ORIGINAL INVESTIGATION

**Guanshan Zhu · Robert H. Lipsky · Ke Xu ·
Sameer Ali · Thomas Hyde · Joel Kleinman ·
Longina A. Akhtar · Deborah C. Mash ·
David Goldman**

Differential expression of human *COMT* alleles in brain and lymphoblasts detected by RT-coupled 5′ nuclease assay

Abstract *Rationale:* A common polymorphism, Val158-Met, alters catechol-*O*-methyltransferase (COMT) enzyme activity and has been linked to psychiatric phenotypes. Bray et al. (2003) reported that *COMT* is subject to differential allele expression in brain, finding modest (13–22%) underexpression of a haplotype containing Val158. However, disparate findings by another group who used the same method, but in lymphoblasts, raise the issues of tissue specificity, magnitude of differential expression, and identity of loci altering expression. *Objectives:* We measured *COMT* allele expression ratios in heterozygous human lymphoblast cell lines and brains. *Methods:* Using transcribed single nucleotide polymorphisms as endogenous reporters, we developed an RT-coupled 5′ nuclease assay for allele expression ratios and applied it to 63 *COMT* rs4818(C>G) heterozygotes and 68 Val158Met [rs4680(G>A)] heterozygotes. *Results:* For rs4818 (C>G), the C allele was overexpressed relative to the G allele in 18 of 27 lymphoblast lines and 23 of 36 brains. For Val158Met, Met158 was overexpressed relative to Val158 in all (29 of 29) lymphoblast lines and all (39 of 39) brains. Each of the 22 rs4818 heterozygotes without differential allele expression was a Val158/Val158 homo-zygote. The Met158 allele was overexpressed by 65–77% when compared with Val158 in lymphoblasts and brain. Haplotype augmented ability to predict expression in brain only. However, the expression of the Val158 allele on the high-expressing haplotype was only 19% higher than Val158 alleles on the other haplotype background. *Conclusions:* COMT alleles are differentially expressed. The Met158 allele predicts higher mRNA expression in both brain and lymphoblasts. As exemplified here, the RT-coupled 5′ nuclease assay is a reliable method for the quantitative evaluation of *cis*-acting regulatory effects.

Keywords COMT · Allele-specific expression · Single nucleotide polymorphism · Haplotype · Lymphoblast · Brain · Human

G. Zhu · R. H. Lipsky · K. Xu · S. Ali · L. A. Akhtar ·
D. Goldman (✉)
Laboratory of Neurogenetics, National Institute on Alcohol
Abuse and Alcoholism, National Institutes of Health,
12420 Parklawn Dr.,
Rockville, MD 20852, USA
e-mail: dgneuro@box-d.nih.gov
Tel.: +1-301-4430059
Fax: +1-301-4438579

G. Zhu
Changhai Hospital,
Shanghai, 200433, People's Republic of China

T. Hyde · J. Kleinman
Clinical Brain Disorders Branch, NIMH,
Bethesda, MD 20892, USA

D. C. Mash
Department of Neurology, University of Miami,
Miami, FL 33136, USA

Introduction

Catechol-*O*-methyltransferase (COMT) is a key enzyme in the metabolism of catecholamine neurotransmitters (Axelrod and Tomchick 1958) and is widely expressed in brain and found elsewhere in the body. COMT enzyme activity measured in erythrocytes and liver was studied as a quantitative genetic trait. COMT activity varies threefold to fourfold between individuals, exhibits a trimodal distribution in human populations and is familially transmitted as if controlled by two alleles at a single genetic locus (Weinshilboum and Raymond 1977; Spiel-man and Weinshilboum 1981). A common *COMT* polymorphism, Val158Met, was identified and predicts COMT enzyme activity (Lachman et al. 1996). Val158Met has been associated with several psychiatric phenotypes and intermediate phenotypes (Karayiorgou et al. 1997; Egan et al. 2001; Malhotra et al. 2002; Bilder et al. 2002; Enoch et al. 2003).

The Val158 and Met158 alleles are both found at high frequencies (0.2–0.8) across diverse human populations (Palmatier et al. 1999). The two alleles appear to have contrasting advantages and disadvantages, potentially maintaining both alleles at these high frequencies by the

mechanism of balanced selection or niche-specific selection. Val158 is associated with schizophrenia risk (Egan et al. 2001) and diminished frontal cognitive function (Malhotra et al. 2002; Bilder et al. 2002). However, the Met158 allele plays a role in vulnerability to anxiety-related phenotypes (Karayiorgou et al. 1997; Enoch et al. 2003) and pain-stress response (Zubieta et al. 2003). This can be thought of as a "warrior versus worrier" mechanism for maintenance of COMT functional diversity.

A recent linkage study suggests that the picture of COMT functional variation is more complex. Shifman et al. (2002) associated a Val158-containing COMT haplotype to schizophrenia but did not observe significant association to the individual Val158Met locus. Bray et al. (2003) recently reported that COMT is subject to differential allele expression in human brain, potentially explaining the Shifman et al. haplotype linkage finding. Bray et al. detected differential COMT expression using single base extension (SBE) of transcribed single nucleotide polymorphisms (SNPs), a method first developed by Yan et al. (2002). Expression of one allele was reduced 13–22% in four-locus COMT haplotypes containing Val158. However, Yan et al. (2002) used the same method and one of the same loci [rs4633(C>T)] but failed to detect differential COMT expression in lymphoblasts of 22 heterozygotes. These findings raise the issue of tissue-specific differential allele expression, the magnitude of differential expression, and the identity of the locus-altering or -predicting expression.

