Progress in Neuro-Psychopharmacology & Biological Psychiatry xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Association of *MB-COMT* polymorphisms with schizophrenia-susceptibility and symptom severity in an African cohort

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ARTICLE INFO

Article history:
Received 8 May 2012
Received in revised form 1 June 2012
Accepted 6 June 2012
Available online xxxx

Keywords:
African population
MB-COMT
Negative symptoms
Schizophrenia
Xhosa

ABSTRACT

The catechol-*O*-methyltransferase (*COMT*) gene is an attractive schizophrenia candidate gene, encoding a catabolic dopamine enzyme. The enzyme exists as two distinct isoforms, with the membrane bound enzyme (*i.e.* MB-COMT) being predominantly expressed in the brain. Since African populations remain underrepresented in genetic/genomic research, we performed an association study to determine whether *MB-COMT* genetic variants are associated with schizophrenia-susceptibility and symptom severity in the South African Xhosa population.

Fourteen candidate polymorphisms were selected by means of a literature search and *in silico* analyses and were subsequently genotyped in a cohort of 238 Xhosa schizophrenia patients and 240 healthy Xhosa controls. Genetic association was tested with schizophrenia-susceptibility as well as symptom severity within the patient group. Polymorphisms of interest were also analysed using functional assays.

Two SNPs, rs2020917 (OR = 0.54, 95% CI 0.37–0.79; $P = 0.0011$) and rs737865 (OR = 0.52, 95% CI 0.36–0.74; $P = 0.0002$), in the P2 promoter region were significantly associated with schizophrenia as well as an increase (increase = 11.2%, 95% CI 3.7%–19.2%; $P = 0.0031$) in reporter gene expression. The minor alleles of these SNPs were underrepresented in the schizophrenia cohort, indicating a possible protective effect. The P2 region also formed part of a haplotype found to be associated with the severity of the negative symptoms of the disorder.

The data generated by this study indicate that genetic variation of *MB-COMT* could be associated with schizophrenia and negative symptom severity in the Xhosa population and may therefore be one of the genomic loci contributing towards the disorder in the South African community. Future large-scale studies in other African schizophrenia populations are required to further elucidate the significance of these findings.

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1. Introduction

Schizophrenia is a chronic psychiatric disorder that is characterised by positive and negative symptoms as well as cognitive disturbances (Van Os and Kapur, 2009). The disorder displays a high heritability (Kety, 1987), yet a comprehensive understanding of the genomic architecture of schizophrenia remains to be elucidated. Complex interactions of numerous genetic variants, combined with the effects of environmental risk factors, as well as phenotypic heterogeneity, are believed to be responsible for this knowledge gap. Several neurobiological hypotheses exist and genes encoding products important within neurotransmitter systems have been the focus of much research. The dopamine hypothesis of schizophrenia is the oldest of these and states that dysregulation of dopamine in the brain leads to the development of schizophrenia and may account for both the positive and negative symptoms of the disorder (Guillin et al., 2007).

Abbreviations: 22qDS, 22q11.2 deletion syndrome; A, adenine; bp, base pairs; C, cytosine; CI, confidence interval; *COMT*, catechol-*O*-methyltransferase; DNA, deoxyribonucleic acid; DSM-IV, diagnostic and statistical manual of mental disorders; G, guanine; gDNA, genomic DNA; HWE, Hardy–Weinberg equilibrium; kb, kilobase pairs; LD, linkage disequilibrium; LQ, lower quartile; MB-COMT, membrane-bound catechol-*O*-methyltransferase; Met, methionine; *n*, individuals; OCD, obsessive–compulsive disorder; OR, odds ratio; PCR, polymerase chain reaction; S-COMT, soluble catechol-*O*-methyltransferase; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SNP, single nucleotide polymorphism; T, thymine; UQ, upper quartile; Val, valine.

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0278-5846/$ – see front matter © 2012 Published by Elsevier Inc.
doi:10.1016/j.pnpbp.2012.06.006

Please cite this article as: Wright GEB, et al, Association of *MB-COMT* polymorphisms with schizophrenia-susceptibility and symptom severity in an African cohort, Prog Neuro-Psychopharmacol Biol Psychiatry (2012), doi:10.1016/j.pnpbp.2012.06.006

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The catechol-*O*-methyltransferase (*COMT*) gene is an attractive candidate for schizophrenia-susceptibility as: (i) it encodes a catabolic dopamine enzyme (Williams et al., 2007); (ii) it is located on chromosome 22q, a genomic area of implied linkage to schizophrenia (Badner and Gershon, 2002); and (iii) the region is deleted in individuals with 22q11.2 deletion syndrome (22qDS), a disorder where approximately 30% of patients go on to develop schizophrenia (Kobrynski and Sullivan, 2007). *COMT* genetic variation has been associated with various diseases/disorders, including schizophrenia, obsessive–compulsive disorder (OCD) and breast cancer (Ji et al., 2008; Pooley et al., 2007; Shifman et al., 2002).

