

Catechol-o-methyltransferase (COMT) and Proline Dehydrogenase (PRODH) mRNAs in the Dorsolateral Prefrontal Cortex in Schizophrenia, Bipolar Disorder, and Major Depression

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ABSTRACT Catechol-o-methyltransferase (COMT) and proline dehydrogenase (PRODH) may both be susceptibility genes for schizophrenia. As part of the evaluation of their roles in psychosis, we used reverse transcription–polymerase chain reaction to measure COMT and PRODH mRNAs in the dorsolateral prefrontal cortex in schizophrenia, bipolar disorder, major depression, and normal controls ($n = 15$ subjects in each group). We also genotyped two common COMT polymorphisms (–287A/G and 158Val/Met) which might affect its expression. Neither COMT nor PRODH mRNA abundance differed between diagnostic groups, nor when controls were compared with all psychotic patients. COMT mRNA levels were unrelated to COMT genotypes. We conclude that any involvement of COMT and PRODH genes in schizophrenia is not accompanied by significant alterations in their overall mRNA expression, at least in dorsolateral prefrontal cortex. As COMT and PRODH are both located on chromosome 22q11, the results also argue against the hypothesis that schizophrenia is associated with a decrease in expression of all 22q11 genes, as had been suggested by the high prevalence of psychosis in people with hemizygous 22q11 deletions. **Synapse 51:112–118, 2004.**

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INTRODUCTION

Several susceptibility genes for schizophrenia have recently been described (for review, see Harrison and Owen, 2003), including catechol-o-methyltransferase (COMT) and proline dehydrogenase (PRODH). COMT is involved in catecholamine metabolism (Mannisto and Kaakkola, 1999; Weinshilboum et al., 1999), especially in the prefrontal cortex (Karoum et al., 1994; Gogos et al., 1998). COMT is therefore relevant to models of schizophrenia involving dopamine and the prefrontal cortex (Weinberger et al., 2001), particularly following the study of Egan et al. (2001). These authors showed that a common single nucleotide polymorphism (SNP) of COMT (Val158Met) is associated with schizophrenia, replicating the finding of Li et al. (2000), and also with dorsolateral prefrontal cortex (DPFC) performance (see also Jooper et al., 2002). Additional evidence linking COMT with schizophrenia is provided by a strong association between a COMT haplotype (which included the Val/Met SNP) and schizophrenia (Shifman et al., 2002).

PRODH is a mitochondrial enzyme which metabolises proline (Chakravarti, 2002). The strongest evidence for a role of PRODH in schizophrenia comes from Liu et al. (2002), who found a robust association between PRODH SNPs and schizophrenia (see also Jaquet et al., 2002). The plausibility of PRODH as a gene relevant to psychosis is enhanced by the deficit in sensorimotor gating in mice lacking the homologue of PRODH (Gogos et al., 1999), and by the role of proline as a modulator of glutamate neurotransmission (Cohen and Nadler, 1997a,b) suggestive of a link to glutamate

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TABLE I. Demographic details of subjects studied

	Controls	Schizophrenia ^a	Bipolar disorder ^b	Major depression
Age (years)	48.1 (10.6)	44.5 (13.1)	42.3 (11.7)	46.5 (9.3)
Sex (M, F)	9, 6	9, 6	9, 6	9, 6
Brain pH	6.27 (0.24)	6.18 (0.24)	6.19 (0.23)	6.18 (0.22)
Autopsy delay (h)	23.7 (9.9)	33.7 (14.6)	32.5 (16.1)	27.5 (10.7)
Freezer storage (months) ^c	11.7 (7.5)	20.8 (7.8)	20.9 (5.6)	14.8 (9.7)
Hemisphere (R, L)	7, 8	6, 9	8, 7	6, 9
Suicides	0	4	9	7
Onset of illness (years)	—	23.2 (8.0)	21.5 (8.3)	33.9 (13.3)
Duration of illness (years)	—	21.8 (11.4)	21.4 (9.2)	12.7 (11.1)
Cumulative antipsychotic dose ^d	0	52,267 (62,061)	20,827 (24,016)	0
Antipsychotics ever	0	14	12	0

Values are mean (SD).

^aSubtype: paranoid (4), disorganised (1), undifferentiated (10).

^bN = 11 with psychotic features.

