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S-adenosylhomocysteine hydrolase over-expression does not alter S-adenosylmethionine or S-adenosylhomocysteine levels in CBS deficient mice

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ABSTRACT

Elevated plasma total homocysteine (tHcy) is associated with a number of human diseases including coronary artery disease, stroke, osteoporosis and dementia. It is highly correlated with intracellular S-adenosylhomocysteine (SAH). Since SAH is a strong inhibitor of methyl-transfer reactions involving the methyl-donor S-adenosylmethionine (SAM), elevation in SAH could be an explanation for the wide association of tHcy and human disease. Here, we have created a transgenic mouse (*Tg-hAHCY*) that expresses human S-adenosylhomocysteine hydrolase (*AHCY*) from a zinc-inducible promoter in the liver and kidney. Protein analysis shows that human AHCY is expressed well in both liver and kidney, but elevated AHCY enzyme activity (131% increase) is only detected in the kidney due to the high levels of endogenous mouse AHCY expression in liver. *Tg-hAHCY* mice were crossed with mice lacking cystathionine β-synthase activity (*Tg-I278T Cbs*$^{-/-}$) to explore the effect to AHCY overexpression in the context of elevated serum tHcy and elevated tissue SAM and SAH. Overexpression of AHCY had no significant effect on the phenotypes of *Tg-I278T Cbs*$^{-/-}$ mice or any effect on the steady state concentrations of methionine, total homocysteine, SAM, SAH, and SAM/SAH ratio in the liver and kidney. Furthermore, enhanced AHCY activity did not lower serum and tissue tHcy or methionine levels. Our data suggests that enhancing AHCY activity does not alter the distribution of methionine recycling metabolites, even when they are greatly elevated by *Cbs* mutations.

1. Introduction

Elevated plasma total homocysteine (tHcy), termed hyperhomocysteinemia (HHcy), is associated prospectively with increased incidence of mortality for many human diseases including coronary heart disease, stroke, dementia, Alzheimer's disease, diabetic retinopathy, osteoporosis, cancer, congenital birth defects and steatosis of the liver [1–9]. The number and variety of diseases associated with elevated tHcy suggests that it may affect very basic cellular functions. Homocysteine (Hcy) is a non-protein incorporated amino acid that is part of the methionine-recycling pathway (Fig. 1). It is catabolized by the action of cystathionine β-synthase (CBS), which initiates the conversion of Hcy to cysteine via the transsulfuration pathway. Hcy is formed via the hydrolysis of S-adenosylhomocysteine (SAH), which is a product of methyl-transfer reactions involving the methyl-donor S-adenosylmethionine (SAM). SAM is the key one-carbon donor in almost all biological methylation reactions. In the human genome, there are at least 53 known or suspected SAM-dependent protein methyltransferases, 13 SAM-dependent RNA methyltransferases, and 5 SAM-dependent DNA methyltransferases (http://www.hprd.org/). Because the chemical structure of SAM and SAH are nearly identical, SAH can bind to the active site of methyltransferase enzymes and act as a potent competitive inhibitor of those enzymes. Kinetic studies have shown that many DNA and protein methyltransferases bind SAH as well, or even slightly tighter than SAM [10–14]. Thus, the ratio of SAM to SAH inside the cell is thought to be critical for proper regulation of methyl transfer reactions.

Elevated tHcy in serum or plasma is strongly correlated with increased tissue SAH and a reduction in tissue SAM/SAH ratio. In plasma, SAH and SAM are present at approximately 1/500th the concentration

Abbreviations: SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; CBS, cystathionine beta synthase; HHcy, hyperhomocysteinemia; Hcy, homocysteine; tHcy, total homocysteine; *Cbs*$^{-}$, CBS knockout allele; *AHCY*, S-adenosylhomocysteine hydrolase; *Tg-I278T*, transgene human CBS containing the I278T mutation; Zn, zinc water; HA, hemagglutinin; CMC, carboxymethylcellulose; Met, methionine

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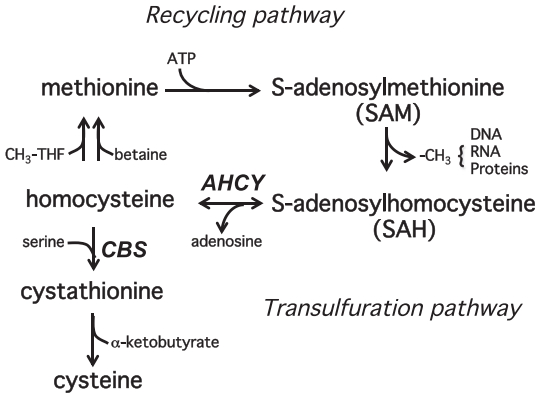

Fig. 1. Methionine metabolism. Figure shows both the methionine recycling pathway and the transsulfuration pathway. The key enzymes for this manuscript, CBS and AHCY are shown in bold by their respective reactions.

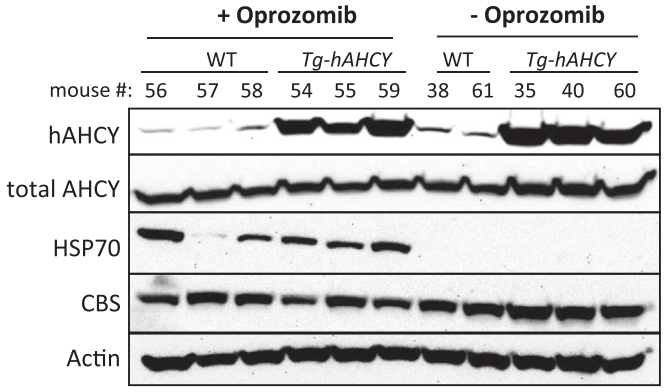

Fig. 3. Effect of proteasome inhibitor on AHCY protein levels. Western blot analysis of transgene negative and *Tg-hAHCY* liver lysates of oprozomib treated mice. Blots were probed with the indicated antibodies.

of tHcy, about 20–80 nM versus 5–15 μM [15]. However, in tissue, such as liver or kidney, the concentration of all four methionine recycling metabolites (methionine, SAM, SAH and homocysteine) are within the same order of magnitude [16–19]. The reason for this is that homocysteine and methionine are membrane permeable, but SAM and SAH are not [20]. Therefore, for a cell to get rid of excess intracellular SAH, it must first convert it to Hcy and only then can it be secreted into the blood. A variety of cellular, animal, and human data supports the idea that plasma tHcy and intracellular SAH levels are strongly correlated [19,21–24].

