Associations between catechol O-methyltransferase genotypes and gene expression in human dorsolateral prefrontal cortex.

Running Title: Catechol O-methyltransferase and gene expression

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Abstract

Having reported associations between catechol O-methyltransferase (*COMT*) genotypes at SNPs rs4818 and rs4680 with levels of soluble COMT (S-COMT) in human dorsolateral prefrontal cortex (DLPFC), we postulated changes in levels of cortical S-COMT could impact on behavioural abilities associated with *COMT* genotype through S-COMT mediated changes in gene expression. To test this hypothesis, we have examined the relationships between *COMT* genotypes and gene expression measured using the Affymetrix™ Human Exon 1.0 ST Array in the DLPFC from 141 individuals, some of whom had had a psychiatric disorder. There were no significant associations between *COMT* genotype and gene expression when heterozygotes at rs4818 and rs4680 were include in our analyses. There were differences in levels of mRNA for 3 genes (*CEP128, EFCAB13* and *FAM133A*) between those who were homozygotic at rs4818 and rs4680; individuals with most significant differences in cortical S-COMT. In addition, levels of mRNA for 13 genes only differed between homozygotes at rs4818 whilst levels of mRNA for 3 genes only differed between homozygotes at rs4680. Our data argues that the association between *COMT* genotype and human cortical functioning may, in part, be related to differences in cortical gene expression with *COMT* genotype.

Introduction

Catechol O-methyltransferase (COMT) catalyzes the transfer of the methyl group from the coenzyme S'-adenosyl-L-methionine to one of the hydroxyl groups of catechols or substituted catechols in a magnesium requiring reaction [1], a critical step in the metabolism of such structures [2]. Thus, whilst COMT is a ubiquitous enzyme [3], having the catecholamines as substrates and having a potential role in inactivating these molecules [2] has made COMT a protein of interest in the area of neuroscience. In considering the ability of COMT to access substrates, it is important that COMT protein exists in two forms, one being soluble (S-COMT) and the other being membrane bound (MB-COMT) [4]. This may be particularly pertinent to the role of COMT in catecholamine degradation as MB-COMT has been shown to have a higher affinity for dopamine than S-COMT and has therefore been proposed to be the form of COMT most involved in the degradation of that important neurotransmitter [3]. By contrast, S-COMT in the cytosol probably cannot target dopamine and the other catecholamines which are predominantly in synaptic vesicles or in the extracellular space [5]. Moreover, it has been reported that neither S- nor MB-COMT are present in dopaminergic or noradrenergic neurons [6] which means neither form of COMT may make a significant contribution to the degradation of catecholamines in the CNS.

In addition to the potential impact of COMT on catecholamine metabolism, it is significant that the *COMT* gene is on chromosome 22q11, an area of the human genome where a 1-2 mega base microdeletion results in velocardiofacial syndrome (VCFS) [7]. Given that 30% of individuals with VCFS develop a serious psychiatric disorder, it was postulated that mutations of genes within that region of the human genome could be associated with an altered risk of developing psychiatric disorders. This made the discovery of a G to A

polymorphism (rs4680) at codon 158 of the *COMT* gene [7] of particular significance to disorders thought to involve aberrations of dopaminergic activity, such as schizophrenia [8], as this change in nucleotide sequence results in a change in amino acid sequence from a valine (Val: G) to a methionine (Met: A) [7]. This change in amino acid sequence appears to result in a change in COMT activity, with the Met substitution associated with lower COMT activity [2]. The change in COMT activity that could occur with genotypes at rs4680, with a subsequent change in catecholamine degradation, was postulated to have a wider significance as dopamine is known to be important in non-disease related CNS functions such as cognition [9]. It was therefore significant that individuals homozygous for the rs4680 Met allele produced significantly fewer perseverative errors when performing the Wisconsin Card Sorting Test compared to those homozygous for the Val allele [10], suggesting an association between COMT activity, dopamine levels and cognition.

Following the demonstration that rs4680 genotype was associated with some aspects of cognitive ability, it became apparent that aspects of cognitive ability were linked to multiple variations in transcribed *COMT* sequence (e.g. SNPs rs737865 and rs165599) as well as variation in the sequence of the *COMT* P2 promoter (SNP rs2097603: -278A>G) [11], which is located upstream of both MB-COMT and S-COMT translation initiation codons [12]. Another study reports that different genotypes at the SNP rs4818 (C>G) are associated with varying ability to complete cognitive tasks, such as the Stockings of Cambridge and the Iowa Gambling Task [13]. This increasing understanding of the diversity of *COMT* genotypes has still not significantly impacted on the dominant view that the association between *COMT* genotype and cognition is via modulation of dopaminergic activity in the CNS [14].

