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Abstract

Several lines of evidence suggest that genetic factors might contribute to drug abuse vulnerability. Recent genomic scans for association demonstrated that the brain-derived neurotrophic factor (BDNF) gene was associated with drug abuse vulnerability. In this study, we analyzed association of two BDNF gene single nucleotide polymorphisms (SNPs), 132C>T (C270T named formerly) in the noncoding region of exon V and 196G >A (val66met) in the coding region of exon XIIIA, with methamphetamine (MAP) abuse in Japan. No significant differences were found in the frequency of the genotype or allele in these two SNPs between MAP abusers and controls (132C>T in exon V: genotype, $p = 0.586$, allele, $p = 0.594$; 196G>A (val66met) in exon XIIIA: genotype, $p = 0.889$, allele, $p = 0.713$). Furthermore, there was no difference between clinical parameters (e.g. prognosis psychosis, spontaneous relapse, or poly-substance abuse) and the two SNPs of BDNF gene. These results suggest that the two SNPs (132C>T in exon V and 196G>A (val66met) in exon XIIIA) of the BDNF gene may not be associated with Japanese MAP abusers.

Key words: Brain-derived neurotrophic factor, Polymorphism, Drug abuse, Methamphetamine

Introduction

Family, twin and adoption studies suggest that genetic factors are implicated in vulnerability of substance abuse (Merikangas et al, 1998; Kendler, 2001; Kendler et al, 2000; Tsuang et al, 2001). The genome-scanning study of poly-substance abuse vulnerability demonstrated that the brain-derived neurotrophic factor (BDNF) gene might be one of the strong candidate genes to drug abuse (Uhl et al., 2001). BDNF is a member of a neurotrophin superfamily mainly expressed within the brain. BDNF interacts with TrkB receptor tyrosine kinase, playing several important roles such as; promotion of survival, differentiation and maintenance of neurons in peripheral nervous system and central nervous system; influences to axonal growth and connectivity; participation in the local responses to various types of neuronal stress or insults (Manji et al, 2003; Mattson et al, 2003). Furthermore, it also has been reported that the gene encoding BDNF might be an important candidate for susceptibility of neuropsychiatric disorders including bipolar disorder (Neves-Pereira et al, 2002; Sklar et al, 2002; Hashimoto et al, 2004), schizophrenia (Krebs et al, 2000), Parkinson's disease (Momose et al, 2002), and Alzheimer's disease (Ventriglia et al, 2002). In the studies reporting possible association of BDNF and these disorders, two SNPs of BDNF gene has been reported. One is 196G>A (val66met) SNP in exon XIIIA (GENBANK: AF411339; at position 95422) located within the propeptide region of BDNF. The A of the ATG-translation initiation codon is denoted

nucleotide +1 in exon XIIIA (GENBANK: AF411339; at position 95227). Sklar et al (2002) reported that BDNF 196G>A (val66met) is significantly associated with bipolar disorder. Interestingly, it has been demonstrated that this SNP (val66met) is strongly suspected to influence human memory and hippocampal function (Egan et al, 2003). Several lines of evidence demonstrated that MAP dependence may cause long-term neural damage in humans, with concomitant deleterious effects on cognitive processes such as memory and attention (Nordahl et al, 2003), suggesting the possible role of BDNF secretion in the memory deficits of MAP abusers. The other SNP frequently analyzed is 132C>T in the noncoding region of exon V (GENBANK: AF411339; at position 53620). This SNP at position 132 of exon V is numbered from the start of exon V (GENBANK: AF411339; at position 53488). It was detected and named C270T by Kunugi et al. (2001) after their searching for a novel nucleotide substitution in the noncoding region of the BDNF gene reported by Shintani et al (1992). It has been reported that the 132C>T in exon V of the BDNF gene was significantly associated with late-onset Alzheimer's disease (Kunugi et al, 2001), Alzheimer's disease lacking the Apolipoprotein E ε4 allele (Riemenschneider et al, 2002), or schizophrenia (Szekeres et al, 2003). In addition, it has been reported that the BDNF 196G>A (val66met) is associated with personality traits in healthy subjects (Itoh et al, 2004; Sen et al, 2003), suggesting the role of BDNF gene in personality traits and temperament. Considering the role of personality traits in substance use disorders

(Howard et al, 1997), it is likely that the BDNF gene may be implicated in the vulnerability of drug abuse.

