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Associations between Variable Number Tandem Repeat Polymorphism in the Monoamine Oxidase A with Cerebrospinal Fluid Levels in Drug Positive Cases

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Abstract

Background: Monoamine oxidase A (MAOA) plays important roles in the metabolism of catecholamines. The MAOA gene is located on the X chromosome, and a polymorphic promoter variable number tandem repeat (VNTR) locus (MAOA-uVNTR) is located approximately 1.2 kb upstream from MAOA exon 1. Functional studies have revealed that MAOA-uVNTR affects MAOA gene expression.

Aims: The associations between MAOA-uVNTR polymorphism and cerebrospinal fluid (CSF) catecholamine concentrations were investigated in autopsy cases in which drugs were detected.

Methods: We examined the frequencies of MAOA-uVNTR alleles in 89 autopsy cases in which psychotropic drugs were detected (PD cases) and 26 autopsy cases in which methamphetamine was detected (MA cases). CSF adrenaline (Adr), noradrenaline (Nad), or dopamine (DA) levels were analyzed in these cases.

Results: In male PD and MA cases, no significant associations between MAOA-uVNTR polymorphism and CSF Adr, Nad, or DA levels were found. In female PD cases, no significant associations between MAOA-uVNTR polymorphism and CSF Nad or DA levels were found. In contrast, female PD cases who were homozygous for the 3-repeat allele (i.e., 3/3 genotype carriers) had higher CSF levels of Adr than individuals who were heterozygous or homozygous for the 4-repeat allele (3/4 and 4/4,

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respectively) ($p = 0.028$).

Conclusion: The results of the present study suggest that MAOA-uVNTR polymorphism influences CSF Adr levels in female PD cases.

Keywords: Psychotropic drugs, Methamphetamine, Monoamine oxidase A, VNTR polymorphism, Catecholamine

INTRODUCTION

The catecholamines, adrenaline (Adr), noradrenaline (Nad), and dopamine (DA), act as neurotransmitters in the central and peripheral nervous systems. Catecholamines are produced in the adrenal medulla, in the brain, and by some sympathetic nerve fibers. Catecholamines are synthesized from the amino acid tyrosine by catecholamine-synthesizing enzymes and inactivated through a combination of reuptake and metabolism.

Monoamine oxidase A (MAOA) is the predominant enzyme responsible for catecholamine deamination (1). MAOA is expressed in most human tissues (2) and is found in catecholaminergic neurons in the human brain (3). The MAOA gene is located on Xp11.3, and various polymorphisms have been described (4). In particular, a functional polymorphism of a variable number tandem repeat (VNTR) in the promoter region of the MAOA gene (MAOA-uVNTR) has been investigated. MAOA-uVNTR consists of a 30-bp repeated sequence present in 2, 3, 3.5, 4, 5, or 6 copies (5). Alleles with 3.5 and 4 repeats are transcribed 2–10 times more efficiently than the 3- and 5-repeat alleles (6). MAOA-uVNTR is associated with emotional instability in women (7), suicide (8), panic disorder among women (9,10), and antisocial behaviors (11).

We previously analyzed levels of Adr, Nad, and DA in cerebrospinal fluid (CSF) from autopsy cases in which amphetamines or psychotropic drugs were not detected (12). Our data suggested that MAOA-uVNTR polymorphism influences CSF Adr and DA levels in females. It has been reported that CSF catecholamine levels are high when the cause of death is drug intoxication (13). It was considered necessary to investigate the effect of MAOA-uVNTR polymorphism on CSF catecholamine concentration in drug detected cases. In the present study, we examined the MAOA-uVNTR genotype in autopsy cases in which psychotropic drugs were detected (PD cases) and autopsy cases in which methamphetamine was detected (MA cases). Several psychotropic drugs target the major neurotransmitter systems in the brain. Amphetamines release neurotransmitters such as catecholamines (14). Therefore, we examined the associations between MAOA-uVNTR genotype with CSF Adr, Nad, and DA levels in PD and MA cases.

MATERIALS AND METHODS

We examined 89 autopsy cases in which psychotropic drugs were detected (PD cases) and 26 autopsy cases in which methamphetamine was detected (MA cases) (Table 1).