^cDiffers between groups (ANOVA, $F(3,56) = 5.21$, $P = 0.003$), being shorter in controls than in schizophrenia and bipolar disorder groups (both $P = 0.02$, Scheffe test).

^dChlorpromazine equivalents (g).

hypotheses of schizophrenia (Olney and Farber, 1995; Harrison and Owen, 2003).

The potential interest in COMT and PRODH in schizophrenia is enhanced by their common location at chromosome 22q11 (Dunham et al., 1999). Hemizygous deletions of 22q11 cause velo-cardio-facial syndrome (VCFS), also called DiGeorge syndrome. Approximately a quarter of adults with VCFS have schizophrenia or a related psychosis (Shprintzen, 1992; Pulver et al., 1994; Karyiourgou et al., 1995; Bassett and Chow, 1999; Murphy et al., 1999). Conversely, the rate of 22q11 deletions is increased in unselected patients with schizophrenia; estimates vary widely (from 2–53%), but even a 2% figure represents a manifold increase over the population rate of 1 in 4,000 (Scambler, 2000).

Hence, a range of evidence supports the candidacy of COMT and PRODH as schizophrenia susceptibility genes. The evidence, though not conclusive, is sufficient to merit consideration of the potential mechanisms which explain the apparent association. Given that the associated alleles and haplotypes for both genes are predominantly intronic or in the promoter (Jacquet et al., 2002; Liu et al., 2002; Schifman et al., 2002), their consequences are likely mediated via quantitative effects on gene expression (e.g., by altering mRNA stability or translation). Even the effect of the COMT Val/Met SNP, which is coding and markedly affects enzyme activity (Lotta et al., 1995; Lachman et al., 1996), may be confounded or counteracted by changes in COMT gene expression; it is already known to affect tyrosine hydroxylase mRNA abundance (Akil et al., 2003). In this study we measured COMT and PRODH mRNAs in the DPFC, using a reverse tran-

scription–polymerase chain reaction (RT-PCR) coamplification method which allows reliable relative quantitation (Burnet et al., 1994; Eastwood et al., 1997; East et al., 2002). As well as comparing schizophrenics and normal controls, we included patients with a history of bipolar disorder and major depression, allowing the issues of diagnostic specificity and psychotropic medication effects to be assessed. We also genotyped the subjects for two common COMT polymorphisms, in case either influenced COMT expression: the 158Val/Met variant, and a promoter SNP (–287A/G; Norton et al., 2002).

MATERIALS AND METHODS

Subjects studied

The study was carried out on the Stanley Medical Research Institute brain series, described in detail by Torrey et al. (2000), and summarised in Table I. Diagnoses were made according to DSM-IV criteria. All brains were free of neuropathological abnormalities and were selected after screening for pH and RNA integrity. A small block of frozen tissue was provided from the dorsolateral prefrontal cortex (Brodmann area 9/46) from each case. Only P.J.H. has access to the series code, with other workers in the group remaining blinded. A spreadsheet containing the data presented here was sent to the Stanley Medical Research Institute prior to data analysis.

RNA extraction and reverse transcription

Approximately 150 mg of tissue was homogenised and total RNA extracted using Tri reagent (Sigma, Poole, UK). The concentration of each sample was determined using absorbance spectrophotometry. Two μg RNA was treated for 1 h at 37°C with 1 U RQ1 RNase-Free DNase (Promega, Southampton, UK), in the presence of 24 U RNasin ribonuclease inhibitor (Promega), and heated to 70°C for 6 min to inactivate the DNase. The DNase-treated RNA was reverse transcribed using 200 U Moloney murine leukaemia virus enzyme (Promega), the recommended reaction buffer (Promega),

Abbreviations

COMT	catechol-o-methyltransferase
DPFC	dorsolateral prefrontal cortex
PRODH	proline dehydrogenase
RT-PCR	reverse transcription-polymerase chain reaction
SNP	single nucleotide polymorphism
VCFS	velo-cardio-facial syndrome

