



Catechol-*O*-methyltransferase (COMT) genotypes are associated with varying soluble, but not membrane-bound COMT protein in the human prefrontal cortex

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Received: 12 June 2018 / Revised: 22 August 2018 / Accepted: 23 August 2018 / Published online: 14 September 2018
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Abstract

Catechol-*O*-methyltransferase (COMT) is an enzyme that catalyses the *O*-methylation, and thereby the inactivation, of catechol-containing molecules. In humans, it has been suggested that COMT modulates cognitive ability, possibly by regulating degradation of dopamine in the prefrontal cortex. Hence, it is significant that two *COMT* SNPs, rs4680 (c.472 G > A, p.Val158Met) and rs4818 (c.408 C > G), have been associated with cognitive ability in humans. We have shown these SNPs to be associated with levels of muscarinic M1 receptor mRNA in human cortex, which is significant as that receptor also regulates cognitive ability. We decided to determine if *COMT* genotype was associated with varying levels of COMT protein, as this could be a mechanism by which *COMT* genotype could be associated with changes in muscarinic M1 receptor mRNA levels. Hence, we measured COMT levels in prefrontal cortex obtained postmortem from 199 subjects, some of whom had a history of schizophrenia, major depressive disorders or bipolar disorders. Our data show, independent of diagnostic status, that genotype at rs4680 and rs4818, but not at rs737865 and rs165599, is associated with differing levels of soluble COMT (S-COMT), but not membrane-bound COMT (MB-COMT). These findings suggest that the association between *COMT* polymorphisms and cognitive functioning could be, at least in part, due to their association with varying levels of S-COMT. This is important as, unlike MB-COMT, the substrates targeted by S-COMT are likely to be intra-cellular rather than, like dopamine, located mainly in the synaptic vesicles or the extra-cellular space.

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Electronic supplementary material The online version of this article (<https://doi.org/10.1038/s10038-018-0511-2>) contains supplementary material, which is available to authorised users.

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Introduction

Catechol-*O*-methyltransferase (COMT) is an enzyme that catalyses the *O*-methylation, and thereby the inactivation, of catechol-containing molecules [1]. In humans, genetic studies suggest that certain *COMT* genotypes are associated with varying cognitive ability [2]. Cognitive impairment is associated with several CNS disorders and is becoming an increasing problem in the aging population, which has led to a strong interest in the role of COMT (MIM:116790) in cognitive function. The most prominent hypothesis as to how COMT can modulate cognitive function is linked to its ability to degrade dopamine, given that a loss in dopaminergic functioning is strongly associated with cognitive decline [3]. This hypothesis also has a genetic underpinning as a common functional polymorphism on the *COMT* gene at SNP rs4680, where the G allele codes for the amino acid valine while the A allele codes for methionine (Val158Met), has been associated with decreased cognitive ability [4, 5] and an altered risk for disorders linked to cognitive deficits such as schizophrenia (MIM:181500) [4, 6].

