

Relationship of MAO-A Promoter (u-VNTR) and COMT (V158M) Gene Polymorphisms to CSF Monoamine Metabolites Levels in a Psychiatric Sample of Caucasians: A Preliminary Report

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Monoamine oxidase A gene promoter (MAOA-uVNTR) and catechol-O-methyltransferase V158M (COMT-V158M) gene functional polymorphisms are reported to be associated with impulsive-aggression, but a biological intermediate effect remains to be determined. This study assessed the association of these polymorphisms with cerebrospinal fluid (CSF) monoamine metabolites as endophenotypes. Ninety-eight Caucasian psychiatric subjects were assessed for Axis I and II diagnosis. Subjects were genotyped for the functional polymorphisms, MAOA-uVNTR and COMT-V158M. CSF was obtained by lumbar puncture. Relationships of the two polymorphism to monoamine metabolites: HVA, 5-HIAA, and MHPG were examined. The higher-expressing MAOA-uVNTR genotype was associated with higher CSF-HVA levels in males only ($n = 46$) (195.80 pmol/ml, $SD = 61.64$ vs. 161.13, $SD = 50.23$, respectively; $P = 0.042$). No association was found with diagnosis. COMT-V158M had no association with CSF monoamine metabolites. The association of MAOA-uVNTR with dopaminergic activity in males is a preliminary finding that needs to be replicated in a larger sample of Caucasian males and relationships sought with clinical phenotypes. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at <http://www.interscience.wiley.com/jpages/0148-7299/1/suppmat/index.html>.

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INTRODUCTION

The two major routes of deactivation of monoamine neurotransmitters in the brain are re-uptake and metabolism by two key enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). These two enzymes are of interest in the study of aggressive behavior in both animals and human subjects. Low serotonergic activity and greater dopaminergic activity has been associated with aggression [Oquendo and Mann, 2000].

The gene for monoamine oxidase A (MAOA) has been mapped to Xp11.23. MAO activity is under partial genetic control and linked to psychopathology such as aggression/impulsivity, schizophrenia, and mood disorders. A functional polymorphism in the MAO A gene promoter has been described [Sabol et al., 1998] and consists of 30-bp upstream repeats (MAOA-uVNTR). Alleles with 3.5 and 4 repeats are transcribed 2–10 times more efficiently than the 3 and 5 repeat alleles. There appears to be a relationship between the MAO A gene and impulsive-aggressive behavior, but the intermediate biological phenotypes of that relationship are unknown. A mutation in the MAO A gene resulting in no enzyme is associated with mental retardation and impulsive-aggressive behaviour in males in a Dutch pedigree [Brunner et al., 1993]. Greater aggression is reported in male transgenic mice lacking MAO A [Cases et al., 1995]. Similarly, higher MAO expression appears to protect against the effects of childhood abuse on development of aggressive behaviors or impulsivity in adulthood in males but not females [Caspi et al., 2002; Huang et al., 2004]. In contrast, Manuck et al. [2000] found an association of the higher expressing 3.5 and 4 repeat alleles with higher impulsivity and aggression in a community sample of males. Both MAO A and MAO B isoenzymes influence monoamine transmission and cerebrospinal fluid (CSF) metabolites. MAO A knockout (KO) mice have increased levels of serotonin, dopamine, and norepinephrine, while MAO B KO mice have increased levels of phenylethylamine [Nagatsu, 2004; Shih, 2004], although some studies suggest that dopamine is also metabolized by MAO B in some regions of the brain [Lakshmana et al., 1998].

COMT is another key enzyme in catecholamine degradation. COMT enzyme activity is regulated by a single nucleotide polymorphism at codon 158 (COMT-V158M). An allele encoding a valine residue at amino acid 158 is associated with higher activity of COMT enzyme (H allele) while the allele encoding a methionine residue is associated with lower activity (L allele) [Weinshilboum and Raymond, 1977]. The L allele was found to be associated with violent traits in patients with schizophrenia [Kotler et al., 1999], violent suicidal attempts in subjects with schizophrenia [Nolan et al., 2000], and aggression in suicidal subjects [Rujescu et al., 2003]. In contrast, Jones et al. [2001]

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found an association of the HH genotype, and not the L genotype, with aggression in 180 patients with schizophrenia.

