorder in having severe myopathy, developmental delay, elevated serum creatine kinase (CK) concentrations, and hypermethioninaemia. Although he has been followed from infancy, the basic enzyme deficiency was established only at age 26 years. The diagnosis was based on markedly elevated plasma concentrations of both AdoHcy and *S*-adenosylmethionine, some 20% of the mean control activity of AdoHcy hydrolase activity in haemolysates of his red-blood cells, and two missense mutations in his gene encoding AdoHcy hydrolase. He had low values of erythrocyte phosphatidylcholine and plasma free choline and marginally elevated excretion of guanidinoacetate, suggesting that the elevated AdoHcy may have been inhibiting methylation of phosphatidylethanolamine and guanidinoacetate. His leukocyte DNA was globally more methylated than the DNA's of his parents or the mean extent of methylation measured in age-matched control subjects.

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Introduction

An association of hypermethioninaemia with severe myopathy, elevated plasma creatine kinase (CK) values and developmental delay was first reported in 1979 (Gaull et al 1979), in a $7\frac{1}{2}$ year-old girl from the former Yugoslavia. At the same time our patient was under investigation for similar findings and was also studied by Gaull and co-workers (Gaull et al 1979, 1981). Extensive studies were done in both cases, but no primary enzyme deficiency was identified. The patient described by Gaull and colleagues (Gaull et al 1979, 1981)

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subsequently died. Our patient is now 26 years old and the cause of his hypermethioninaemia has recently been shown to be *S*-adenosylhomocysteine (AdoHcy) hydrolase (adenosylhomocysteinase, EC 3.3.1.1) deficiency. The present report describes the clinical and biochemical findings in this case.

The present work was prompted by reports by Barić and co-workers of two Croatian siblings (Barić et al 2004, 2005b) who were the first patients with proven AdoHcy hydrolase deficiency. In the proband, who was investigated because of hypotonia, psychomotor delay and elevated plasma CK, hypermethioninaemia as high as 748 μmol/L (normal 13–45 μmol/L) was first detected at age 8 months. At age 11 months, the finding of a plasma AdoHcy concentration some 150-fold above normal suggested the correct diagnosis (Barić et al 2004). The second patient, a younger brother of the proband, has been followed since birth and, like his brother, had hypotonia, psychomotor delay, elevated plasma CK and severely elevated plasma AdoHcy (Barić et al 2005a,b). Plasma methionine was at about the upper limit of the reference range.

AdoHcy is formed by transfer of a methyl group from *S*-adenosylmethionine (AdoMet). Subsequent cleavage of AdoHcy to adenosine and homocysteine occurs in a complex reaction catalysed by AdoHcy hydrolase. Human AdoHcy hydrolase is a cytosolic tetramer of identical subunits requiring NAD^+ as cofactor (Hershfield et al 1985). Amino acid sequences have been highly conserved over a billion years of evolution (Coulter-Karis and Hershfield 1989; Hu et al 1999; Kasir et al 1988). In the human gene a 256G>A transition of unknown prevalence has been identified (Coulter-Karis and Hershfield 1989) and three sequence variants have been reported recently (Gellekink et al 2004). Study of two of these polymorphisms revealed no clear effect on plasma total homocysteine (tHcy) or on the risk of recurrent venous thrombosis (Gellekink et al 2004). Embryo mice homozygous for a deletion overlapping the AdoHcy hydrolase gene die around the time of implantation (Miller et al 1994).

It is not clear how the human enzymatic defect causes either the muscle disease or the neurodevelopmental problems, but the clinical similarity of all three proven cases indicates that the clinical and biochemical abnormalities are related. Whether there are milder or more severe examples of the same disorder awaits further study.