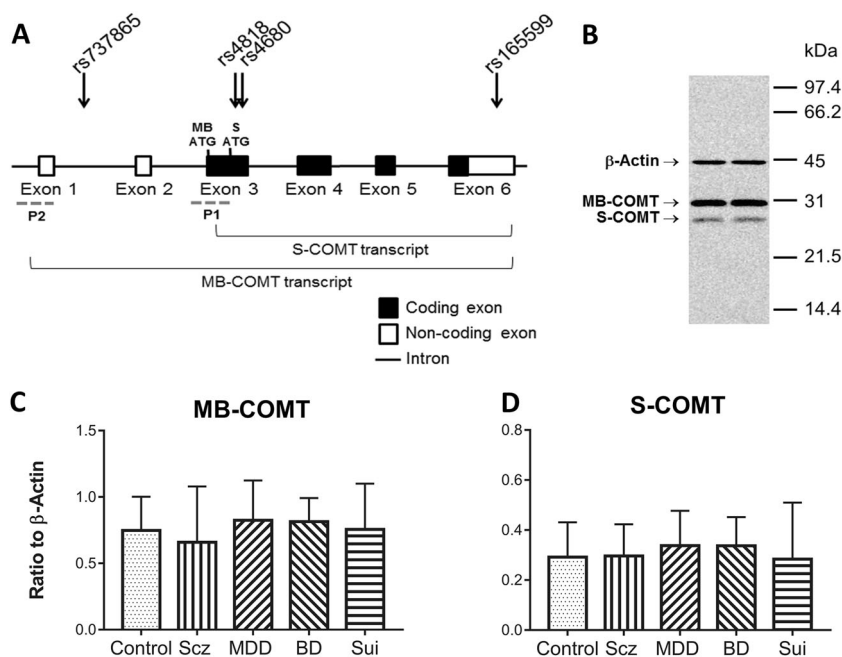


Fig. 1 COMT expression in human brain. **a** Depiction of *COMT* exon and intron arrangement (not to scale), showing relative positions of the P1 and P2 promoter sites, start codons, exon usage of soluble COMT (S-COMT) and membrane-bound COMT (MB-COMT), and locations of the four SNPs genotyped. **b** Twenty microgram human prefrontal cortex homogenate run in duplicate on a 15% SDS-PAGE and probed with antibodies against COMT and β -Actin, detecting bands

corresponding to MB-COMT at 30 kDa, S-COMT at 26 kDa and β -Actin at 43 kDa. MB-COMT (**c**) and S-COMT (**d**) protein levels measured in prefrontal cortex tissue from subjects with schizophrenia (Scz), major depressive disorders (MDD), bipolar disorders (BD), subjects who died of suicide with no history of psychiatric illness (Sui) and controls. Data are represented as median \pm interquartile range

There are two known forms of COMT, a soluble COMT (S-COMT), which is found in the cell cytoplasm and a membrane-bound COMT (MB-COMT), which contains an additional 50 amino acids and is anchored in the membrane of the endoplasmic reticulum and other membranes of mammalian cells [7, 8]. In the CNS, MB-COMT is located on cell bodies, axons and dendrites of neurons [9], where it can have a role in the breakdown of the catecholamines, dopamine and noradrenaline. The 30 kDa MB-COMT displays higher substrate affinity, but lower catalytic activity than 26 kDa S-COMT [10] and is the predominant isoform in the brain [11, 12], whereas S-COMT is the predominant form in other tissues such as liver, adrenal, kidney and duodenum [11, 12]. In most tissues, the two isoforms appear to arise from two transcripts of the same gene, facilitated by the presence of two different promoters, P1 and P2 (Fig. 1a) [11]; however, only the longer transcript, which is able to translate both isoforms [13], has been readily observed in human brain [11, 12, 14].

The functionality of the *COMT* rs4680 genotype arises from the Val variant having increased stability, which contributes to it being present at higher levels in human prefrontal cortex and having higher enzyme activity compared with the Met variant [10, 15, 16]. A synonymous SNP

that sits in a haploblock with rs4680, rs4818, impacts the enzyme activity and cognitive ability, more so than rs4680 (refs. 17, 18). Interestingly, findings in schizophrenia suggest that two non-coding SNPs, rs737865 and rs165599 (ref. 6), are associated with altered cognitive performance [19]. While it is suggested that these SNPs may have this effect by modulating allelic expression in the brain [20], this hypothesis is challenged by data showing that genotypes at these SNPs is not associated with levels of COMT mRNA, protein or enzyme activity [15].

We previously showed that *COMT* genotype at the two SNPs most implicated in cognition (rs4680 and rs4818), but not the two non-coding SNPs rs737865 and rs165599, were associated with varying levels of the cholinergic muscarinic receptor 1 (CHRM1) in the human prefrontal cortex [21]. Notably, cortical CHRM1 is also known to have a role in maintaining cognitive function [22], and therefore our data was the first to suggest a link between levels of CHRM1 expression, COMT and cognition. In this study, to further examine the mechanisms linking *COMT* genotype to cognition, we determined whether *COMT* genotype was associated with levels of cortical MB- and S-COMT in postmortem tissue from 199 subjects, some of whom had been diagnosed with schizophrenia, major depressive disorders or bipolar disorders before death.