We developed a novel, highly accurate method, the RT-coupled 5′ nuclease assay (Zhu et al. 2002) to measure relative expression of alleles at two COMT loci. We detected differential allele expression at both COMT loci. However, the differential expression is several times larger in magnitude than previously reported, occurs in both brain and lymphoblasts, and is predominantly predicted by the Val158Met locus, although 5′ and 3′ loci also have some additional predictive power for COMT expression in brain.

Materials and methods

DNA and RNA samples

From peripheral blood of unrelated individuals (56 Finnish Caucasians, 12 Italian Caucasians, 5 American Caucasians and 4 American Indians), 77 lymphoblastoid cell lines were prepared. Blood was collected after receiving informed consent under human research protocols approved by the Institutional Review Boards of the National Institute of Mental Health, NIH, the National Institute on Alcohol Abuse and Alcoholism, the University of Helsinki, and the University of Pisa. The cell lines were maintained in RPMI 1640 with 15% fetal calf serum (FCS). Seventy-eight cerebellar cortex samples were collected postmortem from unrelated individuals (47 American Caucasians, 25 African Americans, 5 Hispanic Americans, 1 Asian). The human brain samples were collected through PHS DA 06227, the University of Miami Brain Endowment Bank, the National Parkinson Foundation, Inc., Miami, FL, and the GRECC, Miami VAMC. Genomic DNA was isolated and genotyped by 5′ nuclease assay (see below). The genotyping identified 27 cell lines and 36 brain samples heterozygous for rs4818, and 29 cell lines and 39 brain samples heterozygous for Val158Met [rs4680 (G>A)]. Total RNA was isolated from each of these heterozygotes and treated with RNase-free DNase (Ambion, Austin, TX) to remove contaminating DNA.

SNP primers and probes for 5′ nuclease assays

An abundant biallelic SNP, rs4818(C>G) (NCBI dbSNP [http://www.ncbi.nlm.nih.gov/SNP]) located in exon 4, was used as an endogenous reporter for the 5′ nuclease assay. Primers and probes for both genomic DNA typing and cDNA allele quantification were designed using Primer Express software (Applied Biosystems, Foster City, CA) and sequences were as follows: primer-4818-F, 5′-GCCTACTGTGGCTACTCAGCTG-3′; primer-4818-R, 5′-AGC-GAAATCCACCATCCG-3′; probe-4818-C, 5′-FAM-CGAGGCT-CATCACCATCGAGATCA-TAMRA-3′; and probe-4818-G, 5′-VIC-CGAGGCTGATCACCATCGAGATCA-TAMRA-3′.

We also genotyped and compared allele expression of Val158Met, the common missense variant which alters COMT enzyme thermal stability and activity. Val158Met [updated dbSNP ID is rs4680 (G>A) instead of passed ID of rs165688 cited by Shifman et al. (2002) and Bray et al. (2003)] is located 64 bp 5′ to rs4818 in exon 4. Val158Met was in complete linkage disequilibrium (LD) with rs4818 in both Finnish (D′=1, N=56) and US Caucasians (D′=1, N=52). Primer and probe sequences for genotyping Val158Met from genomic DNA were: primer-4680-F1, 5′-CGAGATCAACCCC-GACTGT-3′; primer-4680-R1, 5′-CCCTTTTTCCAGGTCTGA-CAA-3′; probe-4680-G1 [Val158], 5′-FAM-TCCTTCACGC-CAGC-MGB-3′ (antisense); and probe-4680-A1 [Met158], 5′-VIC-TGTCCTTCATGCCAG-MGB-3′ (antisense). Primer and probe sequences for cDNA typing were: primer-4680-F2, 5′-TCGAGATCAACCCCGACTGT-3′; primer-4680-R2, 5′-GGGACGCTCCAACCACAA-3′; probe-4680-G2 [Val158], 5′-FAM-CTTCACGCCAGCG-MGB-3′ (antisense); and probe-4680-A2 [Met158], 5′-VIC-CTTCATGCCAGCG-MGB-3′ (antisense).

Allele-specific control DNAs were created for rs4680. Allele-specific rs4680 amplicons were made using two sets of allele-specific primers. One copy each of allele G and allele A were inserted into the plasmid, pDsRed2-1, to make an artificial 1:1 allele ratio control construct. The sequences of the primers for the allele-specific cloning were: primer-4680-F3, 5′-attgaatTCACCATCGA-GATCAACCC-3′; primer-4680-R3, 5′-tataagctTGTC-CAGTGTGTCCACATCAT-3′; primer-4680-F4, 5′-tataagctTCAC-CATCGAGATCAACCC-3′; and primer-4680-R4, 5′-tatggaTC-CAGTGTGTCCACATCATACTT-3′. Lower case indicates sequence tags for EcoRI, HindIII, HindIII, and BamHI restriction enzymes, respectively.

