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TECHNICAL NOTE

PSYCHIATRY

Aysun Baransel Isir,[1] *M.D.; Alper Ibrahim Dai,*[2] *M.D.; Muradiye Nacak,*[3] *M.D.; and Senay Gorucu,*[4] *Ph.D.*

Study: The Lack of Significant Association of the Catechol-*O*-Methyl Transferase (COMT) Gene Polymorphism in Violent Offenders with Mental Retardation

ABSTRACT: Little is known about criminality of cognitively impaired people and also there have been no reports on the relationship between catechol-*O*-methyl transferase (COMT) and committed Mental Retardation (MR) subjects. In the present study, the association between committed (violent offences) MR subjects and genetic variants of COMT were investigated by using polymerase chain reaction and based restriction fragment length polymorphism methods. During 6 years of follow-up, 36 violent offenders with mild MR were investigated. Thirty-six control volunteers were included in the study as a control group. H/L polymorphism of the COMT gene was investigated in these two groups. In conclusion, the COMT gene genotype distribution and allele frequency is not significantly different between the two groups ($p > 0.05$). This result suggests that the H/L polymorphism of the COMT gene does not show an association with the potential of "commits-violent offense" of Turkish subjects with mental retardation, compared with control group.

KEYWORDS: forensic science, catechol *O*-methyl transferase, DNA polymorphism, polymerase chain reaction, committed, mental retardation

Mental retardation (MR) is one of the most common neuropsychiatric disorders observed among children and adolescents, the etiopathogenesis of which is not completely understood, but which is defined as a weakening in cognitive and adaptive functions (1,2). In the Turkish Penal System a penalty shall not be imposed upon a person who, due to mental disorder, cannot comprehend the legal meaning and/or the consequences of the act he/she has committed, nor shall a penalty be imposed upon a person whose ability to control his/her behavior has been significantly diminished in respect to committing such act. The penalty to be imposed may be reduced when a person's inability to control his/her behavior has a direct relationship to that act. Whether the penalty shall be imposed or be reduced is also dependent upon the severity of the MR. For that reason, MR criminal offenders in Turkey are sent by the Court or Public Prosecutor for clinical evaluation and a determination of their ability to control their own behavior.

Many responsible genes have been found in the detailed analysis of MR. Due to the complex genetics of MR, a system has been developed for classification, by biological function, of the genes responsible for each specific irregularity. A few of the functions included in this classification are metabolic pathways, signal transmission pathways, and transcription. One of the metabolic pathways, catecholamine, plays an important role in the intelligence process. Catechol-*O*-methyl transferase (COMT) is the major enzymatic inactivator of the neurotransmitters: dopamine, epinephrine and norepinephrine (catecholamines) (3, 4). The COMT gene is located on band 22 q11.1 to 22 q11.2 of chromosome 22. There is a common single-nucleotide polymorphism (SNP) in codon158 with valine-to-methionine substitution (Val-158-Met) (5–7).

Studies have shown that carriers of the Met/Met genotype (L/L) have approximately three- to four-fold lower enzyme activity in comparison to carriers of the Val/Val genotype (H/H), whereas Val/Met genotypes (H/L) indicate an intermediate activity enzyme (7–9). This functional polymorphism in the COMT gene plays an important role in disorders like chronic pain (10), obsessive-compulsive disorder (11,12) and bipolar disorder (13,14) in individuals with aggressive and anti-social behavior (15) as well as individuals who have attempted suicide (16), in the pathogenesis of Parkinson's disease (17), in schizophrenia (18–25), in alcohol dependency (26), and in anorexia nervosa (27). Variability according to gender, as well as ethnical difference relative to the above-mentioned disorders was also shown (16,28). Evidence also shows that COMT is related to healthy brain functioning and brain disorders and that COMT activity might be regulated in different parts of the brain, depend upon dopamine and norepinephrine amounts and, for that reason, might be associated with mental disorders. Therefore, in investigations into the effect of this polymorphism on patients with MR, it is important to find the cause of some psychiatric diseases.

