Methionine Adenosyltransferase 2A/2B and Methylation: Gene Sequence Variation and Functional Genomics^S

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ABSTRACT:

Methionine adenosyltransferase (MAT) catalyzes the synthesis of *S*-adenosylmethionine, the major biological methyl donor. *MAT1A* and *MAT2A* encode two distinct MAT isoforms in mammals. *MAT2A* is expressed in nonhepatic tissues, whereas *MAT1A* is expressed in the liver. A third gene, *MAT2B*, encodes a *MAT2A* regulatory protein. We resequenced *MAT2A* and *MAT2B* exons, splice junctions, and flanking regions using 288 DNA samples from three ethnic groups and also imputed additional single nucleotide polymorphisms (SNPs) across both genes using data from the 1000 Genomes Project. For *MAT2A*, resequencing identified 74 polymorphisms, including two nonsynonymous (ns) SNPs. Functional genomic studies of wild type and the two *MAT2A* variant allozymes (Val11 and Val205) showed that the Val11 allozyme had approximately 40% decreases in levels of enzyme activity and immunoreactive protein after COS-1 cell transfection. For

MAT2B, 44 polymorphisms, 2 nonsynonymous, were identified during resequencing. Neither of the two *MAT2B* nsSNPs displayed alterations in levels of protein. Imputation using 1000 Genomes Project data resulted in 1730 additional *MAT2A* and 1997 *MAT2B* polymorphisms within ± 200 kilobases of each gene, respectively. Coexpression of *MAT2A* and *MAT2B* in COS-1 cells resulted in significantly increased MAT enzyme activity that correlated with increased *MAT2A* and *MAT2B* immunoreactive protein, apparently as a result of decreased degradation. Finally, studies of mRNA expression in lymphoblastoid cells showed that 7 SNPs in *MAT2A* and 16 SNPs in *MAT2B* were significantly associated with mRNA expression with $p < 0.01$. These observations provide a foundation for future mechanistic and clinical translational pharmacogenomic studies of *MAT2A/2B*.

Introduction

Methionine adenosyltransferase (MAT) is a critical enzyme in the methionine cycle (see Fig. 1). Two MAT isoforms catalyze the synthesis of *S*-adenosylmethionine (AdoMet), the primary methyl donor for biological methylation reactions (Fig. 1) (Cantoni, 1951, 1953, 1975; Mato et al., 2002). These reactions result in the methyl

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conjugation of many drugs, xenobiotics, hormones, and neurotransmitters as well as DNA, RNA, and proteins (Mato et al., 1997; Halim et al., 1999). The methionine cycle plays a central role in three important pathways: transmethylation, trans-sulfuration, and polyamine synthesis (Fig. 1) (Kotb and Geller, 1993). Alterations in AdoMet concentrations have been implicated in a broad range of human disease (Chamberlin et al., 2000; Mato et al., 2002; Lu and Mato, 2005; Loenen, 2006).

The nomenclature used to describe MAT enzymes can be confusing. There are three major multimeric MAT enzymes: MATI, MATII, and MATIII (Kotb and Geller, 1993; Halim et al., 1999; Mato et al., 2001). MATI is a tetramer and MATIII is a dimer of the protein encoded by *MAT1A*, the "hepatic form" of the enzyme (Halim et al., 1999; Chamberlin et al., 2000; Lu and Mato, 2005). In contrast, MATII is a dimer of the protein encoded by *MAT2A* and is expressed in most extrahepatic tissues (Halim et al., 1999; Chamberlin et al., 2000; Mato et al., 2001; Lu and Mato, 2005). Although *MAT1A* and *MAT2A* share a high degree of amino acid sequence identity (84% in humans) (Chamberlin et al., 2000; Mato et al., 2001), multimers of these enzymes differ in their physical and substrate kinetic properties

ABBREVIATIONS: MAT, methionine adenosyltransferase; AdoMet, *S*-adenosylmethionine; V2, variant 2; kb, kilobase; FR, flanking region; ns, nonsynonymous; LCL, lymphoblastoid cell lines; PCR, polymerase chain reaction; ORF, open reading frame; COMT, catechol *O*-methyltransferase; DBA, 3,4-dihydroxybenzoic acid; RRL, rabbit reticulocyte lysate; PAGE, polyacrylamide gel electrophoresis; DMSO, dimethyl sulfoxide; MG132, carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; 3MA, 3-methyladenine; AA, African American; EA, European-American; HCA, Han Chinese-American; qRT, quantitative reverse transcription; SNP, single nucleotide polymorphisms; WT, wild type.

FIG. 1. The methionine cycle. MT, methyltransferase; AHCY, S-adenosylhomocysteine hydrolase; BHMT, betaine homocysteine methyltransferase; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; MTRR, 5-methyltetrahydrofolate homocysteine methyltransferase reductase.

(Okada et al., 1981; Kotb and Geller, 1993; Kotb et al., 1997; Halim et al., 1999; Mato et al., 2001). A third gene, *MAT2B*, unrelated in amino acid sequence to *MAT1A* or *MAT2A*, encodes a regulatory subunit that physically associates with the *MAT2A* dimer, forming a heterotetramer (LeGros et al., 2000, 2001; Martínez-Chantar et al., 2003). When *MAT2B* is bound to the *MAT2A* dimer, the apparent K_m of the complex for methionine is greatly reduced—from 100 to 20 μ M (Halim et al., 1999; LeGros et al., 2000, 2001; Mato et al., 2001; Martínez-Chantar et al., 2003).

The human *MAT2A* and *MAT1A* genes map to chromosomes 2p11.2 and 10q22, respectively, span 6.1 (*MAT2A*) and 17.8 kilobases (kb) (*MAT1A*), and their structures both include nine exons that encode 395 amino acids (Mato et al., 2001). The human *MAT2B* gene maps to 5q34–35.1 and undergoes alternative transcription initiation and alternative splicing that result in two distinct transcripts, *MAT2B* variant 1 (V1) and *MAT2B* variant 2 (V2), with distinct initial exons that are designated 1b and 1a, respectively (Fig. 2) (Yang et al., 2008). The

structures of both *MAT2B* variants include seven coding exons (with identical exons 2–7) that are translated to form proteins that are 334 (V1) or 323 (V2) amino acids in length (Yang et al., 2008). Like *MAT2A*, *MAT2B* is expressed in extrahepatic tissues (Yang et al., 2008).

Most pharmacogenetic studies of phase II conjugation reactions have focused on the enzymes that catalyze conjugation rather than biosynthesis of the donor cosubstrates for the reactions (Weinshilbourn, 1988, 1999; Crettol et al., 2010). Although the *MAT* genes are required for AdoMet synthesis, no systematic studies of common sequence variation in these genes or characterization of the functional implications of that variation have been reported. Therefore, we performed a study of DNA sequence variation in *MAT2A* and *MAT2B* by resequencing exons, splice junctions, and the 5'-flanking regions (5'-FRs) of these two genes in 288 DNA samples from unrelated individuals of three ethnic groups, followed by imputation across each gene using recent data from the 1000 Genomes Project Consortium,

Human MAT2A and MAT2B Genetic Polymorphisms

FIG. 2. Human *MAT2A* and *MAT2B* genetic polymorphisms observed during resequencing. The figure shows a schematic representation of the human *MAT2A* and *MAT2B* gene structures, with arrows indicating the locations of polymorphisms observed during the gene resequencing studies. Black rectangles represent exons encoding the ORF, and open rectangles represent portions of exons encoding untranslated region sequences. The colors of arrows indicate minor allele frequencies.