We have recently reported an association between genotype at SNPs rs4680 and rs4818, but not rs737865 or rs165599, with levels of S-COMT, but not MB-COMT, in human dorsolateral prefrontal cortex (DLPFC) [12]. This finding is significant both because of the localisation of S-COMT to the cytosol [4], where it is unlikely to be in contact with significant amounts of the catecholamines, and its absence from bouton of the neurons that synthesise catecholamines [6]. Our data on the association between *COMT* genotype and levels of S-COMT in the DLPFC led us to postulate that the ability of COMT to selectively degrade catechol structures that can regulate gene expression may be a mechanism by which the enzyme could affect cognitive ability. To further challenge this hypothesis, we have now investigated whether *COMT* genotype at rs4818 and rs4680, which are associated with varying levels of cortical S-COMT, are associated with variations in levels of gene expression in the human DLPFC.

Methods

Human CNS collection

Tissue collection was approved by the Ethics Committee of the Victorian Institute of Forensic Medicine. CNS tissue from 87 individuals with a prior psychiatric diagnosis and 54 individuals with no psychiatric history (Table 1) was collected postmortem after either a witnessed death or having been seen alive within 5 hours of being found dead. All cadavers were refrigerated soon after discovery to slow the effects of autolysis [15]. After CNS collection at autopsy, the left hemisphere from each donor was processed in a standardized manner, where tissue was frozen to -80˚C within 30 min of autopsy [16]. CNS pH [17] was measured for each individual as an indicator of the quality of tissue preservation. For each individual relevant data from clinical histories and interviews with treating clinicians and relatives was obtained using a standardised instrument, the Diagnostic Instrument for Brain Studies [18]. Controls were individuals where it was agreed the donor had no history of psychiatric illness. For those with a psychiatric diagnosis, diagnoses was made according to DSM-IV criteria by consensus between two senior psychiatrists and a psychologist [19]. Postmortem interval (PMI) was calculated as either the time from witnessed death to autopsy or the time mid-way between an individual 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.

For this study, tissue was dissected from Brodmann's area (BA) 9 on the lateral surface of the frontal lobe from an area that includes the middle frontal gyrus superior to the inferior frontal sulcus [20].

COMT genotyping was performed as described previously [21].

RNA Preparation

RNA preparation and expression array processing was performed as described previously [22]. Hence, total RNA was isolated from approximately 100 mg of frozen gray matter using 1.0 ml TRIzol® reagent (Life Technologies, Australia) and, following homogenization and phase separation as per the manufacturer's instructions, the aqueous phase was added to an equal volume of 70% ethanol. RNA was then isolated using RNeasy mini kits (Qiagen, Cat 74104) with samples being treated with DNase using column digestion with the absence of DNA contamination being confirmed using PCR and primers specific for the detection of the presence of genomic DNA. RNA quantity and quality were analysed by spectrophotometry (NanoDrop; ThermoFisher Scientific) and by obtaining RNA integrity numbers (RINs) using an Agilent 2100 bioanalyser (Agilent Technologies, CA, USA). All samples used for the microarray study had RINs of > 7.00 (Range 7.30 to 9.20) as this is a good predictor of well-preserved RNA [23] which is required for acceptable microarray hybridization.

Microarray Processing

The Affymetrix™ Human Exon 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA) were processed either at the Australian Genome Research Facility or the Ramaciotti Centre for

Genomics at the University of New South Wales using the protocol stipulated by the array manufacturer. Thus, ribosomal RNA was eliminated and random priming used to generate cRNA that was end labelled with biotin using the Affymetrix synthesis and labelling kit. Samples that passed the quality checkpoints were prepared for hybridisation using a standard probe cocktail. Each sample was loaded onto an Affymetrix™ Human Exon 1.0 ST Array and hybridised overnight. Following post-hybridization washes, the chips were scanned and the fluorescent signals converted into a DAT file. After visual confirmation of the scans and quality control analysis, these files were used to generate subsequent cell intensity (CEL) and chip (CHP) files for analysis. Data files are held by the CRC for Mental Health and may be made available to bona fide researchers upon request.

