Tissue-specific Changes in H19 Methylation and Expression in Mice with Hyperhomocysteinemia*

Expression of the imprinted genes H19 and insulin-like growth factor 2 (Igf2), which lie in close proximity on mouse chromosome 7, is regulated by methylation of a differentially methylated domain (DMD) located 5′ to H19. Biallelic expression of H19 has been observed in renal disease patients with hyperhomocysteinemia, a cardiovascular disease risk factor. The present study determined whether hyperhomocysteinemia produces decreased tissue methylation capacity, hypomethylation of the H19 DMD, and altered expression of H19 and Igf2 in adult mice. Mice heterozygous for disruption of the gene for cystathionine-β-synthase (Cbs+/−) and C57BL/6 (Cbs+/+) mice were fed a hyperhomocysteinemic or control diet, respectively, from weaning until 9–12 months of age. Higher plasma total homocysteine (p < 0.001) was found in hyperhomocysteinemic mice than in control mice (95 ± 12 versus 5.0 ± 3 μmol/liter). Hyperhomocysteinemia was accompanied by higher levels of S-adenosylhomocysteine (p < 0.05) and lower S-adenosylmethionine/S-adenosylhomocysteine ratios (p < 0.001) in liver and brain. The effect of hyperhomocysteinemia on H19 DMD methylation was tissue-specific. In liver, hyperhomocysteinemic mice had decreased H19 DMD methylation (p < 0.001). In brain, hyperhomocysteinemia was accompanied by increased H19 DMD methylation (p < 0.001) and a decrease in the ratio of H19/Igf2 transcripts (p < 0.05). In aorta, hyperhomocysteinemia produced an increase in H19 DMD methylation (p < 0.001) and a 2.5-fold increase in expression of H19 transcripts (p < 0.05). Levels of H19 transcripts in aorta correlated positively with plasma total homocysteine concentration (p < 0.05, r = 0.620). We conclude that hyperhomocysteinemia produces tissue-specific changes in H19 DMD methylation and increased vascular expression of H19 in adult mice.

Methylation of DNA is an epigenetic process involved in genomic imprinting, X-chromosome inactivation, and gene expression in cancer (1). Recent evidence suggests that DNA methylation may play a role in the pathology of cardiovascular disease. Global DNA hypomethylation has been observed in advanced atherosclerotic lesions of humans, rabbits, and mice (2–4). Apolipoprotein E-deficient mice have been found to have aberrant DNA methylation patterns involving both hypo- and hypermethylation of DNA in aorta and peripheral blood mononuclear cells but not in liver and skeletal muscle (4). Alterations in DNA methylation profiles were detected prior to the appearance of atherosclerotic lesions, suggesting a pathogenic role for changes in DNA methylation in the progression of atherosclerosis (4). In some models of atherosclerosis, changes in DNA methylation of specific genes, such as extracellular superoxide dismutase (3) and estrogen receptor α (5), have been observed.

Elevation of plasma total homocysteine (tHcy)1 is a cardiovascular disease risk factor, the vascular pathology of which may involve altered DNA methylation (6). Homocysteine is metabolically linked to methylation reactions through the methionine cycle. Within the cycle, methionine is converted to S-adenosylmethionine (AdoMet), which serves as a methyl donor for numerous methyl acceptors, including DNA (7). S-Adenosylhomocysteine (AdoHcy) is produced as a byproduct of methyl donation, and homocysteine is formed through the (reversible) liberation of adenosine from AdoHcy. In human subjects with hyperhomocysteinemia, intracellular AdoHcy levels may increase, resulting in a lower AdoMet/AdoHcy ratio, diminished methylation capacity, and global DNA hypomethylation (8, 9). In mouse models of hyperhomocysteinemia produced by targeted disruption of the cystathionine-β-synthase (Cbs) or methylenetetrahydrofolate reductase (Mthfr) genes, decreased AdoMet/AdoHcy ratios and global DNA hypomethylation have been observed (10–12).

