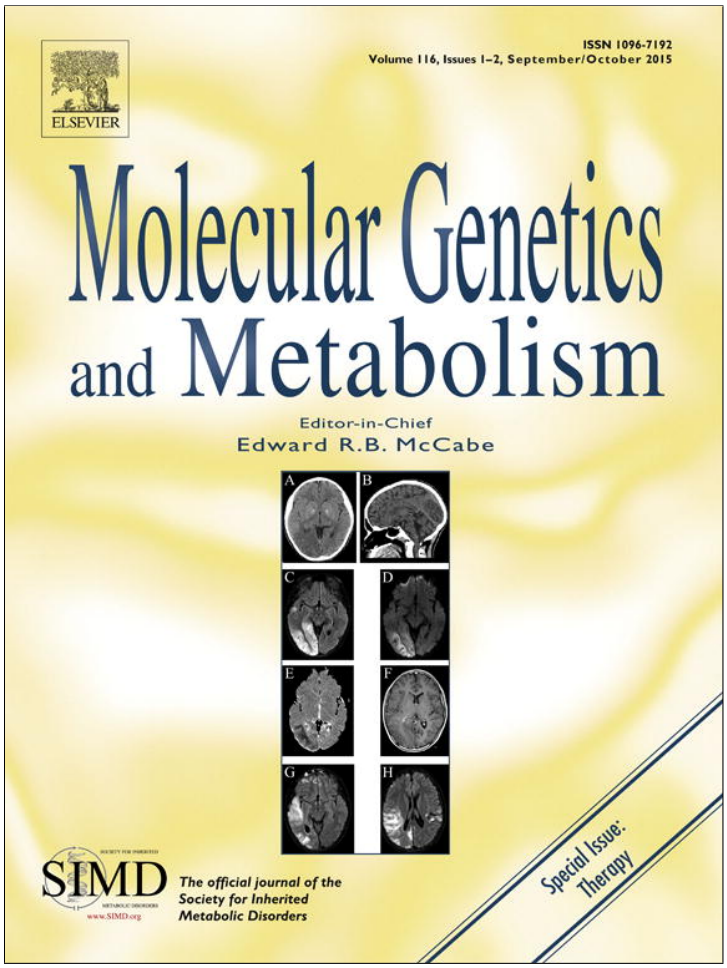

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Liver transplantation for treatment of severe S-adenosylhomocysteine hydrolase deficiency☆

Kevin A. Strauss [a,b,c,*,1], Carlos Ferreira [d,1], Teodoro Bottiglieri [e], Xueqing Zhao [f], Erland Arning [e], Shucha Zhang [f], Steven H. Zeisel [f], Maria L. Escolar [g], Nancy Presnick [a], Erik G. Puffenberger [a,b], Oliver Vugrek [h], Lucija Kovacevic [h], Conrad Wagner [i], George V. Mazariegos [j], S. Harvey Mudd [k,2], Kyle Soltys [j,1]

[a] Clinic for Special Children, Strasburg, PA, USA
[b] Franklin and Marshall College, Lancaster, PA, USA
[c] Lancaster General Hospital, Lancaster, PA, USA
[d] National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA
[e] Center of Metabolomics, Institute of Metabolic Disease, Baylor Research Institute, Dallas, TX, USA
[f] Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, NC, USA
[g] Program for the Study of Neurodevelopment in Rare Disorders and Center for Rare Disease Therapy, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
[h] Translational Medicine Group, Ruđer Bošković Institute, Zagreb, Croatia
[i] Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA
[j] Hillman Center for Pediatric Transplantation, Thomas E. Starzl Transplant Institute and Center for Rare Disease Therapy, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA
[k] Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD, USA

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ABSTRACT

A child with severe S-adenosylhomocysteine hydrolase (AHCY) deficiency (*AHCY* c.428A > G, p.Tyr143Cys; c.982 T > G, p.Tyr328Asp) presented at 8 months of age with growth failure, microcephaly, global developmental delay, myopathy, hepatopathy, and factor VII deficiency. Plasma methionine, S-adenosylmethionine (AdoMet), and S-adenosylhomocysteine (AdoHcy) were markedly elevated and the molar concentration ratio of AdoMet:AdoHcy, believed to regulate a myriad of methyltransferase reactions, was 15% of the control mean. Dietary therapy failed to normalize biochemical markers or alter the AdoMet to AdoHcy molar concentration ratio. At 40 months of age, the proband received a liver segment from a healthy, unrelated living donor. Mean AdoHcy decreased 96% and the AdoMet:AdoHcy concentration ratio improved from 0.52 ± 0.19 to 1.48 ± 0.79 mol:mol (control 4.10 ± 2.11 mol:mol). Blood methionine and AdoMet were normal and stable during 6 months of follow-up on an unrestricted diet. Average calculated tissue methyltransferase activity increased from 43 ± 26% to 60 ± 22%, accompanied by signs of increased transmethylation in vivo. Factor VII activity increased from 12% to 100%. During 6 postoperative months, head growth accelerated 4-fold and the patient made promising gains in gross motor, language, and social skills.

1. Introduction

S-adenosylhomocysteine hydrolase (AHCY), encoded by *AHCY* on chromosome 20, catalyzes the cleavage of S-adenosylhomocysteine (AdoHcy) to adenosine and homocysteine. It plays a pivotal role in the transsulfuration–transmethylation cycle that regulates tissue methionine supply and distributes methyl groups among scores of substrates (Fig. 1) [1–3]. One of three tissue-specific methionine adenosyltransferases converts methionine to S-adenosylmethionine (AdoMet), which is the principal methyl donor in mammalian systems and establishes a complex array of equilibria with AdoHcy via more than 100 mammalian methyltransferase enzymes (Fig. 1).

It is estimated that between 0.6 and 1.6% of all human genes encode AdoMet-dependent methyltransferases [4], which modify diverse substrates (DNA, mRNA, tRNA, rRNA, proteins, lipids) and contribute to key biosyntheses (e.g. heme, ubiquinone, catecholamines, melatonin) [5]. Quantitatively, the highest demand for methyl groups is shared by three enzymes: guanidinoacetate *N*-methyltransferase (GAMT), phosphatidylethanolamine methyltransferase (PEMT), and glycine *N*-methyltransferase (GNMT) catalyze the synthesis of creatine, phosphatidylcholine, and sarcosine, respectively (Fig. 1). Together,

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* Corresponding author at: Clinic for Special Children, 535 Bunker Hill Road, Strasburg PA 17579, USA.
E-mail address: kstrauss@clinicforspecialchildren.org (K.A. Strauss).

[1] Equal contributors.
[2] Posthumous.

