Research report

Catechol-O-methyltransferase gene Val108/158Met polymorphism, and susceptibility to schizophrenia: association is more significant in women

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Abstract

Schizophrenia is a complex disorder with a polygenic inheritance. Catechol-O-methyltransferase (COMT) plays a significant role in the regulation of dopaminergic systems. A polymorphism at COMT Val108/158Met has been identified in association with schizophrenia. We examined the allele and genotype association of the COMT Val108/158Met polymorphism of 297 unrelated schizophrenic patients who strictly met DSM-IV criteria for schizophrenia, and 341 healthy controls. We found significant difference in allele and genotype frequencies between schizophrenic patients and controls ($\chi^2=13.030; P=0.001$). The allele frequency of the COMT-L was 45.79% in the total schizophrenic patients, and 41.50% in controls. The genotype frequency of the COM-LL was 21.2% in the total schizophrenic patients, and 11.4% in controls (OR=2.085; 95% CI=1.350–3.219; $\chi^2=11.293; P=0.001$). With a separate sex analysis, the frequency of the COMT-L allele was moderately distributed in male schizophrenia ($\chi^2=6.177; df=2; P=0.046$). The COMT-LL genotype had a 1.818-fold increased risk for schizophrenia (OR=1.818; 95% CI=1.010–3.273; $\chi^2=4.048; P=0.044$). The frequency of the COMT-L allele was even more significantly distributed in women schizophrenia ($\chi^2=7.797; df=2; P=0.020$). The COMT-LL genotype had remarkably more increased risk for schizophrenia (OR=2.456; 95% CI=1.287–4.687; $\chi^2=7.710; P=0.005$). In conclusion, our results provide strong evidence for a role of the COMT-L allele and LL genotype in the etiopathophysiology of schizophrenia with a sexual difference.

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Topic: Neuropsychiatric disorders
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1. Introduction

Schizophrenia is a complex psychotic disorder with a multiple gene inheritance, and with some genes showing susceptibility at many genetic locations with small penetration, and with a lifetime prevalence of approximately 1%. [27]. Catechol-O-methyltransferase (COMT; E.C. 2.1.1.6) catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. COMT is a candidate gene for schizophrenia due to the fact that it encodes a key dopamine catabolic enzyme and maps to the velocardiofacial syndrome (VCFS) region. The COMT protein is expressed in two distinct forms: a soluble form found in the cell cytoplasm (S-COMT; 221 aa), and a longer, membrane-bound form (MB-COMT; 271 aa). In most tissues, the S-COMT form predominates, is responsible for 95% of the total COMT activity [11,13], whereas the MB-COMT form is the most common form in brain [36]. The COMT gene is polymorphic with a valine-to-methionine (Val/Met) substitution at codons 108 and 158 in the S-COMT
and MB-COMT transcripts, respectively [18]. The Val form (COMT-H) of S-COMT is known to have higher activity and thermostability than the Met form (COMT-L), at least three to four times more [18,21]. The effect of Val–Met substitution on MB-COMT is unknown. The COMT gene is located in chromosome 22q11, a region involved in schizophrenia by linkage studies [4,3,15,25]. And also, a microdeletion in the same chromosomal region is associated with the velocardiofacial (VCFS) syndrome, a syndrome associated with psychotic symptoms in about 20–30% of VCFS cases [24]. Recent studies reported associations between the COMT Val108/158Met gene polymorphisms and Brief Psychiatric Rating Scale (BPRS) scores in schizophrenia subjects [12], and schizophrenia in healthy males [2].

In the present study, we evaluated what role COMTVal108/158Met gene polymorphisms may play in the development of schizophrenia in a case-control study of 297 unrelated schizophrenics with 341 unrelated healthy controls.

2. Materials and methods

The case cohort included 297 unrelated schizophrenic subjects (168 men, 129 women; 41.22±9.43 years) and 341 unrelated healthy controls (192 men, 149 women; 40.94±8.11 years). The cohort was all neuroleptic respondents. The 297 cases were from two outpatient clinics of the Erenkoy Psychiatric and Neurological Disorder Hospital. All participants gave written informed consent after the study had been fully described. The University of Kocaeli Institutional Review Board approved the study. All participants underwent extensive clinical evaluations, which included the Structured Clinical Interview for DSM-IV Personality Diagnosis (SIDP) [30], the Structured Interview for DSM-IV Personality Disorder (SCID) [10], the Structured Interview for DSM-IV Personality Disorder (SIDP) [30], and Family History-Research Diagnostic Criteria (FH-RDC) [1]. All of the patients and controls were unrelated to one other and arose from the same area in Erenkoy, Istanbul, Turkey. Controls were among those who did not have schizophrenia for at least three generation. Patients and controls were sexually matched, and ethnically from the same region.

