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COMT val158met polymorphism and molecular alterations in the human dorsolateral prefrontal cortex: differences in controls and in schizophrenia

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Abstract

The single nucleotide val158met polymorphism in catechol o-methyltransferase (COMT) influences prefrontal cortex function. Working memory, dependent on the dorsolateral prefrontal cortex (DLPFC), has been repeatedly shown to be influenced by this COMT polymorphism. The high activity COMT val isoform is associated with lower synaptic dopamine levels. Altered synaptic dopamine levels are expected to lead to molecular adaptations within the synapse and within DLPFC neural circuitry. In this human post mortem study using high quality DLPFC tissue, we first examined the influence of the COMT val158met polymorphism on markers of dopamine neurotransmission, N-methyl-d-aspartate (NMDA) receptor subunits and glutamatic acid decarboxylase 67 (GAD67), all known to be critical to DLPFC circuitry and function. Next, we compared target gene expression profiles in a cohort of control and schizophrenia cases, each characterized by COMT genotype. We find that the COMT val allele in control subjects is associated with significant upregulation of GluN2A and GAD67 mRNA levels compared to met carriers. Comparisons between control and schizophrenia groups reveal that GluN2A, GAD67 and DRD2 are differentially regulated between diagnostic groups in a genotype specific manner. Chronic antipsychotic treatment in rodents did not explain these differences. These data demonstrate an association between COMTval158met genotype on the molecular profile of DLPFC neural circuitry in the control brain, possibly adaptations to maintain DLPFC function. In

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schizophrenia val homozygotes, these adaptations are not seen and could reflect pathophysiologic mechanisms related to the known poorer performance of these subjects on DLPFC-dependent tasks.

Keywords

dopamine; glutamate; GABA; human; post mortem brain; DLPFC

1. Introduction

Cognitive functions such as executive function and working memory (Sawaguchi and Goldman-Rakic, 1994; Williams and Goldman-Rakic, 1995) are mediated by the prefrontal cortex (PFC) and are critically dependent on dopaminergic neurotransmission. Synaptic dopamine (DA) levels are determined by reuptake by the dopamine transporter (DAT) and/or by neurotransmitter breakdown by COMT. Due to low DAT expression in the PFC (Lewis et al., 2001), COMT may be an important determinant of PFC DA levels. In COMT knockout mice, DA levels are increased by 60% in the PFC, but not striatum, suggesting a particularly important role for COMT in the PFC (Kaenmaki et al., 2010). A functional single nucleotide polymorphism of G → A transition in codon 158 of the human COMT gene results in decreased enzyme activity (Chen et al., 2004; Lachman et al., 1996) by coding for the amino acid methionine (met) instead of valine (val) i.e. the val allele encodes a more active isoform than the met allele (Chen et al., 2004). The val158met polymorphism (hereafter referred to as COMT val/met) has been extensively studied with evidence that this polymorphism influences DLPFC activity (Ceaser et al., 2013; Egan et al., 2001; Eisenberg et al., 2010; Pomarol-Clotet et al., 2010; Takizawa et al., 2009) and executive function, particularly working memory (Barnett et al., 2007; Tsuchimine et al., 2013). COMT inhibition improves cognitive performance on specific tasks in rats (Tunbridge et al., 2004) and humans (Apud and Weinberger, 2007; Farrell et al., 2012). Together, these findings support the notion that COMT val/met can modulate DA neurotransmission in the PFC.

Besides a role in mediating normal cognitive processes, DA neurotransmission in PFC is implicated in the pathophysiology of schizophrenia (Knable and Weinberger, 1997). Individuals with schizophrenia perform poorly on working memory tasks and exhibit altered DLPFC activity while performing such tasks (Callicott et al., 2003; MacDonald et al., 2005; Silver et al., 2003). Given the influence of COMT val/met on DA flux in the PFC, numerous studies have examined the effects of this polymorphism on the cognitive test performance in schizophrenia. A systematic review of 53 studies finds that working memory is one of two neuropsychological tasks strongly associated with COMT val/met polymorphism in individuals with schizophrenia, with Val allele homozygotes demonstrating poorer performance on these tasks (Ira et al., 2013).