2010. Availability of the resequencing data helped to make the imputation possible. During the resequencing studies, we observed 74 and 44 polymorphisms in *MAT2A* and *MAT2B*, respectively, with two nonsynonymous (ns) single nucleotide polymorphisms (SNPs) in each gene. The 1000 Genomes Project imputation resulted in 1730 and 1997 additional polymorphisms within introns and 200 kb 5' and 3' of exons for *MAT2A* and *MAT2B*, respectively. Functional studies of nsSNPs were then performed, as well as studies of the possible association of SNPs with variation in mRNA microarray expression data for the lymphoblastoid cell lines (LCLs) from which the resequenced DNA had been isolated. The results described subsequently define the nature and extent of common genetic variation in *MAT2A* and *MAT2B* and the functional implications of that variation. They also provide novel insights into the interaction between *MAT2A* and *MAT2B*.

Materials and Methods

DNA Samples and Gene Resequencing. DNA samples from 96 European-American (EA), 96 African-American (AA), and 96 Han Chinese-American (HCA) subjects (sample sets HD100CAU, HD100AA, and HD100CHI, respectively) were obtained from the Coriell Cell Repository (Camden, NJ). These samples had been collected and anonymized by the National Institute of General Medical Sciences. All subjects had provided written informed consent for the use of their DNA for research purposes. Our studies were reviewed and approved by the Mayo Clinic Institutional Review Board. These DNA samples were used to resequence the *MAT2A* and *MAT2B* genes. In particular, all *MAT2A* and *MAT2B* exons, intron-exon splice junctions, and portions of their 5'-FRs (~2500 for *MAT2A*; ~1200 base pairs 5' of *MAT2B* exon 1a and ~900 base pairs 5' of *MAT2B* exon 1b, respectively) were amplified using the polymerase chain reaction (PCR), and the amplicons were sequenced using dye terminator sequencing chemistry as described previously (Moyer et al., 2008). Polymorphisms observed only once, as well as any ambiguous sequences, were confirmed by performing independent PCR amplifications, followed by DNA sequencing. Primer sequences used to perform the amplifications are listed in Supplemental Table 1.

Mammalian and Bacterial Expression. The open reading frames (ORFs) of *MAT2A*, *MAT2B* V1, and *MAT2B* V2 were amplified from Origene clones SC116444, SC110397, and SC110398 (Origene, Rockville, MD), respectively, and each amplicon was subcloned into pcDNA3.1D/V5-His-TOPO (Invitrogen, Carlsbad, CA) in frame with the V5-His tag. Sequences of the inserts were verified by DNA sequencing. Site-directed mutagenesis was then used to create variant allozyme expression constructs. Sequences of variant allozymes were verified by DNA sequencing, and transient expression of the constructs in COS-1 cells was performed as described previously (Moyer et al., 2008).

The *MAT2A* and *MAT2B* wild-type (WT) and variant constructs were also cloned into the bacterial expression vectors pET28a(+) and pET51b(+) (Novagen, Madison, WI), respectively, and transformed into BL21 *Escherichia coli* to express *MAT2A*-His and *MAT2B*-Strep fusion proteins for use in substrate kinetic studies. The *MAT2A*-His fusion proteins were purified with a Ni-NTA Superflow column (QIAGEN, Valencia, CA), and the *MAT2B*-Strep fusion proteins were purified with a Strep-Tactin Sepharose column (IBA, St. Louis, MO). The human catechol *O*-methyltransferase (*COMT*) gene ORF for the soluble form of the enzyme (Shield et al., 2004) was cloned into pET28a(+) and was also expressed in BL21 *E. coli*. After purification with a Ni-NTA column, this bacterially expressed protein was used as a "reagent" in the MAT enzyme assay described under *MAT Enzyme Activity Assay*.

MAT Enzyme Activity Assay. Recombinant *MAT2A* allozymes were assayed for MAT enzyme activity using an L-[methyl-¹⁴C]methionine (specific activity 55 mCi/mmol; American Radiolabeled Chemicals Inc., St. Louis, MO) radiochemical *COMT*-coupled reaction (Chou and Lombardini, 1972; Wang et al., 2003b). This assay was a modification of the method described by Wang et al. (2003b). In particular, the [¹⁴C]-labeled methyl group of AdoMet was transferred enzymatically to 3,4-dihydroxybenzoic acid (DBA) in a reaction catalyzed by *COMT* to form [¹⁴C]methyl-labeled radioactive 3-methoxy-4-hydroxybenzoic acid as described previously (Raymond and Weinshilboum, 1975). The reaction mixture contained 5 mM Tris-HCl buffer (pH 7.4), 1 mM

DBA, 2 mM ATP, 15 mM MgCl₂, 150 mM KCl, 5 mM dithiothreitol, 1 mM L-[methyl-¹⁴C]methionine (1 μCi/μmol), 20 μg of purified *COMT* protein, and recombinant enzyme (in 5 mM potassium phosphate buffer, pH 7.4) in a final reaction volume of 248 μl. Blanks consisted of reactions that lacked ATP, DBA, or enzyme. The reaction mixture was incubated for 30 min at 37°C with shaking, and the reaction was stopped by the addition of 80 μl of 1 N HCl. Toluene (2.5 ml) was added, and the mixture was vortexed for 10 s. After centrifugation at 700g for 10 min at room temperature, 1.5 ml of the organic layer was aspirated and added to 4 ml of Bio-Safe II liquid scintillation counting fluid; radioactivity was measured in a LS6500 liquid scintillation counter (Beckman Coulter, Fullerton, CA). For the substrate kinetic experiments, L-methionine concentrations varied from 8 to 1000 μM and concentrations of ATP varied from 8 to 500 μM.

β-Galactosidase Enzyme Activity Assay. β-Galactosidase activity was measured spectrophotometrically using the β-Galactosidase Assay System (Promega, Madison, WI), and levels of recombinant MAT enzyme activity for the COS-1 cell transfection experiments were corrected based on the activity of cotransfected β-galactosidase.

Western Blot Analyses. Levels of *MAT2A* and *MAT2B* immunoreactive protein were determined by quantitative Western blot analysis as described previously (Moyer et al., 2008) using a chicken monoclonal anti-MAT2A antibody (Sigma-Aldrich, St. Louis, MO) and a rabbit polyclonal antibody (Cocalico Biologicals, Inc., Reamstown, PA) directed against residues 202 to 223 of *MAT2B* V1 (residues 191 to 212 of *MAT2B* V2). The Immuno-Star Western C kit (Bio-Rad Laboratories, Hercules, CA) was used to detect bound antibody by enhanced chemiluminescence. The Western blot data were analyzed with the Quantity One 1-D Analysis Software (Bio-Rad Laboratories) using the ChemiDoc XRS (Bio-Rad Laboratories).

In Vitro and In Vivo Protein Degradation Experiments. A rabbit reticulocyte lysate (RRL) system was used to transcribe and translate *MAT2A* and *MAT2B* allozymes in the presence of [³⁵S]methionine (PerkinElmer Life and Analytical Sciences, Waltham, MA) and [³⁵S]cysteine (MP Biomedicals, Solon, OH). The reaction mixture was incubated at 37°C for 90 min, and 5-μl aliquots were used to perform SDS-polyacrylamide gel electrophoresis (PAGE). After transcription and translation, protein degradation experiments were performed as described previously (Wang et al., 2003a). In particular, 10-μl aliquots of in vitro-translated [³⁵S]methionine- and [³⁵S]cysteine-labeled protein were added to 40 μl of untreated RRL. After incubation at 37°C, 10-μl aliquots were removed at 0, 2, 4, 6, 12, and 24 h, followed by SDS-PAGE and autoradiography.