Materials and methods

CNS collection

Tissue used in this study was provided by the Victorian Brain Bank Network. Approval to collect tissue was obtained from the Ethics Committee of the Victorian Institute of Forensic Medicine. Tissue was only collected from donors after either a witnessed death or having been seen alive within 5 h of being found dead. All cadavers were refrigerated soon after discovery to slow the effects of autolysis [23]. The left hemisphere from each donor was then processed in a standardised manner, where the tissue was frozen to -80°C within 30 min of autopsy [24]. Just prior to freezing, a sample of the CNS tissue was collected to measure the pH of the tissue as an indicator of the quality of tissue preservation [25], as previously described [26]. For each case, relevant data from clinical histories and interviews with treating clinicians and relatives were obtained using a standardised instrument, the Diagnostic Instrument for Brain Studies [27]. Cases were assigned as controls where it was agreed that the donor had no history of psychiatric illness, or to a diagnostic group when a diagnosis was made by consensus between two senior psychiatrists and a psychologist according to the DSM-IV criteria [28]. Postmortem interval (PMI) was calculated as either the time from witnessed death to autopsy or the time mid-way between a subject being last seen alive and being found dead until autopsy, and duration of illness (DI) was calculated as the time from first presentation with psychiatric symptoms to death.

Genotyping had previously been performed as described [21]. This study was completed using Brodmann's area (BA) 9 from subjects with schizophrenia, major depressive disorders, bipolar disorders, controls and suicide completers with no identifiable history of psychiatric illness.

Western blotting

Researchers were kept blinded to diagnoses and genotypes during experimentation. Approximately 100 mg of tissue was excised from Brodmann Area (BA) 9 (lateral surface of the frontal lobe, including the middle frontal gyrus superior to the inferior frontal sulcus). The tissue was cut along the full cortical depth with visual guidance by an experienced technician to ensure sampling of all cortical laminae and the exclusion of white matter. Homogenates were prepared at 10% w/v in 10 mM Tris, (pH 7.4) containing 1% w/v sodium dodecyl sulphate and 1 mM sodium orthovanadate (Sigma-Aldrich, St. Louis, MO, USA). Total protein concentrations were measured using a DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) before storing at -80°C .

For each gel, five samples chosen at random were diluted to the desired concentration of $2\ \mu\text{g}/\mu\text{l}$, and the

concentrations were re-measured prior to dilution of the samples with an equal volume of $2\times$ sample buffer (0.5 M Tris-HCl pH 6.8, containing 20% glycerol, 4% SDS, 10% 2- β -mercaptoethanol and 0.05% bromophenol blue), the samples were then denatured for 5 min at 95°C and $20\ \mu\text{g}$ total protein was loaded in duplicate onto a 15% SDS-PAGE resolving gel. Twenty micrograms of protein homogenate was determined to fall within the linear protein concentration range for all proteins analysed (data not shown). Proteins were separated at 150 V, then transferred onto an Amersham Hybond-ECL Nitrocellulose membrane (GE Health Life Sciences, Chicago, IL, USA) overnight at 40 mA. Equal loading and efficient transfer of proteins was confirmed by staining with 0.2% Ponceau S Red in 3% trichloroacetic acid before blocking and probing with 1:5000 anti-COMT antibody (Ab5873, Merck, Kenilworth, NJ, USA) and 1:200,000 anti-Actin antibody (MAB1501, Merck), followed by 1:2000 goat anti-rabbit IgG:HRP-conjugated antibody and 1:5000 goat anti-mouse IgG:HRP-conjugated antibody (Dako Agilent, Santa Clara, CA, USA) for 1 h each in the presence of Tween Tris buffered saline (100 mM Tris pH 7.5, 0.9% sodium chloride and 0.1% Tween 20) containing 5% blotting grade non-fat milk. Bands were detected with ECLTM (Thermo Fisher Scientific, Waltham, MA, USA) imaged at 45 s chemiluminescence exposure on a UVP Biospectrum[®] Imaging System (Analytik Jena, Jena, Germany), and sum pixel intensities for MB-COMT (30 kDa), S-COMT (26 kDa) and β -Actin (43 kDa) were measured using the VisionWorks[®] Image Acquisition Analysis Software (Fig. 1b and Supplementary Figure 1).