Microarray Data Analyses

All CEL files were imported into JMP Genomics 5.1 (SAS, Cary, NS, USA) at the gene level, collapsing the exon level data onto known transcripts. To control for array to array variation, the data was normalized using the Robust Multichip Average (RMA) algorithm. The data was then log2 transformed and normalized for between group comparisons using the "Least Square means" method [24]. All QCs from each array were assessed using a distribution analysis to identify alterations in pattern expression followed by a one-way ANOVA to quantify any variation. A Correlation and Principal Variance Component Analysis was also run on the QC data, including Mahalanobis Distances analysis to identify any outliers. Using data that passed QC, JMP genomics was used to compare data across *COMT* genotypes using an ANOVA. In addition, to reflect the differential relationship between *COMT* genotype and S-COMT [12], unrestricted pairwise comparisons were allowed between levels of RNA in DLPFC of individuals who were homozygotes at SNPs

associated with higher levels of S-COMT versus those with lower levels of S-COMT and results were expressed as a ratio of these relationships (rs4818: GG vs CC; rs4680: GG vs AA).

In this study, the numbers of individuals with certain genotypes were relatively low and therefore false discovery rates were not used to avoid too many true positives being "called" as false positive [25, 26]. Rather, to further reduce the likelihood of the inclusion of false positive results, we followed the recognised standard practice of using a non-stringent group-wise p-value cut off of <0.01 with a fold change of ± Δ 0.2 [27] as the final parameters used to filter genes with changed levels of expression. Using this approach, and two different Affymetrix arrays, we have previously shown that changes in gene expression identified using expression microarrays and postmortem CNS from individuals with schizophrenia and age/sex matched controls are readily validated using quantitative polymerase chain reaction both within [22, 28-31] and outside [29-31] of the cortical region in which expression array experiments were carried out and could be shown to have some diagnostic specificity [29, 30]. The data from these two studies suggests the rate of "false" positive results is < 10% of the total genes identified as being differentially expressed.

Statistics

For the analyses of non-array data, one-way ANOVA was used to detected variation in continuous demographic and CNS collection data with genotype. Chi-squared tests were used to detect variation in frequency in non-continuous variables with genotype and genotype with diagnoses. All analyses were completed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Demographic and CNS collection

RNA of a quality suitable for studies using the Affymetrix™ Human Exon 1.0 ST Arrays was prepared from Brodmann's area 9 from 141 individuals (Table 1); there was neither variation in age, CNS pH or PMI nor frequency of gender or suicide with genotype at rs4818 or rs4680.

COMT Genotype and Diagnoses

Our data did not suggest that any genotype at rs4818 ($\chi^2 = 6.004$, d.f. = 8, p = 0.64) or rs4680 ($\chi^2 = 5.277$, d.f. = 8, p = 0.73) was associated with an altered risk for having a psychiatric disorder. However, the number of individuals within each diagnostic group (schizophrenia, bipolar disorders, major depressive disorders) make our study too underpowered to make these findings of any meaningful value.

COMT Genotype and Gene Expression

For both rs4818 and rs4680, there was no variation in the levels of mRNA for any gene with genotype when heterozygotes were included in the analyses. However, pairwise comparisons between gene expression at rs4818: GG vs CC showed significant differences in the levels of mRNA for 15 genes in the DLPFC (Table 2). Levels of mRNA for only 1 of these genes,

EFCAB13, was higher in the DLPFC from individuals with the rs4818: GG genotype. For rs4680, levels of mRNA for 6 genes differed in the DLPFC when comparing individuals carrying GG compared to AA, with 4 genes being expressed at higher levels in the individuals with GG. Significantly, levels of expression of 3 genes (*CEP128*, *EFCAB13* and *FAM133A*) varied with genotype at both rs4818 and rs4680.

An IPA Core analysis of genes differentially expressed between rs4818: GG vs CC showed that 9 of 15 (60%: *ADGRL4, CEP128, DAXX, KRT82, MGAT4A, MX2, RTF1, THSD4* and *ZNF226*) are in pathways involved in cell death and survival, cellular assembly and organization as well as DNA replication, recombination, and repair. Whilst the number of genes changed between subjects homozygote at rs4840 were too few for a full IPA Core analysis, a partial analysis suggest changed levels of expression of 4 of 6 genes between the homozygotes at rs4680 (75%: *CEP128, DNAH6, GNAT3* and *WASHC5*) could affect pathways involved in behaviour, cardiovascular system development and function as well as cell morphology.