Since association studies of psychiatric clinical phenotypes often generate conflicting results, we assessed the association of these two functional polymorphisms with brain biological endophenotypes. We chose the CSF monoamine metabolites as endophenotypes because they are the product of catabolism of monoamine neurotransmitters and they are correlated with impulsive-aggression, depression, and suicidality [Mann et al., 1996]. This study examined the association of the MAOA-uVNTR and the COMT-V158M polymorphisms with CSF monoamine metabolites in a clinical sample of Caucasian subjects in order to clarify the relationship of these polymorphisms to clinical phenotype by assessing a possible intermediate biological endophenotype.

METHODS

Subjects

Subjects ($n = 98$) were recruited from clinics of a university affiliated psychiatric institute. Only Caucasian subjects of European origin were included in this analysis to reduce ethnic variation and stratification effects [Sabol et al., 1998]. Subjects were physically healthy on medical evaluation, negative for illicit drugs on toxicology screen and off for at least 3 weeks from drugs known to affect monoamine turnover (longer for longer half-life medications, such as 6 weeks for fluoxetine). Toxicology screen included blood and urine. Written informed consent was obtained as approved by the Institutional Review Board. DSM-IV Axis-I and II diagnoses were based on the Structured Clinical Interview SCID-I [Spitzer et al., 1990] and SCID-II [First et al., 1996].

Lumbar Puncture (LP)

LP was performed at about 8:00 AM, after subjects had been kept at bed rest and fasting from midnight. CSF was withdrawn and CSF 5-HIAA, HVA, and MHPG were assayed by high-performance liquid chromatography as described by Placidi et al. [2001].

Genotyping

See the online supplementary material at <http://www.interscience.wiley.com/jpages/0148-7299/1/suppmat/index.html>.

MAOA-uVNTR

DNA was obtained from blood samples and extracted from lymphocytes using our previously published method [Huang et al., 2004].

COMT-V158M

PCR was performed as described by Weinshilboun and Raymond [1977].

Statistical Analysis

Pearson's correlations were performed between the CSF metabolites. The dependence of continuous variables on categorical independent variables was tested by *t*-tests and analysis of variance (ANOVA). CSF metabolite levels in the two MAOA genotype groups (H/L) and three COMT genotypes were compared first for the whole sample and then for each gender separately, since gender differences were found in measures of impulsive-aggression and MAO A is X-linked gene. The MAOA-uVNTR heterozygote (LH) genotype is found only in females and was therefore excluded from the analysis of the total sample [Jonsson et al., 2000; Caspi et al., 2002]. All tests were two-tailed.

RESULTS

The sample consisted of 98 subjects (46 males, 52 females) aged 18–74 years (mean 41.6, SD = 13.7). Married: 30.6% and 84.7% had at least some college education. The primary diagnoses were: 56 (57.1%) major depressive disorder, 11 (11.2%) schizophrenia, 19 (19.4%) bipolar disorder, 4 (4.1%) remitted major depression, 2 (2%) substance use disorder in remission, 1 (1%) other psychosis, and 5 (5.1%) had no Axis I diagnosis. Diagnoses were grouped into three major categories: mood disorders ($n = 79$), psychotic disorders ($n = 14$), and no Axis I group ($n = 5$). No significant differences were found when comparing monoamine metabolites across diagnostic groups and, therefore, diagnosis was not controlled for in the analyses.

Gender

Males had lower CSF 5-HIAA (91.1 pmol/ml, SD = 29.6 vs. 106.4, SD = 32.0 in females; $F = 5.966$, $df = 1,96$, $P = 0.016$) and lower HVA (177.7, SD = 58.0 vs. 210.6 SD = 87.1; $F = 4.721$, $df = 1,96$, $P = 0.032$) compared with females. CSF MHPG levels did not differ between genders.