3. Genotype analysis

A 10-ml peripheral venous blood was collected from all volunteers, and genomic DNA was isolated using the procedure previously described [23]. In the COMT genotype analysis, a 217-bp fragment was amplified using a forward primer 5′-TCG TGG ACC CCG TGA TTC AGG-3′ and a reverse primer 5′-AGG TCT GAC AAC GGG TCA GGC-3′ as described by Yim et al. [40] with some modifications. Subsequently a 10-ul of the 217-bp PCR amplified fragment was further digested by a 5 U of the Hsp 92 II restriction endonuclease (Promega, Madison, WI) overnight at 37 °C. The electrophoresis of the digested fragment was carried out at 20 W for 35 min on 10% polyacrylamide gels followed by silver staining. The gels were scanned using a scanner. Restriction fragments of 114, 83, 20 bp revealed the COMT-H allele, while the 114-bp fragment was cut into 96- and 18-bp fragments in the COMT-L allele.

4. Statistical analysis

Allele and genotype frequencies were compared between cases and controls using the χ² test, and Student’s t-test was employed for age difference. The odds ratio (OR) and 95% confidence intervals (CI) were calculated to evaluate the effects of the different genotypes, and alleles using conditional logistic regression. Significance was set at \( P<0.05 \). SPSS (v11) was used in the statistical analysis. The Hardy–Weinberg equilibrium was verified for all tested populations, and our results were in Hardy–Weinberg equilibrium [16,33].

5. Results

We provide evidence for allele and genotype association between the COMT-L allele, COMT-LL genotype and schizophrenic population from Erenkoy in Istanbul, Turkey.

When we compared them to the control population, we obtained the following data. The COMT allele frequency was significantly distributed (\( \chi^2=13.030; \, df=2; \, P=0.001 \)) between the total schizophrenic cases and healthy controls. The COMT-HL genotype was protective towards schizophrenia (OR=0.641; 95% CI=0.468–0.878; \( \chi^2=7.704; \, P=0.006 \)). The allele frequency of the COMT-L was 45.79% in the total schizophrenic subjects and 41.50% in the controls. The frequencies of the COMT-HH, HL, and LL
genotypes were 29.6%, 49.2%, and 21.2% in the total schizophrenic subjects, and 28.4%, 60.1%, and 11.4% in the healthy controls, respectively (Table 1). After the stratification for both sexes, in men; the COMT Val108/158 Met polymorphism was moderately significantly distributed ($\chi^2=8.206; df=2; P=0.017$). The allele frequency of the COMT-L was 43.15% in the schizophrenia and 41.50% in the controls. The COMT-HL genotype was protective for schizophrenia (OR=2.450; 95% CI=1.451–4.136; $df=1; P=0.003$). The frequencies of the COMT-HH, HL, and LL genotypes were 25.6%, 50.4%, and 24% in the schizophrenia, and 28.4%, 60.1%, and 11.4% in the controls, respectively (Table 2). In women; however, the COMT Val108/158 Met polymorphism was even more significantly distributed ($\chi^2=11.787; df=2; P=0.003$). The frequency of the COMT-L allele was 49.22% in the schizophrenia and 41.50% in the controls. The COMT-LL genotype had an even more increased risk for schizophrenia (OR=2.456; 95% CI=1.451–4.136; $\chi^2=11.712; df=1; P=0.001$). The frequencies of the COMT-HH, HL, and LL genotypes were 25.6%, 50.4%, and 24% in the schizophrenia, and 28.4%, 60.1%, and 11.4% in the controls, respectively (Table 3). When we further stratified the schizophrenic subjects as well as the controls according to the sex differences. We obtained the following data; In men; the COMT-L allele frequency was significantly distributed ($\chi^2=6.177; df=2; P=0.044$). The