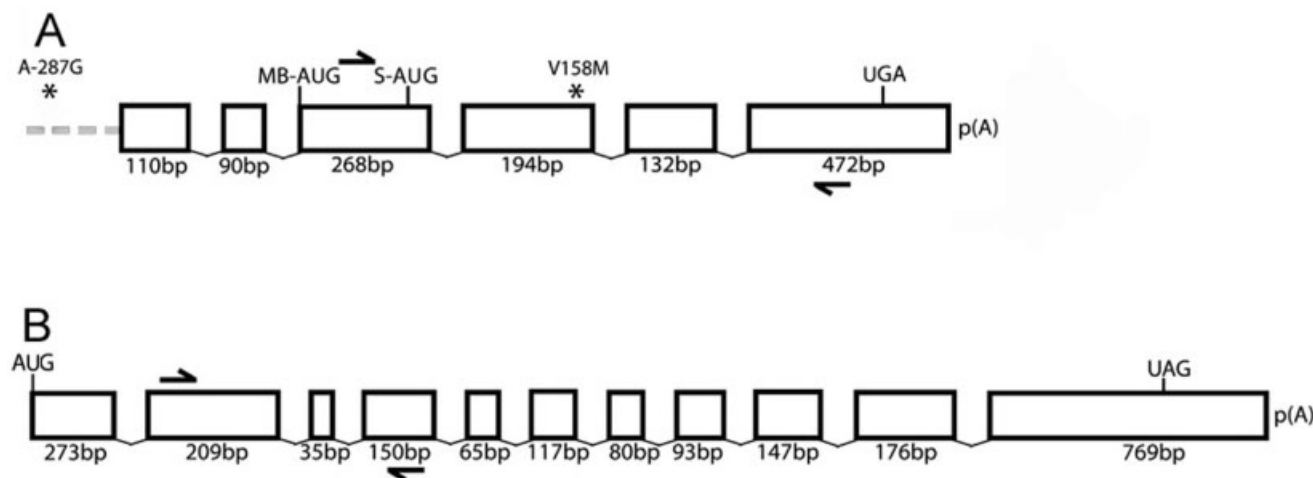


Fig. 1. Structure of (A) COMT mRNA and (B) PRODH mRNA. Exons are shown as rectangles, introns as adjoining lines, and the COMT promoter region as a dashed line. The location of the primers used in this study is shown by half arrows. Note that both primer pairs span introns to avoid amplification of genomic DNA. In (A), the asterisks denote the position of the two COMT polymorphisms genotyped here.

0.9 mM of each deoxynucleotide triphosphate (ABGene, Epsom, UK), and 30 ng dT oligonucleotides (Oswel, Southampton, UK). Reactions were incubated at 42°C for 1 h and the enzyme deactivated at 70°C for 10 min. Sterile water was added to each reaction to produce a final volume of 144 μ l, which was divided into aliquots and stored.

Polymerase chain reaction

COMT and PRODH transcripts were separately co-amplified with cyclophilin as an internal control. Each protocol was optimised in pilot experiments (varying primer concentrations and cycling parameters) to ensure that both the gene of interest and cyclophilin were within the exponential (linear) phase of amplification (not shown). Different cyclophilin antisense primers were used in the COMT and PRODH experiments to ensure adequate electrophoretic separation of the two products. All primers spanned an intron to avoid inadvertent amplification from any residual genomic DNA (Fig. 1).

The COMT sense (5' GAA CGA GTT CAT CCT GCA GCC CAT C 3') and antisense (5' GTG TGT GCA CTC AAA GCA GCT GCT C 3') primers generated a 616 basepair (bp) product. The corresponding cyclophilin primers (sense: 5' GAG CTG TTT GCA GAC AAG GTC CCA AAG 3'; antisense: 5' CCA ACC ACT CAG TCT TGG CAG TGC 3') generated a 301 bp product. Reactions contained 3 μ l cDNA, 1.25 U Biotaq DNA polymerase (Bioline, London, UK), recommended buffer (Bioline), 1.5 mM MgCl₂ (Bioline), 0.4 mM of each deoxynucleotide triphosphate (ABGene) and 125 ng of each COMT primer in a reaction volume of 25 μ l. After nine cycles (94°C, 35 sec; 65°C, 30 sec; 72°C, 60 sec), 125 ng of cyclophilin primers were added and the reactions incubated for a further 15 cycles.

