Kyusyu, Setouchi, Tyukyou, Toukai, and Kantou. Advance approval was obtained for this study from the ethics committees of each institute of JGIDA, and all subjects provided written informed consent for the use of their DNA samples in this research.

The patients were divided into two subgroups according to the latency of their psychosis from first MAP intake: less than three years ($n = 54$, average = 0.83 years) or more than three years ($n = 53$, average = 9.98 years). For the remaining 31 subjects we were not able to determine the latency period.

Genotyping

Genomic DNA was extracted from a peripheral blood sample using the standard phenol extraction protocol. The 5'UTR and 5' flanking regions (up to about 5.6 kbp from the start codon) of the OPRM were separately amplified by the polymerase chain reaction (PCR) as three fragments. Exon 1 and part of intron 1 of the OPRM were amplified by PCR. To find possible polymorphisms in 5'UTR, exon 1, and part of intron 1 of OPRM, the fragments amplified from genomic DNA of 44 control subjects with PCR were sequenced using BigDye terminators (Applied Biosystems). The genomic DNA of the remaining control subjects and the MAP-dependent subjects was then analyzed by automated DNA sequencing in the region of exon 1 and part of intron 1. Primers were designed based on the reference genomic contingency sequence in the National Center of Biotechnology Information (Genbank Accessions no. NT-023451).

Statistical Analysis

For the statistical analysis, the chi-squared test was used; the statistical significance level was chosen as .05. The Hardy-Weinberg (HW) equilibrium was checked in all polymorphisms using the chi-squared test. In the analysis of linkage disequilibrium and estimation of haplotype frequencies, genotypic data from 179 control subjects and 128 MAP-dependent subjects were analyzed using the Arlequin program available from http://anthro.unige.ch/arlequin.[15] To analyze the variation in nucleotide repeats between control and MAP-dependent subjects, the CLUMP program (16) was used to estimate the significant values of the chi-squared test (T1) based on 1,000 Monte Carlo simulations.

RESULTS

To identify polymorphisms in OPRM, we analyzed exon 1, part of intron 1, and part of 5' UTR of control subjects ($n = 44$). Seventeen novel and one previously reported polymorphism were found, and there were no deviations from HW expectations (TABLE 1). SNPs (C12G, C17T, G24A) that have already been reported in the exon 1 cording region (5–14) were not identified in our control subjects. The allelic frequency of A118G was remarkably high in our control subjects (45.3%) compared with African-American or Caucasian populations (7.5–25.8%).[7–14] We found that one pair of SNPs, intervening sequence (IVS) 1-A4980G, and IVS1-A4910G in intron 1 were in a relationship of absolute disequilibrium.

We picked up these two SNPs—A118G, which was higher frequency, and IVS1-A4980G, which represented A4980G and A4910G—for analysis of our samples

TABLE 1. List of SNPs found in control subjects (5'UTR, exon 1, and part of intron 1)

Position	SNP Name	Allelic Frequency	Sample Size
5′ flanking region and 5′UTR	A-5580G	<1.5%	44
	C-5342T	<1.5%	44
	A-5308G	<1.5%	44
	C-5236T	<1.5%	44
	G-5026A	<1.5%	44
	G-4936A	<1.5%	44
	C-4504A	<1.5%	44
	T-2694G	<1.5%	44
	A-2693C	<1.5%	44
	T-2683T	<1.5%	44
	T-2402C	<1.5%	44
Exon 1	A118G	<45.3%	213
Intron 1	IVS1-A4980G	2.7%	187
	IVS1-A4910G	2.7%	187
	IVS1-G4690A	<1.5%	113
	IVS1-T4591G	2.7%	113
	IVS1-T4456G	<1.5%	113

(TABLE 2). No significant differences were found in allele frequencies of either SNP between controls and MAP-dependent subjects. We further tested the linkage disequilibrium in these SNPs. There was no linkage disequilibrium ($D' = 0.13$) between A118G and IVS1-A4980G in our control subjects, but disequilibrium $D' = 1.00$ corresponding to complete linkage disequilibrium in MAP-dependent subjects. IVS1-A4980G in intron 1 is located about 25 kbp downstream from exon 1. It is possible that the linkage disequilibrium block in intron 1 and exon 1 is larger than 25 kb.

We also found one nucleotide repeat downstream of IVS1-A4980G in intron 1, dinucleotide repeats IVS1-4908 $(AC)_{12-20}$. There was significant linkage disequilibrium ($P < .05$ by the Arlequin program) between IVS1-4908 $(AC)_{12-20}$ and the two SNPs tested above. A118G showed weak but significant linkage disequilibrium with IVS1-4908 $(AC)_{12-20}$ (in control subjects $D' = 0.33$, and in MAP-dependent subjects $D' = 0.46$). IVS1-A4980G showed complete linkage disequilibrium ($D' = 1.00$) with IVS1-4908 $(AC)_{12-20}$ in both control and MAP-dependent subjects. We looked for differences in the repeat polymorphism IVS1-4908 $(AC)_{12-20}$ between control and MAP-dependent subjects (TABLE 3), but no significant difference was found ($P = .83$ by CLUMP program (T1)).

