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Betaine-homocysteine methyltransferase: human liver genotype-phenotype correlation

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Abstract

Betaine-homocysteine methyltransferase (BHMT) catalyzes the remethylation of homocysteine. BHMT is highly expressed in the human liver. In the liver, BHMT catalyzes up to 50% of homocysteine metabolism. Understanding the relationship between *BHMT* genetic polymorphisms and function might increase our understanding of the role of this reaction in homocysteine remethylation and in S-adenosylmethionine-dependent methylation. To help achieve those goals, we measured levels of BHMT enzyme activity and immunoreactive protein in 268 human hepatic surgical biopsy samples from adult subjects as well as 73 fetal hepatic tissue samples obtained at different gestational ages. BHMT protein levels were correlated significantly ($p < 0.001$) with levels of enzyme activity in both fetal and adult tissue, but both were decreased in fetal tissue when compared with levels in the adult hepatic biopsies. To determine possible genotype-phenotype correlations, 12 tag SNPs for *BHMT* and the closely related *BHMT2* gene were selected from SNPs observed during our own gene resequencing studies as well as from HapMap data were used to genotype DNA from the adult hepatic surgical biopsy samples, and genotype-phenotype association analysis was performed. Three SNPs (rs41272270, rs16876512, and rs6875201), located 28 kb upstream, in the 5'-UTR and in intron 1 of *BHMT*, respectively, were significantly correlated with both BHMT activity ($p = 3.41E-8$, $2.55E-9$ and $2.46E-10$, respectively) and protein levels ($p = 5.78E-5$, $1.08E-5$ and $6.92E-6$, respectively). We also imputed 230 additional SNPs across the *BHMT* and *BHMT2* genes, identifying an additional imputed SNP, rs7700790, that was also highly associated with hepatic BHMT enzyme activity and protein. However, none of the 3 genotyped or one imputed SNPs displayed a "shift" during electrophoretic mobility shift assays.

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These observations may help us to understand individual variation in the regulation of BHMT in the human liver and its possible relationship to variation in methylation.

Keywords

Betaine-homocysteine methyltransferase; *BHMT*; *BHMT2*; single nucleotide polymorphisms; SNPs; genotype-phenotype correlation; hepatic tissue

Introduction

Betaine homocysteine methyltransferase (BHMT) is a cytosolic zinc metalloenzyme that is highly expressed in the liver, kidney and lens of the eye [1,2]. BHMT catalyzes the transfer of a methyl group from betaine to homocysteine, resulting in the generation of dimethylglycine and methionine. Homocysteine remethylation is a critical step in the synthesis of S-adenosylmethionine (AdoMet), the methyl donor for most methylation reactions [3]. A methionine-deficient diet induces BHMT expression and increases its activity in mice [4], while excess AdoMet can down regulate BHMT expression through an NFkB-dependent mechanism [5]. By competing with transsulfurylation, BHMT-catalyzed remethylation of homocysteine helps to maintain homocysteine homeostasis. Elevated levels of homocysteine are a known risk factor for vascular disease and neural tube defects [6–9]. BHMT transgenic mice are resistant to alcohol-induced hepatic steatosis [10]. This effect is thought to result from increased AdoMet/S-adenosylhomocysteine (AdoHcy) and phosphatidylcholine (PC)/phosphatidylethanolamin (PE) ratios. BHMT has been reported to protect hepatocytes from homocysteine-induced injury [11,12]. Finally, betaine, the methyl donor in homocysteine remethylation catalyzed by BHMT, has been used to treat alcoholic liver disease [13,14].

The human *BHMT* gene maps to chromosome 5q13.1-5q15, spans approximately 20 kb, contains 8 exons and encodes a 406 amino acid protein [4,15]. A closely related gene, *BHMT2*, is located 22.3 kb 5' of *BHMT*, and encodes a protein that is 73% identical to BHMT in amino acid sequence [16]. These two genes are thought to have originated from a tandem duplication event [17], and many SNPs in the two genes are in linkage disequilibrium (LD). *BHMT2* protein is rapidly degraded unless it is bound to BHMT and is stabilized by homocysteine [18]. Little is currently known with regard to the relationship between DNA sequence variation in these genes and variation in their expression.