The imprinted gene H19 is located on mouse chromosome 7 in close proximity to the Igf2 gene, which encodes insulin-like growth factor 2 (IGF2). These two genes are reciprocally imprinted in both mice and humans, with H19 expressed from the maternal allele and Igf2 expressed from the paternal allele (13, 14). Paternal-specific methylation of an imprinting control region located 5′ to H19 is a major regulator of imprinting (13). A differentially methylated domain (DMD) within the imprinting control region is thought to function as a boundary/insulator element (14–16). On the maternal allele, the H19 DMD is generally unmethylated, which allows binding of the zinc finger DNA-binding protein, CCCTC-binding factor (CTCF) (14),

* This work was supported by the Office of Research and Development, United States Department of Veterans Affairs, National Institutes of Health Grants HL63943 and NS24621, and American Heart Association Beginning Grant-in-aid 0455315Z (to A. M. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Present address: Nutrition Research Program, University of British Columbia, BC Research Institute for Children’s and Women’s Health, 950 West 28th Ave., Vancouver, Canada.

‡‡ To whom correspondence should be addressed: Dept. of Internal Medicine, C32 GH, The University of Iowa, IA 52242, Tel.: 319-356-4048; Fax: 319-335-8848; E-mail: steven-lentz@uiowa.edu.

1 The abbreviations used are: tHcy, total homocysteine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Cbs, cystathionine-β-synthase; CpfG, CG dinucleotide; CTCF, CCCTC-binding factor; DMD, differentially methylated domain; HH, hyperhomocysteinemic diet; IGF2, insulin-like growth factor 2; HPLC, high performance liquid chromatography.
15). CTCF is thought to allow distal enhancers to activate H19 transcription while blocking enhancer access to Igf2. On the paternal allele, the H19 DMD remains methylated, which prevents CTCF binding and enhancer activation of H19 transcription. Biallelic expression of H19 has been observed in peripheral blood mononuclear cells of human subjects with hyperhomocysteinemia and renal disease (17), which suggests that changes in cellular methylation capacity during hyperhomocysteinemia may be accompanied by hypomethylation of the H19 DMD and consequent loss of imprinting of H19. The vascular consequences of altered expression of H19 and Igf2 are unknown, but increased expression of H19 transcripts has been observed in a rat model of carotid artery injury (18) and in human atherosclerotic plaques (19), and there is evidence that Igf2 may promote atherosclerosis in mice (20).

The goal of the present study was to test the hypothesis that reduced methylation capacity in Cbs+/− mice with hyperhomocysteinemia is accompanied by hypomethylation of the H19 DMD and altered expression of H19 and Igf2. Cbs-deficient mice were chosen as an animal model for homocysteine-related vascular pathology because they are susceptible to diet-induced increases in plasma tHcy and have homocysteine-related endothelial dysfunction (11, 21, 22). Our findings demonstrate that hyperhomocysteinemic mice have tissue-specific alterations in methylation of the H19 DMD and in expression of H19 and Igf2. The relationship between changes in DMD methylation and expression of H19 and Igf2 was stronger in brain than in liver or aorta. Hyperhomocysteinemia produced a 2.5-fold increase in H19 expression in the aorta, which suggests a possible role for H19 in the vascular pathology of hyperhomocysteinemia.