Two isoforms of the COMT enzyme exist, namely the soluble form (S-COMT) and the membrane-bound form (MB-COMT), which are expressed from the proximal P1 and the distal P2 promoters respectively (Tenhunen et al., 1994; Xie et al., 1999). MB-COMT is the predominant isoform expressed in the brain (Tenhunen et al., 1994), and is therefore believed to be more relevant for psychiatric genetic research. Due to a low density of dopamine transporters in the prefrontal cortex, COMT is believed to play a major role in regulating dopamine levels in this region, an area of the brain which is believed to be involved with schizophrenia pathogenesis (Williams et al., 2007). The majority of research has been performed on a functional nonsynonymous polymorphism, $Val^{108/158}Met$ (rs4680), which causes a significant reduction in COMT enzymatic activity (Lachman et al., 1996). Despite this variant occurring at a high frequency in the majority of populations worldwide, inconsistent results have been obtained in studies examining the association between this variant and many complex diseases, including schizophrenia (Mukherjee et al., 2010). For example, a meta-analysis presented on the SZGene Database of over 60 schizophrenia case–control association studies involving the $Val^{108/158}Met$ polymorphism (representing >35 000 samples), detected no significant association for this variant (OR 0.98; 95% CI 0.94, 1.02) (http://www.szgene.org/meta.asp?geneID=420).

Subsequent studies have attempted to identify additional *COMT* variants that may supplement our knowledge on functional genetic variation in the region, placing focus on variants altering gene expression, mRNA degradation and protein synthesis (Nackley et al., 2006). Research has therefore shifted towards performing haplotype studies, incorporating numerous polymorphisms that span the entire gene locus, including the P2 promoter (Ji et al., 2008; Nackley et al., 2006; Shifman et al., 2002). A population genetic study of the *COMT* gene locus revealed that the linkage disequilibrium (LD) in the region is extremely complex, varying substantially between populations and may be responsible for the inconsistencies in results of genetic association studies (Mukherjee et al., 2010). African populations were found to display the lowest levels of LD and greatest haplotypic diversity at the *COMT* gene locus (Mukherjee et al., 2010). This is due to the fact that these individuals have the highest levels of genetic diversity worldwide due to their ancient history (Tishkoff et al., 2009). Performing association studies in these populations can be advantageous as the low levels of LD present in their genomes enable possible fine-mapping of "causal variants" (Manolio et al., 2009).

In light of the above considerations, we decided to conduct a case–control association study in the South African Xhosa population to elucidate whether genetic variants in *MB-COMT* are associated with schizophrenia-susceptibility. In addition to this primary aim, we decided to conduct an exploratory analysis to test whether genetic variation at this locus may influence schizophrenia symptom severity in this population. To our knowledge, no such study has been performed in an indigenous African population. The Xhosa population are the second largest population group in South Africa, representing ~8 million individuals (http://www.statsonline.gov.za/census01/html/RSAPrimary.pdf). Additionally, the Xhosa schizophrenia population is thought to be ethnically and culturally homogenous (Niehaus et al., 2005a), making them ideal for performing this type of study, as they are less likely to present with phenotypic heterogeneity and other confounding factors (Wright et al., 2011).

2. Methods

2.1. Study population and clinical assessment

This study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. A cohort of 238 Xhosa schizophrenia patients (18% female, mean age: 35.76 ± 11.30 years) and 240 healthy Xhosa controls (21% female, 35.83 ± 11.72 years), matched for age (within five years) were recruited for this study in the Cape Town metropole from local hospitals, clinics and by word of mouth (Table 1). All participants were unrelated and of Xhosa ethnicity (4/4 grandparents of Xhosa origin). Schizophrenia patients required a DSM-IV diagnosis for the disorder and underwent the Diagnostic Interview for Genetic Studies version 2, as well as the Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1983, 1984). "Attention", however, was not included in the global SANS score. Cannabis use/abuse was defined as >21 uses of the drug in a year (Koen et al., 2009). Subjects were excluded from the study if they presented with a significant general medical condition. Interviews were conducted in isiXhosa and ethical approval was obtained from the Committee for Human Research (Stellenbosch University). After complete description of the study to the subjects, written informed consent was obtained.