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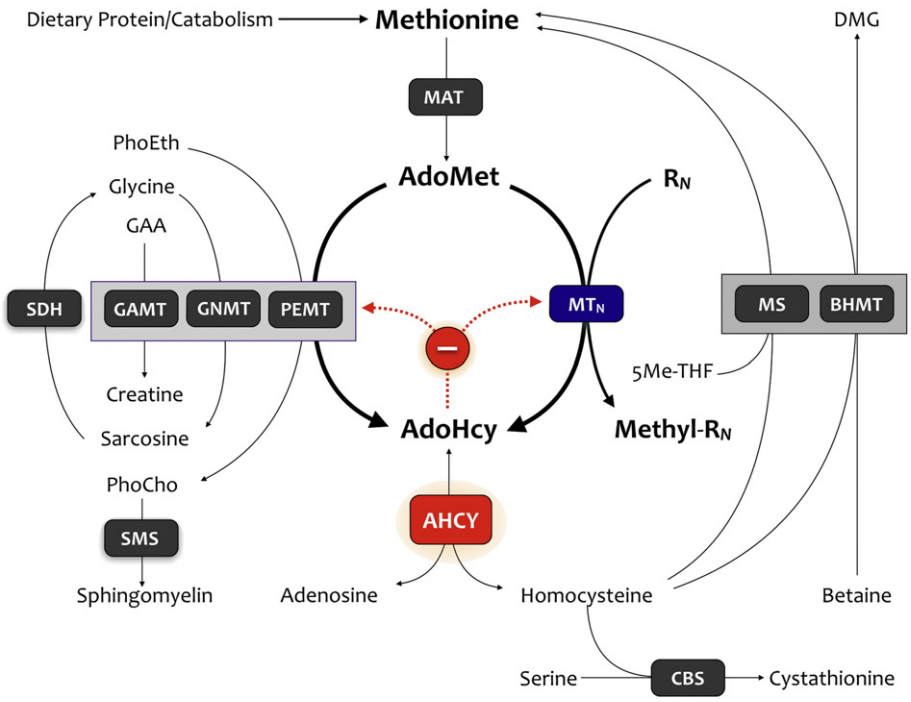

Fig. 1. Transsulfuration–transmethylation cycle and its major intermediates. Methionine is an essential amino acid derived from the diet and, to a more limited extent, endogenous protein catabolism. It can be recycled through the actions of 5-methylfolate (5Me-THF)- and vitamin B_{12}-dependent methionine synthase (MS) or betaine homocysteine methyltransferase (BHMT). Methionine is the source of S-adenosylmethionine (AdoMet), the principal methyl donor for transmethylation reactions in mammalian systems, ~85% of which occur in hepatocytes. Three enzymes—guanidinoacetate *N*-methyltransferase (GAMT), phosphatidylethanolamine methyltransferase (PEMT), and glycine *N*-methyltransferase (GNMT)—account for the majority of total transmethylation flux in humans. The glycine:sarcosine ratio in tissues is separately regulated by sarcosine dehydrogenase (SDH). Phosphatidylcholine is converted to sphingomyelin via the action of sphingomyelin synthase (SMS). More than 100 other methyltransferase enzymes (MT_N) distributed throughout human tissues mediate methyl transfer between AdoMet and diverse cellular substrates (R_N) to yield methyl-R_N and S-adenosylhomocysteine (AdoHcy). The latter is reversibly cleaved by AdoHcy hydrolase (AHCY) to yield adenosine and homocysteine, which is further metabolized by cystathionine beta-synthase (CBS) to produce cystathionine. AdoHcy is an allosteric inhibitor of most tissue transferases (indicated by red dotted lines).

these products account for the majority of total methyl flux in humans (5.7 to 14.0 mmol/m^2/day), but more than 30 other methylated compounds can be recovered from human urine [3,6].

A decade has passed since the first description of human AHCY deficiency [7]. Six additional case reports appeared in the literature between 2005 and 2012 [8–12]. Severe AHCY deficiency presents during infancy with some combination of growth failure, microcephaly, psychomotor delay, epilepsy, hypomyelination, myopathy, hepatopathy, factor VII deficiency, and marked elevations of blood methionine, AdoMet, and AdoHcy. A distinctive biochemical characteristic of AHCY deficiency is reversal of the normal AdoMet:AdoHcy molar concentration relationship, believed to exert control over numerous methyltransferase enzymes that may be regulated by feedback inhibition from AdoHcy (Fig. 1) [13,14]. Despite dietary interventions, developmental outcomes are poor [7–9].

Although AdoMet is synthesized in all mammalian cells, transsulfuration and transmethylation reactions are concentrated in the liver. On a whole body basis, hepatocytes synthesize 50% of AdoMet, mediate 85% of all transmethylation reactions [15,16], and have the highest AdoMet transmembrane gradient (~1400-fold) [17]. AHCY activity is similarly enriched in the liver, which has 4-fold higher *AHCY* mRNA expression than most other tissues and 10-fold higher gene expression than the brain (http://biogps.org/). Hepatic activity of AHCY is therefore a focal point of systemic AdoMet and AdoHcy homeostasis that can influence methylation status and physiological function of tissues throughout the body.

Here we describe a child with severe AHCY deficiency who received a liver segment from a healthy, unrelated living donor at 40 months of age. We observed significant and stable biochemical improvement, accelerated head growth, and developmental progress during 6 months of postoperative follow-up, suggesting a broader role for liver transplantation in treatment of human transsulfuration–transmethylation disorders.

2. Methods

2.1. Clinical and biochemical methods

The study was approved by the Institutional Review Board of Lancaster General Hospital and the proband's parents consented in writing to all procedures and practices. At 39 months of age, just prior to liver transplantation, the proband was evaluated for visual, linguistic, and motor skills using the Mullen Scales of Early Learning (AGS Edition) [18] and Peabody Developmental Motor Scales, 2nd Edition [19], Gross Motor Function Measure. Skill levels were represented as developmental quotients (DQ = developmental age/chronological age). Brain magnetic resonance imaging was obtained at 9, 18, 32, and 39 months of age.

Plasma AdoMet, AdoHcy, and related transsulfuration–transmethylation metabolites were measured by tandem mass spectrometry (Shimadzu Nexera LC System interfaced with a 5500 QTRAP® Sciex) as previously described [20] and quantified using stable isotopes. Total plasma homocysteine and 5-methyltetrahydrofolate (5-MeTHF) were determined by LC–ESI-MS/MS [21]. Choline and its metabolites in plasma were measured by liquid chromatography-stable isotope dilution-multiple reaction monitoring mass spectrometry as previously described [22]. Transsulfuration–transmethylation and choline analytes from the proband were compared to those from 36 healthy control subjects [22]. Plasma concentrations of fluoraldehyde-conjugated amino acids were measured by high-performance liquid chromatography (Agilent 1100 series) and results were compared to values from 51

children who were not affected by disorders of amino or organic acid metabolism.