We set out to test the hypothesis that variation in the sequence of the *BHMT* and/or *BHMT2* genes might play a role in variation in BHMT expression in the liver. Specifically, BHMT protein and enzyme activity levels were determined for 268 adult liver surgical biopsy samples and 73 fetal hepatic tissue samples. Twelve “tag SNPs” from *BHMT* and *BHMT2* were then genotyped using DNA from the adult hepatic biopsy samples. Genotype-phenotype association studies were performed, and SNPs that showed significant correlations with protein expression were studied functionally by performing electrophoresis mobility shift (EMS) assays. In summary, we have identified a series of SNPs that were associated with both levels of BHMT protein and enzymatic activity in these human hepatic biopsy samples. These results represent a step toward understanding the role of genetic polymorphisms in variation in BHMT function.

Materials and methods

Human hepatic surgical biopsy samples

A total of 341 human tissue biopsy samples were included in this study. Two hundred and sixty-eight adult liver samples were obtained from European-American (EA) women who had clinically indicated surgery at the Mayo Clinic, predominantly for the diagnosis and/or treatment of metastatic carcinoma. Hepatic tissue uninvolved with tumor was used to perform these experiments. An additional 73 fetal liver samples were obtained through NICHD-supported tissue retrieval programs, 43 from the Laboratory of Developmental Biology at the University of Washington (Seattle, WA) and 30 from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland (Baltimore, MD). The fetal tissue consisted of samples from 27 females and 33 males. Information on sex was not available for 13 fetal tissue samples. All samples were anonymized, and only information with regard to clinical diagnosis, sex, race, and age was provided. The Mayo Clinic Institutional Review Board reviewed and approved these studies, and collection of the fetal tissues was approved by the Pediatric Institutional Review Board at Children's Mercy Hospitals and Clinics.

Genotyping and gene resequencing

For the 268 adult liver biopsy samples, twelve polymorphisms were selected for genotyping by using the LD-tag selection method of Carlson [19] and the haplotype-tagging (ht-tag) method [20], utilizing both our own gene resequencing results [18] and HapMap data. Specifically, nine *BHMT* SNPs and three *BHMT2* SNPs were genotyped. LD-tag SNPs were required to have a minimum frequency of 5% and an 80% correlation within 'bins'. Ht-tag SNPs were required to have a minimum frequency of 2%, a haplotype frequency of 1%, and an r^2 value of 0.9. Genotyping was performed using the Illumina GoldenGate platform (Illumina, San Diego, CA). All SNPs genotyped had 100% call rates.

Two human liver biopsy DNA samples were also used to resequence the *BHMT* gene because these samples represented "outlier points" for *BHMT* homospecific activity. For these two DNA samples, 9 PCR reactions were performed with primers that hybridized approximately 200 bp on either side of each *BHMT* exon. Approximately 1 kb of the 5'-flanking region (FR) was also amplified and all amplicons were sequenced, as described previously [18].

BHMT enzyme assay

Methyl- ^{14}C -betaine hydrate (specific activity 29.3 mCi/mmol) was synthesized by Perkin-Elmer (Boston, MA) for use in the *BHMT* enzyme activity assay. The assay procedure was a modification of the method described by Garrow et al [1]. Specifically, the 100 μL reaction mixture contained 400 μM D, L-homocysteine, 64 μM (0.16 $\mu\text{Ci}/\mu\text{mol}$) betaine and 50 mM KH_2PO_4 (pH 8.0). The enzyme assay was initiated by the addition of radioactive betaine. Following incubation for 2 h at 37°C, the reaction was terminated by adding 1 ml of ice-cold water to each tube, and the tubes were immediately placed on ice. After vortexing, 1 ml of each reaction mixture was transferred to a Dowex 1-X4 ion exchange column that contained 2 ml of resin (Sigma-Aldrich Corp., St. Louis, MO). The commercially available Cl^- form of the resin had been converted to the OH^- form by pre-treatment with 1 M NaOH, followed by rinsing with 4 volumes of deionized water. Five ml of deionized water was used to elute unreacted betaine from the column. The reaction product was then eluted by adding 3 ml of 1.5 N HCl, and radioactivity was measured in a liquid scintillation counter.

To ensure consistency and accuracy of the enzyme assays, two precautions were taken. All samples were assayed at two different dilutions, 1:20 and 1:40 and a "standard" that

consisted of a pool of liver supernatant from ten samples was included with assays to make it possible to “correct” each assay for small day-to-day variations. The average activity of the pooled sample was 27 ± 3.1 nmol/hr/mg protein (mean $\pm$ SD, N=20).