We also analyzed associations of the SNPs with latency of MAP psychosis. Two groups were set up according to latency of psychosis from first MAP intake: less than three years ($n = 54$, average = 0.83 years) and more than three years ($n = 53$, average=9.98 years) (TABLE 4). The allelic frequency of SNP A118G differed sig-

TABLE 2. Allelic frequencies of SNPs in control and MAP-dependent subjects

SNP Name	Control Subjects				MAP-Dependent Subjects				
		Number	(Percent)	Allelic Frequency		Number	(Percent)	Allelic Frequency	*P* Value
A118G	A	67	(0.31)		A	50	(0.38)		
	A/G	99	(0.46)		A/G	56	(0.43)	40.5%	
	G	47	(0.22)	45.3%	G	G	(0.19)		.43
	Total	213			Total	131			
IVS1-A4980G	A	177	(0.95)		A	130	(0.94)		
	A/G	10	(0.05)		A/G	8	(0.06)	2.9%	
	G	0	(0.00)	2.7%	G	0	(0.00)		.99
	Total	187			Total	138			

TABLE 3. Allelic frequency of dinucleotide repeat in intron 1 (IVS1-4908$(AC)_{12-20}$)

Number of repeat		12	13	14	15	16	17	18	19	20	(T1)
Control	(2n = 374)	1	0	0	63	31	243	27	5	4	
	(%)	(0.3)	(0.0)	(0.0)	(16.8)	(8.3)	(65.0)	(7.2)	(1.3)	(1.1)	
MAP-dependent subjects	(2n = 276)	0	0	1	48	17	175	28	3	4	$P = .83$
	(%)	(0.0)	(0.0)	(0.4)	(17.4)	(6.2)	(63.4)	(10.1)	(1.1)	(1.4)	$x^2 = 2.70$

TABLE 4. Analysis of association between polymorphisms and latency of MAP psychosis

		Control Subjects			MAP-Dependent Subjects					
					Latency of Psychosis					
					< 3 Years			> 3 Years		
SNP Name		Number	(Percent)	Allelic Frequency	Number	(Percent)	Allelic Frequency	Number	(Percent)	Allelic Frequency
A118G	A	67	(0.31)		20	(0.38)		25	(0.46)	
	A/G	99	(0.46)	45.3%	25	(0.47)	38.7%	24	(0.44)	31.5%
	G	47	(0.22)		8	(0.15)		5	(0.09)	
	Total	213			53		$P = .47$	54		$P = .04$*
	A	177	(0.95)		48	(0.91)		51	(0.94)	
IVS1-A4980G	A/G	10	(0.05)	2.7%	5	(0.09)	4.7%	3	(0.06)	2.8%
IVS1-A4910G	G	0	(0.00)		0	(0.00)		0	(0.00)	
	Total	187			53		$P = .58$	54		P=.75

nificantly (chi-squared test, $P = .04$) between control subjects and MAP subjects, with latency less than three years.

Finally, we tested differences in haplotype frequencies between control and MAP-dependent subjects using genotypic data of A118G, IVS1-A4980G, and dinucleotide repeats IVS1-4908 $(AC)_{12-20}$. There was no significant difference in haplotype frequencies between the control group and either MAP group, whether latency was shorter ($P = .57$) or longer ($P = .87$).

DISCUSSION

Various evidence suggests that the μ-opioid receptor is involved in the abuse of both opiate drugs and nonopiate addictive drugs, such as alcohol, nicotine, and cocaine.[17–19] Sequence variations in both human and mouse OPRM have been reported from several groups including our own.[5–7] In humans, some SNPs (C12C, C17T, A118G, C440G, and G779A; the numbers are relative to the ATG start codon) that cause amino acid substitution (respectively, Ser4Arg, Ala6Val, Asn40Asp, Ser147Cys, and Arg260His) have already been reported.[5–7] We did not find the two SNPs (C12C, C17T) in our Japanese population. In particular, the A118G SNP in exon 1 that causes an Asn40Asp substitution is closely examined because it induces a decrease in one of the five glycosylation sites in the amino terminal of the μ-opioid receptor. *In vitro*, A118G increases the affinity of the μ-opioid receptor to one of its endogenous peptides, β-endorphin, but has no influence on any other opioid ligand.[8] Several recent studies have examined the association between frequencies of polymorphisms in OPRM (also A118G and others) and drug and/or alcohol dependence, although the results are not fully consistent.[7–14]

When MAP abusers were divided into two groups according to latency of psychosis, the allelic frequency of A118G was significantly different between the control group and MAP-dependent subjects with a latency less than three years from first MAP intake. Since we analyzed the allelic frequencies of the two SNPs in connection with MAP dependence/psychosis, Bonferroni corrections were performed on the P values. The corrected P values were $P = .08$, suggesting there is no significance.