2.2. Calculations

2.2.1. Cerebral SLC7A5 amino acid influx

Cerebral influx of essential amino acids was estimated using a custom-formatted Excel spreadsheet (Microsoft Corporation) as previously described [23–26]. Briefly, methionine competes with nine other zwitterionic amino acids (leucine, valine, isoleucine, tryptophan, threonine, tyrosine, phenylalanine, histidine, and glutamine) for exchange across a common sodium-independent facilitative blood–brain barrier transporter (SLC7A5; a.k.a. LAT1) [26,27]. Cerebral capillary SLC7A5 is saturated under physiological conditions, such that brain uptake of each amino acid is influenced by ambient plasma concentrations of its competitors [26]. Substrate competition is expressed by an *apparent* K_m, called K_{app} (μM), calculated for each amino acid according to the equation:

$$K_{app} = K_m\left[1 + \sum\nolimits_{1\to n}(C_i/K_i)_n\right]$$

where K_m is the classical Michaelis–Menten affinity constant for the single amino acid of interest, C_i is the plasma concentration (μM) for each of *n* competitors, and K_i is the classical affinity constant of that competitor (μM).

For a given plasma amino acid profile, K_{app} values were determined for each substrate using published Michaelis–Menten parameters for SLC7A5 [26]. The K_{app} value was then used to calculate brain influx (nanomoles per minute per gram of brain tissue; nmol/min/g) of each amino acid in the competing group, according to the equation:

$$\text{Brain Influx} = (V_{max})(C)/(K_{app} + C)$$

where V_{max} and C are maximal transport velocity (nmol/min/g) and plasma concentration (μM), respectively, of each amino acid. Results for estimated brain influx values were compared to calculations from a population of control subjects (N = 51).

2.2.2. Hepatic fractional methyltransferase activities

A second Excel spreadsheet was formatted to estimate the impact of altered AdoMet:AdoHcy molar concentration ratios on equilibrium flux through 23 different AdoMet-dependent tissue methyltransferases (Table S1) [14]. Equilibrium flux was expressed as a percentage of hypothetical control velocity (fractional V_{max}) based on published Michaelis–Menten parameters for each methyltransferase [14]. To estimate the kinetic impact of altered AdoMet and AdoHcy concentrations, we used the formula [14]:

$$\text{Fractional } V_{max} = C_{AdoMet}/\left[K_m + K_m\left(C_{AdoHcy}\right)/Ki + C_{AdoMet}\right]$$

where V_{max} is maximal unidirectional velocity of the enzyme of interest, K_m is the Michaelis–Menten affinity constant for AdoMet, K_i is the inhibition constant for AdoHcy, and C_{AdoMet} and C_{AdoHcy} represent estimated intracellular liver concentrations of AdoMet and AdoHcy, respectively, assuming that 1) hepatocyte AdoMet concentrations are approximately 1400-fold higher than those of plasma [17,28,29], and 2) the extracellular AdoMet:AdoHcy molar concentration ratio is in chemical equilibrium with the AdoMet:AdoHcy ratio of intracellular fluid [17,30,31].

We first calculated fractional V_{max} for control subjects (N = 36) using measured mean values for AdoMet (91 nM), AdoHcy (27 nM), and AdoMet:AdoHcy (4.1 mol:mol) (Table 1), and assuming hepatocyte AdoMet and AdoHcy concentrations of 127 μM and 31 μM, respectively, which align closely with experimentally derived values [17]. Results were then compared to fractional V_{max} calculated for the proband under three conditions: 1) untreated, 2) dietary therapy, and 3) post-liver transplantation. For each condition, methyltransferase activity was expressed as a percentage of control methyltransferase activity, as depicted in Fig. 3.

2.3. Statistics

Biochemical data are represented as mean ± one standard deviation unless otherwise specified. For selected measures, one-way analysis of variance (ANOVA) followed by Tukey post-test for pairwise comparisons was used to search for differences among three groups: 1) control subjects, 2) proband on dietary therapy, and 3) proband post-liver transplantation (Prism 6, GraphPad). Molar concentration ratios were $\log_{10}$ transformed for statistical comparisons. For graphical clarity, calculated cerebral amino acid influx values were converted to standard scores (i.e. z-scores), where z = (patient value–control mean)/control standard deviation.

3. Case report

3.1. Clinical synopsis

The female proband was born by Caesarean section at 37 weeks gestational age to non-consanguineous parents of mixed European Caucasian ancestry. Birth weight was 2557 g and occipitofrontal head circumference was 31.5 cm (z-score −2.5). Results of expanded state newborn screening by tandem mass spectrometry were normal, including a dried filter paper blood spot methionine level of 20 μM. Psychomotor development was delayed in all streams. She rolled at 18 months, sat unsupported at 24 months, and walked independently at age 33 months, by which time she exhibited acoustic hypersensitivity, inattention, self-injurious behavior (head banging), and impulsive aggression (biting and pinching caregivers). Bilateral esotropia was surgically corrected at 17 months. Physical examination was notable for low body weight (weight z-score −2.5; body mass index 13 kg/m^2), microcephaly (occipitofrontal head circumference z-score −3.4) (Fig. 2), diffuse hypotonia, sarcopenia, and hyporeflexia. Brain imaging at age 9 months showed global hypomyelination (Fig. 2).

The diagnosis of AHCY deficiency was established at age 8 months, after the proband had a generalized seizure that prompted deeper investigation. Laboratory testing revealed hypermethioninemia (614 μM; reference 31 ± 9 μM), myopathy (total creatine kinase 1086 IU/L), hepatopathy (alanine aminotransferase 268 IU/L), and coagulopathy (international normalized ratio 1.4) caused by autoantibody-negative factor VII deficiency (28% activity, normal activity 50–150%) (Table 1). Branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) were low relative to their competing SLC7A5 substrates and calculated brain influx of leucine, valine, and isoleucine were reduced 71%, 67%, and 52%, respectively. Plasma AdoMet and AdoHcy were markedly elevated at 5109 nM (reference 91 ± 28 nM) and 8139 nM (reference 27 ± 15 nM), respectively, and their molar concentration ratio (0.60 mol:mol) was only 15% of the control mean (reference 4.10 ± 2.10 mol:mol) (Table 1). Sanger sequencing revealed compound heterozygous mutations of *AHCY* (c.428A > G, p.Tyr143Cys; c.982 T > G, p.Tyr328Asp).

3.2. Dietary therapy

Dietary therapy starting at 22 months consisted of methionine restriction (≤35 mg/kg/day; i.e. ≤2 g total dietary protein per kg/day) and dietary supplements of creatine (300 mg/kg/day) and phosphatidylcholine (200 mg/kg/day) (Fig. 1) [7,8]. Plasma methionine decreased to 74 ± 179 μM (reference 31 ± 9 μM). Diet did not correct persistent deficits of plasma BCAAs and tryptophan (Table 2), such that calculated brain methionine influx remained >2-fold control values and cerebral BCAA and tryptophan influx were reduced to 37% and 23%, respectively (Table 2, Fig. 4).

Table 1
Plasma and serum measurements in child with AHCY deficiency at diagnosis, on dietary therapy, and post-liver transplant.

