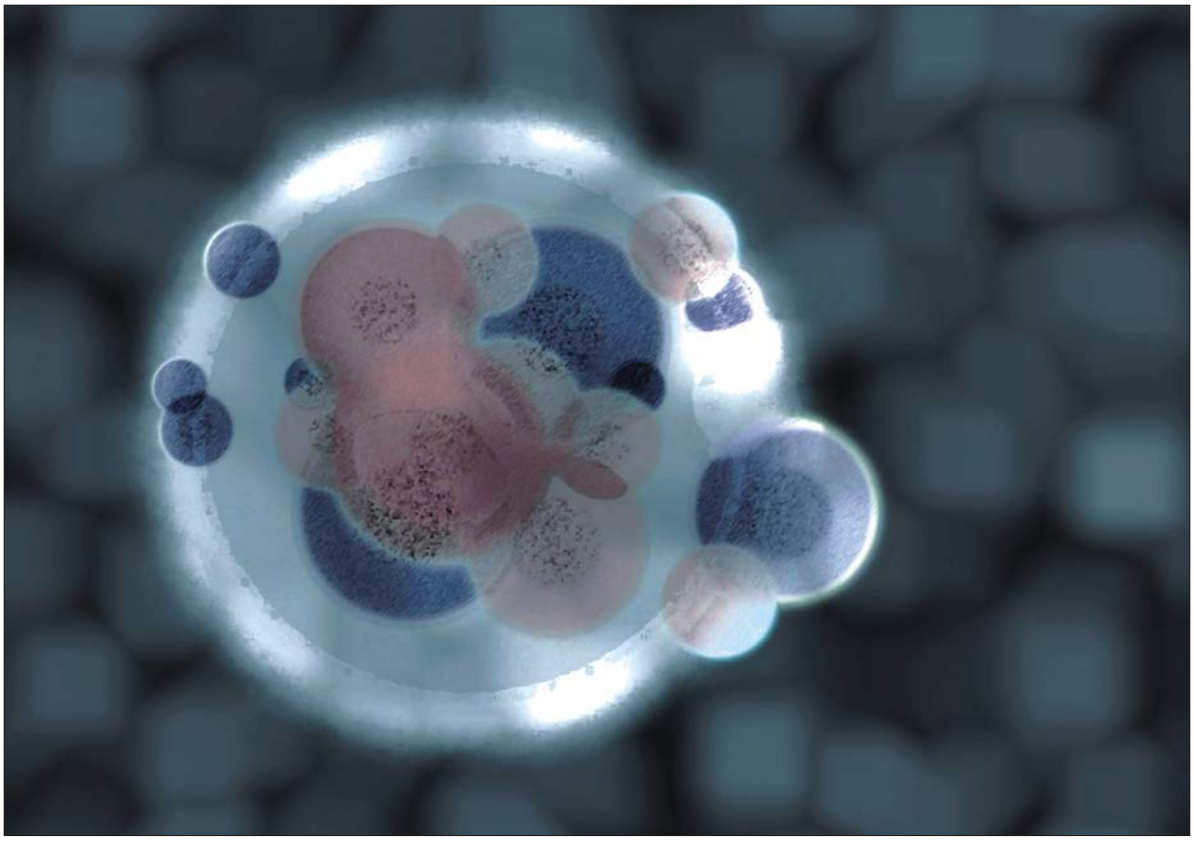

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Genotype Distribution of the Single Nucleotide Polymorphism Val158Met of the *COMT* Gene in the Syrian Population

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Abstract: The transition from G to A at nucleotide 21881 of the *COMT* gene is a functional single nucleotide polymorphism (Val158Met). The produced enzyme which contains Methionine in place of Valine has lower enzymatic activity that has been associated with greater risk for developing psychiatric disorders. The aim of the present study was to determine genotype distribution of the Val158Met polymorphism in a population from Syria as it was not previously determined for a population from the Middle East region. The Val158 Met of the *COMT* gene has been genotyped for a population from Aleppo consisting of 102 healthy subjects randomly selected using a novel optimized RFLP based method. 22.6% of the individuals were found to be homozygous for the Val allele, 28.4% of the individuals were found to be homozygous for the Met allele, while 49% of the individuals were found to be heterozygous. Allele frequencies were calculated and found to be 47 and 53% for the Val and Met allele, respectively. The calculated frequencies were compared to other populations and were found to be closest to those of the Caucasian populations and farthest from those of the East Asian and African populations.

Key words: Catechol-o-methyltransferase, human polymorphism, SNP, Val158/108met

INTRODUCTION

Catechol-O-methyltransferase (COMT) is a widespread enzyme that catalyzes the transfer of the methyl group of S-adenosyl-l methionine (AdoMet) to the catechol substrate. High COMT activity is found in the liver, kidney, gut wall and the brain (Axelrod, 1957; Guldberg and Marsden, 1975). A single *COMT* gene codes for two separate enzymes, soluble (S-COMT) and membrane bound (MB-COMT) forms. S-COMT consists of 221 amino acids. MB-COMT has an additional amino-terminal extension of 50 amino acids in humans (Bertocci *et al*., 1991; Lundstrom *et al*., 1991; Mannisto and Kaakkola, 1999). The human gene for COMT is located at the chromosome 22q11 (Grossman *et al*., 1992). The level of COMT enzyme activity is genetically polymorphic in human tissues. A common polymorphism in the *COMT* gene that has been extensively typed for association studies is the exon 4 functional variant, a G to A nucleotide transition that results in an alteration of the amino acid from Val to Met in the protein. (at codon 108 of S-COMT and codon 158 of MB-COMT). This change in a single amino acid from Val to Met results in a decrease of 67-75% in enzyme activity (Lotta *et al*., 1995; Lachman *et al*., 1996) and is referred to by the L (low activity) allele, in contrast to the high activity allele H. COMT is an obvious candidate gene for a number of neurologic disorders that involve noradrenergic or dopaminergic systems. The case-control association design has been used to study its possible role in many neurological disorders including Parkinson's Disease (PD) (Hoda *et al*., 1996; Kunugi *et al*., 1997; Syvanen *et al*., 1997), Obsessive-Compulsive Disorder (OCD) (Karayiorgou *et al*., 1997), schizophrenia (SZ) Chen *et al*., 1997; Daniels *et al*., 1996; Ohmori *et al*., 1998; Karayiorgou *et al*., 1998), unipolar affective disorder (UPD) (Ohara *et al*., 1998) and bipolar affective disorder (BPD) (Li *et al*., 1997).