Two additional SNPs located in COMT 5′ end P2 promoter region (rs2097603 A>G) and in COMT 3′ end UTR (rs165599 A>G) were selected for 5′ nuclease assay genotyping for haplotype analysis. Primer and probe sequences for the two markers were: primer-2097603-F, 5′-GCCGTGTCTGGACTGTGAGT-3′; primer-2097603-R, 5′-GGGTTCAGAATCACGGATGTG-3′; probe-2097603-A, 5′-FAM-AACAGACAGAAAAGtTTCCCCTTCCCA-TAMRA-3′ (antisense); probe-2097603-G, 5′-VIC-CAGACA-GAAAAGcTTCCCCTTCCCATA-TAMRA-3′ (antisense); primer-165599-F, 5′-GCCAGGGGCACCTGTTAG-3′; primer-165599-R, 5′-CTGGCTGACTCCTCTTCGTTT-3′; probe-165599-A, 5′-FAM-ACGACTGCCaGCCT-MGB-3′; and probe-165599-G, 5′-VIC-AC-GACTGCCgGCC-MGB-3′.

5′ Nuclease assay genotyping

Genomic DNA (2 µl; 50 ng/µl) was added to a 25-µl reaction consisting of 1× universal TaqMan PCR master mix (Applied Biosystems, Foster City, CA), 1 µM forward primer, 1 µM reverse primer, 0.2 µM FAM-labeled probe, and 0.2 µM VIC-labeled probe. Polymerase chain reaction (PCR) conditions were 50°C for 2 min, 95°C for 10 min, and 95°C for 15 s/62°C for 1 min for 40 cycles.

PCR was performed on an ABI 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Endpoint fluorescence signals were detected on the ABI 7700, and data were analyzed using Sequence Detector System software, version 1.6 (Applied Biosystems, Foster City, CA).

RT-coupled 5′ nuclease assay for measurement of COMT allele expression ratios

Total RNA (800 ng) was reverse transcribed in a 20-μl volume reaction following manufacturer's instructions (Invitrogen, Carlsbad, CA). cDNA (2 μl) was added to a 25-μl reaction consisting of 1x universal TaqMan PCR master mix (Applied Biosystems, Foster City, CA), 1 μM forward primer, 1 μM reverse primer, 0.2 μM FAM-labeled probe, and 0.2 μM VIC-labeled probe. For PCR, DNA was incubated at 50°C for 2 min and 95°C for 10 min, and then amplified 40 cycles each consisting of 95°C for 15 s followed by 62°C for 1 min. Control heterozygous genomic DNA (for rs4818) or construct (for Val158Met = rs4680) was also assayed on the same plate. PCR was performed on an ABI 7700 Sequence Detector (Applied Biosystems, Foster City, CA), and real-time fluorescence signals were collected during the PCR process and analyzed using Sequence Detector System software, version 1.6 (Applied Biosystems, Foster City, CA).

Principle and method of computation of allele expression ratios

In the 5′ nuclease assay, an increase in allele-specific fluorescence signal is detected when the probe anneals to the target sequence enabling the 5′ exonuclease activity of DNA polymerase to cleave the probe and uncover the allele-specific fluorescence. In heterozygous genomic DNA, both probes anneal and are cleaved so that two different fluorescent signals are proportionately generated. Assuming that both allele-specific probes anneal with equal avidity and generate a fluorescent signal of similar strength, then the number of PCR cycles before the allele-specific signals cross a predetermined threshold (Ct) will be the same. Ct can be measured fractionally.

If one allele is overexpressed relative to the other, it will reach amplification threshold (Ct) earlier. A one-cycle delay ($\Delta Ct=1$) indicates that the ratio of one allele to the other is approximately 1:2; a two-cycle delay ($\Delta Ct=2$), 1:4; or in general, $1 : 2^{\Delta Ct}$ if amplification efficiency is very close to 100%. Under optimized PCR conditions, PCR amplification efficiency is very close to 100% (Higuchi and Watson 1999), and the exponential parameter is 2. However, when PCR conditions are adjusted (mainly by increasing annealing/extension temperature) to better discriminate allele-specific signals, the efficiency of amplification may be slightly less than 2. Therefore, we measured the actual exponential parameter for both COMT loci. Genomic DNA heterozygous for rs4818 and the construct DNA heterozygous for Val158Met were serially diluted as 1:1, 1:2, 1:4, 1:8 and 1:16, and assayed. The exponential parameter for rs4818 was 1.86 and the parameter for Val158Met was 1.79.