PD cases are autopsy cases in which psychotropic drugs were detected in blood samples but amphetamines were not detected in blood and urine samples. Psychotropic drugs detected were typical antipsychotics, atypical antipsychotics, tricyclic antidepressants, tetracyclic antidepressants, selective serotonin reuptake inhibitors, hypnotics, and antianxiety drugs. MA cases are autopsy cases in which

methamphetamine was detected in blood samples but psychotropic drugs were not detected in blood and urine samples. We previously analyzed autopsy cases in which amphetamines or

Table 1. Case profiles

Cases	Number of cases (male/female)	Age (median)	Postmortem interval (day) (median; h)
Psychotropic drug detected cases	89 (54/35)	14–95 (48)	< 0.5–4 (29)
Methamphetamine detected cases	26 (23/3)	27–80 (45)	< 0.5–4 (33)

Table 2. Cause of death of the forensic autopsy cases

Cause of death	Number of cases
Psychotropic drug detected cases	
Blunt injury	4
Sharp instrument injury (Hemorrhagic shock)	2
Fire fatality	19
Asphyxia	19
Drowning	7
Hypothermia (Cold exposure)	1
Hyperthermia (Heat stroke)	3
Psychotropic drug intoxication	28
Acute alcohol intoxication	2
Acute cardiac death	1
Natural death excluding acute cardiac death	0
Others*	3
Methamphetamine detected cases	
Blunt injury	1
Sharp instrument injury (Hemorrhagic shock)	0
Fire fatality	0
Asphyxia	3
Drowning	2
Hypothermia (Cold exposure)	0
Hyperthermia (Heat stroke)	1
Methamphetamine intoxication	11
Acute alcohol intoxication	0
Acute cardiac death	1
Natural death excluding acute cardiac death	6
Others**	1

* Cervical spinal cord injury (n=1), Undernutrition (n=1), Rhabdomyolysis (n=1)

** Cervical spinal cord injury (n=1)

psychotropic drugs were not detected in blood and urine samples (12). These cases were used as controls, and we compared the allele frequency and CSF catecholamine levels among groups. The causes of death of the examined forensic autopsy cases are summarized in Table 2.

Although vasopressor administration was not clearly indicated in the hospital reports, no significant differences in CSF catecholamine levels were found between cases with and without critical medical care at the time of death. During autopsy, CSF was collected using sterile syringes from openings into the cranial cavities. Blood samples were collected from the right cardiac chambers and external iliac veins. Urine was drawn using an aseptic syringe after opening the abdominal cavities. This study was approved by the medical ethics review board and the institutional ethics committee of the participating institutions.

CSF concentrations of the catecholamines (Adr, Nad, and DA) were determined as described previously (13). Toxicological analyses were performed on right heart blood, peripheral blood, and urine samples using gas chromatography/mass spectrometry and liquid chromatography-tandem mass spectrometry.

The MAOA-uVNTR polymorphism was analyzed as described previously (12). Allele frequencies were estimated, and Hardy–Weinberg equilibrium was determined using SNPAlyze software ver. 8.0.2 (Dynacom, Chiba, Japan). The histograms of CSF catecholamine levels showed a skewed distribution to the right. Therefore, log-transformed data were used for statistical analysis. The association between log-transformed CSF catecholamine concentrations and MAOA-uVNTR polymorphism was analyzed using age-adjusted linear regression analysis with JMP software ver. 13.2.0 (SAS Institute Inc., Cary, NC, USA). Differences were considered significant when $p < 0.05$.

RESULTS

Table 3 shows the results of the MAOA-uVNTR genotype frequency analyses in PD and MA cases. We identified only two alleles (3 and 4 repeats). No significant deviations from Hardy–Weinberg equilibrium for the genotype distribution in the female PD cases were found. Female MA cases could not be statistically analyzed due to the limited number of cases. A comparison among groups (control, PD cases, and MA cases) showed no significant differences in MAOA-uVNTR allele frequencies. The MAOA-uVNTR allele frequencies in this study were similar to those reported previously for Asian populations (15,16).