Specificity of the COMT and β -Actin antibodies had been determined through probing of subcellular fractions (Supplementary Fig. 2A and 2B, respectively). MB-COMT and S-COMT protein levels were expressed as normalised to β -Actin, as β -Actin did not differ with *COMT* genotype at rs4680 ($F_{2,195} = 0.91$, $p = 0.40$), rs4818 ($F_{2,195} = 0.62$, $p = 0.54$), rs737865 ($F_{2,195} = 1.72$, $p = 0.18$) or rs165599 ($F_{2,189} = 0.49$, $p = 0.61$) or with diagnoses ($F_{4,198} = 0.30$, $p = 0.88$) and was therefore an appropriate loading control.

Statistical analysis

Genetics

Hardy–Weinberg equilibrium tests were performed on genotype counts using the OEGE online calculator [29]. Linkage disequilibrium was tested using Haploview [30].

Demographic and CNS collection

All continuous data were tested for normality of distribution using the Kolmogorov–Smirnov normality test (see Supplementary Table 1 and Supplementary Table 2). Potential

Table 1 Summary of demographic data

Demographic	All subjects (<i>n</i> = 199)	Diagnostic group				
		Control (<i>n</i> = 72)	Scz (<i>n</i> = 77)	MDD (<i>n</i> = 25)	BD (<i>n</i> = 17)	Sui (<i>n</i> = 8)
Age (years; Mdn, IQR)	48.0, 34.0–63.0	47.5, 25.0–64.5	47.0, 30.0–63.0	51.0, 36.0–65.5	58.0, 39.5–65.0	48.0, 28.8–53.5
Brain pH (Mdn, IQR)	6.38, 6.19–6.46	6.38, 6.22–6.46	6.27, 6.13–6.42	6.49, 6.38–6.60	6.41, 6.18–6.45	6.49, 6.35–6.58
PMI (Mdn, IQR)	42.3, 32.0–50.0	42.0, 32.4–50.4	43.3, 37.1–50.0	45.0, 28.3–57.1	36.0, 25.5–45.8	31.8, 20.4–47.8
DI (years; Mdn, IQR)	14.5, 6.8–25.5	N/A	12.5, 6.0–30.0	16.0, 6.0–20.2	14.5, 8.0–32.3	N/A
Sex (F/M)	58/141	13/59	25/52	13/12	7/10	0/8
Suicide status (Y/N)	72/127	0/72	34/43	22/3	8/9	8/0
Ancestry (CEU/CHB/ CEU + CHB)	188 / 9 / 2	67/5/0	72/3/2	26/0/0	17/0/0	7/1/0

BD, bipolar disorders; CEU, northern and western European ancestry; CHB, Han Chinese ancestry; DI, duration of illness; F, female; IQR, interquartile range; M, male; MDD, major depressive disorders; Mdn, median; N, no; Y, yes; PMI, postmortem interval; Scz, schizophrenia; Sui, suicide with no history of psychiatric illness

variation in demographic and CNS collection data from different diagnoses cohorts was hence identified using the Kruskal–Wallis test. Chi-squared or Fisher's exact tests were used to test for independence of frequency of non-continuous variables.

COMT protein analyses

Acknowledging that psychiatric diagnoses was a potential confound, variance in the levels of S-COMT and MB-COMT protein with genotype was analysed using Quade's non-parametric one-way rank analysis of covariance [31], with COMT protein levels and *COMT* genotype as the primary measures and diagnoses as a covariant. The potential source of any increase in variance was identified with post hoc analyses using Tukey's test, with correction for multiple comparisons. Spearman's correlation, where a correlation coefficient between 0.71 and 1.0 or -0.71 and -1.0 (>50% variance explained) was taken to indicate a strong correlation, values between 0.31 and 0.70 or -0.31 and -0.70 were considered a moderate correlation, and values between 0 and 0.30 or -0.30 were considered a weak or negligible correlation [32, 33], was used to identify any strong relationships between demographic or CNS collection data and S-COMT or MB-COMT.