Signal efficiency for the two alleles at each locus may still differ slightly. A correction can be made by subtracting the small ΔCt derived from the heterozygous control DNA from the observed ΔCt derived from the test sample, and the corrected measure, designated $\Delta Ct'$, enables computation of the accurate allele expression ratio ($X^{\Delta Ct'}$, where X is 1.86 for rs4818 and 1.79 for Val158Met). Figure 1 b shows COMT rs4818(C>G) allele-specific amplification of genomic DNA sample artificially prepared as a 4:1 ratio of allele G to allele C. The allele C signal crossed the threshold cycle (Ct) at 26.29; whereas the allele G signal crossed the threshold at cycle 24.01. The ΔCt is the difference between the two sets of Ct values; in this example, 2.28 cycles. After correction of this ΔCt for the slight difference in allele-specific Ct in a naturally heterozygous genomic DNA sample (Fig. 1 a, $\Delta Ct=0.07$), $\Delta Ct'$ (2.21) can then be used to calculate an allele ratio ($1.86^{2.21}=3.94$). In this instance, the experimentally measured ratio is thus very close to the expected ratio of 4:1.
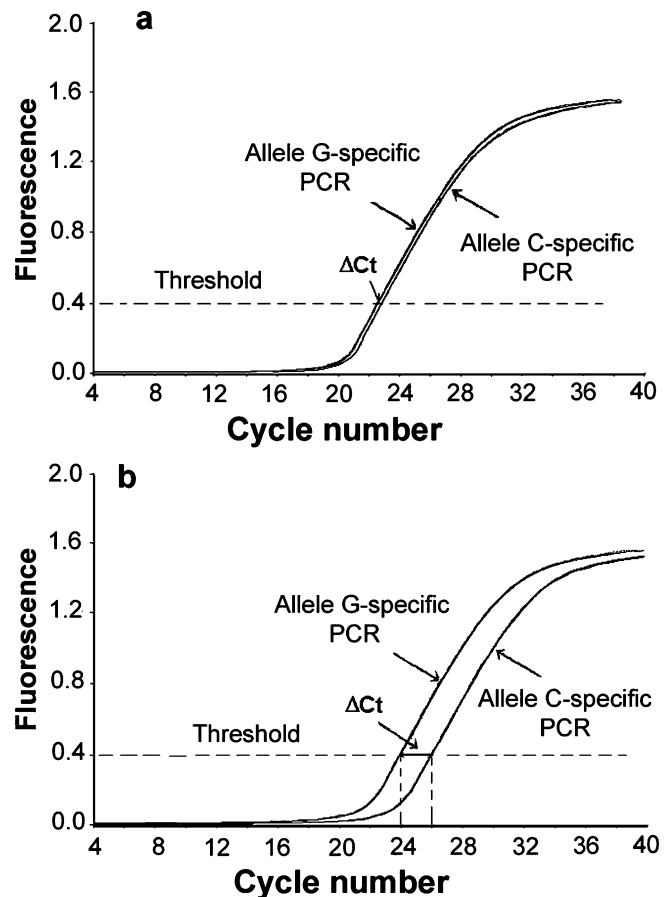
Fig. 1 Allele ratio measurement using the 5′ nuclease assay. Allele-specific amplifications were performed for COMT-rs4818(C>G) in heterozygous (1:1 allele ratio) genomic DNA (**a**) and in a 4:1 G/C mixture of homozygous genomic DNAs (**b**). In each case, ΔCt was calculated. For *panel* **b**, $\Delta Ct'$ was also calculated using the ΔCt in heterozygous DNA as a correction factor. In this example, the ratio of the two alleles in a sample of unknown composition would be computed using the equation $1.86^{\Delta Ct'}$

Statistics

For LD, D′ was calculated using MLOCUS (Long 1999) and haplotype reconstruction was done using PHASE 2.0.2 software (Stephens and Donnelly 2003). Haplotype-based comparisons for differential allele expression were analyzed by means of single-factor analysis of variance (ANOVA).

Results

Accuracy of the 5′ nuclease assay for measurement of allele ratios

To determine the accuracy of relative allele quantification by 5′ nuclease assay, different ratios of COMT-rs4818-C/G alleles were prepared by mixing genomic DNAs homozygous for alleles C and G. Allele ratios were calculated from the $\Delta Ct'$ values as described and using the equation: $1.86^{\Delta Ct'}$ because the amplification efficiency of rs4818 (C>G) was measured as 1.86, rather than 2. As shown in
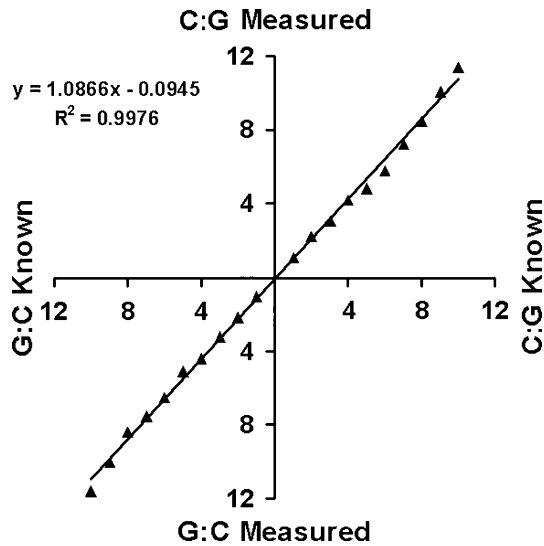
Fig. 2 Measured *COMT* allele ratios by 5′ nuclease assay versus known allele ratios, illustrating the quantitative accuracy of RT-coupled 5′ nuclease assay for allele expression

Fig. 2, the correlation coefficient (*r*) between known and measured allele ratios was greater than 0.99.