### MATERIALS AND METHODS

#### Mice and Experimental Protocol

Mice heterozygous for targeted disruption of the Cbs gene (Cbs+/−) (23), on a C57BL/6 background, and their wild-type C57BL/6 littermates (Cbs+/+) were used in the study. Genotyping for the wild-type Cbs allele was conducted by PCR using the following primers: CbsL, 5′-AAGAGCCCGACAGAT- GAACA-3′, and CbsR, 5′-GTCGCTGAATCCTATGTAGC-3′. Genotyping for the disrupted Cbs allele, Cbsmut, was accomplished by PCR using the P10 primer and a primer corresponding to the neo cassette used in the targeted disruption (23). At weaning, Cbs+/− and Cbs+/+ mice were fed either a control diet (LM485, Harlan Teklad, Madison, WI) or a hyperhomocysteinemic (HH) diet (TD 00205, Harlan Teklad). The HH diet contained double the amount of methionine, minimal folate acid, and lower choline, pyridoxine, cobalamin, and riboflavin (Table 1). The diets contained adequate levels of all nutrients except for folate in the HH diet (12). The HH diet also contained succinyl sulfathiazole to inhibit growth of intestinal bacteria, another source of folate acid. We have shown previously that Cbs+/− fed a control diet and Cbs+/+ mice fed an HH diet have only moderately elevated levels of plasma tHcy (11). We chose, therefore, to perform comparisons between Cbs+/+ mice fed the control diet and Cbs+/− mice fed the HH diet to maximize the difference in plasma tHcy between groups. After 9–12 months on the diets, mice were anesthetized with sodium pentobarbital (150 mg/kg intraperitoneally), and blood was collected by cardiac puncture. Samples of liver and brain were immediately denitrogenated in ice-cold 0.4 mol/liter perchloric acid, homogenized, and centrifuged. The supernatant fraction was immediately frozen and stored at −80 °C for later AdoMet and AdoHcy analysis. Additional samples of liver and brain were flash-frozen and stored at −80 °C for later extraction of genomic DNA and RNA. The aortas were removed, flash-frozen, and stored at −80 °C for later extraction of genomic DNA and RNA. The protocol was approved by the University of Iowa and Veterans Affairs Animal Care and Use Committees.

#### Biochemical Assays

Plasma tHcy, defined as the total concentration of homocysteine after quantitative reductive cleavage of all disulfide bonds (24), was determined by HPLC using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate detection (25). Levels of AdoMet and AdoHcy in liver and brain were determined by HPLC using UV detection (26).

**Bisulfite Treatment**—The protocol used was adapted from Frommer et al. (27). Genomic DNA was extracted from liver and brain using the DNeasy kit (Qiagen, Valencia, CA) and from aorta using TRIzol reagent (Invitrogen). Genomic DNA samples (1–2 μg) were denatured by incubation with 0.2 mol/liter NaOH for 10 min at 37 °C. Samples were treated with 3 μ sodium bisulfite, 0.5 mmol/liter hydroquinone for 16 h at 50 °C. The bisulfite-treated DNA samples were purified with the DNA Wizard clean-up kit (Promega, Madison, WI) and desulfonated with 0.3 mol/liter NaOH followed by precipitation with ethanol and 6 mol/liter NH4Ac using glycerol (20 μl) as a carrier. Precipitated bisulfite-treated DNA samples were resuspended in water and stored at −20 °C.

**Amplification of the H19 DMD and Sequencing**—A region of the H19 DMD shown to be heavily methylated on the paternal allele and hypomethylated on the maternal allele in normal mouse liver (13) was amplified from bisulfite-treated DNA. This region is located between −1968 and −2555 relative to the transcriptional start site (based on accession number AF049801) and contains 26 CpG sites and one CTCF-binding site (14, 15). The reaction was performed using HotStar TaqDNA polymerase (Qiagen). The primers (H19DMD2, 5′-ATGGTCTCCTTACACAGCTAACAGA-3′, and H19DMD3, 5′-CAGCCTCCTGTTTATGCTATGGG-3′) were designed such that only bisulfite-treated genomic DNA would amplify to produce a 588-bp band. Cycling conditions were 95 °C for 15 min followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min with a final extension of 10 min at 72 °C. PCR products were cloned into pCR-TOPO vector (Invitrogen), and plasmid DNA from the clones was purified using the Miniprep spin kit (Qiagen) followed by automated DNA sequencing using an Applied Biosystems model 3730 DNA analyzer (Foster City, CA) available through the DNA Core Facility of the University of Iowa. Twenty to forty clones containing the amplified H19 DMD PCR products were analyzed from brain, liver, and aorta from each animal.