2.2. MB-COMT polymorphism selection and genotyping

Genomic DNA was extracted from whole blood using a standard procedure (Miller et al., 1988). The common genetic variation in the P2 promoter was determined by resequencing this region in 15 randomly selected Xhosa schizophrenia patients. Fourteen single nucleotide polymorphisms (SNPs), spread throughout the *COMT* gene locus, were subsequently selected for genotyping in the Xhosa samples using PCR restriction fragment length polymorphism analyses or Taqman® SNP assays. Briefly, SNPs were selected based on the literature, as well as bioinformatic analyses on polymorphisms in the P2 promoter region. A detailed description of the methods for the resequencing, polymorphism selection and genotyping can be found in the Supplementary Appendix SA1.

Table 1
Demographic and clinical characteristics of the Xhosa schizophrenia sample.

	n	Median	LQ	UQ
Quantitative traits				
Age at interview (years)	237	35	26	44
Age of onset (years)	230	21	19	26
Duration of illness (years)	229	10	4	18
SANS score	233	8	6	11
Counts				
SAPS score	214	7	2	11
Number of hospitalisations	236	3	2	5
Number of episodes	232	3	2	5
Dichotomous		Yes (%)	Count	
Cannabis use/abuse	238	53%	125	
Medication type	238			
Typical		82%	195	
Clozapine		7%	17	
Unknown/no medication		11%	26	

Interquartile range is lower quartile (LQ) and upper quartile (UQ); *n*, individuals where data is available.

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2.3. Reporter gene studies

Polymorphisms that were found to be significantly associated with schizophrenia in the Xhosa population were analysed using luciferase dual reporter assays to determine their respective affect on gene expression. A 2.4 kb P2 promoter fragment, spanning the polymorphisms of interest, was cloned into a promoterless pGL4.10 vector (Promega, Madison, WI, USA) and site-directed mutagenesis, employing circular polymerase chain reactions (PCRs), was subsequently used to insert these polymorphisms into the respective constructs (Supplementary Appendix SA1). Two human cell lines – dopaminergic neuroblastoma BE(2)-M17 cells and hepatocellular carcinoma HepG2 cells (American Type Culture Collection, Manassas, VA, USA) – were cultured to examine possible differences in expression between these tissues. Assays for each construct were performed in quadruplicate in both cell lines and three independent transfection experiments were performed.

2.4. Statistical analyses

Allele and genotype frequencies were determined for genotyped polymorphisms and the pattern of LD between variants was established using Haploview v4.1 (Barrett et al., 2005). Genotypes in both the cases and controls were assessed for deviations from Hardy–Weinberg equilibrium (HWE).

All results are based on linear models for quantitative traits, over-dispersed Poisson models for counts and scores with skewed distributions and logistic regression models for dichotomous characteristics. We used joint models for each outcome-SNP combination, which avoids false positive results due to too many univariate tests, whilst providing more power to detect significant effects. Modelling (rather than univariate tests) outcomes also enable us to adjust for known or suspected confounders, by including them in the models as fixed or random effects. All *P*-values and effect sizes, with 95% confidence intervals, are from these models. As the distributions of several quantitative traits were positively skewed inside the groups being investigated, we summarised all quantitative traits with median and interquartile range in Table 1. Where required, we log-transformed outcomes to symmetry for linear model analysis, or used over-dispersed Poisson models. The primary analysis (*i.e.* schizophrenia-susceptibility) was adjusted for age and gender and the secondary analyses (*i.e.* SANS and SAPS) were adjusted for age, gender, medication type and cannabis use since they have been associated with these traits in this population (Niehaus et al., 2008). We tested haplotype, genotype and allelic association. We report the results corresponding to the model yielding the most significant *P*-value. A linear mixed-effects model was used to analyse luciferase activity as a function of cell-line and construct, including their interaction, adjusting for experiment as random effect.

Results corresponding to P-values<0.05 are described as significant, except for HWE, where we used $P<0.01$. Results were not adjusted for multiple testing, as LD between SNPs may prevent individual polymorphisms being regarded as independent from one another (Nyholt, 2004) and since we analysed variants that have been associated with schizophrenia and the other traits investigated *a priori* (Perneger, 1998). All statistical analyses, except for the generation of LD plots, were done in R (http://www.r-project.org) and R packages genetics version 1.3.6 (http://CRAN.R-project.org/package=genetics) and haplo.stats version 1.5.5 (http://CRAN.R-project.org/package=haplo.stats).