### Discovery of differential expression of COMT alleles

Rs4818 alleles were observed to be differentially expressed in 18 of 27 lymphoblast cell lines and in 23 of 36 brains (Fig. 3). However, Val158Met alleles were differentially expressed in all (29 of 29) lymphoblast cell lines and in all (39 of 39) brains (Fig. 4). The allele ratios among positive individuals were 1.84±0.13 in lymphoblast and 1.90±0.34 in brain for rs4818 (C:G), and 1.65±0.15 in lymphoblast and 1.77±0.26 in brain for Val158Met (A:G). Critically, each of the 22 rs4818 heterozygotes that did not show differential allele expression (all of which had an expression ratio very close to one) was a Val158/Val158 homozygote (Fig. 3).

### Correlation of allele expression ratios of two independent COMT coding SNPs

In brain, the allele expression ratios of the two independent but completely linked SNPs were highly correlated. (*r*=0.9; Fig. 5), indicating reliability of the RT-coupled 5′ nuclease assay for the measurement of allele expression ratios.

COMT haplotype effect on expression of Val158Met alleles

To detect potential effects of loci other than Val158Met on *COMT* expression, we performed a three loci haplotype analysis focusing on differences in allele expression ratio in the context of different haplotype backgrounds. The
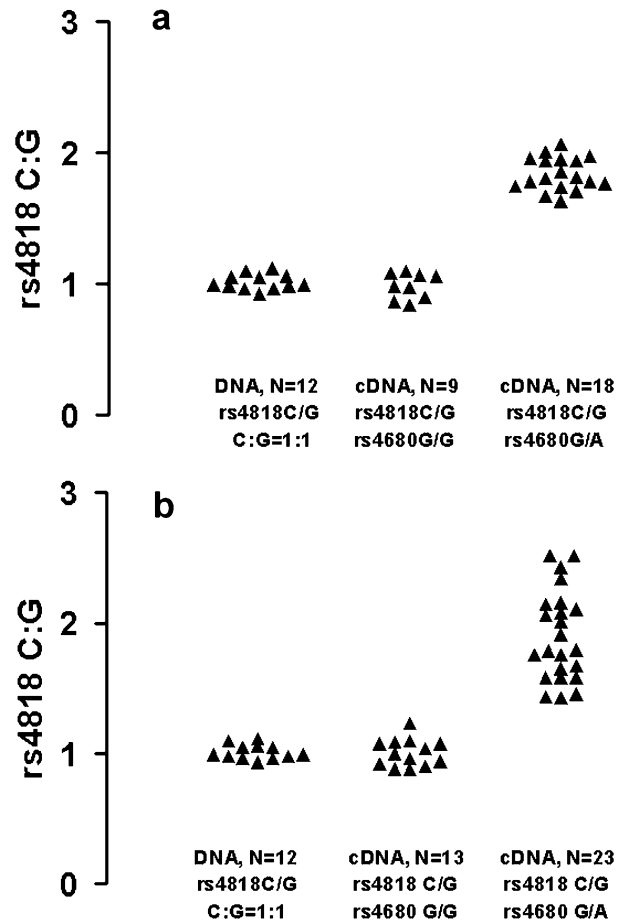

Fig. 3 Differential allele expression of *COMT* rs4818. **a** Lymphoblast; **b** brain
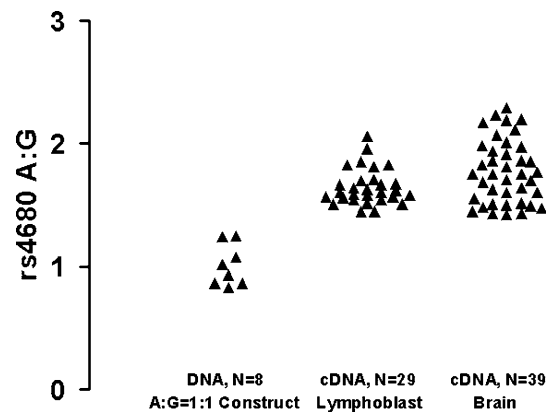
Fig. 4 Differential allele expression of *COMT* Val158Met in human lymphoblast and brain

COMT 5′ end marker rs2097603 (A>G) and Val158Met were in relatively stronger LD in US Caucasians (*D*′=0.64, *N*=50) and relatively lower LD in Finns (*D*′=0.41, *N*=56), while the 3′ end marker rs165599 (A>G) and Val158Met were in relatively lower LD in US Caucasians (*D*′=0.37, *N*=50) but relatively stronger LD in Finns (*D*′=0.84, *N*=56). We were able to identify four major haplotypes (haplotype frequency >0.05) among the study subjects: -A-G-A-, -A-G-G-, -A-A-A-, and -G-A-A- for the marker
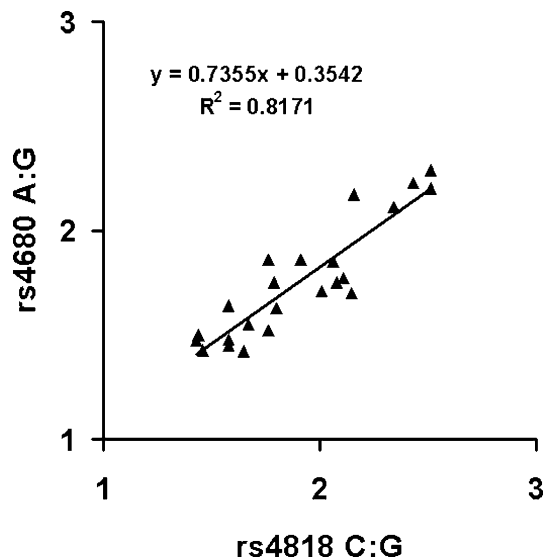
Fig. 5 Correlation of allele expression ratios of two *COMT* coding single nucleotide polymorphisms (SNPs) in 23 human brain samples doubly heterozygous for the two SNPs independently measured by the RT-coupled 5′ nuclease assay

