TABLE 3. Genotype and allele frequencies of the A61C (Gln2Pro) polymorphism in the type 1 sigma receptor gene between subgroups in MAP psychosis

Subgroups in MAP psychosis	Genotype[a] AA	AC	CC	*P* Value	Allele[a] A	C	*P* Value
(a) Age at first MAP use (in years)							
≧ 20	24	27	5		75	37	
(*n* = 56)	42.9%	48.2%	8.9%	0.68	67.0%	33.0%	0.64
< 20	24	25	8		73	41	
(*n* = 57)	42.1%	43.9%	14.0%		64.0%	36.0%	
(b) Duration of MAP use until onset of psychosis							
≧ 3 years	26	24	5		76	34	
(*n* = 55)	47.3%	43.6%	9.1%		69.1%	30.9%	
< 3 years	16	24	7	0.35	56	38	0.16
(*n* = 47)	34.0%	51.1%	14.9%		59.6%	40.4%	
(c) Disappearance type psychosis[b]							
Transient type	27	30	8		84	46	
(*n* = 65)	41.5%	46.2%	12.3%		64.6%	35.4%	
Prolonged type	19	23	5	0.94	61	33	0.97
(*n* = 47)	40.4%	48.9%	10.6%		64.9%	35.1%	
(d) Presence or absence of the spontaneous relapse without MAP use							
Yes	19	16	8		54	32	
(*n* = 43)	44.2%	37.2%	18.6%		62.8%	37.2%	
No	30	36	5	0.12	96	46	0.46
(*n* = 71)	42.3%	50.7%	7.0%		67.6%	32.4%	

[a]*Upper row*, number of subjects; *lower row*, frequency.
[b]*Transient type*: psychotic symptoms improved within one month after discontinuation of METH along with initiation of treatment with neuroleptics; *prolonged type*: psychotic symptoms continued for more than one month even after discontinuation of METH, along with initiation of treatment with neuroleptics.

gene polymorphisms and schizophrenia in the Japanese population. Ishiguro *et al.*[9] first observed a significant association between the presence of the TT/Pro2 haplotype and schizophrenia (odds ratio = 1.27, $P = .04$), and they suggested a possible role in the pathogenesis of schizophrenia. Subsequently, Ohmori *et al.*[10] also observed a weak association between homozygosity for TT/Pro2 and schizophrenia ($P = .045$). However, they reported that this significance did not remain when a Bonferroni's correction was made ($P = .135$) and concluded that the type 1 sigma receptor gene is unlikely to play a major role in the pathogenesis of schizophrenia.

However, considering the previous reports on the close relationship between sigma receptor and psychiatric symptoms, the possibility of some involvement in the sigma receptor gene polymorphism for the development of psychiatric conditions such as MAP psychosis and schizophrenia cannot be completely ruled out. Moreover, because the sample size for the present study is fairly small, there is the possi-

bility of a type II error to detect the significant difference. Therefore, further research is needed to clarify the exact role of these polymorphic sites in determining certain phenotypes, using a larger number of samples, that can be related to some forms of psychosis.

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A Polymorphism of DRD2 Gene and Brain Atrophy in Methamphetamine Psychosis

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ABSTRACT: **Our group, Ujike *et al.*, recently reported that the A1 allele of TaqI A polymorphism of the dopamine receptor D2 (DRD2) gene, associated with transient psychosis, significantly differs from that of patients with prolonged psychosis in methamphetamine psychosis. Therefore, we examined the association between the TaqI A polymorphism of the DRD2 gene and the brain MRI view for patients with methamphetamine psychosis. The subjects underwent brain MRI scans using the FLAIR method. Genotyping was performed by PCR-RFLP methods using genomic DNA extracted from peripheral blood by the phenol method. Ten subjects had the A1/A2 genotype, eleven subjects had the A2/A2 genotype, and no subject had the A1/A1 genotype. The domain size,**

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including the thalamus and basal ganglia that were inside each side of the putamens, did not differ between the three groups (the A1/A2-group, the A2/A2-group, and the young healthy person group). In the comparison based on this domain, the temporal lobe tended to narrow in the A2/A2-group compared to the A1/A2-group ($P = .06$). The other domain (cerebrum, corpus callosum, etc.) showed no difference between the A1/A2-group and the A2/A2-group. It is suggested that in methamphetamine psychosis the TaqI A polymorphism not only regulates prolongation of psychosis symptoms but also influences the form of the temporal lobe.