3. Results

Fig. 1 is a schematic diagram of the location of the genotyped *MB-COMT* SNPs as well as their respective minor allele frequencies in the schizophrenia and control cohorts. Table 2 contains allele and genotype frequencies detected in the Xhosa samples as well as the results of the genetic association analyses of schizophrenia-susceptibility. All results are adjusted as described under statistical methods. All polymorphisms were in HWE. High levels of LD were detected in the distal P2 promoter (Supplementary Fig. S1) and the minor alleles of two polymorphisms in this region, rs2020917 ($OR_{T\text{-allele}}=0.54$, 95% CI 0.37–0.79; $P=0.0011$) and rs737865 ($OR_{G\text{-allele}}=0.52$, 95% CI 0.36–0.74; $P=0.0002$), were significantly underrepresented in the schizophrenia cohort. These two polymorphisms were tightly linked to one another ($D'=0.98$, LOD$=103.62$, $r^2=0.81$). Significant single marker associations were detected for SANS scores with three SNPs, rs45536341 (additive allelic effect of T-allele: score 4.24 less, 95% CI: 1.27–7.22; $P=0.005$), rs6269 (additive allelic effect of G-allele: score 1.11 less, 95% CI: 0.25–1.98; $P=0.026$) and rs4633 (dominant effect of T-allele: T/T-genotype score 2.42 more than C/C and C/T, 95% CI: 0.53–4.07; $P=0.015$). The P2 promoter region also formed part of a haplotype which was associated with SANS severity (Fig. 2; Supplementary Table S6; 4-marker haplotype rs2020917–rs6269, global $P=0.002$). No SNP was associated with SAPS. None of the relevant SNPs reported here showed significant differences between the sexes in either the patient or control cohorts. Finally, the frequently studied $Val^{108/158}Met$ (rs4680) was not associated with any trait analysed in this study.

Luciferase reporter activity ratios in the hepatic cells were approximately 2% of those detected in the neuroblastoma cells ($P<0.0001$; Supplementary Fig. S2), but the patterns of effect for each individual construct compared to wild type, were similar for these cell lines (Supplementary Fig. S3). We therefore analysed the pattern of effects employing a joint model based on all expression data in both cell lines. Differences in relative expression (effects with 95% confidence intervals) of the individual constructs compared to wild type (construct 1) are displayed in Fig. 3, which illustrates that the construct containing the minor alleles of rs2020917 and rs737865 had the most significant effect on basal gene expression (increase = 11.2%, 95% CI 3.7%–19.2%; $P=0.0031$). Further, the potentially functional P2 SNP, rs2075507, also displayed an increase in expression (increase = 8.0%, 95% CI 0.7%–15.8%; $P=0.0305$).

4. Discussion

4.1. MB-COMT polymorphisms and schizophrenia-susceptibility

Analysis of *MB-COMT* polymorphisms in a homogenous clinical sample of Xhosa schizophrenia patients and matched controls revealed two linked SNPs, rs2020917 and rs737865, which could potentially modify susceptibility to the disorder through affecting the expression of the gene from the distal P2 promoter. The minor alleles of these polymorphisms were underrepresented in the Xhosa schizophrenia cohort, conferring a possible protective effect. The first reported association of the rs737865 SNP with schizophrenia was in the Ashkenazi Jewish population (Shifman et al., 2002); although this study found that rather than having a protective effect, the minor G-allele was associated with schizophrenia-susceptibility in these individuals. Meta-analyses have also detected a similar risk-related effect of the G-allele and the disorder (Okochi et al., 2009; http://www.szgene.org/meta.asp?geneID=420).

Inconclusive data have been obtained for this rs737865 allele with regards to its effect on expression (Bray et al., 2003; Chen et al., 2004a; Ji et al., 2008). We therefore decided to evaluate the respective functionality of rs2020917 and rs737865 in a dopaminergic neuroblastoma cell line and to determine whether tissue specific expression patterns exist (Fig. 3). As was expected, results of our luciferase reporter assay analyses observed significantly higher P2-mediated expression in the neuroblastoma cells compared to the hepatic cells, yet when this difference was adjusted for, a similar pattern for the group of constructs was observed in both cell line experiments. Specifically, the construct containing the minor alleles of rs2020917 and rs737865 showed the most significant effect on expression, causing expression levels 11% higher than the wild type. However, it