Quantitative Western blot analysis

A peptide consisting of BHMT amino acids 384-406, an area not represented in the amino acid sequence of BHMT2, was synthesized in the Mayo Proteomics Facility. Rabbit polyclonal antiserum to this peptide was generated by Cocalico Biologicals, Inc. (Reamstown, PA). Specificity of the antibody was tested by Western blot analysis performed with both human liver cytosol and recombinant WT human BHMT and BHMT2 expressed in COS-1 cells. Bound antibody was detected using the ECL Western Blotting system after performing SDS PAGE using 10% gels (Amersham Pharmacia, Piscataway, NJ). Immunoreactive BHMT protein levels were calculated from the intensity of each band compared with serial dilutions of the same pooled human liver cytosol that was used as a standard for the enzyme assays. This step made it possible to create a “standard curve” that consisted of 5 different dilutions. A “unit” of immunoreactive protein was defined in terms of the intensity of bands for the pool which contained 0.85 μ g/ml protein. Finally, for 128 randomly selected samples, separate gels were run and stained for β -actin as a control. The r^2 value for the correlation of β -actin with BHMT was 0.003, indicating that the BHMT results were not due to systematic variation in protein stability in the samples.

Electrophoresis Mobility Shift Assay (EMSA)

EMSA was performed using the Lightshift Chemiluminescent EMSA Kit (Pierce Biotechnology, Rockford, IL). 5'-Biotin-labeled oligonucleotide probes that included 20 to 22 bp of nucleotide sequence surrounding the SNP being studied were synthesized for both WT and variant sequences (see Supplementary Table 1). Nuclear extracts were obtained from HEK293T and HepG2 cells during the log growth phase. Probes were annealed to form double stranded DNA, and binding reactions contained 1X binding buffer, 50 ng/ μ l poly dIdC, 20 fmol Biotin-labeled probe and 9 μ g nuclear extract protein. 500-fold excess (10 pmol) unlabeled probe was used in the competition reactions, and reaction mixtures were run on 6% non-denaturing polyacrylamide gels, with detection by chemiluminescence (Pierce Biotechnology).

Data analysis

Since the distributions of both BHMT enzyme activity and immunoreactive protein levels were slightly skewed, the Spearman rank correlation was used to calculate correlations for the association studies. Correlation analyses for two phenotypes, BHMT protein level and enzyme activity, were performed using the GraphPad Prism software program (GraphPad, San Diego, CA). Genotype-phenotype association analysis was computed assuming an additive genetic model, with genotypes coded as 0, 1 or 2 for the number of minor alleles. Correlations for the phenotypes were assessed with Spearman rank correlation. Haplotype analysis was completed using two statistics in the software *Haplo.Stats* (http://mayoresearch.mayo.edu/schaid_lab/upload/manualHaploStats.pdf); the global score statistics and the maximum score statistic. The maximum score statistic is the maximum of the square of the haplotype-specific score statistics, which has an unknown distribution, so its significance can only be given by the simulated p value. If only one or two haplotypes are associated with the trait, the maximum score statistic should have greater power to detect association than the global statistic. Estimation of p values for haplotype tests was computed using simulation methods. Linkage disequilibrium (LD) was determined by calculating D' and r^2 values for all possible pairwise combinations of polymorphisms. Imputation across *BHMT* and *BHMT2* using MACH v1.0 was also carried out for untyped markers on the 268 human liver biopsies with BHMT phenotype and genotype data. The CEU population of the

“1000 Genomes Project” (N=60) was used as a reference population (including only biallelic markers). The region of interest that was imputed was 78,398,250 to 78,488,033 on chromosome 5. LD plots for all genotyped SNPs and imputed SNPs ($p < 1E-04$) with minor allele frequencies (MAFs) higher than 0.05 were generated using Haploview version 4.1 [21]. Mean values reported subsequently are $\pm$ SD.