### Table 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases Controls</th>
<th>Allele frequency: L of COMT</th>
<th>OR; 95% CI; $\chi^2$; df; P</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>43.15</td>
<td>41.50</td>
<td>$\chi^2=8.206; df=2; P=0.017$</td>
</tr>
<tr>
<td>HH</td>
<td>55</td>
<td>97</td>
<td>1.224; ($0.822–1.825); $\chi^2=0.990; df=1; P=0.320$</td>
</tr>
<tr>
<td>HL</td>
<td>81</td>
<td>205</td>
<td>0.618; ($0.426–0.896); $\chi^2=6.478; df=1; P=0.011$</td>
</tr>
<tr>
<td>LL</td>
<td>32</td>
<td>39</td>
<td>1.822; ($1.095–3.032); $\chi^2=5.431; df=1; P=0.020$</td>
</tr>
</tbody>
</table>

### Table 3

<table>
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<th>Genotype</th>
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<tr>
<td>COMT</td>
<td>49.22</td>
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<td>$\chi^2=11.787; df=2; P=0.003$</td>
</tr>
<tr>
<td>HH</td>
<td>33</td>
<td>97</td>
<td>0.865; ($0.546–1.370); $\chi^2=0.384; df=1; P=0.536$</td>
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<tr>
<td>HL</td>
<td>65</td>
<td>205</td>
<td>0.674; ($0.448–1.013); $\chi^2=3.624; df=1; P=0.057$</td>
</tr>
<tr>
<td>LL</td>
<td>31</td>
<td>39</td>
<td>2.450; ($1.451–4.136); $\chi^2=11.712; df=1; P=0.001$</td>
</tr>
</tbody>
</table>

### Table 4

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<tr>
<th>Genotype</th>
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<th>Allele frequency: L of COMT</th>
<th>OR; 95% CI; $\chi^2$; df; P</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>43.15</td>
<td>41.50</td>
<td>$\chi^2=6.177; df=2; P=0.046$</td>
</tr>
<tr>
<td>HH</td>
<td>55</td>
<td>55</td>
<td>1.212; ($0.774–1.900); $\chi^2=0.707; df=1; P=0.400$</td>
</tr>
<tr>
<td>HL</td>
<td>81</td>
<td>115</td>
<td>0.623; ($0.410–0.947); $\chi^2=4.930; df=1; P=0.026$</td>
</tr>
<tr>
<td>LL</td>
<td>32</td>
<td>22</td>
<td>1.818; ($1.010–3.273); $\chi^2=4.048; df=1; P=0.044$</td>
</tr>
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### Table 5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases Controls</th>
<th>Allele frequency: L of COMT</th>
<th>OR; 95% CI; $\chi^2$; df; P</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>49.22</td>
<td>41.61</td>
<td>$\chi^2=7.797; df=2; P=0.020$</td>
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<tr>
<td>HH</td>
<td>33</td>
<td>42</td>
<td>0.876; ($0.514–1.492); $\chi^2=0.238; df=1; P=0.625$</td>
</tr>
<tr>
<td>HL</td>
<td>65</td>
<td>90</td>
<td>0.666; ($0.413–1.072); $\chi^2=2.811; df=1; P=0.094$</td>
</tr>
<tr>
<td>LL</td>
<td>31</td>
<td>17</td>
<td>2.456; ($1.287–4.689); $\chi^2=7.710; df=1; P=0.005$</td>
</tr>
</tbody>
</table>
COMT-HL genotype was protective towards schizophrenia (OR=0.623; 95% CI=0.410–0.947; \( \chi^2=4.930; df=1; P=0.026 \)) whereas The COMT-LL genotype had a 1.818-fold increased risk for schizophrenia (OR=1.818; 95% CI=1.010–3.273; \( \chi^2=4.048; df=1; P=0.044 \)) (Table 4). In women, the frequency of COMT allele was significantly distributed (\( \chi^2=7.883; df=2; P=0.020 \)). The COMT-LL genotype showed a 2.456-fold increase in risk for schizophrenia (OR=2.456; 95% CI=1.287–4.689; \( \chi^2=7.710; df=1; P=0.005 \)) (Table 5).