order 5′-rs2097603(A>G)-rs4680 (G>A)-rs165599 (A>G)-3′. For these four haplotypes, we classified individuals into the following categories (diplotypes): -A-G-A-/-A-A-A-, -A-G-A-/-G-A-A-, -A-G-G-/-A-A-A-, and -A-G-G-/-G-A-A-. By means of single-factor ANOVA, we found a significant overall difference of allele expression ratio in brain among these four diplotypes (F=3.8, P=0.03) (Fig. 6 b) but not in lymphoblast (Fig. 6 a). In brain samples, the -A-G-G-/-G-A-A- diplotype predicted the largest allele ratio of Val158Met. In other words, the Val158 allele is expressed the lowest in brain when on the -A-G-G- haplotype background. In brain, the -A-G-A- background predicts a 19% higher expression of the Val158 allele. Meanwhile, the Met158 allele is expressed 1.65- to 1.77-fold as high as the Val158 allele in lymphoblasts and brain.

Discussion

In genetically variable organisms, detection of relative differences in expression of transcribed alleles can offer an efficient and widely applicable approach to the discovery and quantitative evaluation of *cis*-acting, gene regulatory polymorphisms (Price et al. 2001). Measurement of allele expression ratios is dependent on the availability of gene polymorphisms in transcribed sequences. Critical issues for measuring allele-specific expression are accuracy and repeatability. Because of the disadvantages of conventional gel and/or isotope-based methods (Mansfield 1993; Ohlsson et al. 1995; Uejima et al. 2000), the advantages of fluorescence labeling, and abundance of transcribed SNPs in the genome (Venter et al. 2001), it is advantageous to use transcribed SNPs as endogenous reporters in assays relying on fluorescent reporters. Both the SBE method developed by Yan et al. (2002) and an Affymetrix HuSNP
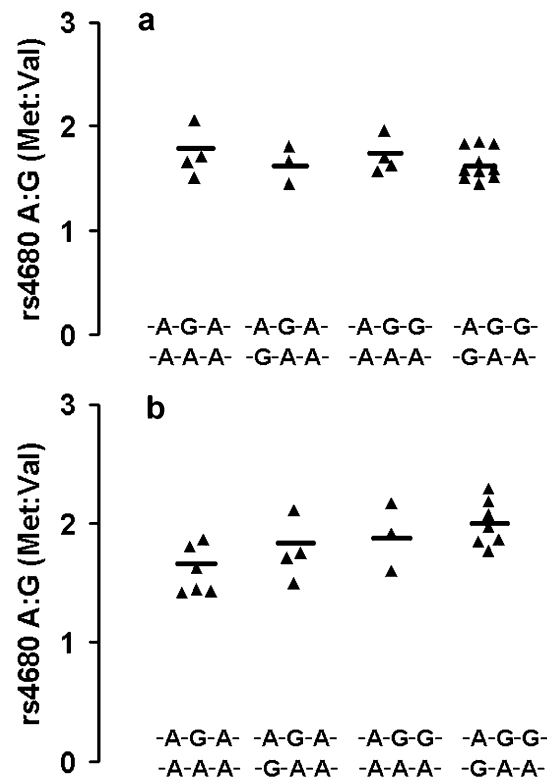
Fig. 6 *COMT* three marker haplotype [-rs2097603(A>G)-rs4680 (G>A)-rs165599(A>G)-] and differential allele expression of COMT in lymphoblast and brain. Haplotype codings: -A-G-A- for rs2097603A-rs4680G-rs165599A haplotype; -A-G-G- for rs2097603A-rs4680G-rs165599G; -A-A-A- for rs2097603A-rs4680A-rs165599A; -G-A-A- for rs2097603G-rs4680A-rs165599A. **a** Effect of haplotype on differential expression of COMT alleles in lymphoblast (*P*=ns). **b** Effect of haplotype on differential expression of COMT alleles in brain (*F*=3.8, *P*=0.03, by single factor ANOVA)

oligo array method developed by Lo et al. (2003) use transcribed SNPs as markers. The recent development shows that non-coding SNPs can also be used for in vivo characterization of differential allele expression, although this approach, named "haploChIP", is a very complicated procedure (Knight et al. 2003). The 5′ nuclease assay was developed for large-scale genotyping of SNPs (Lee et al. 1993) and more recently has been successfully applied to the determination of SNP allele frequencies in pooled DNAs (Xu et al. 2002). We adapted the 5′ nuclease assay to develop a novel RT-coupled 5′ nuclease assay for the measurement of allele expression ratios. The method is arguably simpler than any methods above including SBE, because the RT-coupled 5′ nuclease assay requires no post-PCR processing. The allele expression ratios measured by RT-coupled 5′ nuclease assay are highly accurate, being correlated with the known ratios (Fig. 2) and highly correlated across assays using two independent SNP markers in the same transcript (Fig. 5).