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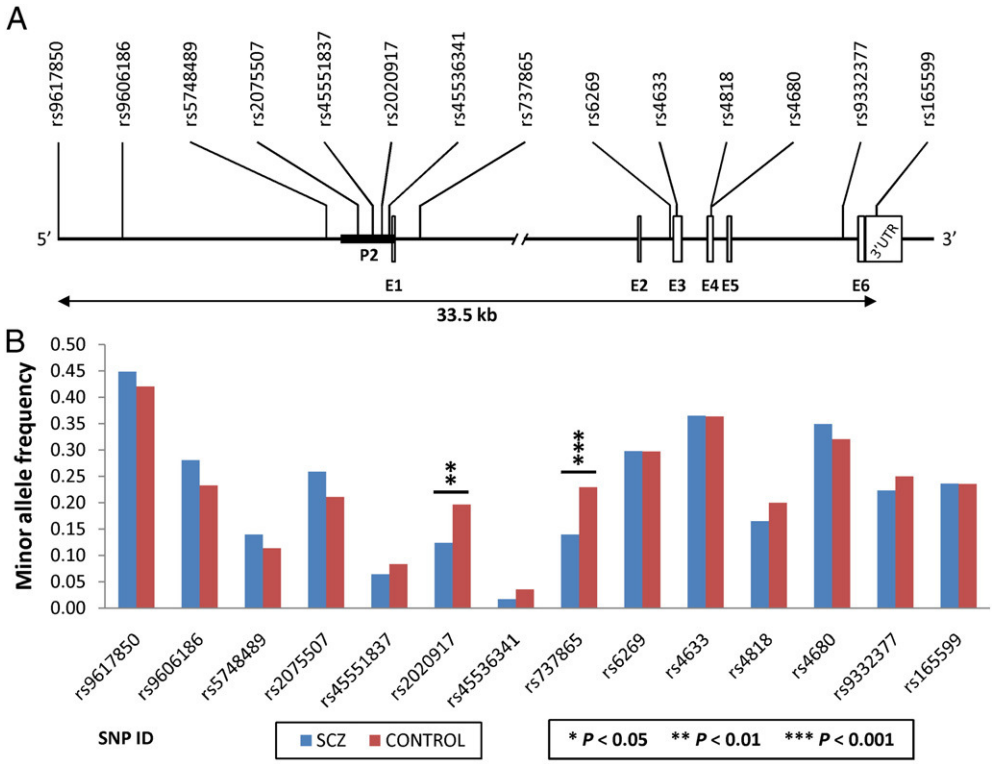

Fig. 1. *MB-COMT* polymorphisms analysed in the Xhosa cohort. (A) Schematic representation of the location of the genotyped *MB-COMT* polymorphisms. (B) Frequencies of the polymorphisms in the Xhosa schizophrenia (blue) and Xhosa control (red) populations, along with respective *P*-values (under an additive genetic model, adjusted for age and gender) of significant associations with schizophrenia. (For interpretation of the references to color in this figure legend, the reader is referred to the web of this article.)

appears that the minor rs737865 G-allele is the main driver of increased expression due to the fact that the construct that exclusively contained the minor rs2020917 T-allele (*i.e.* construct 3) did not show a significantly different expression pattern compared to wild type. Interestingly, the minor alleles of rs2020917 and rs737865 are always linked to the high-activity $Val^{108/158}$ allele, suggesting that the protective effect detected in the Xhosa cohort may be conferred through elevated COMT activities in carriers of these alleles.

4.2. MB-COMT polymorphisms and symptom severity

To assess whether genetic variation of *MB-COMT* affected the severity of schizophrenia symptomatology, in an exploratory study, we assessed SANS and SAPS scores with regards to the genotyped polymorphisms. Three SNPs, one in the P2 promoter (rs45536341) and a further two, which aid in tagging haplotypes that alter mRNA structure (Nackley et al., 2006; *i.e.* intron two rs6269 and synonymous exon three rs4633), were associated with SANS scores (Fig. 2). The P2 promoter SNP was predicted to alter transcription factor binding sites by *in silico* analyses (Supplementary Table S4) and occurred at a low frequency (1.7% in the Xhosa schizophrenia patients). The minor alleles of rs45536341 and rs6269 were associated with lower scores on the SANS, whilst the rs4633 T/T genotype was associated with higher SANS scores. Similar results for the rs4633 SNP were observed in a cohort of Han Chinese schizophrenia patients (Wang et al., 2010), where individuals carrying the rs4633(T)–rs4680(Val) haplotype showed more severe negative symptoms. SANS haplotype analyses in our cohort (Fig. 2), however, implicated the region upstream of rs4633, including the SNPs from the P2 promoter region that were found to be associated with schizophrenia (*i.e.* rs2020917 and rs737865). These analyses yielded the most significant associations with SANS scores and suggest the additional possibility that the genotyped variants may be in LD with unobserved variants that affect severity of the negative symptoms.

The negative symptoms are believed to be associated with prefrontal hypodopaminergia (Guillin et al., 2007) and variants associated with lower or higher SANS may therefore be linked to increased and decreased dopamine levels respectively. Comparable to the study by Wang et al. (2010), no polymorphisms appeared to be associated with SAPS scores. This could have arisen due to the cross-sectional design of this study, as previous work in Xhosa schizophrenia patients has revealed that SANS scores, in contrast to SAPS appraisals, remain relatively constant over time (Niehaus et al., 2005a, 2008). Despite the exploratory nature of our analyses of the symptom dimensions, there has been additional evidence for the involvement of *COMT* genetic variation and negative symptom severity reported in recent studies (Li et al., 2012; Roffman et al., 2011; Wang et al., 2010), providing further support for the findings of the current study.