DA afferents target both GABA fast-spiking interneurons and glutamatergic pyramidal neurons in the PFC (Goldman-Rakic et al., 1989; Sesack et al., 1998). DA innervation of fast-spiking GABA neurons could influence working memory by virtue of the role of fast spiking interneurons in synchronization of pyramidal neuron activity, the coordinated

activity of which is critical for working memory (Lewis and Gonzalez-Burgos, 2008; Lewis et al., 2005; Lewis and Moghaddam, 2006). Thus, interneurons and pyramidal neurons provide cellular substrates through which DA neurotransmission, and therefore the COMT val/met polymorphism, could influence working memory in schizophrenia. Working memory impairment in schizophrenia have been attributed to reduced glutamate neurotransmission NMDA receptors, reduced γ -aminobutyric acid (GABA) transmission through GABA receptors and reduced DA transmission through DRD1 receptors (Lewis and Gonzalez-Burgos, 2006). The influence of COMT val/met on molecular targets in DLPFC has not been extensively examined.

In this human post mortem study, we determined the association between COMT val/met on and expression of markers of dopamine neurotransmission, N-methyl-D-aspartate (NMDA) receptor subunits and glutamatic acid decarboxylase 67 (GAD67) expression in the control human DLPFC and compared the influence of COMT val/met on these neurochemical markers in DLPFC tissue from a cohort of control and schizophrenia cases. Since schizophrenia val/val homozygotes exhibit greatest working memory deficits, we hypothesized that this group would display a distinct gene expression profile that could shed light on the molecular underpinnings of compromised prefrontal function.

2. Materials and Methods

2.1 Human postmortem tissue

Human brain tissue was obtained from the Dallas Brain Collection (Stan et al., 2006). The collection of human brain tissue was approved by the Institutional Review Board of University of Texas Southwestern Medical Center. Briefly, 1.5 cm coronal slabs through the hemisected cerebrum of each human brain were cut and DLPFC tissue (Brodmann area 9) was excised from the middle third of the superior frontal gyrus (Rajkowska and Goldman-Rakic, 1995). A DLPFC block was rapidly frozen in a prechilled dry ice-isopentane slurry bath and stored at -80°C . Frozen tissue ($\sim 100\text{mg}$) was used for RNA extraction. Given that schizophrenia COMT val homozygotes exhibit poorer performance on tests of executive cognition compared to met carriers (val/met and met/met) (Bertolino et al., 2004; Egan et al., 2001) and that met/met homozygotes are the least frequently observed genotype, we designed this study to compare COMT val homozygotes to met carriers (val/met and met/met). DLPFC samples from 53 cases (Table 1) included Val homozygotes (control [Con] $n=13$, schizophrenia [Scz] $n=11$) and met carriers (Con $n=14$, Scz $n=15$). Genomic DNA was used to genotype each case for the COMT val/met polymorphism (details in supplement).

2.2. Chronic Antipsychotic Treatment in Rodents

To determine potential effects of chronic antipsychotic treatment on our genes of interest in the schizophrenia group, Sprague-Dawley rats (Charles River, Wilmington, Mass.) were given a first-generation antipsychotic (haloperidol [$N=13$]), second-generation antipsychotic (risperidone [$N=13$]), or placebo ($N=13$) via drinking water continuously for 6 months as previously described (Ghose et al., 2009).

2.3 Quantitative PCR

Primers were designed for markers of COMT, D1, D2 and D5 (DA related genes), GluN1, GluN2A and GluN2B (NMDA receptor subunits) and GAD 67. qPCR was performed using ABI step one plus real time PCR system and Sybr green PCR mix (Applied Biosystems, CA) (details in supplement).

2.4 Statistical Analysis

Based on genotype and diagnosis, there were four groups – control subjects with val/val genotype (Con Val), control subjects with met/met or val/met genotype (Con Met), schizophrenia subjects with val/val genotype (Scz Val) and schizophrenia subjects with met/met or val/met genotype (Scz Met). Due to skewed distribution of data, non-parametric statistical analyses were used. Ct values for individual mRNA species were correlated with age, PMI or RIN using Spearman Rank Order correlation. For each mRNA species, the results are summarized as median and interquartile range (25th to 75th percentile) and two-way analysis of variance (ANOVA) on ranks (Conover, 1999) used to evaluate the overall effect of genotype and diagnosis and the interaction between genotype and diagnosis. The data were transformed for analysis in order to meet normality homogeneity of variance assumptions. Separate analyses were performed for each gene of interest with Bonferroni-Hochberg correction for the six possible multiple comparisons of the four groups within each analysis. The effects of antipsychotic treatment (vehicle, risperidone) in rodents were analyzed using two tailed t tests for independent samples. SAS 9.2 software was used for statistical analyses.

3. Results

3.1. Gene expression

There were no significant correlations between age, post mortem interval (PMI) or RNA integrity number (RIN) and Ct values of individual mRNA species (all r between -0.30 to 0.34 , all $p > 0.06$).