Tests were performed using GraphPad Prism (GraphPad Software, Inc, La Jolla, CA, USA) or SPSS (IBM, Armonk, NY, USA).

Results

Demographic data

S- and MB-COMT data was available from 199 subjects and a summary of the demographic characteristics of this cohort is given in Table 1. Within this cohort, MB-COMT

and S-COMT protein levels showed no strong correlations with age, brain pH, PMI or DI, and no variance with sex, suicide status or ancestry (Supplementary Table 3).

When separated into diagnostic groups (Supplementary Table 2), there were no significant differences in age ($H_4 = 2.52$, $p = 0.64$) or PMI ($H_4 = 2.99$, $p = 0.20$) between the groups. Brain pH varied significantly with diagnosis ($H_4 = 20.9$, $p < 0.001$; Table 1), with brain pH higher in subjects with MDD compared with controls ($p = 0.03$) and subjects with schizophrenia ($p < 0.001$). However, as there were no strong correlations between brain pH and COMT protein levels (Supplementary Table 3), pH was not considered a potential confound. There was a significant difference in sex ratios ($\chi^2_4 = 15.5$, $p = 0.004$) and number of subjects who died of suicide ($\chi^2_4 = 87.0$, $p < 0.001$; Table 1). However, COMT levels did not differ between males and females or between suicide completers and death by other causes (Supplementary Table 3), and thus neither of these variables were considered a confound.

Genotyping of COMT SNPs

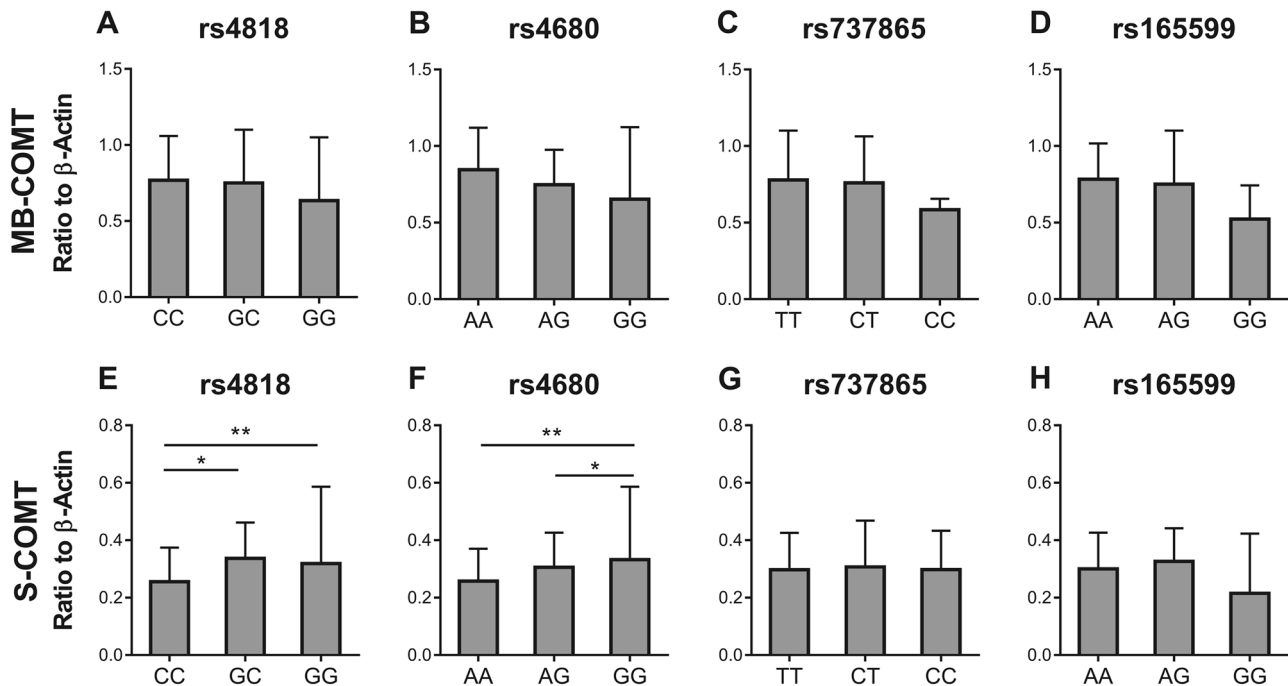
Genotyping failed in one subject at rs4818, one subject at rs4680 and rs737865, and seven subjects at rs165599. Remaining genotype data showed Hardy–Weinberg equilibrium at all four SNPs (rs4680: $\chi^2 = 0.99$, $p = 0.37$; rs4818: $\chi^2 = 2.86$, $p = 0.11$; rs737865: $\chi^2 = 0.11$, $p = 0.92$ and rs165599: $\chi^2 = 3.70$, $p = 0.08$; Table 2). Rs4680 showed high linkage disequilibrium with rs4818 ($D' = 1.0$, $r^2 = 0.66$), but not with rs737865 ($D' = 0.57$, $r^2 = 0.12$) or rs165599 ($D' = 0.20$, $r^2 = 0.02$).