Table 3. Frequency of the variable number tandem repeat allele in the monoamine oxidase A promoter region in forensic autopsy cases

	MAOA-uVNTR	n	Frequency (%)
Psychotropic drug detected cases			
Male	3	34	62.96
	4	20	37.04
Female	3/3	12	34.29
	3/4	13	37.14
	4/4	10	28.57
Methamphetamine detected cases			
Male	3	13	56.52
	4	10	43.48
Female	3/3	2	66.67
	4/4	1	33.33

The CSF levels of Adr, Nad, and DA were analyzed in PD cases and MA cases. In these analyses, the median CSF Adr levels in PD and MA cases were 4.47 and 2.54 ng/mL, respectively; the median CSF Nad levels in PD and MA cases were 28.0 and 19.2 ng/mL, respectively; and the median CSF DA levels in PD and MA cases were 24.5 and 20.1 ng/mL, respectively. Before adjustment for age, PD cases had significantly higher CSF levels of Nad and DA than controls, but after adjustment for age, no significant differences were found among controls, PD cases, and MA cases in CSF Adr, Nad, and DA levels. No significant differences were found between males and females in CSF Adr, Nad, and DA levels in PD case s.

The effects of MAOA-uVNTR polymorphism on the CSF levels of Adr, Nad, and DA were determined in PD and MA cases after adjustment for age. In male PD cases, CSF levels of Adr, Nad, and DA were not associated with MAOA-uVNTR genotypes (Fig. 1).

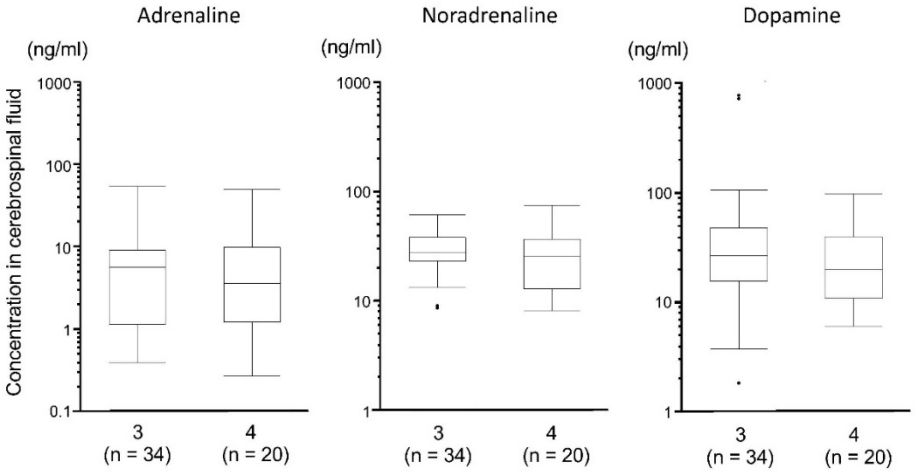

Figure 1. Cerebrospinal fluid levels of catecholamine (ng/mL) according to VNTR polymorphism in the monoamine oxidase A promoter region in male cases in which psychotropic drugs were detected. (Bars, boxes, whiskers, and open circles indicate medians, 25th and 75th percentiles, ranges, and outliers, respectively. CSF: Cerebrospinal fluid)

In female PD cases, CSF levels of Adr, Nad, and DA were not associated with individual MAOA-uVNTR genotypes (Fig. 2A). In contrast, females who were homozygous for 3-repeat alleles (i.e., 3/3 genotype carriers) had higher CSF levels of Adr than females who were heterozygous or homozygous for 4-repeat alleles (3/4 and 4/4, respectively) (Fig. 2B). The median CSF concentrations of Adr in 3/3 genotype carriers and 4-repeat allele (3/4 and 4/4) carriers were 8.70 and 3.26 ng/mL, respectively ($p = 0.028$). In PD cases, CSF levels of Nad and DA were not associated with MAOA-uVNTR genotypes in females (Fig. 2B). In male MA cases, CSF levels of Adr, Nad, and DA were not associated with MAOA-uVNTR genotypes (data not shown).

