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Association of the functional polymorphism in the catechol-O-methyltransferase gene with schizophrenia in the three ethnic groups of the Malaysian population

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ABSTRACT

The catechol-O-methyltransferase (COMT) gene is a candidate gene for schizophrenia as its encoded enzyme is involved in the metabolic inactivation of dopamine and noradrenaline. Several molecular genetic studies thus far have demonstrated that the COMT functional polymorphism of Val158Met is susceptible to schizophrenia. Hence, the present study aims to determine this genetic association of this SNP in the three major ethnic groups of the Malaysian population. A total of 317 patients (79 Malays, 154 Chinese and 84 Indians) meeting DSM-IV criteria for schizophrenia and 417 healthy subjects (160 Malays, 164 Chinese and 93 Indians) were recruited. A PCR-RFLP method was used to determine the genotypes and alleles present. We found no significant association of genotypes within the total pooled samples, as well as in the female subgroup, with a higher frequency of heterozygotes in schizophrenia subjects. However, there were no significant differences in allele and genotype frequency between the schizophrenic patients and normal controls in all three ethnic groups. Our current findings suggest that the Val158Met polymorphism has a weak association with schizophrenia in the Malaysian population and does not play a major role in conferring susceptibility to schizophrenia in any of the three major local ethnicities.

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1. Introduction

Catechol-O-methyltransferase (COMT) is one of the major mammalian methylation enzymes that are widespread in the neurons and glia. It is involved in the metabolic degradation of released catecholamine neurotransmitters (including dopamine, epinephrine and norepinephrine) with the S-adenosyl-methionine-dependent methyl transfer reaction (Weinshilboum et al., 1999). As schizophrenia has been suggested to be related to a dysfunction of dopaminergic neurotransmission (Carlsson, 1988), COMT is a potential candidate gene for this disorder. Furthermore, the COMT gene is located at 22q11.2, a region which has shown significant association in several linkage studies relating to schizophrenia (Pulver et al., 1994).

The COMT gene has a functional nonsynonymous single nucleotide polymorphism (SNP), (rs165688/rs4680) which contains a G to A substitution that changes Valine (Val) into Methionine (Met) at codon 108/158 in exon 4. Although this Val to Met substitution is a common polymorphism in humans, it can dramatically affect COMT enzymatic activity (Lotta et al., 1995). The Met form is thermolabile, resulting in a low activity enzyme (COMT-L), with a four-fold reduction in COMT activity and dopamine catabolism compared with the high activity Val form (COMT-H) (Lotta et al., 1995).

The increase and decrease of COMT activity can influence dopamine neurotransmission in the prefrontal cortex. If the dopamine signalling level is too high or too low, it can reduce the performance of frontally mediated cognitive tasks (Costas et al., 2010). Egan et al. (2001) discovered that the high enzyme activity of the Val form could lead to prefrontal cognitive impairment and poorer memory task performance on the Wisconsin Card Sorting Test (WCST). Thus they argued that Val158Met is potentially associated with susceptibility risk for schizophrenia, in which deficits of prefrontal cognitive function are a common feature. This evidence was also supported by subsequent findings (Rosa et al., 2004; Lafuente et al., 2008; Wrigenes et al., 2010) and more recently, the Val allele was found to relate with poorer performance in attention and in detecting interpersonal problems (Döck et al., 2010). The Met allele has been reported to associate with treatment resistance (Illii et al., 2003) and occurrence of hostility in schizophrenia (Volavka et al., 2004), although it has also been related to better performance in verbal IQ and executive function in adolescent boys (Barnett et al., 2007), as well as increased cognitive stability (Bilder et al., 2004).

The association of Val158Met polymorphism with schizophrenia has been extensively carried out in case-control and family-trio studies. However, the results generated so far are somewhat conflicting, in which significant associations were found in Chinese
Handoko et al., 2005) indicated that gender differences in the Val158Met polymorphism played a susceptible risk for schizophrenia.

Hence, we aimed to test the hypothesis that the Val158Met SNP is associated with a susceptibility risk for schizophrenia in the Malaysian population, investigating both the effects of sex and possible differential effects of the three major local ethnic groups.