**Reverse Transcription of H19 and Igf2 Transcripts**—Total RNA was extracted from liver, brain, and aorta using TRIzol reagent (Invitrogen). Samples were treated with DNase I (Promega), in the presence of RNase inhibitor (Promega), to remove contaminating genomic DNA. Integrity of the RNA was assessed by confirming the presence of 18 S and 28 S RNA on agarose gels. For synthesis of cDNA, DNase I-treated RNA samples from liver (1.5 μg), brain (2.0 μg), or aorta (2.0 μg) were incubated with 1.25 units/μl Multiscribe reverse transcriptase (Applied Biosystems), 0.4 units/μl RNase inhibitor, 2.5 μmol/liter random hexamers, 500 μmol/liter each dNTPs, 5.5 mmol/liter MgCl2, and TaqMan reverse transcription buffer in a final reaction volume of 50 μl. Samples were incubated at 25 °C for 10 min for annealing of random hexamers followed by incubation at 48 °C for 30 min for reverse transcription and 95 °C for 10 min for inactivation of reverse transcriptase.

### Table 1

| Nutrient                  | Control | HHb |
|---------------------------|---------|-----|
| t-Methionine (g/kg)       | 4.0     | 8.2 |
| t-Cysteine (g/kg)         | 3.0     | 3.0 |
| Choline (mmol/kg)         | 17.2    | 7.9 |
| Folic acid (mg/kg)        | 6.7     | 0.2 |
| Pyridoxine (mg/kg)        | 16.9    | 8.5 |
| Cobalamin (μg/kg)         | 91.0    | 25.0 |
| Riboflavin (mg/kg)        | 13.7    | 6.0 |

*The HH diet also contained 5.0 g/kg of succinyl sulfathiazole to prevent intestinal bacterial growth, a potential source of folate acid.*
RESULTS

Plasma tHcy and Methylation Capacity in Liver and Brain—Feeding the HH diet to Cbs+/+ mice produced plasma tHcy levels of 94.7 ± 11.9 μmol/liter, almost 20-fold higher (p < 0.001) than the levels of 5.0 ± 0.3 μmol/liter in Cbs+/+ mice fed the control diet (Fig. 1). The elevation in plasma tHcy in the Cbs+/+ mice fed the HH diet was accompanied by a reduction in tissue methylation capacity, with lower AdoMet/AdoHcy ratios (p < 0.001) in liver and brain when compared with Cbs+/+ mice fed the control diet (Fig. 2, A and B). These differences in methylation capacity were due primarily to higher levels of AdoHcy (p < 0.05) in the liver and brain of Cbs+/+ mice fed the HH diet than Cbs+/+ mice fed the control diet. No significant differences in levels of AdoMet in liver or brain were observed between Cbs+/+ mice fed the HH diet and Cbs+/+ mice fed the control diet. Overall, there was a negative correlation between plasma tHcy and AdoMet/AdoHcy ratios in liver (r = 0.01, r = 0.568) and brain (p < 0.05, r = 0.501).

Methylation of the H19 DMD—Methylation analysis of a region of the H19 DMD containing 26 CpGs was performed by bisulfite sequencing, a method in which incubation of DNA with sodium bisulfite converts all unmethylated cytosine residues to thymidine. Sequencing PCR products amplified from the H19 DMD of bisulfite-treated DNA indicated whether the cytosine residues at each of the 26 CpG sites were methylated (Fig. 3A). In the liver, the percentage of Cbs/− mice fed the HH diet and Cbs/− mice fed the control diet. In Cbs/− mice fed the HH diet, on average, 53.0 ± 0.7% of the CpGs (2757 of 5200 CpGs analyzed) were methylated when compared with 60.6 ± 0.6% (2615 of 4316 CpGs analyzed) in Cbs/− mice fed the control diet.