3.1.1. Dopamine markers—Significant diagnosis x genotype interactions were found for DRD2 (ANOVA $F=6.29$, $df_{1,40}$, $p=0.02$; Fig 1, Supplement Table 1) Post hoc analyses show significant lower expression of DRD2 mRNA in the SczMet group compared to ConMet ($F=11.28$, $df_{1,40}$, $p=0.002$) and compared to SczVal groups ($F=5.13$, $df_{1,40}$, $p=0.03$; Supplement Table 2). We did not observe diagnosis x genotype interactions for any of the other DA markers.

3.1.2. Glutamate markers—Of the three NMDA receptor subunits examined, we found significant diagnosis x genotype interactions for GluN2A ($F=6.95$, $df_{1,47}$, $p=0.01$; Fig 1, Supplement Table 1). Post hoc analyses show that ConMet has significantly lower GluN2A compared to ConVal ($F=6.3$, $df_{1,47}$, $p<0.05$; Supplement Table 2) and the SczVal group have significantly lower GluN2A compared to the ConVal group ($F=6.64$, $df_{1,47}$, $p<0.05$). For GluN2B, we found a significant diagnosis x genotype interactions ($F=3.91$, $df_{1,47}$, $p=0.05$). Post hoc analysis comparing GluN2B in the SczVal group to the ConVal group was not statistically significant ($F=4.92$, $df_{1,47}$, $p=0.13$).

3.1.3. GABA markers—For GAD67 mRNA expression, there was a diagnosis x genotype interaction ($F=4.3$, $df=1,47$, $p=0.04$); Fig 1, Supplement Table 1). Post hoc analyses show that ConMet has significantly lower GAD67 mRNA levels compared to ConVal ($F=4.02$, $df=1,47$, $p<0.05$, Supplement Table 2). The SczVal group expresses significantly lower GAD67 mRNA expression compared to the ConVal group ($F=4.48$ $df=1,47$, $p=0.04$).

3.2. Effects of Chronic Antipsychotic Treatment on DRD2, GluN2A and GAD67 expression

Schizophrenia cases in our cohort were exposed to 2nd generation antipsychotic medications during their lifetimes. To determine if the changes in DRD2, GluN2A and GAD67 could be explained by antipsychotic treatment, we administered a 2nd generation antipsychotic medication, risperidone, to rats continuously for 6 months, using doses that produce plasma levels in the human therapeutic range, as previously described (Ghose et al., 2009). Tissue from the frontal cortex was taken from the treated rats and analyzed for DRD2, GluN2A and GAD 67 RNA expression. There was no effect of antipsychotic treatment on DRD2 ($t=1.2$, $df=1,25$, $p=0.24$), GluN2A ($t=0.13$, $df=1,25$, $p=0.9$) or GAD67 mRNA levels ($t=0.16$, $df=1,25$, $p=0.87$) in the frontal cortex relative to comparison tissue (Fig 2).

4. Discussion

4.1. Gene expression changes associated with COMT val/met in the control brain

Comparisons between homozygous val cases with met carriers show that the former have an increase in GluN2A and GAD67 mRNA levels. There is evidence demonstrating that GluN2A and GAD67 are functionally linked with GluN2A involved in maintenance of PV and GAD67 immunoreactivity (Kinney et al., 2006) (Xi et al., 2009) Chronic systemic injection of an antagonist to the GluN2A subunit (NVP-AAM077) impairs expression of PV and the maturation of fast-spiking interneurons in the mouse cortex (Zhang and Sun, 2011), suggesting a role for GluN2A in the regulation of GABAergic inhibitory circuits. It is possible that the increase in GluN2A and GAD67 observed in Con Val groups could be attempts of cellular molecular pathways and systems to maintain homeostasis (Fig 3, circleB). Upregulation of GluN2A at the postsynaptic DA-GABA synapse could be an attempt to maintain GABA synthesis in PV interneurons and their subsequent role in oscillatory synchronization of pyramidal cells (Sohal et al., 2009).

Higher GluN2A in Con Val individuals can also influence glutamatergic synapses other than those at PV interneurons. Fig 3 shows sites at which higher GluN2A could have potential influence on DLPFC circuitry. (e.g. glutamate afferents to pyramidal neurons). Afferent DA terminals, arising from the mesencephalon, form asymmetric synapses on dendritic spines of pyramidal neurons that are closely apposed to glutamatergic terminals (Smiley and Goldman-Rakic, 1993)(fig 3, circle A). DRD1 receptors are highly expressed on these pyramidal dendrites(Bergson et al., 1995; Fremeau et al., 1991; Glausier et al., 2009; Muly et al., 1998; Paspalas and Goldman-Rakic, 2005). Complex interactions exist between DA and glutamate systems (reviewed in (Wang et al., 2012)). Coordinated neural signaling through the dopamine DRD1 and NMDA glutamate receptor interactions occur via cell signaling pathways (e.g. PKA and DARPP32) or through direct physical interactions(Floresco et al., 2001; Scott et al., 2002). For example, DRD1 receptor C-

terminal tail binds to the GluN1 and GluN2A subunits to modulate NMDA receptor currents (Lee et al., 2002). The high GluN2A in Con Val could be a mechanism by which coordinated signaling in pyramidal neurons is maintained.