We found linkage disequilibrium among A118G in exon 1, IVS1-A4980G, and IVS1-4908 $(AC)_{12-20}$ (from exon 1 to 25 kbp downstream) in the OPRM of our Japanese samples. Linkage disequilibrium between A118G and IVS1-A4980G was found in MAP-dependent subjects but not in control subjects. This finding suggests a correlation between MAP dependence and linkage disequilibrium. Though no significance was found in haplotype analysis, this result called for careful treatment. Hoehe *et al.*[7] identified a combination of variants, consisting mostly of a specific constellation of changes in putative transcription regulatory motifs that are found significantly more frequently in African-American substance-dependent individuals likely to have a significant genetic predisposition to their substance dependence. We did not find their sequence variants in 5′UTR in our present Japanese sample. None of the novel SNPs and dinucleotide repeats in 5′UTR and part of intron 1 in our Japanese population has been reported in African-Americans or European-Americans. Polymorphisms in the functionally relevant 5′ regulatory region of the OPRM in our Japanese population showed differences from other ethnic groups.

The present results suggest that sequence variations in 5'UTR, exon 1, and part of intron 1 of OPRM are not genetic markers for MAP dependence/psychosis. Further studies could usefully look for novel polymorphisms in the downstream sequence of the OPRM gene and for any association between the polymorphisms and MAP dependence/psychosis.

ACKNOWLEDGMENTS

We thank the MAP psychosis/abuser patients and controls for participating in this study. We also acknowledge support from the Japanese Ministry of Health, Labour and Welfare, and the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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Food-Reinforced Operant Behavior in Dopamine Transporter Knockout Mice

Enhanced Resistance to Extinction

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ABSTRACT: **Dopamine (DA) plays roles in circuits that are important for brain reward and in striatal brain regions that are important for certain types of habit learning. These processes in wildtype, heterozygous, and homozygous dopamine transporter knockout (DAT-KO) mice, which were mildly food deprived and allowed to make nose-poke responses for food reinforcement, were studied. The mice were given 20-min sessions of daily (a) baseline exposure to the operant chambers, (b) acquisition of nose-poke responses in which responses were reinforced under a fixed ratio (FR5) schedule, (c) a progressive ratio schedule in which the number of responses required to obtain food was gradually increased, and (d) extinction of responses in which nose pokes were not followed by food. Neither heterozygous nor homozygous DAT-KO mice differed from their wildtype litter mates in the number of nose pokes displayed during baseline exposures to the chambers, the number of sessions required for acquisition, the number of responses under the FR5 schedule, or the number of responses under the progressive ratio schedule. Interestingly, however, in the five extinction sessions in which food was no longer delivered by nose poking, homozygous DAT-KO mice exerted significantly more responses than mice of either of the other two genotypes. These lines of evidence suggest a greater resistance of DAT-KO mice to the elimination of the response and support roles of dopaminergic systems in habit memory.**

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Ann. N.Y. Acad. Sci. 1025: 140–145 (2004). © 2004 New York Academy of Sciences.
doi: 10.1196/annals.1316.018

KEYWORDS: habit learning; extinction; food reinforcement; dopamine transporter

INTRODUCTION

The mesolimbic dopamine (DA) system is known to play a critical role in mediating the reinforcing effects of abused drugs.[1] Recently, the growing evidence indicates that DA in the striatum also plays an important role in habit learning, which is crucial in drug-taking behavior.[2,3] Dopamine transporter knockout mice (DAT-KO) are a valuable model to study the roles of DA in these systems. DAT-KO mice, in which the clearance of DA from the synaptic cleft is about 100 times longer than the normal mice, are known to be hyperactive in a novel environment and insensitive to the motor-stimulating effect of cocaine.[4] Nevertheless, the reinforcing effect of cocaine is manifested in these mice.[5] So far, the behavioral characteristics of DAT-KO mice pertaining to natural reward have not been well documented. In the present study, we have examined the behavior of DAT-KO mice regarding learning and motivation for food reward using the operant conditioning paradigm.

MATERIALS AND METHODS

Animals

A total of 24 female DAT-KO mice (8 homozygous, 8 heterozygous, and 8 wild-types that were 134 to 218 (average 192.7) days old were used. The details of the generation of DAT-KO mice have been described previously.[5] These three genotypes were obtained by crossing adult heterozygotes. Throughout the experimental period, they were housed individually and their food supply was restricted to maintain approximately 90% of their free-feeding body weight. Tap water was freely available in their home cages.