Methods

Metabolite assays

Amino acids were measured by ion-exchange chromatography using a Biochrom 20 plus analyser (Amersham Pharmacia Biotech, Waukesha, WI, USA). Plasma tHcy was analysed using an IMX, Abbott analyser (Abbott Park, IL, USA). Plasma methionine and tHcy were measured also by capillary gas chromatography–mass spectrometry, as were cystathionine, total cysteine (tCys), dimethylglycine and sarcosine (*N*-methylglycine) (Allen et al 1993; Stabler et al 1987, 1993). Plasma and cerebrospinal fluid (CSF) AdoMet and AdoHcy were assayed as previously described (Capdevila and Wagner 1998). Phosphatidylcholine, free choline, and betaine were assayed by high-pressure liquid chromatography–mass spectrometry (Koc et al 2002). Guanidino compounds were measured by cation-exchange chromatography with postcolumn derivatization (Marescau et al 1992; Schulze et al 2001). Purines and pyrimidines were analysed using the method of Morris and Simmonds (1985). Reference ranges listed are those for the laboratory that did the assay in question.

Assays of AdoHcy hydrolase activity

Assays of red blood cell haemolysates were carried out in the direction of synthesis of AdoHcy by a modification of the method of Hershfield and colleagues (Hershfield et al 1979), as described previously (Barić et al 2004).

Global DNA methylation studies

Genome-wide leukocyte DNA methylation was quantified in packed blood cells (Pogribny et al 1999).

Gene analysis

Molecular genetic studies were performed as described (Barić et al 2005b).

MR imaging and ^{1}H MRS studies

The studies carried out included sagittal T1-, axial proton density- T2-, T1- and diffusion-weighted imaging, and coronal FLAIR scans from the foramen magnum to vortex, as well as single voxel (TR/TE 1500/144 ms, 144 NEX) and multivoxel (TR/TE 1000/144 ms, 24 × 16 phase encoding) proton PRESS spectroscopy of the right basal ganglia and right periventricular white matter.

Case history

The patient was born in 1979 after a 43-week gestation to a G1P1 mother. Pregnancy and delivery were normal. Growth parameters were within the normal ranges. Apgar scores were 6/9 but hypotonia and poor feeding necessitated two weeks' hospitalization before discharge. Routine newborn screening on day 3 using a Guthrie bacterial inhibition assay showed mildly elevated blood methionine, 200 μmol/L (cut-off value

of 300 μmol/L in use at that time). State-mandated routine retesting was performed at 2 weeks of age and confirmed a mild elevation. Automatic long-term follow-up evaluation for this abnormality then ensued. Over the next several months, further samples confirmed the permanence of the hypermethioninaemia up to 540 μmol/L. Hypotonia and mild developmental delay persisted.

At 5 months he was first seen at the Metabolic Clinic of Oregon Health & Science University (OHSU). Physical examination showed a nondysmorphic infant who was markedly weak, predominantly in the upper extremities. Reflexes were normal. There was no visceromegaly. The EEG was normal. Plasma methionine was 800 μmol/L; all other amino acids (including tyrosine) were within the normal range. Homocystine was not noted in either plasma or urine during column chromatography. Also elevated were serum CK 1500 IU/L (<250 IU/L), lactate dehydrogenase >500 IU/L (<240 IU/L), and aspartate transaminase 166 IU/L (20–45 IU/L). All other standard blood results, including carnitine, were normal. The acylcarnitine/free carnitine ratio in urine was slightly elevated. The developmental quotient was around 50–60% of normal.

The persistent hypermethioninaemia (135–577 μmol/L) and elevated CK (1400–3040 IU/L) led at 5 months of age to the institution of dietary restriction of methionine to 20 mg/kg and of protein to 1.0–1.4 g/kg. On this diet, the plasma methionine was readily maintained between 10 and 60 μmol/L. This regimen was continued closely for about 5 years but produced no clearly apparent clinical or behavioural improvement, and so was gradually discontinued. By age 6 years, the plasma methionine concentrations were 239–456 μmol/L, all other amino acids being within the normal ranges.

