No association between Val158Met of the \textit{COMT} gene and susceptibility to schizophrenia in the Syrian population

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\textbf{Abstract}

\textbf{Background:} The Val158Met single nucleotide polymorphism of the \textit{COMT} gene has been implicated in the aetiology of schizophrenia, although results from different populations have been conflicting. \textbf{Aims:} The aim of the present study was to investigate possible association between schizophrenia and Val158Met in a novel Arab population from Syria. \textbf{Methods and Materials:} 71 unrelated schizophrenic subjects (45 men) and 102 unrelated healthy controls (62 men) were recruited to take part in this case-control study. The Val158Met of the \textit{COMT} gene was genotyped for patients and controls, using a new optimized PCR-RFLP method. \textbf{Results:} the results demonstrated that there is no statistically significant difference between the two groups. \textbf{Conclusion:} This study does not support that Val158Met has an influence on susceptibility for schizophrenia in this population.

\textbf{Keywords:} Schizophrenia, Polymorphism, COMT, Catechol-O-Methyltransferase, Val158Met.

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\textbf{Introduction}

The heritability of schizophrenia is estimated to be around 80\%, with around 10-fold risk increase in first degree relatives \cite{1}. It is believed that the genetic predisposition to schizophrenia is related to a number of low-penetrance variants, with these interacting with environmental factors. The current view regarding the pathophysiology of schizophrenia strongly implicates the dysfunction in the dopaminergic neurotransmitter system. The catechol-O-methyl-transferase (\textit{COMT}) gene is one of the most significant candidate genes for schizophrenia, since \textit{COMT} catalyzes the transfer of the methyl group of S-adenosyl-l methionine (AdoMet) to the catecholamine neurotransmitters, and thus inactivating them. The human gene that encodes \textit{COMT} is located on the chromosome 22q11 \cite{2}. This region is affected with a microdeletion in the velocardiofacial syndrome, a condition that is frequently associated with schizophrenia. Two co-dominant alleles (G and A) in exon 4 of the \textit{COMT} gene influence the amino acid structure (Val or Met) at codon 158. The \textit{COMT} enzyme activity is genetically polymorphic with a trimodal distribution (high activity in Val/Val genotype, intermediate activity in Val/Met genotype, and low activity in Met/Met genotype). The difference in \textit{COMT} activity is three- to four-fold (Val/Val vs. Met/Met).

The results of the studies on the Val158Met polymorphism of the \textit{COMT} gene are conflicting, with a number of studies suggesting a possible effect of the Val158Met polymorphism in vulnerability to schizophrenia \cite{3-10},...
and others showing no association [11-18].

The aim of this study was to utilise a homogeneous Syrian case-control sample of categorically-defined Schizophrenia to investigate its association with the Val158Met polymorphism of the COMT gene.

**Subjects and Methods**

**Study Cohort**

The study cohort consisted of 71 unrelated schizophrenic subjects (45 men, 26 women; 37± 10 years) and 102 unrelated healthy controls (62 men, 40 women; 40 ± 10 years). Patients were recruited from the Ibn Kaldoon hospital in Aleppo, Syria. All patients met the DSM-IV diagnosis for schizophrenia. Appropriate ethical and governance permission was obtained from the local authorities prior to blood sample collection. Patients and controls were gender-matched and were all from the same ethnic background.