SAH is converted to Hcy by the enzyme S-adenosylhomocysteine hydrolase, which is encoded by the *AHCY* gene. This is a highly evolutionarily conserved enzyme with 71% amino acid identity between the *H. sapiens* and *S. cerevisiae* protein sequences. AHCY catalyzes the hydrolysis of SAH to homocysteine and adenosine. In vitro this reaction is entirely reversible, and the chemical equilibrium constant actually favors the reverse reation, i.e., the formation of SAH from homocysteine and adenosine. However, in vivo the reaction is driven in the forward direction due to the action of adenosine kinase, which keeps the intracellular concentration of adenosine quite low [20,25]. Loss of SAH hydrolase activity leads to an accumulation of SAH and causes developmental and aging phenotypes in diverse organisms including *D. melanogaster*, *A. thaliana*, *M. musculus*, and *H. sapiens* [26–29].

In this study, we have engineered a mouse that ectopically expresses human *AHCY* from a zinc-inducible transgene to see if increasing AHCY enzyme level alters SAM/SAH homeostasis in mice that have greatly elevated serum tHcy due to a mutation in the *Cbs* gene. Our results suggest that increasing AHCY activity does not alter SAM/SAH homoestasis.

2. Methods

2.1. Plasmid construction

In this study, we generated two recombinant plasmids for injection, one containing the mouse *Ahcy* gene (*pTg-mAhcy*) and one the human *AHCY* gene (*pTg-hAHCY*). For the p*Tg-mAhcy* construction, we first performed PCR amplification of the mouse *Ahcy* ORF (Dharmacon cloneID: 424197) using a 5′ primer containing a hemagglutinin (HA) epitope tag (HA-mAhcy-F) and a 3′ primer containing the stop codon (HA-mAHCY-R). The PCR product was cloned into *PCR2.1* and the lack of mutation was confirmed by sequencing. *PCR2.1:mAHCY* was then digested with *EcoR1*, and the insert was then cloned into the *MfeI* site of plasmid *pUC:MT-I:MfeI* [30]. The resultant plasmid, *PUC:MT-I:mAhcy*,

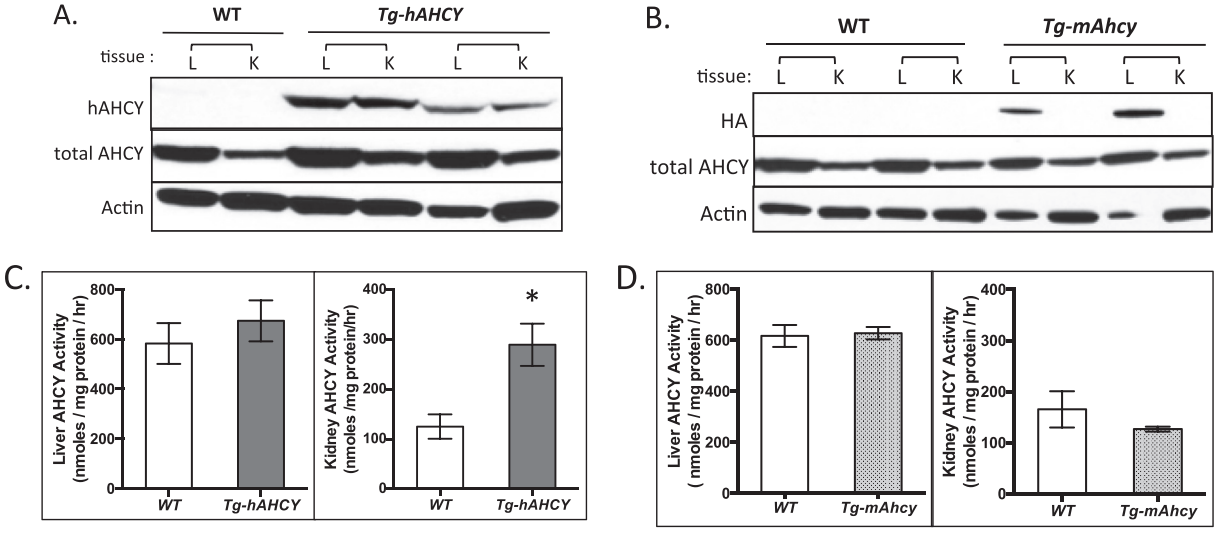

Fig. 2. AHCY expression and activity. (A) Western blot analysis of *Tg-hAHCY* liver and kidney lysates from zinc-induced animals. Lanes labeled WT are from non-transgenic siblings. Top row shows level of human AHCY as determined by human specific antibody. Middle row shows total AHCY as determined by anti-body that recognizes both species. (B) Western analysis of *Tg-mAhcy* liver and kidney. Top row is probed with anti-HA antibody that only recognizes mAHCY expressed from the transgene, middle row shows total AHCY. (C) AHCY enzyme activity in liver (n = 7) and kidney (n = 4) from *Tg-hAHCY* mice and transgene negative controls. (D) AHCY enzyme activity in liver (n = 3) and kidney (n = 4) from *Tg-mAhcy* mice and transgene negative controls. Error bars show SEM. Asterisk indicates $P < 0.05$.