At 14 months of age, biopsies of liver and muscle were performed. Samples of these tissues were sent to Dr Gerald Gaull at the NY State Institute for Basic Research in Mental Retardation and assayed for methionine adenosyltrasferase (MAT) activity using his standard assays (Gaull et al 1979, 1981). No abnormalities were found. Histologically the liver architecture was normal, but in electron microscopy there were extensive cytoplasmic lipid droplets and a paucity of glycogen. Some mitochondria were misshapen with linear crystal-like inclusions (Fig. 1). At the time, these findings were considered nonspecific, possibly related to the restricted diet. Histologically the muscle showed a necrotizing myopathy (Fig. 2) characterized by degeneration of many individual fibres and infiltration by macrophages, but no evidence of inflammation. Numerous cells stained deeply basophilic with Gomori trichrome and there was marked variation in cell size. There were areas of regeneration with basophilic vesicular sarcoplasmic nuclei and nucleoli. Electron microscopy (Fig. 3), showed no pathological subcellular abnormalities, although there were many degenerating fibres with loss of filaments and mononuclear infiltration. Specifically, there were no myelin inclusion bodies. The fibres were of varying size with prominent endomysial connective tissue.

Over the next few years the patient continued to show generalized muscle weakness. Repeated developmental assessments indicated a scattered performance between 50% and 60% of the normal range. He was highly distractible with obsessive behaviour and attention deficit that were described as the worst the clinic had ever encountered. At age 11 years, his overall Stanford–Binet score was 72. CK (1400–3500 IU/L), lactate dehydrogenase (400–850 IU/L), and aspartate transaminase all remained elevated and did not vary consistently with the diet.

He has continued to live at home, being cared for by his parents. He has been seen very intermittently in our clinic, at which times he has continued to show impulsive, perseverative behaviour with echolalia and obsession to certain thoughts and objects that are hardly altered by phenidate or Dexedrine. Muscle strength has been static, but with his increasing size and weight the myopathy is more debilitating; his gait is wide based and waddling and he has had ever-greater difficulty in standing from sitting and was too weak to perform Gower's test. The biochemical tests remain unaltered with plasma methionine 400–900 μmol/L and CK in the range 2000–7590 IU/L.

At 20 years, WAIS testing showed a verbal IQ score of 76, performance IQ 56, and full scale IQ 64. Tests of 'executive function' showed a wide scatter of abilities that would make any long-term employment unlikely to succeed. At 26 years of age he was seen again and his physical examination was unchanged except that movement was even more restricted owing to increased weight. His behaviour was unchanged. At that time the reports by Barić and colleagues (Barić et al 2004, 2005b) stimulated the collaboration that has proved that this patient is also AdoHcy hydrolase-deficient.

Results of studies at age 26 years

Metabolite concentrations (Table 1)

For the patient, methionine, AdoMet and AdoHcy were markedly elevated in plasma, tHcy was near the upper limit of the reference range, and cystathionine was slightly elevated, although sarcosine was not. In single samples of blood and urine, guanidinoacetate excretion was marginally high, but low creatine was not found in either plasma or urine. Phosphatidylcholine was low-normal in plasma, and low in RBC, whereas free choline was low in plasma and normal in RBC. Betaine was elevated in both plasma and RBC. Not listed in Table 1 are normal values for plasma 2-methylcitrate, glycine and serine. Neither parent had an elevation of plasma methionine, AdoMet or AdoHcy.