To test the possibility that autophagy or proteasome-mediated degradation might influence MAT levels, 4 h after the transient transfection of COS-1 cells with expression constructs, the cells were incubated with either dimethyl sulfoxide (DMSO), 10 mM 3-methyladenine (3MA; an autophagy inhibitor; Sigma-Aldrich), or 1 μM carbobenzoxyl-L-leucyl-L-leucyl-L-leucinal (MG132) (a proteasome inhibitor; Sigma-Aldrich), both dissolved in DMSO. The cells were then trypsinized, washed in 1× phosphate-buffered saline (Invitrogen), resuspended in 5 mM K₂HPO₄ (pH 7.4), and homogenized on ice. The homogenate was centrifuged at 100,000g for 1 h at 4°C, the cytosolic fraction was removed, and immunoreactive *MAT2A* was assayed in the cytosol by quantitative Western blot analysis.

In Vivo Protein Translation. To test the possibility that the RRL in vitro coupled transcription-translation system might not accurately reflect the in vivo situation, we also measured the translation of *MAT2A* in COS-1 cells. Specifically, COS-1 cells were transiently transfected with constructs encoding either WT or Val11 *MAT2A*. After 48 h, the transfected cells were grown in methionine- and cysteine-free media for 30 min and were then "pulsed" with media containing 0.1 mCi/ml each of [³⁵S]methionine and [³⁵S]cysteine (PerkinElmer Life and Analytical Sciences). The cells were isolated at various times for 90 min after the "pulse" and were processed with the Classic IP Kit (Thermo Fisher Scientific, Waltham, MA) using a monoclonal mouse anti-V5 antibody (Abcam Inc., Cambridge, MA). Eluant samples from the immunoprecipitation reactions were then subjected to SDS-PAGE. The resulting gels were dried, levels of radioactively labeled protein were determined by autoradiography, and the film was scanned and quantified using Image J software (NIH Image, Bethesda, MD).

mRNA Expression and qRT-PCR. The LCLs from which the 288 DNA samples used for gene resequencing had been obtained were purchased from

TABLE 1
Human MAT2A and MAT2B genetic polymorphisms

Polymorphisms in *MAT2A* and *MAT2B* exons and untranslated regions (UTRs) are numbered relative to the A (nucleotide 1) in the ATG translation initiation codon. In the case of *MAT2B*, we used the ATG in exon 1a. Negative numbers are assigned to positions 5' to that location, and positive numbers are assigned to for positions 3' to that location. Nucleotides located within introns are numbered based on their distance from the nearest splice junction, with distances from 3'-splice junctions assigned positive numbers and distances from 5' splice junctions assigned negative numbers. Polymorphisms within exons are boxed. Amino acid changes in *MAT2B* are numbered for the 334 amino acid protein of *MAT2B* V2. Previously identified polymorphisms are indicated by rs number.

MAT Gene	Polymorphism Location	Nucleotide Sequence Change	Amino Acid Sequence Change	Minor Allele Frequency			rs Number
				AA	EA	HCA	
2A	5'FR (−2453)	del TTTTG		0.000	0.005	0.000	
2A	5'FR (−2442)	T > C		0.000	0.000	0.005	
2A	5'FR (−2405)	T > C		0.229	0.547	0.641	rs3755014
2A	5'FR (−2384)	G > C		0.016	0.047	0.000	rs72844458
2A	5'FR (−2370)	A > G		0.229	0.547	0.641	rs3755015
2A	5'FR (−2338)	T > C		0.000	0.005	0.000	
2A	5'FR (−2237)	A > C		0.010	0.005	0.000	
2A	5'FR (−2205)	C > T		0.010	0.000	0.000	
2A	5'FR (−1976)	T > C		0.000	0.016	0.000	rs13020028
2A	5'FR (−1905)	A > T		0.094	0.005	0.000	rs72940556
2A	5'FR (−1883)	G > T		0.000	0.000	0.005	
2A	5'FR (−1829)	G > A		0.010	0.005	0.000	
2A	5'FR (−1749)	ins A		0.000	0.005	0.000	
2A	5'FR (−1716)	G > A		0.010	0.005	0.000	
2A	5'FR (−1606)	del CT		0.005	0.000	0.000	
2A	5'FR (−1483)	T > C		0.000	0.000	0.005	
2A	5'FR (−1451)	T > G		0.219	0.547	0.641	rs1446668
2A	5'FR (−1445)	A > T		0.406	0.229	0.307	rs1048740
2A	5'FR (−1443)	T > G		0.406	0.229	0.307	rs1048739
2A	5'FR (−1418)	G > A		0.000	0.005	0.000	
2A	5'FR (−1344)	C > T		0.016	0.000	0.000	
2A	5'FR (−1218)	G > A		0.005	0.000	0.000	
2A	5'FR (−1206)	C > A		0.063	0.198	0.005	rs6729475
2A	5'FR (−1169)	G > T		0.026	0.000	0.000	
2A	5'FR (−1157)	T > C		0.000	0.000	0.005	
2A	5'FR (−1040)	ins C		0.005	0.000	0.000	
2A	5'FR (−973)	C > G		0.010	0.000	0.000	
2A	5'FR (−972)	C > T		0.063	0.198	0.005	rs4258795
2A	5'FR (−970 to −969)	ins C		0.406	0.229	0.307	
2A	5'FR (−970)	T > G		0.052	0.016	0.135	rs1446667
2A	5'FR (−940)	G > A		0.094	0.010	0.005	rs72940558
2A	5'FR (−698)	G > A		0.000	0.005	0.000	
2A	5'FR (−678)	C > G		0.000	0.000	0.010	rs17026411
2A	5'FR (−556)	C > T		0.010	0.000	0.000	rs60048404
2A	5'FR (−474)	G > T		0.036	0.000	0.000	rs60749033
2A	5'FR (−458)	C > T		0.000	0.005	0.000	
2A	5'FR (−454)	G > A		0.000	0.000	0.010	rs17026416
2A	5'FR (−440)	G > A		0.010	0.000	0.000	
2A	5'FR (−399)	A > T		0.005	0.000	0.000	
2A	5'FR (−372)	T > G		0.005	0.000	0.000	
2A	5'FR (−140)	G > T		0.000	0.005	0.000	
2A	5'FR (−132)	G > A		0.036	0.000	0.000	rs6733827
2A	5'FR (−129)	C > G		0.016	0.042	0.000	rs2028106
2A	5'UTR (−120)	G > A		0.000	0.005	0.000	
2A	5'UTR (−111)	G > A		0.000	0.005	0.000	
2A	5'UTR (−97)	C > G		0.000	0.005	0.000	
2A	5'UTR (−92)	A > G		0.000	0.000	0.010	rs17026419
2A	5'UTR (−16)	G > A		0.000	0.000	0.005	
2A	Exon 1 (32)	C > T	Ala/Val (11)	0.005	0.000	0.000	
2A	Intron 1 (44)	C > T		0.406	0.234	0.307	rs2289972
2A	Intron 1 (60)	C > T		0.000	0.005	0.000	
2A	Intron 1 (−46)	ins ATAC		0.047	0.000	0.000	
2A	Intron 2 (47)	A > G		0.078	0.000	0.000	rs58507836
2A	Intron 3 (70)	G > A		0.005	0.047	0.000	
2A	Exon 4 (318)	G > T		0.005	0.000	0.000	rs72940560
2A	Intron 4 (11)	G > C		0.000	0.000	0.005	
2A	Intron 4 (32)	T > C		0.047	0.000	0.000	rs62620249
2A	Intron 4 (−19)	G > A		0.010	0.000	0.000	
2A	Exon 5 (501)	A > G		0.005	0.000	0.000	
2A	Intron 5 (−32)	A > G		0.010	0.000	0.000	rs1078005
2A	Exon 6 (613)	A > G	Ile/Val (205)	0.000	0.000	0.005	
2A	Intron 6 (−68)	T > C		0.005	0.000	0.000	
2A	Exon 7 (792)	G > C		0.276	0.542	0.641	rs1078004
2A	Exon 7 (813)	T > C		0.005	0.000	0.026	
2A	Intron 7 (17)	del TA		0.000	0.005	0.000	
2A	Intron 7 (−62)	G > A		0.000	0.005	0.000	
2A	Intron 7 (−49)	A > G		0.406	0.234	0.307	rs2043675
2A	Intron 7 (−19)	G > A		0.010	0.000	0.000	