Discussion

The hypothesis that was tested in our study was that *COMT* genotypes at rs4818 and rs4680, which are associated with increased levels of cortical S-COMT [12], would be associated with changes in gene expression modulated by varying levels of cortical S-COMT. Supporting this hypothesis, we have now shown that there are significant differences in the levels of mRNA in the DLPFC from rs4818 and rs4680 homozygotes. Such changes in gene expression could be the outcome of a number of mechanisms which includes the impact of the Val versus Met allele on COMT activity [2], the impact of changed levels of S-COMT [12] and the impact of rs4818 on COMT mRNA stability [13]. In addition, it would seem possible that the changes in expression of these genes may be contributing to changes in ability to perform tasks such as Stockings of Cambridge and the Iowa Gambling Task [13], as these are also associated with the same *COMT* genotypes.

It is notable that the expression of 3 genes (centrosomal protein 128 (*CEP128*), EF-hand calcium binding domain 13 (*EFCAB13*) and family with sequence similarity 133, member A (*FAM133A*)) were differentially expressed when comparing homozygotes at rs4818 or rs4680 (Figure 1). Importantly, as a ratio of higher S-COMT versus low S-COMT related genotype (GG vs CC and GG vs AA), the direction of change in levels of expression is the same for rs4818 and rs4680, supportive of these changes being caused by the same mechanism of action, which could be that of S-COMT. The functions of these genes are not well characterised in human cortex but CEP128 is a centrosome protein that regulates ciliation and has roles in cell cycle progression [32]. However, for this study it is of interest that CEP128 coordinates cellular signaling pathways in neurons and hence could be ultimately involved in behavioural regulation (Figure 1). *EFCAB13* encodes a protein with a calcium binding

domain but, beyond this structural definition, little is currently known about the function of this gene. Finally, whilst FAM133A is recognised to be selectively expressed in CNS [33] its functional role remains to be elucidated. Based on our new data on COMT genotypes and their association with changed gene expression and levels of S-COMT, we postulate that some changes in gene expression associated with *COMT* genotype will be due to S-COMT and that one impact of S-COMT-mediated changes in gene expression is on neuronal cell signalling (Figure 1).

On demonstrating that levels of S-COMT varied with genotype at rs4818 and rs4680, we argued the very limited access of S-COMT to dopamine lessoned the likelihood that the ability of COMT to modulate cognitive ability was due solely to its ability to metabolise dopamine [12]. Rather, we postulated S-COMT could affect gene expression through oestrogen mediated mechanisms because COMT metabolises catecholestrogens, which are estrogen receptor agonists [34]. It is therefore significant that there is evidence to show levels of expression of *CEP128, DAXX, DNAH6 , FAM133A, GNAT3, KRT82, MGAT4A, MX2, SLC26A7, SLC38A4, THSD4* and *UNKL* is under oestrogen regulation [35] (Figure 1: bolded and italicised). These data support the argument of COMT acting on gene expression through oestrogen related mechanisms, however they do not necessitate that the oestrogen regulation of these genes is mediated solely through S-COMT. This hypothesis may explain why the associations between *COMT* genotype and the ability to perform specific cognitive tasks are more apparent in males, and females after menopause [36]. In these individuals, the low levels of estrogen allow the changes in catecholestrogens, which are metabolised by COMT [2], to become apparent.

The IPA Core analysis of genes with varying expression associated with varying homozygosity at rs4818 showed there was a greater than expected representation of genes involved in cell death and survival, cellular assembly and organization as well as DNA replication, recombination, and repair. Whilst the link between these functions may not readily be associated with variation in the ability of an individual to complete a cognitive task, what might be of relevance is that some of these genes are involved in synaptic plasticity (*ADGRL4* [37], *DAXX* [38] and *MGAT4A* [39]) or attentional behaviour (*MX2* [40] and *THSD4* [41]) (Figure 1). It is also notable that the expression of all these genes is subject to regulation by oestrogen.