Apparatus

A standard operant chamber for mice was used (O'Hara & Co. Ltd.). One wall of the chamber had a hole equipped with a dim light and a photo beam. Poking the hole interrupted the beam and resulted in the delivery of a 20-mg food pellet into the hole. Four identical chambers were used in sound-attenuating boxes. The experiment was controlled by the MED-PC system (MED-Associates, Inc.) using in-house software.

Procedure

The mice were given 20-min sessions of the following five stages daily:

(1) *Baseline exposure to the chambers*: The number of spontaneous nose-poke responses was recorded.

(2) *Training of the food-reinforced response*: A food pellet was delivered contingent on the nose poke. Initially, the response was reinforced under a fixed ratio (FR) 1 schedule, in which each response was followed by the delivery of food. The training continued until the animal got at least 10 reinforcements in a session (the

response criterion). When the criterion was met, the ratio was set to two and finally to five. The number of sessions required to meet the criterion under an FR5 schedule for three consecutive sessions was recorded.

(3) *Progressive ratio (PR) schedule*: The number of responses required to obtain food was gradually increased. Initially, the number was set to five. Every time the animal got the food within 300 s, the ratio was increased to 7, 10, 14, 20, 28, 40, 57, 80, and 113. If the animal failed to get food within the limited time, the test was terminated and the ratio immediately before termination was defined as the breaking point.

(4) *Retraining of the response under an FR5*: Before going to the next stage, it was confirmed that the responses had not deteriorated.

(5) *Extinction of response*: Nose pokes were not followed by food. Five extinction sessions were given.

RESULTS AND DISCUSSION

Throughout the experiment the body weight of the homozygotes was significantly lower than the other two genotypes (mean ± SD: homo: 19.2 ± 1.2 g; hetero: 22.3 ± 1.7 g; wild: 22.5 ± 1.1 g) (ANOVA $F(2, 21) = 14.16$, $P = .0002$, with the *post hoc* Fisher's PLSD test).

Baseline Level

Mean numbers of responses in a 20-min session are shown in the leftmost column of TABLE 1. Although there was little difference among genotypes in the number of responses, the difference was not statistically significant. Thus, the homozygous DAT-KO mice were not spontaneously hyperactive in this particular experimental situation.

Training of the Food Reinforced Response

Two out of eight wildtype mice failed to obtain 10 reinforcements under the FR1 schedule despite the extensive training by more than 15 sessions. The data of these mice were not included in the further analysis. This might be attributable to one of the background strains of DAT-KO (129/sv). The median number of sessions required to meet the criterion under the FR5 schedule was 7.5 (range 5–16) for homozygous, 5 (range 3–12) for heterozygous, and 7.5 (range 3–21) for wildtype mice. The mean numbers of responses under the FR5 schedule are shown in TABLE 1. No statistical difference among genotypes was found in both of these measures.

Progressive Ratio (PR) Schedule

The median value of the breaking point was 34 (range 5–113) for homozygous, 70.5 (range 20–113) for heterozygous, and 40 (range 14–80) for the wildtype mice. There was no significant difference among genotypes. We also calculated the response rate per minute under each response requirement, and again there was no significant difference among genotypes (data not shown). The PR schedule test is known to be a standard method for testing the reward value and/or motivation to get

TABLE 1. Mean numbers of nose-poke responses in a 20-min session, with standard deviation in parentheses

	Baseline response	FR5 (average of 3 days)	Extinction day 1	2	3	4	5
Homozygous ($n = 8$)	43.3 (12.2)	110.3 (30.0)	235.1 (91.9)	221.8 (80.3)	93.6 (52.7)	120.4 (70.0)	110.3 (72.2)
Heterozygous ($n = 8$)	51.3 (12.7)	92 (49.2)	360.8 (154.9)	191 (111.4)	126.3 (49.7)	91.5 (32.9)	83.5 (34.2)
Wildtype ($n = 6$)	35.8* (9.1)	80.7 (18.5)	251.2 (98.5)	112 (50.9)	65.3 (60.2)	45.5 (23.8)	50.7 (28.5)

*$n = 8$.

the reward.[6] Thus, the present data suggest that the DAT-KO mice were the same as the wildtype mice with respect to motivation to get the food reward.

Retraining Under an FR5 Schedule

No response deterioration was found in any of the three genotypes.