	Control (N = 36)[a]		AHCY diagnosis	AHCY diet (n = 10)		AHCY LT (n = 7)		ANOVA P	Tukey pairwise comparisons		
	Mean	SD	(n = 1)	Mean	SD	Mean	SD		Ctl v. diet	Ctl v. LT	Diet v. LT
Transsulfuration intermediates											
Methionine (μM)	31	9	591	74	179	33	15	0.2563			
S-adenosylmethionine (nM)	91	28	5109	1533	778	130	15	<0.0001	****		****
S-adenosylhomocysteine (nM)	27	15	8139	2995	1245	113	57	<0.0001	****		****
Homocysteine, total (μM)	7.5	3.0	23.1	4.2	1.4	3.8	0.9	0.0055	*	**	
Cystathionine (nM)	252	147	791	213	259	382	207	0.1767			
5-Methyltetrahydrofolate (nM)	19.4	8.5	–	68.7	20.6	59.7	17.4	<0.0001	****	****	
Methylation cycle substrates and products											
Choline (μM)	10.3	3.0	–	13.9	12.1	7.4	3.6	0.108			
Phosphatidylcholine (μM)	2146	414	–	1347	371	2606	51	0.0004	**		***
Sphingomyelin (μM)	541	103	–	436	60	635	87	0.0392			*
Guanidinoacetate, plasma (μM)	1.4	0.6	–	6.8	0.4	1.3	0.4	<0.0001	****		****
Creatine, plasma (μM)	63	37	–	56	24	96	11	0.0854			
Betaine (μM)	39	24	–	773	536	43	27	<0.0001	****		****
Dimethylglycine (μM)	5.3	1.6	12.6	8.8	5.1	2.6	1.1	0.0001	**	*	****
Key metabolite concentration ratios[b]											
AdoMet/AdoHcy (mol:mol)	4.10	2.11	0.63	0.52	0.19	1.48	0.79	<0.0001	****	****	**
Met/AdoMet (mol:mol)	0.37	0.19	0.14	0.04	0.08	0.27	0.10	<0.0001	****		****
Phosphatidylcholine/choline (mol:mol)	228	82	–	133	105	313	90	0.0047	*		**
Guanidinoacetate/creatine (mol:mol)	0.022	0.010	–	0.143	0.072	0.014	0.004	<0.0001	****	****	****
Betaine/dimethylglycine (mol:mol)	0.83	0.26	–	1.81	0.48	1.24	0.19	<0.0001	****	*	**
Tissue disease markers											
Alanine transaminase (IU/L)	16	7	268	128	88	132	53	<0.0001	****	****	
Creatine kinase, total (IU/L)	100	38	1086	482	396	1314	44	<0.0001	****	****	****

Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; AHCY, S-adenosylhomocysteine hydrolase; LT, liver transplantation; Met, methionine; SD, standard deviation.
Significance levels: **** < 0.0001;*** < 0.001; * < 0.01; * < 0.05.
[a] "N" indicates number of subjects; "n" indicates number of samples obtained for a single subject.
[b] Molar concentration ratios were $\log_{10}$ transformed for ANOVA testing.

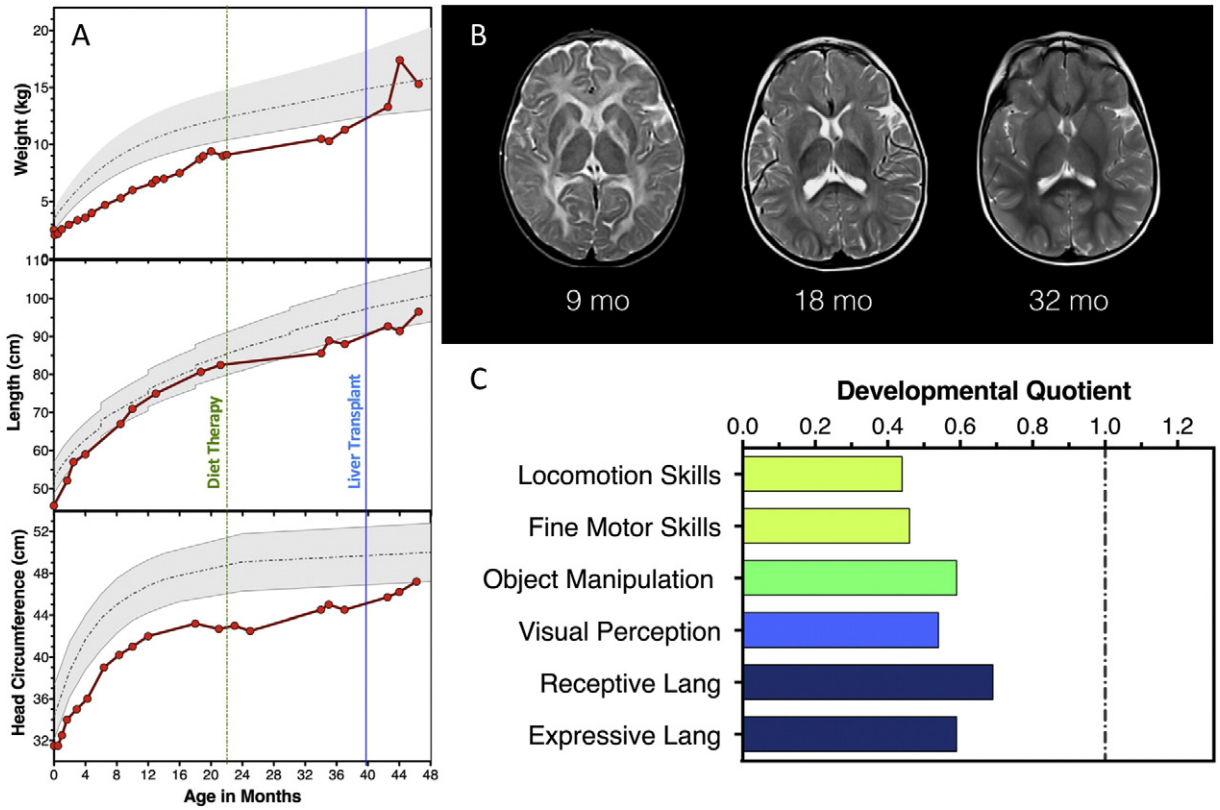

Fig. 2. (A) The proband had a low weight and head circumference at birth. Growth trajectories were not significantly altered by initiation of dietary therapy at 22 months of age (green dotted line). Weight gain increased markedly after liver transplant at 40 months of age (blue solid line) but then stabilized. Head growth increased from 0.11 to 0.41 cm/month during the 6 month post-transplant interval. (B) Comparable axial T2-weighted MRI scans show hypomyelination at age 9 months, which was improving without specific treatment by age 18 months. Cerebral myelination was age-appropriate on follow-up scans at 32 and 39 months. (C) Despite relatively preserved signal intensities of brain tissue, head growth remained poor on dietary therapy and development was severely delayed in all domains tested. Developmental quotients, obtained at age 39 months, represent developmental age/chronological age.