TABLE 1—Continued

MAT Gene	Polymorphism Location	Nucleotide Sequence Change	Amino Acid Sequence Change	Minor Allele Frequency			rs Number
				AA	EA	HCA	
2A	Intron 7 (−3)	ins T		0.010	0.000	0.000	
2A	Intron 8 (55)	G > A		0.000	0.005	0.000	
2A	Intron 8 (57)	ins GA		0.000	0.005	0.000	
2A	3'UTR (1299)	G > A		0.005	0.000	0.000	
2A	3'UTR (1464)	T > C		0.005	0.000	0.000	
2A	3'UTR (1590)	T > C		0.068	0.000	0.000	rs6711750
2B	5'FR (−1043)	A > T		0.104	0.281	0.724	rs10462941
2B	5'FR (−924)	A > G		0.000	0.005	0.000	
2B	5'FR (−544)	T > A		0.000	0.000	0.005	
2B	5'FR (−467)	G > A		0.047	0.000	0.000	
2B	5'FR (−362)	A > C		0.125	0.000	0.000	
2B	5'UTR 1a (−133)	G > A		0.000	0.000	0.005	
2B	5'UTR 1a (−114)	T > G		0.000	0.026	0.000	
2B	5'UTR 1a (−3)	G > A		0.005	0.000	0.000	
2B	Exon 1 1a (28)	C > A		0.042	0.000	0.000	
2B	Intron 1a (8)	C > A		0.000	0.000	0.005	
2B	Intron 1a (981)	C > G		0.000	0.005	0.000	
2B	Intron 1b (990)	A > G		0.112	0.000	0.000	rs7726100
2B	Intron 1b (1288)	C > T		0.199	0.146	0.083	rs7734424
2B	Intron 1b (1337)	G > A		0.112	0.000	0.000	rs7709905
2B	Intron 1b (1598)	del AAC		0.091	0.000	0.000	
2B	Intron 1b (1656)	G > A		0.100	0.000	0.000	rs7710409
2B	Intron 1b (1717)	del TT		0.006	0.000	0.000	
2B	Intron 1b (1751)	C > T		0.011	0.026	0.078	rs28364754
2B	Intron 1b (1760)	A > G		0.100	0.000	0.000	rs10062614
2B	Intron 1b (1767)	del GAGATGTGTCCATTATGCTCTA		0.109	0.000	0.000	rs34602754
2B	Intron 1b (1942)	del A		0.000	0.000	0.005	
2B	Intron 1b (1960)	C > G		0.000	0.000	0.016	
2B	Intron 1b (1978)	C > A		0.005	0.000	0.000	
2B	Intron 1b (2045)	G > T		0.016	0.000	0.000	
2B	Intron 1b (2049)	T > G		0.108	0.000	0.000	rs57122772
2B	Intron 1b (2157)	T > C		0.011	0.000	0.000	
2B	Intron 1b (2376)	del G		0.043	0.000	0.000	
2B	Intron 1b (2392)	T > G		0.183	0.146	0.083	rs299299
2B	Intron 1b (−49)	G > T		0.010	0.000	0.000	
2B	Intron 2 (57)	A > T		0.016	0.031	0.078	
2B	Intron 3 (64)	A > G		0.036	0.000	0.000	rs964945
2B	Intron 3 (70)	G > A		0.052	0.000	0.000	rs7729611
2B	Exon 4 (492)	A > G		0.083	0.135	0.005	rs17061795
2B	Intron 4 (78)	T > C		0.000	0.005	0.000	
2B	Intron 5 (6)	del AAG		0.016	0.000	0.000	
2B	Intron 5 (51)	C > T		0.000	0.010	0.000	
2B	Intron 5 (−104)	C > T		0.016	0.000	0.000	
2B	Exon 6 (781)	C > T	Pro/Ser(261)	0.000	0.000	0.005	
2B	Intron 6 (29)	A > G		0.214	0.635	0.807	rs7733775
2B	Intron 6 (−205)	C > T		0.016	0.000	0.000	
2B	Intron 6 (−202)	G > T		0.005	0.000	0.000	
2B	Intron 6 (−153)	C > A		0.016	0.000	0.000	
2B	Exon 7 (820)	C > T		0.000	0.000	0.005	
2B	Exon 7 (872)	C > T	Thr/Ile (291)	0.000	0.000	0.031	

rs, reference SNP IDs.

the Coriell Cell Repository. These cell lines were used to perform basal microarray mRNA expression assays. Specifically, total RNA was extracted and mRNA expression analysis was performed as described elsewhere (Moyer et al., 2008). Data for the MAT2A probe set 200768_s_at and MAT2B probe set 217993_s_at were then used to perform association analyses between SNPs in the two genes and mRNA expression array data.

For some experiments, quantitative reverse transcription (qRT)-PCR was performed after mRNA isolation using the RNeasy Mini Kit (QIAGEN). qRT-PCR was performed with the one-step Brilliant SYBR Green qRT-PCR Kit (Stratagene, La Jolla, CA) using primers purchased from QIAGEN. All of these experiments were performed in triplicate.

Structural Analysis. Crystal structures of human MAT2A (hMAT2A, [2P02](#), unpublished), human MAT1A (hMAT1A, [2OBV](#), unpublished), and rat MAT1A (rMAT1A, [1O9T](#)) (González et al., 2003) were used to evaluate the possible structural consequences of the two MAT2A variant allozymes. Superimposition and analysis of these structures and the computational “mutation” of Ile205 to Val205 in human MAT2A were performed with the graphics program COOT (Emsley and Cowtan, 2004). No structural models for MAT2B

or for homologous proteins were available in the Protein Data Bank, so it was not possible to use a similar approach to study MAT2B.

Data Analysis. Gene sequencing chromatograms were analyzed using Mutation Surveyor software (SoftGenetics, State College, PA). Linkage disequilibrium was determined by calculating r^2 and D' values (Hedrick, 2000; Hartl and Clark, 2007), and intragene haplotypes were inferred as described by Tajima (Schaid et al., 2002). Imputation using HapMap2 and pilot 1000 Genomes Project data were performed using the software package Mach 1.0 (Li et al., 2006). Untyped SNP genotypes were estimated using 1000 Genomes Project (pilot phase release October 2010) and HapMap (phase 2 release 22) data as the reference sets, and the resequencing data plus genome-wide SNP genotype data for the lymphoblastoid cell line DNA used to perform resequencing were used as the genetic background. Variants within 200 kb of each gene were included in the imputation. To account for the three racial groups represented in the LCLs, we imputed within each group with appropriate reference data for that ethnic group. Imputation quality estimates were determined by masking 10% of the genotypes at random and imputing the masked genotypes to compare the original and imputed masked genotypes. Estimated

allelic dosage values for the imputed genotypes were then used to perform association analysis with expression array data as the phenotype. Specifically, genotype-phenotype correlations for *MAT2A* and *MAT2B* SNPs with log₂-transformed mRNA expression array data were calculated separately for each ethnic group using PLINK (Purcell et al., 2007). Possible transcription factor binding sites were predicted using TFSEARCH version 1.3 (Heinemeyer et al., 1998). Apparent *K_m* values were calculated using Prism 4 (GraphPad Software Inc., San Diego, CA). Average levels of allozyme enzyme activity and protein levels were compared using the two-sample Student's *t* test (Microsoft Excel, Redmond, WA).