CSF Monoamine Metabolites

There were positive correlations between CSF 5-HIAA and HVA levels (Pearson's $r = 0.597$, $P < 0.001$), 5-HIAA and MHPG ($r = 0.234$, $P = 0.021$), and between MHPG and HVA ($r = 0.256$, $P = 0.011$).

MAOA Genotype and CSF Monoamine Metabolites

Thirty-nine subjects had the MAOA-uVNTR H genotype (22 males, 17 females), 34 had the L genotype (24 males, 10 females), and 25 females had the LH heterozygote genotype. The genotypes were in Hardy–Weinberg equilibrium.

CSF levels in subjects with the H genotype were: CSF MHPG = 44.2 pmol/ml (SD = 19.7); HVA = 200.7 (SD = 72.4); and 5-HIAA = 99.9 (SD = 25.6). Subjects with the L genotype had monoamine metabolites levels comparable to those with the H genotype ($P > 0.05$): MHPG level = 40.6 (SD = 16.7), HVA = 180.5 (SD = 69.3) and 5-HIAA = 95.6 (SD = 37.2) pmol/ml, respectively. Examining genders separately, a difference in CSF HVA levels was found between H and L genotypes in males (195.8 pmol/ml, SD = 61.6 vs. 161.1, SD = 50.2, respectively, $t = 2.099$, $df = 44$, $P = 0.042$) (Fig. 1), but not between H and L genotype in females (207.0 pmol/ml SD = 85.9 vs. 227.1 SD = 88.1, respectively; $T = 0.579$, $df = 25$, $P = 0.567$). The difference in the means was 121.5% in males compared with 109% in females. ANOVA for the three genotypic groups in females (LL/LH/HH) was also not significant ($F = 0.213$, $df = 2,49$; $P = 0.808$). Genotype was not associated, in males or females, with CSF MHPG ($P = 0.398$, 0.850, respectively) or 5-HIAA ($P = 0.296$, 0.359, respectively).

COMT Genotype and CSF Monoamine Metabolites

COMT-V158M LL genotype (9 males, 9 females), LH heterozygote genotype (25 males, 33 females), and HH genotype (12 males, 10 females) were in Hardy–Weinberg equilibrium. Mean CSF HVA levels did not differ between the three genotypic groups ($F = 0.076$, $df = 2,95$; $P = 0.927$). Neither the differences were seen in CSF 5-HIAA ($F = 0.168$, $df = 2,95$, $P = 0.845$) nor in MHPG levels ($F = 1.227$, $df = 2,95$, $P = 0.298$). This was also the case when genders were examined separately (data not shown).

DISCUSSION

We found a possible association of the high-expression allele of the MAOA-uVNTR polymorphism with higher CSF-HVA

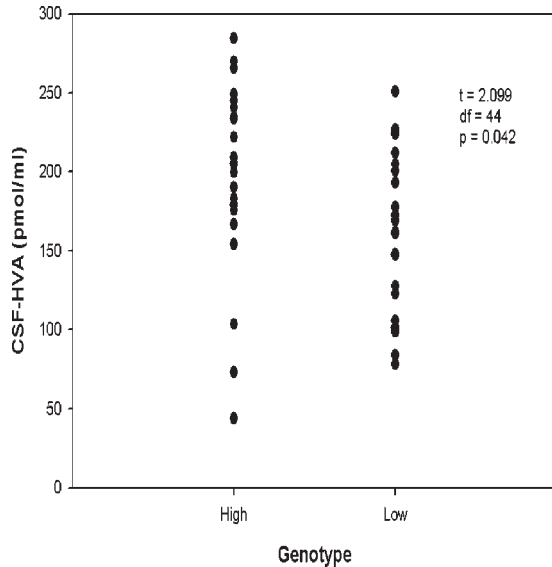


Fig. 1. MAO-A functional polymorphism and CSF-HVA levels in males.

