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No association between Val158Met of the *COMT* gene and susceptibility to schizophrenia in the Syrian population

Bassam Lajin[1*] , Amal Alachkar[2*] , Abdul Rezzak Hamzeh[3], Roula Michati[4], Hamid Alhaj[5]

Departments of Analytical Chemistry[1], Pharmacology[2] and Biochemistry[3],
Faculty of Pharmacy, University of Aleppo, Aleppo, Syria.
[4]Ibn Khaldoon Psychiatric Hospital, Aleppo, Syria.
[5]Academic Psychiatry, Newcastle General Hospital, Newcastle NE4 6BE, United Kingdom.
*These authors contributed equally to this work as first authors.

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Abstract

Background: The Val158Met single nucleotide polymorphism of the *COMT* gene has been implicated in the aetiology of schizophrenia, although results from different populations have been conflicting. **Aims:** The aim of the present study was to investigate possible association between schizophrenia and Val158Met in a novel Arab population from Syria. **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 *COMT* gene was genotyped for patients and controls, using a new optimized PCR-RFLP method. **Results:** the results demonstrated that there is no statistically significant difference between the two groups. **Conclusion:** This study does not support that Val158Met has an influence on susceptibility for schizophrenia in this population.

Keywords: Schizophrenia, Polymorphism, COMT, Catechol-O-Methyltransferase, Val158Met.

Correspondence to: Bassam Lajin, Department of Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria. Email: BassamL7@yahoo.co.uk

Introduction

The heritability of schizophrenia is estimated to be around 80%, with around 10-fold risk increase in first degree relatives [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 (*COMT*) gene is one of the most significant candidate genes for schizophrenia, since 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 COMT is located on the chromosome 22q11 [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 *COMT* gene influence the amino acid structure (Val or Met) at codon 158. The 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 COMT activity is three- to four-fold (Val/Val vs. Met/Met).

The results of the studies on the Val158Met polymorphism of the *COMT* gene are conflicting, with a number of studies suggesting a possible effect of the Val158Met polymorphism in vulnerability to schizophrenia [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 Khaldoon 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 ethnical background.

Genotype Analysis

The Val158Met of the *COMT* gene was genotyped by a new optimized PCR-RFLP method using the restriction enzyme *Nla*III. 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 ^{o}C for 5 minutes followed by 31 cycles of 94 ^{o}C for 30 seconds, 62 ^{o}C for 30 seconds and 72 ^{o}C for 10 seconds, and a final extension step at 72 ^{o}C for 5 minutes. The primer sequence for the forward and reverse primers were 5′-CGAGGCTCATCACCATCGAGATC-3′ and 5′-CTGACAACGGGTCAGGAATGCA-3′, respectively. The PCR product was digested with *Nla*III (FastDigest® *Nla*III enzyme, Fermentas®, Lithuania) according to the manufacturer instructions. The resulting fragments were separated on agarose gels (2.5%). Digestion of the amplified fragment with *Nla*III 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: $\chi2$=1.18; df=2 ; p=0.55 ;and for controls: $\chi2$=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 ($\chi2$=0.905, df=2, p=0.64 and $\chi2$=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), $\chi2$=2.19, df=1, p=0.13).

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

	n	Genotype (%)			Allele (%)	
		Val/Val	Val/Met	Met/Met	Val	Met
Schizophrenia	71	14 (19.7)	40 (56.3)	17 (23.9)	68 (47.9)	74 (52.1)
Male	45	7 (15.6)	28 (62.2)	10 (22.2)	42 (46.7)	48 (53.4)
Female	26	7 (26.9)	12 (46.1)	7 (26.9)	26 (50)	26 (50)
Control	102	23 (22.6)	50 (49)	29 (28.4)	96 (47.1)	108 (52.9)
Male	62	14 (22.5)	30 (48.3)	18 (29)	58 (46.7)	66 (53.3)
Female	40	9 (22.5)	20 (50)	11 (27.5)	38 (47.5)	42 (52.5)

No significant difference was observed in genotypic distribution or allele frequencies between the patients and controls ($\chi2$=0.905, df=2, p=0.64 and $\chi2$=0.023, df=1, p=0.88, respectively).

Discussion

Few studies have reported a positive association between the COMT-L 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 heterozugosity 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.

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