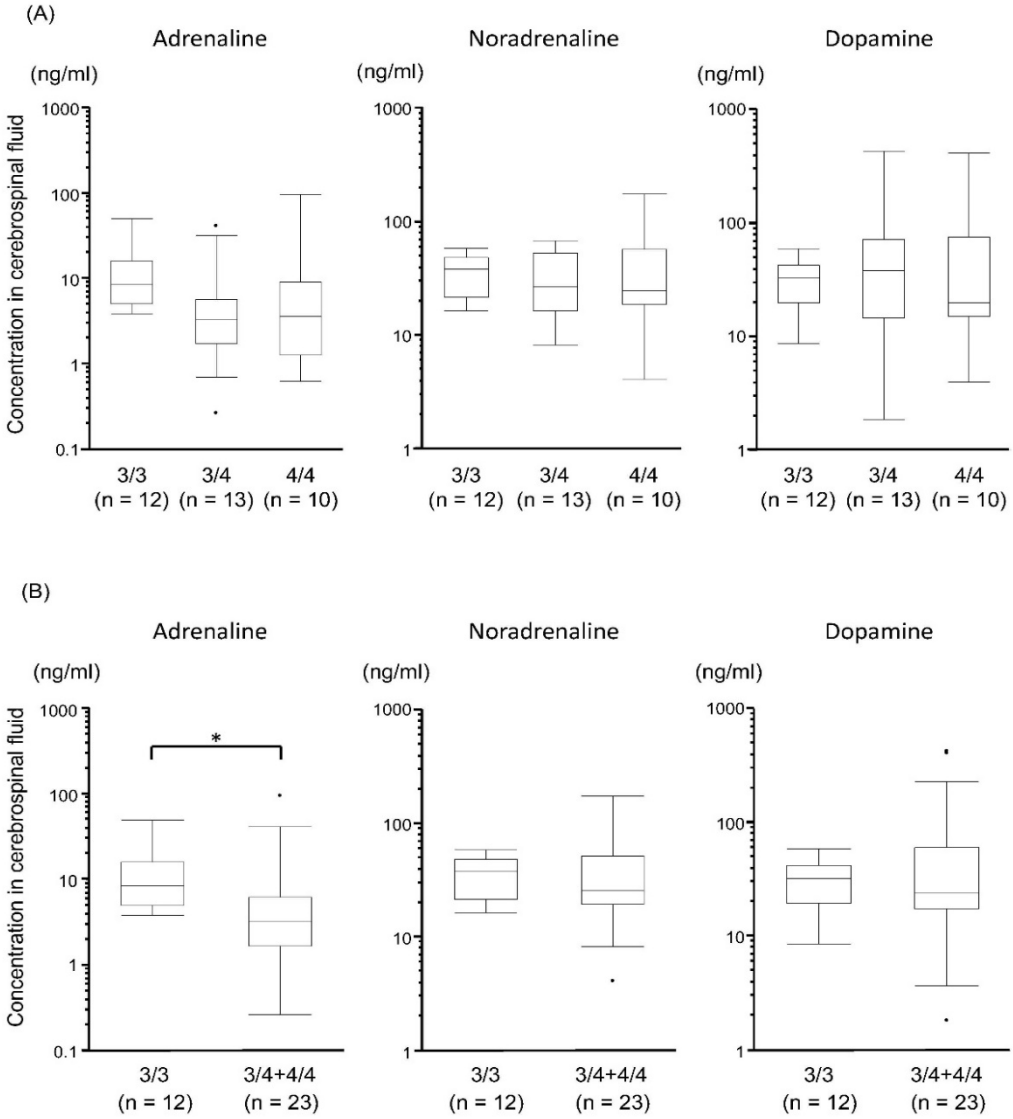

Figure 2. Cerebrospinal fluid levels of catecholamine (ng/mL) according to VNTR polymorphism in the monoamine oxidase A promoter region in female cases in which psychotropic drugs were detected. (A) Comparison of 3/3, 3/4, and 4/4 genotypes. (B) Comparison of 3/3 versus 3/4 + 4/4 genotypes.