COMT protein levels with genotype

With diagnosis as a covariate, MB-COMT levels showed no variance with genotype at any of the four SNPs (rs4680:

Table 2 Observed genotype counts at four SNPs

rs4680		rs4818		rs737865		rs165599	
Genotype	n (%)	Genotype	n (%)	Genotype	n (%)	Genotype	n (%)
AA	54 (27)	CC	79 (40)	TT	106 (53)	AA	76 (39)
AG	92 (46)	CG	83 (42)	CT	79 (40)	GA	99 (50)
GG	52 (26)	GG	36 (18)	CC	13 (7)	GG	17 (9)

**Fig. 2** Cortical COMT levels with *COMT* genotype. Levels (median \pm interquartile range) of membrane-bound COMT (MB-COMT; **a–d**) and soluble COMT (S-COMT; **e–h**) in human prefrontal cortex with rs4818, rs4680, rs737865 and rs165599 genotype (* $p < 0.05$, ** $p < 0.01$)

$F_{2,195} = 0.85$, $p = 0.43$; rs4818: $F_{2,195} = 0.21$, $p = 0.81$; rs737865: $F_{2,195} = 0.81$, $p = 0.45$; rs165599: $F_{2,189} = 2.11$, $p = 0.12$) (Fig. 2a–d). By contrast, S-COMT levels show variance with rs4680 ($F_{2,195} = 6.10$, $p = 0.003$) (Fig. 2e) and rs4818 ($F_{2,195} = 5.69$, $p = 0.004$) (Fig. 2f) genotype. S-COMT did not show variance with rs737865 ($F_{2,195} = 0.56$, $p = 0.57$) (Fig. 2g) or rs165599 ($F_{2,189} = 1.34$, $p = 0.26$) (Fig. 2h) genotype. Post hoc analyses showed that at least some of the variance at rs4818 was due to lower S-COMT protein in subjects who were homozygous CC genotype (median \pm IQR: 0.26, 0.14–0.37) compared with those who were heterozygous GC (median \pm IQR: 0.34, 0.20–0.46, $p = 0.049$), and homozygous GG (median \pm IQR: 0.32, 0.02–0.59 $p = 0.005$; Fig. 2e). Post hoc analyses similarly revealed that variance at rs4680 was due to lower levels in subjects who were homozygous AA (median \pm IQR: 0.26, 0.14–0.37) compared with those who were homozygous GG (median \pm IQR: 0.33, IQR 0.22–0.59, $p = 0.002$), and those who were heterozygous AG (Mdn 0.31, IQR 0.18–0.43) compared to those who were homozygous GG (median \pm IQR: 0.33, 0.22–0.59, $p = 0.03$; Fig. 2f).

COMT protein levels with diagnoses

MB-COMT and S-COMT levels did not vary with diagnoses (MB-COMT: $H_4 = 0.83$, $p = 0.96$; S-COMT: $H_4 = 0.89$, $p = 0.93$; Fig. 1c, d).