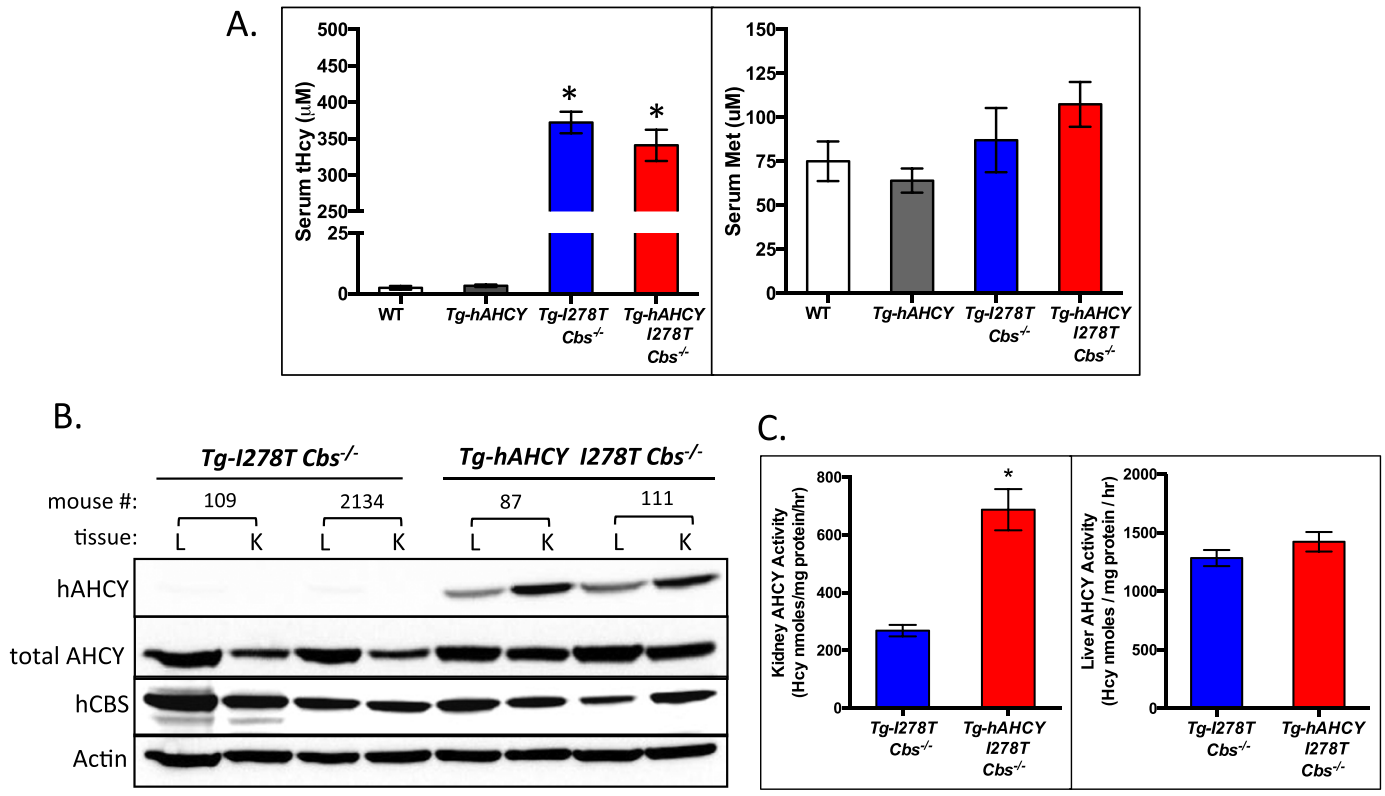

Fig. 4. Analysis of *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ mice. (A) Serum tHcy and methionine from wild-type (n = 5), *Tg-hAHCY* (n = 4), *Tg-I278T Cbs*$^{-/-}$ (n = 4) and *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ mice (n = 7). Error bars show SEM. Asterisk indicates P < 0.0001 compared to WT. (B) Western blot analysis of liver and kidney lysates from zinc-induced *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ and *Tg-I278T Cbs*$^{-/-}$ mice. (C) Kidney and liver AHCY enzyme activity from *Tg-I278T Cbs*$^{-/-}$ (n = 4) and *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ mice (n = 8). Error bars show SEM. Asterisk indicates P < 0.003.