Intensive genetic research has revealed the relationship between COMT polymorphisms and the cognitive ability of people with psychiatric mental disorders (16,29–32). Studies have shown that

[1]Department of Forensic Medicine, Faculty of Medicine, University of Gaziantep, Gaziantep-Turkey.
[2]Department of Pediatrics, Faculty of Medicine, University of Gaziantep, Gaziantep-Turkey.
[3]Department of Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep-Turkey.
[4]Department of Medical Biology and Genetics, Faculty of Medicine, University of Gaziantep, Gaziantep-Turkey.

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genetic components to some extent underlie behavioral disorders such as impulsive aggression and violence. However, the characteristics and criminal behavior in mentally retarded individuals remains largely unstudied. In the current literature, there has been no report dealing with polymorphism of the COMT gene in committed MR subjects. Under the light of this information, we hypothesize that there may be association between violent offenders with MR and genetic variants of COMT gene.

In this study, the association between violent offenders with MR and genetic variants of COMT were investigated by using polymerase chain reaction (PCR) and based restriction fragment length polymorphism (RFLP) methods. We investigated a total of 36 violent offenders with mild MR and tried to find out if there is an association between H/L polymorphism of the COMT gene and the potential of "commits-violent offense" individuals with MR, in comparison to control group.

Materials and Methods

During the 6 years (between 1999 and 2005) of follow-up, we identified 36 violent offenders with mild MR. Subjects offended against the person, especially against life or physical integrity, with intentional injury.

Thirty-six violent offenders with mild MR (11 female and 25 male) and 36 control subjects (16 female and 20 male) were included in the study. All criminal offenders were sent by the Prosecutors to the Department of Forensic Medicine Medical Faculty Gaziantep University for the purpose of assessing the legal aspects of their case/cases as they relate to punishment to be administered. Control subjects were selected from healthy volunteers. Formal consent forms were obtained from all subjects participating in the study. The violent offenders with MR and healthy control subjects were all from the same Turkish geographic regions and were all of the same Turkish ethnic origin. The mean age for the MR subjects was 13.58 years, and for the control group, 14.34 years. After a complete description of the study was explained to the subjects, written formal consent was obtained. Individuals with a known genetic cause of retardation such as Fragile-X, Down's syndrome, etc. were excluded the study.

The pediatric neurology and forensic medicine specialist interviewed each subject, and also consulted to child psychiatry. Subjects were tested with the Turkish-Wechsler Intelligence Scale for Children (WISC-R) by psychological experts. The subjects were screened using the Adaptive Scale of Infant and Children (1998). Using these scales, each subject was given a social adaptive score or a mental handicap score. The MR diagnosis standard was recommended by the WHO in 1995. The conclusive diagnosis of MR was made on the basis of interviews, physical examination, and medical records in accordance with DSM-IV diagnostic criteria. Blood samples were taken to obtain complete blood count and blood chemistry as well as to determine the molecular analysis of the COMT gene polymorphism.

The subjects had no comorbid major medical illnesses and no psychiatric illnesses other than MR. The control subjects also were applied the above-mentioned formal intelligence testing to make sure they were not mildly retarded. The mean IQ scores for the MR subjects was 60.64 and for the control group, 92.45.

Genomic DNA was extracted from peripheral blood leucocyte and a PCR-based RFLP assay was performed to detect the presence of the G > A transition at position 1947 in the COMT gene (gene map locus: chromosome 22 q11.2, G/A 1947 polymorphism in HSCOMT gene/gene bank accesion number Z26491- dbSNP: rs165388). PCR was used to amplify a 185 bp fragment of genomic DNA containing the polymorphism.