Results

Phenotyping of adult hepatic biopsy samples

BHMT immunoreactive protein and enzyme activity levels were measured in 268 adult human liver cytosol preparations. A single pooled liver cytosol sample served as a standard for these assays, and an identical quantity of this standard preparation was assayed with all samples. In these adult human liver samples, the average BHMT enzyme activity was 17.5 ± 6.30 nmol/hr/mg protein and ranged from 3.58 to 44.4 nmol/hr/mg protein (Figure 1A). BHMT immunoreactive protein was calculated as “units” relative to the pooled standard, and averaged 6.86 ± 3.29 units BHMT protein/mg protein in these 268 samples, ranging from 0.34 to 24.6 units BHMT protein/mg protein (Figure 1B).

An additional phenotype, activity per unit BHMT protein, i.e., homospecific activity, was also determined, and the frequency distribution histogram for this phenotype is shown in Figure 1C. While most of the samples clustered in the 0 to 5 nmol/hr/unit BHMT protein range, two samples showed significantly higher activity per unit protein, as indicated by the arrow. To pursue this observation, we resequenced all exons and the 5'-FR of *BHMT* using DNA from these two samples in an attempt to identify novel SNPs. One new SNP was identified in the 5'-FR, 399 bp upstream from the *BHMT* start codon [C(−399)T]. However, no novel nonsynonymous SNPs – the most likely explanation for a polymorphism related to alteration in homospecific activity – were identified in either of these samples.

None of the three phenotypes studied showed a significant correlation with age in samples from adults (Figure 2) and, since all of the adult samples were obtained from women, neither age nor gender were taken into account as covariants in the association studies described subsequently. Correlations were also determined between activity and protein levels for the adult samples (Figure 3A). There was a significant correlation between BHMT enzyme activity and immunoreactive protein in these liver biopsy samples (Figure 3A, $r_s = 0.55$, $p < 0.0001$). Therefore, variation in BHMT activity in these adult liver samples appeared to depend mainly on variation in the quantity of protein.

Phenotyping of fetal hepatic biopsy samples

Seventy-three fetal liver biopsy samples were also phenotyped, using the same pooled hepatic cytosol as an internal standard. BHMT enzyme activity in these samples averaged 8.4 ± 5.68 nmol/hr/mg protein and immunoreactive protein averaged 2.69 ± 2.09 units BHMT protein/mg protein (Figure 3B); in both cases significantly ($p < 0.0001$) lower than in the adult tissue samples. These two phenotypes were also significantly correlated in fetal hepatic samples, with $r_s = 0.61$, $p < 0.0001$ (Figure 3B). Values for the fetal samples clustered at the lower end of the distribution of values for adult sample (Figure 3C). The combined sample set, including both adults and fetal samples, had an $r_s = 0.69$, $p < 0.0001$ for the correlation between enzyme activity and protein (Figure 3C).

The possible correlation of intrauterine age with BHMT activity and/or protein levels in fetal hepatic tissue was also determined. In these fetal liver samples, gestational age correlated significantly with BHMT activity (Figure 4) but was not significant for protein ($p = 0.055$). Rao and co-workers have reported that the expression of BHMT in the epithelium and cortical region of the lens is developmentally regulated [22]. Our data indicate that the same

appears to be true of the fetal liver enzyme. No gender difference was observed in either fetal liver BHMT activity or immunoreactive protein levels (data not show).

Genotyping of adult liver samples

A total of twelve tag SNPs in the *BHMT* and *BHMT2* genes were selected for genotyping. Ten of the SNPs were selected from HapMap data (<http://hapmap.ncbi.nlm.nih.gov/>), and two were selected based on data from our previous *BHMT* gene resequencing study [18]. Nine SNPs from the *BHMT* gene, and three from *BHMT2* were included on the basis of the linkage disequilibrium (LD) structures of the two genes. In order to have adequate power, genotyping was performed only with the 268 adult liver samples. Three of the DNA samples failed, so the final analysis included 265 samples. Genotype and minor allele frequencies (MAFs) were determined for the 12 genotyped SNPs and are listed in Table 1. All SNPs had 100% call rates in the 265 DNA samples studied, and MAFs obtained during genotyping compared favorably to those in the HapMap database for European subjects.