We have previously reported an association between genotype at rs4818 and rs4680 and levels of expression of the muscarinic M1 receptor in the DLPFC [21] which we did not replicate in our microarray data (rs4818: ratio GG vs CC = 1.015, p = 0.18; rs4680: ratio GG vs AA = 1.010, p = 0.16). There are several factors that could have contributed to such different outcomes. First, whilst the earlier study was completed using a similar number of individuals (n = 145) the individuals in that study were either controls or individuals with schizophrenia, whereas this study included individuals with schizophrenia, bipolar disorders and major depressive disorders. However, as in neither study did *COMT* genotype vary with diagnoses it is difficult to postulate how the number of psychiatric disorders in the two studies would be such a confounding variable. Notably, in our earlier study levels of expression of the muscarinic M1 receptor was measured using *in situ* hybridisation. Therefore, the difference in outcomes of the two studies could be because two different methods of measuring levels of RNA were used and our single gene study did not have such a strong impact from multiple measures. Given these methodological concerns it would not be overly surprising if studies using different approaches to measuring gene expression

discovered the expression of more genes varying with *COMT* genotype than so far detected. Given the variation in outcome of associating *COMT* genotype with gene expression it would also be of value to carry out a similar study examining the cortical proteome.

In conclusion, our study of the associations between *COMT* genotype and gene expression add weight to the argument that variation in S-COMT with genotype may be acting through oestrogen-regulated gene expression. In addition, our data raise the possibility that the changes in gene expression associated with *COMT* genotypes at rs4818 may impact on cortical synaptogenesis and attention; *COMT* genotype associated changes in synaptogenesis could certainly be a mechanism that could impact on cortical ability, which has been shown to be associated with cortical synaptic homeostasis [42]. Our data would therefore suggest that a better understanding of how different forms of COMT impact on gene expression may eventually lead to a better understanding as to why *COMT* genotype is a predictor of cognitive ability.

Conflicts of Interest

The authors have no conflict of interest.

References

1. Axelrod, J. & Tomchick, R. Enzymatic O-methylation of epinephrine and other catechols. *J. Biol. Chem.* **233**, 702-705 (1958).
2. Chen, J., Song, J., Yuan, P., Tian, Q., Ji, Y., Ren-Patterson, R. et al. Orientation and cellular distribution of membrane-bound catechol-O-methyltransferase in cortical neurons: implications for drug development. *J. Biol. Chem.* **286**, 34752-34760 (2011).
3. Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I. et al. 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 (1995).
4. Assicot, M. & Bohuon, C. Presence of two distinct catechol -O- methyltransferase activities in red blood cells. *Biochimie.* **53**, 871-874 (1971).
5. Rao, F., Friese, R., Wen, G., Zhang, L., Chen, Y., Das, M. et al. in *Comprehensive Hypertension* (eds. Lip, G.Y.H. & Hall, J.E.), Ch. 72, 895-911, (Mosby, Philadelphia 2007).
6. Schendzielorz, N., Oinas, J.-P., Myöhänen, T.T., Reenilä, I., Raasmaja, A. & Männistö, P.T. Catechol-O-Methyltransferase (COMT) Protein Expression and Activity after Dopaminergic and Noradrenergic Lesions of the Rat Brain. *PLoS One.* **8**, e61392 (2013).
7. Lachman, H.M., Papolos, D.F., Saito, T., Yu, Y.M., Szumlanski, C.L. & Weinshilboum, R.M. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics.* **6**, 243-250 (1996).
8. Carlsson, A. Does dopamine play a role in schizophrenia? *Psychol. Med.* **7**, 583-597 (1977).

9. Dickinson, D. & Elvevåg, B. Genes, Cognition and Brain through a COMT Lens. *Neurosci.* **164**, 72-87 (2009).

10. Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E. et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 6917-6922 (2001).

11. Funke, B., Malhotra, A.K., Finn, C.T., Plocik, A.M., Lake, S.L., Lencz, T. et al. COMT genetic variation confers risk for psychotic and affective disorders: a case control study. *Behavioral and Brain Functions.* **1**, 19 (2005).

12. Parkin, G.M., Udawela, M., Gibbons, A., Scarr, E. & Dean, B. Catechol-O-methyltransferase (COMT) genotypes are associated with varying soluble, but not membrane-bound COMT protein in the human prefrontal cortex. *J. Hum. Genet.* (2018).

13. Roussos, P., Giakoumaki, S.G., Pavlakis, S. & Bitsios, P. Planning, decision-making and the COMT rs4818 polymorphism in healthy males. *Neuropsychologia.* **46**, 757-763 (2008).

14. Schacht, J.P. COMT val158met moderation of dopaminergic drug effects on cognitive function: a critical review. *The Pharmacogenomics Journal.* **16**, 430 (2016).