Results

***MAT2A* and *MAT2B* Gene Resequencing.** Sanger DNA sequencing was used to resequence the exons, splice junctions, and the 5'-FRs of *MAT2A* and *MAT2B* using 288 DNA samples—96 each from AA, EA, and HCA subjects. After the resequencing studies were performed, 1000 Genomes Project pilot Next-Generation DNA sequence data were released, so we also used those data to impute polymorphisms across both genes, including introns, and out to 200 kb 5' and 3' of *MAT2A* and *MAT2B*. The Sanger resequencing studies identified 74 polymorphisms in *MAT2A* (45 in AA subjects, 24 unique; 36 in EAs, with 16 unique to

FIG. 3. *MAT2A* allozyme functional genomics. A, *MAT2A* allozyme enzyme activity was assayed with 2 mM ATP and 1 mM methionine as substrates. EV, empty vector. Activities are expressed as a percentage of WT. Values shown are mean $\pm$ S.E.M. for three determinations. *, *p* < 0.04 by Student's *t* test. B, *MAT2A* allozyme immunoreactive protein levels expressed as a percentage of WT. Values shown are mean $\pm$ S.E.M. for three determinations. *, *p* < 0.04 by Student's *t* test.

TABLE 2

MAT2A allozyme apparent *K_m* values

Values are mean $\pm$ S.E.M. for three independent determinations. Neither of the values for variant allozymes differed significantly from that for the WT allozyme.

Allozyme	<i>K_m</i> Methionine	<i>K_m</i> ATP
	μ M	μ M
WT	555 $\pm$ 135	233 $\pm$ 55
Val11	494 $\pm$ 35	192 $\pm$ 47
Val205	427 $\pm$ 135	194 $\pm$ 37

EA subjects; and 24 in HCAs, with 10 unique to that group), including 2 nsSNPs (C32>T, A11V, and A613>G, I205V), and 44 polymorphisms in *MAT2B* (31 in AAs, 24 unique; 12 in EAs, with 5 unique to that ethnic group; and 15 in HCAs, 8 unique), including 2 nsSNPs (C781>T, P261S and C872>T, T291I) (Fig. 2; Table 1). Our resequencing data were deposited in the National Institute of Health-supported pharmacogenetics database PharmGKB (submission identification: PS208974 for *MAT2A* and PS208973 for *MAT2B*).

***MAT2A* Functional Characterization.** Functional genomic studies were performed to evaluate the possible functional effects of the *MAT2A* nsSNPs identified during the gene resequencing studies. Specifically, WT *MAT2A* and its two variant allozymes were transiently expressed in COS-1 cells. A mammalian cell line was used to perform these experiments to ensure the presence of mammalian post-translational modification and protein degradation processes. After correction for transfection efficiency, the *MAT2A* Val11 allozyme showed a significant decrease in enzyme activity to 59.9 $\pm$ 7.8% (mean $\pm$ S.E.M., *p* = 0.032) of the WT value (Fig. 3A). One mechanism by which changes in encoded amino acids might alter enzyme activity is through changes in substrate kinetics. Therefore, using bacterially expressed and purified protein, we determined the apparent *K_m* values for ATP and methionine, the two *MAT* substrates, for the *MAT2A* WT and variant allozymes. There were not significant differences between the two variant allozymes and WT *MAT2A* in the apparent *K_m* values for either substrate (Table 2).

The alteration in level of enzyme activity that we observed for the

FIG. 4. *MAT2A* allozyme RRL degradation studies. Average levels of ³⁵S-labeled recombinant *MAT2A* allozyme protein remaining at 1, 2, 4, 6, and 24 h after incubation in an untreated RRL are shown. Each time point represents the average value of protein remaining relative to the initial protein level for three independent assays. Values shown are mean $\pm$ S.E.M. *, *p* < 0.005 by Student's *t* test; **, *p* < 0.001 by Student's *t* test compared with degradation in the absence of MG132. The rapidly degraded TPMT*3A protein (Wang et al., 2003c) was included as a positive control.

FIG. 5. MAT2A WT and Val11 expression in COS-1 cells treated with 10 mM 3MA (A) or 1 μ M MG132 (B). Quantitative Western blot analysis was then performed with anti-MAT2 antibody. EV, empty vector. All values are mean $\pm$ S.E.M. for three determinations. *, $p < 0.002$; **, $p < 0.007$ by Student's t test.

Val11 allozyme might also be due to an alteration in protein quantity, as reported previously for many genetically polymorphic enzymes (Weinshilboum and Wang, 2004; Ji et al., 2007; Moyer et al., 2008). Therefore, we also performed quantitative Western blot analysis of recombinant MAT2A allozymes using the same cytosol preparations that we had used to assay enzyme activity. MAT2A Val11 displayed significantly less immunoreactive protein than did the WT allozyme, a reduction to $58.4 \pm 4.9\%$ of the WT value ($p = 0.036$) (Fig. 3B). Several possible mechanisms might explain changes in cytosolic immunoreactive protein levels, but accelerated degradation has been the most common cause during previous studies of the functional effects of genetic polymorphisms (Wang et al., 2003c; Weinshilboum and Wang, 2004; Hildebrandt et al., 2007).

To determine whether proteasome-mediated degradation might be responsible, *in vitro* translation and degradation experiments were performed using a RRL system. We were able to synthesize radioactively labeled protein for the WT and Val11 MAT2A allozymes using this system. There was a $30.1 \pm 3.3\%$ ($p = 0.015$) decrease in MAT2A WT protein after 24-h incubation with untreated RRL, but the Val11 variant allozyme decreased almost exactly the same amount ($29.9 \pm 3.6\%$, $p = 0.007$) (Fig. 4). A positive control, the TPMT*3A variant allozyme (Wang et al., 2003a), was rapidly degraded with $91.2 \pm 0.5\%$ lost in 24 h ($p = 0.0004$) in the same RRL system. When

FIG. 6. MAT2A WT and Val11 allozyme translation in COS-1 cells. Average levels of 35 S-labeled recombinant MAT2A allozyme protein at 5, 10, 30, 60, and 90 min after incubation in [35 S]methionine and [35 S]cysteine media. The mean level of protein relative to the WT value at 5 min is shown at each time point. Each value is mean $\pm$ S.E.M. of independent assays. *, $p < 0.01$; **, $p < 0.002$; ***, $p < 4 \times 10^{-5}$.

the proteasome inhibitor MG132 was added to the RRL system, a $9.0 \pm 1.0\%$ ($p < 0.05$) decrease was observed for MAT2A WT after 24 h, with a nearly identical decrease of $9.1 \pm 2.3\%$ ($p < 0.01$) for the Val11 allozyme (Fig. 4). When MG132 was present, the TPMT*3A control only decreased $18.1 \pm 1.4\%$ ($p < 0.002$). These results indicate that, in the RRL system, proteasome-mediated degradation occurred for WT and Val11 MAT2A, but there was no apparent difference between the two allozymes. The next step was to test the *in vivo* degradation of the two allozymes in a cell-based system.

We attempted to determine whether autophagy or proteasome-mediated degradation might play a role in the degradation of MAT2A allozymes after the transfection of COS-1 cells with WT and Val11 MAT2A in the presence or absence of 3MA, an autophagy inhibitor, or MG132, a proteasome inhibitor. Cytosol from these cells was then

FIG. 7. Human MAT2A crystal structure in the region surrounding Ile205 (Protein Data Bank accession code 2P02, unpublished). The Ile205 side chain is shown as a ball-and-stick structure in magenta (atoms that are retained in Val205) and green (methyl group that is present in Ile205 but absent in Val205). Side chains of surrounding hydrophobic residues are shown in orange. AdoMet (yellow carbon atoms) is bound at the dimer interface between two monomers (cyan and gray backbone ribbon structures). The figure was prepared by using PyMOL (DeLano, 2004).