KEYWORDS: **methamphetamine psychosis; gene polymorphism; TaqI A; MRI; dopamine; atrophy**

INTRODUCTION

In drug-induced psychosis, cocaine, organic solvents, and alcohol, for example, have all been reported to cause specific brain form changes. In contrast, other studies on methamphetamine psychosis have concluded that there are no specific changes in the brain image.[1] However, many clinicians have the impression that anomalies in the brain image of patients with methamphetamine psychosis are not rare. Moreover, the existence of a necrotizing vasculitis has been suggested in a pathological tissue study of intracerebral bleeding due to methamphetamine abuse,[2–7] and in medication experiments of methamphetamine on animals, the existence of nerve cell toxicity in which apoptosis is involved has been suggested.[8] Based on these facts, we consider it unlikely that methamphetamine abuse does not influence cerebral form.

Turning now to recent molecular biology research on methamphetamine psychosis, the existence of some form of gene polymorphism that affects the susceptibility to methamphetamine psychosis has been proposed. Ujike *et al.* state that TaqI A polymorphism and −141 Ins/Del polymorphism of dopamine receptor D2 gene (DRD2), which alter DRD2 density and function, and VNTR polymorphism of 3′UTR of dopamine transporter gene (hDAT1), have a significant effect on prolongation of psychotic symptoms of methamphetamine psychosis. Regarding the TaqI A polymorphism of the DRD2 gene, for patients who have the A1/A1 genotype it takes longer for the onset of psychosis to occur after the methamphetamine abuse is initiated, and after medical treatment they do not present prolonged psychosis and flashback more easily than those who have other genotypes.[9–11] From these facts it has been concluded that there exists a gene polymorphism that influences the susceptibility to methamphetamine psychosis and the progress of its symptoms.

The purpose of this study is to clarify the influence of methamphetamine abuse/psychosis on cerebral form, by carrying out a comparison examination of the MRI view of the brain among the groups that were distinguished by the gene polymorphism.

MATERIALS AND METHODS

Subjects

All subjects were patients in the psychiatry hospital in Fukuoka prefecture and met DSM-IV criteria for methamphetamine psychosis. The young, healthy subjects

also underwent brain MRI and theyhad a comparison examination. All subjects were unrelated and all were Japanese.

This study was approved by the Ethics Committee of Kurume University. After a complete description of the study was provided, written informed consent was obtained from all subjects before the study commenced.

Twenty patients were in the experimental group (all males), and the average age was 48.3 (SD ± 10.1) years. There were twelve young, healthy subjects (all males), and their average age was 38.1 (± 5.5) years.

Genotypings

The gene polymorphisms analyzed were the TaqI A polymorphism of the DRD2 gene (TaqI A/DRD2) and the VNTR polymorphism of the 3'UTR of the hDAT1 gene (VNTR/DAT), which Ujike *et al.* have reported influence the condition and the prognosis of methamphetamine psychosis. Genomic DNA was extracted from peripheral blood by the phenol method, and the restriction fragment length polymorphism (RFLP) method and/or DNA sequencing after PCR were used for genotyping the polymorphism.

MRI Analysis

The subjects underwent MRI brain scans by a GE Signa 1.5T LX system using the fluid attenuated inversion recovery (FLAIR) method. MRI data were input as 3-dimensional data to a workstation (GE Medical Systems Volume Analysis/Advantage Workstation 4.0), and after reconstruction of the cerebrum, the cerebral ventricle, the thalamus, and so forth, in the workstation, they were measured by the line-segment method. Statistical analyses were performed using two group *t*-tests.