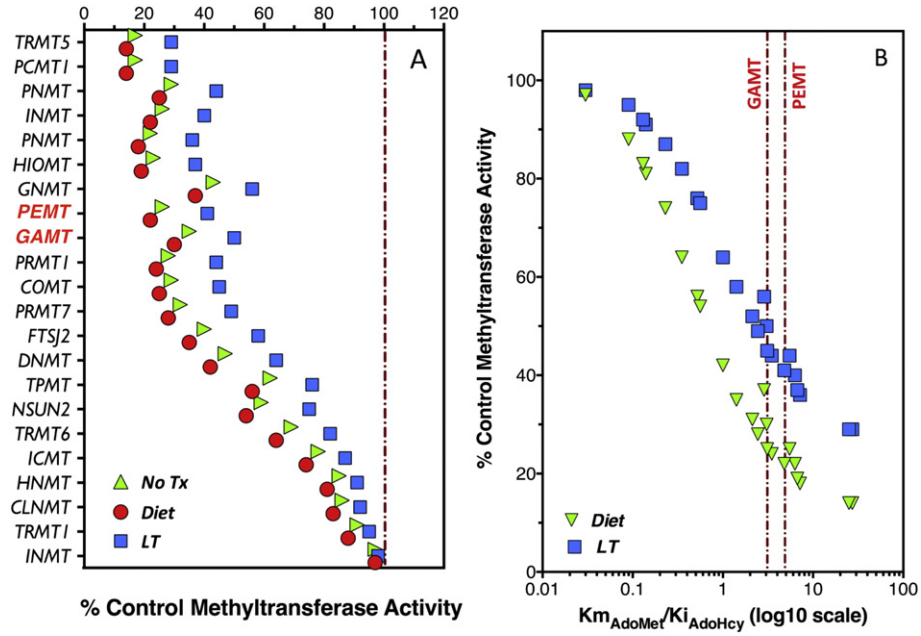

Fig. 3. Calculated tissue methyltransferase activities. (A) Calculated enzyme activity as a percentage of control is shown for tissue methyltransferases with diverse target substrates, including nucleotides (DNA, tRNA, rRNA), lipids, proteins, and small molecules (See text and Table S1 for details of calculation method). The red dotted line indicates 100% control enzyme activity. Predicted transmethylation rates prior to treatment (red circles) were 47 ± 26% (range 17–97%) of control values and did not change appreciably with institution of dietary treatment (green triangles). Liver transplantation (blue squares) was associated with a 3-fold increase in the circulating AdoMet:AdoHcy ratio (from 0.52 ± 0.19 to 1.48 ± 0.79 mol:mol), which increased calculated global transmethylation rates to 60 ± 22% (range 29–98%). (B) The sensitivity of an enzyme to inhibition by AdoHcy is dependent on the ratio between the Michaelis–Menten constant for AdoMet (K_m) and inhibition constant for AdoHcy (K_i). Although *global* calculated transmethylation rates increased only 28% after liver transplantation, predicted activity increased 65% to 85% for enzymes such as GAMT and PEMT (red font, red dotted lines), which have a high Km_{AdoMet}/Ki_{AdoHcy} ratio. These predictions are consistent with observed postoperative increases of plasma creatine and phosphatidylcholine.

On dietary therapy, plasma AdoMet and AdoHcy decreased 70% and 63%, respectively. However, AdoHcy remained >100-fold elevated and plasma AdoMet:AdoHcy molar concentration ratio (0.52 ± 0.19 mol:mol) was essentially unchanged at 87% below the reference value (4.10 ± 2.10 mol:mol) (Table 1). While on dietary therapy, calculated average reaction velocities of 23 different tissue methyltransferases (Table S1) were 43 ± 26% (range 14–97%) of $V_{control}$ and did not differ from calculated pre-treatment values (47 ± 26%, range 17–97%) (Fig. 3). Dietary supplements did not correct plasma creatine or phosphatidylcholine levels (Table 1), reflecting persistent in vivo inhibition of GAMT and PEMT, respectively (Table S1, Fig. 1). Betaine levels remained markedly elevated (773 ± 536 μM; reference 39 ± 24 μM) (Table 1), accompanied by a

Table 2
Amino acid concentrations and calculated cerebral amino acid influx associated with treatment of AHCY deficiency.

	Control (N = 51)		AHCY no tx (n = 4)		AHCY diet (n = 12)		AHCY LT (n = 10)		ANOVA P	Tukey pairwise comparisons[a]		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		Ctl v. diet	Ctl v. LT	Diet v. LT
Plasma concentration (μM)												
Leucine	119	38	47	48	57	33	92	35	<0.0001	****		
Phenylalanine	55	18	48	29	47	26	75	24	0.0055		*	**
Tryptophan	50	18	42	33	28	7	36	5	<0.0001	***	*	
Tyrosine	63	24	74	75	50	36	74	21	0.0992			
Isoleucine	65	25	25	21	34	22	63	29	0.0011	***		*
Histidine	87	29	74	15	91	27	86	23	0.8904			
Valine	207	61	146	107	117	55	184	64	<0.0001	****		*
Glutamine	527	146	670	102	752	199	736	131	<0.0001	****	***	
Methionine	31	10	657	469	81	172	35	15	0.0826			
Threonine	120	44	124	68	89	38	149	67	0.0140			*
Cerebral influx (nmol/min/g)												
Leucine	12.35	2.25	3.58	2.86	7.47	2.14	9.29	2.02	<0.0001	****	***	
Phenylalanine	10.45	1.93	7.00	3.99	11.85	2.85	14.06	2.25	<0.0001		****	*
Tryptophan	9.61	2.95	4.78	1.13	7.44	2.31	6.89	1.33	0.0031	*	*	
Tyrosine	4.81	1.29	3.23	1.89	4.68	1.43	5.58	0.75	0.1771			
Isoleucine	3.53	0.92	1.16	1.11	2.32	0.69	3.31	1.17	0.0006	***		*
Histidine	2.78	0.89	2.06	1.42	3.91	1.05	2.65	0.42	0.0004	***		**
Valine	2.50	0.48	1.19	0.45	1.83	0.38	2.14	0.41	<0.0001	****		
Glutamine	1.42	0.57	1.85	1.91	2.71	0.94	1.91	0.51	<0.0001	****		*
Methionine	1.00	0.32	11.39	7.31	2.35	3.80	1.06	0.34	0.0265	*		
Threonine	0.48	0.14	0.35	0.09	0.46	0.07	0.57	0.21	0.1495			

Abbreviations: AHCY, AdoHcy hydrolase deficiency; Ctl., control population (N = 51); LT, liver transplant; N, population size; n, number of data individual data points for analysis.
[a] Significance levels: **** < 0.0001;*** < 0.001; * < 0.01; * < 0.05.