levels in a sample of Caucasian males with a psychiatric disorder. There was no association of the genotype with diagnosis or gender. We found no significant association of the COMT-V158M polymorphism with CSF monoamine metabolites. This possible association of the MAOA-uVNTR polymorphism and greater dopamine turnover needs to be replicated to rule out a false positive. Manuck et al. [2000] reported that subjects with high transcription alleles of MAO A were more aggressive and had impaired serotonergic activity. They did not assess dopamine activity. That finding is counter to the reports that lower MAO A activity is associated with more aggression. Our finding of higher CSF HVA in subjects with these alleles may explain greater aggression, but we did not find a positive correlation of CSF HVA with lifetime aggression severity in this small sample of subjects.

Less MAO A enzyme associated with low-expressing MAOA-uVNTR genotype can be expected to favor lower dopamine metabolite levels in brain and CSF [Nagatsu, 2004; Shih, 2004]. One other study assessed the relationship of MAO-A uVNTR genotype with CSF metabolites in healthy volunteers [Jonsson et al., 2000]. In 88 healthy subjects, they found a similar association of higher expressing MAO-uVNTR polymorphisms with higher CSF HVA, in females and, in addition, with higher 5-HIAA, but not with MHPG. They found a trend in the opposite direction in males. Our study is the first to examine such relationships in a psychiatric population. In agreement with their findings in healthy volunteers, we found an association of the higher expressing allele with higher CSF HVA. However, our finding is in males and not females. Moreover, we did not find a relationship with 5-HIAA. Since CSF HVA and 5-HIAA are correlated, it is possible their association with HVA [Jonsson et al., 2000] contributes to the relationship identified with 5-HIAA. However, both study samples are small and thus prone to type I error and limited statistical power. For example, their finding in females depended on only five females with the low expressing genotype. Moreover, with females it is hard to predict the overall expression level in heterozygotes and, hence, to interpret the relationship of MAO gene expression to monoamines. Oral contraceptives and smoking were highly prevalent among the female subjects in the Jonsson study and may alter MAO activity and, thereby, may alter CSF HVA differently in females compared with males.

The relationship of CSF HVA to an aggression phenotype was not assessed in this preliminary study. However, it was a part of other studies with mixed results. For example, we [Sher et al., 2003] found lower CSF HVA levels in depressed aggressive alcohol abusers and the HVA:5-HIAA ratio is associated with psychopathic aggressive traits [Soderstrom et al., 2003]. Others found correlations of aggression with 5-HIAA and MHPG but not with HVA [Brown et al., 1979; Virkkunen et al., 1994]. These contradictory findings may indicate more complex relations between dopamine and serotonin in the regulation of aggression. For example, since MAO A affects both serotonin and dopamine degradation in the same direction, increased dopamine might be expected to have a pro-aggressive effect, while increased serotonin would be expected to diminish aggression [Oquendo and Mann, 2000]. Alternatively, correlational studies between brain biology and aggression are difficult to compare because the subject populations range from prison populations to subjects with mood disorders. Since clinical phenotypes are too complex and diverse to readily identify relationships with gene polymorphisms that explain such a small part of the variance, the genetic basis of complex psychiatric traits such as impulsive-aggression, must move towards identification of more specific biological endophenotypes (e.g., CSF HVA).

Limitations

The major limitation of this analysis is the sample size. CSF is difficult to obtain from clinical sample subjects, especially with the requirement for a long medication washout period and a negative toxicology screen. These exclusion criteria limited the sample size. The results of this study should be regarded with reservations, since association studies with limited sample size are prone to both type I and II errors, especially with multiple comparisons and stratification by gender. The lack of influence of the MAO A-uVNTR and COMT polymorphisms on the other two CSF metabolites needs explanation. Although correlated with each other and under partial genetic control, many other factors regulate CSF monoamine metabolite levels [Rogers et al., 2004]. If the preliminary possible association reported here is replicated in a larger, independent homogeneous sample of males and can be extended to show a relationship with an aggression behavior phenotype, it may add to our understanding of the role of these two genes in impulsive-aggression.

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