4.2. Gene expression changes associated with COMT val/met in schizophrenia

We find that GluN2A and GAD67 expression, both influenced by the COMT val/met polymorphism in Controls, is significantly altered in schizophrenia. The increase in GluN2A and GAD67 seen in ConVal cases is not seen in the SczVal cases. i.e. GluN2A and GAD67 mRNA levels stayed low in SczVal cases (at the same level seen in met cases). It is possible that the inability to appropriately regulate gene expression leads to a failure to maintain homeostatic balance within the DLPFC neural circuit and eventually leads to the known greater functional deficits such as impairments in working memory seen in SczVal cases.

NMDA receptors at glutamatergic synapses on interneurons are currently postulated to be important molecular mediators of the DLPFC pathophysiology in schizophrenia (Rotaru et al., 2012). There is evidence of decreased GluN2A in the schizophrenia DLPFC (Beneyto and Meador-Woodruff, 2008). Further, reduction in GluN2A subunit expression has been specifically observed in GABAergic neurons in schizophrenia, albeit in the anterior cingulate (Woo et al., 2004). These data suggest a specific disruption in GluN2A-containing NMDA receptors in the DLPFC in schizophrenia which we predict, based on our current data, are more pronounced in Scz Val cases. The absence of an upregulation in GluN2A in SczVal cases could lead to reduced GAD67 levels and also D1-NMDA disruptions. Prior PET studies have found reduced DRD1 receptor-like binding in the prefrontal cortex of drug-naïve patients with schizophrenia with the decrease in DRD1 binding correlating with the severity of cognitive dysfunction (Okubo et al., 1997).

We also found that SczMet cases express lower levels of DRD2 when compared to ConMet or SczVal cases. DRD2 receptors have been shown to modulate working memory in primates (Wang et al., 2004; Zhang et al., 2007) with systemic administration of DRD2 agonists in non-human primates affects working memory (Arnsten et al., 1995). PFC involvement has been demonstrated by infusion of DRD2 agonists and antagonists into the PFC with modulating effects on working memory and set-shifting in rodents (Druzin et al., 2000; Floresco et al., 2006; St Onge et al., 2011). Consistent with these animal studies, genetic variation in DRD2 receptors modulates prefrontal activity and working memory in humans (Zhang et al., 2007). The relative lower expression of DRD2 in SczMet could, therefore be relevant to cognitive deficits in this group of subjects. This observation, however, appears contrary to the observed differential response to antipsychotic medications (DRD2 blockade) with carriers of the met allele showing significant improvement on working memory task and prefrontal physiology (Bertolino et al., 2004; Weickert et al., 2004). Since DRD2 is expressed on both GABA interneurons and pyramidal neurons (Negyessy and Goldman-Rakic, 2005), it will be important to determine the cellular and synaptic localization of the reduction in DRD2 in SczMet cases.

COMT mRNA is expressed at higher levels in neurons compared to than glial cells in the human DLPFC (Matsumoto et al., 2003a) and electron microscopic studies report COMT protein is present in dendritic processes of neurons (Kastner et al., 1994). COMT has a