The converse effect on H19 DMD methylation was observed in the brain and aorta, where the percentage of CpG sites that were methylated was higher (p < 0.001) in Cbs/− mice fed the HH diet than in Cbs/− mice fed the control diet (Fig. 3B). In Cbs/− mice fed the HH diet, on average, 53.0 ± 0.7% of the CpGs (2757 of 5200 CpGs analyzed) were methylated when compared with 60.6 ± 0.6% (2615 of 4316 CpGs analyzed) in Cbs/− mice fed the control diet.
accompanied by tissue-specific alterations in the expression of \(H19\) or \(Igf2\), levels of \(H19\) and \(Igf2\) transcripts were quantified by real-time PCR (Table II). In the liver and brain, levels of \(H19\) transcripts did not differ significantly between \(Cbs^{+/+}\) mice fed the control diet and \(Cbs^{+/−}\) mice fed the HH diet. In the aorta, higher levels of \(H19\) transcripts (\(p = 0.02\)) were found in \(Cbs^{+/−}\) mice fed the HH diet than in \(Cbs^{+/+}\) mice fed the control diet. Overall, there was a positive correlation (\(p < 0.05, r = 0.619\)) between levels of \(H19\) transcripts in aorta and plasma tHcy concentration.

Levels of \(Igf2\) transcripts tended to be lower in the liver and higher in the brain of \(Cbs^{+/−}\) mice fed the HH diet when compared with \(Cbs^{+/+}\) mice fed the control diet, but these differences did not reach statistical significance (Table II). No differences in levels of \(Igf2\) transcripts were seen in the aortas of \(Cbs^{+/+}\) mice fed the control diet versus \(Cbs^{+/−}\) mice fed the HH diet.

Because \(H19\) and \(Igf2\) are reciprocally imprinted in the mouse, we compared the ratios of \(H19\) transcripts with those of \(Igf2\) transcripts in liver, brain, and aorta in each group of mice. The ratio of \(H19/Igf2\) transcripts in liver and aorta did not differ significantly between \(Cbs^{+/+}\) mice fed the control diet and \(Cbs^{+/−}\) mice fed the HH diet (Fig. 4, A and C). In the brain, which exhibited a large increase in \(H19\) DMD methylation in \(Cbs^{+/−}\) mice fed the HH diet when compared with \(Cbs^{+/+}\) mice fed the control diet (Fig. 3C), there was a corresponding decrease in the ratio of \(H19/Igf2\) transcripts in \(Cbs^{+/−}\) mice fed the HH diet (\(p = 0.006\)) (Fig. 4B).

**DISCUSSION**

This work sought to determine whether the reduced methylation capacity in hyperhomocysteinemic mice is accompanied by hypomethylation of the \(H19\) DMD and altered expression of \(H19\) and \(Igf2\) transcripts. We chose to study the effects of hyperhomocysteinemia in the liver because it is the primary site of homocysteine metabolism, and also in the aorta and brain, which are sites of pathology in humans with hyperhomocysteinemia (6).