Using the RT-coupled 5′ nuclease assay, we confirmed that *COMT* alleles are differentially expressed in human brain and are also differentially expressed in lymphoblasts. These results exclude the possibility of tissue-specific differential allele expression of human *COMT*. The smaller

(13–22%) effects of *COMT* alleles on mRNA expression detected by Bray et al. and the inability of Yan et al. (2002) to detect an effect raise questions about the sensitivity of SBE for detection of differences in allele expression.

Both loci we evaluated showed differential expression; however, one of them, Val158Met, in every case showed higher (65–77%) expression of Met158 than Val158. Critically, each of the 22 rs4818 heterozygotes that did not show differential allele expression (all of which had an expression ratio very close to one) was a Val158/Val158 homozygote (Fig. 3). Because the magnitude of differential expression was substantially larger than that seen by Bray et al., we underline their observation of *COMT* differential expression. Haplotype analysis showed that additional *COMT* loci would not add additional information on the level of *COMT* expression in lymphoblast, but did add a minor amount of additional information in brain, which indicates a potential tissue-specific haplotype effect on *COMT* mRNA expression.

Bray et al. associated differential *COMT* allele expression with a haplotype. However, the uniformity of Val158Met differential expression indicates that haplotype analysis captures very little additional information on expression of *COMT*, at least in the populations we studied. These results argue that the Val158Met locus either determines *COMT* expression, in a *cis*-acting fashion, or that Val158Met is in allelic identity with a locus that does, again, in the populations we studied.

COMT has two promoters, leading to expression of either membrane-bound MB-COMT or a soluble S-COMT truncated by 50 amino acids. Transcription of MB-COMT is directed by the P2 promoter. Both lymphocytes and brain predominately express MB-COMT mRNA (Tenhunen et al. 1994; Jiang et al. 1998; Matsumoto et al. 2003), so that mRNA expression signals detected in our study and that of Bray et al. are largely due to MB-COMT. Furthermore, while S-COMT enzyme activity is high in various other tissues, MB-COMT levels are thought to be most important for synaptic catecholamine levels. COMT Val158 allele is more thermostable than the Met158 allele even at physical temperature (37°C) (Lotta et al. 1995), establishing that at least part of the effect of the Val158Met locus on COMT activity is at the mechanistic level of protein structure. The findings reported here and, to a lesser degree, the earlier report of Bray et al. add the information that the higher activity Val158 allele is less expressed in brain. The higher level of expression of Met158 apparently only partly mitigates the impairment of enzyme activity seen with this allele. This is further supported by the result of a recent study in which COMT enzyme activity in human brain of Val/Val individuals was only about 30% higher than enzyme activity in Met/Met individuals, rather than threefold to fourfold higher as had previously been reported in studies of hepatic and erythrocyte COMT (Jingshan Chen and Daniel Weinberger, personal communication).

The locus responsible for *COMT* differential expression (if not Val158Met) is unknown. A requirement would be very strong LD with Val158Met and other markers in this region of the *COMT* gene, and, thus far, loci in the P2 promoter region and 3′ region of *COMT* have not been found to be in very strong LD with Val158Met. For example, rs2097603 (G>A) located in the P2 promoter is not in very strong LD with Val158Met in either Finns (D' =0.23) or US Caucasians (D'=0.53) (DeMille et al. 2002, and as we have replicated). Bray et al. (2003) reported modest differential allele expression of rs165599 (G>A), located in the 3′ UTR, and suggested that this locus or one in very strong LD is responsible for *COMT* differential expression. However, rs165599 is not in very strong LD with Val158Met (D'=0.46) (Bray et al. 2003, and as we have replicated), ruling out rs165599 as well as others in strong LD with it.

Bray et al. correlated a schizophrenia high-risk haplotype (Shifman et al. 2002) with *COMT* differential allele expression. Their three-locus haplotype contains Val158. Other investigators (Egan et al. 2001) had found associations with the Val158 allele itself. Our data indicate that *COMT* differential expression does not explain the Shifman et al. haplotype association finding. Like us, Bray et al. also observed lower expression of Val158. Our results indicate that the magnitude of *COMT* allelic expression is predominantly predicted by Val158Met genotype. Meanwhile, the Val158Met locus is the only common missense variant capable of altering COMT enzyme function. If the *COMT* haplotype is more predictive for behavior, it is likely that some other inherited property of *COMT* is being better tracked by the haplotype, for example the ratio of S-COMT to MB-COMT might be altered by some unknown functional locus.

To summarize the integrated effect of Val158Met differential mRNA expression and differential enzyme activity, we believe that COMT enzyme activity is a product of effects on expression at both the RNA and protein levels. Val158Met predicts both mRNA expression and enzyme activity but in opposing directions. The net result, as indicated by the unpublished data of Chen and Weinberger, is 30% lower enzyme activity with the Met158 allele. However, 5′ end and 3′ end *COMT* loci also have minor additional predictive value for *COMT* expression in brain, but not in lymphoblast *COMT* haplotype. In brain, approximately 15% of the time the Val158 allele resides on a haplotype -A-G-A- which is over-expressed 19% relative to Val158 alleles residing on the other common Val158 haplotype background -A-G-G-. It appears most likely that lower expression of Val158 mRNA modulates the effect of the enzyme functional polymorphism. A need for further studies on regulatory mechanisms and effects of genotype on COMT expression, particularly the control of expression of soluble versus membrane-bound COMT, remains.