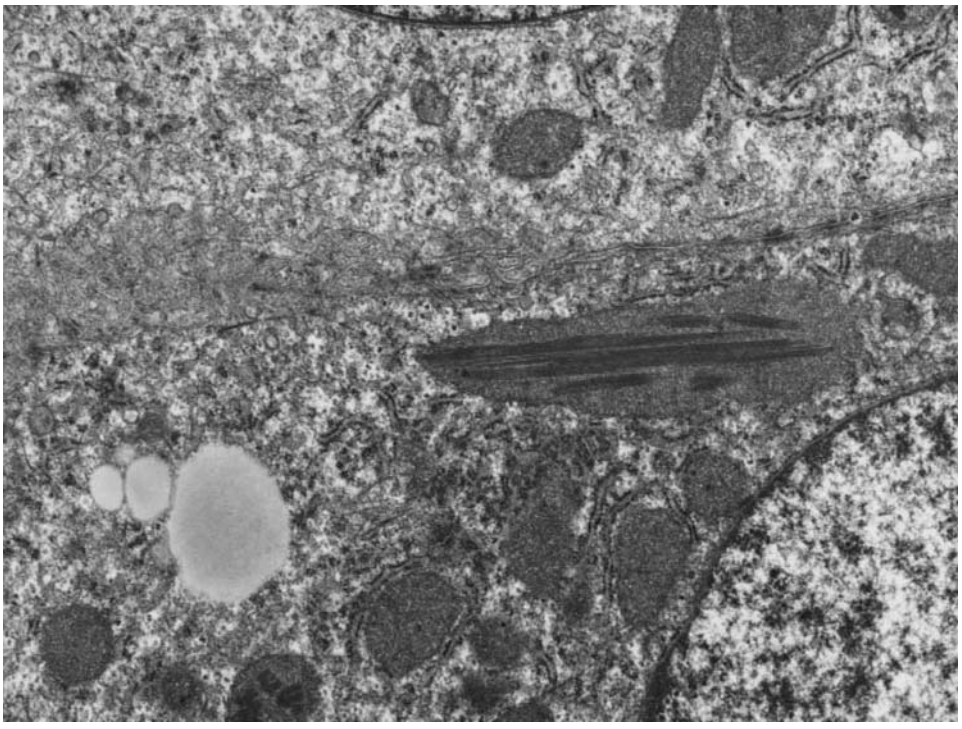

Fig. 1 Electron photomicrograph of liver at age 14 months showing a giant misshapen mitochondrion with paracrystalline inclusions

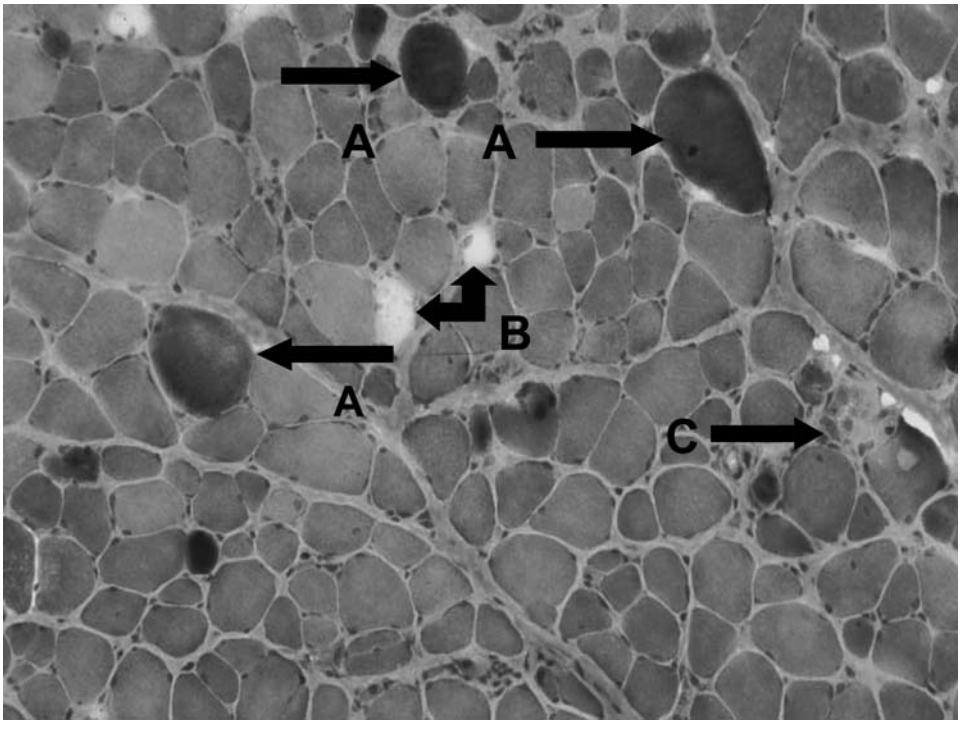

Fig. 2 Gomori trichrome staining of cross-section of quadriceps muscle at age 14 months. Note the normal appearance of most of the cells. A, deeply staining cells, most of which are larger than normal; B, two partially degenerated myocytes; C, a degenerated myocyte infiltrated with macrophages