There are three major findings of this study. First, we found that the effect of hyperhomocysteinemia on \(H19\) DMD methylation is tissue-specific. In the liver, the reduced tissue methylation capacity (AdoMet/AdoHcy ratio) in \(Cbs^{+/−}\) mice with hyperhomocysteinemia was accompanied by a significant decrease in methylation of the \(H19\) DMD. In the brain, hyperhomocysteinemia also produced a decrease in the AdoMet/AdoHcy ratio, but the lower methylation capacity was paradoxically associated with an increase in methylation of the \(H19\) DMD. Hyperhomocysteinemia in \(Cbs^{+/−}\) mice was also associated with a significant increase in \(H19\) DMD methylation in the aorta. The second major finding is that the reduced methylation capacity and increased \(H19\) DMD methylation in the brain of mice with hyperhomocysteinemia was associated with
from Cbs HH diet (\(H11002/H11001\) Cbs \(H11005\) (n transcripts in brain from script levels in mice with hyperhomocysteinemia. A transcripts in liver from \(H19/Igf2\) \(H19\) in regulating which suggests that DNA methylation plays a major role in a significant decrease in the ratio of \(H19\) to that seen in human patients with hereditary hyperhomocysteinemia. Cbs \(H11001\) \(H11002\) Cbs \(H11005\) mice fed the HH diet (95 (n transcripts in aorta. This finding suggests a possible role for \(H19\) in the vascular pathology associated with hyperhomocysteinemia. We used an animal model that combined a genomic approach (Cbs+/−) with a dietary intervention (the HH diet) to induce hyperhomocysteinemia. The level of plasma tHcy achieved in Cbs+/− mice fed the HH diet (95 ± 12 μmol/liter) was similar to that seen in human patients with hereditary CBS deficiency (28). As expected, Cbs+/− mice fed the HH diet had decreased AdoMet/AdoHcy ratios in both liver and brain (Fig. 2), indicative of reduced methylation capacity. The effect of hyperhomocysteinemia on AdoMet/AdoHcy ratio was principally caused by a significant increase in the level of AdoHcy in both liver and brain. We had predicted that the reduced methylation capacity in hyperhomocysteinemia might result in decreased methylation of the \(H19\) DMD and loss of imprinting at the \(H19\) locus. In the liver, the hyperhomocysteinemic animals did have a modest but significant decrease in methylation of the \(H19\) DMD (Fig. 2B). In the brain, however, these animals had a large increase in \(H19\) DMD methylation (Fig. 2C). It was not possible to determine whether there was differential loss of \(H19\) genomic imprinting because the Cbs+/− and Cbs+/− mice were of the same background strain (C57BL/6). This question could be addressed in future studies using control and hyperhomocysteinemic mice that differ in a sequence polymorphism at the \(H19\) locus.

Interestingly, the increase in \(H19\) DMD methylation in the brain was accompanied by a significant decrease in the ratio of \(H19\) transcripts to \(Igf2\) transcripts (Fig. 4B). This observation fits well with the boundary/insulator model of the \(H19\) DMD in which CTCF binds to the unmethylated \(H19\) DMD, allowing distal enhancers to activate \(H19\) transcription whereas simultaneously inhibiting access of these enhancers to \(Igf2\) (14–16). The mice with hyperhomocysteinemia did not, however, have an altered ratio of \(H19\) transcripts to \(Igf2\) transcripts in liver. This finding suggests that control of \(H19\) and \(Igf2\) expression in liver involves other regulatory factors besides \(H19\) DMD methylation.

We also found a significant increase in the levels of \(H19\) transcripts in aorta from Cbs+/− mice fed the HH diet. The increased expression of \(H19\) transcripts was likely unrelated to the methylation status of the \(H19\) DMD because these mice had a modest increase in methylation of the \(H19\) DMD in aorta. The effect of hyperhomocysteinemia on levels of \(H19\) transcripts in aorta may be a consequence of homocysteine-related changes in the expression and/or ability of CTCF to bind to the \(H19\) DMD, changes in the methylation status of other sites within the imprinting control region, or an effect on some other yet uncharacterized factor required for \(H19\) transcription.

The finding of increased levels of \(H19\) transcripts in aorta of Cbs+/− mice fed the HH diet may have implications for the vascular pathology of hyperhomocysteinemia. \(H19\) belongs to a family of imprinted genes that encode untranslated RNA transcripts. Some studies suggest that \(H19\) mRNA may function as a tumor suppressor, whereas others have shown increased expression of \(H19\) in tumors (29, 30). Increased expression of \(H19\) transcripts has been demonstrated in rat carotid arteries following balloon injury (18) and in human atherosclerotic plaques (19). Overexpression of \(H19\) in a bladder carcinoma cell line was associated with increased expression of tumor necrosis factor-α, a factor implicated in the vascular pathology associated with hyperhomocysteinemia (31, 32).