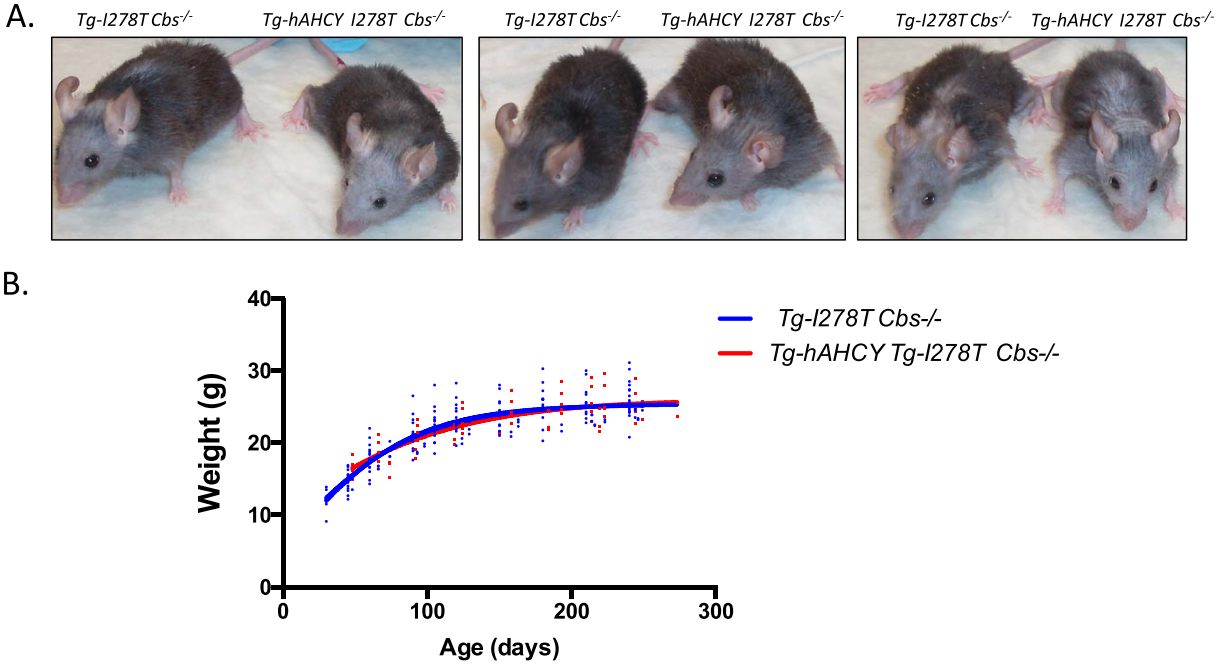

Fig. 5. Phenotype of zinc-treated *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ mice. (A) Photograph of three sibling pairs of 130 day old mice. Genotype of each pair is shown above. (B) Weight gain of mice.

was then cut with *EcoR1* and the insert was cloned into pLW3 [30]. This plasmid, *pTg-mAhcy*, contains the mouse *Ahcy* cDNA under control of the mouse *MT-I* promoter flanked by two locus control regions. The *pTg-hAHCY* construct was made nearly identically using the pPROK AdoHcyase clone as the source of the human cDNA [31]. In this case, no HA tag was added, and an *EcoR1* fragment containing *hAHCY* was cloned directly into *pUC:MT-1:MfeI*.

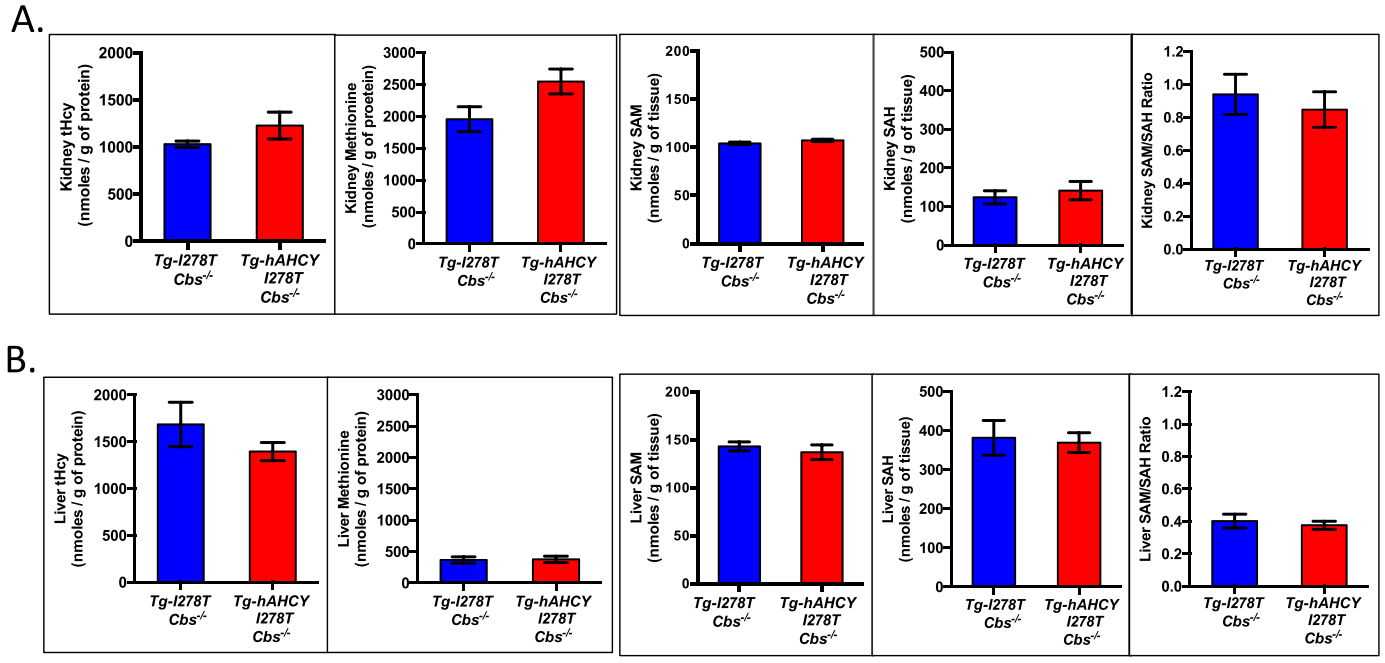

Fig. 6. Concentration of methionine recycling metabolites. (A) Concentration of tHcy, methionine, SAM, and SAH in kidney. The SAM/SAH ratio is also shown. Error bars show SEM. (B) Concentration of tHcy, methionine, SAM, and SAH in liver.

2.2. Mouse generation

Transgenic mice containing *pTg-mAhcy* and *pTg-hAHCY* were created as previously described [30]. DNA was digested with *Sal*I to release bacterial sequences, and the purified fragment was microinjected into the pronuclei of day 0.5 C57BL/6JxC3H/HeJ F2 embryos. For *pTg-hAHCY,* we obtained 63 pups, of which two were transgene positive, as determined by PCR of mouse tail DNA. The two positive pups were then backcrossed to C57BL6 to confirm germline transmission, and some of the progeny of this cross were then put on zinc water and screened for transgene expression by Western blot. Only the offspring of progeny 19 showed inducible expression and this line was designated *Tg-hAHCY*. For *pTg-mAhcy,* we obtained 13 pups and two were transgene positive by PCR. One of the two lines showed good transgene expression (progeny 82)

Tg-hAHCY mice were bred to *Tg-I278T Cbs*$^{+/-}$ mice [32] to obtain *Tg-hAHCY Tg-I278T Cbs*$^{+/-}$ mice. These animals were then intercrossed to obtain *Tg-hAHCY Tg-I278T Cbs*$^{-/-}$ mice. All crosses were done on Zn water (25 mM $ZnSO_4$) to induce the *Tg-I278T* transgene, which is required for viability after the neonatal period [32]. Genotyping of offspring was generally done between 10 and 14 days of age as previously described [30]. Animals were fed standard rodent chow (Teklad 2018SX) containing 0.6% methionine by weight.