The important single nucleotide polymorphism has been genotyped in all population around the world except for populations in the Middle East region (Palmatier *et al*., 1999).

MATERIALS AND METHODS

The research has been conducted at the university of Aleppo, Faculty of Pharmacy from March 2009 to December 2009. The research was fully funded by the university of Aleppo.

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Subjects: The population studied consisted of 102 white healthy subjects living in Aleppo, Syria. The participants did not have any history of neurologic disorders. All subjects were native Syrians. Informed consent was obtained from each subject. Blood Samples were collected in EDTA tubes and anonymously coded and stored.

RFLP detection of Val158Met: We have developed an optimized novel protocol for the detection of Val158Met based on the restriction fragment length polymorphism Method (RFLP) using *Nla*III as the restriction enzyme. 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 40 μL containing 100-150 ng genomic DNA as the template, 1 μM of each primer (synthesized by VBC-Biotech, Austria), 1.8 mM $MgCl_2$, 75 μM of each dNTP, 5% DMSO, 1X Taq buffer (10 mM Tric-HCl pH 8.4, 50 mM KCl) and 1.5 units of Taq DNA polymerase (Fermentas, Lithuania). The PCR amplification was carried out in a MasterCycler® thermal cycler (Eppendorf, Germany) with an initial denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 10 sec and a final extension step at 72°C for 5 min. The primer sequences for the forward and reverse primers were 5'-CGAGGCTCATCACCATCGAGATC-3' and 5' CTGACAACGGGTCAGGAATGCA-3'. 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 AND DISCUSSION

A total of 102 native subjects living in Aleppo City were genotyped for the Val158Met COMT polymorphism. Among the 102 subjects studied, 50 (49%) were found to be heterozygous, 23 (22.6%) were found to be homozygous for the Val allele and 29 (28.4%) were found to be homozygous for the Met allele.

Allele frequencies were found to be 53 and 47% for the Met allele and the Val allele, respectively.

The results obtained following genotype distribution determination of the Val158Met polymorphism in the sample population from Syria show a balance between the Met allele and the Val allele in contrast to the populations from Eastern Asia (Japanaese and Chinese) and Africa where the Val allele is clearly dominant (over 70%). On the other hand, the results seem to be in homology with those previously reported for the Caucasian populations (48% for the Val allele and 52% for the Met allele). (Palmatier *et al*., 1999).

The study can serve as a control study for association studies between the Val158Met and different cases involving neurologic disorders in the Syrian population and probably in the Middle East region (Syria, Lebanon, Jordan, Iraq) given the fact that the all the native Middle Eastern populations are of the same origin.

The suggested protocol for the detection of Val158Met shows clear advantages over the RFLP protocols commonly used in the literature (Strous *et al*., 2006; Doyle *et al*., 2004; Harris *et al*., 2005). The difference in length between the distinctive fragments in the suggested protocol (36 bp) is significantly larger than that found in the RFLP protocols commonly used in the literature (18 bp) which is a result of the elimination of the constant recognition sequence of *Nla*III (CATG) found in close proximity to the SNP studied using the novel primers designed (Fig. 1).

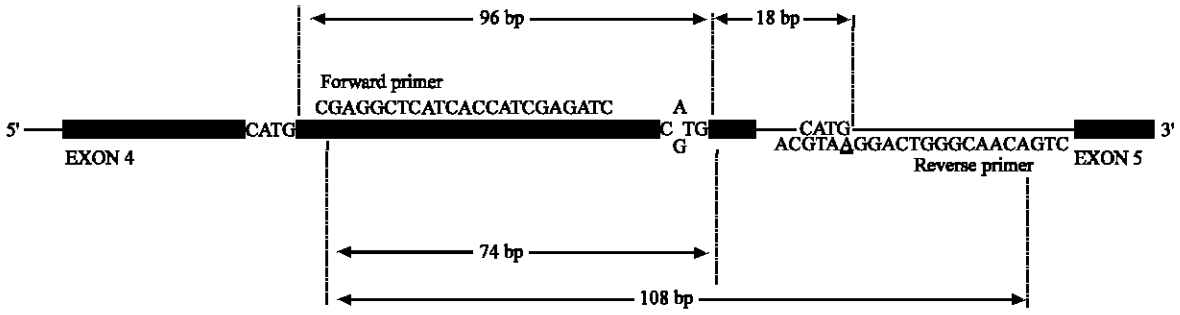

Fig. 1: A schematic illustration of the *COMT* gene showing the location of *Nla*III recognition sequences (CATG) relative to primer annealing sites. Fragments are not drawn at scale. The underlined base indicates the introduced mismatch. Exons are shown as black boxes whereas introns are shown as lines

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