In summary, this study demonstrated that Cbs+/− mice with hyperhomocysteinemia have reduced tissue methylation.
capacity accompanied by tissue-specific changes in the methylation and expression of H19. In this model, changes in methylation of the H19 DMD did not strictly correlate with changes in the expression of H19 or Igf2 transcripts. These findings suggest that other factors besides H19 DMD methylation contribute to the regulation of H19 and Igf2 expression in hyperhomocysteinemia. The tightest correlation between H19 DMD methylation and regulation of H19 and Igf2 expression was seen in the brain, which suggests that H19 DMD methylation is a stronger regulator of H19 expression in the brain. Despite the variable relationship between H19 DMD methylation and H19 expression in different tissues, it is clear that hyperhomocysteinemia can have profound effects on the methylation and expression of imprinted genes in adult animals. Additional work will be required to define the exact mechanism(s) by which homocysteine affects expression of H19 and the role of H19 in the vascular pathology of hyperhomocysteinemia.

**Acknowledgments**—We thank Dr. Sanjana Dayal, Ryan McCaw, and Sara Rozen for technical assistance.

**REFERENCES**

1. Herman, J. G., and Baylin, S. B. (2003) *N. Engl. J. Med.* 349, 2042–2054
2. Laukkana, M. O., Manninen, S., Hiltnenen, M. O., Attomaki, S., Airenne, K., Janne, J., and Yla-Herttuala, S. (1999) Arterioscler. Thromb. Vasc. Biol. 19, 2171–2178
3. Hiltnenen, M. O., Turunen, M. P., Hakkinen, T. P., Ratanen, J., Hedman, M., Makinen, K., Turunen, A. M., Alto-Setala, K., and Yla-Herttuala, S. (2002) Vasc. Med. 7, 5–11
4. Lund, G., Andersson, L., Lauria, M., Lindholm, M., Fraga, M. F., Villar-Garea, A., Ballester, E., Esteller, M., and Zaina, S. (2004) *J. Biol. Chem.* 279, 29147–29154
5. Post, W. S., Goldschmidt-Clermont, P. J., Wilhide, C. C., Heldman, A. W., Sussman, M. S., Owang, P., Milliken, E. E., and Issa, J. P. (1999) *Cardiovasc. Res.* 43, 365–391
6. Homocysteine Studies Collaboration (2002) *J. Am. Med. Assoc.* 288, 2015–2022
7. Finkelstein, J. D. (1990) *J. Nutr. Biochem.* 1, 228–237
8. Yi, P., Melnyk, S., Pogribny, I. P., Hine, R. J., and James, S. J. (2000) *J. Biol. Chem.* 275, 29318–29323
9. Castro, R., Rivera, I., Struys, E. A., Janssen, E. E., Ravasco, P., Camilo, M. E., Blom, H. J., Jakobs, C., and Tavares de Almeida, I. (2000) *Clin. Chem.* 46, 1292–1296
10. Chen, Z., Karaplis, A. C., Ackerman, S. L., Pogribny, I. P., Melnyk, S., Lusser-Cacan, S., Chen, M. F., Pai, A., John, S. W. M., Smith, R. S., Bottiglieri, T., Ragley, P., Selhub, J., Rudnicki, M. A., James, S. J., and Rozen, R. (2001) *Hum. Mol. Genet.* 10, 433–443
11. Dayal, S., Bottiglieri, T., Arning, E., Maeda, N., Malinow, M. R., Sigurd, C. D., Heistad, D. D., Faraci, F. M., and Lentz, S. R. (2001) *Circ. Res.* 88, 1203–1209
12. Devlin, A. M., Arning, E., Bottiglieri, T., Faraci, F. M., Rozen, R., and Lentz, S. R. (2004) *Blood* 103, 2693–2698
13. Thiovattelsen, J. L., Duran, K. L., and Bartolomei, M. S. (1998) *Genes Dev.* 12, 3693–3702
14. Bell, A. C., and Felsenfeld, G. (2000) *Nature* 405, 482–485