15. Ferrer, I., Santpere, G., Arzberger, T., Bell, J., Blanco, R., Boluda, S. et al. Brain protein preservation largely depends on the postmortem storage temperature: implications for study of proteins in human neurologic diseases and management of brain banks: a BrainNet Europe Study. *J.Neuropathol.Exp.Neurol.* **66**, 35-46 (2007).

16. Dean, B., Pavey, G., Chai, S.Y. & Mendelsohn, F.A.O. in *Using CNS Tissue in Psychiatric Research: A Practical Guide* (eds. Dean, B., Kleinman, J.E. & Hyde, T.M.), 67-83, (Harwood Academic, Amsterdam 1999).

17. Kingsbury, A.E., Foster, O.J., Nisbet, A.P., Cairns, N., Bray, L., Eve, D.J. et al. Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res.Mol.Brain Res.* **28**, 311-318 (1995).

18. Hill, C., Keks, N., Roberts, S., Opeskin, K., Dean, B., MacKinnon, A. et al. Problem of diagnosis in postmortem brain studies of schizophrenia. *Am J Psychiatry.* **153**, 533-537 (1996).

19. Roberts, S.B., Hill, C.A., Dean, B., Keks, N.A., Opeskin, K. & Copolov, D.L. Confirmation of the diagnosis of schizophrenia after death using DSM-IV: a Victorian experience. *Aust N Z J Psychiatry.* **32**, 73-76 (1998).

20. Garey, L.J. *Bordmann's 'Localisation in the cerebral cortex'*. (Smith-Gordon: London, 1994).

21. Dean, B. & Scarr, E. COMT genotype is associated with differential expression of muscarinic M1 receptors in human cortex. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **171**, 784-789 (2016).

22. Scarr, E., Udawela, M., Thomas, E.A. & Dean, B. Changed gene expression in subjects with schizophrenia and low cortical muscarinic M1 receptors predicts disrupted upstream pathways interacting with that receptor. *Mol. Psychiatr.* **23**, 295-303 (2018).

23. Stan, A.D., Ghose, S., Gao, X.M., Roberts, R.C., Lewis-Amezcua, K., Hatanpaa, K.J. et al. Human postmortem tissue: what quality markers matter? *Brain Res.* **1123**, 1-11 (2006).

24. Kirst, M., Myburg, A.A., De León, J.P.G., Kirst, M.E., Scott, J. & Sederoff, R. Coordinated Genetic Regulation of Growth and Lignin Revealed by Quantitative Trait Locus Analysis of cDNA Microarray Data in an Interspecific Backcross of Eucalyptus. *Plant Physiol.* **135**, 2368-2378 (2004).

25. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statisitcal Society. Series B.* **57**, 289-300 (1995).

26. Pawitan, Y., Michiels, S., Koscielny, S., Gusnanto, A. & Ploner, A. False discovery rate, sensitivity and sample size for microarray studies. *Bioinformatics.* **21**, 3017-3024 (2005).

27. Maqc Consortium & Shi, L. & Reid, L.H. & Jones, W.D. & Shippy, R. & Warrington, J.A. et al. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat. Biotechnol.* **24**, 1151-1161 (2006).

28. Narayan, S., Tang, B., Head, S.R., Gilmartin, T.J., Sutcliffe, J.G., Dean, B. et al. Molecular profiles of schizophrenia in the CNS at different stages of illness. *Brain Res.* **1239**, 235-248 (2008).

29. Udawela, M., Money, T.T., Neo, J., Seo, M.S., Scarr, E., Dean, B. et al. SELENBP1 expression in the prefrontal cortex of subjects with schizophrenia. *Transl Psychiatry.* **5**, e615 (2015).

30. Scarr, E., Udawela, M., Greenough, M.A., Neo, J., Seo, M.S., Money, T.T. et al. Altered expression of the zinc transporter SLC39A12 suggests a breakdown in zinc cortical homeostasis as part of the pathophysiology of schizophrenia. *NPJ Schizophrenia.* **2** (2016).

31. Udawela, M., Scarr, E., Boer, S., Um, J.Y., Hannan, A.J., McOmish, C. et al. Isoform specific differences in phospholipase C beta 1 expression in the prefrontal cortex in schizophrenia and suicide. *NPJ Schizophr.* **3**, 19 (2017).