The PRODH sense (5' CCA GGA AAC TTC TAG GAC AG 3') and antisense (5' GTA GAA GTA GGT GCG GGC ACT G 3') primers generated a 320 bp product. These primer sequences are specific for PRODH and are not found in the PRODH pseudogene, also located on chromosome 22 (Dunham et al., 1999). PRODH was coamplified with cyclophilin sense (5' GAG CTG TTT GCA GAC AAG GTC CCA AAG 3') and antisense (5' CCT GAG CTA CAG AAG GAA TGA TCT GG 3') primers, which generated a 484 bp product. Each reaction contained 3 μ l cDNA, 1.25 U Biotaq DNA polymerase (Bioline) and recommended buffer (Bioline), 2 mM MgCl₂ (Bioline), 0.4 mM of each deoxynucleotide triphosphate (ABGene), and 125 ng of each PRODH primer in a reaction volume of 25 μ l. After nine cycles (94°C, 35 sec; 60°C, 30 sec; 72°C, 60 sec), 125 ng of cyclophilin primers were added, after which the reactions were incubated for a further 19 cycles.

Alkali blotting

Seven μ l of each coamplification PCR product was loaded onto TBE agarose gels (3% for COMT/cyclophilin and 1.5% for PRODH/cyclophilin; Promega), separated by electrophoresis, and transferred to Hybond N+ membrane (Amersham, Arlington Heights, IL) by capillary action using a modified Southern blotting protocol (Koetsier et al., 1993). Previously amplified COMT, PRODH, and cyclophilin (310 and 484 bp) PCR products from initial studies were gel-purified using a kit (Qiaex II, Qiagen, Crawley, UK) and used as probes for Southern hybridisation. The identity of the probe sequences was confirmed by sequencing. For the COMT/cyclophilin study, ~100 ng of each probe was labelled with Redivue α ³²P-dATP (Amersham) at 37°C for 2 h using 10 U Klenow fragment (Promega), 0.02

TABLE II. COMT and PRODH mRNA expression in the dorsolateral prefrontal cortex measured using RT-PCR

	COMT/CYC mRNA (ratio)	COMT mRNA (O.D. units)	PRODH/CYC mRNA (ratio)	PRODH mRNA (O.D. units)
Controls (n = 15)	0.529 (0.085)	0.136 (0.042)	0.694 (0.148)	0.197 (0.013)
Schizophrenia (n = 15)	0.557 (0.040)	0.177 (0.050)	0.690 (0.186)	0.217 (0.014)
Bipolar disorder (n = 15)	0.625 (0.080)	0.149 (0.053)	0.842 (0.131)	0.224 (0.199)
Major depression (n = 15)	0.573 (0.050)	0.199 (0.056)	0.535 (0.113)	0.208 (0.008)
All psychotic patients (n = 26) ^a	0.586 (0.048)	0.168 (0.040)	0.749 (0.127)	0.206 (0.013)

Results are shown with and without normalisation to coamplified cyclophilin (CYC) mRNA.

Values are mean (SEM). There are no significant differences between diagnostic groups (ANOVA; all $P > 0.5$).

^aThe 15 schizophrenia subjects and 11 psychotic bipolar disorder patients grouped together (see Methods).

mM dCTP, 0.02 mM dTTP, 0.02 mM dGTP and 1 μ g random hexamers (Promega). For the PRODH/cyclophilin study, ~25 ng of each probe was labelled with Redivue α ³²P-dCTP (Amersham) using the Klenow-based Rediprime II kit (Amersham). The radiolabelled probes were hybridised to the membranes using standard methods. Hybridised membranes were apposed to autoradiography film (Kodak BioMax MS). After development, films were digitised at a resolution of 300 dpi and the optical density of the blots measured using Image J v. 1.24 (National Institute of Mental Health, Bethesda, MD). Each experiment was carried out in duplicate and the mean value used for the analyses. The experiments were also repeated in their entirety, and essentially identical results obtained as those presented here (data not shown).

Genotyping of COMT polymorphisms

Genomic DNA was extracted using the Nucleon ST protocol for soft tissues (Tepnel, Manchester, UK). Approximately 200 mg tissue was homogenised in lysis buffer and processed according to the manufacturer's instructions.