AdoHcy hydrolase activities

In an initial assay of RBC haemolysates, AdoHcy hydrolase activities (assayed in duplicate, and expressed in nmol/h per mg protein were 1.2 and 1.0 (patient), 3.8 and 3.4 (mother), 5.1 and 4.9 (father), and 6.2 and 6.5 (control subject). A repeat RBC sample from the patient, drawn 9 months later, was assayed four times, giving a mean value of 1.2 ± 0.6 (SD) nmol/h per mg protein ($n = 4$). Samples from four control subjects, each assayed four times along with the patient sample, had an overall mean activity of 5.9 ± 1.1 (SD) nmol/h per mg protein ($n = 16$). The patient's red cell AdoHcy

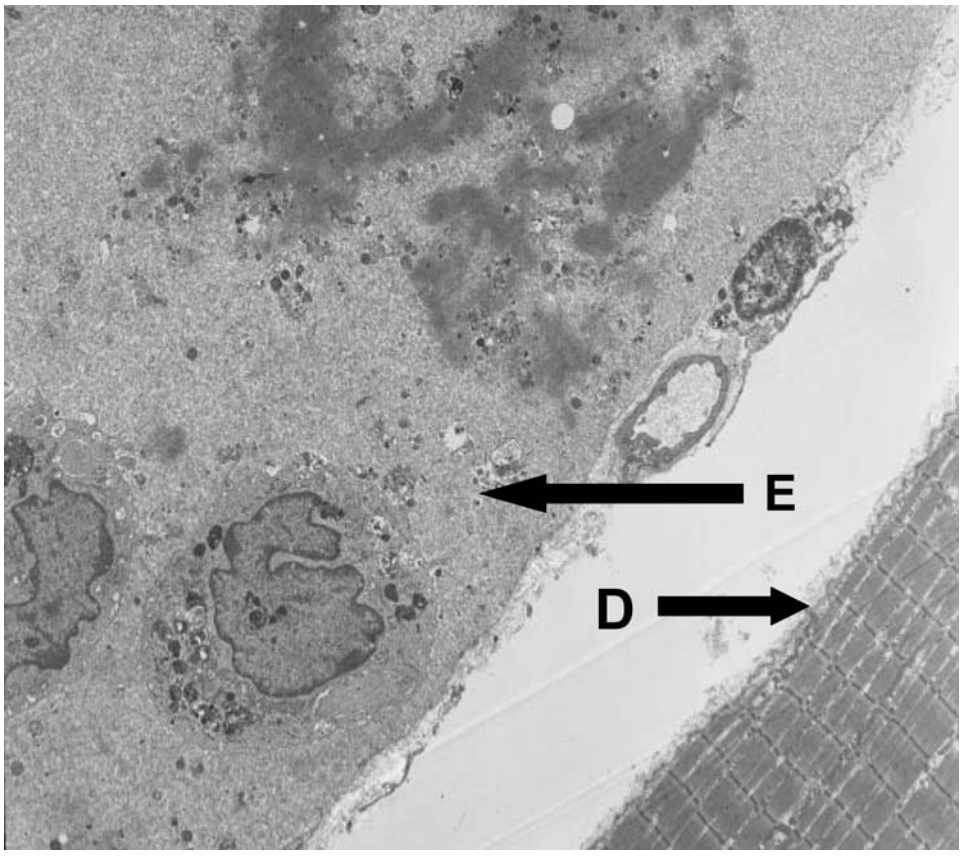

Fig. 3 Electron photomicrograph of quadriceps muscle at age 14 months showing one normal myocyte (D), and one necrotic one (E)