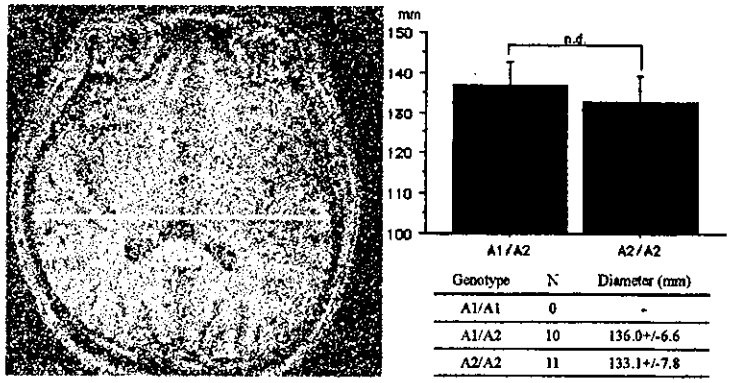
RESULTS

Genotype

For VNTR/DAT polymorphism, 10/7-repeat and 10/9-repeat numbered one patient, respectively, while the remaining nineteen patients were 10/10-repeat. There were no patients with A1/A1 genotype of TaqI A/DRD2 polymorphism, 10 patients with A1/A2 genotype (average age (± SD) 51.2 (± 11.8) years), and 11 patients with 2A2 genotype (45.3 (± 7.7) years). Since the number of non-10-repeat allele of VNTR/DAT was not sufficient to perform a statistical analysis, this subject was not examined in relation to a MRI brain view.

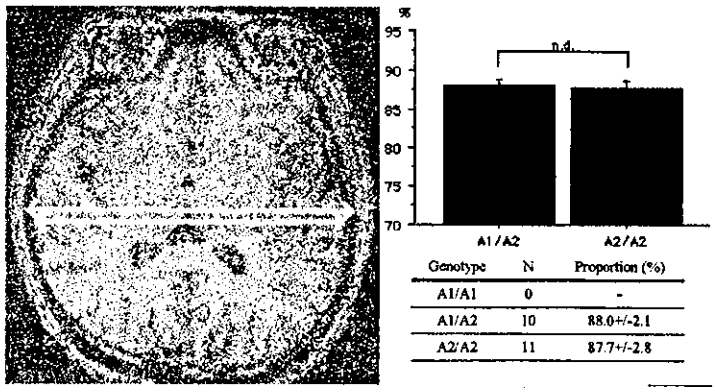
MRI Brain View

No significant difference was observed between the A1/A2 group and the A2/A2 group in [the transverse diameter of the cerebrum] in the horizontal slice at the top of the anterior commissure at the interhemispheric fissure (FIG. 1), [(the transverse diameter of the cerebrum)/(the inner breadth of the skull)] in the horizontal slice at the top of the anterior commissure at the interhemispheric fissure (FIG. 2), [the maximum (front–posterior) length between the caudate nucleus and the pulvinar] in the



Genotype	N	Diameter (mm)
A1/A1	0	-
A1/A2	10	136.0+/-6.6
A2/A2	11	133.1+/-7.8

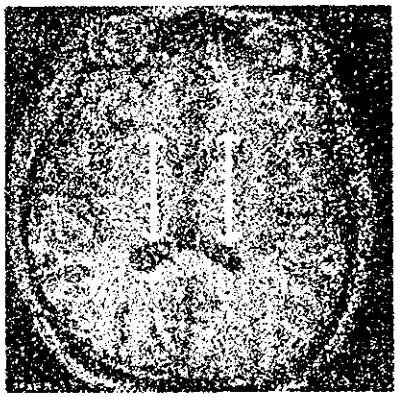
FIGURE 1. The transverse diameter of the cerebrum in a horizontal slice at the height of anterior commissure at the interhemispheric fissure.