Methamphetamine (MAP) is the most popular abused drug in Japan. Use of MAP induces a strong psychological dependence, and repeated usage frequently results in psychotic states, which symptoms are similar to those of paranoid-type schizophrenia (Sato et al, 1992; Ujike, 2002). It has been demonstrated that BDNF plays a role in the survival and differentiation of midbrain dopaminergic neurons *in vivo* (Hyman et al., 1991) and *in vitro* (Spina et al., 1992), and that chronic BDNF treatment enhances locomotor activity and conditioned reward to cocaine (Horger et al., 1999). In addition, it is likely that BDNF could modulate the release of dopamine through the activation of TrkB receptors (Blochl and Sirrenberg, 1996). Furthermore, it has been reported that locomotor behaviors by amphetamine was increased to a greater degree in the BDNF heterozygous (+/-) knock-out mice, and that striatal dopamine concentrations were significantly higher in the BDNF heterozygous (+/-) knock-out mice (Dluzen et al, 2001). Moreover it has been reported recently that pretreatment with intra-nucleus accumbens injection of BDNF antibody or TrkB antibody suppressed significantly the release of dopamine and dopamine-related behaviors induced by administration of MAP, suggesting the implication of BDNF in MAP-induced dopamine release and MAP-induced abnormal behaviors (Narita et al, 2003). Taken together, it is of interest to study the influences of the BDNF gene SNPs in MAP

abuse vulnerability. In this study, we analyzed the frequency of two known SNPs (196G>A (val66met) in exon XIIIA and 132C>T in exon V) of BDNF gene between MAP abusers and healthy subjects in Japan.

MATERIALS AND METHODS

Subjects and samples

This study was performed after obtaining the approval of the ethics committees of each affiliated institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA). All subjects provided written informed consent for the use of their DNA samples for this study. The subjects were 189 patients (150 males and 39 female; age, 36.6 ± 11.9 (mean ± S.D.)) with MAP dependence and a psychotic disorder meeting the ICD-10-DCR criteria (F15.2 and F15.5) who were outpatients or inpatients of psychiatric hospitals of the JGIDA. Two hundred and two volunteers were recruited as healthy controls. All controls have no significant lifetime history of use of any addictive substance (158 males and 44 females; 37.2 ± 10.6 (mean ± S.D.)), the majority of whom were medical staff with no past history and no family history of drug dependence or psychotic disorders. Diagnoses were made by two trained psychiatrists by interview and available information including hospital records. Patients were excluded if they had a clinical diagnosis of schizophrenia, another psychotic disorder, or an organic mental syndrome as reported previously. All subjects were Japanese, born and living in restricted areas

of Japan including northern Kyushu, Setouchi, Tyukyou, Toukai, and Kantou.

The patients were divided into subgroups by some characteristic clinical features. The patients were divided by poly-substance abuse status, 56 patients abuse MAP only in their lifetime, and 122 patients abuse some other drugs besides MAP in present or past. Organic solvent was most frequently abused besides MAP, followed by marijuana. Cocaine and heroine were rarely abused in the present study. Prognosis of MAP psychosis was various among patients, and some patients showed continuous psychotic symptoms even after MAP discontinuance as previously reported (Sato et al, 1992; Ujike, 2002). Therefore, patients were divided into two categories of prognosis, transient-type and prolonged-type, based on duration of psychotic state after MAP discontinuance. Thus, patients with transient-type whose psychotic symptoms improves within one month after discontinuance of MAP consumption and beginning of treatment with antipsychotic drugs, and those with prolonged-type whose psychosis continues for more than one month even after discontinuance of MAP consumption and beginning of treatment. In this study, patients with transient- and prolonged-types of MAP psychosis were 94 and 66, respectively. It has been well documented that once MAP psychosis has developed, patients in remission state becomes reliable to spontaneous relapse without re-consumption of MAP (Sato et al, 1992; Ujike, 2002). It is postulated that sensitization phenomenon induced by repeated consumption of MAP should be developed in the brain of

MAP psychosis patients which result in neural basis for enhanced susceptibility to relapse. Therefore, the patients were divided into two groups according to presence or absence of spontaneous relapse. The patients with and without spontaneous relapse were 64 and 116, respectively.