Individuals were genotyped for COMT G158A (Val158Met) using the method previously described (Lachman et al., 1996). Briefly, a 169 bp fragment of COMT containing the SNP was amplified using sense (5' ACT GTG GCT ACT CAG CTG TG 3') and antisense (5' CCT TTT TCC AGG TCT GAC AA 3') primers. The 169 bp product was digested with *Hsp92II* (an *NlaIII* isoschizomer; Promega) and the products separated by electrophoresis on a 3% agarose gel. After digestion, the G158 allele produces a 114 bp fragment (along with 27 and 28 bp fragments, which are not seen in this assay); the A158 allele introduces an additional *Hsp92II* restriction site, producing a fragment 96 bp in length, which can be identified on an agarose gel (along with unobserved 27, 28, and 18 bp fragments). A G158A heterozygote is thus identified by the presence of bands at both 96 and 114 bp.

The COMT -287A/G SNP was genotyped as described previously (Norton et al., 2002). Briefly, a 353 bp product of COMT was amplified using sense (5' TAG TAA CAG ACT GGG CAC GAA 3') and antisense (5' GTT CAA AGG GCA TTT ATC ATG 3') primers. The amplicon was digested with *HindIII* (Promega) and

the products separated by electrophoresis on a 1.5% agarose gel. The -287A allele remains undigested, whereas the -287G allele introduces a *HindIII* restriction site, resulting in 278 and 75 bp fragments. A heterozygote for this SNP thus has bands at 353 and 278 bp. All genotypes for both polymorphisms were confirmed by repetition.

Data analysis

The primary outcome variable for each individual was the abundance of COMT and PRODH mRNAs relative to the coamplified cyclophilin mRNA. This provides an internal control for variability in RNA loading, amplification efficiencies, etc. However, we also analysed the 'raw' optical density data for COMT and PRODH transcripts. Expression of the genes was compared between the four diagnostic groups using one-way ANOVA. We also examined for possible gender differences, which have been reported for functional and genetic aspects of COMT (Gogos et al., 1998; Karayiorgou et al., 1999; Shifman et al., 2002). Because of the evidence mentioned in the Introduction that 22q11 deletions are associated with a broader range of functional psychoses than just with schizophrenia, we also examined whether COMT or PRODH expression differed in the 26 patients with a history of psychosis (the 15 schizophrenia subjects and 11 of the bipolar disorder subjects grouped together) compared to the healthy controls. Possible effects of age, pH, autopsy delay, and medication exposure (Table I) were investigated using Pearson's coefficient.

RESULTS

COMT and PRODH mRNAs were successfully amplified and coamplified with cyclophilin mRNA from all subjects. Expression of COMT and PRODH mRNAs correlated with each other ($R = 0.703$, $P < 0.001$); this remained significant after partialling for cyclophilin mRNA ($R = 0.741$, d.f. 57, $P < 0.001$).

Table II summarises the data relating COMT and PRODH mRNAs to diagnostic categories. There were no group differences, nor trends, for either mRNA, when expressed relative to cyclophilin mRNA, or when taken in isolation. Neither did cyclophilin mRNA levels differ between groups. Southern blots from two ran-