Extinction of Response

During the course of extinction, the number of responses decreased within a session and also across sessions in all of the animals. However, the homozygous mice exerted more responses relative to other genotypes. TABLE 1 shows the mean daily

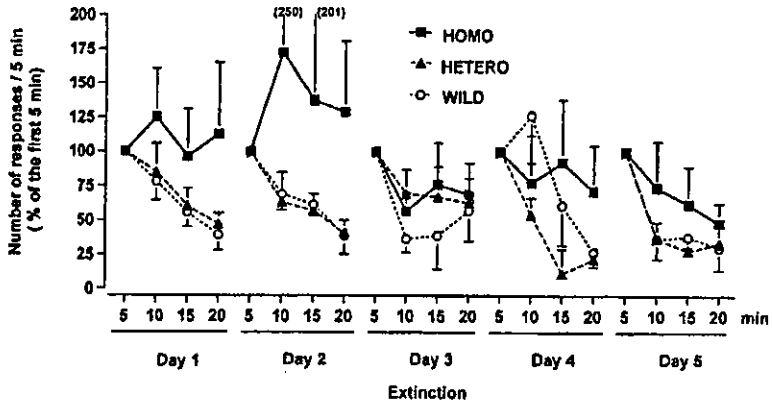

FIGURE 1. Nose-poke response of homozygous ($n = 8$), heterozygous ($n = 8$), and wildtype ($n = 6$) DAT-KO mice during five extinction sessions. Number of responses in a 5-min period was recorded and converted into the percentage of the number of responses in the first 5-min period in each day. Homozygous mice showed greater resistance to resonse elimination of response than other genotypes, especially on days one and two.

number of responses for each genotype. There was a statistically significant interaction between genotypes and the number of extinction sessions (ANOVA with repeated measures, $df = 8$, $F = 3.161$, $P = .0038$), though the main effect of genotype was not significant ($df = 2$, $F = 2.300$, $P = .1275$). The *post hoc* Fisher's PLSD test revealed that the homozygotes exerted significantly more responses than the wildtype mice during extinction days two, four, and five. There was no significant difference between homozygotes and heterozygotes on these days, and between heterozygotes and wildtype mice due to the large individual difference among heterozygotes. When we looked at the response decrement within the day, the decrement was not apparent in homozygotes except for day three (FIG. 1). These data indicate that the homozygotes were resistant to extinction of response. Thus, these mice showed a stronger habit. Another measure confirmed this point. If we employ the arbitrary criterion of extinction of response as no response for five consecutive minutes, only one homozygous mouse out of eight met the criterion. In contrast, six out of eight heterozygous and six out of six in wildtype mice met the criterion.

SUMMARY

Homozygous DAT-KO mice showed no clear evidence of hyperactivity in this operant conditioning situation. Acquisition and maintenance of responses for food reinforcement as well as the motivation to get food tested by the PR schedule were not markedly altered in these mice. However, greater resistance to extinction was found in these mice. Although this study was preliminary in nature, the results indicate that the DA system is involved in the habit memory system when we used food as a reward. Since resistance to extinction of response induced by environmental cues is important for drug-seeking behavior,[7] further behavioral phenotyping of DAT-KO mice related to learning and extinction might provide useful information concerning drug dependence.

ACKNOWLEDGMENT

This study was conducted at the RIKEN, Brain Science Institute and was supported by RIKEN and NIDA-NIH.

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Study of Association between α-Synuclein Gene Polymorphism and Methamphetamine Psychosis/Dependence

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ABSTRACT: **Methamphetamine (MAP) dissipates proton gradients across the membranes of synaptic vesicles, enhances cytoplasmic dopamine (DA) concentrations, and causes calcium-independent, nonvesicular DA release into synapses. MAP is taken into the cytosol by the dopamine transporter (DAT) on the synaptic terminals of DA neurons, and endogenous DA is concurrently released through the transporter by carrier exchange mechanisms, resulting in a robust increase in DA concentration in the synaptic clefts. The enhanced DA release through DAT by MAP is the main mechanism for the reinforcing ef-**

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Ann. N.Y. Acad. Sci. 1025: 325–334 (2004). © 2004 New York Academy of Sciences. doi: 10.1196/annals.1316.040

fects of MAP. The complexes of α-synuclein and DAT facilitate membrane clustering of the DAT, thereby accelerating DA uptake *in vitro*. α-Synuclein has been shown to be overexpressed in the midbrain DA neurons of chronic cocaine abusers. The present study was performed to study the association between the α-synuclein gene polymorphisms and MAP psychosis/dependence in Japanese population. Since the T10A7 polymorphic site at the 5′ end of the noncoding exon 1′ in the α-synuclein gene is highly polymorphic, we analyzed the noncoding exon 1′ and intron 1, including this polymorphic site by sequencing. We confirmed four single nucleotide polymorphisms (SNPs) within 1.38 kbp of the T10A7 polymorphic site. No significant difference was found in genotype or allele frequencies in the T10A7 polymorphic site between MAP psychotic/dependent and control subjects. We found significant association between three SNPs in the vicinity of this polymorphic site in intron 1 and MAP psychosis/dependence in female subjects, but not in males. These results suggest an association of the α-synuclein gene polymorphisms with MAP psychosis/dependence in our female subjects. Further analyses are necessary to clarify the gender difference, by using a larger sample size and/or different ethnic groups, as well as functional variations in the α-synuclein gene.