PCR product was amplified by using the oligonucleotid primers 5′-GGAGCTGGG GGCCTACTGTG-3′ (forward) and 5′-GGCC-CTTTTTCCAGGTCTGACA-3′ (reverse). PCR was performed in a 50 μL volume with 20–100 ng DNA, 100 μm dNTPs, 20 pmol of each primer, 1 mM $MgCL_2$, 20 μM Tris–HCL, pH = 8.6, 50 μM KCl, and 1 U Taq polymerase (Sigma) (11). Amplification was performed on an automated thermal cycler (MG-Research, Waltham, MA). PCR conditions were 3 min for initial denaturation at 94°C, 1 min for denaturation, 1 min at 60°C for annealing and 1 min at 72°C for extension followed by 7 min at 72°C for final extension (11). PCR reaction products were resolved at 100 V for 20–30 min on a 2% agarose (Sigma) containing 0.5 μg/mL ethidium bromide and PCR reaction product was digested by the restriction enzyme NlaIII (New England Biolabs, Beverly, MA) for 3 h at 37°C. The digest products were resolved at 100 V for 20–30 min on a 3% agarose (Sigma) containing 0.5 μg/mL ethidium bromide. A marker, pUC 18 DpnI digest, was used as a size standard for each gel line. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, Marne le Vallee, France).

Restriction fragments of 114, 36, and 35 bp revealed the $COMT^{HH}$ allele, and 96, 35, 36, and 18 revealed the $COMT^{LL}$ allele, and 114, 96, 36, 35, and 18 bp revealed the $COMT^{HL}$ allele.

The genotype distribution of Hardy–Weinberg equilibrium was tested with the chi-square χ^2-test for fitness quality and suitability. Comparisons of the genotype or allele frequencies between groups were performed with a χ^2-test. The *p*-value was considered to be statistically significant if < 0.05. Odds ratios (OR) as the estimates of the relative risk for the disease were calculated using a 95% confidence interval (CI). χ^2 and a one-way analysis of variance testing were also used for the statistical analysis of data. Analyses were run on SPSS software, version 11.5.

Results

Our sample group consisted of 36 violent offenders with MR (11 female and 25 male) and 36 control subjects (16 female and 20 male). Representative samples from the committed MR subjects are shown in Fig. 1.

The genotype and allele frequency of the COMT Val158 Met polymorphism in violent offenders with MR as well as an ethnically matched control group are shown in Table 1. The genotype frequencies of COMT-HH, HL and LL were 38.9, 52.8, and 8.3% in violent offenders with MR and 44.4, 38.9, and 16.7% in the controls respectively. The allele frequencies were 65.3% for the H and 34.7% for the L allele in violent offenders with MR, and 63.9% for the H and 36.1% for the L allele in controls.

We have not noticed any significant difference either in the allele frequency or in the genotype distribution of the H/L

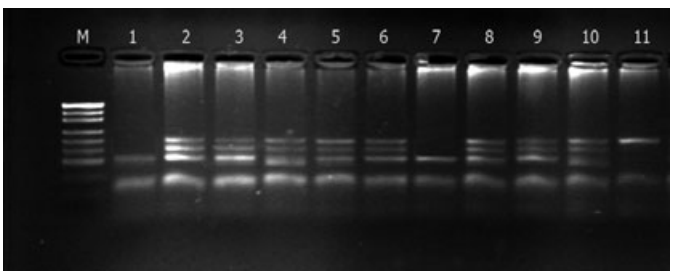

FIG. 1—*COMT Vall58 Met restriction enzyme results. M: Marker; Line 1, 7 are COMTLL; line 2-6, 8–10 are COMTHL genotype; line 11 COMTHH genotype.*

TABLE 1—*The distrubution of COMT Val158 Met polymorphism allele and genotype between violent offenders with mental retarded and ethnically matched control subjects.*

	Patients *n* (%)	Control *n* (%)	Odds Ratio (CI %)*	*p*
Genotype				
HH	14 (38.9)	16 (44.4)	0.8 (0.31–2.03)	>0.05
HL	19 (52.8)	14 (38.9)	1.8 (0.69–4.48)	>0.05
LL	3 (8.3)	6 (16.7)	0.5 (0.1–1.98)	>0.05
Allele				
H	47 (65.3)	46 (63.9)	1.1 (0.54–2.1)	>0.05
L	25 (34.7)	26 (36.1)	0.9 (0.48–1.86)	>0.05