Genotype	N	Proportion (%)
A1/A1	0	-
A1/A2	10	88.0+/-2.1
A2/A2	11	87.7+/-2.8

FIGURE 2. (The transverse diameter of the cerebrum)/(the inner breadth of the skull) in a horizontal slice at the height of anterior commissure at the interhemispheric fissure.

horizontal slice (FIG. 3), and [the vertical thickness of the genu, body, and splenium of the corpus callosum] 5 mm outside the interhemispheric fissure (midline)(FIG. 4). Furthermore, although we compared right and left laterality, plus the average about [the maximum length between the caudate nucleus and the pulvinar] and [the vertical thickness of the genu, body, and splenium of the corpus callosum], no significant difference was detected (data not shown). In [the maximum breadth between the outside of each side of the putamens] in the horizontal slice, no difference was found in comparison between the A1/A2 group and the A2/A2 group, or in comparison with the young healthy person group (FIG. 5). It was thought that the form (size) of the domain, including the thalamus and basal ganglia that were inside each side of the

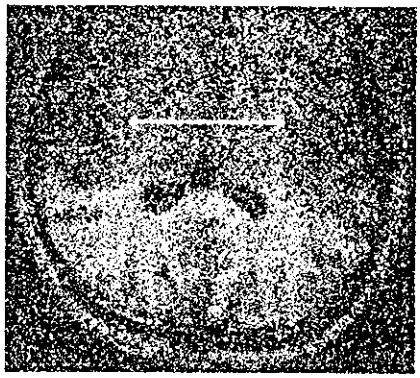


Genotype	N	Length (mm)
A1/A1	0	-
A1/A2	10	48.3+/-2.7
A2/A2	11	46.6+/-2.9

FIGURE 3. The maximum (front–posterior) length between the caudate nucleus and the pulvinar (in a horizontal slice).

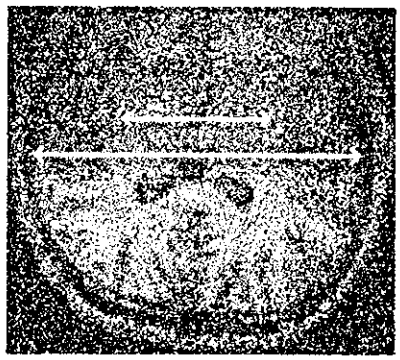
Genotype	N	r-genu	l-genu	r-body	l-body	r-splenium	l-splenium
A1/A1	0	-	-	-	-	-	-
A1/A2	10	9.5+/-1.4	10.7+/-2.5	5.2+/-1.0	5.3-/-1.4	9.9-/-1.4	10.2+/-1.7
A2/A2	11	10.0+/-2.2	10.6+/-1.9	5.2+/-1.4	5.3-/-1.1	10.5+/-1.8	10.7+/-1.3

FIGURE 4. The vertical thickness of genu, body, and splenium of the corpus callosum, 5 mm outside the interhemispheric fissure (midline).



Genotype	N	Breadth (mm)
A1/A2	10	65.0+/-2.0
A2/A2	11	65.2+/-3.8
y.h.o.	12	65.4+/-2.5

FIGURE 5. The maximum breadth between the outside of each side of the putamens (in a horizontal slice).



Genotype	N	Proportion (%)
A1/A1	0	-
A1/A2	10	47.6+/-1.6
A2/A2	11	49.1+/-1.9

FIGURE 6. (The maximum breadth between the outside of each side of the putamens)/(the transverese diameter of the cerebrum) (in a horizontal slice).

putamens, was not easily influenced by either methamphetamine psychosis/abuse or by aging and the genotype. In [(the maximum breadth between the outsides of each side of the putamens)/(the transverse diameter of the cerebrum)] in the same slice, we found that the A2/A2 group tended ($P = .06$) to show a lower value than the A1/A2-group, and it was shown that the temporal lobe (the insula), which exists on the outside of the putamens, had a tendency to be narrow in the A2/A2 group (FIG. 6). Although examination of the lateral ventricle (body and anterior horn), third ventricle, fourth ventricle, and Sylvian fissure was attempted, since the form change by the difference in one slice (1.5-mm step) was steep and complicated, the measured values could mot be reproduced, so we are still considering a measuring method.