Genotype-phenotype association studies

Genotype-phenotype association studies were performed using these data for samples from the adult subjects. Specifically, genotype results were correlated with BHMT enzyme activity and immunoreactive protein levels. The results of that analysis are listed in Table 2, with data for SNPs with significant p values highlighted. Among the 12 SNPs genotyped, rs41272270, rs16876512, and rs6875201 were all associated with both BHMT enzyme activity and immunoreactive protein levels, with p values of 3.41E-08, 2.55E-09 and 2.46E-10, respectively, for activity and 5.78E-05, 1.08E-05 and 6.92E-06, respectively, for protein levels. rs41272270 maps to *BHMT2*, while rs16876512, rs6875201 are located in *BHMT* (see Figure 5). All three of these SNPs were in LD (r^2 values from 0.92 to 1.0). As anticipated, BHMT levels of enzyme activity and immunoreactive protein levels were both significantly correlated with genotype for these SNPs. The correlation of BHMT activity and protein for the rs6875201 SNP with the lowest p values for both phenotypes are depicted graphically in Figure 6.

Haplotype analyses were also performed for both phenotypes. Because of their high LD, it seemed likely that the single SNP correlations observed resulted from their inclusion in common haplotypes. As anticipated, the “AAG” haplotype for rs41272270, rs1687651 and rs6875201 was significantly associated with higher enzyme activity and higher protein levels (Table 3C), while the “GGA” haplotype for these SNPs was associated with decreased enzyme activity (Table 3B) and protein levels (Table 3C). The GGA haplotype for these SNPs was designated as “wild type” (WT) because it was the most common haplotype in our samples.

Functional validation

Genetic variation can result in alteration of transcription factor binding, and, as a result, variation in gene expression [23]. None of the three significant genotyped SNPs was located within the open reading frames of either *BHMT* or *BHMT2*. However, since we only genotyped 12 SNPs, and since they were selected to be “tag” SNPs, we also utilized the latest data from the “1000 Genomes Project” to impute 230 additional SNPs across *BHMT* and *BHMT2* (see Figure 5). The figure shows that, in addition to the 3 genotyped SNPs with low p values for association with the two phenotypes studied, one additional SNP, rs7700970, located in *BHMT*, was also identified. This imputed SNP displayed apparent p values of 5.14E-07 and 1.56E-05 for enzyme activity and protein levels, respectively. As the next step, EMSA was performed for each of the these 4 SNPs, 3 genotyped and 1 imputed, to determine whether any nuclear extract protein(s) might bind to motifs altered by the SNPs. The sequences of the EMSA probes are listed in Supplementary Table 1.

Unfortunately, none of the EMS assays displayed a clear and reproducible “shift”, leaving the mechanism responsible for the associations that we observed unresolved.

Discussion

BHMT catalyzes the folate independent remethylation of homocysteine, a reaction that lies at the convergence of the folate and the methionine cycles [14,18]. BHMT transfers a methyl group from betaine to homocysteine, resulting in the formation of methionine and dimethylglycine. The methionine formed by this reaction can then be converted to AdoMet, the methyl donor for most methylation reactions [24,25]. Because the reaction that it catalyzes generates AdoMet and removes homocysteine, BHMT is thought to play an important role in protecting the liver from hepatotoxicity [11,12]. Elevated AdoMet concentrations have been shown to down-regulate BHMT in HepG2 cells [5]. Analysis of 2.7 kb of the *BHMT* 5'-FR has revealed up to forty different possible transcription factor binding sites [5]. Understanding the relationship between *BHMT* gene sequence variation and the regulation of BHMT expression will not only help us to understand mechanisms involved in maintaining methionine and folate cycle homeostasis, but could also contribute to our understanding of hepatotoxicity. However, despite its potential importance, there have not been previous systematic studies of BHMT genotype-phenotype correlations.

In the present study, we set out to test the hypothesis that DNA sequence variation in or near the *BHMT* gene might be related to variation in its expression. Three phenotypes, BHMT enzyme activity, BHMT immunoreactive protein level and BHMT activity per unit protein, were determined for 268 adult liver biopsy samples and 73 fetal liver tissue samples. All of these phenotypes varied widely among individuals (see Figures 1–3). The distribution of BHMT activity per unit protein showed two outlier samples (Figure 1). However, resequencing of all *BHMT* exons using DNA from these two samples failed to reveal any novel nonsynonymous SNPs that might explain these observations. There was a significant positive correlation between BHMT enzyme activity and protein levels in samples of both adult and fetal hepatic tissue. Therefore, the major mechanism associated with the wide individual variation in BHMT enzyme activity which we observed appeared to be alteration in protein quantity. Phenotyping of BHMT in fetal biopsy samples showed that BHMT protein and enzyme activity increased – in parallel – during fetal development (Figure 3).