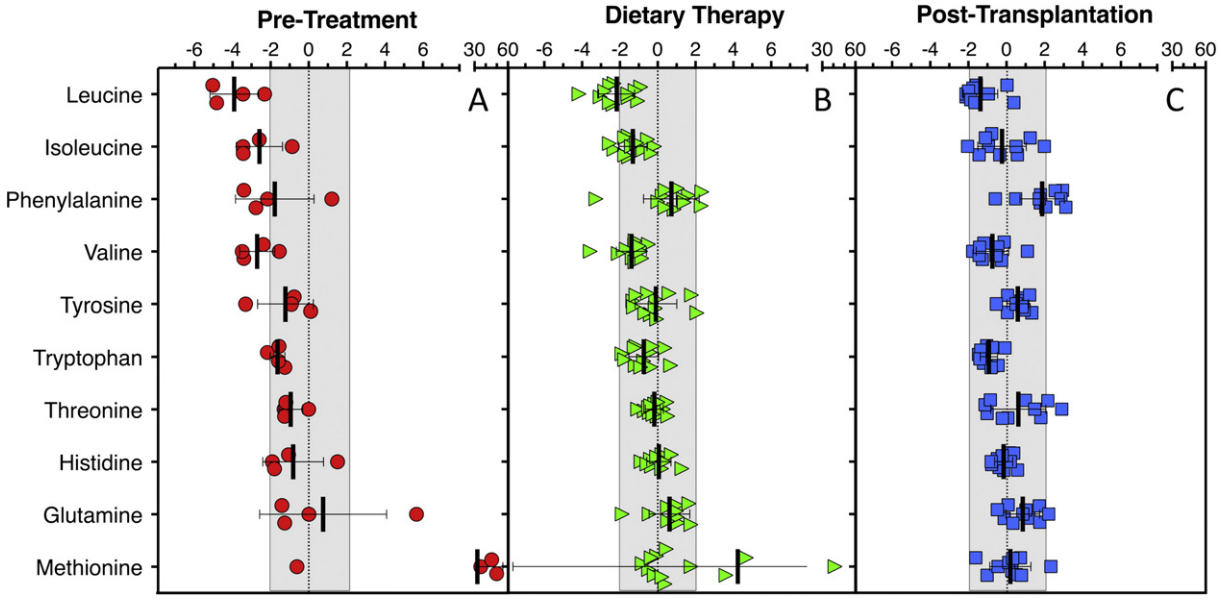

Fig. 4. Calculated cerebral influx rates for SLC7A5 amino acid substrates are depicted as z-scores, where z = (patient value–control mean)/control standard deviation, where >90% of normal values fall between z of −2 and +2 (gray shading) (see text for details of calculation method). Individual values are represented for the proband before treatment (A, red circles), during dietary therapy (B, green triangles), and after liver transplantation (C, blue squares) (error bars, mean ± 1SD). Prior to treatment, calculated brain influx of leucine, valine, and isoleucine were reduced 71%, 67%, and 52%, respectively. Only cerebral leucine influx remained significantly (i.e. 25%) lower than control following liver transplantation. Cerebral phenylalanine influx increased.

5-fold increase of the betaine:dimethylglycine ratio, indicating impaired recycling of homocysteine to methionine via hepatic betaine homocysteine methyltransferase (BHMT) (Fig. 1).

Dietary therapy did not accelerate weight gain or head growth, and psychomotor development remained stagnant (Fig. 2). At age 39 months, developmental level was lowest for gross (DQ = 0.44) and fine (DQ = 0.46) motor skills, but also substantially delayed in domains of visual reception (DQ = 0.54), adaptive object manipulation (DQ = 0.59), receptive language (DQ = 0.69), and expressive language (DQ = 0.59). Myopathy and hepatopathy improved but did not resolve (Table 2) and factor VII activity reached a nadir of 12% (reference 50–150%) prior to liver transplantation (Fig. 5).

3.3. Liver transplantation

Severe AHCY deficiency as a new indication for liver transplantation was approved by the Children's Hospital of Pittsburgh's Innovative Procedure Committee and both parents consented to the operation. At 40 months of age, the proband received a 428 g left lateral segment liver allograft from a 35 year-old living unrelated donor. The allograft was placed in orthotopic position after uneventful piggyback hepatectomy during a total intraoperative time of 437 min. Warm and cold ischemic times were 60 and 31 min, respectively. Immunosuppression included 10 mg/kg methylprednisolone prior to reperfusion, a standard recycle to 1 mg/kg daily over five days, then maintenance tacrolimus (goal trough level 12 ng/dL). The patient was discharged postoperative day 31. Recovery was complicated by acute cellular rejection on postoperative day 43 treated with glucocorticoid and anti-thymocyte globulin. Hepatic arterial stenosis was diagnosed postoperative day 56 and successfully dilated using a percutaneous balloon.

Plasma methionine and AdoMet normalized within 24 h of transplantation and remained stable on an unrestricted diet (protein intake ≥ 2 g/kg/day) during 6 months of follow-up (Table 1, Fig. 6). AdoHcy decreased 96% to 113 ± 57 nM and the AdoMet:AdoHcy concentration ratio increased 2.8-fold to 1.48 ± 0.79 mol:mol, predicting a 28% increase of average tissue methyltransferase reaction velocities (Fig. 3). These stable biochemical changes were accompanied by evidence of increased GAMT and PEMT activity in vivo, including restoration of normal plasma creatine and phosphatidylcholine levels and a 10-fold decrease of the guanidinoacetate:creatine concentration ratio *off* all dietary supplements (Table 1, Fig. 5). Betaine, an alternative methyl donor for methionine recycling (Fig. 1), decreased 96% to reach normal levels after transplantation (Table 1, Fig. 5). Improved systemic amino acid homeostasis restored calculated cerebral methionine, isoleucine, and valine influx to control ranges, but cerebral leucine and tryptophan influx remained 25% and 28% below normal, respectively (Table 2, Fig. 4). Cerebral phenylalanine uptake increased. Post-transplantation creatine kinase and aldolase levels remained elevated indicating a persistent myopathy. Factor VII activity increased within 2 post-operative months from 12% to a stable and functional level of 100% (Fig. 5).

During 6 post-transplant months, our patient's head growth accelerated from 0.11 to 0.41 cm per month and head circumference z-score increased from −2.9 to −1.3 (Fig. 2). We observed gains in gross motor, language, and social skills. The proband's pre-transplant vocabulary of 10–12 single words (e.g. “mommy”, “daddy”, “hi”, “bye”) rapidly expanded during the post-transplant interval, allowing her to communicate in phrases and short sentences (e.g. “Mommy, water please”), execute more complex commands (“Please turn up the radio.”), and sing along with familiar music. She first began to run, learned to catch and throw a ball, and developed new age-appropriate peer behaviors such as reciprocal play, sharing, and taking turns. Post-transplant improvements of fine motor skill were less noticeable, but included better control of eating utensils and the ability to use writing implements (crayons, markers, etc.).