4.3. Associations in the Xhosa population

African populations, due to their remarkable genetic diversity, display complex levels of genetic structure; yet language has been shown to be a strong predictor of the genetic clustering of these populations (Tishkoff et al., 2009). Our cohort consisted of first-language isiXhosa speakers to minimise the chances of population stratification. Additionally, the Xhosa schizophrenia population is believed to be homogenous, representing a well characterised clinical cohort (Niehaus et al., 2005a). The relationship between *MB-COMT* genetic polymorphisms and schizophrenia in the Xhosa population reflects the complexities of studying the genetic architecture of the disorder. The G-allele of rs737865 was overrepresented in the Xhosa control group, where studies in non-African populations have suggested that this allele may be associated with schizophrenia-susceptibility (Okochi et al., 2009; Shifman et al., 2002; http://www.szgene.org/meta.asp?geneID=420). The so-called "flip-flop" associations can be caused by population-related LD differences, if the genotyped polymorphisms are non-causal (Lin et al., 2007).

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Table 2
Genotype and minor allele counts (frequencies) for the *COMT* SNPs in the Xhosa schizophrenia and control samples. *P*-value, and OR (95% confidence interval) from additive allelic association logistic regression models, adjusted for gender and age.

SNP	*n*	Genotype			Minor allele	*P*-value	OR (95% CI)
rs9617850		G/G	G/A	A/A	A		
Cases	233	69 (29.6)	119 (51.1)	45 (19.3)	209 (44.8)	0.2408	1.18 (0.90–1.55)
Controls	240	77 (32.1)	124 (51.7)	39 (16.3)	202 (42.1)		
rs9606186		G/G	G/C	C/C	C		
Cases	226	120 (53.1)	85 (37.6)	21 (9.3)	127 (28.1)	0.1375	1.26 (0.93–1.72)
Controls	238	135 (56.7)	95 (39.9)	8 (3.4)	111 (23.3)		
rs5748489		C/C	C/A	A/A	A		
Cases	236	175 (74.2)	56 (23.7)	5 (2.1)	66 (14.0)	0.3161	1.23 (0.82–1.85)
Controls	238	185 (77.7)	52 (21.8)	1 (0.4)	54 (11.3)		
rs2075507		A/A	A/G	G/G	G		
Cases	230	127 (55.2)	87 (37.8)	16 (7.0)	119 (25.9)	0.1732	1.25 (0.91–1.73)
Controls	239	144 (60.3)	89 (37.2)	6 (2.5)	101 (21.1)		
rs45551837		G/G	G/A	A/A	A		
Cases	234	205 (87.6)	28 (12.0)	1 (0.4)	30 (6.4)	0.2358	0.74 (0.44–1.22)
Controls	239	200 (83.7)	38 (15.9)	1 (0.4)	40 (8.4)		
rs2020917		C/C	C/T	T/T	T		
Cases	238	183 (76.9)	51 (21.4)	4 (1.7)	59 (12.4)	**0.0011**	**0.54 (0.37–0.79)**
Controls	234	148 (63.2)	80 (34.2)	6 (2.6)	92 (19.7)		
rs45536341		C/C	C/T	T/T	T		
Cases	234	226 (96.6)	8 (3.4)	0 (0.0)	8 (1.7)	0.0694	0.47 (0.19-1.06)
Controls	239	223 (93.3)	15 (6.3)	1 (0.4)	17 (3.6)		
rs737865		A/A	A/G	G/G	G		
Cases	236	176 (74.6)	54 (22.9)	6 (2.5)	66 (14.0)	**0.0002**	**0.52 (0.36–0.74)**
Controls	240	139 (57.9)	92 (38.3)	9 (3.8)	110 (22.9)		
rs6269		A/A	A/G	G/G	G		
Cases	230	113 (49.1)	97 (42.2)	20 (8.7)	137 (29.8)	0.9685	1.01 (0.75–1.34)
Controls	239	119 (49.8)	98 (41.0)	22 (9.2)	142 (29.7)		
rs4633		C/C	C/T	T/T	T		
Cases	230	92 (40.0)	108 (47.0)	30 (13.0)	168 (36.5)	0.9626	0.99 (0.76–1.30)
Controls	239	103 (43.1)	98 (41.1)	38 (15.9)	174 (36.4)		
rs4818		C/C	C/G	G/G	G		
Cases	236	164 (69.5)	66 (28.0)	6 (2.5)	78 (16.5)	0.1318	0.77 (0.54–1.08)
Controls	240	153 (63.8)	78 (32.5)	9 (3.8)	96 (20.0)		
rs4680		G/G	G/A	A/A	A		
Cases	236	95 (40.3)	117 (49.6)	24 (10.2)	165 (35.0)	0.4208	1.12 (0.85–1.50)
Controls	240	110 (45.8)	106 (44.2)	24 (10.0)	154 (32.1)		
rs9332377		C/C	C/T	T/T	T		
Cases	235	141 (60.0)	83 (35.3)	11 (4.7)	105 (23.3)	0.2974	0.85 (0.63–1.15)
Controls	240	142 (59.2)	76 (31.7)	22 (9.2)	120 (25.0)		
rs165599		G/G	G/A	A/A	A		
Cases	235	141 (60.0)	77 (32.8)	17 (7.2)	111 (23.6)	0.9037	0.98 (0.73–1.32)
Controls	240	144 (60.0)	79 (32.9)	17 (7.1)	113 (23.5)		