Table 1 Values for the patient, his parents, and a control subject

	Mother	Father	Patient	Patient[a]	Control	Reference range
Plasma values						
Methionine	26	28	542	424	22	3.3–42.7 μmol/L
AdoMet	52	98	1933	1794	76	92.8 ± 16.2 nmol/L
AdoHcy	11	14	772	532	14	27.8 ± 7.9 nmol/L
tHcy	6.0	6.5	14.2	16.6	9.6	5.1–13.9 μmol/L
Cystathionine	108	220	457	379	101	44–342 nmol/L
tCys	309	372	306	315	354	203–369 μmol/L
Dimethylglycine	3.5	6.2	5.2	8.9	3.8	1.42–5.27 μmol/L
Sarcosine	2.0	2.6	2.9	2.3	2.1	0.60–2.67 μmol/L
Methylmalonate	286	170	343	339	181	73–271 μmol/L
Guanidinoacetate	2.39	2.55	2.08	n.a[b]	3.08	0.87–3.64 μmol/L
Creatine	69	32	85	n.a.	14	13–97 μmol/L
PtdCho	1881	2825	1630	n.a.	1922	1500–2559 μmol/L
Free choline	9.3	13.9	6.3	n.a.	11.8	10.7–19.7 μmol/L
Betaine	46	56	664	n.a.	58.5	26–67 μmol/L
Urinary values						
Creatine	n.a.	n.a.	242	n.a.	n.a.	3.4–191 mmol/mol Cr
Guanidinoacetate	n.a.	n.a.	84	n.a	n.a.	25 ± 14 mmol/mol Cr
RBC samples						
PtdCho	1277	1519	989	n.a.	1480	1500–2559 μmol/L
Choline	17.5	15.5	12.2	n.a.	13.6	10.7–19.7 μmol/L
Betaine	37.8	29.5	447	n.a.	25.2	26–67μmol/L

[a]Repeat sample obtained approximately 9 months after the prior sample. [b]n.a., not analysed.

hydrolase activity was therefore about 20% of the latter mean control.

Molecular genetic studies

Sequence analyses of the complete coding region of the AdoHcy hydrolase gene, including all exon splice sites, revealed two point mutations in the patient. The first was a paternally derived exon 4 mutation identical to that described in the two AdoHcy-deficient brothers (Barić et al 2004, 2005b) changing tyrosine to cysteine (c.428A > G; p.Y143C). The second, which was not found in either parent, was an exon 3 mutation changing alanine to valine (c.266C > T; p.A89V). Preliminary data from recombinant protein expression studies show that the alanine to valine exchange decreases AdoHcy hydrolase activity to less than 10% that of the wild-type protein.

Brain MRI and MR spectroscopy

MRI studies of the brain showed normal cerebral hemispheres, brainstem, cerebellum and ventricles without evidence of white-matter changes. Proton spectroscopy showed normal peak heights for creatine, choline and *N*-acetylaspartate.

DNA methylation

Global DNA methylation was assayed by determination of the extent of [^{3}H]dCTP incorporation by single nucleotide extension into DNA of packed blood cells pretreated with a methylation-sensitive restriction enzyme that left guanine overhangs at cleaved methylated sites. The patient's DNA incorporated 2532 dpm/μg DNA, compared to values of 3470 and 3128 into the DNAs of his parents, and 3960 into DNA of a control subject during the same assay. In a subsequent assay, the mean incorporation for five age-matched control subjects was 3208 ± 220 (SD) dpm/μg DNA (range 2965–3480), whereas the incorporation for a second sample of the patient's cells was 2323 dpm/μg DNA, some four SDs below the mean control value. Because in this assay [^{3}H]dCTP incorporation is directly proportional to the number of unmethylated sites, these results indicate the patient's leukocyte DNA was more methylated than were the comparison samples

Serum enzyme activities and albumin

Serum CK was markedly elevated at 3130 U/L (38–397). Alanine transaminase, 69 U/L (10–60), and aspartate transaminase, 66 U/L (10–42), activities were each very slightly elevated. Serum albumin was low-normal at 36 g/L (35–50).

Discussion

Our patient was born just before publication of an article that described a 7½-year-old mentally retarded girl with severe myopathy, elevated plasma methionine and CK, but normal homocystine (Gaull et al 1979). Initially both patients were suspected to have hepatic MAT deficiency, but neither patient showed low activity in liver extracts, and it was concluded they both had a novel type of hypermethioninaemia (Gaull et al 1981). Following the recent identification of AdoHcy hydrolase deficiency (Barić et al 2004), the present patient was studied by the same team. In addition to hypermethioninaemia and elevated CK, the severe elevations of plasma AdoHcy and AdoMet were similar to those in the first two patients (Barić et al 2004, 2005b). The elevated AdoHcy is attributed to defective removal of this compound by AdoHcy hydrolase; the elevated AdoMet to generalized inhibition by AdoHcy of AdoMet-dependent methyltransferases; and the elevation of methionine to decreased flux of methionine to AdoMet when the latter accumulates abnormally (Barić et al 2004, 2005b).