Discussion

We show that *COMT* genotypes at SNPs rs4680 and rs4818, but not rs737865 or rs165599, were associated with altered levels of S-COMT, but not MB-COMT, in the human dorsolateral prefrontal cortex (DLPFC). As the levels of COMT protein have been shown to be directly related with COMT enzyme activity [15, 34], our data would suggest that *COMT* genotype is also associated with increased S-COMT activity in the human DLPFC. Furthermore, given genotypes at rs4680 and rs4818 have a greater association with cognition than rs737865 and rs165599; our findings suggest that S-COMT plays a more important role in cognitive function than MB-COMT.

Our findings of altered S-COMT, but not MB-COMT, levels with genotype at rs4680 are consistent with a previous study [15]; however our additional findings at rs4818 reveal a stronger association between *COMT* genotypes and S-COMT than previously reported. Furthermore, our data on rs4680 genotype and S-COMT levels differs slightly from the earlier study, which also found significantly lower levels of S-COMT between the Met/Met polymorphism (AA) and the Val/Met (AG) [15], whereas we found a significant difference between Val/Met (AG) and Val/Val (GG). There could be a number of explanations for this disparity. For example, the previous study did not specify which brain hemisphere and which Brodmann's area was used, hence our data could be reflecting differences due to hemispheric or regional selection if genotype is not the sole controller of levels of cortical COMT. Furthermore, the previous study consisted of a high proportion of African-American subjects, whereas our cohort had mostly Caucasian subjects, such variation in race could be a confound between the two studies.

While our study was significantly underpowered for a genetic study, it is of note that we saw no evidence for an association between any genotype and a changed risk of a psychiatric disorder (data not shown). Therefore, our study is more in line with those that did not find an altered risk of schizophrenia with *COMT* genotype [35–37].

The function of S-COMT and MB-COMT is to eliminate biologically active catechols and some hydroxylated metabolites from cells [1]. Hence S-COMT and MB-COMT have many substrates that include L-dopa, dopamine, noradrenaline, adrenaline (plus their hydroxylated metabolites), catecholestrogens, ascorbic acid, dihydroxyindolic intermediates of melanin, triphenols, substituted catechols, dobutamine, isoprenaline, rimiterol, α -methyl-dopa, benserazide, carbidopa, dihydroxyphenyl serine, flavonoids and dihydroxy derivatives of tetrahydroisoquinolones [1, 38]. Changes in S-COMT and MB-COMT levels and activity would therefore have a major impact on CNS function. Significantly, S-COMT and MB-COMT are derived from the same gene and their catalytic sites are therefore identical; this means any difference in function is due to their cellular localisation. It is therefore significant that S-COMT is localised to the cell cytosol [1], whereas MB-COMT, due to its hydrophobic N terminus, is localised to the membranes of the cell [7, 9, 39]. Importantly, given that S-COMT is in the cell cytoplasm, while dopamine, noradrenaline and adrenaline are predominantly located either in the extra-cellular space or in the synaptic vesicles, it is difficult to postulate how S-COMT could have a major effect on the levels of those catecholamines as it would not readily be able to access these molecules to increase their degradation. Moreover, as MB-COMT is the predominant isoform in the human brain with an affinity for dopamine up to 100-fold that of S-COMT [10], MB-COMT is more likely to play a role in dopamine degradation.

The hypothesis that the association between *COMT* genotype and altered cognition is due to COMT's ability to degrade dopamine [40] is mainly supported by increased dopamine levels in the prefrontal cortex of *COMT* knockout mice [41]. However, *COMT* heterozygous knockout mice displayed impaired recognition memory, whereas homozygous deletion improved spatial memory [42]. These data suggest that the impact of the absence of COMT protein and activity, as would occur after gene knockout, differ from that when COMT levels are modulated, as occurs in the *COMT* heterozygous mouse [41]. This is noteworthy because modulating levels of COMT in the mouse is more analogous to what is observed in human CNS with regards to *COMT* genotype impacting on COMT levels.