n, number of samples successfully genotyped.

There has been support for the fact that the haplotypic association generated in this Ashkenazi cohort may have been caused by resultant extended LD (Chen et al., 2004b), especially when considering that the original study to detect an association with this variant was performed in a founder population (Shifman et al., 2002). However, despite analysing 14 SNPs in the Xhosa cohort, no haplotype explained this association better than the single locus analyses. Further, our luciferase reporter assay analyses indicated that the minor rs737865 G-allele could be functional, strengthening the case for this SNP being responsible for the significant association with the disorder observed in this study.

The Xhosa population have undergone similar evolutionary pressures due to their shared history and it has been suggested that brain-enriched genes may be more prone to undergo selection to adapt to unique environmental exposures (Niculescu and Le-Niculescu, 2010). Although speculative, an alternative explanation for the seemingly opposite effect of rs737865 in the Xhosa population could be related to differences in the schizophrenia phenotype, possibly involving dopaminergic profiles, between Xhosa patients and other schizophrenia populations. For example, despite high frequencies of comorbidity of OCD observed in schizophrenia patients of other ethnicities (mean prevalence: 12.1%; Achim et al., 2011), a low prevalence of comorbidity has been observed in Xhosa schizophrenia patients (0.5%; Niehaus et al., 2005b). These data, combined with findings that *COMT* alleles have been strongly associated with obsessive–compulsive disorder in men (Pooley et al., 2007), indicate a possible interaction that should be investigated in future research.

Finally, the following limitations of our study should be noted: (i) although functional analyses were performed, the results reported here should be treated with caution until independently replicated in another indigenous African population and (ii) it is possible the multiple statistical analyses that were performed may have resulted in more than the expected 5% false positive results.

5. Conclusions

The difference in the observed effect of *MB-COMT* SNP alleles on schizophrenia in the Xhosa population warrants further attention, especially since reporter gene studies indicated that the polymorphisms could have a functional impact on gene expression. This study highlights the importance of performing psychiatric genetic studies in African populations, employing comprehensive genotyping panels that include non-coding polymorphisms. This was emphasised by the fact that the $Val^{108/158}$Met polymorphism was not associated with any trait in the Xhosa cohort. Next-generation sequencing should aid in determining whether differences in LD architecture could account for these findings, as it will become feasible to characterise the entire gene locus for novel and known variation. This is especially important for understudied regions (*e.g.* ~19 kb intron one), which may contain undocumented functional variation. The findings from the current study suggest that the relationship between *COMT* genotype and different symptom clusters will provide interesting avenues for future research.

Role of funding source

The funding bodies did not play a role in the study design; collection, interpretation and analysis of data; writing the manuscript; or in the decision to submit this work for publication.

Acknowledgements

The authors would like to thank the Xhosa individuals for participating in this study as well as the sponsor-id="gs1" id="gts0005">South African National Research Foundation (NRF) for providing funding. Additionally, we would like to thank Dr S Bardien (Division of Molecular Biology and Human Genetics, Stellenbosch University) and Ms M Bosman

Please cite this article as: Wright GEB, et al, Association of *MB-COMT* polymorphisms with schizophrenia-susceptibility and symptom severity in an African cohort, Prog Neuro-Psychopharmacol Biol Psychiatry (2012), doi:10.1016/j.pnpbp.2012.06.006