For the oprozomib treatment experiment, mice were treated under zinc water for 10 days. The proteasome inhibitor oprozomib (40 mg/Kg/Day, Onyx Pharmaceuticals) was then administered as a suspension in 1% (w/v) carboxymethylcellulose (CMC). Animals were dosed daily by oral gavage for 4 days. After the final dose, animals were sacrificed 5 h after last dose.

All experiments were approved by the Fox Chase Cancer Center Institutional Animal Use and Care Committee.

2.3. Immunoblots

All mice were given zinc water for 10 days before any analysis. Tissue homogenates were prepared in 10 mM Tris-HCl (pH 7.5) supplemented with protease inhibitors (Roche). Twenty μg of lysate was separated by 4–12% SDS-PAGE (Invitrogen) under reducing conditions and transferred to nitrocellulose. In this study, blots were probed with two different AHCY antibodies. One antibody (ab151734, Abcam) is a rabbit polyclonal that only recognizes only human AHCY, while the other (H00000191-M07A, Abnova) is a mouse monoclonal that recognizes both human and mouse AHCY. Other antibodies used included a mouse anti-HA (H9658, Sigma), rabbit anti-hCBS [33], and mouse anti-actin (A5441, Sigma). Gel images were captured and quantified using the FluorChem SP system (Alpha Innotech). The relative intensities of each protein are assigned as arbitrary units obtained by calculating the ratio of a test sample divided by a control.

2.4. CBS activity, tHcy and met, and tissue SAM and SAH

CBS activity in the tissue was measured using a Biochrom 30 amino acid analyzer (Cambridge, UK) as described previously [30]. One unit of activity is defined as nmoles of cystathionine formed/mg of tissue lysate protein/hour. Serum and tissue tHcy (a sum total of free and disulfide-bonded homocysteine) and methionine levels were measured using the Biochrom 30 amino acid analyzer as performed previously [19].

SAM and SAH quantitative measurements from liver and kidney tissues were performed using LC MS/MS as described previously [24].

2.5. AHCY activity

We measured AHCY activity in the forward direction using a modification of the method in [34]. This method relies on the addition of adenosine deaminase to remove adenosine from the reaction, thereby, driving the reaction in the forward direction. Reaction (45 μl) containing 20 μg of tissue lysates, 50 mM K-phosphate (pH 7.2), 1 mM EDTA, and 4 units of adenosine deaminase (Roche) was incubated at 37 °C for 2 min prior to the initiation of the enzyme reaction, followed by the addition of 5 μl of 5 mM SAH (Sigma). Sample mixture (50 μl) was incubated at 37 °C for 10 min and stopped by adding an equal volume of 10% sulfosalicylic acid. Followed by one hour incubation at 4 °C and centrifugation, the supernatant was measured for Hcy level on a Biochrom 30 amino acid analyzer. Peaks were identified and quantitated by comparing to a known standard. One unit of activity is

defined as nmoles of Hcy/mg of tissue lysate protein/h.

2.6. Statistical analysis

Values in text are mean ± SEM. Differences between two groups were analyzed by the Mann–Whitney *U* test (unpaired and non-parametric test). Significance between more than two groups were determined using one-way ANOVA followed by Tukey's multiple comparison tests employing GraphPad Prism 6.0 software. Statistical significance was accepted at the value of $P < 0.05$. For growth curves, data was fitted using Gompertz growth model in GraphPad Prism.

3. Results

3.1. Creation and characterization of Tg-hAHCY and Tg-mAhcy mice

To determine the consequences of overexpression of AHCY in vivo, we constructed two different expression plasmids. One contained the mouse *Ahcy* cDNA (*Tg-mAhcy*) and the other contained the human *AHCY* cDNA (*Tg-hAHCY*). Both constructs were made by cloning the respective cDNAs downstream of a mouse *MT-1* promoter that contains locus control regions in a manner identical to that used by our lab to express human CBS in mice [30]. For the *Tg-mAhcy* plasmid, we attached a HA-epitope to the N-terminus to distinguish protein expression between the mouse transgene and the endogenous mouse locus. For the *Tg-hAHCY* plasmid, human AHCY can be distinguished by the use of a human specific antibody. For each construct, we obtained a single founder line that showed clear expression of the transgene in liver when the animals' water was supplemented with zinc (Supplemental Fig. 1).

The *Tg-hAHCY* line exhibits good transgene-driven protein expression in both kidney and liver, while the *Tg-mAhcy* line only has detectable transgene expression in the liver (Fig. 2). Surprisingly, using an antibody that detects both human and mouse AHCY, we failed to see an increase in total liver AHCY in either line. This was confirmed by measuring AHCY activity in liver lysates. However, in the *Tg-hAHCY* line, we were able to detect an increase in total AHCY protein and activity in the kidney. This finding was confirmed by AHCY enzyme activity assays, which reveals a 131% increase in kidney AHCY activity in *Tg-hAHCY* mice compared to sibling Tg^{-} mice (125 vs. 289 units, $P < 0.016$). Because we failed to detect any overexpression *Tg-mAhcy* line, we focused our efforts on characterizing the *Tg-hAHCY* line.