References

Axelrod J, Tomchick R (1958) Enzymatic O-methylation of epinephrine and other catechols. J Biol Chem 233:702–705

Bilder RM, Volavka J, Czobor P, Malhotra AK, Kennedy JL, Ni X, Goldman RS et al (2002) Neurocognitive correlates of the COMT Val(158)Met polymorphism in chronic schizophrenia. Biol Psychiatry 52:701–707

Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, O'Donovan MC (2003) A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. Am J Hum Genet 73:152–161

DeMille MMC, Kidd JR, Ruggeri V, Palmatier MA, Goldman D, Odunsi A, Okonofua F et al (2002) Population variation in linkage disequilibrium across the COMT gene considering promoter region and coding region variation. Hum Genet 111:521–537

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D et al (2001) Effect of COMT Val108/158Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA 98:6917–6922

Enoch MA, Xu K, Ferro E, Harris CR, Goldman D (2003) Genetic origins of anxiety in women; a role for a functional COMT polymorphism. Psychiatr Genet 13:33–41

Higuchi R, Watson RM (1999) Kinetic PCR analysis using a CCD-camera and without using oligonucleotide probes. In: Innis MA, Gelfand DH, Sninsky JJ (eds) PCR applications: protocols for functional genomics. Academic, San Diego, pp 263–284

Jiang H, Ho SL, Xie T, Young LP (1998) Distribution of catechol-O-methyltransferase expression in human central nervous system. Neurochemistry 9:2861–2864

Karayiorgou M, Altemus M, Galke BL, Goldman D, Murphy DL, Ott J, Gogos JA (1997) Genotype determining low catechol-O-methyltransferase activity as a risk factor for obsessive-compulsive disorder. Proc Natl Acad Sci USA 94:4572–4575

Knight JC, Keating BJ, Rockett KA, Kwiatkowski DP (2003) In vivo characterization of regulatory polymorphisms by allele-specific quantification of RNA polymerase loading. Nat Genet 33:469–475

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250

Lee LG, Connell CR, Bloch W (1993) Allelic discrimination by nick translation PCR with fluorogenic probes. Nucleic Acids Res 21:3761–3766

Lo HS, Wang Z, Hu Y, Yang HH, Gere S, Buetow KH, Lee MP (2003) Allelic variation in gene expression is common in the human genome. Genome Res 13:1855–1862

Long JC (1999) Multiple Locus Haplotype Analysis, version 2.0. Software and documentation distributed by the author. Section on Population Genetics and Linkage, Laboratory of Neurogenetics, NIAAA, National Institutes of Health, Bethesda, MD

Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J (1995) Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34:4202–4210

Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, Goldberg T, Goldman D (2002) A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. Am J Psychiatry 159:652–654

Mansfield ES (1993) Diagnosis of down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms. Hum Mol Genet 2:43–50

Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE et al (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116:127–137

Ohlsson R, Hall K, Ritzen M (1995) Parental Imprinting: causes and consequences. Cambridge University, Cambridge

Palmatier MA, Kang AM, Kidd KK (1999) Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. Biol Psychiatry 46:557–567

Price SJ, Greaves DR, Watkins H (2001) Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 276:7549–7558

Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I et al (2002) A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 71:1296–1302

Spielman RS, Weinshilboum RM (1981) Genetics of red cell COMT activity: analysis of thermal stability and family data. Am J Med Genet 10:279–290

Stephens M, Donnelly P (2003) A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73:1162–1169

Tenhunen J, Salminen M, Lundström K, Tiviluoto T, Savolainen R, Ulmanen I (1994) Genomic organization of the human catechol-O-methyltransferase gene and its expression from two distinct promoters. Eur J Biochem 223:1049–1059

Uejima H, Lee MP, Cui H, Feinberg AP (2000) Hot-stop PCR: a simple and general assay for linear quantitation of allele ratios. Nat Genet 25:375–376

Venter CJ, Adams MD, Myers EW, Li PW, Mrual RJ, Sutton GG et al (2001) The sequence of the human genome. Science 291:1304–1351

Weinshilboum RM, Raymond FA (1977) Inheritance of low erythrocyte catechol-O-methyltransferase activity in man. Am J Hum Genet 29:216–218

Xu K, Lipsky RH, Mangal W, Ferro E, Goldman D (2002) Single-nucleotide polymorphism allele frequencies determined by quantitative kinetic assay of pooled DNA. Clin Chem 48:1605–1608

Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW (2002) Allelic variation in human gene expression. Science 297:1143

Zhu G, Lipsky RH, Xu K, Ali S, Hyde H, Kleinman J, Akhtar LK et al (2002) RT-coupled 5′ nuclease assay—a general and high throughput method for detection of gene differential allele expression using transcribed SNPs as endogenous reporters. Am J Hum Genet 71[Suppl 4]:401

Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D (2003) COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 299:1240–1243