KEYWORDS: **methamphetamine; dopamine transporter; α-synuclein; mesolimbic dopaminergic pathway**

INTRODUCTION

α-Synuclein is a major component of nigral Lewy bodies in Parkinson's disease.[1,2] α-Synuclein is a soluble presynaptic protein and is abundant in neurons,[3] but its function is yet to be elucidated. Lee and colleagues found that complexes of α-synuclein and dopamine transporter (DAT) facilitate membrane clustering of the DAT, thereby accelerating dopamine (DA) uptake *in vitro*.[4] Excess α-synuclein potentiates production of reactive oxygen species by DA, which may cause cell death.[5–8] Modulation of DA transmission by α-synuclein is probably involved with neurodegenerative and neuropsychiatric disorders such as drug dependence.

The mesolimbic dopaminergic pathway has an important role in addiction to psychostimulants and reinforcement. [^{3}H]-WIN 35428 binding sites, which reflect DAT protein amount and/or function, were increased in postmortem brains of cocaine abusers.[9] Mash and colleagues found overexpression of α-synuclein protein in DA neurons in cocaine abusers.[10] These findings provide further support for the involvement of α-synuclein in regulating dopaminergic neurons.[9,10] Methamphetamine (MAP) dissipates proton gradients across the membranes of synaptic vesicles, enhances cytoplasmic DA concentrations, and causes calcium-independent, nonvesicular DA release into synapses. MAP is taken into cytosol by DAT on the synaptic terminals of DA neurons, and endogenous DA is concurrently released through the transporter by carrier exchange mechanisms, resulting in a robust increase of DA concentration in the synaptic clefts. The enhanced DA release through DAT by MAP is the main mechanism for the reinforcing effects of MAP.[11,12] It has been reported that long-term MAP abuse induced development of psychosis. These findings suggested the importance of α-synuclein on MAP abusers and prompted us to study the association between the α-synuclein gene and MAP psychosis/dependence in Japa-

nese population. A highly polymorphic sequence variation (T10A7) has been reported at the 5′ end of the noncoding exon 1′ of α-synuclein gene.[13] In the present study, we have investigated whether the polymorphic sites in the noncoding exon 1′ and intron 1, including T10A7, are associated with MAP psychosis/dependence in Japan.

MATERIALS AND METHODS

Subjects

This study was performed following approval from the ethics committees of each 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 research. The subjects were 170 unrelated patients with MAP-dependence disorder meeting ICD-10-DCR criteria (F15.2 and F15.5), who were outpatients or inpatients of psychiatric hospitals of JGIDA, and also 161 geographical origin-matched healthy controls, mostly medical staff who had no past or family history of drug dependence or psychotic disorders. Patients were excluded if they had a clinical diagnosis of schizophrenia, another psychotic disorder, or an organic mental syndrome. All subjects were Japanese, born and living in certain areas of Japan, including northern Kyusyu, Setouchi, Chukyou, Toukai, and Kantou. Blood samples were drawn for DNA extraction from 170 patients (male 138, female 32) and 161 controls (male 83, female 78). The mean age of the patients was 37.6 ± 12.0 years (male: 39.5 ± 12.0 years; female: 29.4 ± 7.4 years). The mean age of the controls was 38.6 ± 12.0 years (male: 38.2 ± 11.1 years; female: 39.1 ± 12.9 years). Genomic DNA was extracted from peripheral blood by the phenol/chloroform method.

Defining Variation with the α-Synuclein Gene

The 5′ end of the noncoding exon 1′ in the α-synuclein gene (accession no. AF163864) was amplified by PCR, and the products were sequenced in both directions using BigDye terminators (Applied Biosystems). Amplification primer pairs were 11F: CAT CTC CCA TCC ATC TTG GC and 12F: AGA AGC TCT GAC AAA TCA GCG GTG. The PCR product was 1.38 kbp and was sequenced using four primers (11F, 11R: AAA TCT GTC TGC CCG CTC TC, 12F, 12R: ACC CGG TGT TCT CCA GGA TTT CCA). Genotyping and sequencing were performed on an ABI3100 Genetic Analyzer (Applied Biosystems). The position numbers of polymorphic variants are quoted with respect to the National Center for Biotechnology Information (NCBI) single nucleotide polymorphism (SNP) consortium database.