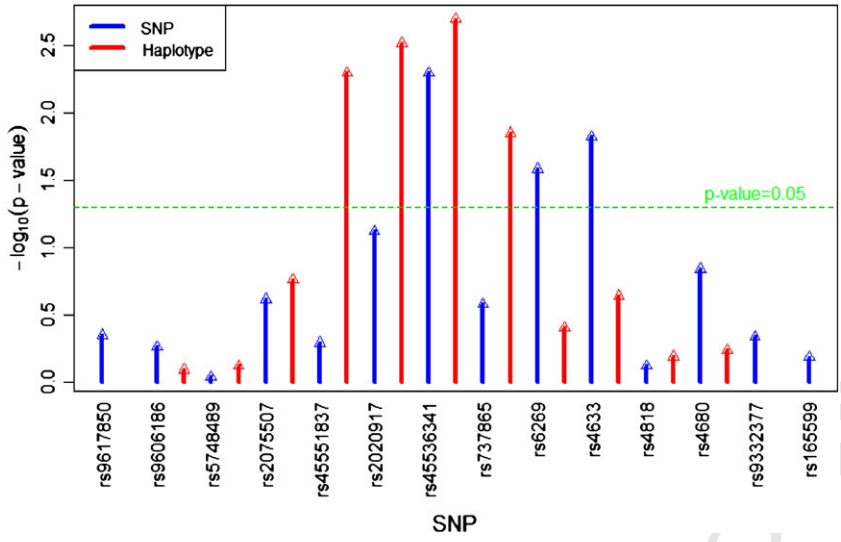

Fig. 2. The analysis of the effect of *MB-COMT* polymorphisms on SANS score in the Xhosa population. Representation of allelic (blue) and four-SNP haplotype (red) association analyses results for the *COMT* polymorphisms and SANS scores, displayed as the negative base 10 logarithm of the respective *P*-values [−log(*P*-value)], adjusted for age, gender, medication type and cannabis use/abuse. *P*-values for haplotype data are plotted in the middle of the relevant four-SNP haplotype. As can be seen in the graph, four-SNP haplotypes covering the distal P2 promoter were additionally found to be associated with SANS scores (most significant four-marker haplotype rs2020917-rs6269, global $P = 0.002$). (For interpretation of the references to color in this figure legend, the reader is referred to the web of this article.)

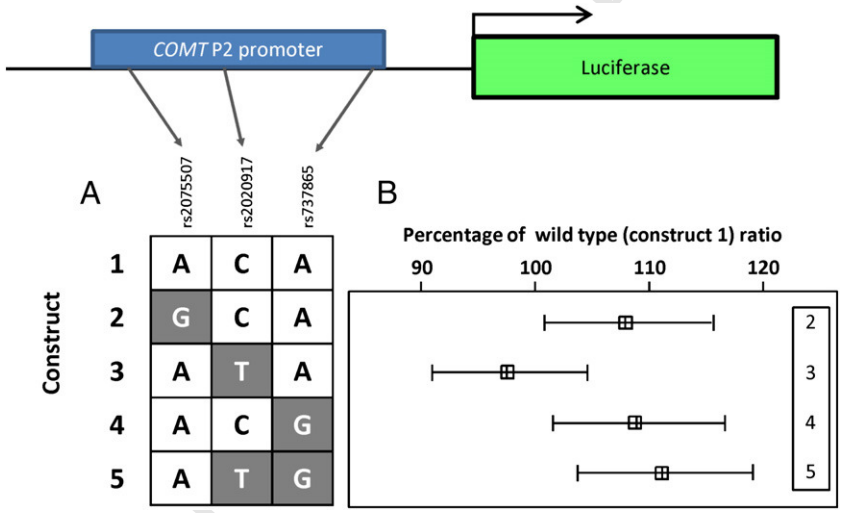

Fig. 3. Functional analysis of the *MB-COMT* P2 promoter variants underrepresented in the schizophrenia cohort (A) pGL4.10 luciferase dual reporter constructs employed to investigate the functionality of the polymorphisms of interest in dopaminergic neuroblastoma BE(2)-M17 cells and hepatic HepG2 cells. Minor alleles are shaded in grey boxes. The rs2020917 T-allele and rs737865 G-allele were overrepresented in the Xhosa control group, whilst the rs2075507 SNP was also included as there is evidence that this polymorphism may be functional (Chen et al., 2004a). (B) Estimated/modelled mean (block) and the simultaneous 95% confidence intervals for the percentage of wild type ratio for the other constructs, independent of cell line. Constructs 2, 4 and 5 showed significant increases in expression, compared to wild type ($P = 0.0305$, 0.0171, 0.0031 respectively).

from the Department of Genetics (Stellenbosch University) for assistance with the tissue culture experiments and reporter gene assays respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pnpbp.2012.06.006.

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Please cite this article as: Wright GEB, et al, Association of *MB-COMT* polymorphisms with schizophrenia-susceptibility and symptom severity in an African cohort, Prog Neuro-Psychopharmacol Biol Psychiatry (2012), doi:10.1016/j.pnpbp.2012.06.006