*Cl = confidence interval.

polymorphism at COMT loci between violent offenders with MR and the control group ($p > 0.05$). The frequency distribution was made by Hardy–Weinberg equilibrium. When subjects were examined by gender diagnosis with regard to H–L alleles there was no significant association between violent offenders with MR and control group (Table 2). There was also no association between the age of onset of the disease and COMT variation ($p > 0.05$)

Discussion

In the human genome, many genes were defined (33) which are believed to have an influence on intelligence. The definition of numerous genes effective in human cognition was initiated as well by the sequence analysis in the human genome. Association studies were carried out to reveal the relationship between a single gene and cognition.

In several studies it was suggested that COMT activity might be associated with mental disorders considering that it regulated active dopamine and norepinephrine amounts in different parts of the brain (5,18). It was also suggested that, in many functional brain disorders: in alcoholism, bipolar disorder, and migraine headache, $COMT^{LL}$ genotype is effective. On the other hand, disorders such as anorexia nervosa and polysubstance abuse are associated with $COMT^{HH}$ genotype (28). In the case-control study by Zhang et al. there was no association between the frequencies of COMT genotypes and alleles in MR children and normal children (34). In another study by Zhang, a positive association was suggested between the genetic variants of the COMT gene and MR in the Chine Han population (31).

This study, the first in the literature, was designed to identify possible H/L polymorphism of COMT gene in violent offenders with MR. In this study, we have shown that the COMT gene genotype distribution and allele frequency were not statistically different between the two groups ($p > 0.05$). There are, however, important individual differences in the COMT mechanism and the association of genes with MR that should be determined accurately. One of these associations is X-linked MR (2). For this reason, subjects on whom Fragile X testing was performed, but who were also shown as not having this disorder, were recruited for the present study group.

TABLE 2—*COMT allele frequency tabulated by diagnosis-sex.*

	Patients *n* (%)	Control *n* (%)	Odds Ratio (CI %)*	*p*
Male allele				
H	32 (38.9)	26 (44.4)	1.0 (0.4–2.28)	>0.05
L	18 (52.8)	14 (38.9)	1.0 (0.44–2.59)	>0.05
Total	50	40		
Female allele				
H	15 (65.3)	20 (63.9)	1.3 (0.41–4.05)	>0.05
L	7 (34.7)	12 (36.1)	0.8 (0.25–2.45)	>0.05
Total	22	32		

*Cl = confidence interval.

The COMT polymorphism exhibits ethnical differences in terms of the neuropsychiatric disorders that it causes. In Chinese, North American, and British population-based case-control studies, no relationship with schizophrenia was found. However, an association with the high-activity (HH) COMT allele was found in the Japanese population. In the Spanish population, no relationship was found with bipolar disorder while in the Chinese population a relationship with high activity (HH) COMT allele was found (4) and while heterozygosity of this gene is high among the European, South-East Asian, and Turkish populations (4), our findings are concordant with these studies.

In a previous study (1), it had been revealed that MR is seen more frequently in males than females. The number of sex chromosomes differs between the two sexes and the existence of numerous X-linked single gene mutations are an effective cause of MR. While gender-associated differences were found for polymorphism of the COMT (4), no relationship with gender was found in this study.

In conclusion, our results suggest that H/L polymorphism of the COMT gene is not associated with violent offenders with MR in Turkey. However, further studies are needed to confirm these findings. This methodology can be applied to other ethnic populations and further studies may identify other genetic factors and/or polymorphism that play a role in the development of MR.

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Additional information and reprints requests:
Aysun Baransel Isir, M.D.
Assistant Professor
University of Gaziantep
Faculty of Medicine
Forensic Medicine Department
27100 Gaziantep
Turkey
E-mail: aybaransel@yahoo.com