32. Wang, T.-M., Shen, G.-P., Chen, M.-Y., Zhang, J.-B., Sun, Y., He, J. et al. Genome-Wide Association Study of Susceptibility Loci for Radiation-Induced Brain Injury. *JNCI: Journal of the National Cancer Institute.* djy150-djy150 (2018).

33. Condomines, M., Hose, D., Reme, T., de Vos, J., Requirand, G., Rossi, J.-F. et al. Gene Expression Profiling and Real-Time PCR Analyses Make It Possible To Identify Novel Potential Cancer-Testis Antigens in Multiple Myeloma. *Blood.* **110**, 1793-1793 (2007).

34. Schutze, N., Vollmer, G. & Knuppen, R. Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. *J. Steroid Biochem. Mol. Biol.* **48**, 453-461 (1994).

35. Hah, N., Danko, C.G., Core, L., Waterfall, J.J., Siepel, A., Lis, J.T. et al. A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells. *Cell.* **145**, 622-634 (2011).

36. Papaleo, F., Sannino, S., Piras, F. & Spalletta, G. Sex-dichotomous effects of functional COMT genetic variations on cognitive functions disappear after menopause in both health and schizophrenia. *Eur. Neuropsychopharmacol.* **25**, 2349-2363 (2015).

37. Duman, J.G., Tu, Y.K. & Tolias, K.F. Emerging Roles of BAI Adhesion-GPCRs in Synapse Development and Plasticity. *Neural Plast.* **2016**, 8301737 (2016).

38. Salomoni, P. The PML-Interacting Protein DAXX: Histone Loading Gets into the Picture. *Front. Oncol.* **3**, 152 (2013).

39. Kippe, J.M., Mueller, T.M., Haroutunian, V. & Meador-Woodruff, J.H. Abnormal N-Acetylglucosaminyltransferase Expression in Prefrontal Cortex in Schizophrenia. *Schizophr. Res.* **166**, 219-224 (2015).

40. dela Peña, I., Bang, M., Lee, J., de la Peña, J.B., Kim, B.-N., Han, D.H. et al. Common prefrontal cortical gene expression profiles between adolescent SHR/NCrl and WKY/NCrl rats which showed inattention behavior. *Behav. Brain Res.* **291**, 268-276 (2015).

41. Wang, K.-S., Liu, X., Zhang, Q., Aragam, N. & Pan, Y. Parent-of-origin effects of FAS and PDLIM1 in attention-deficit/hyperactivity disorder. *Journal of Psychiatry & Neuroscience : JPN.* **37**, 46-52 (2012).

42. Henstridge, C.M., Sideris, D.I., Carroll, E., Rotariu, S., Salomonsson, S., Tzioras, M. et al. Synapse loss in the prefrontal cortex is associated with cognitive decline in amyotrophic lateral sclerosis. *Acta Neuropathol.* **135**, 213-226 (2018).

Figure Legends:

Figure 1: A schematic showing associations between differences in levels of gene expression in the human dorsolateral prefrontal cortex (DLPFC) between individuals who are homozygotes at the SNPs rs4818 or rs4680 in the catechol O-methyltransferase (*COMT*) gene. It is proposed that changes common to genotypes at both SNPs may be modulated through differences in levels of soluble COMT (S-COMT) which differs with genotype at those SNPs. There is evidence to suggest that at least one gene could link *COMT* genotype to neuronal cell signalling through S-COMT. In addition, changes in cortical gene expression between individuals who are homozygote at rs4818 include genes involved in synaptic plasticity and attention. These genes could link *COMT* genotype to behaviour through non-S-COMT mechanisms. Expression of genes bolded and underlined have been shown to be regulated by oestrogen and therefore the association between *COMT* genotype and gene expression in the DFPLC may be subject to oestrogen-related mechanisms.

Gene	Ratio GG/CC
DAXX	↓
ADGRL4	↓
KRT82	↓
MGAT4A	↓
MX2	↓
RTF1	↓
SLC26A7	↓
SLC38A4	↓
THSD4	↓
TSG1	↓
UNKL	↓
ZNF226	↓

SYNAPTIC PLASTICITY

ATTENTION

Gene	Ratio GG/AA
DNAH6	↑
GNAT3	↑
WASHC5	↑

rs4818
rs4680

COMT gene

Exon 1 Exon 2 Exon 3 Exon 4 Exon 5 Exon 6

S-transcript

S-COMT protein
rs4818 & rs4680

rs4818 Ratio CC/GG	Gene	rs4680 Ratio GG/AA
↓	***CEP128***	↓
↑	*EFCAB13*	↑
↓	***FAM133A***	↓

NEURONAL CELL SIGNALING

Table 1: A summary of demographic and CNS collection data relating to the case from whom Brodmann's area 9 was obtained for this study.