Statistical Analysis

Data for each locus were used to estimate allele and genotype frequencies and to test for Hardy-Weinberg equilibrium (HWE), using the chi-squared method or the Arlequin program available from http://anthropologie.unige.ch/arlequin.[14] The allele and genotype frequencies of patients and control groups were compared using the chi-squared method and the Monte Carlo type CLUMP analysis program.[15]

RESULTS

Our subjects were 170 MAP psychotic/dependent patients and 161 controls. DNA samples from 16 of the patients were sequenced in 1.38 kbp around the T10A7 polymorphic site at the 5′ end of the noncoding exon 1′ of the α-synuclein gene. We confirmed four SNPs (rs#1372520, 3756063, 2870027, 3756059) in these patients in addition to the T10A7 polymorphic site. All these four SNPs were in intron 1. The genotype and allele frequencies of these four SNPs were all in Hardy-Weinberg equilibrium (HWE), indicating no sample bias in our case and control samples. These four SNPs showed no association in genotypic or allelic analysis according to the chi-squared test (TABLE 1a). We found four allelic variations in the T10A7 polymorphic site (TABLE 2a). The genotype frequencies of the MAP psychotic/dependent group and control group were in HWE (control $P = .73$, MAP $P = .77$). Genotype frequencies were compared using the CLUMP analysis program and showed no association ($P = .677$, T1 = 4.00). Allele frequencies also showed no association based on the chi-squared test ($P = .622$, chi-squared = 1.77).

Since there were many more MAP psychotic/dependent males than females, we analyzed the associations in each gender. In males, there was no difference in the four SNPs and the T10A7 polymorphic site between patients and control samples (TABLES 1b and 2b). In females, genotype frequencies were significant in rs#1372520 ($P = .03$), rs#3756063 ($P = .03$), and rs#3756059 ($P = .03$) (TABLES 1c and 2c).

DISCUSSION

We have analyzed the sequence variation (T10A7) at the 5′ end of the noncoding exon 1′ in the α-synuclein gene and found no significant difference in genotype or allele frequencies between MAP psychotic/dependent subjects and controls. We confirmed four SNPs in intron 1 and found a significant difference of genotype in three SNPs in MAP psychotic/dependent females, but not in males. Association in the T10A7 polymorphism was first studied by Autere and colleagues. They found no statistically significant differences in Parkinson's disease patients in Finland.[13] Since the T10A7 polymorphic site has many variations, this site is thought to be a good marker for an association study of the α-synuclein gene. Our results at this site do not suggest any role for the α-synuclein gene in MAP psychosis/dependence. We nevertheless found significant association between three SNPs in the vicinity of this polymorphic site and MAP psychosis/dependence in female subjects, though not males. The reason for this gender difference is not clear, although recent evidence suggests women and men differ in their progression to dependence and abuse. In preclinical and clinical studies, it has been suggested that ovarian hormones, particularly estrogen, are involved in gender differences in drug abuse.[16] Koizumi and colleagues also found a correlation between glutathione S-transferase M1 gene deletion and MAP abuse by females (Koizumi and Iyo, unpublished data). The data in our study should be carefully treated, as the samples were divided into two groups by gender. The significance was corrected to $P = .025$ by Bonferroni corrections, and the P value of these sites was .03, suggesting weak association.

The functional alterations caused by these SNPs are not clear in the present study, but there are several possibilities. First, the SNPs or relating linkage disequilibrium

TABLE 1. Genotype and allele distribution of α-synuclein gene SNPs in patient and control groups

SNP	Group	*N*	Genotype (Percent)			*P*	Allele (Percent)		*P*
a. Male and Female									
rs#1372520			GG	GA	AA		G	A	
	Control	161	141 (88)	18 (11)	2 (1)		300 (93)	22 (7)	
	MAP	170	142 (84)	27 (16)	1 (1)	.389	311 (92)	29 (5)	.502
rs#3756063			CC	CG	GG		C	G	
	Control	161	2 (1)	18 (11)	141 (88)		22 (7)	300 (93)	
	MAP	170	1 (1)	29 (17)	140 (82)	.263	31 (9)	309 (91)	.348
rs#2870027			GG	GA	AA		G	A	
	Control	161	55 (34)	75 (47)	31 (19)		185 (58)	137 (43)	
	MAP	170	60 (35)	88 (52)	22 (13)	.281	208 (61)	132 (39)	.371
rs#3756059			CC	CT	TT		C	T	
	Control	161	2 (1)	18 (11)	141 (88)		22 (7)	300 (93)	
	MAP	170	1 (1)	28 (17)	141 (83)	.323	30 (9)	310 (91)	.420
b. Male									
rs#1372520			GG	GA	AA		G	A	
	Control	83	71 (86)	11 (13)	1 (1)		153 (92)	13 (8)	
	MAP	138	119 (86)	18 (13)	1 (1)	.932	256 (93)	20 (7)	1.000
rs#3756063			CC	CG	GG		C	G	
	Control	83	1 (1)	11 (13)	71 (86)		13 (8)	153 (92)	
	MAP	138	1 (1)	20 (14)	117 (84)	.909	22 (8)	254 (92)	.888
rs#2870027			GG	GA	AA		G	A	
	Control	83	27 (32)	40 (48)	16 (19)		94 (57)	72 (43)	
	MAP	138	46 (33)	76 (55)	16 (12)	.274	168 (61)	108 (39)	.435
rs#3756059			CC	CT	TT		C	T	
	Control	83	1 (1)	11 (13)	71 (86)		13 (8)	153 (92)	
	MAP	138	1 (1)	19 (14)	118 (86)	.932	21 (8)	255 (92)	.920