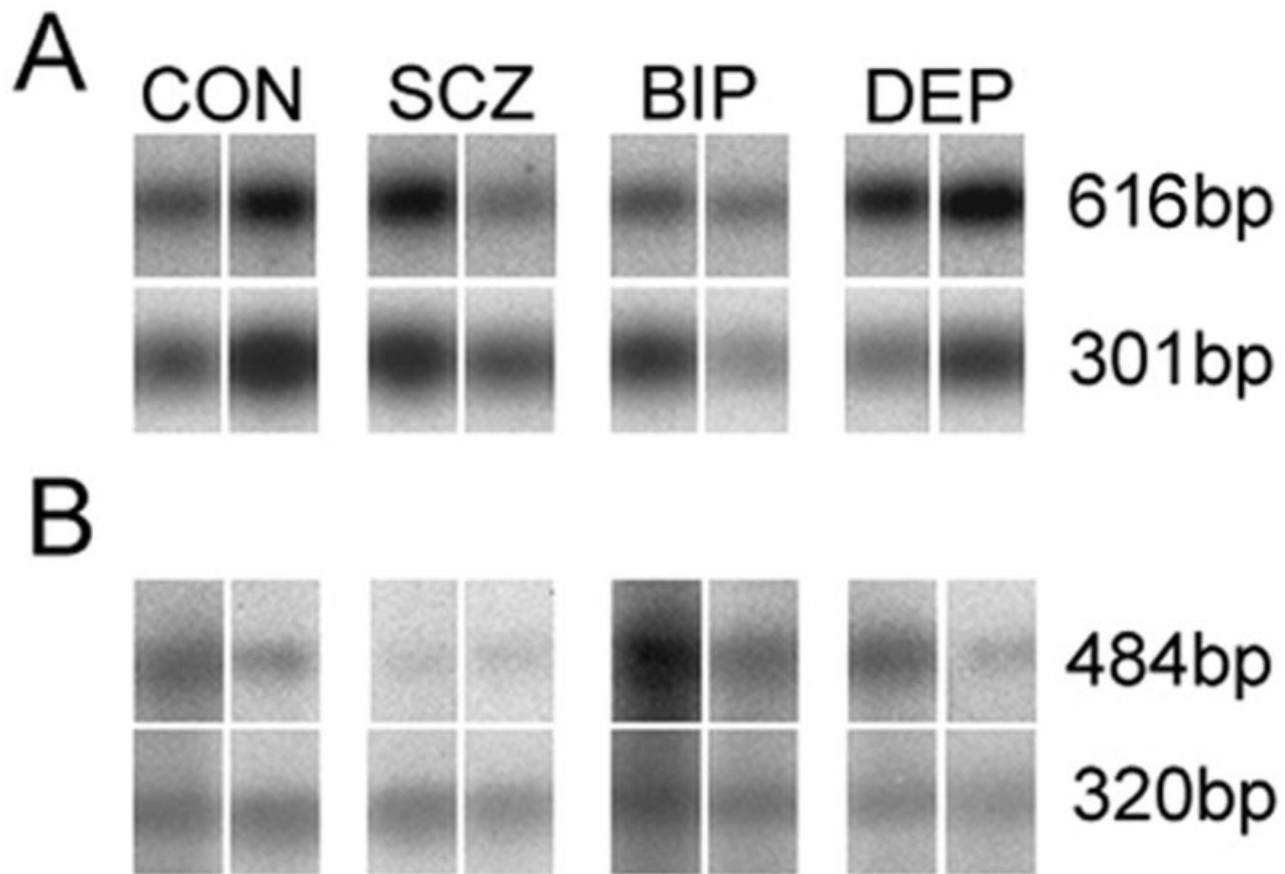


Fig. 2. Coamplification of COMT and PRODH with cyclophilin. Southern blots showing the coamplification of (A) COMT (616 bp) and cyclophilin (301 bp) and (B) PRODH (320 bp) and cyclophilin (484 bp). For each coamplification, two randomly selected controls (CON), subjects with schizophrenia (SCZ), subjects with bipolar disorder (BIP), and subjects with major depression (DEP) are shown.

TABLE III. COMT genotype and COMT gene expression

	COMT/CYC mRNA	COMT mRNA
-287A/G		
A/A (n = 18)	0.573 (0.041)	0.195 (0.049)
A/G (n = 31)	0.597 (0.057)	0.159 (0.035)
G/G (n = 11)	0.494 (0.046)	0.135 (0.050)
Val158Met		
Val/Val (n = 14)	0.592 (0.045)	0.215 (0.061)
Val/Met (n = 30)	0.583 (0.050)	0.168 (0.036)
Met/Met (n = 16)	0.530 (0.071)	0.116 (0.036)

Results are shown with and without normalisation to coamplified cyclophilin (CYC) mRNA.

Values are mean (SEM). There are no significant differences (ANOVA, all $P > 0.3$).

domly chosen subjects within each diagnostic category are shown in Figure 2. As well as illustrating the lack of group differences, the figure also indicates the inter-subject variability of the transcripts.

COMT mRNA abundance was similar in men (0.56 ± 0.04 , $n = 36$) and women (0.59 ± 0.06 ; $n = 24$, $P = 0.7$) in the whole sample and also in the control group (men: 0.53 ± 0.09 , $n = 9$; women: 0.53 ± 0.18 , $n = 6$, $P = 0.52$). Age, brain pH, and postmortem interval did not correlate significantly with any of the indices, and re-

sults remained negative when these factors were included as covariates. Within the patient groups there were no correlations of COMT or PRODH mRNAs with lifetime antipsychotic exposure, or with a history of treatment with antidepressants or mood stabilisers.

Table III shows COMT gene expression categorised by COMT genotype. Although the mean abundance of COMT mRNA did appear to relate to genotype at both alleles, especially when COMT mRNA was considered without reference to cyclophilin, none of the differences approached significance (all $P > 0.3$). There were no interactions between diagnosis and genotype (not shown).