One possible explanation for why there was no significant increase in AHCY protein and activity in the liver of *Tg-hAHCY* mice is that there may be a homeostatic mechanism that limits the amount of total AHCY protein that can be expressed, perhaps mediated at the level of protein stability. To see if there was evidence for this, we treated both *Tg-hAHCY* and *Tg-* controls with the proteasome inhibitor oprozomib (Fig. 3). We observed elevated Hsp70 in treated compared to untreated mice indicating that the drug was effective at inhibiting the proteasome. However, we observed no increase in either hAHCY or total AHCY steady state -protein levels. These findings suggest that AHCY protein levels are not controlled by the ubiquitin/proteasome system.

3.2. Tg-hAHCY Tg-I278T $Cbs^{-/-}$ mice

To study the effects of AHCY overexpression in the context of severe HHcy, we created *Tg-hAHCY* mice that also lacked functional *Cbs*. Mice homozygous for a *Cbs* null allele ($Cbs^{-/-}$) normally die of liver failure between four and six weeks of age [35]. However, $Cbs^{-/-}$ mice that contain a transgene that expresses a mutant human CBS protein (*Tg-I278T* $Cbs^{-/-}$) survive this neonatal period and have extreme elevations in serum tHcy. We constructed Tg-*hAHCY Tg-I278T* $Cbs^{-/-}$ mice by repeated breeding of *Tg-hAHCY* mice to *Tg-I278T* $Cbs^{+/-}$ mice followed by genotyping for the three alleles. Serum tHcy in Tg-*hAHCY Tg-I278T* $Cbs^{-/-}$ mice was over a 100-fold higher than in *Tg-hAHCY* animals (340 μM vs. 3.3 μM, $P < 0.0001$), while serum methionine was only slightly elevated (107 vs. 64 μM, $p = 0.087$) (Fig. 4*A*). Like the *Tg-hAHCY* line, Tg-*hAHCY Tg-I278T* $Cbs^{-/-}$ mice had expression of the hAHCY protein in the liver and kidney, although we did notice more protein in the kidney than in the liver in this line (Fig. 4*B*). In addition, Tg-*hAHCY Tg-I278T* $Cbs^{-/-}$ mice had increased kidney AHCY protein and activity, but no increase was observed in the liver (Fig. 4*C*). Phenotypically, Tg-*hAHCY Tg-I278T* $Cbs^{-/-}$ mice were indistinguishable from *Tg-I278T* $Cbs^{-/-}$ mice in terms of hair loss and weight gain (Fig. 5).

To determine the metabolic consequences of AHCY overexpression, we compared methionine, tHcy, SAM, SAH, and the SAM/SAH ratio in liver and kidney tissues from *Tg-I278T* $Cbs^{-/-}$ and *Tg-hHCY Tg-I278T* $Cbs^{-/-}$ mice (Fig. 6). Surprisingly, none of the metabolites showed statistically significant differences in concentrations in any of the tissues examined. There was also no difference in liver or kidney CBS activity (Supplemental Fig. 2). These findings indicate that the steady-state pools of methionine recycling metabolites are not altered by increasing the amount of AHCY protein.

4. Discussion

This study was motivated by our desire to determine whether the phenotypes observed in patients with severe hyperhomocysteinemia (or homocystinuria) could be ameliorated by overexpression of AHCY. The overarching idea was that elevated tHcy in blood reflected elevated SAH in cells, resulting in inhibition of cellular methylation reactions due to inhibition of SAM-dependent methyltransferases. We hypothesized that overexpression of AHCY, the enzyme that catalyzes the conversion of SAH to homocysteine, would lower SAH levels and therefore lessen tHcy associated phenotypes.

To test this idea, we created at transgenic mouse that expressed human *AHCY* from the mouse *MT-I* promoter flanked by locus control regions. Previously, we had used this vector to overexpress human CBS in mice, where we observed good protein expression in both liver and kidney, resulting in a 116% and 260% increase in CBS activity, respectively [30]. For human AHCY, we observed good protein expression in liver and kidney, but only found a 16% increase in activity in liver (p = ns) and a 131% increase in kidney. The lack of overexpression in liver made us wonder whether there might be a post-translational regulatory mechanism involving proteasomal-mediated AHCY degradation that might limit the amount of AHCY protein that could accumulate in liver. However, treatment of *Tg-hAHCY* mice with the proteasome inhibitor oprozomib did not enhance AHCY levels, suggesting this was not the case. We also considered the possibility that even though human and mouse AHCY proteins are 98.4% identical, these few differences might be important for optimal protein-folding and function in mouse tissues. Therefore, we made a second construct that contained the mouse *Ahcy* gene. However, this line also did not show increased *Ahcy* activity in the liver and, unfortunately, did not express the transgene in kidney. Given the above results, the most likely explanation for the apparent lack of increase in liver AHCY activity is that the endogenous AHCY levels in liver are simply too high to detect the difference. Consistent with that idea is proteomics data showing that AHCY is the 19th most abundant protein (out of 5421) in mouse liver, but is only the 212th most abundant in mouse kidney [36]. In comparison, CBS was ranked 444 in liver and 2063 in kidney [36].

To study the effects of AHCY overexpression in the context of severe hyperhomocysteinemia, we engineered a mouse that contained the *Tg-hAHCY* transgene but lacked CBS activity (*Tg-hAHCY Tg-I278T* $Cbs^{-/-}$). These mice had over a 100-fold increase in tHcy compared to control mice, and the presence of *Tg-hAHCY* had no significant effect on this level. Like the parent *Tg-hAHCY* mice, we observed statistically significant increase AHCY activity in the kidney, but not in the liver. When we aged sibling *Tg-hAHCY Tg-I278T* $Cbs^{-/-}$ mice and compared them to *Tg-I278T* $Cbs^{-/-}$ mice, we noted no beneficial phenotypic effects of having AHCY overexpression in kidney. At the metabolic level,

we found that there was no difference in the steady state levels of any of the methionine recycling metabolites in either liver or kidney.