2. Methods

2.1. Study subjects

A total of 317 schizophrenic patients, comprising 79 Malays (51 males, mean ± S.D. mean age 36.07 ± 11.11 years; 28 females, mean age 38.27 ± 11.01 years); 154 Chinese (88 males, mean age 38.08 ± 11.84 years; 66 females, mean age 42.56 ± 12.43 years) and 84 Indians (42 males, mean age 41.43 ± 10.05 years; 42 females, mean age 47.03 ± 11.27 years) were recruited from the psychiatric clinic of the University Malaya Medical Centre (UMMC). Certified psychiatrists interviewed all the patients directly, while diagnoses were assigned using a standard procedure defined by the Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) criteria. All four grandparents of each subject were of one ethnic origin: either Malay, Chinese or Indian. Schizophrenia-related chronic psychoses, including psychotic disorders due to a general medical condition; substance-induced psychotic disorders; mood disorders with psychotic features; schizophrenia spectrum disorders; schizoaffective disorders; mood and anxiety disorders with psychotic features; schizotypal disorders; schizoid disorders and paranoid personality disorders were excluded. In the present study, a total of 417 healthy controls consisting of 160 Malays (74 males, mean age 23.45 ± 5.30 years; 86 females, mean age 21.88 ± 3.18 years), 164 Chinese (112 males, mean age 30.21 ± 9.92 years; 52 females, mean age 27.05 ± 9.14 years) and 93 Indians (61 males, mean age 41.43 ± 10.05 years; 32 females, mean age 36.13 ± 11.22 years) were collected from volunteers during blood donation campaigns with permission from the Blood Transfusion Unit of UMMC. All participants in the study gave written informed consent corresponding to an Institutional Review Board-approved protocol from the Ethics Committee of UMMC (ethics committee reference number: 609.24).

2.2. Genotyping assays

Genomic DNA was prepared from blood samples through use of the QiaGen DNA Extraction Kit. PCR was carried out using forward oligonucleotide 5′-TCT TGG ACG CCG and reverse primers 5′-AGC GAC CAG TCA GCC GGC-3′ (Bioneer, Seoul, Korea), as described by Yim et al. (2001). Taq polymerase (Vivantis Technology, Shah Alam, Malaysia) was used in 35 amplification cycles of PCR (30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C). Subsequently, the PCR product was digested with MnlI restriction enzymes (New England Biolabs, Hertfordshire, UK) for 3 and 1/2 h at 37 °C. After electrophoresis at 120 V for 35 min, 3 agarose gels containing GelRed™ (Biotium, Hayward, CA) were photographed under UV light. Restriction fragments of 114, 83 and 20 bp revealed the Val allele, whereas in the presence of Met allele, the 114 bp fragment was cut into 96 and 18 bp fragments. Random sequencing assays were undertaken to confirm accuracy of the genotyping.

2.3. Statistical analysis

The association with schizophrenia was tested by comparing genotype and allele frequencies in patients and controls using SPSS software Version 16.0. The two-tailed estimation of significance used in the analyses was defined as P<0.05. We estimated the common odds ratio (OR) with SPSS software programme (Shi and He, 2005). The presence of Hardy–Weinberg equilibrium (HWE) was tested by chi-square test ($\chi^2$) for goodness of fit. Sample power to detect a significant association ($\alpha<0.05$) with effect size index of 0.2 was calculated using the G*Power programme (Faul and Erdfelder, 1992).

3. Results

None of the genotype distribution in the patient and healthy controls of the three ethnic groups deviated from HWE ($P>0.05$), with control subjects exhibiting: Malays: $\chi^2=1.207, d.f.=1, P=0.227$; Chinese: $\chi^2=1.127, d.f.=1, P-value=0.288$; Indians: $\chi^2=0.561, d.f.=1, P=0.454$ and patients: Malays: $\chi^2=0.706, d.f.=1, P-value=0.401$; Chinese: $\chi^2=1.215, d.f.=1, P-value=0.270$; Indians: $\chi^2=2.599, d.f.=1, P-value=0.107$.

The overall results are reported in Table 1, in which significance was detected in the genotype frequencies, although allele frequencies were not significantly different. This reflected fewer Val/Val homozygotes and more Val/Met heterozygotes in the patient group than in the control group. The pooled sample group still demonstrated a significant effect ($P=0.016$) after investigating ethnic stratification by binomial regression with ethnic group included as a covariate during the analysis. Within the female cohort of samples, the effect was found to be significant in terms of genotype frequency (Table 1). Meanwhile, low Met/Met homozygote frequencies were observed in both sex-subgroups of patients and controls.