4. Discussion

4.1. Pathophysiology and treatment of AHCY deficiency

AHCY deficiency is a multisystem disease with severe neurodevelopmental consequences. The extant literature about the disorder is largely descriptive, documenting its complex impact on transsulfuration and transmethylation pathways, organ function,

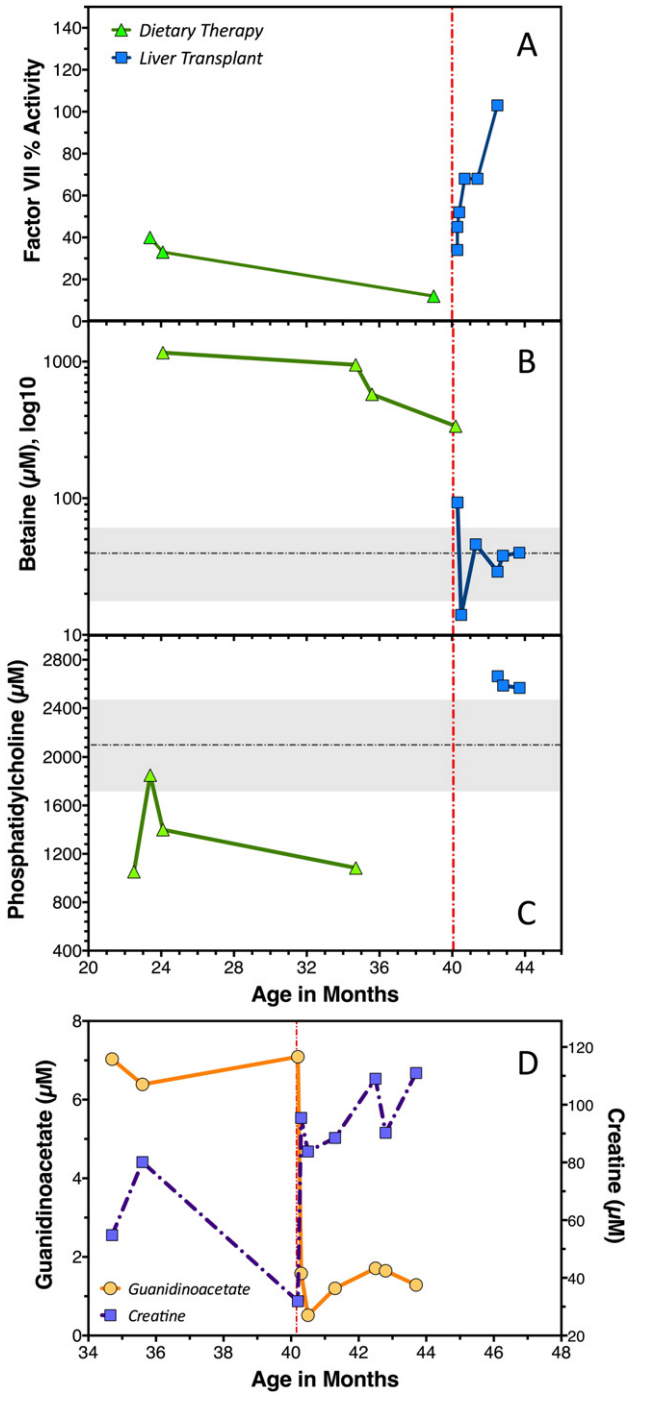

Fig. 5. (A) At the time of diagnosis, prothrombin time was increased (international normalized ratio 1.4). Factor VII activity was 40% and continued to decrease to 12% despite dietary treatment (green triangles). Following liver transplantation at 40 months of age (red dotted lines), factor VII activity (blue squares) increased over 3 months to a level of 100%. (B) Betaine, an alternative substrate for methyl transfer from homocysteine to methionine, was markedly elevated at the time of diagnosis and did not change appreciably during dietary therapy, but normalized promptly following liver transplantation. (C) Biosynthesis of phosphatidylcholine and creatine via PEMT and GAMT, respectively, comprise a large proportion of whole body methyl flux in humans. Both analytes remained low despite dietary supplementation. Levels normalized after liver transplantation and supplements were discontinued. (D) The post-transplant increase of plasma creatine (purple squares) was mirrored by a decrease of its precursor guanidinoacetate (orange circles), indicating increased flux through GAMT in vivo.

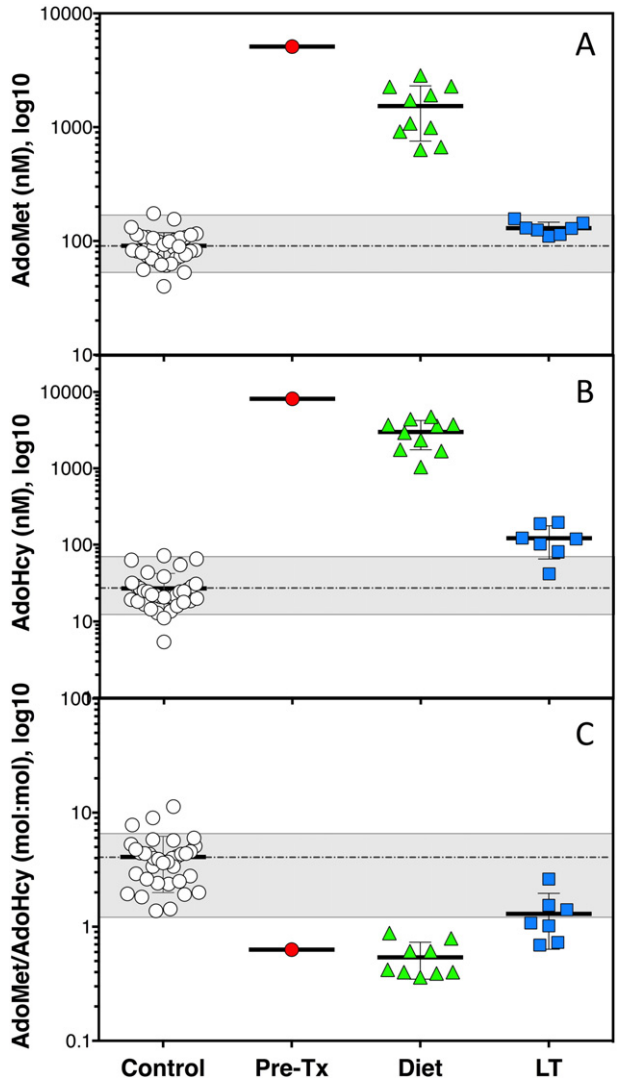

Fig. 6. S-adenosylmethionine (AdoMet; panel A), S-adenosylhomocysteine (AdoHcy; panel B), and the AdoMet:AdoHcy concentration ratio (panel C) are represented for 36 healthy control subjects (white circles, control mean ± 1SD gray shaded areas) and the proband before treatment (red circle), during dietary therapy (green triangles), and after liver transplantation (blue squares). All values are plotted on a $\log_{10}$ scale.

and development as well as response to empirical dietary therapies [7–9,11]. Mechanisms at the core of the disease process remain unknown.

Conventional dietary therapy reduced our patient's plasma AdoMet and AdoHcy levels by 69% and 63%, respectively, but AdoHcy remained elevated two orders of magnitude and its concentration relationship with AdoMet was unchanged (0.63 versus 0.52 mol:mol) (Table 1). More stringent dietary protein restriction entailed risk of clinically significant cerebral BCAA deficiency (Table 2, Fig. 4), ultimately limiting the safety and utility of this strategy, whereas creatine and phosphatidylcholine supplements did not correct systemic deficiency of these metabolites (Table 1).