soluble form (S-COMT) and a membrane-bound form (MB-COMT) (Tenhunen and Ulanen, 1993), with the latter being the predominant form found in the human brain MB-COMT is generally localized intracellularly (Ulanen et al., 1997) suggesting that its function in dopamine (DA) metabolism is secondary to DA uptake in the postsynaptic terminal which may be served by either the organic cation transporter or the plasma membrane monoamine transporter (PMAT) located in the postsynaptic neuron or glia (Godar and Bortolato, 2014). A prior human post mortem study by Matsumoto et al. reported DLPFC laminar specific changes in COMT mRNA with low levels in pyramidal neurons of the superficial layers (II/III) and higher levels in the intermediate/deep layers (IV/V) pyramidal neurons in schizophrenia while control subjects did not show such laminar variation (Matsumoto et al., 2003b). These laminar changes were not a result of prior antipsychotic treatment. In our study, we did not find a COMT genotype X disease interaction on COMT mRNA levels but did observe group (diagnosis) changes with higher COMT levels in schizophrenia compared to controls. Schizophrenia involves both abnormalities in prefrontal dopamine signaling and in nigrostriatal dopamine activity. A reciprocal relationship between cortical hypo-dopaminergia and subcortical hyper-dopaminergic state have been demonstrated in animal studies (Lipska and Weinberger, 1998; Pycock et al., 1980) and may contribute to cognitive and psychotic symptoms, respectively, in schizophrenia (Bilder et al., 2004; Weinberger et al., 1988). Our observations are consistent with the postulation put forth by Matsumoto et al. that increased DLPFC COMT expression could contribute to a relative hypo-dopaminergic state in the DLPFC resulting in a subsequent hyper-dopaminergic state at the level of striatal function in schizophrenia. Akil et al determined that control subjects with the COMT val/val genotype express higher levels of tyrosine hydroxylase (TH) in mesencephalic DA neurons (Akil et al., 2003), suggesting an increase in DA synthesis. TH was increased primarily in the ventral tier of the substantia nigra (SN) compacta that sends projections to the striatum and amygdala. The reciprocal effects of COMT genotype on DA signaling in the prefrontal cortex and on mesencephalic TH gene expression suggests a mechanism by which the COMT val allele mediates effects relevant to schizophrenia. In this current study, we report increases in GluN2A and GAD 67 in the DLPFC of control val/val cases, possibly a downstream compensatory response to DLPFC hypo-dopaminergia, to maintain cognitive function in these control subjects. In schizophrenia val/val cases, however, we do not observe the changes in GluN2A and GAD67. We speculate that this failure to compensate to a low DA state in the DLPFC may underlie the poorer cognitive function seen in schizophrenia val/val cases.

There are several limitations of this study. Firstly, we report mRNA levels of our target genes and it is possible that this might not reflect receptor levels or enzyme activity. Second, DLPFC tissue was obtained from the whole gray matter cortex. It is possible that expression of gene targets found to be non-significant in our study may be differentially regulated in a laminar specific manner. On the other hand, this might also suggest that the transcript changes we report from the whole cortex might actually reflect larger laminae-specific changes than reported (due to dilution effects of other laminae where transcript changes might not occur). Third, we recognize that the relatively small sample size (n=11–15) in this study raises the possibility of type I or type II errors. Thus, replication of findings using a larger sample is warranted. Another factor to take into consideration is that the COMT

val158met polymorphism is not found in species other than humans (Palmatier et al., 1999) and, while we do not observe an effect of antipsychotic medications on genes of interest in chronically treated rats, it is possible that there is a differential effect of antipsychotic treatment between COMT val and met schizophrenia cases. Our observations, however, are still very relevant to real world patients with schizophrenia on treatment with antipsychotic medications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Graphs showing mRNA expression of target genes. Significant genotype x diagnosis interactions were observed for DRD2, GluN2A and GAD67. Data shown are medians with 25th and 75th percentile bars. Con = Controls, SCZ = schizophrenia, Met = met carriers, Val = val homozygotes. * indicates $p < 0.05$

Figure 2.

Effect of Antipsychotic Treatment on DRD2, GluN2A and GAD67 mRNA expression.

Graphs illustrate the absence of an effect of chronic treatment in rodents given risperidone (6 mg/kg/d) compared with water (placebo) [n=13/group] on gene expression in the frontal cortex. Bars represent the standard deviation of mean values.

Figure 3. Schematic representation of DLPFC neural circuitry showing glutamate and DA afferents targeting pyramidal neuron dendrites (circle A) and GABA interneurons (circle B) and synaptic distribution of gene targets differentially associated with COMT val/met.

Table 1

Demographics		Sex	Age (years) mean (S.D.)	Race	PMI (hours) mean (S.D.)	RIN mean (S.D.)
CON	VAL (N=13)	13M	50.92 (16.94)	12C/1H	18.03 (8.49)	7.88 (1.33)
	MET (N=14)	10M/4F	54.23 (17.6)	13 C/1AA	19.36 (6.25)	7.06 (2.3)
SCZ	VAL (N=11)	7M/4F	46.7 (9.48)	7C/4AA	17.11 (7.17)	7.77 (2.06)
	MET (N=15)	10M/5F	43.93 (13.82)	14C/1AA	15.77 (5.49)	7.28 (2.64)

CON= controls, SCZ = schizophrenia, C= Caucasian, AA=African American, H = Hispanic, PMI = postmortem interval, RIN = RNA integrity number.