Genotyping

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assay were performed to genotype the DNA sequence variants of the BDNF gene. PCR was carried out in a total volume of 25 μl with 1 units of Ex Taq DNA polymerase (Takara Shuzo Ltd., Kyoto, Japan) in the reaction mixture. The primer sequences for analysis of val66met (196G>A) (GENBANK: AF411339; at position 95422) in exon XIIIA (position 95206-98892) were forward: 5'-GGTGAGAAGAGTGATGACCA-3' (position 95214-95233) and reverse: 5'-GCCAGCCAATTCTCTTTTTG-3' (position 95892-95911). The former contains the first met and the second thr (MT), while the latter contains 223lys, 224lys, 225arg, 226ile, 227gly and 228trp (KKRIGW). The primer sequences for analysis of 132C>T (GENBANK: AF411339; at position 53620) in exon V (position 53488-53644) were the forward primer 5'-CAGAGGAGCCAGCCCGGTGCG-3' (position 53458-53478) and the reverse primer 5'-CTCCTGCACCAAGCCCCATTC-3' (position 53660-53680).

The amplification conditions were initiated at 94˚C for 4 min, followed by 32 cycles consisting of denaturation at 94˚C for 30 s, annealing at 58˚C for 30 s and extention at 72˚C for 30 s, with a final extention step of 7 min at 72˚C. The PCR products were digested at 37˚C with restriction enzyme *Pma*C I (Takara Shuzo Ltd., Kyoto, Japan) for analysis of 196G>A (val66met) in exon XIIIA or *Hinf*I (Takara Shuzo Ltd., Kyoto, Japan) for analysis of 132C>T in exon V, followed by 2 % or 4 % agarose gel- electrophoresis with ethidium bromide staining, respectively.

Statistical analysis

Fisher's exact test was used for categorical comparisons, and Student's t-test was employed for age difference. Significance for the results was set at $p < 0.05$.

RESULTS

Both the genotype and allele frequencies for the patients and controls are shown in Table 1 and Table 2. The genotype distribution for patients groups and control groups did not deviate significantly from the Hardy-Weinberg equilibrium. No significant differences were found in the frequency of the genotype or allele in these two SNPs between patients and controls (132C>T in exon V: genotype, $p = 0.586$, allele, $p = 0.594$; 196G>A (val66met) in exon XIIIA: genotype, $p = 0.889$, allele, $p = 0.713$). As for the 132C>T substitution, there was no individual who was

homozygous for the 132T allele in exon V. Within patients, we analyzed the effects of prognosis psychosis (transient or prolonged), spontaneous relapse (positive or negative), and poly-substance abuse (yes or no) on the BDNF gene SNPs (132C>T in exon V and 196G>A in exon XIIIA). The genotypic and allelic distribution of two SNPs was not significantly different between transient type of psychosis and prolonged type of psychosis (Table 1 and 2). Furthermore, the genotypic and allelic distribution of two SNPs was not significantly different between positive spontaneous relapse and negative spontaneous relapse (Table 1 and 2). Moreover, the genotypic and allelic distribution of two SNPs was not significantly different between poly-substance abuse and non-poly-substance abuse (Table 1 and 2). In addition, we found that two SNPs were not in linkage disequilibrium with each other.

DISCUSSION

The present study suggests that two SNPs (132C>T in exon V and 196G>A (val66met) in exon XIIIA) of the BDNF gene may not be susceptible to MAP-abuse in Japanese samples. Using a European American sample and an African American sample, it has been reported that the BDNF gene could contribute to vulnerabilities to poly-substance abuse (Uhl et al, 2001). It is possible that difference in ethnicity might contribute to discrepancy between our study and other study. Frequency of A allele of 196G>A (val66met) in Japanese population (Momose et al,

2002; Nakata et al, 2003; Itoh et al, 2004; this study) is higher than that of Caucasian population (Ventriglia et al, 2002; Egan et al, 2003; Hakansson et al, 2003; Sen et al, 2003), suggesting the ethnic difference in this SNP (val66met)(Shimizu et al, 2004). First, it has been reported that the 196G>A (val66met) of the BDNF gene is associated with Parkinson's disease in Japanese subjects (Momose et al, 2002). However, lack of association between the BDNF 196G>A (val66met) and Parkinson's disease in a Swedish population was reported (Hakansson et al, 2003). Second, it has been reported that the 196G>A (val66met) of the BDNF gene is associated with bipolar disorder in Caucasian (Neves-Pereira et al, 2002; Sklar et al, 2002). However, no association between 196G>A (val66met) of the BDNF gene and bipolar disorder in Japanese population was detected (Nakata et al, 2003), suggesting that the BDNF gene may confer a susceptibility to bipolar disorder in Caucasian, but not in Japanese population. Thus, it is likely that ethnic differences may contribute to inconsistent findings between Caucasian sample and Japanese sample.