The results of the allele and genotype frequencies according to the three ethnic groups are presented in Table 2. We found no significant difference in the allele and genotype frequencies between the schizophrenic patients and healthy controls in the Malay, Chinese and Indian ethnic groups; although notably each ethnic group showed the same pattern as seen in the overall sample i.e. greater heterozygote but...
lower homozygote frequencies in the schizophrenia group. The Indians tended to have a higher frequency of homozygous Met/Met (0.14) compared with the Malays and Chinese. The OR results obtained in each ethnic group illustrated that the control group is more likely to carry the Val allele than the schizophrenia group.

4. Discussion

The statistical significant association reported between the Val158Met polymorphism with pooled samples showed that this SNP is associated with elevated risk for schizophrenia in the Malaysian population. This positive correlation of the Val158Met genotype in the female group within our samples contrasts with the studies carried by Kremer et al. (2003), Sazci et al. (2004) and Joo et al. (2005), who previously reported that women who carry the Val allele possessed a greater risk for developing schizophrenia. Gender differences in brain development and sex hormones have been related to vulnerability to schizophrenia (Hoenicka et al., 2010). Moreover, COMT activity reduction in erythrocytes of schizophrenia patients may be influenced by estrogen hormones (Xie et al., 1999), in which women generally have 20–30% lower COMT activity compared to men (Boudikova et al., 1990). Additionally, women who carry the Met allele also demonstrate better performance of executive function and verbal memory than males (Matu et al., 2008). Recently, Coman et al. (2010) found that gender moderated effects of the Val158Met polymorphism during the processing of emotional stimuli in which women activated the cingulate gyrus more often than males. All these indicate that sex hormones could play a factor as to why more females are more vulnerable to schizophrenia.

Our results indicate that the significant association was also primarily driven by an excess of heterozygotes in the schizophrenia group. However, this functional polymorphism might not be the only locus that accounts for increased risk of schizophrenia, as the presence of protective or modifying alleles at other loci or additional variations within the COMT gene might also have an significant influence on the illness. For example, rs737865 (Shifman et al., 2002), rs2075507, rs2097063 (Funke et al., 2005), rs4633 (Handoko et al., 2005) and rs6267 (Lee et al., 2005), as well as two recently identified SNPs of rs2020917 and rs165815 (Pal et al., 2009) in the COMT gene region, have been reported as risk factors for schizophrenia. The high frequency of heterozygotes within the schizophrenia group may reflect the possible influence of other risk loci adjacent to the Val158Met polymorphism. Therefore, vulnerability of the Val158Met polymorphism to schizophrenia might also be manifested by the linkage disequilibrium (LD) with other causal variants, e.g., rs4633–rs4680 (Bray et al., 2003) and rs737865–rs4680–rs4633 (Handoko et al., 2005). The haplotype found by Shifman et al. (2002) has been demonstrated to associate with low expression of COMT mRNA (Bray et al., 2003). Hence, despite the LD effect, it is also plausible that haplotypes of the polymorphisms within the COMT gene contribute to the susceptibility of schizophrenia. The G–A–A–A haplotype which consisted of rs2097063(A/G)–rs737865(A/G)–ValMet(A/G)– rs165815(A/G) (Funke et al., 2005) has revealed a significant association with schizophrenia. The rs2097063–Val158Met has been found to associate with hippocampal gray matter volume, a region where the membrane-bound form of COMT is predominantly expressed (Honea et al., 2009). Hence, we can see that there are many other variants that can come into play within the COMT gene itself when influencing structural brain changes that have been related to schizophrenia. However, it is also conceivable that the Val158Met polymorphism may potentially increase the overall risk for susceptibility to schizophrenia, but other external conditions such as environmental factors and daily life stressors act in a secondary manner to manifest the effect. In addition, significant correlation between Val158Met polymorphism with aggressive behaviour (Strous et al., 1997), homicide tendency (Kotler et al., 1999), positive and negative symptomatology (Goghi and Sponheim, 2008) of schizophrenia indicates that this SNP might have greater effects on schizophrenia symptoms rather than its phenotype.