FIG. 8. MAT2B allozyme functional genomics. A, MAT2B allozyme immunoreactive protein levels expressed as a percentage of WT. EV, empty vector. Values shown are mean $\pm$ S.E.M. for three determinations. B, coexpression of MAT2A and MAT2B in COS-1 cells. MAT enzyme activity is expressed as a percentage of that observed when MAT2A was expressed alone. Values shown are mean $\pm$ S.E.M. for three determinations. *, $p < 0.04$ by Student's t test. C, MAT2B expression in COS-1 cells. Endogenous MAT enzyme activity is expressed as the percentage of values after transfection with EV.

used to perform quantitative Western blot analysis. After correcting for transfection efficiency, there were significant increases in MAT2A protein in the presence of 3MA (WT: $32.4 \pm 1.9\%$, $p = 0.0007$; Val11: $29.8 \pm 1.8\%$, $p = 0.0012$) and MG132 (WT: $63.1 \pm 4.0\%$, $p = 0.0015$; Val11: $60.6 \pm 2.9\%$, $p = 0.0001$), but, once again, there was not a significant difference between WT and Val11 allozymes in the proportional increase of immunoreactive protein (Fig. 5, A and B, respectively). Therefore, although it appeared that autophagy and the proteasome contributed to MAT2A degradation, an important observation for the MAT2A/2B interaction experiments described subsequently, these processes did not appear to be primarily responsible for the decreased level of MAT2A Val11 compared with the WT allozyme because no difference was seen between the two allozymes in their rates of degradation by these processes.

Although results obtained with the RRL system showed no differences between WT and Val11 MAT2A in translation, a recent publication studying the functional genomics of COMT (a drug-metabolizing enzyme) reported that apparent translation rates for certain COMT haplotypes differed between the in vitro RRL system and the in vivo situation (Tsao et al., 2011). Therefore, we transiently expressed MAT2A WT and Val11 in COS-1 cells and determined the rate of translation for [35 S]methionine/cysteine-labeled protein. At each of the time points studied, the MAT2A Val11 allozyme showed significantly less translated protein, with $69.9 \pm 0.87\%$ (mean $\pm$ S.E.M., $p < 2.3 \times 10^{-7}$) of the WT value at 90 min (Fig. 6). These results indicated that decreased translation might contribute to the decrease in the level of MAT2A Val11 protein shown graphically in Fig. 3B.

However, we also wanted to determine whether differences in mRNA concentrations, perhaps as a result of an alteration in mRNA stability, might contribute to the decrease observed in MAT2A Val11 expression. Specifically, we performed qRT-PCR using total mRNA isolated from transiently transfected COS-1 cells that were cotransfected with *lacZ* to control for transfection efficiency. mRNA levels for Val11 MAT2A did not differ significantly from those for the WT allozyme (see Supplemental Fig. 1). In summary, the mechanism responsible for the decrease in the MAT2A Val11 protein, compared with the WT allozyme, after the transfection of COS-1 cells with expression constructs, appears to result, at least in part, from an alteration in translation. In addition, although neither proteasome- nor autophagy-mediated degradation appeared to contribute to the decreased level of MAT2A Val11 protein, we observed that WT and Val11 MAT2A were degraded through these processes. We next attempted to determine whether structural data for MAT2A might help to explain our functional genomic results.

MAT2A Variant Allozyme Structural Analysis. Examination of X-ray crystal structures for MAT2A showed that amino acids N-terminal to Gly16 in human MAT2A/human MAT1A or N-terminal to Gly17 in rat MAT1A were not represented in their respective crystal structures; as a result, they were presumed either to be very flexible or to have been proteolyzed during crystallization. Because these crystal

TABLE 3

MAT2A coexpression with MAT2B apparent K_m values

Values are mean $\pm$ S.E.M. for three independent determinations.

Construct	K_m Methionine μM	K_m ATP μM
MAT2A	187 ± 32	158 ± 34
MAT2A/2B V1	30 ± 3^a	72 ± 9^a
MAT2A/2B V2	31 ± 4^a	68 ± 8^a

^a $p < 0.0001$ compared with MAT2A.

FIG. 9. MAT2A coexpression with MAT2B in COS-1 cells. A, MAT2A immunoreactive protein levels expressed as a percentage of MAT2A alone. EV, empty vector. Values shown are mean $\pm$ S.E.M. for three determinations. *, $p < 0.007$ by Student's t test. B, endogenous MAT2B immunoreactive protein levels expressed as a percentage of EV. Values shown are mean $\pm$ S.E.M. for three determinations. *, $p < 0.05$ by Student's t test. C, MAT2B immunoreactive protein levels expressed as a percentage of MAT2B WT. All values are mean $\pm$ S.E.M. for three determinations. *, $p < 0.04$ by Student's t test.

structures did not include the Ala11 residue, it was not possible to perform a structural analysis of the Val11 variant allozyme. Ile205 in the WT protein is located in a β -strand for which the opposite face is adjacent to AdoMet bound in the human MAT2A active site (Fig. 7). The Ile205 side chain is buried in a hydrophobic environment provided mainly by the side chains of Leu244 in an adjacent β -strand and by Leu222, Val226, and Ile227 in a nearby α -helix. Ile205 and its hydrophobic neighbors are conserved in the three crystallized human

and rat MAT2A/MAT1A proteins. The Ile205Val substitution removes a methyl group from the branched side chain, therefore a small void may be created. An analysis of human MAT2A and the 100 most similar protein sequences as identified by BLAST (ranging from 75–99% sequence identity) revealed that, although the hydrophobic nature of residues at position 205 and those surrounding it is conserved, these amino acids are often conservatively substituted. At position 205, most sequences have Ile, but Val is also present in some proteins. Position 222 is usually occupied by Leu, Ile, or Val; whereas positions 226 and 227 can be either Val or Ile, Leu, Ile, and Met residues are found at position 244. The sequence variability observed for Ile205 and the surrounding hydrophobic residues in MAT2A and its most closely related sequences suggests that this region of the MAT2A protein fold can accommodate modest changes in the size of these hydrophobic residues without significantly compromising protein structure or function, consistent with our observations that the

FIG. 10. MAT2A coexpression with MAT2B in COS-1 cells transfected with empty vector (EV), MAT2A, MAT2A + MAT2B, or MAT2B followed by treatment with DMSO or 10 mM 3MA (A) or 1 μ M MG132 (B)—both dissolved in DMSO. Quantitative Western blot analysis was then performed with anti-MAT2A antibody. All values are mean $\pm$ S.E.M. for three determinations. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.001$ by Student's t test.

Val205 allozyme levels of enzyme activity and protein were similar to those of the WT allozyme (Fig. 3).

MAT2B Functional Characterization. Functional genomic studies were also performed with *MAT2B* variant allozymes. To characterize the effect of nsSNPs on function, we performed quantitative Western blot analysis of recombinant MAT2B V1 and V2 WT and MAT2B variant allozymes expressed in COS-1 cells. Neither of the variant allozymes for either MAT2B V1 or MAT2B V2 showed significant differences in total protein levels when compared with WT MAT2B V1 and V2 (Fig. 8A). Because *MAT2B* encodes a regulatory protein for MAT2A, we also determined whether MAT2B nsSNPs might alter MAT2A activity after coexpression of the two proteins. It was previously reported that coexpression of MAT2B with MAT2A increased MAT2A enzyme activity (Halim et al., 1999). Therefore, we coexpressed WT MAT2A with WT and variant allozymes for MAT2B V1 and MAT2B V2 in COS-1 cells and assayed WT MAT2A enzyme activity. As anticipated, MAT2A activity increased when coexpressed with MAT2B. However, we did not observe significant differences in enzyme activities between the WT MAT2B or variant MAT2B allozymes during coexpression or differences between V1 and V2 (i.e., the two MAT2B isoforms had similar effects on MAT2A-catalyzed enzyme activity, as did the variant MAT2B allozymes) (Fig. 8B).