4.2. Tissue distribution and physiological impact of AdoMet:AdoHcy equilibria

Several lines of evidence indicate that tissue methylation reactions are influenced by the cellular AdoMet:AdoHcy concentration ratio, which in turn equilibrates with extracellular concentrations of AdoMet

and AdoHcy in humans ($r = 0.70$, $P < 0.01$) [17,28]. Intracellular concentrations of AdoMet and AdoHcy exceed plasma concentrations by 700 ± 350-fold (Table S2), and liver has the highest observed transmembrane concentration gradient (~1400-fold) [17]. These cellular AdoMet:AdoHcy equilibria are influenced by diet and disease. In rats, increasing dietary methionine intake from 0.3% to 3% is associated with a 5-fold increase of hepatic AdoMet but a 20-fold increase of AdoHcy, such that the tissue AdoMet:AdoHcy ratio decreases from 7.1 to 1.6 mol:mol (Table S2) [16].

In principle, changes of the tissue AdoMet:AdoHcy ratio should alter activity of various methyltransferases [13,14,32], with the magnitude of this effect dependent on each enzyme's Michealis–Menten affinity constant for AdoMet (K_m) relative to its inhibition constant for AdoHcy (K_i); i.e. enzymes with a higher K_m/K_i are more sensitive to inhibition when AdoMet:AdoHcy decreases (Fig. 3) [14]. There is evidence of this phenomenon in vitro and in vivo. Cell cultures exposed to an 8-fold decrease of AdoMet:AdoHcy show a 50% reduction of C-terminal methyl "capping" of small G-protein Ras family proteins, indicating reduced isoprenylcysteine *O*-methyltransferase activity [32,33]. In humans with chronic renal failure, AdoHcy accumulates in plasma and tissues, and a 4- to 8-fold decrease of the AdoMet:AdoHcy ratio in erythrocytes correlates with a 50% reduction of protein L-isoaspartate (D-aspartate) *O*-methyltransferase activity [13,34]. Stable isotope studies in uremic patients show a 24% decrease of whole body transmethylation [35].

Based on these lines of evidence, an 8-fold reduction of the systemic AdoMet:AdoHcy ratio associated with severe AHCY deficiency could reflect quantitatively similar or even larger intracellular reductions of this ratio, inhibiting methyltransferase activities 50% of more in tissues throughout the body. We thus conceptualized liver transplantation as a strategy designed to correct the systemic AdoMet:AdoHcy ratio with aims to: 1) normalize transmethylation fluxes in this organ, 2) restore normal balance between circulating AdoMet and AdoHcy, 3) protect the proband from hazards associated with long-term dietary protein restriction, 4) improve transmethylation homeostasis in non-hepatic tissues, and 5) correct life-threatening factor VII deficiency.

Within 24 h of cross-clamping the donor liver segment, plasma methionine and AdoMet returned to normal and were not influenced by unrestricted protein intakes within an age-appropriate range during 6 months of follow-up. AdoHcy decreased 96% and the AdoMet:AdoHcy ratio doubled, accompanied by compelling in vivo evidence of restored GAMT and PEMT activity (Table 1, Figs. 3 and 5). Although longer-term follow-up is necessary to document the stability of this biochemical repair, our experience with other metabolic conditions suggests that these changes should remain durable over time provided the graft remains healthy [36,37].

The deeper and more important clinical question is whether sustained improvement of hepatic and circulating AdoMet and AdoHcy homeostasis can influence cerebral methyltransferase activities in a way that alters the child's developmental trajectory. At present, there is insufficient evidence to extrapolate changes in plasma AdoMet and AdoHcy to changes of these metabolites or transmethylation rates in brain tissue. One study suggests that intracellular AdoMet concentrations are relatively uniform throughout the human brain and low (1.6 ± 0.4 μM) relative to levels in the rat brain (31.6 ± 4.1 μM) and other rat organs (63.3 ± 32 μM) [17,38]. The brain is nevertheless critically dependent on AdoMet-dependent reactions that methylate lipids, proteins, and neurotransmitters and can be affected by changes of the cerebral AdoMet:AdoHcy ratio [39,40]. A 4-fold acceleration of head growth and strong postoperative developmental progress in the proband suggest that stable changes of the circulating amino acid pattern and AdoMet:AdoHcy ratio favorably affect brain chemistry; we plan to subject these provisional observations to more rigorous neurocognitive testing at 12 and 24 postoperative months.

4.3. Liver transplantation for the treatment of metabolic disorders

Metabolic disorders comprise the second largest category of indications for pediatric liver transplantation [41]. As liver transplantation outcomes continue to improve at centers of excellence, the strategy has been successfully applied to a growing number of conditions such as maple syrup urine disease (MSUD), for which efficacy is now established [37,42]. For the indication of AHCY deficiency, our multidisciplinary teams supported proceeding with liver transplantation based on the serious neurological morbidity associated with the disorder (Fig. 2) [7–9], the central role of the liver in systemic transsulfuration–transmethylation homeostasis [3,15,16,29], and a concomitant diagnosis of factor VII-deficient coagulopathy.

As survival after pediatric liver transplantation has improved over the past decade [41], attention has turned to preventing post-transplant medical complications and optimizing long-term quality of life. Despite progress on this front, there remains a finite potential for abrogating all morbidity and mortality after transplant. Our experience underscores the types of complications that may occur, and should enter into any discussion about the risks and benefits of pediatric liver transplant for a metabolic disorder.

But the stakes are high: families who choose liver transplantation for a metabolic indication must understand that risk–benefit considerations differ from those of children who undergo liver transplantation for end-stage liver disease. In contrast to the more obvious benefit of transplant to prevent death from liver failure, the transplantation strategy is principally applied to metabolic disorders to prevent neurological damage and safeguard long-term well being [7,8,36,37]. Our preliminary observations at 6 postoperative months suggest that liver transplantation stabilizes the biochemical profile of AHCY deficiency, eliminates the need for protein restriction and dietary supplementation, and might improve brain growth and development. However, as with any neurometabolic condition, proof of efficacy in principle does not insure good outcomes in practice [24]; timing is critical. To the extent that biochemical correction of AHCY deficiency improves neurological outcome, it is axiomatic that earlier treatment is better. This underscores the importance of newborn screening, which remains a central challenge for human transsulfuration–transmethylation disorders.

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Dedication

The authors dedicate this work to the late S. Harvey Mudd, who pioneered the study of transsulfuration and its relevance to human well-being. Harvey possessed a rare combination of humility and wonder that allowed him to wear his scholarship lightly and share it openly. He had a reputation for training his keen intellectual focus on people with real problems, and remained a tireless healer to the end of his life. Harvey taught by example to strive with clarity, alleviate suffering, and always be kind.

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