We then selected 12 tag SNPs in *BHMT* and the closely related *BHMT2* gene for genotyping of DNA extracted from the same 268 adult liver biopsy samples. Genotype-phenotype correlation analyses showed that three SNPs, all in LD, were significantly associated with the BHMT expression phenotypes. Two of the highly associated SNPs were present in the *BHMT* gene, rs16876512 in the 5'-FR and rs6875201 in intron 1, while the third SNP, rs41272270, was located in intron 5 of *BHMT2*. We also used “1000 Genomes Project” data to impute 230 additional SNPs across *BHMT* and *BHMT2* (see Figure 5) and identified an additional SNP, rs7700970, that also displayed a high degree of association with the two phenotypes. Since SNPs 5' of a gene, within the gene, or 3' of a gene can all influence transcription [26,27], we then performed EMS assays using probes that spanned each of these SNPs, but none of the four SNPs displayed a clear “gel shift” – leaving the mechanisms responsible for the association of these SNPs with BHMT expression unresolved.

In summary, we performed a genotype-phenotype correlation analysis with 268 adult human liver biopsy samples and identified three SNPs that were significantly associated with BHMT expression in the human liver. The four SNPs identified during genotyping, as well as the one imputed SNP, represent biomarkers that could potentially be used to help predict variation in BHMT expression, function and – ultimately – its role in human disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations

BHMT betaine-homocysteine methyltransferase

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Figure 1. Frequency distribution histograms of BHMT phenotypes in adult human liver samples. One sample with undetectable protein was excluded from panel (C).

Figure 2.

Correlations between *BHMT* phenotypes and age for adult liver biopsy samples.

* Indicates that samples were excluded from these figures because we lacked patient age information. One additional sample with undetectable protein was excluded from panel (C).

Figure 3.
Correlations between BHMT protein and enzyme activity levels.

Figure 4.
Correlations between gestational age and BHMT phenotypes in the fetal liver samples.

Figure 5. BHMT genotyped and imputed SNP associations with hepatic BHMT enzyme activity and protein levels. Association of genotyped (red circles) and imputed SNPs (black triangles) with (A) hepatic BHMT enzyme activity and (B) protein levels. (C) The LD structure of *BHMT* and *BHMT2* was plotted using genotyped SNPs and imputed SNPs ($p < 1E-04$). SNPs with the lowest p values are indicated by red rectangles around the rs number.

Figure 6.
Genotype-phenotype relationships for rs6875201.

Table 1*BHMT* Genotyping Results.

SNP marker	Genotype	Genotype count	Genotype %	MAF
rs57906668	GG	7	2.6	0.20
	AG	91	34.3	
	AA	167	63.0	
rs59866108	AG	7	2.6	0.01
	GG	258	97.4	
rs16876512	AA	2	0.8	0.10
	AG	51	19.2	
	GG	212	80.0	
rs16876527	AA	241	90.9	0.05
	AG	24	9.1	
rs3733890	AA	14	5.3	0.28
	AG	122	46.0	
	GG	129	48.7	
rs41272270	AA	2	0.8	0.10
	AG	49	18.5	
	GG	214	80.8	
rs506500	AA	25	9.4	0.33
	AG	127	47.9	
	GG	113	42.6	
rs542852	AA	37	14.0	0.40
	AG	137	51.7	
	GG	91	34.3	
rs567754	AA	24	9.1	0.33
	AG	128	48.3	
	GG	113	42.6	
rs585800	TT	19	7.2	0.30
	AT	119	44.9	
	AA	127	47.9	
rs682985	GG	41	15.5	0.42
	AG	139	52.5	
	AA	85	32.1	
rs6875201	GG	2	0.8	0.11
	AG	53	20.0	
	AA	210	79.2	

Table 2

Single SNP Correlations with BHMT Phenotypes

SNP marker	BHMT activity Spearman rho	BHMT activity p value	BHMT protein level Spearman rho	BHMT protein level p value
rs57906668	0.044	0.475	0.030	0.621
rs682985	0.075	0.225	0.078	0.205
rs41272270	0.331	3.41E-08	0.244	5.78E-05
rs16876512	0.356	2.55E-09	0.267	1.08E-05
rs542852	0.017	0.779	0.055	0.369
rs6875201	0.376	2.46E-10	0.272	6.92E-06
rs16876527	0.073	0.236	0.029	0.634
rs506500	0.058	0.345	0.035	0.570
rs567754	-0.070	0.259	-0.090	0.146
rs59866108	0.020	0.746	-0.050	0.413
rs3733890	0.031	0.617	0.104	0.092
rs585800	-0.015	0.803	0.008	0.892

Table 3

Haplotype Analysis of BHMT in Adult Liver Samples

(A) Overall haplotype summary.