MAT2B has also been reported to lower the apparent K_m value of MAT2A for methionine and ATP (Halim et al., 1999; LeGros et al., 2001; Yang et al., 2008). To determine whether the regulation of MAT2A substrate kinetics might differ between MAT2B V1 and V2, we bacterially expressed and purified WT MAT2A and the WT MAT2B to determine apparent K_m values for ATP and methionine, the two cosubstrates for the reaction. We found, as anticipated, that MAT2B significantly lowered the apparent K_m values of MAT2A (Table 3), consistent with previous reports, but there was not a significant difference between the effects of MAT2B V1 and V2 on the apparent K_m values for the complex.

MAT2A and MAT2B Interaction. MAT2A forms a heterotetrameric complex with its MAT2B regulatory protein (Halim et al.,

1999). As described previously, we observed that coexpression of WT MAT2A with WT MAT2B V1 or V2 resulted in an increase in WT MAT2A activity (Fig. 8B). The increases in activity shown in Fig. 8B ranged from 28.9 to 35.5% of WT activity. Because COS-1 cells, like virtually all nonhepatic mammalian cell lines, express MAT2A, we determined whether expression of human MAT2B V1 or V2 in COS-1 cells might result in an increase in endogenous COS-1 MAT2A activity compared with “empty vector” transfection. We observed a significant increase (206.5 ± 8.1 and $210.0 \pm 9.0\%$) in endogenous MAT2A activity in COS-1 cells transfected with human *MAT2B* V1 or V2, respectively, compared with cells transfected with empty vector ($p < 0.003$; Fig. 8C).

Although it had been reported previously that MAT2B increased the activity of MAT2A (Halim et al., 1999), that study had not determined whether the increase in enzyme activity might be due, in part, to an increase in MAT2A protein. Therefore, we also performed quantitative Western blot analysis of cytosolic preparations from our coexpression studies. WT MAT2A/MAT2B cotransfected COS-1 cytosol showed an increase in MAT2A immunoreactive protein that ranged from 40.0 to 45.0% ($p < 0.007$; Fig. 9A). Furthermore, MAT2B transfected COS-1 cytosol showed a significant increase in endogenous MAT2A immunoreactive protein that ranged from 189.3 to 216.9% ($p < 0.001$; Fig. 9B). Finally, there were also significant increases in levels of immunoreactive MAT2B V1 and V2 after coexpression with WT MAT2A (56.8 to 64.3%, $p < 0.04$; Fig. 9C).

In an attempt to understand mechanisms responsible for these increases in MAT2A and MAT2B protein during coexpression, we performed qRT-PCR with total mRNA isolated from transiently transfected COS-1 cells using cotransfected *lacZ* to control for transfection efficiency to test the hypothesis that cotransfection might increase mRNA stability. However, *MAT2A* mRNA levels, when coexpressed with *MAT2B*, were not significantly different from those seen without cotransfection (data not shown).

We next attempted to determine whether coexpression might alter the degradation of MAT2A/2B by autophagy and/or proteasome-

FIG. 11. *MAT2A* and *MAT2B* mRNA expression. A and B, *MAT2A* and *MAT2B* mRNA expression in the lymphoblastoid cells from which the resequenced DNA was obtained. Each bar represents an individual sample, and the height of the bar represents mRNA expression level as determined by Affymetrix U133 2.0 Plus GeneChip analysis. Data are color-coded by ethnic group. C and D, box plots of *MAT2A* and *MAT2B* mRNA expression by ethnic groups. Mean expression values are shown for each ethnic group. C, *MAT2A* mRNA expression. *, $p < 0.04$ for AA versus HCA. D, *MAT2B* mRNA expression. **, $p < 0.0004$ for AA versus EA and AA versus HCA.

mediated processes. In earlier studies of MAT2A, we had observed that approximately 30% of MAT2A was degraded by the RRL system within 24 h (Fig. 4). Therefore, we synthesized radioactively labeled WT MAT2A protein and nonradioactive MAT2B V1 and V2 protein, and we added increasing quantities of MAT2B to the RRL system, adding from 1 to 5 times the quantity of MAT2A present. TPMT*3A was once again included as a rapidly degraded control. After a 24-h incubation, there was no evidence that MAT2B altered MAT2A degradation in the RRL system (Supplemental Fig. 2).

The next series of experiments was designed to determine whether autophagy and/or proteasome-mediated degradation might be involved in the increases in MAT2A and MAT2B protein that we had observed after coexpression. Specifically, COS-1 cells were transiently transfected with empty vector, WT MAT2A, WT MAT2B, or both WT MAT2A and MAT2B in the presence or absence of 3MA, an inhibitor of autophagy, or MG132, an inhibitor of proteasome-mediated degradation. Quantitative Western blot analysis showed that, in the presence of 3MA, WT MAT2A protein levels increased significantly ($33.1 \pm 1.1\%$, $p = 0.001$) over those in untreated cells, and that coexpression of MAT2A with MAT2B resulted in MAT2A protein levels that were similar to those observed after incubation with 3MA (Fig. 10A). Furthermore, when we assayed MAT2B protein levels, we observed that there was no significant difference after 3MA treatment. In the presence of MG132, WT MAT2A protein levels increased $65.6 \pm 0.31\%$ ($p = 0.00006$) over those of nontreated cells, and coexpression of MAT2A with MAT2B resulted in MAT2A protein levels similar to those seen after MG132 treatment (Fig. 10B). MAT2B protein levels were not affected by treatment with MG132. These results suggest that MAT2B may prevent degradation of MAT2A by autophagy and proteasome-mediated processes.

Polymorphisms and MAT2A/2B Transcription. In addition to characterizing the functional implications of nsSNPs observed during our resequencing of the *MAT2A* and *MAT2B* genes, we also tested the possibility that these SNPs, as well as SNPs in introns or SNPs within 200 kb of the gene that we imputed using recently published 1000 Genomes Project data, might be associated with variation in basal mRNA expression for these genes. Expression array data for the 288 LCLs are shown graphically in Fig. 11, A and B. In those figures, each bar represents results for a single cell line—color-coded by ethnic group. Box plots for *MAT2A* and *MAT2B* mRNA expression by ethnic group are shown in Fig. 11, C and D. A slight but significant difference in *MAT2A* expression among the three ethnic groups was observed ($p = 0.04$) (with HCA having the highest expression and AA having the lowest). There was also a significant ($p = 0.004$) difference in *MAT2B* expression among ethnic groups, with the highest expression in HCA subjects and the lowest in the AAs. The genotype-phenotype correlations for imputed SNPs, sequenced SNPs, and genotyped SNPs for *MAT2A* and *MAT2B* are shown in Fig. 12, C and D, respectively. The black filled circles show the limits of the lengths of the two genes depicted schematically in Fig. 2. For *MAT2A*, the most significant association between SNPs and basal expression array data ($p = 0.0018$) using the imputed SNP data exceeded the most significant SNP-expression association identified using the gene resequencing data ($p = 0.008$) (Fig. 12A), and two of the top nine *MAT2A* SNPs mapped to an ENCODE-identified putative transcription factor binding sites for Brg1 and Ini1 (Rosenbloom et al., 2010). The most significant SNP-expression association for *MAT2B* ($p = 0.0017$) was for an imputed SNP ($p = 0.0012$) (Fig. 12B). All SNPs with p values $< 10^{-2}$ for association with mRNA expression are listed in Table 4.