DISCUSSION

Although recent research on evaluating the change of the form of the brain has used the technique of measuring capacity directly, such as Voxel-based morphometry (VBM),[12] in this research we used a technique based on a 2-dimensional picture that was extracted from 3-dimensional data. The reasons for this were that this latter method has the advantage that object domains can be chosen intuitively, and that it cannot be said that the validity of VBM has been established. However, it is thought that the conventional technique of measuring the picture directly on MRI or CT film has a serious problem in that reproducibility is low, as the measurement of ventricles in this research made clear. That is, since a measurement value can change considerably with only a shift of several millimeters in a slice, there is a problem in reproducibility and reliability. A research method combining the technique used in this research and the VBM technique may be effective in the future.

Although it appears that the temporal lobe having turned slightly contradicts the conclusion that there is no difference in (the transverse diameter of the cerebrum) and [(the transverse diameter of the cerebrum)/(the inner breadth of the skull)], we would speculate that the change in size of the temporal lobe was lost through deviations in cerebrum or skull size. On the other hand, there is an indication that the domain is suitable as a standard for evaluating changes in brain form, in that the value of (the maximum breadth between the outsides of each side of putamens) deviates little among all groups, including the young healthy person group. The reason that we were able to find the cerebral form change in this research is that this measurement/comparison method was used in addition to the classification procedure based on the gene polymorphism.

The objection will no doubt be raised that the reason that the size of the temporal lobe is narrow is not the result of atrophy but of nature. However, considered in the light of the previous report about neurotoxication of methamphetamine, we suggest that atrophy caused this narrowness.

The tendency shown by [(the transverse diameter of the cerebrum)/(the maximum breadth between the outsides of each side of the putamens)], that is, the tendency for the temporal lobe to be narrow in the A2/A2 group cannot necessarily be called a strong association. However, since the mean age of the A1/A2 group (51.2 ± 11.8 years) is older than the A2/A2 group (45.3 ± 7.7 years), and since brain atrophy progresses according to aging, the results as $P = .06$ mean a strong difference. Furthermore, it is also expected that a strong association will be found in patients with the A1/A1 genotype, of which there were none in this research. Ujike *et al.* state that in patients who have the A1/A1 genotype there is a longer gap between the beginning of methamphetamine abuse and the onset of psychosis, and after medical treatment they do not present prolonged psychosis and flashback as easily as those with other genotypes.[9,10] The result of this research is not only in agreement with the report of Ujike *et al.*, but shows that even if the A1 allele exists as a homozygote, it has an influence on cerebral form in patients with methamphetamine psychosis.

The distribution (A1/A1: A1/A2: A2/A2 = 0:10:11) of the TaqI A/DRD2 genotype polymorphism has clearly deviated from the equilibrium of Hardy-Weinberg. It can be presumed that in this research, since the objects were collected from inpatients, patients with the A1/A1 genotype were not included because, for them, there is a longer gab between the beginning of methamphetamine abuse and the onset of

psychosis, and after medical treatment they do not present prolonged psychosis more easily than those who have other genotypes, as Ujike *et al.* have reported. The previously stated facts suggest that TaqI A polymorphism of the DRD2 gene influences not only the symptoms and progression of methamphetamine psychosis/abuse but also changes in brain form.

We would now like to develop these findings by extending our research to patients who have the A1/A1 genotype. The results of this research will change the foregoing characterizations on methamphetamine psychosis/abuse and should prove interesting.