15. Harla, A. T., Schoenherr, C. J., Katz, D. J., Ingram, R. S., Leverose, J. M., and Tilghman, S. M. (2000) *Nature* 405, 486–489
16. Kaffer, C. R., Srivastava, M., Park, K. Y., Ives, E., Hsieh, S., Batlle, J., Grinberg, A., Huang, S. P., and Pfeifer, K. (2000) *Genes Dev.* 14, 1908–1919
17. Ingrosso, D., Cimmino, A., Perna, A. P., Masella, L., De Santo, N. G., De Bonis, M. L., Vaccu, M., D’Esposito, M., D’Urso, M., Gallietti, P., and Zappia, V. (2003) *Lancet* 361, 1693–1699
18. Kim, D. K., Zhang, L., Duan, V. J., and Pratt, R. E. (1994) *J. Clin. Investig.* 93, 355–360
19. Han, D. K., Khaing, Z. Z., Pollock, R. A., Haudenschild, C. C., and Liau, G. (1996) *J. Clin. Investig.* 97, 1267–1268
20. Zaina, S., Pettersson, L., Ahrens, B., Brazen, L., Hasson, A. B., Lindholm, M., Mattsson, R., Thibyng, J., and Nilsson, J. (2002) *J. Biol. Chem.* 277, 4505–4511
21. Eberhardt, R. T., Forgione, M. A., Cap, A., Leopold, J. A., Munn, M. R., Malinow, M. R., Heyrick, S., Kings, E. S., Moldovan, N. I., Yaghoubi, M., Goldschmidt-Clermont, P. J., Fair, H. W., Cohen, R., and Luykz, J. (2000) *J. Clin. Investig.* 106, 483–491
22. Dayal, S., Arning, E., Bottiglieri, T., Bocker, R. H., Sigmund, C. D., Faraci, F. M., and Lentz, S. R. (2000) *Stroke* 31, 957–962
23. Watanabe, M., Osada, J., Arasani, Y., Khulkin, K., Reddick, R., Malinow, M. R., and Maeda, N. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 1585–1589
24. Mudd, S. H., Finkelstein, J. D., Refsum, H., Ueland, P. M., Malinow, M. R., Lentz, S. R., Jacobone, D. W., Brattstrom, L., Wilcken, B., Wilcken, W. D., E., Blom, H. J., Stable, S. P., Allen, R. H., Selhub, J., and Rosenberg, I. H. (2000) *Arterioscler. Thromb. Vasc. Biol.* 16, 2015–2022
25. Martin, S. C., Hilton, A. C., Bartlett, W. A., and Jones, A. F. (1999) *Biochem. Chromatogr.* 13, 81–92
26. Bottiglieri, T. (1990) *Biochem. Chromatogr.* 4, 239–241
27. Frommer, M., McDonald, L. E., Miller, D. S., Collins, C. M., Watt, F., Grigg, G. W., Millroy, P. L., and Paul, C. L. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 1827–1831
28. Yap, S., Naughten, E. R., Wilcken, B., Wilcken, D. E., and Boers, G. H. (2000) *Semin. Arterioscler. Hemostasis* 26, 335–340
29. Hao, Y., Crenshaw, T., Moulton, T., Newcomb, E., and Tycko, B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 89, 239–241
30. Ariet, I., Miao, H. Q., Ji, X. R., Schneider, T., Roll, D., de, G. N., Hochberg, A., and Ayesh, S. (1999) *J. Biol. Chem.* 274, 21–25
31. Ayesh, S., Matouk, I., Schneider, T., Ohana, P., Laster, M., Al-Shafer, W., de-Groot, N., and Hochberg, A. (2002) *Mol. Carcinogen.* 35, 63–74
32. Ungvari, Z., Csiszar, A., Edwards, J. G., Kalmann, M., Wolin, M. S., Kaley, G., and Koller, A. (2003) *Arterioscler. Thromb. Vasc. Biol.* 23, 418–424
Tissue-specific Changes in H19 Methylation and Expression in Mice with Hyperhomocysteinemia

Angela M. Devlin, Teodoro Bottiglieri, Frederick E. Domann and Steven R. Lentz

J. Biol. Chem. 2005, 280:25506-25511.
doi: 10.1074/jbc.M504815200 originally published online May 17, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M504815200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 15 of which can be accessed free at http://www.jbc.org/content/280/27/25506.full.html#ref-list-1