**Genotype Analysis**

The Val158Met of the COMT gene was genotyped by a new optimized PCR-RFLP method using the restriction enzyme NlaIII. A 108 bp fragment containing the single nucleotide polymorphism studied was amplified using the PCR method. The PCR reaction was carried out in a total volume of 20µl containing 100-150ng genomic DNA as the template, 0.5 µM of each primer (synthesized by VBC-Biotech, Austria), 2.3mM MgCl2, 200µM of each dNTP. 1X Taq buffer (10 mM Tric-HCl pH 8.4, 50 mM KCl) and 0.75 units of Taq DNA polymerase (Fermentas, Lithuania). PCR amplification was carried out in a MasterCycler® thermal cycler (Eppendorf, Germany) with an initial denaturation step at 94 °C for 5 minutes followed by 31 cycles of 94 °C for 30 seconds, 62 °C for 30 seconds and 72 °C for 10 seconds, and a final extension step at 72 °C for 5 minutes. The primer sequence for the forward and reverse primers were 5’-CGAGGCTCATCACCAGTAGTC-3’ and 5’-CTGACACGGGTCCAGGAATGCA-3’, respectively. The PCR product was digested with NlaIII (FastDigest® NlaIII enzyme, Fermentas®, Lithuania) according to the manufacturer instructions. The resulting fragments were separated on agarose gels (2.5%). Digestion of the amplified fragment with NlaIII showed 3 bands in heterozygotes (108, 72 and 36 bp). The amplified fragment remained intact in Val homozygotes after digestion with the restriction enzyme, with agarose gel electrophoresis showing a single 108 bp band. In Met homozygotes 2 bands were produced (72 and 36 bp).

**Results**

Genotypes in the patient and control populations were in Hardy-Weinberg equilibrium (for patients: χ²=1.18, df=2 ; p=0.55 ;and for controls: χ²=0.03; df=2 ; p=0.98). As summarized in Table 1, no statistically significant difference was observed in genotypic distribution or allele frequencies between the total patients and controls (χ²=0.905, df=2, p=0.64 and χ²=0.001, df=1, p=0.97, respectively). Analysis by sex was avoided due to the unavailability of sufficient number of samples. However, Table 1 shows that heterozygosity is higher in the male patients group (62.2%) than that in the control group (49%) (OR (95%) =1.7, CI (0.83-3.5), χ²=2.19, df=1, p=0.13).

**Table 1** Genotypic distributions and allele frequencies of the Val158Met polymorphism of the COMT gene.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Genotype (%)</th>
<th>Allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia</td>
<td>71</td>
<td>Val/Val (19.7)</td>
<td>Val (40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Val/Met (56.3)</td>
<td>Met (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met/Met (23.9)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>Val (28)</td>
<td>42 (50)</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>Val (12)</td>
<td>26 (50)</td>
</tr>
<tr>
<td>Control</td>
<td>102</td>
<td>Val (29)</td>
<td>96 (50)</td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>Val (30)</td>
<td>58 (66)</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>Val (20)</td>
<td>38 (47)</td>
</tr>
</tbody>
</table>

No significant difference was observed in genotypic distribution or allele frequencies between the patients and controls (χ²=0.905, df=2, p=0.64 and χ²=0.023, df=1, p=0.88, respectively).

**Discussion**

Few studies have reported a positive association between the COMT allele and schizophrenia [5, 13]. Ohmari et al. [5] 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. [13] 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. On the contrary, other studies reported that the COMT-H allele may be associated with schizophrenia [14, 15]. Wondoi et al. [14] found that the frequency of the COMT-H allele was significantly higher in schizophrenia cases compared to controls (62.0% vs. 50.6%; P=0.043). A meta-analysis by Glatt et al. [15] showed significant association between the COMT-H allele and schizophrenia in European populations (odds ratio=2.2, 95% CI=1.4–3.4, P=0.001).

To our knowledge, this is the first study to investigate the association between the Val158Met polymorphism of the COMT gene and schizophrenia in a Syrian population. This study failed to demonstrate any statistically significant genetic association between neither the COMT-L allele nor the COMT-H allele and schizophrenia. These negative results of our study are in agreement with several other studies and meta-analyses [16-22].
Although the results do not support the hypothesis that a link exists in this population, the higher heterozygosity in the male patients group than that in the control group suggests that heterozygosity is a susceptibility factor in males. This is in sharp contrast with a recent paper suggesting that heterozygosity has a protective effect against schizophrenia based on the overdominance modal [9]. One possible cause of our results is type 2 error, due to a relatively small sample size. Hence, replication in a larger sample, especially for males, may be warranted.

**Conclusion**

We investigated the role of Val158Met polymorphism of the COMT gene in Schizophrenia susceptibility in an Arab population from Syria. The overall results indicate little effect of the studied polymorphism on the susceptibility for developing schizophrenia in the population studied.

**References**