The finding that overexpression of AHCY had no effect on the concentrations of any of the methionine recycling metabolites was unexpected. We assumed that increasing AHCY levels would lower tissue SAH levels in much the same way the overexpression of CBS lowered to plasma tHcy levels [30]. However, our results show that increasing the level of AHCY in the kidney did not alter the ratio of SAH to Hcy. Thus, AHCY levels do not seem to be rate limiting in vivo. A possible explanation for the difference between the effects of CBS and AHCY overexpression may be related to the fact that CBS only works in the forward direction, while the reaction catalyzed by AHCY is entirely reversible, and the equilibrium actually favors the reverse reaction, i.e., the synthesis of SAH. In vivo, the reaction is pushed in the forward direction because adenosine levels inside the cell are quite low due to the action of adenosine kinase. Our results suggest that there is enough AHCY in vivo to create a situation where the flux is limited by the law of mass action as opposed to the level of the AHCY enzyme. Our findings are also consistent with studies in HEK-293 cells, which showed that overexpression of AHCY does not affect the level of global DNA methylation [37].

In summary, our findings show that increasing the amount of AHCY enzyme by 131% in mouse kidney did not have any effect on the steady-state concentrations of intracellular methionine recycling metabolites, even in in the context of extreme hyperhomocysteinemia. Our studies suggest that enhancing AHCY activity is probably not a viable strategy to ameliorate the phenotypes of hyperhomocysteinemia.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2018.01.002.

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Author contributions

HOL - Designed research, performed research, analyzed data, wrote paper.

LW - Performed research.

YMK - Performed research.

AJA - Contributed new reagents or analytic tools.

SG - Performed research, analyzed data.

WK - Designed research, analyzed data, wrote paper.

References

[1] Homocysteine Studies Collaboration, Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis, JAMA 288 (2002) 2015–2022.

[2] Y. He, Y. Li, Y. Chen, L. Feng, Z. Nie, Homocysteine level and risk of different stroke types: a meta-analysis of prospective observational studies, Nutr. Metab. Cardiovasc. Dis. 24 (2014) 1158–1165.

[3] S. Seshadri, A. Beiser, J. Selhub, P.F. Jacques, I.H. Rosenberg, R.B. D'Agostino, P.W. Wilson, P.A. Wolf, Plasma homocysteine as a risk factor for dementia and Alzheimer's disease, N. Engl. J. Med. 346 (2002) 476–483.

[4] H. Chen, S. Liu, L. Ji, T. Wu, F. Ma, Y. Ji, Y. Zhou, M. Zheng, M. Zhang, G. Huang, Associations between Alzheimer's disease and blood homocysteine, vitamin B12, and folate: a case-control study, Curr. Alzheimer Res. 12 (2015) 88–94.

[5] C. Xu, Y. Wu, G. Liu, X. Liu, F. Wang, J. Yu, Relationship between homocysteine level and diabetic retinopathy: a systematic review and meta-analysis, Diagn. Pathol. 9 (2014) 167.

[6] H. Zhang, X. Tao, J. Wu, Association of homocysteine, vitamin B12, and folate with bone mineral density in postmenopausal women: a meta-analysis, Arch. Gynecol. Obstet. 289 (2014) 1003–1009.

[7] D. Zhang, X. Wen, W. Wu, Y. Guo, W. Cui, Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals, PLoS One 10 (2015) e0123423.

[8] V. Iacobazzi, V. Infantino, A. Castegna, G. Andria, Hyperhomocysteinemia: related genetic diseases and congenital defects, abnormal DNA methylation and newborn screening issues, Mol. Genet. Metab. 113 (2014) 27–33.

[9] Y. Dai, J. Zhu, D. Meng, C. Yu, Y. Li, Association of homocysteine level with biopsy-proven non-alcoholic fatty liver disease: a meta-analysis, J. Clin. Biochem. Nutr. 58 (2016) 76–83.

[10] D. Patnaik, H.G. Chin, P.O. Esteve, J. Benner, S.E. Jacobsen, S. Pradhan, Substrate specificity and kinetic mechanism of mammalian G9a histone H3 methyltransferase, J. Biol. Chem. 279 (2004) 53248–53258.

[11] H.G. Chin, D. Patnaik, P.O. Esteve, S.E. Jacobsen, S. Pradhan, Catalytic properties and kinetic mechanism of human recombinant Lys-9 histone H3 methyltransferase SUV39H1: participation of the chromodomain in enzymatic catalysis, Biochemistry 45 (2006) 3272–3284.

[12] H.W. Lee, S. Kim, W.K. Paik, S-adenosylmethionine: protein-arginine methyltransferase. Purification and mechanism of the enzyme, Biochemistry 16 (1977) 78–85.

[13] T. Yokochi, K.D. Robertson, Preferential methylation of unmethylated DNA by mammalian de novo DNA methyltransferase Dnmt3a, J. Biol. Chem. 277 (2002) 11735–11745.

[14] J. Flynn, N. Reich, Murine DNA (cytosine-5-)-methyltransferase: steady-state and substrate trapping analyses of the kinetic mechanism, Biochemistry 37 (1998) 15162–15169.

[15] C. Wagner, M.J. Koury, S-adenosylhomocysteine: a better indicator of vascular disease than homocysteine? Am. J. Clin. Nutr. 86 (2007) 1581–1585.

[16] S.F. Choumenkovitch, J. Selhub, P.J. Bagley, N. Maeda, M.R. Nadeau, D.E. Smith, S.W. Choi, In the cystathionine beta-synthase knockout mouse, elevations in total plasma homocysteine increase tissue S-adenosylhomocysteine, but responses of S-adenosylmethionine and DNA methylation are tissue specific, J. Nutr. 132 (2002) 2157–2160.