In this study, we investigated two SNPs; one (132C>T in exon V) in the noncoding region and the other (196G>A (val66met) in exon XIIIA) in the coding region. Whereas BDNF 196G>A (val66met) SNP does not affect the function of a mature BDNF protein, it has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF, and, thus, the regulated secretion of the mature BDNF protein (Egan et al, 2003). At cellular levels,

marked deficits were observed in the intracellular distribution, processing, and secretion of met-BDNF, suggesting that pro-BDNF may play a critical role in synaptic targeting and activity-dependent secretion at synapses (Egan et al, 2003). Remarkably, healthy human subjects with the met allele exhibit impaired hippocampal activity and memory function (Egan et al, 2003). However, it is currently unknown whether the BDNF 132C>T SNP could affect on the function, synthesis or secretion of BDNF. There are still other known SNPs in the BDNF gene sequences, and it is possible that there are more unknown SNPs. Further studies of other SNPs and unknown SNPs should be done to clarify the involvement of the BDNF gene in substance abuse vulnerability.

In conclusion, we failed to detect evidence for a role of two SNPs (196G>A (val66met) in exon XIIIA and 132C>T in exon V) of the BDNF gene in the pathogenesis of MAP abusers in our Japanese sample. Therefore, it is unlikely that the two SNPs (196G>A (val66met) in exon XIIIA and 132C>T in exon V) of BDNF gene are associated with Japanese MAP abusers.

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Table 1. Genotype and allele frequencies of the BDNF 132C>T (in exon V) gene polymorphism of in controls and MAP abusers

132C>T	n	Genotype			p	Allele		p
		CC	CT	TT		C	T	
Control	202	183 (90.6 %)	19 (9.4 %)	0 (0 %)		385 (95.3 %)	19 (4.7 %)	
Abuser	189	175 (92.6 %)	14 (7.4 %)	0 (0 %)	0.586	364 (96.3 %)	14 (3.7 %)	0.594
Prognosis of Psychosis								
Transient	94	87 (92.6 %)	7 (7.4 %)	0 (0 %)	0.664	181 (96.3 %)	7 (3.7 %)	0.671
Prolonged	66	62 (93.9 %)	4 (6.1 %)	0 (0 %)	0.612	128 (97.0 %)	4 (3.0 %)	0.620
Spontaneous Relapse								
Positive	64	60 (93.8 %)	4 (6.3 %)	0 (0 %)	0.611	124 (96.9 %)	4 (3.1 %)	0.619
Negative	116	107 (92.2 %)	9 (7.8 %)	0 (0 %)	0.685	223 (96.1 %)	9 (3.9 %)	0.692
Poly-substance Abuse								
No	56	51 (91.1 %)	5 (8.9 %)	0 (0 %)	1	107 (95.5 %)	5 (4.5 %)	1
Yes	122	114 (93.4 %)	8 (6.6 %)	0 (0 %)	0.414	236 (96.7 %)	8 (3.3 %)	0.424

Statistical analysis was performed by a Fisher's exact test (vs Control).

Table 2. Genotype and allele frequencies of the BDNF 196G>A (val66met)(in exon XIIIA) gene polymorphism in controls and MAP abusers

196G>A (val66met)	n	Genotype			p	Allele		p
		GG	GA	AA		G	A	
Control	202	70 (34.7 %)	107 (53.0 %)	25 (12.4 %)		247 (61.1 %)	157 (38.9 %)	
Abuser	189	70 (37.0 %)	96 (50.8 %)	23 (12.2 %)	0.889	236 (62.4 %)	142 (37.6 %)	0.713
Prognosis of Psychosis								
Transient	94	32 (34.0 %)	53 (56.4 %)	9 (9.6 %)	0.778	117 (62.2 %)	71 (37.8 %)	0.856
Prolonged	66	25 (37.9 %)	30 (45.5 %)	11 (16.7 %)	0.472	80 (60.6 %)	52 (39.4 %)	0.918
Spontaneous Relapse								
Positive	64	27 (42.2 %)	30 (46.9 %)	7 (10.9 %)	0.571	84 (65.6 %)	44 (34.4 %)	0.403
Negative	116	39 (33.6 %)	62 (53.4 %)	15 (12.9 %)	0.972	140 (60.3 %)	92 (39.7 %)	0.866
Poly-substance Abuse								
No	56	21 (37.5 %)	27 (48.2 %)	8 (14.3 %)	0.791	69 (61.6 %)	43 (38.4 %)	1
Yes	122	47 (38.5 %)	60 (49.2 %)	15 (12.3 %)	0.762	154 (63.1 %)	90 (36.9 %)	0.676

Statistical analysis was performed by a Fisher’s exact test (vs. Control).