TABLE 1. (*continued*) **Genotype and allele distribution of α-synuclein gene SNPs in patient and control groups**

SNP	Group	*N*	Genotype (Percent)			*P*	Allele (Percent)		*P*
c. Female									
rs#1372520			GG	GA	AA		G	A	
	Control	78	70 (90)	7 (9)	1 (1)		147 (94)	9 (6)	
	MAP	32	23 (72)	9 (28)	0 (0)	.030	55 (86)	9 (14)	.077
rs#3756063			CC	CG	GG		C	G	
	Control	78	1 (1)	7 (9)	70 (90)		9 (6)	147 (94)	
	MAP	32	0 (0)	9 (28)	23 (72)	.030	9 (14)	55 (86)	.077
rs#2870027			GG	GA	AA		G	A	
	Control	78	28 (36)	35 (45)	15 (19)		91 (58)	65 (42)	
	MAP	32	14 (44)	12 (38)	6 (19)	.719	40 (63)	24 (38)	.671
rs#3756059			CC	CT	TT		C	T	
	Control	78	1 (1)	7 (9)	70 (90)		9 (6)	147 (94)	
	MAP	32	0 (0)	9 (28)	23 (72)	.030	9 (14)	55 (86)	.077

TABLE 2a. Genotype and allele frequencies of the T10A7 polymorphism of the α-synuclein gene in MAP psychosis/dependence: male and female

Subjects	*N*	Genotype (Percent)						
		T10A7/ T10A7	T10A8/ T10A8	T11A6/ T11A6	T10A7/ T10A8	T10A7/ T11A6	T10A8/ T11A6	T11A6/ T12A5
Control	161	18 (11)	7 (4)	31 (19)	30 (19)	46 (29)	29 (18)	0 (0)
MAP	170	20 (12)	10 (6)	22 (13)	29 (17)	53 (31)	35 (21)	1 (1)
		Allele (Percent)						
		T10A7	T10A8	T11A6	T12A5			
Control	322	112 (35)	73 (23)	137 (43)	0 (0)			
MAP	340	122 (36)	84 (25)	133 (39)	1 (0)			

NOTE: *N*, number of genotypes and alleles in MAP psychotic/dependent subjects and controls. MAP and control genotypes of both sexes were in Hardy-Weinberg equilibrium (control $P = .73$, MAP $P = .77$). CLUMP analysis for genotype frequencies: $P = .677$ (T1 = 4.00). Chi-squared test for allele frequencies: $P = .622$ (chi-squared = 1.77).

TABLE 2b. Genotype and allele frequencies of the T10A7 polymorphism of the α-synuclein gene in MAP psychosis/dependence: male

Subjects	*N*	Genotype (Percent)						
		T10A7/ T10A7	T10A8/ T10A8	T11A6/ T11A6	T10A7/ T10A8	T10A7/ T11A6	T10A8/ T11A6	T11A6/ T12A5
Control	83	7 (8)	4 (5)	16 (19)	16 (19)	23 (28)	17 (20)	0 (0)
MAP	138	14 (10)	9 (7)	16 (12)	22 (16)	45 (33)	31 (22)	1 (1)
		Allele (Percent)						
		T10A7	T10A8	T11A6	T12A5			
Control	166	53 (32)	41 (25)	72 (43)	0 (0)			
MAP	276	95 (34)	71 (26)	109 (39)	1 (0)			

NOTE: *N*, number of genotypes and alleles in MAP psychotic/dependent subjects and controls. MAP and control genotypes of males were in Hardy-Weinberg equilibrium (control $P = .82$, MAP $P = .34$). CLUMP analysis for genotype frequencies: $P = .682$ (T1 = 3.96). Chi-squared test for allele frequencies: $P = .748$ (chi-squared = 1.22).