AdoHcy hydrolase deficiency was confirmed by the low activity in RBC haemolysates, as well as by the demonstration of a missense mutation in each of his alleles encoding AdoHcy hydrolase. Among other causes of hypermethioninaemia, cystathionine β-synthase (CBS) deficiency is ruled out by the very minimal elevation of plasma tHcy and by the elevated plasma cystathionine; MAT I/III deficiency by the elevated plasma AdoMet; tyrosinaemia type I by the clinical picture and normal plasma tyrosine concentrations; glycine *N*-methyltransferase (GNMT) deficiency by the elevated plasma AdoHcy (not present in GNMT deficiency (see Augoustides-Savvopoulou et al 2003; Mudd et al 2001)); and citrin deficiency (citrullinaemia type II) by the absence of hypercitrullinaemia and persistence of the hypermethioninaemia (Ohura et al 2003).

The exon 4 p.Y143C missense mutation is paternally derived; it is identical to one of the two found in both brothers from Croatia (Barić et al 2004, 2005b). It seems highly likely the patient described in 1979, also from the former Yugoslavia (Gaull et al 1979, 1981), had the same disorder (see further discussion below). There is no recognized connection of our patient's father to central/eastern Europe, but the known family history does not extend beyond three generations. The exon 3 p.A89V missense in the patient is presumably from a gonadal mutation in the mother. Expression studies show that it is severely inactivating.

The similarities between the three proven cases of AdoHcy hydrolase deficiency are striking and suggest a clear phenotype for this enzyme defect. In view of the severe neonatal hypotonia and the early elevations of CK, muscle (and possibly CNS) damage may originate prenatally. The histological features of the muscle in our patient show striking similarities

between this case, the initial proven case of AdoHcy hydrolase deficiency reported by Barić and colleagues (2004), and the patient described by Gaull and colleagues (Gaull et al 1979, 1981). In all three, the destructive myopathy was obvious, being patchy with some cells apparently intact while others were markedly necrotic. As in our case, both Gaull and colleagues (1981) and Barić and colleagues (Barić et al 2004, 2005a,b) reported varying sizes of myocytes and occasional cells with deeply staining cytoplasm. Gaull and colleagues (1981) noted that on electron microscopy nearly every fibre had myelin body inclusions in the I-band region and elsewhere. The mitochondria were reportedly normal. In the histology reported by Barić and colleagues (2004), electron microscopy (supplemental Fig. 4 in Barić et al 2004) also revealed numerous myelin figures that seem to be associated with the I bands, as well as elsewhere. In some cells many mitochondria were enlarged and abnormally shaped. In our case, the mitochondria did not appear to be abnormal and, unlike the other cases, no myelin inclusion bodies were seen.

Similarities were also apparent in the liver biopsies from the cases of Gaull and colleagues (1981), Baríc and colleagues (2004), and the present patient. All three were thought to show minor histological changes that Barić and colleagues (2004) described as normal architecture with mild chronic active hepatitis. Both in Barić's case and in this one, there was a large increase in lipid droplets**.** Increased smooth endoplasmic reticulum was noted in all three with variable changes in the rough endoplasmic reticulum. In both our case and the one described by Gaull and colleagues (1981), but not in that reported by Barić and colleagues (2004), abnormal mitochondria were seen, some with massive crystalline inclusions. Together with the similar clinical and biochemical results, these histological resemblances strengthen the evidence that the girl reported by Gaull (Gaull et al 1979, 1981) did indeed have AdoHcy hydrolase deficiency.