We have shown that *COMT* genotype is associated with S-COMT, but not MB-COMT, protein levels, and therefore it is imperative that the two isoforms be considered individually. Given the possible intracellular separation of S-COMT and dopamine, our data, which shows an association between *COMT* genotype and levels of S-COMT protein, argues that the link between *COMT* genotype and cognition is not necessarily due to S-COMT directly regulating the dopamine levels by altering the rates of degradation of the neurotransmitter. Rather, increased S-COMT activity could act to increase the degradation of L-DOPA, which is present in the cytosol, to cause a decrease in dopamine synthesis, which would likely cause the relative hypodopaminergic state thought to be associated with poorer cognitive functioning [4]. However, this outcome clearly does not readily fit with the association between *COMT* genotype and cognition, perhaps suggesting that another biological mechanism might be the link to this association.

Given the number of substrates in the cytosol that would be available for S-COMT, there would be other mechanisms by which *COMT* genotype could be associated with cognitive ability. For example, catecholestrogens are a substrate for COMT and are agonists at the oestrogen receptor [43]; increased levels of S-COMT could therefore act to induce a relatively hypoestrogenic state by lowering the oestrogen receptor occupancy by degrading catecholestrogens. With regards to our data on the association between *COMT* genotype and CHRM1 expression [21], levels of CHRM1 have been shown to be higher in ovariectomised rats and to return to expected levels after treatment with 17 β -estradiol [44]. It is therefore possible that an S-COMT-mediated hypoestrogenic state would cause increased levels of CHRM1 in human CNS. Significantly, when an agonist binds to an oestrogen receptor, the receptor–agonist complex moves to the nucleus where it can regulate gene expression [45]. Hence it could be possible that the association between *COMT* genotype and levels of CHRM1 (ref. 21) is due to S-COMT acting to change oestrogen-mediated gene expression. This hypothesis has credence because our studies on S-COMT and CHRM1

expression by *COMT* genotype have used Brodmann's area 9 from the same cohort of subjects, the CHRM1 is known to be important in cognition [22, 46], and because of evidence showing that the association between *COMT* genotype and cognition in humans is only detectable in males and in females after menopause [47], individuals in which oestrogen levels are low. However, it must be acknowledged that oestrogen-mediated changes in gene expression are not limited to CHRM1 and there are many more substrates for S-COMT, and therefore we cannot exclude other mechanisms as also being important in the link between *COMT* genotype and cognition.

In summary, the findings that the *COMT* genotypes associated with cognition have stronger effects on S-COMT than MB-COMT protein levels in the human prefrontal cortex suggests that the association is linked to the levels and activity of S-COMT. This proposal challenges the primary link between *COMT* genotype and cognition being due to the effects on dopamine degradation, as S-COMT should not be able to readily access the neurotransmitter, which is mainly in synaptic vesicles or the extracellular space. Our data suggests that the association between *COMT* genotype and cognition involves increased degradation of S-COMT substrates in the cell cytosol. One such substrate is the catecholestrogens; changes in the levels of this substrate could affect oestrogen receptor-mediated changes in gene expression. In particular, we suggest a role for catecholestrogen–oestrogen receptor-mediated effects on cholinergic neurotransmission through the CHRM1 as a mechanism by which *COMT* genotype can be associated with cognitive ability.

Acknowledgements The authors would like to thank Mr. Geoff Pavey for careful dissection of the tissue that was used in this study. This project was supported by the National Health and Medical Research Council (NHMRC; Australia; project grant 566967), the Cooperative Research Centre (CRC) for Mental Health and the Victorian Government's Operational Infrastructure Support Programme. BD is a recipient of NHMRC Fellowship APP1002240, ES was supported by the Australian Research Council (Future Fellowship FT100100689) and GMP is an awardee of an Australian Government Research Training Program Scholarship. The Victorian Brain Bank Network is supported by The Florey Institute of Neuroscience and Mental Health and the Alfred and the Victorian Forensic Institute of Medicine, and funded in part by Parkinson's Victoria and MND Victoria and Mental Health.

Funding This project was supported by the National Health and Medical Research Council (NHMRC; Australia; project grant 566967), the Cooperative Research Centre (CRC) for Mental Health and the Victorian Government's Operational Infrastructure Support Programme. GMP is an awardee of an Australian Government Research Training Program Scholarship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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