(Bars, boxes, whiskers, and open circles indicate medians, 25th and 75th percentiles, ranges, and outliers, respectively. *$p < 0.05$ for the difference between genotypes. CSF: Cerebrospinal fluid)

DISCUSSION

MAOA-uVNTR genotype was associated with CSF Adr levels in female PD cases. We previously reported that MAOA-uVNTR polymorphism influences CSF Adr and DA levels in female control cases (12). In human neuroblastoma and human placental choriocarcinoma cell lines transfected with MAOA-uVNTR constructs containing 3 repeats, observed transcriptional activity was lower than in cells transfected with constructs containing 4 repeats (6,9). Therefore, MAOA activity in carriers of the 3/3 genotype was expected to be lower than that in carriers of the 4/4 genotype. However, we found no significant difference in CSF catecholamine levels between 3/3 genotype carriers and 4/4 genotype carriers (Fig. 2A). This discrepancy may reflect the limited number of cases examined in the present study. In contrast, we identified a significant difference in CSF Adr levels between 3/3 genotype carriers and individuals harboring 4-repeat alleles (i.e., 3/4 and 4/4 genotype carriers) (Fig. 2B). Because no marked difference was seen in CSF Adr levels between PD cases and controls, an effect of MAOA-uVNTR genotype on CSF Adr levels was present in the PD cases. MAOA-uVNTR is related to an increased risk of violent and aggressive behavior (17), reactive impulsive experimental aggressiveness in healthy men and women (18), sex-dependent psychopathological disorders, such as anxiety (19), and enhanced vulnerability to suicide in males with depression (20). MAOA-uVNTR may affect MAOA activity and catecholamine levels, resulting in psychological and physical effects. Adr is associated with psychologic states produced by environmental or physiologic stresses (21). Adr increases heart rate, blood pressure, and blood glucose levels. High or low concentrations of Adr affect a wide variety of health conditions.

MAOA-uVNTR genotype was not associated with CSF DA levels in female PD cases unlike the female control cases. The CSF DA levels were not significantly different between controls and PD cases after age adjustment, but PD cases tended to have higher CSF DA levels than controls. Various psychotropic drugs were detected in PD cases such as DA antagonists. The pharmacological action of these drugs may also affect the CSF DA levels. For these reasons, the effect of MAOA-uVNTR genotypes on CSF DA levels may have disappeared in the female PD cases. CSF Nad levels were not associated with MAOA-uVNTR genotypes in female PD cases or in controls. The Km of MAOA for Nad is higher than that for Adr and DA in homogenates of human cerebral cortex (22). An enzyme with a high Km has a low affinity for its substrate and requires a greater concentration of that substrate to achieve Vmax. Therefore, MAOA-uVNTR genotypes may have little effect on CSF Nad levels.

The MAOA-uVNTR genotype was not associated with CSF Adr, Nad, and DA levels in male PD cases, male MA cases, or male control cases. Females have two X chromosomes, whereas males have only one. Thus, the

correlation between MAOA-uVNTR genotype and MAOA gene expression is expected to be simpler in males. The specific activity of MAOA differs 515-fold among skin fibroblast cultures from different males (23), and thus varies considerably by individual. In the present study, CSF Adr, Nad, and DA concentrations in males varied widely. This wider variance may explain the failure to detect a significant difference in males.

The following points are limitations of this study. Various types of psychotropic drugs were detected in the PD cases, and many individuals had taken multiple drugs. Further studies are needed to examine the effect of MAOA-uVNTR genotypes on CSF catecholamine levels by classifying the cases according to the pharmacological action of psychotropic drugs. Moreover, we could not investigate female MA cases because of a limited number of cases. In female controls and female PD cases, MAOA-uVNTR genotypes were associated with CSF catecholamine levels. Thus, more female MA cases need to be examined in the future. In addition, catecholamine levels are affected by various factors such as stress response, circadian rhythm, and exercise (24). In forensic autopsy cases, antemortem information is often not available. The cause of death, time of death, conditions before death, drug amounts, and drug intake history varied among subjects. Subjects were also highly likely to be under great stress before or at the time of death. Many factors other than MAOA-uVNTR may influence CSF catecholamine concentrations in autopsy cases. To overcome these limitations, increasing the number of cases and analyzing cases in which the conditions are matched as much as possible are necessary.

CONCLUSIONS

In the present study, we identified MAOA-uVNTR genotypes in autopsy cases, and examined the relationships between MAOA-uVNTR genotypes and CSF Adr, Nad, and DA levels in PD and MA cases. The CSF Adr levels were significantly higher in carriers of the 3/3 genotype than 4-repeat-allele (3/4 and 4/4) carriers, but only in female PD cases. Our results suggest that MAOA-uVNTR genotypes affect CSF Adr levels in female PD cases.

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Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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