	Genotype	n	Gender (m / f)	Age (yr)	pH	PMI (hr)	Brain Weight (gms)	Suicide (Y / N)	Psychiatric Diagnoses (Y / N)
Total Population		141	104 / 37	49 ± 1.4	6.34 ± 0.22	43 ± 1.2	1390 ± 14	48 / 93	87 / 54
rs4818	CC	55	47 / 10	47 ± 2.3	6.39 ± 0.19	41 ± 2.0	1400 ± 22	21 / 36	32 / 23
	GC	59	39 / 20	50 ± 2.2	6.33 ± 0.03	43 ± 1.7	1382 ± 21	20 / 39	23 / 36
	GG	25	18 / 7	48 ± 3.0	6.29 ± 0.05	46 ± 3.4	1385 ± 40	7 / 18	17 / 8
	χ^2 of F		4.055	0.323	1.997	0.91	0.18	0.606	5.959
	d.f.		2	2,138	2,137	2,138	2,122	2	2
	p		0.13	0.72	0.14	0.4	0.83	0.73	0.05
rs4680	GG	34	25 / 9	46 ± 2.7	6.30 ± 0.04	44 ± 2.6	1375 ± 31	11 / 23	23 / 11
	GA	68	47 / 21	50 ± 2.1	6.33 ± 0.03	44 ± 1.8	1402 ± 20	18 / 50	36 / 32
	AA	39	31 / 8	49 ± 2.8	6.41 ± 0.03	39 ± 2.2	1380 ± 27	18 / 21	28 / 11
	χ^2 of F		1.359	0.69	2.52	1.422	0.358	4.34	4.4
	d.f.		2	2,138	2,136	2,138	2,122	2	2
	p		0.51	0.5	0.08	0.24	0.7	0.11	0.11

Abbreviations: f = female, hr = hour, m = male, N = no, PMI = postmortem interval, Y = yes, yr = year,

Table 2: Differences in levels of mRNA expressed as a ratio of GG vs CC for rs4818 and GG vs AA for rs4680 in the human DLPFC.

SNP	Gene Name	Symbol	Chromosome	Ratio	p
rs4818	centrosomal protein 128	*CEP128*	14q31.1	0.730	0.0062
	EF-hand calcium binding domain 13	*EFCAB13*	17q21.32	1.205	0.0067
	death-domain associated protein	*DAXX*	6p21.3	0.779	0.0064
	adhesion G protein-coupled receptor L4	*ADGRL4*	1p33-p32	0.787	0.0024
	family with sequence similarity 133, member A	*FAM133A*	Xq21.32	0.775	0.0015
	keratin 82	*KRT82*	12q13	0.762	0.0011
	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A	*MGAT4A*	2q12	0.755	0.0002
	MX dynamin like GTPase 2	*MX2*	21q22.3	0.757	0.0038
	Rtf1, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)	*RTF1*	15q15.1	0.719	0.0096
	solute carrier family 26, member 7	*SLC26A7*	8q23	0.780	0.0092
	solute carrier family 38, member 4	*SLC38A4*	12q13	0.762	0.0015
	thrombospondin, type I, domain containing 4	*THSD4*	15q23	0.777	0.0043
	tumor suppressor TSG1	*TSG1*	6q16.1	0.780	0.0014
	unkempt family like zinc finger	*UNKL*	16p13.3	0.762	0.0044
	zinc finger protein 226	*ZNF226*	19q13.2	0.790	0.0094
rs4680	centrosomal protein 128	*CEP128*	14q31.1	0.790	0.0053
	EF-hand calcium binding domain 13	*EFCAB13*	17q21.32	1.315	0.0006
	dynein, axonemal, heavy chain 6	*DNAH6*	2p11.2	1.223	0.0002
	family with sequence similarity 133, member A	*FAM133A*	Xq21.32	0.795	0.0001
	G protein subunit alpha transducin 3	*GNAT3*	7q21.11	1.289	< 0.0001
	WASH complex subunit 5	*WASHC5*	8q24.13	1.210	0.0054

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