6. Discussion

It has been previously reported that a positive association between the COMT-L allele and schizophrenia has been demonstrated by two independent studies [28,29] in which Ohmori et al. [28] showed that The COMT-L allele had a 1.47-fold increased risk for schizophrenia (95% CI=1.04–2.09; \( P=0.028 \)). They also observed a significant difference in genotype distribution (\( P=0.026 \)). The frequency of COMT-L allele was 27% in the healthy Japanese population, and 36% in the schizophrenics. The other study by Park et al. [29] also showed a positive association. The COMT-L allele had a 1.7-fold increased risk for schizophrenia (95% CI=0.9–3.1), when they stratified schizophrenics by family history, they found a 4-fold increased risk for schizophrenia compared with controls. The frequency of the COMT-L allele was 19.90% in the healthy Korean population and 27.18% in the schizophrenics. In our study, the frequency of the COMT-L allele was 41.50% in the total healthy controls, 45.79% in the total schizophrenics, 43.15% in the men schizophrenics, and 49.22% in the women schizophrenics. In Caucasian subjects, it is more than 50% in the healthy population [7,35].

In the present study, the COMT-LL genotype showed a 2.085-fold increased risk for schizophrenia, and alleles were significantly distributed (\( \chi^2=13.030; df=2; P=0.001 \)) (Table 1). The COMT-HL genotype was protective for schizophrenia (OR=0.641; 95% CI=0.468–0.878; \( \chi^2=7.704; df=1; P=0.006 \)). The COMT-L allele and COMT-LL genotype were significantly distributed in men and women schizophrenics (OR=1.818; 95% CI=1.010–3.273; \( \chi^2=4.048; df=1; P=0.044 \); and OR=2.456; 95% CI=1.287–4.689; \( \chi^2=7.710; df=1; P=0.005 \)), respectively (Tables 4 and 5).

The findings of Ohmori et al. [28], Park et al. [29], and together with ours may explain the classic hyper-dopaminergic theory of schizophrenia. In that COMT is implicated in the degradation of dopamine by methylation, low COMT activities could result in a hyperdopaminergic state that has been hypothesized to participate in the pathogenesis of schizophrenia. In the original transmethylation hypothesis, it was suggested that a deficiency in methyl synthesis or methylation may result in schizophrenia [19,32]. These findings are also compatible with the reduced expression of COMT by its deletion that might underlie the increased risk of schizophrenia seen in VCFS. Bray et al. [5] have reported that the haplotype associated with schizophrenia was associated with low COMT expression rather than with high COMT expression. Their results suggested that the haplotype implicated in schizophrenia susceptibility may exert its effect by down-regulating COMT expression. Moreover, Shifman et al. [34] found a highly significant association between COMT and schizophrenia, using a COMT haplotype analysis in a large control sample. In their study, the COMT Val108/158Met polymorphism showed only a modest or no risk effect on schizophrenia, other functional polymorphisms in the COMT gene contributed to disease risk, particularly in women.

Nevertheless, there are a number of studies that failed to show a significant association between COMT and schizophrenia [7,6,8,26,35,38]. It has been previously reported that the COMT Val allele may be associated with schizophrenia [9,17,20,22,39]. The Val allele has been more convincingly associated with reduced performance in tests of frontal lobe function [14,20]. It has been recently reported that the COMT-LL genotype was also associated with hostility [37].

Philippu et al. [31] reported the reduced COMT activity in erythrocytes in schizophrenic patients, they also indicated that COMT activity in erythrocytes is influenced by peripheral factor, such as hormones. Actually, our findings support their observations since COMT-L allele and COMT-LL genotypes are more frequent in women schizophrenics. The frequency of COMT L allele was 49.22% in the women schizophrenics compared to male schizophrenics 43.15%, and healthy controls 41.50%. Moreover, The frequency of COMT-LL genotype was 24% in women schizophrenics, 19% in male schizophrenics, and 11.4% in the healthy controls (Tables 1–3). In women, COMT is required for the degradation of dopamine concomitantly. Hence, the individuals with the COMT-LL genotype may not remove excess amount of catecholestrogens and dopamine. As a consequence, the low COMT activities could result in hyperdopaminergic and hypercatecholestrogenic state that may be involved in the pathogenesis of schizophrenia and even breast cancer.

In conclusion, hence, the COMT-L allele, and COMT-LL genotype are genetic risk factors for schizophrenia in Turkey. The extent of the association was even more pronounced in women schizophrenia.

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References