While we observed a significant association of genotype with the pooled schizophrenic patients, this was not the case in any of the three ethnic subgroups studied. However, the pattern of genotype distribution (i.e., greater heterozygosity in schizophrenia) in the full sample was apparent in the individual ethnicities, indicating that this weak association with schizophrenia is not accounted for by an effect within any one single ethnic group. This is also true within the female sub-population studied here (data not shown). Nor does it indicate that there is any difference in the contribution of the COMT polymorphism to susceptibility to schizophrenia between the ethnic groups, although substantially larger sample sizes would be required to confirm this. The Val allele frequency in the Indian ethnicity (65.1%) is in concordance with the average allele frequency suggested by Fan et al. (2003). Hence, despite the LD effect, it is also plausible that haplotypes of the polymorphisms within the COMT gene contribute to the susceptibility of schizophrenia. The G–A–A–A–A haplotype which consisted of rs2097063(A/G)–rs737865(A/G)–ValMet(A/G)– rs165815(A/G) (Funke et al., 2005) has revealed a significant association with schizophrenia. The rs2097063–Val158Met has been found to associate with hippocampal gray matter volume, a region where the membrane-bound form of COMT is predominantly expressed (Honea et al., 2009). Hence, we can see that there are many other variants that can come into play within the COMT gene itself when influencing structural brain changes that have been related to schizophrenia. However, it is also conceivable that the Val158Met polymorphism may potentially increase the overall risk for susceptibility to schizophrenia, but other external conditions such as environmental factors and daily life stressors act in a secondary manner to manifest the effect. In addition, significant correlation between Val158Met polymorphism with aggressive behaviour (Strous et al., 1997), homicide tendency (Kotler et al., 1999), positive and negative symptomatology (Goghi and Sponheim, 2008) of schizophrenia indicates that this SNP might have greater effects on schizophrenia symptoms rather than its phenotype.

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contradiction with the meta-analysis of Glatt et al. (2003), who found the Val allele to act as a risk factor for individuals of European ancestry. Most notable in respect of the current results are the recent findings of Costas et al. (2010), who also reported an association between COMT heterozygosity and schizophrenia. Their findings from meta-analyses showed that heterozygosity contributed to a weak protective effect, although this was primarily due to the contribution from subjects with Southwest European ethnicity.

Thus many empirical studies of the role of the functional Val158Met polymorphism in schizophrenia still remain controversial. It is possible that differences in the genetic background of the different studies led to these conflicting results. The three ethnic groups from Malaysia are very distinct and unique, i.e., each with their own mongoloid-ancestral background. Malaysian Malays are reported to be of mixed ancestries, such as Acehnese, Arab, Banjarese, Bugis, Javanese and Minangkabau (Sainuddin, 2003); while, the majority of Malaysian Chinese originated from Southern China during the fifteenth, late-eighteenth and early-twentieth centuries (Li et al., 1998). The Indians, on the other hand, migrated from Southern India, Sri Lanka, Pakistan and Bangladesh since the eleventh century (Sindhur, 1993). In a comparison between the allele and genotype frequencies of our Chinese control samples with the Han Chinese from China (Fan et al., 2005), we observed very close correspondence of the Val allele and Val/Val genotype with our findings: 79.5% vs. 73.7% (Val allele) and 59.1% vs. 54.4% (Val/Val genotype). The small differences might be due to genetic selection or drift that occurs during population migration and with adaptation to a new environment.

There are several other limitations in our study that may have influenced our results including unstructured interviews for the diagnosis of the healthy controls, in terms of the history of psychiatry illness and the pharmacy status of their parents, and small sample sizes. The study was adequately powered (at 85%) to identify differences in the combined samples with a small effect size. However, it was not powered to identify other than strong differences within the ethnic subgroups. The low statistical power in the ethnic sub-groups would increase the chances of type II error and thus contribute to the negative results.

The Val158Met polymorphism did not play a major role in the susceptibility to schizophrenia development in the three main ethnic groups of the Malaysian population. However, minor risk effects of this functional polymorphism on schizophrenia cannot be excluded. Thus, more detailed analyses of this functional polymorphism in the COMT gene should involve the use of larger sample sizes, as well as the further assessment of other possible COMT variants.

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