FIG. 12. *MAT2A* and *MAT2B* genotype-phenotype associations for LCL mRNA expression. The figure shows $\log_{10} p$ values for SNP associations with *MAT2A* (A) and *MAT2B* (B) mRNA expression using GWAS genotyped SNPs, resequenced SNPs, and 1000 Genomes imputed SNPs. The chromosomal regions (chromosome 2 for *MAT2A* and chromosome 5 for *MAT2B*) shown display sequenced SNPs across each gene (●) as well as imputed (△) and genotyped (○) SNPs within 200 kb of the 3'- and 5'-ends of each gene. The y-axis is the $-\log_{10} p$ value for association with mRNA expression. Association analyses were performed with all three ethnic groups combined (All Subjects) as well as for individual ethnic groups.

Discussion

MAT catalyzes the synthesis of AdoMet, the most important biological methyl donor (Mato et al., 2002). As a result, MAT is a key enzyme in one-carbon metabolism and methylation. However, little is known with regard to common genetic variation in the human MAT genes or the functional consequences of common variation in the sequences of these genes. We have applied a genotype-to-phenotype strategy to identify common *MAT2A* and *MAT2B* polymorphisms and then to study the possible functional implications of those SNPs. As a first step, Sanger resequencing of *MAT2A* and *MAT2B* was performed using 288 DNA samples from three ethnic groups, followed

TABLE 4
MAT2A and MAT2B SNPs with $p \leq 10^{-2}$ for association with LCL mRNA expression

Gene	SNP	All Populations Probe Set Correlation	All Populations Probe Set p Value	Base Pairs	Gene Location	SNP Type	Minor Allele Frequency		
							EA	HCA	AA
MAT2A	rs72844410	0.183	0.0018	85,576,517	5'-FR	i	0.1764	0.0469	0.1408
MAT2A	rs72844411	0.174	0.0032	85,577,370	5'-FR	i	0.1797	0.0469	0.1333
MAT2A	rs72844412	0.173	0.0034	85,577,520	5'-FR	i	0.1797	0.0468	0.1332
MAT2A	rs72844414	0.172	0.0036	85,577,614	5'-FR	i	0.1839	0.0506	0.1331
MAT2A	rs56015515	0.172	0.0036	85,578,865	5'-FR	i	0.1797	0.0468	0.1329
MAT2A	rs6704863	-0.271	0.0079	85,627,152	5'-FR	i	— ^a	—	0.156
MAT2A	rs116481102	-0.293	0.0039	85,785,526	3'-FR	i	—	—	0.1858
MAT2B	rs744022	-0.159	0.0070	162,700,884	5'-FR	i	0.4427	0.0521	0.3309
MAT2B	rs7703237	-0.153	0.0097	162,707,304	5'-FR	i	0.4427	0.0718	0.3158
MAT2B	rs114887858	0.282	0.0054	162,734,696	5'-FR	i	0.0246	—	—
MAT2B	rs10050871	-0.177	0.0027	162,739,955	5'-FR	i	0.4325	0.0468	0.2738
MAT2B	rs1592778	0.157	0.0077	162,753,473	5'-FR	i	0.1125	0.0106	0.1481
MAT2B	rs9313969	-0.167	0.0045	162,765,343	5'-FR	i	0.4241	0.0447	0.2898
MAT2B	rs2069347	0.155	0.0085	162,799,773	5'-FR	o	0.4635	0.1533	0.3105
MAT2B	rs299283	0.157	0.0075	162,829,434	5'-FR	o	0.2917	0.416	0.4368
MAT2B	rs116745026	0.262	0.0099	162,908,654	5'-FR	i	0.0343	—	—
MAT2B	rs115759359	0.265	0.0091	162,933,917	Intron	i	0.0444	—	—
MAT2B	rs76990151	0.270	0.0077	162,947,880	3'-FR	i	0.0343	—	—
MAT2B	rs172389	0.265	0.0092	162,953,337	3'-FR	i	0.0476	—	—
MAT2B	rs6878378	0.171	0.0038	162,978,522	3'-FR	o	0.1251	0.1338	0.3426
MAT2B	rs17062061	0.162	0.0060	162,980,057	3'-FR	i	0.1248	0.1788	0.3426
MAT2B	rs11949278	0.167	0.0046	162,984,895	3'-FR	o	0.1249	0.1719	0.342
MAT2B	rs116239680	0.293	0.0040	163,045,314	3'-FR	i	—	—	0.1031

rs, reference SNP IDs; MAF, minor allele frequency; i, imputed SNP; o, observed SNP (either by resequencing or genotyping).
^a Dashes indicate that the variant allele was not observed in that population.

by imputation using recently released 1000 Genomes Project pilot data. We then determined the possible functional implications of nsSNPs and also attempted to determine whether any SNPs in or near these genes might be associated with alteration in transcription in the lymphoblastoid cell lines from which the resequenced DNA had been obtained. In addition, we studied the functional consequences of MAT2A-MAT2B interaction.

Specifically, after transient expression in a mammalian cell line, the MAT2A Val11 variant allozyme displayed a significant decrease in MAT enzyme activity (Fig. 3A) that was due to a decrease in immunoreactive protein (Fig. 3B). Although the SNP responsible for this variant allozyme is rare, the decreased MAT2A associated with the SNP in vivo might result in decreased AdoMet synthesis and, as a result, decreased methylation. Subsequent functional genomic studies identified a decrease in translation as the mechanism responsible, at least in part, for the decreased level of protein that we observed for this variant allozyme. Substrate kinetic studies also failed to show differences between WT and Val11 allozymes for MAT2A. MAT2B variant allozymes showed no apparent functional consequences of their changes in amino acid sequence. We also investigated noncoding SNPs in MAT2A and MAT2B and found that several MAT2A SNPs were associated with changes in basal MAT2A mRNA expression in lymphoblastoid cell lines (Fig. 12, A and B).

To study the possible effects of genetic variation on the known regulatory properties of MAT2B for MAT2A, we coexpressed WT MAT2A with WT and variant MAT2B V1 and V2 in COS-1 cells. Enzyme activity was significantly higher for all WT MAT2A/MAT2B combinations than for WT MAT2A expressed alone (Fig. 8B). The increase in activity observed during coexpression was apparently due to an increase in MAT2A protein (Fig. 9A). We also observed an increase in MAT2B protein during cotransfection (Fig. 9C). A similar phenomenon was observed for endogenous COS-1 cell MAT2A enzyme activity and protein levels when MAT2B was expressed in those cells. In an attempt to understand the underlying mechanism for this phenomenon, we performed in vitro and in vivo experiments to determine whether protein degradation might play a role. We found

that autophagy and proteasome-mediated degradation were involved in MAT2A degradation, but that MAT2B could impede this process (Fig. 10, A and B). These observations provide insight into mechanisms for the functional consequences of the MAT2A-MAT2B protein-protein interaction and highlight the importance of MAT2B as a regulatory protein.

In summary, we resequenced the human MAT2A and MAT2B genes, followed by imputation using 1000 Genomes Project pilot data, to identify and characterize common genetic variation in MAT2A, a gene that encodes a critical enzyme for the nonhepatic generation of AdoMet, and MAT2B, a gene encoding a protein that regulates MAT2A enzymatic activity—in part by stabilizing both proteins and impeding their degradation. These observations will serve as a basis for future translational and mechanistic studies of these genes encoding proteins that play a critical role in a major Phase II reaction, methyl conjugation.

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Authorship Contributions

Participated in research design: Nordgren, Peng, Moon, Abo, Feng, Yee, Wieben, and Weinshilboum.

Conducted experiments: Nordgren, Peng, Pellemounter, Moon, Abo, and Eckloff.

Contributed new reagents or analytic tools: Peng and Yee.

Performed data analysis: Nordgren, Peng, Pellemounter, Moon, Abo, Feng, Eckloff, Yee, Wieben, and Weinshilboum.

Wrote or contributed to the writing of the manuscript: Nordgren, Peng, Abo, Yee, and Weinshilboum.

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