[17] M.A. Caudill, J.C. Wang, S. Melnyk, I.P. Pogribny, S. Jernigan, M.D. Collins, J. Santos-Guzman, M.E. Swendseid, E.A. Cogger, S.J. James, Intracellular S-Adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice, J. Nutr. 131 (2001) 2811–2818.

[18] A.M. Devlin, T. Bottiglieri, F.E. Domann, S.R. Lentz, Tissue-specific changes in H19 methylation and expression in mice with hyperhomocysteinemia, J. Biol. Chem. 280 (2005) 25506–25511.

[19] S. Gupta, J. Kühnisch, A. Mustafa, S. Lhotak, A. Schlachterman, M.J. Slifker, A. Klein-Szanto, K.A. High, R.C. Austin, W.D. Kruger, Mouse models of cystathionine β-synthase deficiency reveal significant threshold effects of hyperhomocysteinemia, FASEB J. 23 (2009) 883–893.

[20] D.R. Hoffman, D.W. Marion, W.E. Cornatzer, J.A. Duerre, S-adenosylmethionine and S-adenosylhomocystein metabolism in isolated rat liver. Effects of L-methionine, L-homocystein, and adenosine, J. Biol. Chem. 255 (1980) 10822–10827.

[21] H. Wang, M. Yoshizumi, K. Lai, J.C. Tsai, M.A. Perrella, E. Haber, M.E. Lee, Inhibition of growth and p21ras methylation in vascular endothelial cells by homocysteine but not cysteine, J. Biol. Chem. 272 (1997) 25380–25385.

[22] P. Yi, S. Melnyk, M. Pogribna, I.P. Pogribny, R.J. Hine, S.J. James, Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation, J. Biol. Chem. 275 (2000) 29318–29323.

[23] S. Dayal, T. Bottiglieri, E. Arning, N. Maeda, M.R. Malinow, C.D. Sigmund, D.D. Heistad, F.M. Faraci, S.R. Lentz, Endothelial dysfunction and elevation of S-adenosylhomocysteine in cystathionine beta-synthase-deficient mice, Circ. Res. 88 (2001) 1203–1209.

[24] H.O. Lee, L. Wang, Y.M. Kuo, S. Gupta, M.J. Slifker, Y.S. Li, A.J. Andrews, W.D. Kruger, Lack of global epigenetic methylation defects in CBS deficient mice, J. Inherit. Metab. Dis. 40 (2017) 113–120.

[25] G. De La Haba, G.L. Cantoni, The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine, J. Biol. Chem. 234 (1959) 603–608.

[26] A.A. Parkhitko, R. Binari, N. Zhang, J.M. Asara, F. Demontis, N. Perrimon, Tissue-specific down-regulation of S-adenosyl-homocysteine via suppression of dAhcyL1/dAhcyL2 extends health span and life span in drosophila, Genes Dev. 30 (2016) 1409–1422.

[27] L. Mull, M.L. Ebbs, J. Bender, A histone methylation-dependent DNA methylation pathway is uniquely impaired by deficiency in arabidopsis S-adenosylhomocysteine hydrolase, Genetics 174 (2006) 1161–1171.

[28] M.W. Miller, D.M. Duhl, B.M. Winkes, F. Arredondo-Vega, P.J. Saxon, G.L. Wolff, C.J. Epstein, M.S. Hershfield, G.S. Barsh, The mouse lethal nonagouti (a(x)) mutation deletes the S-adenosylhomocysteine hydrolase (Ahcy) gene, EMBO J. 13 (1994) 1806–1816.

[29] N.R. Buist, B. Glenn, O. Vugrek, C. Wagner, S. Stabler, R.H. Allen, I. Pogribny, A. Schulze, S.H. Zeisel, I. Baric, S.H. Mudd, S-adenosylhomocysteine hydrolase deficiency in a 26-year-old man, J. Inherit. Metab. Dis. 29 (2006) 538–545.

[30] L. Wang, K.H. Jhee, X. Hua, P.M. DiBello, D.W. Jacobsen, W.D. Kruger, Modulation of cystathionine beta-synthase level regulates total serum homocysteine in mice, Circ. Res. 94 (2004) 1318–1324.

[31] D.E. Coulter-Karis, M.S. Hershfield, Sequence of full length cDNA for human S-adenosylhomocysteine hydrolase, Ann. Hum. Genet. 53 (1989) 169–175.

[32] L. Wang, X. Chen, B. Tang, X. Hua, A. Klein-Szanto, W.D. Kruger, Expression of mutant human cystathionine beta-synthase rescues neonatal lethality but not homocystinuria in a mouse model, Hum. Mol. Genet. 14 (2005) 2201–2208.

[33] W.D. Kruger, A yeast system for expression of human cystathionine beta-synthase: structural and functional conservation of the human and yeast genes, Proc. Natl.

Acad. Sci. U. S. A. 91 (1994) 6614–6618.
[34] R. Hudec, K. Hamada, K. Mikoshiba, A fluorescence-based assay for the measurement of S-adenosylhomocysteine hydrolase activity in biological samples, Anal. Biochem. 433 (2013) 95–101.
[35] W.D. Kruger, Cystathionine beta-synthase deficiency: of mice and men, Mol. Genet. Metab. 121 (2017) 199–205.
[36] T. Geiger, A. Velic, B. Macek, E. Lundberg, C. Kampf, N. Nagaraj, M. Uhlen, J. Cox, M. Mann, Initial quantitative proteomic map of 28 mouse tissues using the SILAC mouse, Mol. Cell. Proteomics 12 (2013) 1709–1722.
[37] M. Hermes, H. Osswald, R. Riehle, C. Piesch, D. Kloor, S-adenosylhomocysteine hydrolase overexpression in HEK-293 cells: effect on intracellular adenosine levels, cell viability, and DNA methylation, Cell. Physiol. Biochem. 22 (2008) 223–236.