DISCUSSION

Several complementary lines of evidence implicate COMT and PRODH genes in schizophrenia: linkage and association data, location of the genes on 22q11, their putative functions, and the phenotype of knock-down or knockout mice (see Introduction). Anatomically, the DPF is the region in which any influences in schizophrenia would seem most likely to be mediated

(Gogos et al., 1998, 1999; Egan et al., 2001; Weinberger et al., 2001).

The cellular and molecular mechanisms underlying the putative involvement of COMT and PRODH in schizophrenia remain unknown. One plausible process could be a difference in gene expression, hence the study reported here. In the event, we did not find alterations of either COMT or PRODH mRNAs in the disorder (Table II). The finding for COMT mRNA in schizophrenia is replicated by a recent *in situ* hybridization study (Matsumoto et al., 2003b), and our data show that it also applies to bipolar disorder and major depression subjects. Unaltered COMT mRNA in schizophrenia is also consistent with an earlier finding regarding COMT activity (Cross et al., 1978). Equivalent antibody-based studies would allow evaluation of COMT protein abundance in schizophrenia, and thereby complete the picture from gene to enzyme activity, taking into account the existence of membrane and soluble forms of COMT (Lotta et al., 1995), a complexity which might be relevant in schizophrenia and which cannot be addressed by mRNA studies. Furthermore, the existing data in schizophrenia are not entirely negative. Matsumoto et al. (2003b) found a partial redistribution of DPFPC COMT mRNA from superficial to deeper lamina pyramidal neurons (see also Matsumoto et al., 2003a). This finding illustrates that, whilst overall DPFPC COMT expression in schizophrenia is unaffected, more subtle but potentially functionally important changes may occur.

There was no significant effect of either the Val158Met or -287A/G SNPs upon COMT mRNA abundance (Table III). The lack of effect of the Val158Met SNP agrees with an earlier study (Matsumoto et al., 2003b) and contrasts with its influence upon tyrosine hydroxylase mRNA in the ventral tegmental area (Akil et al., 2003); the latter finding suggests there may be an interaction between pre- and postsynaptic regulation of dopaminergic mesocortical transmission in which the COMT Val158Met SNP plays a role. However, the data in Table III are somewhat equivocal, since there was a considerable, albeit nonsignificant, difference in mean COMT mRNA abundance between Val158 and Met158 carriers, and between -287A and -287G carriers. Indeed, a recent study indicates that the COMT haplotype associated with schizophrenia (Schifman et al., 2002) may influence COMT mRNA abundance (Bray et al., 2003). The latter study also showed allelic differences in COMT expression, influenced in part by a novel SNP in the 3' untranslated region. Therefore, genetic effects on COMT expression should continue to be evaluated, using larger samples and more complex analyses.

There are gender differences in the strength of COMT genetic associations with several disorders (e.g., Karayiorgou et al., 1999; Shifman et al., 2002), and in the behaviour of heterozygous COMT knockout mice

(Gogos et al., 1998). These differences may relate to the roles of COMT in metabolism of oestrogens (Weinshilboum et al., 1999). We found no male–female differences in COMT mRNA expression, either in the whole sample or in controls. Hence, either the gender differences are not mediated via mRNA abundance or they occur in other areas of the brain.

The finding of unaltered PRODH mRNA in schizophrenia is novel. As with COMT, the observation implies that any involvement of the PRODH gene in the disorder is not accompanied by, or manifested in, its overall expression in DPFPC. The regional and cellular expression profile of PRODH in human brain is unknown. Such information will be needed to help establish the roles of PRODH in the neural circuitry and its relevance for schizophrenia.

Chromosome 22q11 has attracted considerable interest as a locus harbouring genes for psychosis because of the increased rates of psychosis in individuals with hemizygous deletions of this region, and vice versa (Bassett and Chow, 1999; Murphy, 2002). Two basic mechanisms might explain this relationship, and by extrapolation, underlie the relevance of this region for psychosis in general. One option is that there is a gene or genes within the region which predispose(s) to psychosis; the other is that there is a downregulation of expression of 22q11 genes in line with the halved gene dosage (haploinsufficiency). Our negative results, showing no reduction in COMT or PRODH expression in schizophrenia or bipolar disorder, argue against the latter hypothesis.

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