Phenotype	Global test of haplotype association ¹	Test of largest haplotype effect ²
Protein level	0.023	0.020
Enzyme activity	0.0016	0.0186

(B) Haplotype analysis for BHMT enzyme activity.

rs57906668	rs682985	rs41272270	rs16876512	rs542852	rs6875201	rs16876527	rs506500	rs567754	rs59866108	rs3733890	rs585800	Hap-Freq	Hap-Score	p-value
A	A	A	A	G	G	A	G	G	G	A	A	0.03084	3.25699	0.00082
A	A	A	A	G	G	A	A	G	G	T	A	0.02281	2.66864	0.00658
A	A	G	G	G	A	A	G	A	G	T	A	0.27859	-2.36256	0.01974
A	A	G	G	G	A	A	G	G	G	A	A	0.0379	-2.07265	0.03834
A	A	G	G	A	A	A	G	G	G	A	A	0.02088	-1.71641	0.081
A	A	A	A	G	G	A	A	G	G	T	T	0.01062	1.64793	0.11112
A	A	A	A	G	G	A	G	A	G	T	A	0.02834	1.61042	0.11194
A	A	G	G	G	A	A	A	G	G	T	T	0.11589	-1.42933	0.15399
A	A	G	G	G	A	A	G	G	G	T	A	0.01879	-1.32689	0.1837
A	G	G	G	G	A	G	G	G	G	T	A	0.02073	0.76239	0.45076
G	G	G	G	A	A	A	G	G	G	A	A	0.1781	0.59642	0.56219
A	G	G	G	G	A	A	A	G	G	T	A	0.01282	-0.41139	0.68452
A	G	G	G	A	A	G	A	G	G	T	T	0.01534	0.37383	0.71073
A	G	G	G	A	A	A	A	G	G	T	T	0.13483	0.17011	0.86852

(C) Haplotype analysis for BHMT protein level.

rs57906668	rs682985	rs41272270	rs16876512	rs542852	rs6875201	rs16876527	rs506500	rs567754	rs59866108	rs3733890	rs585800	Hap-Freq	Hap-Score	p-value
A	A	A	A	G	G	A	G	G	G	A	A	0.03084	3.32181	< 0.001
A	A	G	G	G	A	A	G	A	G	T	A	0.27859	-2.55085	0.012
A	A	A	A	G	G	A	A	G	G	T	T	0.01062	2.44239	0.016
A	A	A	A	G	G	A	G	A	G	T	A	0.02834	1.99501	0.053
A	A	G	G	G	A	A	G	G	G	T	A	0.01879	-1.32619	0.18
A	A	G	G	G	A	A	A	G	G	T	T	0.11589	-1.23016	0.194

(C) Haplotype analysis for BHMT protein level.

rs57906668	rs682985	rs41272270	rs16876512	rs542852	rs6875201	rs16876527	rs506500	rs567754	rs59866108	rs3733890	rs585800	Hap-Freq	Hap-Score	p-value
G	G	G	G	A	A	A	G	G	G	A	A	0.1781	1.03721	0.315
A	A	G	G	G	A	A	G	G	G	A	A	0.0379	-0.98676	0.318
A	G	G	G	A	A	G	A	G	G	T	T	0.01534	0.70565	0.474
A	G	G	G	A	A	A	A	G	G	T	T	0.13483	0.54181	0.566
A	G	G	G	G	A	G	G	G	G	T	A	0.02073	-0.4716	0.603
A	A	G	G	A	A	A	G	G	G	A	A	0.02088	-0.45823	0.637
A	A	A	A	G	G	A	A	G	G	T	A	0.02281	0.47948	0.647
A	G	G	A	G	A	A	A	G	G	T	A	0.01282	0.15124	0.873

1

Simulation based p values for the global test of haplotype association using a score test.

2

Simulation based p values for maximum score statistic.