For our patient, in 1979 dietary restriction of methionine was the only logical treatment available. It was used in the hope that the muscle and developmental problems might be ameliorated. The diet was followed fairly rigorously for about 5 years but with lack of any overt benefits; the family gradually began to relax until, by the age of 6 years, it was *de facto* stopped. It was clear that the plasma methionine was easy to control, but the CK and the clinical manifestations were not improved. It was hard to argue for continuing a restrictive regimen in the absence of any other supportive clinical reports. However, the delayed brain myelination present in both of Barić's young patients before treatment (Barić et al 2004, 2005b) contrasts with that of the present patient. While no MRI images are available from earlier, those done at age 26 years showed no evidence of abnormal myelination. Major myelination is normally completed by 18 months of age, except in a few areas (Kucharczyk et al 1990). With hindsight, it seems possible that methionine restriction in the early years might have been beneficial during the time of rapid myelination. Although this remains unproven, it adds incentive to consider methionine restriction for other AdoHcy-deficient patients at least during the early years.

Methionine restriction in Barić's two cases clearly resulted in decreases in plasma AdoHcy, AdoMet and methionine. In mammals, at least 39 AdoMet-dependent methyltransferases are known, almost all of which are inhibitable by AdoHcy (Clarke and Banfield 2001). Prior to treatment, the initial patient had abnormally low plasma concentrations of phosphatidylcholine and free choline and elevated guanidinoacetate, suggesting inhibition of at least two methyltransferases, phosphatidylethanolamine and guanidinoacetate methyltransferases (Barić et al 2004). These abnormalities were not observed in the second patient, perhaps because he was younger when studied and started sooner on treatment (Barić et al 2005b). The products formed by phosphatidylethanolamine and guanidinoacetate methyltransferases – phosphatidylcholine and creatine, respectively – were provided as dietary supplements to both patients. These compounds can be ingested safely and deficiency of either or both might play a role in retarded brain and/or muscle development. In both patients during treatment there were improvements in muscle strength and in brain myelination (Barić et al 2005b), affording a cogent reason to initiate similar therapy for our patient.

Other forms of hypermethioninaemia are not associated with necrotizing myopathy, indicating that this elevation does not, *per se*, play an causative role, at least in the muscle damage. Although in the first two patients dietary supplementation with phosphatidylcholine and creatine was accompanied by marked gains in muscle strength, their CK concentrations remained severely elevated (Barić et al 2004, 2005b), suggesting that the pathophysiology of the myopathy is complex and may involve other mechanisms. The lack of involvement of cardiac muscle must indicate a significant difference in the metabolism of cardiac and skeletal muscle.

The results reported here extend to all three proven AdoHcy hydrolase-deficient patients' increased global methylation of blood cell DNA. For further discussion of this finding, see Baric and colleagues (2005b) and the paper by Devlin and colleagues (2005) that reports tissue-specific increased DNA methylation of gene *H19* in brain and aorta of experimental mice accumulating elevated AdoHcy while being fed a diet leading to hyperhomocysteinaemia. The three known patients also had elevated plasma cystathionine (due possibly to diversion of any available homocysteine towards cystathionine synthesis by stimulation of CBS activity by AdoMet, and/or inhibition of cystathionine gamma-lyase by methionine). Two of the three patients also have elevations of betaine – previously attributed to inhibition of betaine-homocysteine methyltransferase by methionine (Barić et al 2005b).

With regard to the possible detection of AdoHcy hydrolase deficiency by newborn screening for hypermethioninaemia, the present patient did have mildly elevated blood methionine detected by a newborn Guthrie screen (methionine 200 μmol/L at age 3 days). However, since the value was not very high, he would not automatically have fallen into a follow-up protocol if Oregon did not routinely repeat testing between 2 to 6 weeks. The first patient was shown retrospectively not to have sufficient hypermethioninaemia to be detected by a newborn screen (Barić et al 2004), and in the younger brother plasma methionine was only 31 μmol/L at age 3 days and rose no higher than 74 μmol/L (at age 0.8 months) before methionine restriction was started around 3 months of age (Barić et al 2005b). Thus newborn screening for elevated methionine is not a reliable way to detect AdoHcy hydrolase deficiency. Assay of plasma AdoHcy might be a more definitive approach.

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