CONCLUSION

We examined the association between the TaqI A polymorphism of the DRD2 gene and the brain MRI view in patients with methamphetamine psychosis. The results indicate that the size of the domain encompassing the thalamus and basal ganglia was not influenced by methamphetamine abuse/psychosis, differences in the genotype, or aging. On the other hand, the temporal lobe tended to narrow in patients with the A2/A2 genotype of the TaqI A polymorphism of the DRD2 gene compared to patients with the A1/A2 genotype. This suggests that in methamphetamine psychosis the TaqI A polymorphism not only regulates prolongation of psychosis symptoms, but also influences the form of the temporal lobe.

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Gene Polymorphisms of the Mu Opioid Receptor in Methamphetamine Abusers

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ABSTRACT: **In drug addiction, the opioid system is thought to mediate motivational effects through dopamine-independent mechanisms. We have investigated associations of the μ-opioid receptor gene (OPRM) variations with methamphetamine (MAP) dependence/psychosis. The allelic frequency of A118G (Asn40Asp) in exon 1 of ORPM was 45.3% in our control subjects, but only 7.5–25.8% in the Caucasian or African-American population of previous studies. We have identified several novel polymorphisms in intron 1 and the 5′ untranslated region (5′UTR) of OPRM. Polymorphisms in the functionally rel-**

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evant 5′ regulatory region of OPRM were different in our Japanese population from Caucasian or African-American populations. No significant differences between controls and MAP abusers were found in either genotype or allele frequency at any single nucleotide polymorphism (SNP) or (AC)n dinucleotide repeat in intron 1. A subdivision of our MAP group revealed that A118G of OPRM shows a significant association with MAP psychosis having latency less than three years. Further analysis should be capable of identifying associations between the OPRM variations and MAP dependence/psychosis.

KEYWORDS: **single nucleotide polymorphism; nucleotide repeats; human μ-opioid receptor gene; methamphetamine; dependence; psychosis**

INTRODUCTION

Methamphetamines (MAPs) and other psychostimulants produce their effects by potentiating monoaminergic transmission, in which dopamine is believed to be directly related to the reinforcing effect.[1] It is generally believed that dopaminergic nerve systems interact with opioid nerve systems. Opioid receptor agonists regulate dopamine metabolism in nerve endings, regulate dopamine release into the synaptic cleft,[2] and attenuate methamphetamine-induced alterations in dopamine neurotransmission.[3,4] It is therefore possible that variations in the opioid receptor function could give rise—depending on differing susceptibilities among individuals—to the development of MAP dependence and/or psychosis.

Several single nucleotide polymorphisms (SNPs) of the μ-opioid receptor gene (OPRM) that cause amino acid substitution and other SNPs in noncoding regions or silent mutations have been reported, mostly in African-American or Caucasian populations.[5,6] Association between frequencies of polymorphisms in OPRM and opioid, alcohol, or polydrug dependence has been studied in African-American, Caucasian, Hispanic, and Han Chinese populations.[7–14] In the present study, we have screened the coding and functionally relevant regulatory regions of the OPRM for genetic variation in a Japanese population, and examined the association between novel and reported polymorphisms in OPRM and MAP abusers in Japan.

MATERIALS AND METHODS

Study Subjects

The subjects were 138 unrelated patients exhibiting MAP dependence and/or psychotic disorder (107 males and 31 females, average = 35.7 ± 1.1) meeting ICD-10-DCR criteria (F15.2 and F15.5). They were outpatients or inpatients at psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA). As controls we used 213 age-, gender- and geographical origin–matched normal individuals (158 males and 55 females, age 34.4±1.6). Most were medical staff who had no past history and no family history of drug dependence or psychotic disorders. Diagnoses were made by two trained psychiatrists based on an interview and all available information, including hospital records. Patients who had a clinical diagnosis of schizophrenia, another psychotic disorder, or an organic mental syndrome were excluded. All subjects were Japanese, born and living in Japan, including northern

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

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

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